Regional Oral History Office
The Bancroft Library

University of California
Berkeley, California

Stanley N. Cohen

SCIENCE, BIOTECHNOLOGY, and RECOMBINANT DNA: A PERSONAL HISTORY

With an Introduction by
Stanley Falkow, Ph.D.

Interviews conducted by
Sally Smith Hughes, Ph.D.
in 1995

Since 1954 the Regional Oral History Office has been interviewing leading participants in or well-placed witnesses to major events in the development of Northern California, the West, and the nation. Oral History is a method of collecting historical information through tape-recorded interviews between a narrator with firsthand knowledge of historically significant events and a well-informed interviewer, with the goal of preserving substantive additions to the historical record. The tape recording is transcribed, lightly edited for continuity and clarity, and reviewed and corrected by the interviewee. The corrected manuscript is bound with photographs and illustrative materials and placed in The Bancroft Library at the University of California, Berkeley, and in other research collections for scholarly use. Because it is primary material, oral history is not intended to present the final, verified, or complete narrative of events. It is a spoken account, offered by the interviewee in response to questioning, and as such it is reflective, partisan, deeply involved, and irreplaceable.

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It is recommended that this oral history be cited as follows:

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Stanley Cohen, 1995
Photo courtesy of University of Pennsylvania

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INTRODUCTION—by Stanley Falkow

There is no doubt that Stanley N. Cohen played an important role in the history of American biomedical science. His landmark publication with Herbert W. Boyer on a direct way to cut and splice genes from different biological sources revolutionized how we do research. As Cohen explains in his interviews, this was not a unique idea that occurred solely to him or to Boyer. Rather, there were many different laboratories working towards a similar goal. Where Cohen and Boyer triumphed was in developing a method that was straightforward and, most of all, worked surprisingly well.

Stan Cohen's description of the history of this discovery is notable for his direct, some might say blunt, description of the events as he saw them unfolding before him. It provides some fascinating reading and perhaps insights into the ecstasy of discovery and the unexpected turmoil that followed in subsequent years. I have written about some of these events, especially the legendary evening snack in a Jewish deli run by Koreans on Waikiki in November, 1972. Participants in fast moving, exciting and anxiety-provoking events do not make the best nor the most accurate or objective witnesses. However, I suppose this is what historians must tackle—how do different individuals view the same events?

Stan Cohen's memories and thoughts collected by Sally Smith Hughes are a milestone in her quest to document one of the most important events in the history of science. Cohen provides a detailed eyewitness account of a singular event in scientific history where he played a pivotal role. I believe that Stan's words and the interviews of the other participants in this drama document a paradigm shift in how working biological scientists interfaced with the public-at-large, with the press, with politicians at all levels of government, and with entrepreneurs. These interactions, which took place over a relatively short span of time, forever changed the character of biological research. I have often stated that the events surrounding the discovery of recombinant DNA technology, the public furor that followed, and the subsequent, rapid emergence of biotechnology resulted in a kind of loss of innocence by those of us in the biological sciences. I presume that the physical scientists had preceded us in this respect by several decades or more.

The one thing I can perhaps add to the account that follows is the perspective of Stanley Cohen as a person distinct from his scientific *persona*. Cohen documents our first meeting when I was at Walter Reed studying plasmids and especially R-factors. The first thing that strikes anyone meeting Stan Cohen is his intensity. It is apparent in his look, his demeanor, and even in the way he walks. He characteristically asks penetrating questions. Stan has very wide scientific interests. The assertive man revealed in this series of interviews often speaks in a surprisingly soft tone. When he hears something that is new, he says with enthusiasm, "Now isn't that interesting," almost always accompanied by a smile that mirrors his delight. On the other side of the coin, it is easy to tell when Stan is angry. If Stan says to you, "Listen Chief…", you're in trouble. His debating skills, which he developed while a university student, come to play during discussions at meetings. He argues with the data from his own lab but can turn the tables on you by using the data from your lab to make his point. The reader may note this while reading this interview.

I have known Stan Cohen for close to 40 years. We have been friends, but there were times when we were scientific competitors as well, and we passionately disagreed with one another. Yet, when I think of him, there are two events that always jump into my mind. The first is a story he shared one evening when our wives joined us for an after dinner drink shortly after my arrival at Stanford. Stan and his wife Joan recalled a time when they were struggling during Stan's medical school years to make ends meet. Stan told us the only food they could afford was chicken livers, and they bought large bags of them from the butcher. As he began to describe the various ways they tried to disguise and modify each meal to deflect the fact they were eating chicken livers for every meal, he was suddenly racked

with uncontrollable laughter until tears were running down his face. It seemed to me this story reveals the depth of Stan's desire to successfully complete his education. It was the cornerstone of his early life, and it shaped his work ethic. The other side of Stan that most people do not know, he actually reveals in his reminiscences. Stan is an accomplished musician, and he likes to sing and play the banjo. I have watched him perform, usually in the evening following a scientific meeting. He obviously derives much pleasure from this activity. Those listening to him, view him in an entirely different light thereafter. I think this is indicative of another feature of Stan Cohen that is to some extent also obvious in his recollections. He is very good at almost everything he attempts. He was a successful songwriter, and there were a number of paths he could have followed during his medical education. He was a marvelous physician, but he chose instead to concentrate on basic research. His first academic experiences put him into medical disciplines that were new to him. He became head of a Division of Clinical Pharmacology and could have become one of the leaders of that new discipline particularly in the application of computers to understanding drug interactions. Indeed, at one point in his career, he was faced with choosing between the teaching of clinical medicine or pursuing the molecular basis of bacterial plasmids. As you read below, you will see that he chose the right path.

Many of the players on the recombinant DNA stage shared a common legacy of ideas and seminal discoveries handed down from those who participated in what Salvatore Luria described as "the Golden Age of Molecular Biology." I shared this legacy and was a participant in several of the events described by Stan Cohen and was, as well, a collaborator of Herb Boyer. Thus, I am not the person to attempt to provide an objective view or historical perspective on the scientific contributions described by Stanley Cohen and his interactions with others. However, Stan's words provide an intimate glimpse for the non-scientist about the serendipitous observations that often pervade all research. The simplicity of the recombinant DNA technique may surprise some, but more often than not the great scientific discoveries are marked by their simplicity. I suspect that Stanley Cohen's thoughts and recollections will be read, pondered, and analyzed by people all over the world in years to come.

Stanley Falkow
Professor
Department of Microbiology and Immunology
Stanford University

Stanford, California
August 2009

INTERVIEW HISTORY—by Sally Smith Hughes

There are myriad aspects to this long and rich oral history with Stanley Norman Cohen,[1] best known in the scientific world (and beyond) as the inventor, with Herbert Boyer, of recombinant DNA technology. The interviews provide the most complete history to date of the three sets of experiments (1973-1974) that form the basis of the technology, a set of techniques that transformed basic bioscience and became a pillar of the biotechnology industry. Cohen also details his central role in the recombinant DNA political controversy of the 1970s over the potential hazards arising from recombinant research, including his oppositional vote at the Asilomar Conference of 1975, his experiment describing genetic recombination as a natural process, and his lobbying activities at the federal and state levels to thwart pending legislation aimed at regulating recombinant DNA research. An intriguing focal point of these interviews is Cohen's frank and carefully referenced comments on the relationship—if any—of the Cohen-Boyer method to that of Paul Berg and his laboratory, also at Stanford. Of related interest are Cohen's thoughts on Berg's receipt of the 1980 Nobel Prize in Chemistry for contributions to recombinant DNA research, an award that made no mention of the Cohen-Boyer work. Readers may wish to consult Paul Berg's, Arthur Kornberg's, and Herbert Boyer's oral histories in this series, and the wealth of scientific and historical documents presenting varying perspectives on this scientifically portentous and politically troubled period in recent biological research.[2]

In the 1970s, while actively developing and applying recombinant DNA technology in his laboratory, Cohen also had clinical duties as a Department of Medicine physician and also somehow found time to collaborate on devising and publishing a computerized drug-interaction system. He tells of his close involvement with the prosecution of the Stanford-University of California patent application on the basic Cohen-Boyer procedure and the contention surrounding that effort at a time when patenting in academic biomedical research was uncommon and the recombinant DNA controversy was escalating. In 1980, the U.S. Patent Office issued the first Cohen-Boyer patent (there are three), the first major patent in biotechnology and the subsequent generator of enormous revenues for the universities and the inventors.

The interviews also provide accounts of Cohen's research before and after the invention of recombinant DNA technology, research in which he takes rightful pride but which that key invention tends to overshadow. In 1978, he became the somewhat reluctant chairman of the Department of Genetics, succeeding his colleague and friend Joshua Lederberg and serving for eight years. Individuals who have only known Cohen as a serious and accomplished molecular geneticist may be surprised to meet in these pages a young Stan who wrote and recorded songs, one of which reached the Hit Parade, and who made his way across Europe one summer, playing his banjo and singing in cafes.

Oral History Process

The process began with a review of Cohen's extensive personal archives in his office at Stanford's School of Medicine, followed by fifteen interviews conducted over a seven-month period in 1995.[3] A scientist not given to fancy or speculation, who operates on the basis of what he considers

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[1] Stanley Norman Cohen, a Stanford University molecular geneticist, and Stanley Cohen, a Vanderbilt biochemist and Nobel laureate, are two different individuals.

[2] The oral histories are online at: http://bancroft.berkeley.edu/ROHO/projects/biosci/ For earlier interviews related to recombinant DNA science and politics, conducted by Charles Weiner and others, see the recombinant DNA collection at MIT.

[3] After Cohen completes his autobiography, he plans to donate his correspondence to the National Library of Medicine.

solid fact, Cohen spoke carefully and cautiously, sometimes stopping the recording to flip through his reprint binder or to review other documents. I edited the transcripts for clarity and sent them to Dr. Cohen for review. There they remained more or less untouched for almost fourteen years. Then in 2009 Cohen made room in his busy schedule and with characteristic care and dedication not only thoroughly reviewed and corrected the transcripts but also hired a student to prepare an index and add references to his and others' scientific publications. We are both grateful to Cohen's Stanford colleague and friend Stanley Falkow for his generous effort in writing an introduction. We also acknowledge Stanford's Green Library and Office of Technology Licensing for their financial support.

This oral history is the most complete account available thus far of the upbringing, education, and professional life of this private, sensitive, and very accomplished scientist. One hopes that the autobiography Dr. Cohen is writing will soon accompany it.

Sally Smith Hughes
Historian of Science

The Bancroft Library
University of California, Berkeley
August 2009

CURRICULUM VITAE
Stanley N. Cohen

Birthdate: February 17, 1935
Birthplace: Perth Amboy, New Jersey

Education

Rutgers University, New Brunswick, NJ	B.S., 1956
University of Pennsylvania School of Medicine, Philadelphia, PA	M.D., 1960

Postgraduate Training

Intern, Mt. Sinai Hospital, New York, NY	1960-61
Assistant Resident in Medicine, University Hospital, Ann Arbor, MI	1961-61
Clinical Associate, Arthritis & Rheumatism Branch, National Institute of Arthritis & Metabolic Diseases, Bethesda, MD	1962-64
Senior Resident in Medicine, Duke University Hospital, Durham, NC	1964-65
American Cancer Society Postdoctoral Fellow, Dept. of Molecular Biology & Dept. of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, NY	1965-67

Academic Positions (all at Stanford University School of Medicine)

Kwoh-Ting Li Professor in the School of Medicine	1993-present
Chairman, Department of Genetics	1978-1986
Professor of Genetics	1977-present
Professor of Medicine	1975-present
Associate Professor of Medicine	1971-1975
Head, Division of Clinical Pharmacology	1969-1978
Assistant Professor of Medicine	1968-1971

Professional Societies

American Society for Biochemistry and Molecular Biology, Genetics Society of America, American Society for Microbiology, American Society for Pharmacology and Experimental Therapeutics, Association of American Physicians

Selected Extramural Advisory Committees

Chemical/Biological Information Handling Review Committee, Division of Research Resources, NIH (1970-1974); International Committee on Plasmid Nomenclature (1970-1973); Committee on Recombinant DNA Molecules, National Academy of Sciences, National Research Council (1974); American Cancer Society Scientific Review Committee on Microbiology and Virology (1979-1982); Committee on Genetic Experimentation (COGENE), International Council of Scientific Unions (1977-1995); Albert Lasker Medical Research Awards Jury (1981 - 1988;
2006 -); Scientific Advisory Board, Life Technologies, Inc., (1984 - 2000); Committee on Biotechnology Nomenclature, National Research Council (1986); Scientific Advisory Board, Palo Alto Medical Research Foundation (1987-1990); Member, Board of Trustees and Board of Overseers, University of Pennsylvania Medical Center (1989 - 1997); Member, Burroughs Wellcome Fund Experimental Therapeutics Advisory Committee (1992 - 1997); Advisory Board, Program in the

History of the Biological Sciences and Biotechnology, The Bancroft Library, University of California-Berkeley (1996 - present); University of Pennsylvania Board of Trustees (1997 – 2002); Hong Kong Council of Advisors on Innovation and Technology – Committee on Biotechnology (2000 – 2001); Singapore Economic Development Board – Biomedical Sciences International Advisory Council (2000 – 2004)

Current Editorial Board Positions

Proceedings of the National Academy of Sciences
Current Opinion in Microbiology

Selected Honors and Awards

Baldouin Lucke Research Award, Univ of Pennsylvania School of Medicine	1960
Research Career Development Award of U.S. Public Health Service	1969
Burroughs-Wellcome Scholar Award in Clinical Pharmacology	1970
Josiah Macy, Jr. Foundation Faculty Scholar Award	1975
Guggenheim Foundation Fellowship Award	1975
V.D. Mattia Award, Roche Institute of Molecular Biology	1977
Fellow, American Academy of Arts and Sciences	1978
Harvey Society Lecturer	1979
Member, National Academy of Sciences (Chair, Genetics Section 1988-91)	1979
California Inventor of the Year Award	1980
Albert Lasker Basic Medical Research Award	1980
Marvin J. Johnson Award, American Chemical Society	1981
Wolf Prize	1981
California Inventors Hall of Fame	1982
Distinguished Service Award, Miami Winter Symposium	1986
Distinguished Graduate Award, Univ of Pennsylvania School of Medicine	1986
American Society for Microbiology/Cetus/Chiron Award	1988
LVMH Institut de la Vie Prize	1988
Institute of Medicine of the National Academy of Sciences	1988
National Medal of Science	1988
City of Medicine Award	1988
National Biotechnology Award	1989
National Medal of Technology	1989
American Chemical Society Special Award	1992
Fellow, American Academy of Microbiology	1992
Helmut Horten Research Award	1993
Fellow, American Association for the Advancement of Science	1994
Hall of Distinguished Alumni, Rutgers University	1994
Sc.D., *honoris causa*, Rutgers University	1994
Sc.D., *honoris causa*, University of Pennsylvania	1995
Lemelson-MIT Prize	1996
National Inventors Hall of Fame	2001
Albany Medical Center Prize in Medicine and Biomedical Research	2004
The Shaw Prize in Life Science and Medicine	2004
The Economist Innovation Award in Bioscience	2005
Einstein Professor, Chinese Academy of Sciences	2006
Member, American Philosophical Society	2006
John Stearns Award for Lifetime Achievement in Medicine, NY Academy of Medicine	2007

Interview 1: January 11, 1995

FAMILY BACKGROUND AND EDUCATION

Parents

Hughes: Dr. Cohen, I'd like to begin at the beginning, namely your birth on February 17, 1935, in Perth Amboy, New Jersey. Perhaps you could start by telling me something about your parents.

Cohen: My father was Bernard Cohen and my mother was Ida (Stolz) Cohen. My father had a particularly important influence on my life as a scientist. He had always been interested in science and, in fact, at one point had started a post-high school education at the Pratt Institute of Technology in New Jersey, but for financial reasons couldn't continue that education. During World War II, he worked in a defense plant near Perth Amboy and after World War II, he established a small business.

He had an innate curiosity about all kinds of things and was especially interested in understanding how things worked. He was employed as an electrician for part of his career, and during my childhood was involved in a number of entrepreneurial enterprises to supplement our income. I provided help in some of these. When fluorescent lighting was first commercialized, he assembled and sold fluorescent fixtures and I wired the transformers to the “starter” and transformer components in our basement. At one point, he sold electric fans, and I assembled the fans in the basement of our home. I guess I was around ten or twelve years old at the time.

My mother and father were both graduates of Perth Amboy High School. My mother worked as a secretary during my early childhood. We were not well off financially, but somehow we always managed to do things that needed to be done and to buy things we needed, and to take family vacations. In her younger years, my mother was active in several community organizations. There was a social service organization called the Golden Chain that she was particularly involved with, and both of my parents had many, many friends. My father was viewed as Mr. Nice Guy, and as I got older, I realized that because he was seen in this way, sometimes people took advantage of him.

My mother was ambitious for her family and for herself, and both of my parents were very hard working people throughout most of their lives.

Hughes: That ethic was instilled in their children?

Cohen: Yes, in both children. I have a sister, Wilma Probst, who is almost ten years younger than I. Since the age difference is so great, we grew up in very different environments. I was almost like a third parent to her.

After the Second World War, my father started a small electrical supply business. Shortly after that, his mother died, leaving a retail yarn business in Perth Amboy to my father and his brother. The two brothers became partners in both the yarn business and the electrical business. The two businesses didn’t fit very well together, and neither did well, although both families made a living.

Childhood Interests and Activities

Cohen: At one point, I became interested in hydroponics. Do you know about hydroponics?

Hughes: Very little.

Cohen: It's the science of growing plants using nutrient solutions. Throughout most of my childhood, we lived half a block from the Raritan River and near a small park along the river. We lived in a fourplex house, and I spent a lot of time with my friends hanging out around the water. There was a sand beach not far down the river, and I built some wooden boxes, filled them up with sand from the beach, and started ordering chemicals to make solutions to grow plants hydroponically. The boxes were set up on our flat-roofed garage. I think my parents took a lot of kidding that their son was growing tomatoes on the garage roof. But they encouraged me anyway, and I enjoyed it. It was one of a number of science-related activities I was involved in.

Hughes: And did the hydroponics work?

Cohen: Many of the plants died, but I did get some tomatoes. They weren't the largest or juiciest tomatoes I've seen.

Hughes: Were you in high school at this point?

Cohen: No, that was, maybe, just before high school

Hughes: Was it a disciplined household?

Cohen: Not really. It took a lot to get my father angry, and to discipline his kids. When he became angry, he sometimes became really angry and on more than one occasion, took off his belt and gave me a whop on the backside with it. But, fundamentally he was a gentle person. My mother was much more emotional. Neither parent was a strict disciplinarian, although there certainly were times when they disciplined me. When I used language that my mother thought wasn't appropriate, she would sometimes force a cake of soap against my teeth to "wash out" my mouth. I suppose that overall I wasn't much of a wayward kid, so there really wasn't a lot of need for discipline.

At one point, a group of other boys and I went down to the river and were fooling around with some of the boats that were tied up there. We accidentally set ourselves adrift and were drifting out towards Raritan Bay, which empties into the Atlantic Ocean. It was in the late winter or early spring, and we knew that if we drifted out further, it would be a while before we would be found. So we jumped overboard—I guess that was in March, and the water was really cold—and we swam to the shore. I came home dripping wet. I don't remember the excuse I gave to my parents, but it was clear to them that I hadn't told them just what had gone on. However, they were willing to let it pass.

Hughes: You essentially were raised as an only child?

Cohen: I was until I was ten or so.

Family, and Family Religion, Politics, and Ambitions

Hughes: What about religion and politics?

Cohen: My father came from a very religious family. In Jewish tradition there is a group called the Kohanim, who are descended from Moses' brother Aaron, and who served as the priests of the Jerusalem temple. Although we were descendants of this line, my father was not an observant Jew. My grandfather, Samuel Cohen, was a strict disciplinarian, and when his father died, my father rejected a lot of the religion associated with his traditional upbringing. Although overall my parents, sister, and I weren't highly observant religiously, we went to the synagogue on the High Holy Days, Rosh Hashanah and Yom Kippur, and on some other occasions. I had a Bar Mitzvah at age 13. In fact, somewhere in that file [which I lent to you] is my bar mitzvah talk, which my mother kept a copy of until her death.

I remember both of my grandparents on my father's side. My grandfather died when I was still quite young, around four or five. He worked as a butcher and had been raised in England. Some of his brothers had moved to South Africa, where they pursued medical careers. My grandfather and his wife, Bertha Samuels, who was my father's mother, had not divorced but they lived separately for many years, and she ran the small yarn business I've mentioned. My grandmother on my father's side lived until I was nine or ten, so I remember her well. On my mother's side, my grandmother, Sarah Wolf Stolz, had died in the influenza epidemic of 1918, and my mother was raised by my grandfather with the help of a neighboring family. A child of the neighboring family was my mother's lifelong friend, and I grew up thinking of her as my aunt and her children as my cousins.

My mother had a brother, Michael Stolz, who lived in Pennsylvania and whom we saw occasionally.

Politically, my parents were Democrats, and they were involved in small-town New Jersey politics in the sense that everyone knew just about everyone else in town and people with similar political views would band together to support their favorite candidates. My mother worked on election days to help supporters of the party get out the vote, but I don't think of my parents as political people.

Hughes: You said your mother was ambitious. Was she also ambitious for you?

Cohen: Oh yes.

Hughes: Your parents wanted you to rise beyond their level?

Cohen: From my mother it was obvious. I think that my father wanted that also, but he was more subtle about it. Their hopes were apparent to me, but at the same time they never were pushy.

I did well academically throughout public school in Perth Amboy, and during that time became interested in writing. I won some writing contests and other awards, and my parents were always very pleased when this happened. You could see their pleasure and pride, but they weren't ambitiously aggressive about this.

I can remember only one occasion of open parental ambition much later, after I established my laboratory here at Stanford, and my mother visited. I brought her to my lab and she looked at the door. There was a sign saying “Stan Cohen,” and she said that it should read “Dr. Stan Cohen.”

Interest in Science

Hughes: In the interview you did for MIT you said that it was in high school that your interest in science switched from the physical to the biological sciences.[4]

Cohen: That's true. When I was ten, the first atom bomb was exploded. As an eighth grader, I had entered an essay contest and had written an essay on atomic energy, which won first prize. As a result of doing research for the essay, I became interested in atomic energy, and during the next year I read a lot about atoms. I thought at that time that I wanted to be a physicist.

When I got to high school, I took the first year course in general science that was part of the normal curriculum, and during my second year took a biology course. There was a very stimulating high school biology teacher named Mrs. Florence Eggemann, who made biology exciting to me. I thought it would be more interesting to work with living things rather than with cyclotrons. So at that point my focus morphed to the biological sciences. To my high

[4] Interview with Stanley Cohen by Rae Goodell, May 19, 1975, Stanford, California. Project on the Development of Recombinant DNA Research Guidelines, MIT Oral History Program.

school career advisor, that meant being a physician. Later in high school, I decided to become a premed student and applied to college as a premed. It wasn't clear to me whether I wanted to practice medicine, but I was convinced that premedical curriculum would give me flexibility to move to other areas of biology. Whether I wanted to do basic science or take care of patients was something that I hadn't determined, and as you'll see, this uncertainty existed for a long time afterward.

Hughes: In high school, you were already thinking about biological research as a possible career?

Cohen: Yes, I was. But I was almost equally interested in writing. I was Editor of the high school paper, and Associate Editor for the yearbook. I enjoyed writing and was repeatedly told that I wrote well. For a while I thought, well, maybe I want to do scientific writing.

Hughes: Were you running with a group of friends that was planning for future careers?

Cohen: Some were; some were not. We were a bunch of high school kids fooling around, going to movies on weekends, doing sports and just having fun in various other ways.

I never excelled at sports, although I played baseball, basketball, and football, touch tag stuff, and was good at sprinting. I tried out for the high school track team, and did reasonably well. I became a member of the team but didn't run fast enough to win races. I remember having to miss one particular match because I developed a wart on the bottom of my foot and had it removed. When I brought in a note from my doctor saying that I couldn't run for a couple of weeks, I remember our coach making a big thing out of it, joking loudly, "Cohen, have you been walking on toads?" And although I was tempted to tell him that I didn't think toads had anything to do with warts in humans, I decided to keep my mouth shut.

UNDERGRADUATE, RUTGERS UNIVERSITY, 1952-1956

Choosing Rutgers

Hughes: Why did you choose Rutgers?

Cohen: Well, for a number of reasons. The principal one was that they offered me the most scholarship support.

Hughes: Which was the only way you could go to college?

Cohen: Well, that was one reason, but there were also others. I was involved at that point with a synagogue-based youth organization [United Synagogue Youth]. I was supporting myself partly by leading multiple youth groups and getting paid for that, and these groups were in Northern New Jersey. More importantly, my father had developed diabetes and neuritis and his health was poor, so I wanted to remain in the area. Overall, I was happy at Rutgers, although not initially.

Hughes: Were you living at home?

Cohen: I lived in a dormitory at the college.

The way I got involved with synagogue youth group activities is sort of interesting in retrospect. Although my family wasn't steeped in religious practices, as a teenager I had joined a youth group at a local synagogue and, in my last year of high school, was sent to a convention aimed at forming a national organization of such groups. I ended up being elected national vice president and was asked by the organization to visit various cities around the country, essentially giving pep talks to other teenagers that were starting synagogue-based youth groups. I realized that I needed to learn more about Jewish history and tradition, and I traveled into

New York from Rutgers weekly for a few months to take a course at the Jewish Theological seminary. For a while people thought I might become a rabbi.

Hughes: Was that ever an idea that you had?

Cohen: No, but some others thought that.

Hughes: Why were you unhappy that first year?

Cohen: Initially, I didn't feel a bond with many of the other students. But Rutgers is a fine university, and I found that by the end of the first year I was with a group of friends that had common interests, and in fact I still have close relationships with some of them.

Hughes: How strong was the premed curriculum?

Cohen: It was a very good premed curriculum, and I learned a lot there.

Hughes: Did you know that before you applied?

Extracurricular Activities at Rutgers

Cohen: I did, yes. But one of the things that bothered me is that many of the premeds were grinds. I worked hard at my studies as well, but I also liked to do other things. I found that wasn't the case with my premed classmates. But it sorted itself out. I ended up becoming heavily involved in extracurricular activities there as well, and was especially involved with the Rutgers debating team. Even though I ran fast enough for the high school track team, I didn't think my speed as a runner would make it on the college track team. I tried out for the debating team, and found that I was good at it. I enjoyed debating enormously. Our debating team had some excellent members during the time I was at Rutgers and we did extremely well in national tournaments.

One of the things about debating is that it's important to anticipate the opposing arguments. A debater must be prepared to debate either the negative or positive side of an issue. Sometimes you don't know until you get to a tournament which side of the issue you're going to argue, and during the same day you could be arguing for or against the same proposition. Debating helps one see both sides of an issue more clearly. During the recombinant DNA controversy, when I had to make arguments to support my position, I think that my debating background made it easier to see the opposing point of view.

Interest in Music

Hughes: During your undergraduate years, you made quite a successful foray into music. Did your interest in music begin in college?

Cohen: I had learned to play the piano as a child, although not very well. I also taught myself to play the ukulele and then subsequently picked up the guitar, which was more fun for a college student than the ukulele. I had written a few songs that friends thought sounded pretty good, and in college recorded two of them with a classmate, a guy named Bob Sileo, who had a very big and nice voice, as the vocalist, together with a group of other college musicians. We did this at the recording studio of a local radio station, and we actually had some vinyl records pressed, using a “label” I called Stanton Records. The recording quality wasn’t great, which I knew at the time, but I was foolish enough to continue with the project anyway. Maybe 25 records were sold to our friends and families. I think that I still have some of the remaining discs.

Subsequently I decided to try to get one of my songs, which was called “Only You”, recorded by a professional vocalist and was able to find a music publisher who liked the song. A New

York photographer named Jimmy Kriegsman, who was a leading photographer of pop music recording artists, also became interested in the song. With Kriegsman as a co-author, the song was published and recorded by Billy Eckstine, who was a very well-known vocalist at the time, and by two other groups. It did reasonably well.

Hughes: Well, from those news clippings that you let me look at this morning, I understand that you used at least some of the royalties from that record to fund your schooling.

Cohen: Yes, that's true. The song actually began to take off on the Hit Parade. However, the rise in popularity was aborted because of another song, initially called “Only You and You Alone,” which was recorded and released about the same time. The title of the other song was then shortened, and it also became “Only You.” Both songs were in the same style and it was confusing to radio disc jockeys. The other “Only You” was a better song, and even though the recordings of my song did reasonably well, the other “Only You” recorded by the Platters was a number one hit. Those days were a lot of fun, although I spent many hours walking the halls of the Brill Building trying to peddle my song. The building, I still remember the address, 1619 Broadway, housed most of the major music publishers of the time. George Levy from Lowell Music, which published my “Only You,” tried to interest me in staying in the music publishing business, but I didn't at all consider that.

Hughes: Do you continue your interest in music?

Cohen: When I was in medical school, I worked during one of the summers at a resort singing and playing the banjo. My daughter, Anne, is a solid musician who has perfect pitch. She points out to me that I sing a bit off key and I know that. However, I've learned that if you play the banjo loudly enough, people don't notice off-key singing.

Hughes: I know that you worked under a pseudonym, which was Norman Stanton, when you were writing songs. Why did you choose to do it that way?

Cohen: Well, not for any sound reason. Lots of songwriters had pseudonyms at the time and it was sort of fun to do. Norman is my middle name and Stanton is from Stan.

MEDICAL STUDENT, UNIVERSITY OF PENNSYLVANIA, 1956-1960

Choosing Penn

Hughes: The next step is medical school. Why did you choose the University of Pennsylvania?

Cohen: Well, I was influenced a lot by the feeling I got about a medical school when I went for an interview. I liked the feeling at the University of Pennsylvania.

Hughes: What was there about it?

Cohen: I liked the quality of the students I talked with during the interview day. I liked the way that the administrators and the interviewing faculty interacted with me. I was also attracted to some other medical schools, but there were some that didn’t appeal to me at all. I had an interview at one medical school where the person who interviewed me happened to be a psychiatrist. I knocked on the door of his office, and heard, “Come in.” I entered the room and he was standing with his back towards me, looking out the window. After standing there for a few moments, I said, “Dr. —,” whatever his name was. No response. Then after half a minute or so he spun around and said, “Well, sit down. What the hell are you waiting for?” I didn't particularly like this style of interviewing and quickly decided that I would not go there. Penn was a school that I liked and wanted to attend, and also they offered me the very substantial scholarship support that I needed.

During medical school, I also received financial assistance from the Robert Wood Johnson Foundation, which is associated with the Johnson and Johnson Company in New Jersey. With the scholarship I received from Penn plus the funds from Robert Wood Johnson, I had most of my expenses paid for nearly all of medical school.

I liked being in Philadelphia, although there were all kinds of bad jokes about the city. I had a few rocky times during my first year in medical school. On my first examination in biochemistry, I wrote answers to the essay questions up to the time limit, and at the end of the exam the instructor came around to collect the blue notebooks containing the student responses. I scribbled my name quickly on a notebook and handed it in. A few days later, the instructor came around to my lab bench and told me that the book I handed in was blank. I went running down to my locker to retrieve the lab coat I had been wearing and found the correct blue book crumpled up in the pocket. I brought up my lab coat with the crumpled notebook and handed the book to him apologetically. He said, “Well, I don't know whether we can accept it at this point.” I said, “Well, I understand, but I hope that you can.” A few days later the instructor told me jokingly with a deadpan face that the faculty had discussed the matter and concluded that if I were trying to cheat on the exam, this would be a very clever way of cheating and that I probably wasn't smart enough to concoct such a scheme, and so they accepted the exam book. The instructor later became a friend and mentor. This was a very supportive faculty. I liked the school and I liked the students. I enjoyed Penn.

Research with Charles Breedis

Hughes: Were you still considering research?

Cohen: I considered it as a possibility, but didn’t get involved in a serious way in research until my second year of medical school, when I worked as a student researcher in the laboratory of Dr. Charles Breedis in the Department of Pathology. I had become interested in transplantation immunity and was intrigued by reports in the literature about transplanted tumors being rejected for immunological reasons.

Hughes: How had you picked up on that subject?

Cohen: In the second-year Pathology course lectures, I learned about immunity to skin grafts that had come from foreign sources. I also learned that foreign cells and tissues implanted into the cheek pouch of the Syrian hamster survived better than implants made elsewhere in the animal—especially when cortisone was given to the recipient—but conflicting results had been reported by different groups. I thought this observation was interesting, and I wanted to learn whether the hamster cheek pouch was really an immunologically privileged environment, and if so, why. I designed experiments that showed that normal adult skin grafts from rabbits could survive and grow in the hamster cheek pouch, while rabbit skin implanted elsewhere was promptly rejected, even in cortisone-treated animals. These experiments resulted in my first scientific publication.[5] Then, I planned a simple experiment to try to understand the basis for the observation. I put rabbit skin grafts into cheek pouches and later grafts from the same rabbits onto the backs of the same hamsters that had received the cheek pouch implants. I saw that not only were the grafts on the backs of the animals rejected, but in some animals, the cheek pouch grafts—which had been growing well up until then—were also rejected. This suggested that once animals were sensitized by an orthotopic graft, the rejection mechanism acted throughout the animal. But the number of animals was small and the results weren't

[5] Cohen, SN. Comparison of autologous, homologous and heterologous normal skin grafts in the hamster cheek pouch. *Proceedings of the Society of Experimental Biology and Medicine*. 1961; 106: 677-680.

definitive enough to publish them.

Hughes: Had you come to Breedis with this specific research project in mind?

Cohen: I was interested in immunological rejection when I first approached him, but at that point hadn't actually worked out the details of how I would investigate this. Breedis' lab had been studying the Shope papilloma virus in rabbits, but didn't work in the area of transplantation immunity. I proposed the specific experiments after he agreed to let me work in his lab.

Hughes: How unusual was it for a second-year medical student to be doing laboratory research?

Cohen: There were many students at Penn doing that, even before the medical scientist training programs that are now so prevalent. I continued that project during the summer between my second and third years of medical school. Some of my Penn classmates were also working on research projects and spending the summer in Philadelphia. We were given modest stipends, and together rented a small house near the medical school. During warm summer evenings, we sat on the front porch of the house drinking gin with tonic after a day of lab work, and discussed our experiments. Both the science and the social interaction were a lot of fun. That was the first serious scientific research that I did.

Across the street from Penn Medical School were the Wistar Labs. Rupert Billingham, who worked at Wistar and was an expert in the field of transplantation immunity, became an additional source of advice. Billingham had trained with Peter Medawar, who had done pioneering work at University College in London on how animals react to implants from foreign sources, and Billingham himself had become well recognized as a leader in the field.

Research in Peter Medawar's Laboratory, 1959

Cohen: I thought that Medawar's lab in London would be a great place to do further work on the cheek pouch project during my last summer as a medical student, and Breedis was supportive of this idea. I had never been outside of the U.S., except for a couple of childhood trips to Canada with my family, and wrote to Medawar in early 1959 asking whether he would accept me as a summer student. Although initially he said that he didn't have space, I persisted, and Billingham wrote a letter supporting my request. Medawar decided to take me on to work in his lab and I did that at the end of my third year at Penn medical school, extending the stay into the first part of my final year [May to September 1959]. Medawar's lab was very active scientifically at the time, and the work he had carried out earned him a Nobel Prize.

Hughes: A year later in 1960. Did you have much contact with him?

Cohen: Yes, he was very accessible, although he wasn't the person in the lab most directly involved in mentoring me. Peter Brent, an associate of Medawar's, was the primary scientist that supervised my project.

While I was in Europe, I spent some time traveling around the British Isles and the continent. I supported myself by playing the banjo and singing off-key in cafes. It was a wonderful time.

Hughes: And did the research go well?

Cohen: It didn't go as well as I would have liked. I got results, but they were still not definitive enough for an additional publication. The questions I was trying to answer in Medawar's lab were answered by Billingham and his co-workers a few years later, who established the mechanism underlying the failure of the cheek pouch grafts to initiate immunity. But my time in London was a great learning experience and I enjoyed it.

EARLY PROFESSIONAL CAREER

Decision to Go to NIH

Cohen: Early in my senior year in medical school, I applied for an internship. I had decided that I was more suited to an academic career involving medical research than to the clinical practice of medicine. I was quite interested in immunology because of my work with skin grafts, and I imagined myself going on and taking an internship and residency in internal medicine, and then doing immunologically related research and teaching in a university department of medicine.

But another event affected my career very substantially. The Berlin Wall crisis occurred during my last year in medical school. Physicians were being drafted (the "Selective Service") by the Army to care for troops that were stationed in Germany. I had decided that the clinical practice of medicine was not my career goal and was able to arrange to serve instead at the NIH [National Institutes of Health].

Cohen: At Penn, I had encountered Colin MacLeod, who was one of three scientists (Oswald T. Avery, Maclyn McCarty, and MacLeod) who discovered 15 years earlier that DNA, rather than proteins, contain the genetic information of cells.[6] It has been puzzling to many people why the group did not win a Nobel Prize for this enormously important discovery. Avery died a few years after that discovery and MacLeod died some years later. McCarty is still alive and in fact just…

Hughes: Got the [Albert] Lasker [Award in Medical Research].

Cohen: Yes, and I think it's been long overdue.

Hughes: Do you think that because the paper was couched in conservative terms, there might have been some doubt as to whether they recognized the significance of their discovery?

Cohen: No, I don't think so. The data are absolutely convincing and the conclusions were clearly stated. Conservatively stated, yes, but unequivocally. It's a classic paper and much has been written subsequently about the Nobel committee's decision not to recognize its importance.

Clinical Associate, National Institute of Arthritis and Metabolic Diseases, 1962-1964

Cohen: In any case, MacLeod was a Research Professor at Penn and a friend of Joseph Bunim, who was head of the clinical branch of the Arthritis Institute [National Institute of Arthritis and Metabolic Diseases], and there was a lot of immunological research going on at the NIH related to arthritis. So, with a recommendation from MacLeod, who knew about my hamster cheek pouch work, I had the opportunity to do research at the NIH as a Public Health Service officer to satisfy my military obligation. I made arrangements to do that following an internship at Mount Sinai Hospital [1960-1961] and a year of residency in internal medicine at the University of Michigan [1961-1962]. My plan was to continue immunological work at the NIAMD.

But a couple of months before I was scheduled to arrive, the person that I had been assigned to work with decided to temporarily leave the NIH. My appointment at the NIH was for a specific two-year period [1962-1964] as a Clinical Associate in the Arthritis and Rheumatism Branch, so I looked around at other labs.

[6] Avery, OT, MacLeod, CM, McCarty, M. Studies on the chemical nature of the substance inducing transformation of pneumococcal types: Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type. III. *Journal of Experimental Medicine*. 1944; 79: 137-158.

Clinical Associates at the NIH spent most of their time in the lab, but also took care of patients that were brought to the NIH Clinical Center for investigations of new therapeutic approaches. A number of young scientists who were Clinical Associates or Research Associates at the NIH during the time I worked there later accepted university faculty positions and made some very important scientific discoveries.

Research on the Interaction of Chloroquine with DNA

Cohen: I ended up in the laboratory of K. Lemone Yielding, who had done beautiful work with a more senior NIH scientist whom you may know of, Gordon Tomkins.[7]

Hughes: Oh yes.

Cohen: Everyone knew that Gordon was one of the smartest people around at the NIH and I thought Lemone was pretty smart, too. Lemone and Gordon were working collaboratively on allosteric enzymes. These are enzymes that change their molecular conformation and substrate specificity. Lemone and Gordon were studying glutamic/alanine dehydrogenase. I wasn't especially interested in allosteric enzymes but Lemone was willing to give me a place to work in his lab and to support work on a project that wasn't along the main lines of his research, but which I was eager to carry out. I will always be grateful for that.

I had become interested in the mechanism of action of chloroquine, an anti-malarial drug that also was being used to treat arthritis. I ended up studying the interaction between chloroquine and DNA, the specificity of the interaction, what promoted it, what inhibited it. I found that chloroquine affects the functions of DNA and RNA polymerases, which were newly discovered enzymes at the time, as a result of its ability to bind to the DNA template used by these enzymes.

Hughes: Was this your first taste of molecular biology?

Cohen: Yes it was. In fact, "molecular biology" was a relatively new term then. I think I first became aware of the term when the first issue of *Journal of Molecular Biology* appeared in 1959, just a few years prior to my appointment to the NIH position.

Colleagues at the NIH

Cohen: The NIH was an idyllic environment to work in. I was a novice, but could walk down the corridor and find people that I could readily get scientific advice from. Some of the major researchers of the period were there, people like Leon Heppel who helped to educate me about RNA biochemistry. In the next lab was Victor Ginsberg who was a polysaccharide chemist but was always ready to talk about any area of science and give advice when he could. Further on down the hall was a scientist named Art Weissbach, who was a bona fide DNA polymerase maven. He had a lot of experience working with DNA and I used to depend a lot on Art for advice and guidance. A Research Associate training in his lab, David Korn, subsequently came to Stanford as Chairman of Pathology and is currently Dean of the School of Medicine here. We first became friends at the NIH.

It's funny the kinds of things you remember: The first time that I isolated DNA, I used a protocol I had gotten from David. And, in order to help the DNA precipitate at one particular

[7] Gordon M. Tomkins, M.D., Ph.D., [1926-1975] was chief of the Laboratory of Molecular Biology at the National Institute of Arthritis, Metabolism and Digestive Diseases from 1962 to 1969. In 1969 he became professor and vice chairman of the Department of Biochemistry and Biophysics, UCSF.

step, the protocol said to scratch the tube. I held the tube in one hand and scratched the outside of the tube with the other, but no precipitate formed, and I went to discuss this with David. He said, “Stan, you're supposed to scratch it on the inside with a glass rod.” That’s how inexperienced I was, but I subsequently got my DNA preparation.

The research that I did in Lemone's lab was quite productive. It led to a paper in the *Journal of Biological Chemistry*,[8] which was the premier biochemistry journal, and to a *Proceedings of the National Academy of Sciences* paper,[9] and then a couple of less important papers.

But I realized I needed to learn more biochemistry. I had taken a biochemistry course as a medical student but didn’t have any serious training in the field. I had read a lot and had learned from attending seminars at the NIH, and certainly had picked up some practical knowledge about DNA through experiments I did in Lemone's lab and by talking with other scientists at the NIH about my experimental results.

Hughes: Was there such a thing as a practical course in molecular biology?

Cohen: Not at the time. But, it was clear that if I wanted to pursue work in this area, I would need to know much more biochemistry and genetics. My interests were taking me further and further from clinical medicine and yet my formal training had been as a physician. I had taken an internship and residency in internal medicine and enjoyed the challenge of making the right diagnosis and the satisfaction of helping sick people. I found that the satisfaction that I got from clinical medicine was complementary to the satisfaction I got out of research. In research, there is nothing better than the high that comes from discovering something new and important, and also nothing more depressing than times when things aren't going well. If I were to plot out satisfaction from a research career over time, the curve would resemble the profile of mountain peaks in the Pinnacles National Monument, which is located about 70 miles south of here. There were no highs in clinical medicine as satisfying to me as when things are exciting in the lab. But for me at least, clinical medicine provided a steady level of satisfaction.

Through Art Weissbach, I was able to arrange to train as an American Cancer Society postdoctoral fellow [1965-1967] in the laboratory of Jerry Hurwitz who was a young biochemist focusing on RNA polymerase and other enzymes that interact functionally with DNA. His lab was at the Albert Einstein College of Medicine in New York. But notwithstanding my increasing interest in basic research, I planned to also use my training as a physician and decided to complete my clinical training by having a senior residency year in internal medicine at Duke University Hospital [1964-1965] before going to Jerry’s lab.

3

Senior Resident in Medicine, Duke University Hospital, 1964-1965

Duke was a place that was quite flexible in wanting to support individualized career plans and I was able to arrange to spend two-thirds of my senior residency year doing clinical work, while spending the remainder of the year beginning postdoctoral training in Jerry’s lab. So I moved from the NIH to Duke in late June 1964, and then left for New York at the end of February 1965.

Hughes: Did Duke have a policy that encouraged physicians to take basic science training?

Cohen: Good question. Duke had a very strong focus on basic science training for its physician trainees. The chairman of Medicine was Eugene Stead, who was known as a strict

[8] Cohen, SN, Yielding, KL. Spectrophotometric studies of the interaction of chloroquine with deoxyribonucleic acid. *Journal of Biological Chemistry*. 1965; 240: 3123-3131.
[9] Cohen, SN, Yielding, KL. Inhibition of DNA and RNA polymerase reactions by chloroquine. *Proc Natl Acad Sci USA*. 1965; 54: 521-527.

disciplinarian who expected a lot from his students and medical housestaff. Many of the housestaff found him intimidating, but I felt that he was a really warm person, and I liked him very much. We worked for six days a week and had every other Sunday off. Some weeks we worked seven days a week. Even on the Sundays that we had off, Dr. Stead held “Sunday School,” which meant that we would all arrive at the hospital at 8 A.M. Dr. Stead—everyone called him “Dr. Stead” and we used to joke that we thought even his wife probably called him “Dr. Stead”—conducted “Sunday School,” and one of the residents or clinical fellows would be assigned to give a talk about a new scientific advance. We would be there from eight until eleven or so, and at the end of Sunday School if it was your day off, you'd have the rest of the day away from the hospital, probably to sleep.

Dr. Stead expected directness. Of course he knew the realities of medical practice, but he simply did not tolerate excuses for sub-optimal performance. For example, if he asked about a lab test result for a patient, and if an intern said that he didn’t have a chance to do the test, Dr. Stead would look at the intern and in a soft, southern drawl, he'd say something like, “Well son, what you're telling me is that life is difficult. You don't have to tell me life is difficult; I already know that. What you're trying to tell me is that it's hard work being a good doctor.” He was tall—and sometimes it seemed as though he was about seven feet tall. The hospital beds at Duke had circular metal curtain supports around the top, and Dr. Stead would extend his arms upward and sometimes reach up to those railings. I enjoyed Dr. Stead, and learned a lot from him about life as well as about rigorous thinking in clinical medicine.

A number of years later, when Herb Boyer and I received the City of Medicine Research Award [1988], [Gene Stead] also won that year’s Lifetime Achievement Award for his clinical accomplishments and contributions to medical education. I admired him enormously and it was a thrill for me to be getting an award along with my old Chairman of medicine.

POSTDOCTORAL RESEARCH FELLOW, ALBERT EINSTEIN COLLEGE OF MEDICINE, 1965-1967

Cohen: At the end of February 1965, I left Duke to begin postdoc training in Jerry Hurwitz's lab. One of the first people I encountered there was a young graduate student named Lucy Shapiro, who is now a colleague here at Stanford and is chairperson of the Department of Developmental Biology. Lucy has always been very outspoken and was quick to say that she had told Jerry that she felt that he should not have accepted me to his lab. She expected that because I am a physician, I would not actually be using the scientific training I would receive in his lab, and it would be wasted. There's not a whole lot one can say in response. But, soon after that rocky beginning, Lucy and I became good friends and have remained close friends over many years. I've teased her occasionally about that conversation.

Research on Lambda Phage Development

Cohen: Most people in Jerry’s lab were working on enzymatic methylation of nucleic acids or on other biochemical projects. Possibly because of my limited background in biochemistry, Jerry assigned me to a partly genetic project that wasn't mainstream in his lab. He wanted me to try to learn something about the basis for transcription selectivity by RNA polymerase during development of bacteriophage lambda. Jerry was one of the discoverers of RNA polymerase. The project was related to Jerry’s interest in factors that affect the RNA polymerase interactions with DNA, but the genetic component was new for Jerry and no one else in the lab had been working on anything similar.

It had been found earlier by several labs that fragments of lambda DNA produced by mechanical shearing could be physically separated by centrifugation in cesium salt gradients. The genes responsible for the phage functions expressed early in the life cycle mapped genetically to approximately one half of the lambda genome, the left half on a genetic map, whereas the genes involved in later functions mapped to the right side of the genome. Jerry was interested in learning whether there was differential transcription of these two sets of genes by purified RNA polymerase *in vitro*.

And so I set out to mechanically shear lambda DNA and separate its two halves using the centrifugation approach that had been reported previously, and I tested the ability of purified *Escherichia coli* [*E. coli*] RNA polymerase to differentially transcribe the genes on the two lambda DNA fragments. The hypothesis from the genetic experiments done *in vivo* was that the bacterial RNA polymerase might be able to transcribe only the "early" genes and that proteins encoded by these genes would then facilitate transcription of the "late" genes. My experimental results showed that the early genes were, in fact, preferentially transcribed by the polymerase.

Hughes: Did your previous experience in molecular biology at the NIH give you the tools that you needed for this research?

Cohen: No. I had isolated DNA before and had done work with RNA polymerase and DNA polymerase at the NIH, but I had never purified any protein myself. In Jerry's lab I spent time in the cold room and learned to actually purify enzymes. I learned a lot, not only from Jerry, but also from the other postdocs and students that were in his lab.

My experiments showed that transcription was initiated preferentially at promoters located on the left half of lambda DNA, and then set out to ask questions about strand specificity and directionality of transcription on lambda DNA. Results published a short while earlier by others showed that DNA strands could be physically separated by gradient centrifugation using a particular reagent [polyguanilic acid] that can bind preferentially to the two strands and enables their separation in cesium chloride density gradients. And so I set out to do that with lambda DNA.

I learned additional DNA separation techniques and carried out experiments that produced a map of transcripts made *in vitro* on the bacteriophage DNA template. My results showed that transcription of some lambda genes is initiated on one DNA strand while some lambda genes are transcribed from the other strand. This work yielded publishable results that I was happy about, but similar experiments were being done concurrently by other groups of scientists and I was scooped on the publication of some of the findings.

Since the genetic and biochemical techniques I was using were totally new to me and most were also new to Jerry's lab, I needed a lot of advice from people outside of the lab. Some of the advice came from Julius Marmur, who was a faculty member in the Department of Biochemistry at Albert Einstein, and from Carl Schildkraut in that department. Advice on lambda phage genetics came from Betty Burgee, who was at Cold Spring Harbor and had worked for many years with Al Hershey, who had done pioneering work on the exchange of genetics information by viruses. A former student of Jerry's named Anne Skalka, who was a close friend of Lucy Shapiro and also has become a good friend of mine, was working at Cold Spring Harbor, collaborating with Waclaw Szybalski at the University of Wisconsin in studies of lambda gene expression. It was an area of very active investigation.

Hughes: You liked the activity?

Cohen: Well, yes and no. I felt that the competition in the area of lambda biology was a bit too intense, but I certainly liked the excitement. I was invited to meetings to present my results and was invited to give seminars at universities.

It was during one of these seminars that I first met Jim Watson. Mark Ptashne, who was

working with lambda and lambda repressor, invited me to give a talk at Harvard, where Watson was a department chair, and Watson came to the seminar. During my entire talk, he sat in the first row and read the *New York Times*. Presenting my work in that setting as just a postdoc was a big event for me, and I was depressed that Watson seemed to find the work boring. But then at the end of the seminar he asked a number of insightful questions, so it was clear that he had been listening. I suppose that one of his minds was on the *New York Times* and another was focusing on my presentation.

Interview 2: January 18, 1995

Developing an Interest in Antibiotic Resistance

Cohen: Because there was little expertise in viral genetics in Jerry's lab, he arranged for me to take courses at Cold Spring Harbor during the summer of 1966. Each course offered total immersion in lectures and lab work for a few weeks.

I spent essentially the entire summer at Cold Spring Harbor taking two courses sequentially, one in phage genetics and one in bacterial genetics. During both, there were visiting speakers. One of the speakers in the bacterial genetic course was Richard Novick, who had started an independent lab at the Public Health Research Institute in New York City after completing postdoctoral fellowship training with Rollin Hotchkiss at Rockefeller University. Richard was studying staphylococcal plasmids.

At that time, there was general awareness that antibiotic resistance was becoming a serious problem. In fact, during my training at Penn, I had learned about a medical resident who died from antibiotic resistant staphylococcal pneumonia; the microbe that caused his death was resistant to every known antibiotic that was available at the time and his infection was not treatable. But there wasn't much known about the genetic basis for resistance. Richard's seminar made the connection between antibiotic resistance and plasmids.

About the same time, two papers were published in the *Journal of Molecular Biology* on the molecular nature of antibiotic resistance plasmids: one by Stanley Falkow and his collaborators[10] and a second by Bob Rownd's group.[11] These papers were published in succeeding issues of the journal. What interested me especially about those papers was that the plasmids that Falkow and Rownd groups were studying could be physically separated from chromosomal DNA in some species of bacteria that they had been transferred to, using differences in buoyant density in cesium chloride gradients.

A few years before then it was discovered that resistance traits could be transferred between closely related bacteria. The work by Falkow and Rownd showed that multiple new bands of DNA were sometimes detectable in the recipient bacteria after transfer of resistance. What led to the occurrence of multiple bands wasn't known, although it had been hypothesized that the bands were resistance gene components and transfer gene components of plasmids.

Antibiotic resistance was an important medical problem, and I thought that some of the background and tools that I was using in my lambda studies might be applicable to studying

[10] Falkow, S, Citarella, RV, Wolhheiter, JA. The molecular nature of R-factors. *J Mol Biol*. 1966; 17 (1): 102-116.

[11] Rownd, R, Nakaya, R, Nakamura, A. Molecular nature of the drug-resistance factors of the Enterobactericeae. *J Mol Biol*. 1966; 17 (2): 376-393.

plasmids. The approaches I had worked out to separate the halves of mechanically-sheared bacteriophage lambda DNA might be used to separate plasmids and plasmid DNA fragments from each other. My interest was in learning how resistance plasmids had evolved and how the resistance and transfer components of plasmids interacted functionally. Doing this would require identifying and mapping the genes that determine different plasmid functions.

Decision to Study Plasmids

Hughes: Was anybody else taking that particular approach?

Cohen: Well, I found out later that a couple of other groups were, but overall, plasmid biology was a very quiet area. Much of the molecular biology world was focused on phage. An important reason was that by using phage, it was possible to make identical copies—clones—of the progeny of a single DNA molecule: the phage genome. A cell infected by a phage makes thousands of replicas of the infecting virus during the normal viral life cycle. And so, it was possible to study the effects of a mutation in a single virus by producing a large population of viruses identical to the mutated one. But it wasn't possible to make clones of individual plasmids, and there weren't many scientists interested in plasmids anyway.

The fact that plasmid research was a sort of backwater of molecular biology was to me an attractive aspect of working on plasmids. I had been trained as a physician and had spent years learning clinical medicine, and I planned to look for a job in a Department of Medicine. I thought if I tried to compete with the hotshot labs working on phage, it would be difficult to do because I expected to also have clinical responsibilities. Antibiotic resistance was certainly a medically relevant area, and I thought that with only a few labs working on plasmids, and only a few papers being published every year, I could contribute something meaningful in an area that was very quiet—at least at that time.

Hughes: In your M.I.T. Oral History, you say you got in touch with Falkow, which was an obvious thing to do. He was interested in plasmid epidemiology as much as he was in their molecular biology and I gather that was an unusual combination of interests.[12]

Cohen: Right. His overall interests were largely in understanding how bacteria cause disease. He had interests in molecular biology but he viewed himself principally as a microbiologist. And he told me that he was planning to end his molecular studies of plasmids.

Falkow was encouraging and helpful to me in entering the field. We'll talk in a little while about how I went about a job search, but Jerry Hurwitz, my advisor, advised me not to move to a Department of Medicine. He thought it would be difficult to do serious research in a clinical department and tried his best to persuade me to take a job in a basic science department. Of course I considered his advice seriously, but decided in the end that I had invested so much of my life being trained in clinical medicine that I would try to combine clinical activities with basic research. I also had the concern that my experience in basic genetics and biochemistry was relatively limited, but I knew that I was a competent physician.

Hughes: One could argue that you could have had a basic science appointment and then practiced medicine.

Cohen: Not really. It just doesn't work that way in medical schools. Faculty in basic science departments usually do basic science research and teaching full time. If someone wants to also treat patients and teach clinical medicine, it usually means having a primary appointment in a

[12] Interviews with Stanley Falkow by Charles Weiner, May 20, 1976 and February 26, 1977. MIT Oral History Program.

Department of Medicine, Pediatrics, or another clinical department. But the academic environment at that time was very conducive towards doing basic research in clinical departments, and many clinical departments were trying to attract young physicians who had been trained scientifically. The hope was that these faculty members would provide a connection between clinical medicine and the basic sciences and would introduce more science into medical practice. So it turned out that my career goals were consistent with what many leaders in medical education were thinking.

Hughes: Did the NIH support that model?

Cohen: Definitely. And subsequently that model morphed to the medical scientist training programs implemented at many or most medical schools. Many physicians who received training in the basic sciences at the NIH did move to faculty appointments in clinical departments, but some have not.

In 1967, about a year before I left Jerry's lab, Falkow organized a symposium at Georgetown on antibiotic resistance plasmids. I attended the symposium and afterwards asked Stanley for some of the bacterial strains that I would need to begin my work. He was very generous and that was important in getting my plasmid experiments going.

Initial Postdoctoral Plans

Cohen: I suppose that I should say something more about the job hunt that brought me to Stanford. When I was at Duke as a senior resident in medicine, I got to know Jim Wyngaarden, who subsequently became Chair of the Department of Medicine at the University of Pennsylvania. Jim knew of my career plans and recruited me for an assistant professor position in Medicine at Penn. The prospect of returning to Penn was very attractive to me. As I've already said, I liked being a medical student there and I had good feelings about the place. Being a member of the Penn faculty would be sort of like "going home."

I accepted Jim's offer, and while still a postdoctoral fellow in Jerry's lab, traveled on weekends from New York to Philadelphia with my wife Joan to look for a house to live in. After several months, Joan and I found one that we were interested in buying in a suburb of Philadelphia. I phoned Jim at his office to let him know, and he said that he had just then been trying to reach me in New York tell me that he had decided to return to Duke. My appointment at Penn had been approved, I could still go there, but Jim said he hoped that I would join him in the Department of Medicine at Duke, which he would be leading. I hadn't especially liked living in North Carolina during my residency at Duke, but Duke was an excellent place medically and scientifically, and the offer was attractive. I didn't think it made sense to move to a chairman-less department at Penn.

I visited Durham to look at the lab that Jim was offering and to make a decision. There was a heavy smell of freshly harvested tobacco, and the heat and humidity of the Durham area were particularly oppressive at the time of my visit. When I opened the door to the air-conditioned car I was traveling in, my eyeglasses fogged up. I realized that I just wasn't happy about the prospect of returning to North Carolina. But deciding not to accept Jim's offer was difficult, since I liked and respected him, and I didn't have another job. Jerry Hurwitz offered to let me stay on as a postdoc and generously arranged for an interim appointment as an Assistant Professor of Developmental Biology and Cancer at Albert Einstein. I held this position for most of a year [1967-1968], while I searched for a permanent job.

Hughes: You didn't consider staying at Albert Einstein?

Cohen: I did briefly, but felt that it wasn't wise to continue with a career at the same institution as my

mentor. Jerry was a very well known scientist, and I thought that it was important for me to be in a separate place and work independently. If I stayed at Einstein we would continue to publish together and my research program wouldn't be viewed as being independent. I was ready to start my own laboratory.

Decision to Move to Stanford

Cohen: Jerry has a lot of friends in the field of biochemistry. He had been in the same department at Wash U. [Washington University in St. Louis] as Arthur Kornberg and he was a friend of Paul Berg. Paul was working on RNA polymerase, partly in competition with Jerry, but he and Paul interacted very amicably and on a relatively frequent basis. Jerry also knew Dale Kaiser and other former members of the Biochemistry Department at Wash U. that Kornberg had brought to Stanford. One day Dale was visiting Jerry's lab, and Jerry told him that I was looking for a job. Dale and I knew each other because of my work with lambda. Dale was one of the leaders in the lambda field; he and I had been at several scientific meetings together and had talked pretty extensively about lambda biology. Dale suggested that I consider moving to Stanford, and I thought that was an interesting idea. Subsequently, Jerry had a discussion about this with Paul Berg, and the Stanford possibility progressed a little further.

Hughes: Berg was chairman?

Cohen: No, Kornberg was Chairman of Biochemistry. But, because of Paul's interest in RNA polymerase, he also knew of my work. He offered to speak with Halstead Holman who was Chair of Medicine. Shortly afterwards, I received an invitation to give a seminar at Stanford, essentially a job seminar. This was an interview for a possible appointment in the Department of Medicine here.

Hughes: Stanford was one of the places where the clinical departments were interested in a basic science orientation?

Cohen: You bet. Holman was strongly focused on that notion.

So I traveled to Stanford, and gave two seminars, one for the Department of Biochemistry in its library, and one for the Department of Medicine. The Department of Biochemistry seminar was very well attended, in fact the room was packed, and it was clear that a lot of faculty, students and postdocs were interested in the work that I had been doing with lambda. There were good questions and enthusiastic discussion, and I enjoyed that very much. It was apparent that I had passed this biochemistry test; I knew that Paul and his colleagues were going to support my appointment.

I also gave a seminar in the Department of Medicine, in what I think was possibly the smallest lecture room at Stanford. There were only ten or so members of the Department of Medicine faculty that attended, and even though the room was small, it seemed empty. I think that the few people who were there had come because they had been asked, or felt obliged, to appear. Most of them were members of the Department of Medicine division that I was being interviewed for. I was disappointed that there were not more people in Medicine that had an interest in the work, since this was said to be a department that was strongly basic science oriented.

Anyway, after my visit I was offered a Stanford position as Assistant Professor of Medicine. Hal Holman, who was recruiting me, knew that I had not been to the West Coast before coming for the initial interview. He proposed that I come out for a second visit and said that he would provide a rental car and several days of expenses for my wife and me to travel around Northern California and decide whether this was a place where we wanted to live. We drove to Yosemite

and up the coast, and the beauty of California helped to attract us to come here.

JOINING THE STANFORD FACULTY

Starting a Research Program and Trying to Become a Hematologist

Cohen: The job opportunity itself had its pluses and minuses. I knew many of the biochemistry faculty and they were interested in me and my work, and I felt that I would have strong scientific support from them. I expected that I would be able to discuss science with the Biochemistry Department faculty readily, and I liked that idea.

The negative aspect was that the Department of Medicine position I was being recruited for was in the Division of Hematology and I had not been trained at all as a hematologist. At the NIH, where I had been a Clinical Associate, I learned something about clinical arthritis and immunology, but not hematology. And yet the department was interested in having a molecularly oriented hematologist and they wanted me to become one. Despite my misgivings about this plan, the overall attractiveness of the offer convinced me to accept. But after a couple of months of participating in clinical hematology rounds and conferences, I began to have concerns as to whether I had made the right decision. Looking at blood cells under the microscope and deciding whether the granules they contained were “big” or “small” or were stained pink or blue, which was an important and necessary part of hematological diagnosis at the time, was not something that I enjoyed. But I had made a commitment to try to learn to be a hematologist and I expected to do that.

Hughes: Had you also made provisions to protect your research time?

Cohen: Yes, and I was very hard-nosed about this issue. In fact, I had a discussion with Arthur Kornberg about this shortly after coming to Stanford. I had known Arthur from his visits to Jerry’s lab in New York, and I visited him soon after moving here. Arthur, who has always been very direct said, “Well, I hear you're going to be a hematologist.” I said, “Yes, I’m planning to try.” He said, “Well, that's a very demanding medical subspecialty,” and said that he didn't imagine that I would do significant research if I had a lot of clinical responsibilities. I told him that I was eager to protect my research time and I expected to seriously pursue studies of plasmids. He then said, “Well, if you're going to be seriously involved in research, then you're going to neglect your clinical responsibilities, and that's not appropriate either.”

Arthur was pointing out, and it's true, that it is difficult to pursue a career in clinical medicine and basic science at the same time unless the basic science is very closely related to the clinical activities. Some of my colleagues on the Stanford faculty—Hugh McDevitt is one—have done that very successfully. Hugh has made major contributions in the area of immunology, and his clinical involvement has been in rheumatology and clinical immunology. But I was proposing to work on plasmids and at the same time was trying to learn to be a hematologist, and perhaps that wasn't a very smart way to proceed.

Hughes: Kornberg was probably also speaking from his own personal experience, was he not?

Cohen: Arthur had been trained as a physician. After some difficult clinical experiences during World War II, following his graduation from medical school, Arthur decided to abandon clinical medicine. But I've never discussed that decision with him.

Arthur also told me he thought that plasmids were not a very interesting area to be working in. He said that most of the things that were important to know about plasmids were probably already known, and that I should study something meaningful, like phage. So this wasn't a very comforting introduction to Stanford. I should say, in fairness to Arthur, that he was making

points that he felt strongly about, and his style is to leave little room for uncertainty. In retrospect, he was right about the difficulties of successfully pursuing a career in both clinical medicine and basic science, but wrong about plasmids.

Interactions with Faculty in Departments of Biochemistry and Medicine

The other faculty in the Biochemistry Department were quite helpful to me in many ways. When I began my laboratory here, I continued for a while to study bacteriophage lambda gene expression, essentially extending some experiments I had started in Jerry's lab, while beginning work on plasmids. The laboratory that was assigned to me by Holman wasn't ready to be occupied at the time of my arrival, and Dave Hogness, who also worked with lambda, generously provided temporary space in his laboratory for me to use for a few months. As a result, I became friends with additional people in the Biochemistry Department, especially with the postdoctoral fellows and students. Most of the Biochemistry faculty were considerably older than I was, or at least it seemed that way, and I viewed the students and postdocs more as my contemporaries. Out of those interactions came relationships that were important in my later work.

Hughes: So perhaps it was a blessing in disguise that you did not immediately get lab space in the Department of Medicine.

Cohen: That's right.

I interacted heavily with two separate groups of people during my first years at Stanford. One group included the biochemistry postdocs and students, and Peter Lobban was one of those students. We'll talk about his work in a little while. Lou Reichardt, another biochemistry student that I talked with a lot, shared a lab with Peter. Fred Welland was a young physician who had been trained in oncology and was receiving research training in Hogness' lab during the time that Hogness let me use one of his lab benches. Fred was especially helpful to me during my early months here. Just a short while later, Fred tragically developed an incurable cancer and, as an oncologist, he knew the prognosis and killed himself.

I also became close to a group of young faculty in the Department of Medicine, all of whom had very solid clinical training and also significant training in basic science. Holman had recruited all of us within a relatively short space of time. Our laboratories were located near each other on the first floor of the S-wing of the medical school. They were Hugh McDevitt, who had discovered the genetic basis for the immune response and has become a world-class leader in the field of immunology, Tom Merigan, who became the head of the Infectious Disease Division shortly after his arrival at Stanford and has done important work on interferon and HIV, Frank Stockdale, who was a medical oncologist as well as a first-rate basic scientist who now has shifted his appointment primarily to the biology department, Bill Robinson, who was an excellent virologist, and me. We all had received rigorous basic science training and were here in clinical department appointments. We became friends and colleagues in the enterprise that Holman was trying to create: a Department of Medicine that was oriented towards the basic sciences.

Hughes: Now were any of these people that you just mentioned in medicine interested in a molecular approach?

Cohen: Yes, they all were in different ways.

Hughes: Were you doing such things as attending lectures and seminars in the Department of Biochemistry?

Cohen: Well, I was, but Merigan and McDevitt and Robinson were more closely aligned with the

Department of Microbiology and Immunology. Bacterial plasmids were certainly related to microbiology, but my basic science connections were largely with the Department of Biochemistry because of my earlier research, and that department was where the lambda people were. A lot of work with viruses was being done in the Biochemistry Department, and you might ask, why not in microbiology? Well, the department name isn't necessarily related to the area of faculty scientific interest.

Hughes: I have heard it said, and it's been from UCSF scientists who may have an axe to grind, that Stanford is more insular than UCSF, that the departments are more hierarchically structured than is true at UCSF.

Cohen: I don't know that the correct term is "hierarchically structured." I wouldn't necessarily agree with that, but it's true that departments at Stanford historically have existed as discrete units. That's probably part of the Kornberg legacy. Kornberg brought his colleagues from Wash U. to Stanford and created an elite Department of Biochemistry. The department faculty was a very distinguished one, and frankly, some of the faculty had an elitist attitude. I was accepted as a young Department of Medicine colleague and my science was respected by the Biochemistry department, but I think that some members of that department had different standards to evaluate science being done in a medical department and expected less. I had initially thought that a joint appointment in Biochemistry might be a reasonable way to formally recognize the scientific relationships that existed *de facto*, but soon recognized that this was not likely to happen.

Hughes: Joint appointments were not very common?

Cohen: They were quite uncommon at the time at Stanford. In fact, I wasn't aware of anyone who had a joint appointment in a clinical department and a basic science department when I first came here, and soon realized that the biochemistry department especially wouldn't be likely to make joint appointments, at least at that time.

Hughes: Is that largely an issue of power?

Cohen: I can't really assess that. I was a assistant professor just trying to get my research program started, and I appreciated whatever scientific help and advice I could get from members of the Biochemistry Department. We interacted amicably and closely. And whether there was some formal relationship with the biochemistry department was not an issue for me. The question I was trying to deal with was whether I wanted to continue to do hematology and make clinical rounds on patients who had hematological diseases.

Clinical versus Research Activities

Cohen: Caring for hematology patients was challenging. Most of them were very ill, and while I had been well trained in clinical medicine, I hadn't treated patients during the years I spent in the Hurwitz lab, and some relearning was necessary. Secondly, I found that even though I had assurance that I would have a large fraction of my time protected for research for at least the first year or two, there were increasing clinical incursions and requests to spend more and more time clinically. I knew that if I did this, my research program wouldn't get off the ground. I wasn't inclined to be more involved with patient care, and yet I wanted to do a good job clinically.

By early 1969, my plasmid work had progressed to the point where I had evidence that multiple molecular classes of resistance plasmids can exist concurrently in *E. coli* as DNA circles. These

results were published in *Nature* in the last issue of the year.[13] Chris Miller, a young Berkeley graduate I had hired as a research assistant, was included as a co-author. Some months later, Chris and I extended these findings in a long paper in the *JMB*, and in October 1970 reported the isolation of the separate transfer unit of plasmid DNA.[14] My reading of the literature was mostly in molecular biology, biochemistry, and microbiology and not so much in clinical medicine, except during the time when I was assigned to the attending or consulting service. I found that continued clinical involvement was becoming increasingly difficult. As an attending physician on the medical service, I phoned the medical resident the night before to learn what patients would be presented to me by medical students for discussion on the following day, so I could refresh my knowledge of those diseases and have something meaningful to teach to the students.

Starting the Division of Clinical Pharmacology

Hughes: I think this is the time to bring in the Division of Clinical Pharmacology.

Cohen: In 1969, after trying for more than a year to become interested in clinical hematology, I decided that it just wouldn't happen. I discussed this with Hal Holman. He said, "Well, we'd like to keep you here on the faculty. What would you like to do medically?" I thought it was a remarkably open and generous response. I said I was potentially interested in the emerging field of Clinical Pharmacology. There was a need for better research on drug effects in patients, and for better teaching of rational drug therapy, and I had some ideas about developing computer-based systems to provide advice to physicians in this area. Particularly about drug interactions.

Hughes: Now was that an idea whose time had come in medicine?

Cohen: Well, I don't know that its time had come, but it was an idea that I had at the time. Possibly it was a little premature. At the NIH I had studied the mechanism of action of chloroquine, and during my clinical work at Stanford, I became interested in the ability of concurrently administered drugs to affect the actions of another drug. Hal Holman said, "Okay, if you want to start and lead a Clinical Pharmacology division, go ahead."

Ken Melmon, whom I knew from the NIH and who at that time was head of Clinical Pharmacology at UCSF, was a great help in letting me visit UCSF to observe the workings of the Clinical Pharmacology program there. I spent several weeks traveling back and forth to UCSF. During that time, Ken and I, who had only been casual friends previously, got to know each other much better. Ken subsequently came to Stanford as Chairman of Medicine when I was Chair of Genetics and we worked closely as department chairpersons. We have continued to have a close friendship for many years.

Holman's go-ahead enabled me to leave Hematology and start the Division of Clinical Pharmacology in the Department of Medicine. An important factor in establishing my credibility in Clinical Pharmacology was that I received a Burroughs Wellcome Scholar Award, which was a very prestigious award in the field. The Burroughs Wellcome Fund was, and still is, a charitable foundation that supports the development of clinical pharmacology programs at universities in the U.S. I was nominated by Stanford as a candidate for the 1970 Burroughs Wellcome award, and I guess on the basis of my research work and the clinical pharmacology program that I proposed to establish, I was selected. That award provides funds intended to free

[13] Cohen, SN, Miller, CA. Multiple molecular species of circular R-factor DNA isolated from *Escherichia coli*. *Nature*. 1969; 224: 1273-1277.

[14] Cohen, SN, Miller, CA. Non-chromosomal antibiotic resistance in bacteria. II: Molecular nature of R-factors isolated from *Proteus mirabilis* and *E. coli*. *Journal of Mol. Biol*. 1970; 50: 671-687.

up more time for laboratory research by recipients. I also applied for and received a Career Development Award from the NIH, which is also intended to provide salary support to enable recipients to focus on their research. So Stanford didn't have to pay my salary, and with the approval of Burroughs Wellcome, I was able to use the funds I received from them to help support my research program more directly. So I got off to a good start in clinical pharmacology, as well as in my lab. But the Pharmacology Department here did not like the notion of having a Division of Clinical Pharmacology within the Department of Medicine, and was not especially supportive during those early years.

Hughes: They saw you as a competitor?

Cohen: Well, that's an inference I wouldn't want to make. I think that perhaps they felt that I really didn't know a whole lot of pharmacology. They saw me as a biochemist, molecular biologist, or microbiologist, rather than a "card-carrying" pharmacologist; I was working with phage lambda and on plasmid biology, and it was hubris for me to begin a program in clinical pharmacology. In reality, I was more of a Clinical Pharmacologist than hematologist, but without the credibility that resulted from the Burroughs Wellcome Scholar Award, it would have been impractical to make the transition. Holman and I both thought that I could contribute to the field of clinical pharmacology, and the Burroughs Wellcome Fund thought that also.

Hughes: And how long did that last?

Cohen: The Division of Clinical Pharmacology that I started continues to exist. Initially, I was the only member of the Division. Then, I was able to recruit the support of Leo Hollister who had a lab at the VA [Veterans Administration hospital] at the time. Leo was a *bona fide* clinical pharmacologist who was doing very nice research on psychoactive drugs. His research contributions were well recognized outside of Stanford, but hadn't been adequately acknowledged at this university. He had an appointment in the Department of Medicine, but wasn't part of the "mainstream." I think he didn't even have a tenure line appointment at that time. Yet, he was more senior than I was and certainly was more experienced as a clinical pharmacologist.

Hughes: Do you think that lack of acknowledgment had something to do with his location at the VA?

Cohen: I think in part it did. I also think that Leo was a very low-key person, and many people had the impression that he was less capable than he really was. I thought he was a smart and very able guy and was happy to have his participation in the development of a Clinical Pharmacology Division, and he was happy to be brought into the mainstream of Stanford faculty. Together, we were the nucleus of what became a successful Division of Clinical Pharmacology. Soon afterwards, I recruited another faculty person, Terry Blashke, to the Division faculty, and the Division continued to expand. I remained head of Clinical Pharmacology until I shifted my principal appointment to the Department of Genetics in 1978.

Computer-Based Research On Drug Interactions and Antimicrobial Therapy

Cohen: In my role as a Clinical Pharmacologist, I worked on developing a computer-based reporting system to alert physicians about possible drug interactions. The drug interaction project attracted an extraordinarily capable first year medical student, Ted Shortliffe [Edward H. Shortliffe], who was the driving force in still another project in my lab: the development of a computer-based expert system to provide advice about antimicrobial therapy. Ted had done undergraduate work in medical computing at Harvard, and after considering different research options at Stanford, asked to work with me, and I was very happy about this. Ted applied to the MSTP (Medical Science Training Program), and although it took some persuasion from me to convince the selection committee that computer-based medical research was really "science,"

he was selected as an MSTP student. Ted's research was interdisciplinary and didn't fit into any particular departmental program, so an interdepartmental committee was put together to eventually determine whether he qualified for a Ph.D. degree. That worked, and was in fact necessary because I did not have an appointment in a Ph.D.-granting department. Ted subsequently has had a remarkable career in academic medicine, and has become one of the leaders in the medical use of artificial intelligence methods. He's now at Stanford as a Professor of Medicine.

We established a collaboration with Tom Merigan and Stan Axline [Stanton G. Axline], another young faculty member in the Division of Infectious Diseases, and began to develop a system that Ted named MYCIN. We used a rule-based approach to provide medical advice about antimicrobial therapy. This meshed well with my interest in resistance to antimicrobial drugs. MYCIN and the drug interaction reporting system, which was called MEDIPHOR [Monitoring and Evaluation of Drug Interactions by Pharmacy-Oriented Reporting], were both very successful projects, and over a period of years I had substantial grant support for both projects from the NIH.

As the size of the group I put together to do computer-based research in clinical pharmacology increased, we outgrew our space. I was able to get funds from the NIH to rent a small, pre-fabricated building, which was installed in a Medical Center parking lot. It included offices, a little conference room, and a room that contained several computers. This was before the days of PCs [personal computers], and we used small mainframe computers to do our analyses. So in the early 1970s, I was heavily committed to both my basic research on plasmids and to clinically-related, computer-based research. All of this provided a lot of intellectual stimulation and fun, but it became increasingly difficult for me to concurrently pursue two careers, one as a clinical pharmacologist and another as a basic scientist working on plasmids.

Hughes: How did you balance those two lives?

Cohen: With difficultly.

Hughes: Did one or the other suffer?

Cohen: I don't know for certain. In principle, yes, one or the other probably suffered, but it wasn't apparent. Things seemed to be going well in both areas. I was working very hard and getting data that I was being invited to present at scientific meetings, and I was publishing our findings in first-tier journals.

There was also some time spent writing a book on drug interactions.[15] That came about through a situation where I had agreed to write the book in collaboration with a clinical pharmacology postdoctoral fellow, Marsha Armstrong. I subsequently became so involved with my research activities that I wasn't eager to proceed, but she was very insistent. I ended up deciding to do the book, and it turned out to be a useful contribution to the field at the time.

Joining the Department of Genetics

Cohen: Other than the work I was doing in Clinical Pharmacology, my research activities took me further and further from clinical medicine. But before we talk about my lab research, I should tell you that by early 1975, it was becoming increasingly clear that I couldn't continue to spend so much time in clinically-related activities and still pursue the scientific opportunities that my lab research had opened up. I left on sabbatical leave in mid 1975, and this postponed the need

[15] Cohen, SN, Armstrong, MF. *Drug Interactions.* Baltimore: Williams & Wilkins, 1974.

to deal with the issue. But returning to clinical responsibilities in after the sabbatical year ended brought it to the fore again.

At that point Hal Holman was no longer chairman of the Department of Medicine. So, I went to speak with his successor, Dan Federman (Daniel D. Federman), to ask whether I could, at least during the next couple of years, be assigned fewer clinical responsibilities. He said that he was unwilling to do this and told me that if I wanted to have a full time appointment in the Department of Medicine, it would be necessary for me to continue to pull my weight clinically. Joshua Lederberg, who was Chairman of the Genetics Department, suggested that I consider a joint appointment in Genetics, and I was very attracted to this idea. I liked and admired Josh enormously. Josh was also very much involved with computer-based research and had been one of the developers of DENRAL, a computer-based expert system for analysis of DNA data obtained by mass spectrometry, and he was familiar with my computer-based research in clinical pharmacology. We had multiple common research interests, and Josh was very supportive of my science. Having an appointment in a basic science department as well as one in Medicine was very appealing, and I thought that taking on teaching responsibilities in Genetics might allow me to reduce my clinical role. The joint appointment in Genetics began in mid 1977.

Hughes: A primary appointment has a stipulation about time commitment?

Cohen: No, but it defines which department the faculty member has a principal relationship with, which department the research space comes from, which department sets the salary, which department provides administrative support, *et cetera*.

Hughes: Is there a difference in salary between the clinical and basic science appointments?

Cohen: There is.

Hughes: So that was a consideration as well?

Cohen: It was certainly a fact. But the salary difference wasn't something that I was considering in making a decision.

Hughes: Was the disparity considerable? I ask that knowing the history at UCSF where the disparity between clinical and basic science salaries had been a bone of contention.

Cohen: I think that the disparity is very considerable. There’s even a significant disparity between clinical departments in the surgical specialties vs. medical specialties. The Department of Medicine and the Department of Pediatrics are two of the lower paying clinical departments. That has to do with the compensation that physicians receive in private practice in the different areas of clinical medicine.

Hughes: Yes, it's all competitive.

Cohen: Right. Although I continued to hold a Department of Medicine appointment and did not take a salary cut immediately when I switched my primary appointment, I expected that I would not have an increase in salary for a while until the basic sciences salary level caught up.

Hughes: The context of your basic science research up until this point was biochemistry.

Cohen: And scientifically, also in genetics and microbiology.

Congruency of Research Interests with Lederberg

Hughes: Well, we'll develop that because we haven't heard much about genetics.

Cohen: Formally, the disciplines of genetics and biochemistry are quite different. However, faculty in biochemistry departments and genetics departments often carry out similar research. Molecular

biology was a child of both disciplines, and a reason that we've been talking so much here about biochemistry is that the Biochemistry Department at Stanford had people doing molecular biology in areas related to my scientific interests. Aside from Lederberg himself and another scientist named A.T. Ganesan, who worked on the bacterium *B. subtilis* and is now deceased, there was no faculty person in Genetics working on bacteria. There was a faculty person named Luca Cavalli-Sforza, who was, even then, one of the world's leading human geneticists. Although Luca began his career in microbial genetics, his scientific interests had shifted. There was, Len Herzenberg, an immunogeneticist that Lederberg had recruited soon after he started the department, and Eric Shooter, whose work was primarily in neurobiology and who later became the first chair of a newly established Neurobiology Department at Stanford. There were also a couple of non-tenure-line appointments, but Genetics was a very small department at that time.

Lederberg's continuing interest and involvement in plasmids went back for many years. His early work on bacterial conjugation and recombination, which earned him the Nobel Prize, was dependent on plasmids, and Luca Cavalli had independently worked with conjugation in the 1940s. They began their interactions in those days. Lederberg is the person who invented the term "plasmid"; he coined the term in a *Physiological Reviews* article in *1952*.[16] As a member of the U.S. National Academy of Sciences, he later communicated to the *PNAS* [*Proceedings of the National Academy of Sciences*] two of the papers describing the early DNA cloning experiments from my lab.

Lederberg's scientific interests have always been extraordinarily broad. During the 1970s, he wrote a weekly science column for the *Washington Post,* and he also had played a major role in the development of artificial intelligence activities here at Stanford. Lederberg had established a computer system called ACME to carry out of his activities his research in computer sciences. It was the predecessor of the SUMEX-AIM "Artificial Intelligence In Medicine" system. When Lederberg left Stanford to become president of Rockefeller University, because of my own work with expert systems on the MYCIN project, I agreed to server as the Principal Investigator for the SUMEX-AIM grant for a year. After that, Ted Shortliffe, whose primary interest was in computer-based research, joined the Stanford faculty and took on that responsibility.

Hughes: Another connection of Lederberg's with your subsequent work on recombinant DNA was an application that he made in the late 1960s to NIH for support on joining DNA from different sources.[17] Do you remember talking about it early on with Lederberg?

Cohen: I don't. But, he had interests in a lot of different areas and that wouldn't surprise me. After the initial experiments, Lederberg was one of the first people I discussed the results with.

Interview 3: February 1, 1995

EARLY LABORATORY RESEARCH AT STANFORD: SCIENTIFIC BACKGROUND AND INITIAL EXPERIMENTS

Plasmid History

[16] Lederberg, J. Cell genetics and hereditary symbiosis. *Physiological Reviews*. 1959; 32: 403-430.
[17] Wright, S. *Molecular Politics: Developing American and British Regulatory Policy for Genetic Engineering, 1972-1982.* Chicago: University of Chicago Press, 1994, p. 71. Hereafter, *Molecular Politics.*

Hughes: We mentioned plasmid research in past sessions, but I thought today we should talk in greater detail and perhaps you'd like to start with the history of the field.

Cohen: Of plasmids?

Hughes: Of plasmids.

Cohen: Okay. Much of molecular biology in its early stages had to do with phage for reasons that I mentioned the last time we spoke, primarily because it was possible to make identical copies of individual phage particles. But a key aspect of Lederberg's early work on recombination in bacteria in the 1940s involved a genetic element that was then called a fertility (F) factor and promoted gene transfer. Additional studies showed that the F factor is a plasmid that can sometimes integrate into the bacterial chromosome. Work with the F factor was also important in the development of the concept of the operon by Jacob and Monod. The *E. coli lac* gene, which provided the model for these experiments, had been picked up by the F plasmid when F inserted itself into the chromosome. I don't know the extent that…

Hughes: Yes.

Cohen: …I should go into the technical details here.

Hughes: No this is fine.

Cohen: So, plasmids had an important role in the early years of molecular biology. They were also important to the concept of the "replicon" developed by Jacob and his colleagues. Autonomously replicating extrachromosomal elements were sometimes called "episomes" at that time. As I've mentioned, Lederberg coined the term "plasmid" in his *Physiological Reviews* article in 1952.

Hughes: Where did he get—why plasmid?

Cohen: Because, as he mentioned in the article, they contribute to the genetic fluidity of the organism.

For many years the molecular nature of plasmids was not at all clear. Some of the earliest work on this was done in the laboratory of Paul Doty at Harvard in the early 1960s. Stanley Falkow and Bob Rownd, whose work with R factor DNA a few years later was important in exciting my interest in plasmid biology, were collaborating with Julius Marmur and Doty to study DNA isolated from bacteria containing F factors, and the group found that F-factor DNA could be detected as a discrete entity. They transferred the F factor into bacterial species that contain a chromosome having a different nucleotide composition, and showed that the F-factor DNA formed a band at a different position in cesium chloride gradients.[18]

After the discovery of antibiotics in the 1940s, there was the prevalent view that these drugs would end infectious diseases caused by bacteria. Of course, that has not happened, and the reason was the advent of antibiotic resistance. Initially, the question of how resistance develops was controversial, and some workers in the field proposed that exposure to an antibiotic induced resistance. Others argued that bacteria acquire spontaneous mutations that make them insensitive to the antibiotics, and that resistant bacteria are given an advantage, in a Darwinian sense, by the widespread clinical use of antibiotics—which kill or restrict the growth of antibiotic-sensitive bacteria in microbial populations. But the resistant bacteria survive and propagate themselves. That controversy was resolved experimentally, and Lederberg and his first wife, Esther [Lederberg], did a crucial experiment using a procedure called replica plating.[19] Would you like me to go into the details of the procedure?

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[18] Marmur, J, Rownd, R, Falkow, S, Baron, LS, Schildkraut, C, Doty, P. The nature of intergeneric episomal infection. *Proc Natl Acad Sci USA*. 1961 July; 47 (7): 972-979.

[19] Lederberg, J, Lederberg, E. Replica plating and indirect selection of bacterial mutants. *Journal of Bacteriol*. 1952 March; 63 (3): 399-406.

Hughes: Well, yes.

Cohen: Essentially, a population of bacteria was plated on a petri dish that lacked any antibiotic. Then, a replica of this population, made by taking a sterile piece of velvet and pressing it against the top of the petri dish and then against the top of another petri dish, was created. But the second petri dish contained an antibiotic. This prevented the growth of most of the bacterial cells into colonies, but the Lederbergs found that there were some antibiotic-resistant colonies that grew on the second dish. They went back to the initial antibiotic-free petri dish and found antibiotic resistant bacteria at locations corresponding to the positions of these colonies, whereas the bacteria in other locations were sensitive. These experiments, plus others, helped to establish the mutational basis for antibiotic resistance.

Early on, it was also thought that antibiotic resistance was entirely a chromosomal phenomenon. If the frequency of mutation to resistance to a single antibiotic in a bacterial population was 10^{-6}, one in a million, then the chance of having the cell become concurrently resistant to two different antibiotics was 10^{-12}. This led to the notion of circumventing resistance by using multiple antibiotics to treat infections. But in the mid 1950s there began to appear, initially in Japan and in England, instances of bacteria that were resistant to not just one antibiotic but were concurrently resistant to two or three or four drugs.

Role of Plasmids in Antibiotic Resistance

Cohen: As work continued with these resistant microorganisms, another very important phenomenon was discovered: resistance could be transferred between bacteria. Some resistant bacterial cells not only expressed resistance to multiple antibiotics, but also could transfer the multidrug resistance to other bacteria by cell-to-cell contact. The recipients of resistance could reproduce and generate a population of resistant offspring. In this way, resistance can spread rapidly through bacterial populations, and be transferred from a less pathogenic bacterial host to one that is more pathogenic.

The genetic element responsible for antibiotic resistance in bacteria was termed an “R factor.” And, studies in the laboratory of Dr. Tsutomu Watanabe, in Japan, and by other scientists as well, particularly in Japan and in England—Naomi Datta and Guy and Eleanor Meynel, and E. S. Anderson—genetically mapped the locations of R factor genes. It was found that one locus of the R factor contains genetic information that enables transfer, and this was called the “RTF” or “resistance transfer factor,” whereas genes encoding antibiotic resistance were mapped genetically to another segment. Studies from the labs of Falkow and Rownd showed that R factors could be detected in cesium chloride gradients as bands located at a different position from chromosomal DNA. This work implied that the R factors are discrete units, but it was not known at that time that they consist of circular DNA molecules.

Hughes: Is this idea of the extrachromosomal nucleic acid something that's happening on a broad basis or is this unique to—I'm thinking of [Barbara] McClintock's work with the jumping genes, for example.

Cohen: Yes. Extrachromosomal DNA occurs in many types of organisms, but what I’m describing is different from jumping genes.

Hughes: Which, if I understand the history right, was not a concept that was readily accepted. And I guess what I'm really asking is how acceptable is it to be thinking about genetic material that is outside the chromosome?

Cohen: Oh, I think that was well recognized, at least genetically. Work by Lederberg and others had provided genetic evidence of “episomes.”

Hughes: And we are now talking about the sixties?

Cohen: Now we're up to the mid-sixties.

Molecular Nature of R Factors

Cohen: In 1967 and 1968, other observations relevant to my planned work with plasmids were reported. One was the finding by Radloff, Bauer and Vinograd at Caltech that closed circles of DNA from a virus that infects mammalian cells can be separated from noncircular DNA using the dye ethidium bromide. The dye molecules insert themselves between coils of the DNA helix and change the spacing between those coils. If the duplex DNA exists as a closed circle, its ability to adjust to the change in spacing is constrained, and this causes the DNA circle to form a tightly twisted coil. The change in conformation alters the buoyant density of DNA when it is centrifuged in cesium salt gradients, so that coiled circular DNA can be separated from non-circular DNA. Vinograd's lab originally did this with polyoma virus, and were able to separate polyoma virus circular DNA from noncircular DNA molecules.[20]

This advance was important. It provided me and others with a method for separating circular plasmids from bacterial chromosomes efficiently.

Other relevant discoveries were made in Don Helinski's lab at UC San Diego. This work, which was published by Helinski and his collaborators, Michael Bazaral and Don Clewell, in late 1968 and 1969, provided the first molecular evidence that the DNA of small colicinogenic plasmids is circular.[21] [22]

Hughes: May I ask you a question about that though? Are you emphasizing the circularity of it?

Cohen: Yes. It had been well established that plasmids consist of DNA, and genetic evidence had suggested that F factors were circular. But Helinski's work provided the first physical evidence of circularity, least for some small bacterial plasmids.

My subsequent work and the work of others showed that large R factors are also circular DNA molecules, but the circularity of plasmids remained somewhat controversial. After I had been working on plasmids at Stanford for a little more than a year, I presented my lab's evidence for the existence of R factor circles at a scientific meeting sponsored by the American Society for Microbiology in Miami Beach, I guess in mid-1969. A postdoc from Bob Rownd's laboratory, which argued that R factors were not circular, stood up in the discussion period to question my conclusions and said, "Well, you know, Dr. Rownd believes that R-factor circles are an experimental artifact." I was taken a little aback by this put-down; it was my first talk as an independent scientist.

Hughes: But what is the real significance, though, about worrying about that fact?

Cohen: Well, there was a fundamental uncertainty at that time about the structure of R factors. The circularity of R-factor DNA also had practical significance in terms of my subsequent work.

By 1969 it was accepted that R factors are extrachromsomal elements, and that autumn,

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[20] Radloff, R, Bauer, W, Vinograd, J. A dye-buoyant-density method for the detection and isolation of closed circular duplex DNA: the closed circular DNA in HeLa cells. *Proc Natl Acad Sci USA*. 1967 May; 57 (5): 1514-1521.

[21] Clewell, DB, Helinski, DR. Supercoiled circular DNA-protein complex in *Escherichia coli*: purification and induced conversion to an open circular DNA form. *Proc Natl Acad Sci USA*. 1969 April; 62 (4): 1159-1166.

[22] Bazaral, M, Helinski, DR. Circular DNA forms of colicinogenic factors E1, E2 and E3 from *Escherichia coli*. *J Mol Biol*. 1968 September 14; 36 (2): 185-194.

Helinski and his co-workers reported that colicinogenic factors are circular DNA; but colicinogenic factors are small genetic elements and there was evidence that R factors are much larger. There were disparate views about the molecular nature of R factors, and they were viewed by some as being linear DNA molecules. Even as late as the early 1970s, arguments were being made that R-factor circles were an artifact of the isolation procedure: that they became circularized during the course of isolation.

But let's go back a step. When I began at Stanford in March 1968, I was a young assistant professor eager to begin my research. I had received an NIH grant and wanted to start the research. The goal of the project was to elucidate how R factors had evolved and to learn whether they were formed by association of independent sub-units, as had been suggested by genetic data. I needed access to an analytical ultrcentrifuge to proceed with my experiments but hadn't requested funds to purchase this instrument, which even in the late 1960s cost about $40,000, in the budget I had submitted to the NIH. Jerry Hurwitz had advised me to limit the amount of support I requested in what was my first research grant application; $40,000 was a lot of money for a single piece of equipment, and certainly a lot for an assistant professor to be asking for. There were a few analytical ultracentrifuges in the labs of other Stanford faculty, and Holman had indicated that I could make part-time use of those instruments in my research. However, it turned out that these centrifuges weren't as readily available as we had hoped. So I needed additional research funds.

As I've already mentioned, when I arrived at Stanford I found that my assigned space was still occupied by others that Hal Holman had loaned it to on a temporary basis. Holman pointed out that I didn't yet have funds to purchase an analytical ultrcentrifuge and wondered whether I actually needed to occupy the lab prior to obtaining the centrifuge. Through the generosity of several pharmaceutical companies that I made appeals to, I soon pulled together enough funds to purchase the centrifuge and move into my assigned space. But in the interim, I was able to begin doing experiments using borrowed laboratory space in the Department of Biochemistry.

Isolation of Circular R-factor DNA and Resistance Transfer Factor (RTF)

In a surprisingly short time after my move to Department of Medicine space in mid-1968, Chris Miller, who was a technician in my lab, and I worked out methods to isolate circular R-factor DNA using nitrocellulose. The nitrocellulose method enabled us to isolate circular plasmid DNA and analyze it by electron microscopy and by centrifugation. The results showed that *E. coli* bacterial cells carrying an R factor named RI contain multiple molecular species of circular DNA. The nitrocellulose method proved to be less efficient than the ethidum bromide method, but the data we obtained resulted in my first paper on R-factor DNA structure.[23]

My next goal was to try to isolate the individual components of R factors. If R factors truly come apart, perhaps there was a way to isolate the resistance transfer factor [RTF] as a separate circular DNA molecule. I worked out a scheme for doing this, which involved multiple rounds of plasmid transfer. The notion was that by using multiple rounds of transfer, together with a screen that did not select for antibiotic resistance, it might be possible to isolate cells that contain only the RTF unit.

And the strategy worked. Chris and I showed in a paper published in the *PNAS* in 1970 that the RTF unit of resistance plasmids could be isolated as a discrete, autonomously replicating

[23] Cohen, SN, Miller, CA. Multiple molecular species of circular R-factor DNA isolated from *Escherichia coli*. *Nature*. 1969; 224: 1273-1277.

genetic element.[24]

Okay. So at that point we went on to study, in some detail using cesium chloride centrifugation methods, the conditions that affect the relative amounts of DNA components of large antibiotic resistance plasmids. Eventually these experiments led to a long paper in the *Journal of Molecular Biology* on the molecular nature of resistance plasmids isolated from *E. coli* and *Proteus mirabilis*.[25] Similar studies of R-factor DNA by electron microscopy were going on in the laboratory of Roy Clowes, who was a professor at the University of Texas at Dallas and who subsequently became a good friend.

It seemed to me that in order to further understand the functions of R-factor components, we needed to have a way of introducing the plasmid DNA into bacterial cells. Up until then, we were taking plasmid DNA out of bacteria and had isolated the transfer unit as well as the whole R factor. But we also wanted to learn what the other resistance-associated DNA bands we saw in gradients were. We had isolated the RTF as an independent replicon, but didn't know whether the resistance gene component(s) would also be able to exist as separate replicons. We wanted to correlate each of the DNA bands with particular biological functions.

Hughes: So in essence, were you moving from studies that emphasized structure into ones that are now emphasizing structure and function?

Cohen: Yes. I think that's a fair way of putting it.

During the first year or so of my work at Stanford on antibiotic resistance plasmids, I also was following up some of the earlier results that I had gotten in Jerry Hurwitz's lab on bacteriophage lambda. This became a sort of "bread and butter" project, while I was beginning the new research.

Hiring Lab Personnel: Annie Chang and Chris Miller

Cohen: I'd like to backtrack and say something now about the people working in my lab early in my career at Stanford; they were doing much of the actual bench work for the experiments I've been describing. A few days after my arrival here in March 1968, I started interviewing candidates for a research assistant position. The first person I interviewed was Annie Chang. Annie was born in China and received her undergraduate degree from McGill University. She had a reasonable background in biology, but she knew nothing about DNA isolation or about plasmids. She previously had worked on a protein-related project, but in the course of the interview, I began to feel that she might be very suitable for the job I had available.

Hughes: Why?

Cohen: Well, she was very direct and she asked good questions. When she didn't know the answer to a question I asked her, she said so. It was clear that she had worked hard in the past, as I had, and I thought that she would be motivated to learn. When I began to tell her about plasmids and why I was interested in them, she made the point that plasmid research seemed to be an obscure area of biology. I agreed with her, but pointed out that what may be considered obscure to one person can be exciting and interesting to someone else. As an example, I noted that someone might be interested in the mechanism of joint articulation in an insect's knee, and I would find that area a bit obscure. And she smiled and didn't say anything. I learned later that her brother was an entomologist and had research interests somewhat akin to the example that I had

[24] Cohen, SN, Miller, CA. Non-chromosomal antibiotic resistance in bacteria. III: Isolation of the discrete transfer unit of the R-factor RI. *Proc Natl Acad Sci USA*. 1970; 67: 510-516.

[25] Cohen, SN, Miller, CA. Non-chromosomal antibiotic resistance in bacteria. II: Molecular nature of R-factors isolated from *Proteus mirabilis* and *E. coli*. *Journal of Mol. Biol*. 1970; 50: 671-687.

chosen.

I was advised by colleagues to interview several other candidates before filling the job opening, and I did that. But, I offered Annie the position, and she accepted and helped me to set up my laboratory. She still works in my laboratory, having gotten her Ph.D. degree subsequently, and is now in a Senior Research Associate position here at Stanford. Another person I hired a few months later—I had funds for two technicians—was Chris Miller, who also still works in my laboratory.

I met Chris in May or June of 1968 when she was about to graduate from Berkeley. She started in my lab soon after her graduation and worked here for several years before moving to Chicago to be with someone she was seeing at that time. Periodically I stopped in Chicago en route to or back from the East Coast, and invited Chris to lunch or dinner, and tried to persuade her to come back to work in my lab at Stanford. Eventually, she did that, persuading her husband to accept a position in the Bay Area.

Hughes: So Annie, at least originally, came with a biochemical background?

Cohen: Yes, she came with some biochemical background. Chris also had been a biochemistry major at Berkeley. Both of them had undergraduate degrees but little or no subsequent training or research experience. I didn't anticipate being able to find anyone who had experience working specifically with plasmids, but my feeling was that I would search for smart and motivated people and train them in the area I was interested in. During those first years, I was still able to work at the lab bench myself a fair amount of the time, and was able to provide hands-on training on how to do things.

Hughes: Were you thinking at that stage of the work you were doing as essentially biochemistry?

Cohen: Yes, that's basically correct. There really was not a lot of genetics involved. The genetics of R factors had been investigated by Japanese scientists and others in England, and I was interested mainly in trying to learn about the molecular nature of resistance plasmids.

Hughes: Is there any story behind the concentration—from what you were saying—of the research in three countries, namely in Japan, Britain, and the United States?

Cohen: Historically, the phenomenon of multi-drug resistance was discovered in Japan in the mid 1950s and then was observed in England. But I hadn't read those papers, at least not at the time of their publication. There was a later article in *Scientific American* by Tsutomo Watanabe in 1963, which brought antibiotic resistance plasmids, or antibiotic resistance factors, as they were called then, to wider attention in Western countries. I also hadn't seen that article, but after hearing the seminar by Novick at Cold Spring Harbor and reading the papers in the *JMB* by Falkow and Rownd, I went back and read Watanabe's *Scientific American* paper, and my reaction was, "Wow!" Collectively, all of these things made me want to learn more about bacterial antibiotic resistance.

Expanding the Lab Group

I'd like to say something else about my lab group during the early years. As a faculty member starting work in the Department of Medicine in 1968, I had no access to graduate student trainees. There was no graduate student training program in the Department of Medicine, and as I've mentioned, I didn't have a joint appointment in a basic science department. So there was no prospect of having graduate students working in my lab, except through *ad hoc* programs like the one established later for Ted Shortliffe, and I didn't yet have the publication record I thought would be needed to attract postdocs.

But relatively soon after my arrival at Stanford, two things happened that expanded the size of

my lab group. One was an inquiry by a young physician named Arnold Brown. He said that he was interested in pursuing a career that includes research and, wanted to receive basic science training. He had attended a seminar talk that I had given to a Department of Medicine group, and liked what he heard. He knew that it was relatively late in his career to start to learn molecular biology, but asked nevertheless to train in my lab. Although Arnie had no prior experience at all in laboratory research, and I knew that a lot of effort from me would be required to provide him with lab bench training, he was very motivated and I thought that he would learn. It was an opportunity to expand my lab group, and that was fine with me. I offered Arnie a postdoc position, and he accepted.

As I began to report some of my lab's initial observations on plasmids, one of Stanley Falkow's graduate students named Richard Silver decided that he wanted to come to my lab for postdoctoral training. Rich was interested in my work and in the approaches I was using. He had been trained in Falkow's lab, and he wanted to learn more about the molecular structure of plasmids. Falkow had told him that he thought my lab would be a good place to do this. So Rich applied for a postdoctoral position, and I was happy to accept him into my lab.

So during those first few years, I was able to put together a laboratory group of four people—Rich, Arnie, Chris, and Annie—and I was working at the bench myself for part of the time, so it was a five-person lab group, and it was very closely knit. The group was small, but we worked on several different projects. Arnie and Annie were finishing up some of the work I had been doing with bacteriophage lambda. Rich was working on a plasmid project. Later, when Chris moved to Chicago, I hired Annette McCoubrey as a replacement. After the work with lambda was completed, I also switched Annie to a plasmid project.

Organization of Lab Activities During the Early Years

Cohen: It was a good start for my lab and I enjoyed those years. I had clinical responsibilities also, as I've mentioned, but my research progressed nicely.

Hughes: Was there any pattern to your day or your week? Were there times when you could count on being in the lab?

Cohen: It depended on my clinical assignments. During my initial stint as a hematologist, I participated in a weekly hematological patient review session, which I think was on Wednesday afternoons. It lasted from noon or one o'clock until six o'clock in the evening. We reviewed the clinical status of each patient that had been on the hematology service during the week. I also saw hematology patients at other times, and made attending rounds on the general medical service. Most Fridays, in the mid-afternoon, I went out to the local Baskin-Robbins store to get ice cream for the people in my lab. I brought back ice cream and we would sit around together for a while and eat and chat. It kind of became a lab tradition.

Hughes: Do you carry it on?

Cohen: No, I don't. I can do without the calories and cholesterol, and now the laboratory is much bigger and I have stopped working at the bench. But, I have tried to maintain a small lab atmosphere and we still have a mom-and-pop-shop-type lab. I should say something more about this before we get too far away from the point. These days in molecular biology, many larger labs, and even medium-sized labs such as mine, often have specialization within the lab, so that one person performs the same type of work, for example DNA sequencing, for a number of projects. Someone else is doing the plasmid construction and someone else is doing whatever. My lab has always been a place where a person works on multiple aspects of a project, learning the various techniques and concepts necessary for it. I think that this is probably not the most productive way to organize a lab, but I have always felt that it is the best

way to train young scientists to do research.

Research assistants in my lab almost always have been assigned to their own projects, serving as my "hands," and have been authors on papers. When a paper reports work that a research assistant has had the primary role in carrying out, the RA has been listed as first author. Over the years, this has sometimes been problematical, when research assistants and postdocs have together collaborated on experiments. For example, I can remember one instance where a postdoc complained that first authorship was justified for her because she would soon be looking for faculty position, whereas the research assistant on the project didn't have that pressing need. But the research assistant had made and recognized the importance of most of the key observations. Authorship should be determined by scientific contributions.

Hughes: Do you want to say something now about communication? I mean, what means did you have to stay in touch with these various projects that were going on in the lab?

Cohen: When my office was a little cubbyhole off of a single laboratory that contained only five or six benches, I could talk to people in the lab about their experiments everyday. We planned experiments together and students and postdocs usually would come into my office right after they got a result and discuss the data with me. It wasn't a large operation.

Hughes: That's less possible now with a larger lab?

Cohen: Well, it's less convenient. That's true. I don't have the day-to-day contact with everyone in my lab that I did in those days, but I stay in pretty close touch. In addition to the general lab meetings we have on a weekly basis, I try to schedule individual meetings with people in my lab every few weeks. The timing depends on the person and the stage of the project. I've learned over the years that if it's been a while since a student or postdoc has stuck their head through the doorway of my office to talk to me about results, this can indicate a problem with the way the project is going. When students are getting good results in the lab, they're usually eager to communicate those results. If students haven't stopped in to talk for a while, I'll arrange a time to speak with them.

The office that I currently have is located across the corridor from my lab. Before I moved into this lab space, it was used by the medical center to house small animals. It's in a part of the building surrounded by a three-foot thick windowless "shearwall" that I was told was designed to withstand earthquakes. When the space was assigned to me, I redesigned it for use as a lab. I was able to get approval to put three windows in the thick outer wall facing the courtyard. I had to make the decision about whether the bench space or my office would have the windows, and I decided it was more important for the windows to be in the lab. I put my office, which was a little cubbyhole about half the size of the office that we're in now, off of a corridor in the lab. That worked well for communication because lab people had to pass my office to go almost anywhere, and would stick their heads into my office regularly, sometimes multiple times during the course of the day when my door was open.

I eventually became a little claustrophobic in my small, windowless office and persuaded the Dean to give me some additional space so I could have a window. But this office is located across the corridor from my lab space and one of the issues I had to face was being physically separated from my laboratory. I didn't like that idea, but I don't think it has significantly affected communication with my students and postdocs. Walking across the corridor is not quite as easy as passing my office door 10 times a day, but it works.

Okay, let's get back to the 1970s, unless we want to stop here.

Hughes: I'm willing to go.

RESEARCH FINDINGS BY VARIOUS LABS PRIOR TO THE INVENTION OF

RECOMBINANT DNA

Uptake of Bacteriophage DNA by *E. coli*: the Work of Mandel and Higa

Cohen: Okay, let's go on.

My lab's analysis of R-factor structure had gone well, but I realized that to make further progress I needed a way to introduce plasmid DNA into bacteria and have the plasmid genes expressed there. Bacterial transformation by DNA wasn't a novel idea. It goes back to the work of Avery, McCarty and MacLeod who showed, by transforming *Pneumococcus*, that genetic information resides in DNA.

After the Avery work, genetic transformation by DNA was shown also in other species of bacteria: *Haemophilus influenza*, and *Bacillus subtilis*, for example, but at that point no one had been able to genetically transform *E. coli*, which was the organism that I was working with.

Around 1960, Dale Kaiser and David Hogness were introducing fragments of lambda DNA into *E. coli* to learn whether the genetic map is co-linear with the lambda genome. To get lambda DNA into the bacteria, they needed a live "helper" virus. A few years later, Rich Calendar at Berkeley found that using a buffer containing calcium ions increased lambda DNA uptake in the helper phage system. Then in 1970, [Morton] Mandel and [Akiko] Higa, who were working at the University of Hawaii in Honolulu, reported an important observation: treatment of *E. coli* with calcium chloride enabled the uptake of lambda DNA by *E. coli* even in the absence of helper phage, and the bacteria produced viable phage particles. They also tried to transform calcium chloride-treated *E. coli* genetically with chromosomal DNA but they did not get transformants, even though viable phage particles were made by the bacteria. Their work was described in a Note in the *Journal of Molecular Biology*.[26]

Peter Lobban was a graduate student in Dale Kaiser's lab in the Department of Biochemistry, and he was using Mandel and Higa's calcium chloride procedure to try to get uptake of DNA of the bacterial virus, P22 by *E. coli*. Peter got viable P22 phage when he transfected the DNA, as Mandel and Higa had found for lambda DNA. P22 DNA was taken up by the bacteria and virus particles were formed. I knew about Peter's results, and wondered whether calcium chloride treatment would also work to get R-factor DNA into *E. coli*. But, unlike phage production by transfected bacteria, which requires only that the bacterial cells serve as a bag of enzymes to assist the phage in proceeding through its reproductive cycle, genetic transformation requires that transfected bacteria make copies of themselves, and of the genes that have been taken up.

Mandel and Higa's efforts to genetically transform calcium chloride-treated *E. coli* had failed, so why did I think that I might be able to get genetic transformation by genes carried by R factors? Well, to be propagated in calcium chloride-treated bacteria, the incoming chromosomal DNA that Mandel and Higa used had to recombine genetically with the resident chromosome. But my work had shown that R factors were autonomous replicons. Like phage, R factors can replicate on their own, and I thought that genes carried by these plasmids might be able to transform bacteria without entering the chromosome. It seemed worth trying.

Cohen Lab's Development of a System for Genetic Transformation for *E. coli* Using Plasmid DNA

[26] Mandel, M, Higa, A. Calcium-dependent bacteriophage DNA infection. *Journal of Mol. Biol*. 1970; 53: 159-162.

There was another event that occurred around this time, and that was the addition of a new person to my research group: Leslie Hsu. Leslie had graduated from Stanford in 1970 with an undergraduate degree in biology. She had done very well academically and had been accepted to Harvard as a graduate student in the Biolabs, but decided to delay her entry into graduate school for a year and to travel in Europe for with friends for several months. In January 1971, she returned to Palo Alto to look for a temporary job for five or six months before leaving for graduate school in Boston.

I needed a secretary at that time. Up until then, I shared a secretary with several other junior faculty, but my research activities and backed-up manuscripts that I wanted to submit for publication were growing to the point where I needed additional office help. Funds to get this help were available from my Burroughs Wellcome Fund Clinical Pharmacology Scholar Award. So, I hired Leslie as my secretary. And she was absolutely great. First of all, she had been a pianist and had very adept hands and could type at an unbelievable speed. She was also very smart. Thirdly, she was interested in the work we were doing because she had been trained as a biologist. And after a few weeks, she asked if she could attend my lab meetings. That was an unusual request from someone working in an office, but certainly she was welcomed at our lab meetings. So she became even more interested in my lab work.

As I've mentioned, in addition to the bench research going on in my lab, I had non-bench work going on with the computer-based drug interaction reporting system I was trying to develop. Leslie was interested in not only the research going on in the lab but also in the clinically-related drug interaction project. After a few months, she told me that she was thinking about going to medical school and pursuing a career in both clinical medicine and basic research. She thought that what I was doing was a good model. To her, this was a more appealing plan than going to graduate school and pursuing a career fully in basic research.

So by the end of May or the beginning of June, Leslie decided to apply to medical school. She had great grades as an undergraduate and excellent recommendations from her undergraduate advisors. And it was clear to me also that she was an intellectually gifted person whom I could recommend highly for admission to medical school and I did that. There was a space available in the entering class, and in June or early July, she was accepted to Stanford Medical School to begin classes a couple of months later, in September.

Leslie wanted to pursue a research project in my laboratory as a medical student. The project that I assigned to her was to try to transform *E. coli* genetically with R-factor DNA. She and I got very helpful technical advice from Peter Lobban, who had the Mandel and Higa calcium chloride procedure working for P22 DNA, and Leslie found that she could introduce R-factor DNA into bacteria, and that cells taking up these plasmids could reproduce and could express antibiotic resistance genes carried by the R factor. Antibiotics were used selectively to allow the growth of cells that had taken up and were propagating the plasmids, and bacteria that expressed the antibiotic resistance genes grew into colonies. Initially, the efficiency of genetic transformation was low, but we made procedural modifications that improved it. The discovery that bacteria could be genetically transformed by R-Factor DNA was very exciting to me because it made possible the cloning of individual plasmid DNA molecules.

Later, [S.] Cosloy and [M.] Oishi[27] found that the ends of linear chromosomal DNA fragments, which Mandel and Higa were using in their unsuccessful transformation experiments, are degraded by exonucleases present in *E. coli* cells. When mutants of *E. coli* lacking exonuclease activity are used, chromosomal DNA can also transform *E. coli.* So a key factor underlying the success of our transformation experiments was the circularity of the R-factor DNA we were

[27] Cosloy, SD, Oishi, M. Genetic Transformation in *Escherichia coli* K12. *Proc Natl Acad Sci USA*. 1973; 70: 84-87.

using.

I think that our paper, which was published in the *Proceedings of the National Academy of Science* in August 1972, was viewed as "interesting,"[28] but most people working in molecular biology at the time didn't realize that we could now do with plasmids what could be done previously only with phage: namely clone entire extrachromosomal genomes. Although scientists working with plasmids were turned on by our publication, the most important aspect of this work---the ability to make clones of cells containing individual autonomously replicating DNA molecules, was missed by most of the scientific community. And the lack of greater realization of the implications of the R factor transformation work was fine with me. It gave me time to proceed further without the pressure of intense scientific competition.

Hughes: Why did they miss that?

Cohen: Well, I don't really know. I suppose partially because there weren't many researchers working with plasmids at the time. Genetic transformation had been demonstrated previously in other organisms using chromosomal DNA, so the introduction of genetic traits into bacteria wasn't new. However, in the genetic transformation that had been shown for *B. subtilis*, the introduced DNA had to recombine into the chromosome to be propogated, and of course that requires homology between the introduced DNA and the chromosomal DNA. It wasn't immediately apparent to many people that transformation by plasmid DNA was different from previous genetic transformation because plasmids were autonomous replicons that could be stably inherited without being integrated into the chromosome. No homology with chromosomal DNA is required. Also the title of our paper was, "Nonchromosomal antibiotic resistance in bacteriogenetic transformation of *Escherichia coli* by R-factor DNA," and there wasn't a lot of interest in R factors. I think most scientists didn't grasp the significance of being able to take a plasmid out of a cell, introduce it into another cell, and than select cells that contain plasmids that are the progeny of a single DNA molecule taken up by a particular cell.

But, being able to clone individual plasmid replicons was important to me because, as I explained earlier, I wanted to learn the genetic contents of the multiple R factor DNA bands that were being detected. By isolating the extrachromosomal DNA and using it to transform a population of bacteria, I hoped to obtain bacterial clones that contained different plasmid DNA species. I was very excited about our results, and, as you see, I'm still excited about the finding twenty years later.

Hughes: Yes.

Cohen: One of the questions I wanted to answer was whether the large R factors that we were working with were composed of multiple replicons that had been joined together. The R factors were very large DNA molecules—up to 100 kilodaltons. I thought that if we could mechanically shear these large DNA molecules and then introduce the fragments into bacteria, we might get recircularization of some of the fragments of the plasmid intracellularly. I should say something at this point about DNA ligases. In fact, let's stop here.

Interview 4: February 7, 1995

End-To-End Joining of DNA Molecules by DNA Ligase

[28] Cohen, SN, Chang, ACY, Hsu, L. Nonchromosomal antibiotic resistance in bacteria: Genetic transformation of *Escherichia coli* by R-factor DNA. *Proc Natl Acad Sci USA*. 1972; 69: 2110-2114.

Hughes: Dr. Cohen, when we stopped last time, we were just about to enter the subject of ligases. So do you want to start there this time?

Cohen: Yes, let's do that. DNA ligases were discovered in the late 1960s. In fact, Jerry Hurwitz's lab, where I was a postdoctoral fellow at the time, was one of several labs competing in a search for enzymes that can join together pieces of DNA end-to-end. The first report of discovery of a DNA ligase activity in *E. coli* came from the lab of Martin Gellert. Marty was someone I had known for several years. When I was at the NIH in Lemone Yielding's lab, Marty, who was then working with Gary Felsenfeld at the NIH, helped me with technical advice during my spectrophotometric studies of chloroquine interactions with DNA. Anyway, Marty had the insight to use the complementary ends of bacteriophage lambda in his search for DNA-joining activity. It had been known for some time that the linear DNA of bacteriophage lambda has ends that can join together to make covalently closed DNA circles during the lambda life cycle. This occurs because lambda DNA ends are single-stranded and the nucleotides at each end are complementary to the nucleotides at the other end. Because of the complementarity, the two ends of lambda DNA can pair with each other, and be held together by hydrogen bonds. At a particular stage of the lambda life cycle, the lambda DNA ends come together to form circular molecules containing nicks, which are then sealed *in vivo* by an enzyme that forms covalent bonds between the nucleotides at the DNA ends. Marty used hydrogen-bonded lambda DNA circles to search for an activity in *E. coli* extracts that converted hydrogen-bonded circles to covalently closed ones, and he found it.

Using lambda DNA circles, Malcolm Gefter, a graduate student in the Hurwitz lab, confirmed Gellert's results and highly purified the *E. coli* DNA ligase to almost homogeneity. Other laboratories, including Bob Lehman's laboratory and Arthur Kornberg's laboratory here at Stanford, isolated ligase from *E. coli* about the same time. The fact that cohesive-ended molecules had been used as a substrate in these experiments was important in causing the scientific community to focus on using complementary nucleotides held together by hydrogen bonding to join together DNA ends.

Hughes: Had there been talk before that date of the benefits of joining different types of DNA?

Cohen: Not in the Hurwitz lab. If there was such talk elsewhere at that time, it's unlikely that I'd have known about it.

Hughes: The discovery of ligases prompted thinking along those lines?

Cohen: Well, ligases certainly did provide a tool for linking DNA ends together.

Work on DNA end Joining in the H. Gobind Khorana Lab

Cohen: One of the first uses of ligase for DNA end joining was by [H. Gobind] Khorana and his collaborators. Khorana is a biochemist who in the mid 1960s developed methods for synthesizing small oligonucleotides that have a defined sequence. By linking together synthetic deoxyribonucleotides, he produced an intact gene for alanine transfer RNA, and in 1968 won the Nobel Prize for his role in elucidating the genetic code and establishing its function in protein synthesis. The sequence of the tRNA was known, and Khorana and his colleagues synthesized short DNA oligonucleotides containing overlapping ends to re-create the sequence encoding the tRNA. The sequence at the end of the deoxyribonucleotide chain was complementary to the sequence at the end of an adjacent one. Khorana used DNA ligase to covalently join DNA oligonucleotides that were held together by hydrogen bonding of base pairs in the overlapping regions and, in a laborious way, synthesized a gene. That work

involved both DNA ligation and complementarity between DNA ends.

Vittorio Sgaramella, a postdoc in Khorana's lab, made the observation together with J.H. van de Sande that a DNA ligase encoded by the genome bacteriophage T4 can join together not only cohesive-ended molecules but even blunt-ended synthetic DNA molecules. That work was reported in a paper in the *PNAS* in 1970.[29] But, the scientific community was so focused on cohesive-ended DNA molecules that for a while, probably until 1974 or so, some scientists working on DNA end joining expressed doubt that DNA ends that are not complementary could actually come together and be joined. But the data in the Sgaramella paper are clear, and they showed that complementarity at the ends of DNA chains is not necessary for joining.

Work by Peter Lobban, by Jackson, Symons, Berg, and by Jensen *et al*. on DNA End Joining

Cohen: In 1969, Peter Lobban, who was a graduate student in Dale Kaiser's lab, proposed, as part of an examination to qualify him for the next stage of his PhD training, a strategy to add complementary nucleotides to the ends of DNA so that different DNA fragments could be joined together. Instead of laboriously adding nucleotides one at a time to create a complementary DNA sequence as Khorana had done, Peter proposed using the enzyme terminal transferase which added a series of identical nucleotides to the 3' terminus of a non-duplexed DNA strand or of a strand of a DNA duplex, adding a stretch, for example, of polyAs [adenines] to one population of DNA molecules. He then would add polyTs [thymidines] to another population of DNA molecules. By mixing the two DNA species, Peter expected that hydrogen bonding between the As and Ts would hold the DNAs together, and that he could then use *E. coli* DNA ligase to covalently link the molecules. Although this strategy was initially proposed for a hypothetical project, he then decided to develop the method as an actual thesis project. I was able to obtain a copy of Peter's proposal in the mid 1970s when I wrote a *Scientific American* article about this overall technology.[30]

Hughes: That idea was unique?

Cohen: Well, it was very clever but not actually unique. There was another group that had, so far as I can determine, independently used the same approach. These were three scientists who worked for an industrial organization, the International Minerals and Chemicals Company, and most others in molecular biology probably are not aware of this group of scientists. And in mid 1971, these scientists, Jensen, Wodzinski, and Rogoff, published a paper [31] reporting use of the same strategy; they added a stretch of Ts to one batch of DNA molecules and a stretch of As to another. I think they used the DNA of bacteriophage T7 or another phage as a substrate. They showed by sedimentation in gradients that DNA molecules were held together by dA-T [deoxyadenine-thymidine] tails. Then, they incubated the molecules with DNA ligase, but when they then heated the mixture, the DNA molecules came apart, so their attempts to join the DNA molecules covalently were not successful.

So by 1971 there were three groups using complementary dA-T tails added biochemically to hold DNA fragments together: Jensen and his colleagues, Peter Lobban and Dale Kaiser, and the Jackson, Symons and Berg group. Lobban was trying to join together segments of the genome of a bacteriophage, P22, and Jackson, Symons and Berg were trying to join lambda *dv*,

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[29] Sgaramella, V. Enzymatic oligomerization of bacteriophage P22 DNA and of linear simian virus 40 DNA. *Proc Natl Acad Sci USA*. 1970 November; 67 (3): 1468-1475.

[30] Cohen, S.N. The manipulation of genes. *Scientific American*. 1975; 233: 25-33.

[31] Jensen, RH, Wodzinski, RJ, Rogoff, MH. Enzymatic addition of cohesive ends to T7 DNA. *Biochem Biophys Res Commun.* 1971 Apr 16; 43 (2): 384-392.

which is a circular variant of bacteriophage lambda, to DNA of the mammalian virus SV40. How Jackson, Symons, and Berg began using the dA-T joining approach has never been entirely clear to me. When I wrote my *Scientific American* article in 1975, I tried to determine who in the Stanford Biochemistry Department first had the idea for using that method to link DNA molecules together. Peter Lobban presented this strategy in his qualifying exam proposal in 1969. Paul Berg has said that he didn't learn of Lobban's proposal or thesis project until after Dave Jackson had begun his SV40 experiments, which was not until sometime in 1970, according to Jackson's account in his M.I.T. oral history.[32] Paul signed Peter's final thesis dissertation as a member of the examining committee, but has indicated that he was not a member of the faculty committee that reviewed and evaluated Lobban's qualifying exam proposal.

In any case, the work on dA-T joining was carried out in the Department of Biochemistry concurrently by David Jackson and Bob Symons in Paul Berg's lab, and by Peter Lobban in Dale Kaiser's lab using different kinds of DNA. Both groups have said that they shared information. The Jackson, Symons, and Berg[33] paper credits Peter Lobban for having discovered two crucial steps in the dA-T joining procedure that allowed Berg and his colleagues to link the molecules covalently, and which presumably prevented Jensen *et al*. from being successful in their earlier attempts at *in vitro* ligation. One of these steps increased the efficiency of adding tails to DNA; terminal transferase adds nucleotides to 3' extensions and if the extensions are longer, the enzyme works better. Peter used lambda exonuclease to trim back the 5' ends of the duplex DNA, which increased the single-strand length of the 3' extension and made it easier to add tails.

The second step, which I think is probably the more crucial one, was Peter's discovery that to get good ligation, phosphates had to be removed from the terminus of the DNA molecules that were to be linked together, and he used a specific *E. coli* exonuclease [exonuclease III] to do this. My understanding from the publications is that there was a contaminant in the terminal transferase so that the DNA fragments had mixed phosphate and hydroxyl termini. A way was needed to remove the 3' terminal phosphate, and this was important in being able to seal the nick and get covalent joining of DNA ends. Lobban was successful in splicing together segments of DNA from bacteriophage P22; and using information that they said was received from Lobban, the Jackson, Symons, and Berg group linked SV40 DNA to lambda *dv* DNA using the dA-T method.

Lobban's Priority

Hughes: Were you following closely what was happening in the two respective labs?

Cohen: Well, I certainly knew about Peter Lobban's work, and I also knew more generally about Dave Jackson's work with SV40. I knew that Peter had joined monomers of P22 using dA-T tails, and that Berg's group was doing similar experiments to join lambda *dv* and SV40. I think that most people at Stanford viewed the method as Lobban's and considered the lambda *dv*-SV40 work to be another application of the approach that Peter was using for his thesis project.

Hughes: Which is not the tilt that one gets by reading Lear's book on the history of recombinant DNA, where he puts Lobban forward as being the neglected student who didn't receive proper

[32] Jackson, D. Recombinant DNA Oral History Collection. MIT.

[33] Jackson, DA, Symons, RH, Berg, P. Biochemical method for inserting new genetic information into DNA of Simian Virus 40: circular SV40 DNA molecules containing lambda phage genes and the galactose operon of *Escherichia coli*. *Proc Natl Acad Sci USA*. 1972; 69 (10): 2904-9.

credit.[34]

Cohen: Well, I think that Lear's take is correct. Peter's role was clearly recognized at Stanford, but he hasn't received proper credit outside of Stanford. That's exactly the view that those of us who were here have, and it's different from the view of most of the rest of the world. I've talked about this point with Lou Reichardt, who worked at a bench in the same lab as Peter, and with others in the Department of Biochemistry. All of us knew that Peter had worked out the technical problems in dA-T joining to make the procedure work, and this was acknowledged in the Jackson, Symons and Berg paper. Although Peter proposed his project the year before Dave Jackson started working on dA-T joining and Peter worked out the bugs in the procedure, it's sad that Peter was viewed by much of the outside world as having simply having used a method designed and developed by Berg group. After his postdoctoral fellowship, Peter was not able to get a suitable faculty position and he ended up leaving the biological sciences and studying engineering. I think that he currently works as an engineer and lives in the Bay Area.

Hughes: Do you think that his status as a graduate student at the time of this work made a difference?

Cohen: No, the authors of his paper were Lobban and Kaiser; Dale Kaiser was a senior faculty person at the time. Just as Jackson and Symons were postdocs in Berg's lab, Lobban was a graduate student in Kaiser's lab. In recognition for Dale Kaiser's role, the year [1980] that Boyer and Berg and I received the [Albert] Lasker [Basic Medical Research] Award, Dale shared the award with us. So there are some in the scientific community who are aware of this history.

Gene Splicing versus Recombinant DNA

Cohen: So as you see, splicing of synthetic and natural DNAs had been done in various ways before my collaboration with Boyer. The covalent linkage of deoxribonucleotide chains had first been accomplished by Khorana and his collaborators. The linkage of separate pieces of natural DNA by adding complementary "tails" was reported first by Jensen and his colleagues, although this joining was not covalent. Peter Lobban and Dale Kaiser had achieved covalent joining of duplex DNAs of a phage, and Jackson, Symons, and Berg had done this with SV40 and lamda *dv*. In the Jackson, Symons and, Berg work, the DNA came from different sources; one was an animal virus and one was a bacterial virus. But chemically, the same *in vitro* procedure, which depended on the actions of terminal transferase, lambda exonuclease and exonuclease III was used for both. I think there is the notion among the general public, and maybe even among some scientists, that recombinant DNA is equivalent to gene splicing. But the splicing together of DNA molecules *in vitro* is only part of "recombinant DNA". A key additional step is the *in vivo* propagation and cloning of the DNA molecules that have been biochemically joined.

Hughes: From what I understand, Berg's group had conceptually carried the research a bit further in that by the summer of 1972, they were thinking of introducing the chimera into *E. coli*.

Cohen: I think that occurred even prior to 1972. He was planning on trying to use SV40 to introduce DNA into animal cells.

Hughes: My point is that Berg indeed seemed to be thinking about what you are calling gene cloning. As we know, he decided not to do that experiment because of the potential biohazards. Am I right?

Cohen: Berg's writings about this point indicate that his goal was to see whether SV40 could work as a mammalian version of a transducing bacteriophage virus, and you could certainly say that transduction is a biological way of cloning genes. Actually, the concept of gene cloning by viral

[34] Lear, J. *Recombinant DNA: The Untold Story*. New York: Crown Publishers, 1978, p. 44-46.

transduction goes back to the early work of Lederberg, and was also considered by Peter Lobban in his 1969 proposal.

Hughes: Ah. So Lobban was interested in more than just splicing?

Cohen: I think that his interests were more general. Peter was working with P22, which was the bacteriophage that Norton Zinder and Joshua Lederberg had used in their discovery of atransduction, which involves the picking up of chromosomal genes during the normal phage growth cycle.[35] Peter's 1969 proposal and the paper that Lobban and Kaiser published[36] on their dA-T joining work with P22 DNA recognized that it might be possible to generate transducing phages biochemically by inserting blocks of genes from other organisms into the phage genome. And Lederberg and others have indicated that they also had been thinking about gene cloning. This was a natural outcome of the Zinder and Lederberg transduction experiments. So, the concept of gene cloning wasn't novel at that point; it preceded my own work, Berg's work with SV40, and Lobban's work.

At a Cold Spring Harbor Lab course in 1971, discussion with Berg's student, Janet Mertz, led Robert Pollack, a CSH scientist, to raise concerns about possible biohazards of such hybrid molecules, and as you've just said, Berg decided not to continue working with them.[37] But putting aside any such biohazard concerns, there wasn't, so far as I know, yet a way to infect mammalian cells with SV40 DNA—so it would have been necessary to develop such a method before being able to actually use SV40 to propagate and clone genes in mammalian cells. Berg indicated later [34] that he also planned to try to introduce the SV40 DNA into *E. coli* to determine if the SV40 DNA genes would be expressed there. But how he planned to do this isn't clear: Mandel and Higa had reported their inability to genetically transform *E. coli* cells using the calcium-chloride procedure, and prior to Leslie's Hsu's experiments with R-factor DNA, which I talked about earlier, it wasn't known that genetic transformation of *E. coli* was achievable.

Hughes: Scientifically, it wouldn't be terribly interesting to splice pieces of DNA together?

Cohen: Oh sure it would be.

Hughes: Why?

Cohen: Well, Khorana spliced together deoxyribonucleotide chains to create a whole gene, and these experiments helped to provide an understanding of the genetic code and of mechanisms of protein production. In fact, there were lots of people interested in mechanisms of DNA repair and DNA end joining per se, including Gellert, Hurwitz, and others. Two papers by Sgaramella around this time were about DNA end joining.[38] [39] The Jackson, Symons, and Berg paper focuses on the biochemical joining reaction. Even today, there is a lot of scientific interest in the biochemistry of DNA end joining and, more generally, in DNA repair and in the enzymes that do this.

I think that there's an important point here that is often overlooked: It's sometimes stated that the reason the Berg lab wasn't the first to show that DNA cloning is possible is that biohazard

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[35] Zinder, ND, Lederberg, J. Genetic exchange in salmonella. *J. Bacteriol*. 1952; 64 (5): 679-699.
[36] Lobban, PE, Kaiser, AD. Enzymatic end-to-end joining of DNA molecules. *J. Mol. Biol*. 1973; 78: 453-471.
[37] Berg, P. Dissections and Reconstructions of Genes and Chromosomes. Nobel lecture. 1980 December 8; Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305.
[38] Sgaramella, V. Studies on polynucleotides: A novel joining reaction catalyzed by T4 polynucleotidal ligase. *Proc Natl Acad Sci USA*. 1970; 87: 1468-75.
[39] Sgaramella, V. Enzymatic oligomerization of P22 DNA in a linear simian virus SV40 DNA. *Proc Natl Acad Sci USA*. 1972 November; 69 (11): 3389-3393.

concerns led Berg to abandon further experiments with SV40 and lambda *dv* hybrids. But if Paul believed he had an approach for actually propagating foreign DNA in *E. coli* using lambda *dv*, why not use another piece of DNA instead of SV40? There was no general concern about propagating DNA in bacteria at the time, and the issues of concern to Robert Pollack and some others related specifically to the fact that SV40 is a tumor virus.

Hughes: Right.

Cohen: I posed this question at different times to both Bob Symons and Dave Jackson; I don't think I've ever asked Paul. Bob and David said they weren't thinking in those terms at the time. But after it was shown by Leslie Hsu that *E. coli* can be genetically transformed, and we published this in August 1972, the Berg group could, in principle, have tried to use lambda *dv* to introduce some other DNA fragment in bacteria without having to worry about the biohazard issues that were raised by SV40. But if Berg had tried such an experiment, the experiment wouldn't have worked. The reason is that they were adding dA-T tails to lambda *dv* DNA that had been cut with the *Eco*RI restriction enzyme to linearize the DNA. The *Eco*RI cleavage site in lambda *dv* is in the O gene, which is required for replication of the bacteriophage, and constructs containing inserted DNA fragments at that site would not replicate in *E. coli*, and therefore can't be propagated. That was not known in 1971 or 1972. So it would have been problematical to clone any DNA in bacteria using the system that Berg and his colleagues described.

Hughes: The line of research using SV40 was temporarily stopped because people were thinking in terms of potential biohazard, not in terms of generalizing this technique and using viruses that did not have a potential biohazard.

Cohen: Or not using mammalian viruses.

Hughes: Right.

Cohen: Anyway, if Jackson *et al*. had tried DNA cloning in bacteria using lambda *dv*, they would have gotten a negative result, and this might have been interpreted as indicating that DNA hybrids made *in vitro* can't be propagated.

Hughes: In terms of acceptance by the scientific community, did it make a difference that Peter Lobban was a graduate student and his research findings appeared in his dissertation? A dissertation does not normally have wide readership.

Cohen: It's not that Peter's findings weren't accepted. They were published in a leading scientific journal, the *Journal of Molecular Biology*.[40] But by the time Lobban's work was published (August 1973), the complementary nature of DNA ends generated by cleavage with the *Eco*RI endonuclease had been shown by several labs, and making complementary DNA ends this way was much easier than adding dA-T tails. Also, my work with Annie Chang, Herb Boyer, and Bob Helling had shown that *Eco*RI-generated DNA fragments could be cloned in bacteria using plasmids. Boyer mentioned these experiments at a Gordon conference on nucleic acids in June 1973 and word of the results had spread. So interest began to turn from phage to plasmids as possible vehicles for propagating DNA, and from using dA-T tail addition, to using restriction enzymes to generate complementary DNA ends. Anyway, my point was that given Paul's statements about his intent,[41] it's puzzling that he didn't try DNA other than SV40.

Hughes: That was a conceptual block?

Cohen: Conceptual block in what sense?

Hughes: That line of research was not pursued in the sequential way that it might have been if the

[40] Lobban, P, Kaiser, D. Enzymatic End-to-End Joining of DNA Molecules. *J Mol. Biol.* 1973; 78: 453-471.

[41] Berg, P. Dissections and reconstructions of genes and chromosomes. Nobel lecture. 1980 December 8; Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305.

biohazard issue hadn't come up.

Cohen: Well, maybe the block was conceptual, or possibly experimental. The biohazard concerns raised were about just tumor viruses and the concerns didn't preclude cloning of other DNAs.

DNA CLONING: THE INVENTION OF RECOMBINANT DNA

Leading Up to the First Cohen-Boyer Experiment

Hughes: Well, could I clarify the connection between this work that you've been describing—the Lobban and Kaiser, and Jackson, Symons and Berg work—and your own? Am I understanding correctly that you were pursuing your interest in plasmid science which made it of great importance to you to be able to clone the materials that you were interested in studying and in a sense it was almost incidental that this other line of research was going on? To put it very simplistically, you didn't look at what Lobban and the Berg group were doing and decide, "I'm going to pursue a different approach."

Cohen: What you've said is exactly correct. What they were doing was incidental to my work. As I've said, my goal was to isolate plasmid DNA molecules and re-introduce them into *E. coli*, and we worked out a procedure for doing this. The next step was, okay, can we take plasmid DNA molecules apart and isolate the replication region? We were trying to do this by shearing plasmid DNA molecules into pieces mechanically, and I hoped to get rejoining of some of these fragments by DNA recombination in cells after the fragments were introduced into calcium chloride-treated bacteria. The work by Lobban and Kaiser, by Berg's group, and by Jensen was focused on the biochemical joining of DNA ends rather than on separating or isolating genes. My focus was as much on taking plasmids apart as well as on putting them together in order to identify individual plasmid genes. Restriction enzymes offered a possible way for me to do both. Mechanical shearing broke different DNA molecules differently; restriction enzymes cut DNA molecules in the population uniformly.

Early on, my lab showed that multiple resistance plasmids can coexist in bacteria as separate pieces of circular DNA.[42] [43] [44] The need to separate and isolate different R-factor DNA species was the driving force that led me to try to genetically transform *E. coli* with this DNA. Once we found that we could transform *E. coli* cells with R-factor DNA and showed that transformed bacteria acquire DNA circles having all of the properties of the parent R factor,[45] I thought, well okay, here is an autonomously replicating DNA molecule that we can take out of a bacterial cell, put back into another cell, and propagate and clone it. Would it be possible to attach other plasmid DNA fragments to the plasmid replication region so that segments containing specific plasmid genes can be identified? I knew from the heteroduplex experiments that Phil Sharp, Norman Davidson, and I had reported that large plasmids probably evolved in nature by genetic recombination events that joined resistance genes to replication regions.

At that time, I was thinking just about studies of *E. coli* plasmids. It was known, as Herb Boyer

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[42] Cohen, SN, Miller, CA. Multiple molecular species of circular R-factor DNA isolated from *Escherichia coli*. *Nature*. 1969; 224: 1273-1277.

[43] Cohen, SN, Miller, CA. Non-chromosomal antibiotic resistance in bacteria. III: Isolation of the discrete transfer unit of the R-factor RI. *Proc Natl Acad Sci USA*. 1970; 67: 510-516.

[44] Cohen, SN, Miller, CA. Non-chromosomal antibiotic resistance in bacteria. II: Molecular nature of R-factors isolated from *Proteus mirabilis* and *E. coli*. *J Mol Biol.* 1970; 50: 671-687.

[45] Cohen, SN, Chang, ACY, Hsu, L. Nonchromosomal antibiotic resistance in bacteria: Genetic transformation of *Escherichia coli* by R-factor DNA. *Proc Natl Acad Sci USA*. 1972; 69: 2110-2114.

has probably told you in discussing restriction enzymes,[46] that bacteriophage propagated on one *E. coli* strain can be restricted in its ability to grow on a different strain. So propagation of phage between strains of even the same species of bacterium is sometimes difficult, and there was a general belief that "natural barriers" would preclude DNA exchange between unrelated biological species.

The Species Barrier Issue

Hughes: Jumping ahead, the obstacle you confronted with the *Xenopus* work, even if you were successful in transforming bacteria, was: Would a gene function in a foreign host?

Cohen: Right, would it function? And could you propagate it?

Hughes: And the feeling was that it probably wouldn't?

Cohen: Well, there was evidence that the mechanisms and signals governing gene expression and DNA replication in prokaryotes and eukaryotes are different. And, eukaryotic mRNA molecules contain stretches of A nucleotides at their 3' ends, and these hadn't been thought to occur, at least then, in bacteria. DNA isolated from different species was often different in nucleotide composition. There were multiple reasons to doubt that simply linking a DNA fragment biochemically to an *E. coli* plasmid replicon would enable propagation of the foreign DNA in *E. coli* and that chimeric DNA molecules made biochemically would be viable and functional in cells. Certainly, many of my colleagues at Stanford originally thought that the biological crossing of species barriers would not be successful.

Scientific Goals in the Development of Recombinant DNA Methodology

Hughes: Another point, which is inherent in what we've been saying but perhaps should be stated explicitly, is that you were really not focusing on the methodology. I mean, it was not your idea to develop what later became recombinant DNA technology.

Cohen: That's correct.

Hughes: What you were trying to do was to pursue your science, and for your science you needed this particular method.

Cohen: Right.

Hughes: Is that the order of priority?

Cohen: Yes.

Hughes: I think we now look back and tend to see the technology as being the dominant thing, where in actuality it was the science.

Cohen: I think that's an important point, Sally, and that was also probably true for at least some of the other people that were working [in the field] as well. In my case, the technology was developed out of necessity so that we could study antibiotic resistance plasmids.

Restriction Enzyme History

[46] See the oral history with Herbert W. Boyer in this Bancroft Library series.

Hughes: We started this session with the idea of pulling together the different strands that went into what eventually became recombinant technology. According to your Harvey lecture,[47] there were four elements that you felt were necessary.

Cohen: Right.

Hughes: We've got the ligases and we've got the cloning vehicle.

Cohen: Yes, which was the plasmid.

Hughes: And we've got the procedure for introducing hybrid molecules into a cell.

Cohen: Transformation.

Hughes: Right.

Cohen: And we've got the joining [ligation].

Hughes: And we talked a little about the restriction enzymes. Do you want to say more?

Cohen: Okay, let me tell you my understanding of the history of these enzymes, which were discovered almost a decade prior to the experiments we've been discussing. As I've mentioned, restriction of bacteriophage growth by some bacterial strains had been known for some time, largely from the early work of Werner Arber. But some phage escape the restriction mechanisms. It was found that specific enzymes restrict phage growth by cleaving the phage DNA, and these are called "restriction enzymes." Other enzymes can modify the phage DNA to make it unsusceptible to cleavage by the cognate restriction enzymes, and these are called "modification enzymes." Restriction and modification enzymes commonly work in pairs. This was the phenomenon that Herb was interested in studying.

An early worker in the field of restriction/modification was Daisy Dussoix, who as a graduate student in Arber's lab in Switzerland, had made observations central to the discovery of the restriction phenomenon. Later, Dussoix, whose name had become Roulland-Dussoix, moved to UCSF and collaborated with Boyer. Also at UCSF was a graduate student named Robert Yoshimori who, as I understand it, was initially a student of Dussoix. When Dussoix left UCSF in the early 1970s, Herb inherited Yoshimori as a student. Yoshimori had identified an enzyme that came from an *E. coli* strain isolated from a patient hospitalized at UCSF. It was encoded by an antibiotic resistance plasmid,[48] so the history forms a circle, in a sense. And as I've mentioned, Tsutumo Watanabe in Japan, who had done some of the early major work with antibiotic resistance plasmids, had also found that certain resistance plasmids encode restriction/modification systems. The plasmid that Yoshimori had identified and isolated encoded a restriction enzyme called *Eco*RI (*E. coli* restriction enzyme I). This was the enzyme used in the initial experiments that Boyer and I did.

Inviting Boyer to the Honolulu, Hawaii Meeting on Plasmid Biology, November 1972

As I've mentioned, Don Helinski and Watanabe and I organized an NSF (National Science Foundation)-sponsored plasmid DNA meeting in Hawaii in November 1972 [November 13-15, 1972]. Watanabe was quite ill in the months prior to the meeting and died just a few days before it began. Don phoned me a couple weeks before the meeting to tell me he had learned about some recent work that Herb Boyer had been doing with restriction enzymes encoded by

[47] Cohen, SN. The transplantation and manipulation of genes in microorganisms. *The Harvey Lectures.* New York: Academic Press. 1980; 74: 173-204.

[48] Yoshimori, R, Roulland-Dussoix, D, Boyer, HW. R factor-controlled restriction and modification of deoxyribonucleic acid: Restriction mutants. *J Bacteriol.* 1972 December; 112 (3): 1275-9.

plasmids. I didn't know Boyer personally and I knew relatively little about his work, although I had seen some of his papers. He had published a review on restriction enzymes and several other papers in that area. At that point he hadn't published anything on *Eco*RI. Since this was a meeting about plasmid biology, Don suggested that we invite Herb as a participant, and I thought that was a good idea. So, I wrote to Herb extending a formal invitation on behalf of the two of us as the co-organizers, and also on behalf of Watanabe. So Herb showed up at the meeting.

Work by Sgaramella and by Mertz and Davis Showing that the *Eco*RI Restriction Enzyme Generates Complementary DNA Termini

About the time that the meeting was held, some additional relevant papers were published. These were all in the November 1972 issue of the *PNAS*. There was a paper by Joe Hedgepeth, Howard Goodman, and Herb Boyer in which they showed that the *Eco*RI enzyme-generated DNA ends have a unique sequence and that DNA ends generated by *Eco*RI cleavage were complimentary.[49] They did this by using DNA sequencing of the ends. There were two other papers on the complementarity of *Eco*RI generated ends that were published in the same PNAS issue: one was Vittorio Sgaramella's paper on covalent joining of DNA molecules[50] and the other was by [Janet] Mertz and [Ronald] Davis.[51]

I'd like to back up a bit here and provide some background information. Sgaramella, who earlier had trained in Khorana's lab and had participated there in studies of the chemical joining of DNA segments, had come to Joshua Lederberg's lab as a postdoctoral fellow, I think in 1970. He was working in Josh's lab with bacteriophage P22 and was using *Eco*RI enzyme to cleave P22 DNA. To examine the DNA fragments generated by cleavage, Sgaramella needed an electron microscope, and there was no electron microscope in the Department of Genetics at the time. But the Biochemistry Department had an E.M. that was being used primarily by Ron [Ronald W.] Davis, who just had been recruited to Stanford as an assistant professor after completing training in Norman Davidson's lab at Caltech. Ron is an extraordinarily creative scientist, and already had made major contributions in working out heteroduplex techniques to identify regions of similarity in different DNA molecules.

Vittorio was given use of the Biochemistry Department's E.M. The department had generously also allowed me to use that electron microscope to examine R-factor DNA molecules. I had learned how to prepare DNA preparations for examination by electron microscopy from Phil [Phillip A.] Sharp and others in Norman Davidson's lab at Caltech, and I came back here and used the Biochemistry Department's electron microscope to look at plasmid DNA structure.

My understanding of this part of the history is that while Sgaramella was using the biochemistry department's electron microscope, he found that P22 DNA fragments generated by *Eco*RI cleavage joined together end-to-end to form oligomers when *E. coli* DNA ligase was added. Unlike the T4 ligase, which Sgaramella had previously shown, while in Khorana's lab, can join blunt-ended DNA molecules, *E. coli* ligase was known to require complementarity to join DNA ends. This led Sgaramella to conclude that *Eco*RI generates complementary ends during its cleavage of DNA.

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[49] Hedgpeth, J, Goodman, HM, Boyer, HW. The DNA nucleotide sequence restricted by the RI endonuclease. *Proc Natl Acad Sci USA*. 1972; 69: 3448-3452.

[50] Sgaramella, V. Enzymatic oligomerization of bacteriophage P22 DNA and of linear Simian virus 40 DNA. *Proc Natl Acad Sci USA*. 1972 Nov; 69 (11): 3389-93.

[51] Mertz, JE, Davis, RW. Cleavage of DNA by R 1 restriction endonuclease generates cohesive ends. *Proc Natl Acad Sci USA*. 1972 November; 69 (11): 3370-4.

Sgaramella also observed that *Eco*RI-cleaved SV40 DNA, which John Morrow, a graduate student in Berg's lab, had found is cleaved by the *Eco*RI enzyme at a single site,[52] also forms oligomers. Morrow's discovery of the ability of *Eco*RI to cleave SV40 DNA at a single site was in contrast to what had been observed for a *Hemophilus influenzae* restriction enzyme,[53] which [Kathleen] Danna and [Daniel] Nathans had shown cleaves SV40 into multiple fragments.[54] After learning of Sgaramella's results, Janet Mertz, who of course knew also of Morrow's finding, tested whether the ends generated by *Eco*RI cleavage of SV40 DNA could join together to regenerate duplex covalently-closed DNA molecules when *E. coli* ligase was added. The molecules were examined by her and Ron Davis on the E.M., and they found that cleaved SV40 could in fact recircularize.

Janet and Ron published a paper showing that *Eco*RI cleavage generates complementary ends in SV40,[55] in the same issue of the *PNAS* that reported Vittorio Sgaramella's conclusion that complementary ends are generated in phage P22 and SV40 DNA by this enzyme. This *PNAS* issue also contained the report of the sequence of *Eco*RI-generated DNA ends by Hedgepeth, Goodman, and Boyer.[56]

So who first made the discovery that *Eco*RI generates complementary DNA ends that can be joined by *E. coli* ligase? I think that most people credit Mertz and Davis for that finding, even though the papers by Mertz and Davis and Vittorio Sgaramella were published in the same issue of the *PNAS*. Paul Berg, who credits Mertz and Davis for this discovery, indicates[57] that his name was not included as an author of the Mertz and Davis paper because the *PNAS* does not allow an author's name to appear on more than one paper in a single issue of the journal, and he already had authored a paper with John Morrow in that same issue. The paper by Sgaramella, which was communicated to the *PNAS* by Lederberg and was published under his tutelage, cites the similar findings by Mertz and Davis and acknowledges the use of Biochemistry Department facilities and expertise. There is no mention of Sgaramella's findings in the Mertz and Davis paper, which was communicated to the *PNAS* by Berg about a week later. But some years ago, Vittorio showed me a statement that he said had been drafted, he thought by Paul, to acknowledge Sgaramella's priority in the discovery. Vittorio told me that the statement was intended for inclusion in the Mertz and Davis paper. However, the published paper does not contain it.

The Sgaramella and the Mertz and Davis papers, along with the Hedgpeth, Goodman, and Boyer paper, were pul

At the Honolulu Meeting: Beginning the Collaboration with Boyer

Cohen: I went to the meeting in Honolulu and saw the sequence data that Joe Hedgepeth and Herb Boyer had obtained for *Eco*RI cleavage sites. From the six base pair sequence that Herb

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[52] Morrow, JF, Berg, P. Cleavage of simian virus 40 DNA at a unique site by a bacterial restriction enzyme. Proc *Natl Acad Sci USA*. 1972 November; 69 (11): 3365-9.

[53] Kelly, TJ, Smith, HO. A restriction enzyme from *Hemophilus influenzae*. II. *J of Mol Biol*. 1970; 51 (2): 393-409.

[54] Danna, K, Nathans, D. Specific cleavage of simian virus 40 DNA by restriction endonuclease of *Hemophilus influenzae*. *Proc Natl Acad Sci USA*. 1971 December; 68 (12): 2913-2917.

[55] Mertz, JE, Davis, RW. Cleavage of DNA by R 1 restriction endonuclease generates cohesive ends. *Proc Natl Acad Sci USA*. 1972 November; 69 (11): 3370-4.

[56] Hedgpeth, J, Goodman, HM, Boyer, HW. DNA nucleotide sequence restricted by the RI endonuclease. *Proc Natl Acad Sci USA*. 1972 November; 69 (11): 3448-52.

[57] Paul Berg, Ph.D., "A Stanford Professor's Career in Biochemistry, Science Politics, and the Biotechnology Industry," an oral history conducted in 1997 by Sally Smith Hughes, Regional Oral History Office, The Bancroft Library, University of California, Berkeley, 2000.

disclosed, I estimated that the large antibiotic resistance plasmids I was studying, which were about 100,000 nucleotides in length, would be cleaved into perhaps 20 fragments, on average once every 5000 nucleotides. The plasmids would be cut specifically and reproducibly, and each of these fragments would likely contain only a few genes. This would certainly be better than the mechanical shearing methods I had been using for taking plasmids apart, and the number of DNA fragments would probably be low enough to separate them by centrifugation and determine the size. And because the sequences at the ends of the multiple plasmid DNA fragments likely to be generated by *Eco*RI would be complementary, I thought that individual plasmid DNA fragments in the mixture might join to each other in different combinations. If cleavage left the replication functions of the plasmid intact, the replication region might join to different antibiotic resistance genes in the mix and form DNA circles containing different fragment combinations. Ligase could be added to seal the circles, and we could try to genetically transform calcium chloride-treated *E. coli* cells with the ligated DNA. Maybe we could isolate cells containing plasmids containing different combinations of antibiotic resistance genes.

During an evening walk along the street that parallels Waikiki Beach, Herb and I had a lengthy discussion about the experiments that his lab and mine were doing. The people present were Stanley Falkow, Charles Brinton, a University of Pittsburgh microbiologist who was on sabbatical leave in my lab at the time, and Charlie's wife, Ginger. Charlie had been working with pili, which are hairlike projections on the surface of bacteria; they're encoded by plasmid genes and are involved in plasmid transfer. Charlie and I were doing some collaborative experiments at the time at Stanford. His wife Ginger was with him here at Stanford and she came along to Hawaii. So Stanley, Charlie, Herb, Ginger, and I were taking this long walk and chatting. We ended up at a delicatessen and continued to talk, over sandwiches and beer.

Herb initially was not very interested in looking at plasmid genes, and offered to provide the enzyme as a gift for the experiments I wanted to do. He said he had given *Eco*RI to various other people at Stanford, and he'd be willing to give some to me. I said, "Well, that doesn't seem quite fair. Your lab has spent a lot of time isolating the enzyme and we should really do this as a collaboration." And that's the way we decided to do it.

Caveats About the Feasibility of DNA Cloning

Something that's often missed by people looking at this episode through a "retrospectroscope" is there was no assurance that any of this experimentation would work. We knew even from the Khorana lab's experiments published several years previously that pieces of DNA could be linked together biochemically: biochemical joining wasn't revolutionary. And we knew, because we had done it in my lab, that we could genetically transform *E. coli* with plasmid DNA and could use antibiotic resistance genes to identify cells that acquire the plasmids. We expected from the sequence at the *Eco*RI cleavage site that this restriction enzyme would cut the DNA of our large plasmids reproducibly into multiple fragments. And we knew at that point that *Eco*RI generates cohesive DNA ends. So these components were there.

But the crucial question was whether biochemically linked DNA fragments could be propagated in living *E. coli* cells and would function there, and the answer was not known. The joining of fragments at *Eco*RI sites would be bringing together DNA sequences non-biologically, whereas transduction and other forms of genetic recombination occurring in cells were biological processes that had evolved in nature. As Falkow said at the time,[58] "If it works,

[58] Interview with Stanley Falkow by Charles Weiner, May 26, 1976 and February 26, 1977, MIT Oral History Program.

let me know." There are some people who think that once a method of biochemical joining DNA ends was worked out, it was obvious that the chimeric DNA could be cloned. That's easy to say in retrospect, but in actuality it was not the case—especially for DNA molecules that contain components derived from different biological species. I'll say more about this a little later.

Initial DNA Cloning Results

In the first experiments of my collaboration with Boyer, we took a large plasmid, the R6-5 plasmid, which carries multiple resistance genes, and cut it up into pieces using *Eco*RI. An experimental procedure that became available at the time facilitated experiments, and that was the ability to analyze DNA fragments by agarose gel electrophoresis. [Joe] Sambrook, [Phillip] Sharp, and [William] Sugden had worked this out.[59] Herb had learned about the procedure from Joe Sambrook. The procedure made it convenient to fractionate and characterize DNA fragments by size. The fragments could be stained by ethidium bromide, which was the same DNA-binding dye that we had been using to separate circular plasmid DNA from chromosomal DNA. The fluorescent dye makes the fragments visible when they're exposed to UV [ultraviolet] light so the DNA can be seen in the gels. The cleaved DNA was electophoresed on a gel and stained. We saw that *Eco*RI had cut the plasmid into fragments of defined sizes; we could see eleven of them, rather than the 20 that I had estimated.

We introduced the *Eco*RI cleaved plasmid DNA into *E. coli*, both with and without ligation and isolated bacteria expressing resistance to different combinations of antibiotics. We wanted to see whether we could get reconstituted plasmids that express only some of the resistance genes present on R6-5 and include only some of the eleven DNA fragments. One goal of the work was to identify the fragment that contains the replication machinery of R6-5, so we also wanted to determine whether any DNA fragment was common to all of the plasmids. We also wanted to see whether certain fragments were correlated with specific resistance traits. And, in fact, we did see new combinations of resistance genes and DNA fragments. But, we also saw new combinations of *Eco*RI-generated fragments in bacteria receiving DNA that hadn't been treated with ligase. This showed that single-strand nicks in the DNA can be sealed by ligation *in vivo* after introduction of the DNA into bacteria.

Hughes: Now were you an original observer of the phenomenon?

Cohen: Which phenomenon?

Hughes: That when plasmid fragments were introduced into a cell the ligation occurred naturally.

Cohen: Yes. That was an unexpected observation. We initially had thought that it would be necessary to ligate the DNA molecules *in vitro*. DNA splicing occurred *in vivo* at a lower efficiency, but it occurred. This [finding] became important a few years later in some of the biohazard controversy issues.

By transforming cells with a mixture containing all of the *Eco*RI-generated fragments of R6-5 and selecting for kanamycin resistance, we recovered a smaller kanamycin resistance plasmid, and when we isolated that plasmid and cleaved it with *Eco*RI, we found that it contained several fragments identical in size to the *Eco*RI-generated fragments generated by cleavage of R6-5 DNA.

[59] Sharp, PA, Sugden, B, Sambrook, J. Detection of two restriction endonuclease activities in *Haemophilus parainfluenzae* using analytical agarose-ethidium bromide electrophoresis. *Biochemistry*. 1973; 12 (16): 3055-3063.

pSC101: the First Vector for Recombinant DNA

Hughes: Now, is that pSC101?

Cohen: No, not yet. pSC101 was not yet part of the experiments.

I need to explain here that pSC101, which we had isolated earlier after mechanical shearing of R6-5 DNA,[60] didn't actually originate from R6-5, as we had originally thought. We showed, a couple of years later, that it was a separate *Salmonella panama* plasmid that had contaminated the transformation mix.[61] We had originally named the plasmid "Tc6-5," and changed it to "pSC101," in keeping with impending recommendations about plasmid nomenclature.[62]

The heteroduplex investigations that Sharp, Davidson and I had done suggested that large R factor plasmids may have been formed in nature by the addition of antibiotic resistance genes to regions for replication and transfer. The kanamycin resistance plasmid [named pSC102] we recovered in our initial DNA cloning experiments included three fragments from R6-5. We wanted to learn which one of these contained the kanamycin resistance gene, and one way to find out was to try to clone the resistance gene fragment. To do this, we wanted a small plasmid replicon carrying a resistance gene that would allow us to select cells that contain it, but which did not express kanamycin resistance—and we wanted it to be cleaved only once by *Eco*RI. We tested several plasmids and found that pSC101 had these properties.

We cleaved both pSC101 DNA and pSC102 DNA with *Eco*RI, mixed the two DNAs together, and added the mixture to calcium chloride-treated *E. coli* cells. By including tetracycline in the growth medium, we could select cells that harbor the pSC101 backbone, and we than tested these cells for resistance to kanamycin to identify bacterial clones that expressed both resistance genes. We isolated plasmids from these bacteria and found one, pSC105, that included the pSC101 DNA fragment plus one of the three fragments of pSC102. pSC102 had been formed by the rearrangement *in vitro* of fragments of the same DNA molecule, the R6-5 plasmid, but pSC105 was the first DNA ever to be propagated that contained fragments of different DNA molecules that had been joined together outside of cells.

We later found how close the *Eco*RI cleavage site is to a location where insertion of another DNA fragment would have prevented the experiment from being successful. The promoter for the tetracycline resistance gene on pSC101 begins just 35 or 40 base pairs away from this cleavage site. If cleavage had occurred in the gene or its promoter, the selection for tetracycline resistance wouldn't have worked. Our backup plan was to use pSC102 to clone other fragments of R6-5, but it was very convenient to have a small tetracycline-resistance replicon that worked as a vector. We then joined pSC101 to a plasmid we had obtained from Stanley Falkow, RSF1010, which we found also contained a single *Eco*RI cleavage site, making a two-replicon plasmid that we showed could be propagated in *E. coli*. We published these experiments in the first of the three *PNAS* papers[63] that reported the cloning of DNA from different sources.

Measuring Success in the Experiments

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[60] Cohen, SN, Chang, ACY. Recircularization and autonomous replication of a sheared R-factor DNA segment in *Escherichia coli* transformants. *Proc Natl Acad Sci USA*. 1973; 70: 1293-1297.

[61] Cohen, SN, Chang, ACY. Revised interpretation of the origin of the pSC101 plasmid. *J. Bacteriol.* 1977; 132: 734-737.

[62] Novick, RP, Clowes, RC, Cohen, SN, Curtiss, R, Datta, N, Falkow, S. Uniform nomenclature for bacterial plasmids: A proposal. *Bacteriological Reviews*. 1976; 40: 168-189.

[63] Cohen, SN, Chang, ACY, Boyer, HW, Helling, RB. Construction of biologically functional plasmids *in vitro*. *Proc Natl Acad Sci USA*. 1973; 70: 3240-3244.

Hughes: There must have been a moment when you realized that the experiment had worked. What was that moment?

Cohen: That moment was elation. [Laughter.]

Hughes: Based on which particular part of the experiment?

Cohen: Actually, it was a series of moments. The first was when we found that the large R6-5 plasmid was cut into multiple fragments. Second was when we found that the plasmid fragments could be propagated in tranformants, and that different bacterial cell clones expressed different combinations of antibiotic resistance. Third was when we actually analyzed the plasmid DNA from these cells and found that the different cells contained different plasmids, and that the DNA fragments were all derived from the R6-5 parent. Fourth was when we found that pSC101 was cleaved only once by the enzyme. The most crucial moment was, I suppose, when we linked the kanamycin resistance fragment of pSC102 to pSC101, which showed that pSC101 would actually work as a carrier to propagate another DNA fragment in bacteria. The fact that we could take a non-replicating piece of DNA, link it to a plasmid vector, and replicate it in bacteria was especially exciting. So there were a series of exciting discoveries. I suppose that's the best way of putting it.

Hughes: All right, the experiment worked. Then where did your thinking go?

Cohen: Well, in part my thinking went to, "Hey, now I can study plasmids in the way that I've wanted to study plasmids." That was the original motivation for doing these experiments. [Interruption to find reprint of the paper.] But in the summary of our paper reporting these experiments we also said, "The general procedure described here is potentially useful for insertion of specific sequences from prokaryotic or eukaryotic chromosomes or extrachromosomal DNA into independently replicating bacterial plasmids. The antibiotic resistance plasmid pSC101 constitutes a replicon of considerable potential usefulness for the selection of such constructed molecules, since its replication machinery and its tetracycline resistance gene are left intact after cleavage by the *Eco*RI endonuclease." So yes, at the time, we certainly realized the potential utility for using this method to clone DNA from other sources. But we were really quite cautious about what we said in the paper because the generality of what we had done was not yet determined. Yes, the restriction modification systems of *E. coli* did not destroy the new *E. coli* plasmid constructs we had made, but we had no idea what would happen if we tried taking DNA from another species and putting it into *E. coli*.

Contributions of Individual Team Members

Hughes: Is this the time to talk about who was doing what? The work was going on in your lab and Boyer's lab, and you were obviously doing different things.

Cohen: Well, that's right.

At that time, Annie Chang, who was a research technician in my lab, lived in San Francisco. And that was very convenient because she was able to be a courier between Boyer's lab and mine in addition to doing many of the day-to-day experiments. She had been a co-author on the transformation experiments with Leslie Hsu. In the collaboration with Herb, the plasmids were isolated and the DNA was purified in my lab. The DNA was then taken up to UCSF by Annie where Herb and/or Bob Helling, who was on sabbatical leave in his lab, cut it with *Eco*RI and did the ligation, and then sent the DNA back to us. We did the transformation and selection for the cells that expressed the resistance phenotype in my lab; we isolated the plasmid DNA from these cells and it was characterized in various ways. The characterization by electron

microscopy and centrifugation was done here. The characterization by gel analysis was done largely by Bob Helling in Boyer's lab. So it was really a project in which both labs were contributing very substantively towards the experiments. There were skills from both of our labs that were important.

Hughes: Was this research a central focus in each lab or was it just one of several projects?

Cohen: It was one of several projects going on in each lab at the same time. My lab was also studying the role of tranposons in plasmid evolution. And, we were studying plasmids in other ways as well. Annie was the one person in my lab who was working on the DNA cloning project. Other people in the lab were working on other projects. I felt I could afford to put a research technician on a project that had a high risk of not leading anywhere. If it wasn't successful, okay, well that's the way it goes. The postdocs who needed papers to get them faculty jobs had projects that I felt had a higher likelihood of producing publishable results; but Annie had a permanent job in the lab and if the project didn't work out, she wasn't at risk. And there were also a number of projects going on in Herb's lab at the time. You've talked with Mary Betlach and others about these.

Hughes: Yes.[64] Did Boyer consider it a risky experiment as well?

Cohen: As far as I know, he did. From the discussion that we had in Hawaii, he also felt that these experiments had exciting potential but, again, it was very uncertain whether they would work.

Hughes: Over what period of time did these experiments go on?

Cohen: We began the work just shortly after the New Year in 1973, and by early March, two months later, we had shown the basic feasibility of the method. So the experiments went very quickly.

Hughes: Were there any surprises?

Cohen: Well, one of big surprises was that normally non-replicating fragments of DNA could actually be propagated in this way.

Hughes: But other than that?

Cohen: I was also surprised to find that non-ligated fragments could be joined together *in vivo* after they were taken up by cells. But other than that, the experiments were basically planned out at the beginning of the collaboration and we were happy to find that they progressed according to plan. As you know, even experiments that are carefully planned can run into unforeseen obstacles, but these just worked out extremely well. Bob Helling and Herb had the *Eco*RI cleavage conditions worked out and the agarose gel technique going; I had the electron microscope heteroduplex methods, transformation methods, and other plasmid procedures worked out. The experiments proceeded quickly, and it was an extremely exciting time. We often wished that the bacteria would grow faster so that we could get the result of an experiment sooner. We came into the lab each morning to look at the [culture] plates. We would hurry to isolate the plasmid DNA and Annie would carry some of it up to Herb's lab where it would be analyzed by agarose gel electrophoresis. At the same time, we'd look at it here by centrifugation and by EM. It was really a continual high.

Recognizing Potential Industrial Applications

Hughes: Was there any thought at this point that there might be industrial applications? It's certainly not in the paper, but was it in your thinking?

[64] Oral histories with Mary Betlach and with Axel Ullrich are available online in this Bancroft Library series..

Cohen: It's hard to think back and pinpoint the moment that this thought first occurred. To me, it probably was not before the initial positive results, but certainly very soon after that. But industrial applications were dependent on the ability to clone DNA from other species, and as I've said, we hadn't shown that yet. That's why we didn't go beyond the conservative statement we made in the discussion: "...potentially useful for insertion of specific sequences from prokaryotic or eukaryotic chromosomes...." But even if it turned out that animal cell genes couldn't be propagated in bacteria, the point of this statement was that it might be possible to take genes that were native to other bacterial species and introduce them into more-easily grown *E. coli*.

Hughes: The idea was in the wind. I haven't read Peter Lobban's thesis. Apparently, he made specific reference to the fact that this procedure might have industrial applications.[65]

Cohen: I read Peter's thesis dissertation when I wrote my *Scientific American* article in 1975, but don't remember any mention of that. Jensen *et al.*, the industrial group I mentioned earlier, probably were thinking in terms of such applications in undertaking the work they published in 1971, since they worked for a company. But in starting the initial gene cloning experiments, we weren't thinking, or at least I wasn't, about putting animal cell DNA into bacteria. And when I did start thinking about this, most colleagues I discussed it with thought that it was unlikely that animal cell DNA could survive the restriction systems of prokaryotes.

There's another point that I should make about the cloning of animal cell DNA. The problem of how to select bacteria that contain foreign DNA fragments was, at that time a formidable one. In our initial experiments, it was possible to identify the bacteria containing recombinant plasmids because the fragments that we joined to the pSC101 vector included a resistance gene that we could test for, or select for. I knew that even if eukaryotic DNA could in fact be propagated in bacteria, it would be necessary to work out ways to identify the bacteria that acquired recombinant plasmids, versus those bacterial clones that taken up the vector only.

Interview 5: March 1, 1995

WRITING THE FIRST COHEN-BOYER PAPER

Hughes: Dr. Cohen, last time we talked about the scientific aspects of the first recombinant experiment. Maybe today we want to talk about the actual writing up of that work.

Cohen: Okay. Well, there were several issues related to the reporting of it. As I mentioned last time, by March of 1973 we had the initial data and knew that the strategy was successful. But we still needed additional data for some of the control experiments, and also to find out whether DNA fragments from other plasmids could be ligated and propagated in the same way. Getting that data took a couple of months, from March through May. Herb and I met, I think in late in May, to outline the paper. The notes that I took at the time are in a notebook that I no longer have: it's at the Smithsonian [Museum of Natural History],

Order of Authorship

[65] Wright, S. *Molecular Politics: Developing American and British Regulatory Policy for Genetic Engineering, 1972-1982*. Chicago: University of Chicago Press, 1994, p. 72.

Cohen: I was the person who had initially proposed the experiments and their design, and I would be doing most of the writing of the paper. Herb agreed that the authorship order should reflect that. We discussed what other people would be authors. Now, at the time of this meeting, Herb raised, for the first time, an issue related to his colleague at UCSF, Howard Goodman. He said that because he had been collaborating with Howard in his work on *Eco*RI, he thought Howard should be an author of this paper. My reaction was, "Well come on, Herb, Howard has had absolutely nothing to do with this work, either experimentally or conceptually." I didn't think it was appropriate, and I didn't care what kind of private arrangement he had with Howard. So far as I was concerned, I just wouldn't have any part of it. Herb was not happy about that because he had to go back and argue with Howard. But he recognized the validity of the points that I raised, and there was agreement that the authors would be me and Annie Chang, as the group at Stanford, and Herb and Bob Helling as the group at UCSF, in that order.

Hughes: You explained why you were first author. But why the next sequence?

Cohen: Because there were two groups involved with these experiments, and I was the primary person in the first group, and Herb was the primary person in the second group.

Hughes: Is that *pro forma*?

Cohen: Not really *pro forma*. It might take us a couple of hours to discuss how the order of authors is determined for papers in the biological sciences. But the customary order of authorship is that the name listed last is the senior person in whose laboratory the work is done, and is the "corresponding" author for the paper. In this instance, Herb and I both were pretty junior at the time, and the work was done in both of our laboratories. So the question was whether I wanted to be listed first or to be listed in the position usually reserved for the corresponding or senior author. One possible way of doing it was to list Annie in the first position and me in the last one, with Boyer and Helling in between, but Herb was not enthusiastic about that and I agreed that this order was problematical. Annie was a technician who was working very explicitly under my direction. Technicians aren't necessarily included as authors, but Annie had made important technical contributions and gotten experiments to work. So I felt that she should be an author, although I agreed with Herb that putting Annie in the first author position wasn't appropriate. We also agreed that the Stanford contributors would be listed first. So, as I've mentioned, the authorship order we settled on was myself and Annie in the first two positions, and then Herb and Bob as the second two authors. Herb and I would be listed first for each of our two groups, respectively. Helling worked on the project in Herb's lab, but so far as I knew, he had not made any conceptual contributions. The experimental plan was already worked out by the time Herb and I had left Hawaii. Herb can tell you more about Bob's actual role, I'm sure.

Hughes: Was there any discussion about including some of the technical staff in Boyer's laboratory?

Cohen: No. At least not in any discussion that I was part of. Bob Helling was the "technical staff" in the UCSF experiments. Bob was working full time at the bench in Herb's lab in much the same way that Annie was working full time at the bench in mine. When a professor goes on sabbatical leave, it's a wonderful opportunity to return to work at the bench, and Bob had come to Herb's lab to do this. I'm not sure whether Bob had a role in purifying the *Eco*RI preparations we used. I think he probably did not, but he did at least some of the ligations at UCSF, and he did gel analyses. Annie and I knew most of the other people in Herb's lab, but Bob was the only other person at UCSF that we interacted with over these experiments, and Herb had never raised any questions concerning authorship of any other members of his lab. The only issue that was raised about co-authorship was the one about Howard Goodman.

But regarding Helling, I should say here that later on in the course of the patents pursued by

Stanford and UCSF, Bob claimed that he should be included as an inventor.[66] Who the inventors were from a legal perspective was not up to me to say. The Stanford patent attorney concluded, based on the information that he had gathered, that the inventors were myself and Herb, and that Annie and Bob Helling were not inventors. Bob disputed that determination and the matter was then re-reviewed by the Stanford and UCSF lawyers; I was told that inventorship is a legal issue that is independent of authorship and that their conclusion was that Bob was not an inventor.

In any case, the manuscript was put together in June [1973], and I sent it to Norman Davidson who is a member of the National Academy [of Sciences]. He was someone I knew well, and I had collaborated with him on the electron microscope heteroduplex experiments I've mentioned. Norman was an expert on DNA and familiar with plasmids So, I asked him whether he would consider communicating this manuscript to the *PNAS* in our behalf. He agreed to consider doing this and sent the paper out for peer review. The reviewers raised some small points, which wasn't unusual, and we addressed them by minor revisions. And the manuscript was accepted for publication.

Disclosure of Results at Gordon Conference and the Singer-Söll Letter

Cohen: Herb and I had agreed that our results shouldn't be talked about publicly until all of the controls were done and we had pulled the data together in a manuscript. But the results were very exciting, and he couldn't help telling others about them at the Gordon [Research] Conference [on Nucleic Acids] that he went to in June 1973. As you've learned from reading through the MIT oral histories, he reported on our experiments as an informal add-on talk. And as often happened during the first year or so following our work, the importance of the findings was initially lost on many of the people who heard about them. But the next day, I've forgotten who [William Sugden][67] realized the implications of being able to clone DNA and said, "Well, now maybe we can put together any kind of DNA." The discussion that followed led to some of the biohazard concerns, which were then raised in the form of a letter from Dieter Söll and Maxine Singer, the co-chairs of that particular Gordon conference.[68] It was that letter that eventually led to the formation of the National Academy of Sciences committee, the Berg *et al.* committee. So in any case, the Gordon conference talk was the initial disclosure of the results to a broader group of scientists.

Hughes: How did you feel about that?

Cohen: Well, I had mixed feelings. On one hand, I wasn't very happy about what had happened because Herb and I had agreed not to talk yet about our work, which was at least several months away from publication. On the other hand, I realized that it is difficult to avoid telling others about results that are so exciting. I also wanted to let others know about the results. When you have an exciting finding, you want to let colleagues know about it. But we had not even submitted a manuscript at that point, and there had been no peer review of our data.

Hughes: Now, would you have had that attitude about any research that hadn't been published?

Cohen: Not necessarily, but this wasn't just any research.

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[66] The patenting process is discussed in detail in later interview sessions.

[67] Lear, J. *Recombinant DNA: The Untold Story*. New York: Crown Publishers, 1978, p. 70.

[68] Maxine Singer, Dieter Söll to Philip Handler, July 17, 1973. In: J.D. Watson and J. Tooze. *The DNA Story: A Documentary History of Gene Cloning.* San Francisco: W.H. Freeman and Co, 1981, p. 5. Hereafter, Watson and Tooze.

Publication Delay

Cohen: Now there was another matter regarding publication: the delay between submission and publication. *PNAS* usually publishes a paper two-to-three months after it is accepted. So, if a paper is accepted and communicated by a member on behalf of someone else, or is contributed by a member who is an author, publication usually occurs within two to three months. However, in 1973 there were some problems at the *PNAS* that resulted in a much extended publication schedule. So, even though our paper was completed in June and communicated after peer review to the *PNAS* office by Norman Davidson in July, instead of being published in September or early October, as would have normally happened, the paper wasn't published until November, which was about five months after the Gordon conference. By that time, we had also discussed the work with a lot of colleagues, and word about our results had gotten around fairly extensively. I was excited about the work, and Herb was excited about the work, and although we didn't give, as I recall, any outside seminars on our findings during this period, everyone here at Stanford knew that we had been able to clone genes using plasmids.

Hughes: Was Boyer likewise not deliberately talking about this work?

Cohen: It wasn't that we were deliberately not talking about the work. We were talking about it openly with colleagues.

Hughes: You weren't going on a lecture circuit.

Cohen: No, we were not. The approach and findings hadn't yet become a topic for the "lecture circuit." I don't remember giving any seminars on our DNA cloning results prior to publication of the paper in November. I don't know definitely whether Herb did, but somehow I don't think so, aside from the Gordon conference talk.

To jump ahead a little, I guess it was in the winter of '74, I was invited by Bill Robinson, a colleague at Stanford, who chaired a Keystone meeting on animal cell viruses, to speak at that meeting. These scientific meetings started as the Squaw Valley Symposia; now they're known as the Keystone Symposia. Bill was excited about my work and I was asked to give a talk. My presentation was scheduled for the next to last day of the meeting. It was an add-on talk for one of the late afternoon sessions. At that point, we had done the *Xenopus* work; we had put the frog DNA into *E. coli* and had cloned it and had shown its ability to be transcribed using *E. coli* promoters,[69] and that's what I talked about. There weren't a lot of people present at the session and there didn't seem to be a lot of interest in my talk. After the talk, someone came up from the audience and said, "Well, these experiments are kind of cute, but why in the world would anyone want to put DNA from a frog into bacteria?" I mention this because even after we had published the plasmid paper and after we had done the work on *Xenopus*, it still wasn't apparent to many people what one could do with this methodology. But clearly, it was evident to some, and these scientists jumped right in and wanted to do experiments using the DNA cloning methods we had developed.

INTERSPECIES GENE TRANSPLANTATION

The *Staphylococcus* DNA Experiments

[69] Morrow, JF, Cohen, SN, Chang, SCY, Boyer, HW, Goodman, HM, Helling, RB. Replication and transcription of eukaryotic DNA in *Escherichia coli. Proc Natl Acad of Sci USA*. 1974, 71: 1743-1747.

I'm looking now at the date of communication of that initial paper on plasmids. It was communicated by Norman Davidson on July 18, 1973. Immediately after completing the experimental work for this initial paper, which was sometime in May of 1973, Annie Chang and I began experiments to learn whether DNA from another species of bacteria could be transplanted to *E. coli* by linking it to pSC101. The goal was to try to clone and express genes from a plasmid taken from *Staphylococcus aureus*, a bacterial species totally unrelated to *E. coli*, using the pSC101 replicon.[70] The *Staphylococcus* plasmid, which was named pI258, carries a gene that encodes an enzyme called beta-lactamase which destroys penicillin and which we expected would also destroy *ampicillin*, a similar antibiotic that is highly effective in killing *E. coli*. By using ampicillin, we had a way of selecting for bacteria that were propagating and expressing the gene.

The experiment was a simple one. It was to use the *Eco*RI enzyme to cut up the DNA of the staphylococcal plasmid into individual fragments—we found that there were four—and then to take the cleaved DNA and mix it with pSC101, carry out the ligation and transformation procedures, and then select for cells that were resistant to penicillin/ampicillin and tetracycline. As the staph plasmid can't replicate in *E. coli*, these steps enabled us to isolate composite plasmids that contained and expressed both the tetracycline resistance gene carried by pSC101 and the beta lactamase [ampicillin/penicillin] resistance gene that originated on the staphylococcal plasmid. We didn't know before doing the experiments whether *Eco*RI endonuclease would interrupt the penicillin resistance gene—if it did, we couldn't select for it—or whether the staph gene could survive in and be expressed in *E. coli*. The two bacterial species are very different. But we found plasmids that expressed resistance to both antibiotics. And, by using heteroduplex methods and ultracentrifugation and agarose gel analysis, we showed that these plasmids included DNA fragments from both bacterial species. Some plasmids also carried additional fragments that had not been selected for, just as we had found in our earlier *E. coli* plasmid experiments.

The discovery that a staph gene could be transplanted to *E. coli* and be propagated there surprised a lot of people. The earlier experiments we did all involved genes isolated from *E. coli*, and this was DNA from an unrelated organism. Even though we had suggested the potential general utility of these methods in our earlier paper, the work with the staphylococcal plasmids provided evidence that we could actually use these methods to clone very foreign DNA in *E. coli*.

Hughes: Was that in your thinking when you chose to do that experiment?

Cohen: Oh yes, that was the thinking. And in that paper we suggested that since it could be done with a staphylococcal DNA, we might possibly be able to introduce useful genes, such as photosynthesis genes or antibiotic production genes that were indigenous to other bacterial species, into *E. coli* using these methods. The restriction mechanisms that people had thought were likely to limit the general utility of these methods didn't prevent the cloning of staphylococcal DNA. We said in this paper's discussion that our results supported the earlier view that antibiotic resistance plasmids such as pSC101 may be useful for putting DNA from eukaryotic organisms into bacteria. We also said that the cloning methods we had reported might be applied for studying the organization of eukaryotic genes. And the eukaryotic DNA experiments began before the staph work was submitted for publication.

Cloning of Eukaryotic Genes: the *Xenopus* DNA Experiments

[70] Chang, ACY, Cohen, SN. Genome construction between bacterial species *in vitro:* Replication and expression of *Staphylococcus* plasmid genes in *Escherichia coli. Proc Natl Acad Sci USA*. 1974, 71: 1030-1034.

Cohen: The way the eukaryotic gene experiments began is sort of interesting. Boyer had run into John Morrow, who was a graduate student of Paul Berg's, at the 1973 Gordon conference where Herb had described our joint experiments on plasmid DNA cloning. John was planning to study *Xenopus* gene expression and DNA structure as a postdoc in Don Brown's lab at the Carnegie Institution near Baltimore. John had finished his thesis work in Paul's lab, and I think for family reasons, couldn't yet leave the Palo Alto area; he had some time on his hands prior to moving to Baltimore. John had been using *Eco*RI from Herb to cleave SV40 DNA and he and Herb knew each other. John also had obtained *Xenopus* ribosomal DNA from Don Brown, and Herb and John discussed whether this might be a good eukaryotic DNA to try to clone in bacteria. I wasn't there, but my understanding is that the suggestion to use *Xenopus* DNA came from John. If Paul's lab had had the capabilities for DNA cloning in bacteria using the lambda *dv* system, I imagine that John would have turned to people in the Berg lab, where he had been working for several years, instead of turning to Boyer.

Anyway, when Herb returned to his lab, he phoned to ask if I thought that *Xenopus* DNA would be a good eukaryotic DNA to try to clone, and if I did, to invite my participation. We didn't have a way to selectively identify bacterial cells that took up recombinant plasmids containing the *Xenopus* DNA, as we did for DNA fragments containing antibiotic resistance genes. But I expected that if such plasmids were formed, we would be able to show, by the centrifugation and heteroduplex approaches Anne Chang and I were using to identify *Staphylococcus* DNA in *E. coli*, that the plasmids included eukaryotic DNA. I thought that we probably would have to screen a lot of cell clones for recombinant plasmids, but it was worth a try. There was also the larger concern that eukaryotic DNA might not be propagated by a vector plasmid in *E. coli*, but the preliminary inter-species gene transplantation results we had obtained using *Staphylococcus* DNA were encouraging. Don Brown gave permission for us to use his *Xenopus* DNA in these experiments, and after the Gordon conference, John and Herb and I got together and mapped out a strategy for cloning this DNA and identifying hybrid molecules.

Experimental Strategy for *Xenopus* DNA Cloning

Cohen: Because we couldn't select directly for the *Xenopus* DNA, we used the strategy of just doing shotgun DNA cloning, selecting for cells that expressed the tetracycline resistance gene of pSC101, and isolating the plasmids and analyzing the plasmid DNA in gels for the presence of restriction-endonuclease-generated fragments that were the same size as those fragments that were generated by *Eco*RI cleavage of the original *Xenopus* ribosomal DNA. We found such plasmids, and then showed that they included DNA fragments from both *Xenopus* and *E. coli*.

Hughes: Strictly by fragment length?

Cohen: That was one criterion, but we realized that we couldn't make such a conclusion simply on the basis of fragment size. So, we showed several other things. Fortunately, ribosomal DNA has a high different buoyant density when centrifuged in cesium chloride gradients; it has a different A+T/G+C [adenine thymine, guanine, cytosine] ratio than *E. coli* plasmid DNA. We could take the plasmid DNA, digest it with the *Eco*RI endonuclease, and examine the DNA both in cesium chloride gradients and in agarose gels, as we had done in the staphylococcal work. We showed that we got fragments that had the buoyant density you would expect from *Xenopus* ribosomal DNA, as well the same size.

Hughes: Now you knew that before you began the experiment, so that became one of the rationales for using *Xenopus*?

Cohen: Yes, from my perspective it certainly did. It wasn't just that the *Xenopus* ribosomal RNA gene

was eukaryotic; it had properties that we could use to find out whether we were actually propagating chimeric plasmids in cells. A key rationale for my own decision to proceed with these experiments was that the *Xenopus* DNA had been characterized by Don Brown and his collaborators and we knew that it had a different buoyant density from *E. coli* plasmid DNA. Annie and I had been using buoyant density differences to help identify *Staphylococcus* DNA that had been cloned in *E. coli* and I expected that the buoyant density would be a useful feature for identification of the *Xenopus* DNA. And there was an additional way we could identify *Xenopus* DNA isolated from *E. coli*. Using the methods of heteroduplex analysis I had learned from Sharp and Davidson, I thought that we might be able to detect heteroduplexes between the hybrid plasmids and the original purified *Xenopus* DNA if there was a region of homology.

In fact, that strategy worked out much better than we had hoped, because we found not only that heteroduplexes formed between the chimeric plasmid DNA and the purified ribosomal DNA, but we also saw some molecules in which two separate plasmid DNA molecules were heteroduplexed with the same piece of *Xenopus* DNA. The hybridization of chimeric plasmids with *Xenopus* ribosomal DNA at two separate sites suggested the ribosomal DNA contains repeats of segments having the same sequence. This was a feature of the ribosomal DNA that helped to establish unambiguously that eukaryotic DNA had been cloned.

Hughes: *Xenopus* DNA had these characteristics but I'm surmising that there were other types of DNA available at that time which also could have been differentiated from the *E. coli* DNA, right?

Cohen: Eukaryotic ones? I guess that the same experiments could have been done with ribosomal genes from any eukaryote, and, in retrospect, there probably were other genes that could have been used.

Hughes: How fortuitous was it that Boyer encountered John Morrow who offered the use of his DNA?

Cohen: Well, I do think that the encounter was fortuitous. At that time there weren't a lot of eukaryotic genes that had been highly purified and characterized. Because ribosomal genes were amplified during *Xenopus* development, Don Brown was able to isolate a lot of the ribosomal gene DNA and separate it from other DNA of *Xenopus*.

Hughes: Now, do you know how Brown's lab had actually characterized that DNA? Were the Sanger and Gilbert sequencing methods available yet?

Cohen: Oh no, that was a couple of years later [1975-1976].[71]

Hughes: So how had Brown's lab characterized the DNA?

Cohen: By hybridization methods and by analysis of its buoyant density and other chemical characteristics. Frankly, I've forgotten the details at this point.

Hughes: It hadn't become a vehicle for laboratory experimentation?

Cohen: No, it had not, although there were other people that had worked out the molecular size of the repeat unit.

There was another possible strategy for isolating chimeric plasmids containing eukaryotic DNA fragments that couldn't be selected for. I thought that chimeric plasmids containing foreign DNA fragments could be enriched in a population of plasmid DNA molecules by running the plasmid DNA in a centrifuge, taking the very forward edge of the DNA peak—which contains the largest molecules—retransforming a population of *E. coli* cells, and then repeating the experiment. We showed that after several cycles we could purify recombinant plasmids this way.[72]

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[71] Wright, S. *Molecular Politics: Developing American and British Regulatory Policy for Genetic Engineering, 1972-1982*. Chicago: University of Chicago Press, 1994, p. 80.

[72] Cohen, SN, Chang, AC. Chang and Cohen method for selective cloning of eukaryotic DNA fragments in

This approach turned out to be unnecessary in the *Xenopus* work, because we got a high frequency of recombinants in our primary screen. And the plasmid enrichment method became quickly outmoded because of a procedure developed by Mike Grunstein the following year in David Hogness' lab. Grunstein and Hogness showed that it was possible, using hybridization methods, to identify colonies that contain a particular fragment of DNA.[73]

Transcription of Eukaryotic DNA in *E. coli*

Hughes: Were you expecting to find transcription in the *Xenopus* work?

Cohen: I really didn't know whether or not we would. There wasn't much known about eukaryotic transcription signals or how they worked. We found that RNA complementary to the *Xenopus* DNA was made in bacteria, but the experiments didn't tell us whether the transcripts were initiated in the pSC101 plasmid DNA segment and extended into the *Xenopus* DNA, or whether eukaryotic transcription start signals were being recognized in *E. coli*.

In subsequent experiments that Annie and I carried out collaboratively with Dave Clayton and Bob Lansman at Stanford the following year, we took entire mitochondrial genomes from mouse cells and cloned them in *E. coli* by inserting them in different orientations relative to the pSC101 plasmid. That was done so that we could specifically determine whether eukaryotic gene transcription starts occurred in bacteria.[74] We expected that if transcription was initiated in the prokaryotic DNA segment, the strand serving as the template would depend on the direction of insertion of the mitochrondrial genome into bacterial plasmid vector. If, on the other hand, transcription was initiated in the mouse mitochondrial DNA signal, the same strand would be transcribed independently of the insert orientation relative to the plasmid. Also, transcription initiated by mitochondrial signals would be independent of the site of the joining of the two DNAs. It's a long story, but the bottom line is that on the basis of those experiments, we concluded that mouse mitochondrial RNA was being made by read-through transcription.

So bacterial promoters could initiate transcripts extending into animal cell DNA inserted adjacent to these promoters. These findings led to the strategy of trying to use prokaryotic gene promoters to express eukaryotic genes in bacterial cells. But at that point, no eukaryotic proteins had yet been made. That was done later, initially as fusion proteins by other laboratories—by Herb and Genentech, for example, for somatostatin—and by Wally Gilbert. The first eukaryotic proteins made in bacteria were fusion proteins, where bacterial genes and eukaryotic genes were fused in the same translational reading frame so that there was no stoppage of translation. But the first synthesis of a discrete and functional eukaryotic protein in bacteria didn't occur until 1978 in a collaboration between my lab and Bob Schimke's for the mouse dihydrofolate reductase. I'll tell you about that when we get further along in the story.

Hughes: What was your reaction to the results of the *Xenopus* work?

Cohen: I was both surprised and not surprised. I was surprised that the efficiency of ligation was great enough for us to be able to isolate clones containing *Xenopus* DNA without having selected for them. But after the finding that staphylococcal DNA, which was from a bacterial species totally unrelated to *E. coli*, could be replicated and expressed in *E. coli* to produce a functional gene product, I was optimistic that bacteria might also be able to tolerate and propagate eukaryotic

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Escherichia coli by repeated transformation. *Mol Gen Genet*. 1974; 134 (2): 133-41.

[73] Grunstein, M, Hogness, DS. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. *Proc Natl Acad Sci USA*. 1975 October; 72 (10): 3961-3965.

[74] Chang, ACY, Lansman, RA, Clayton, DA, Cohen, SN. Studies of mouse mitochondrial DNA in *Escherichia coli:* Structure and function of the eucaryotic-procaryotic chimeric plasmids. *Cell.* 1975; 6: 231-244.

DNA. But the *Xenopus* and *E. coli* DNAs were so very different in base composition, and some colleagues thought, as we ourselves said in some of our own discussions, "Well, so it works with *Staph*, but that's a bacterium, and it doesn't mean that eukaryotic DNA can be propogated by *E. coli* plasmids."

Hughes: So for you the bigger conceptual breakthrough occurred between the original experiments with the two plasmids and the experiments with the *Staph*?

Cohen: I'm not sure I would say that; I'd say it was more of a continuum. The fact that we could clone various plasmid DNA fragments by linking them to a replicon was the initial crucial demonstration. True, the fact that we could get *Staph* genes expressed in *E. coli* was more of a surprise, and you can describe it perhaps as the bigger conceptual breakthrough, but again the DNA was still bacterial. The general view was that a significant biological barrier existed between the animal, plant, and bacterial kingdoms. And little was known about how differences in DNA sequence might affect the ability to propagate DNA in foreign organisms. But certainly I was encouraged by the staphylococcal DNA cloning results, and they were an important step that showed that interspecies recombination was attainable. But that did not necessarily mean that the eukaryotic DNA experiments would work.

Individual Contributions to the *Xenopus* Work[75]

Hughes: I want to establish, since there are a number of names on the paper, who did exactly what.

Cohen: Well, the paper was published with the authors [in order] Morrow, myself, Annie, Herb, Howard Goodman and Bob Helling. Herb said that Howard, who as I mentioned hadn't done any work at all in the first collaboration I had with Boyer, had now run a couple of gels in Herb's lab for the *Xenopus* story. Herb felt that this was the basis for his being included an author. I was not too happy about that because running the gels was a simple task and could have been done easily by someone else. It was clear that Herb was using this as a way to justify including Howard as an author. Howard certainly wasn't conceptually involved in contributing anything. And Howard's inclusion as an author became an even bigger issue later on because while the work was in press, Howard made a lecture tour around Europe giving talks about the *Xenopus* DNA cloning work. He was the first one to present the work to anyone outside of Stanford, and I later discovered that he had talked about the experiments in a way that led listeners to think that the work was primarily his—although he was the fourth author on the paper and had been included only for the reason I've mentioned.

Hughes: Was it an unusual arrangement that he and Boyer had?

Cohen: Well, yes, it was. I've always viewed scientific collaborations as situations where two or more parties contribute to a project meaningfully. Legitimate authorship is derived from contributions, not from some prior decision that people will publish together, and then manipulate the situation in order to justify authorship. But apparently Herb and Howard had entered that agreement and it worked for them. I really don't know the details of what prompted them to enter the agreement. They obviously felt that it was mutually beneficial.

Now Herb and Howard came to the meeting with the proposal that they be the first group, the leading group, on this paper. John Morrow who was also there said, "Well, let's count the figures that will be included and see." In our discussion, we had, at that point, outlined what the paper would report, and the experiments performed in my lab by John and Annie Chang represented the bulk of the material for the paper. When we looked at the figures that formed

[75] For better continuity, this section was moved from the transcripts of interview #6.

the basis for the paper, it was evident to everyone that it wasn't appropriate for the UCSF people to be the first group. John would be the first author, since he had done more experiments than anyone else among the six of us. I had my choice of being the second author or being the last author. And that was an interesting decision. Had I been the senior member of a group, and had the work been done entirely in my lab, or had it been initiated by me, it would have been appropriate for me to be the last author. But I wasn't the most senior of the group, and I hadn't initiated the *Xenopus* collaboration, although it turned out that I had made many of the key contributions to the success of the project. Howard was a tenured associate professor and was the most senior member of the group.

Hughes: Herb Boyer was an associate professor.

Cohen: Yes, Herb was as well. And I also had recently become an associate professor, but had not yet been given tenure. We all realized that this was an important paper and the order of authorship was important. So the question was, did I want to be in the last author position, which was commonly reserved for the conceptual "father" of the work and the senior member of the group, or was another authorship position more appropriate. I thought about that for a couple of days and decided that I should be positioned as second author because my actual contributions to the paper and to the work were, at that point, second to John's. So there was the first group [Stanford] and then the other group [UCSF]. Annie was the third author in the Stanford group. It's a weird order of authorship when you think about it, because Morrow had been a graduate student in biochemistry but was working on experiments in my lab in the Department of Medicine.

Among the UCSF group, Herb was the principal person. Howard wanted to be the last author listed on the paper; he was the most senior person in the group and I felt that if this were done, it would appear that he was the conceptual guru. I objected to that; it just didn't reflect reality. Howard had less to do with the work than any of the other authors, and I felt that the basis for even including him as an author was contrived.

In retrospect, I probably should have asked to be the last author. Perhaps, a more appropriate order of authorship would have been John Morrow, Annie Chang, and then the group from UCSF, and myself.

Hughes: Is this kind of debate common?

Cohen: Well, I think it was more of a discussion than debate. But I should say that sometimes authorship is a difficult thing to resolve when multiple people and multiple labs are involved. But sometimes, it's done very easily. For example, in the later collaborative work done between my lab and Dave Clayton's laboratory on the cloning of mouse mitochondrial DNA, the work was done equally in my lab, largely by Annie Chang, and in Dave's lab by a student of his named Bob Lansman. And when we got together to decide on authorship, we concluded that both Annie and Bob had contributed equally to the work, and there was no way to make a distinction. The first authorship was decided by tossing a coin and putting, in a subscript on the first page, that the order of authorship was arbitrarily determined. It came out Chang, Lansman, Clayton and Cohen.

Postdocs in my lab have sometimes worked collaboratively on a project where each one would like to be listed as the first author on the paper, and if the work had been done entirely in my lab, it's been my responsibility for making the decision. I've always tried to do this fairly by looking at the individual contributions and seeing who has contributed more to the work. That person has a more prominent authorship position.

Other Research on Eukaryotic Genes Begins in the Cohen Lab

Hughes: Stan, did the fact that your interest was in prokaryotic DNA mean that the *Xenopus* work, while exciting from the standpoints that you've mentioned, was less relevant to your specific scientific interests?

Cohen: No, it didn't, Sally, because the ability to clone DNA broadened my interests. It was clear to me that there were important opportunities to use these methods for learning about eukaryotic genes, and my lab proceeded, during the next several years, to collaborate in studies with scientists working with eukaryotic cells. One such collaboration was the work I've already mentioned with mitochondrial DNA with Dave Clayton at Stanford. A second was with histone-encoding genes from sea urchins with Larry Kedes.[76] Histone genes had also been well characterized and they were available in Kedes' lab. These were the first protein-encoding genes that were put into *E. coli*. The *Xenopus* work encoded for ribosomal RNA and not protein. But we found that histone proteins were not produced in *E. coli* from the gene we introduced.

A continuing question was whether it was possible to make functional eukaryotic proteins in bacteria, and my lab collaborated with Bob Schimke's to try to answer this question. I had heard Bob give a talk about his work with the DHFR (dihydrofolate reductase) gene and saw a possible way to select for eukaryotic gene function in bacteria. DHFR is an enzyme that causes resistance to trimethoprim in both bacteria and eukaryotic cells, and was being used as an antimicrobial agent in bacteria. But trimethoprim treatment of bacterial infections doesn't prevent growth of mammalian cells because the eukaryotic DHFR enzyme is less sensitive to the drug. I thought that trimethoprim resistance might offer a way to select for bacteria that express the eukaryotic DHFR gene. If we could express the mouse DHFR in bacteria and get it to function there, we would get bacteria that are resistant to normally inhibitory levels of trimethoprim. We could select for bacteria that had become highly resistant to trimethoprim after introducing the mammalian gene expressed from a bacterial promoter. If we got any clones that showed resistance, we could determine what genetic signals had allowed the mammalian protein to be expressed. This strategy worked, and DHFR was the first functional eukaryotic protein expressed in bacteria.[77]

By 1977, the DNA cloning procedure was widely recognized as an important tool for investigating eukaryotic genes. Shosaka Numa, a Japanese neuroendocrinologist, saw it as an opportunity to learn about a gene that he was interested in. This was the gene encoding the pituitary hormone, proopiomelanocortin.[78] Earlier experiments by Numa and others had suggested that the proopiomelanocortin precursor protein is processed into multiple individual pituitary hormones. Numa saw DNA cloning as a way of elucidating the structure of the gene and determining what hormones it actually encodes. The other collaborations I've talked about thus far, except for the one involving the cloning of *Xenopus* DNA, were initiated by me. But the plan to study pituitary hormone genes was initiated by Numa, who had known Schimke, and Schimke and I agreed that these would be worthwhile experiments. Numa sent a scientific associate, Shigitada Nakanishi, to Stanford to clone the gene with us, and we began the collaboration. The work ended up predicting the existence of a previously unknown component of proopiomelanocortin, γMSH, and it was the first instance where gene cloning had led to the discovery of a novel eukaryotic protein.

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[76] Kedes, LH, Cohn, RH, Lowry, JC, Chang, ACY, Cohen, SN. The organization of sea urchin histone genes. *Cell*. 1975, 6: 359-369.

[77] Chang, ACY, Nunberg, JH, Kaufman, RJ, Erlich, HA, Schimke, RT, Cohen, SN. Phenotypic expression in *E. coli* of a DNA sequence coding for mouse dihydrofolate reductase. *Nature*. 1978; 275: 617-624.

[78] Nakanishi, S, Inoue, A, *et al*. Construction of bacterial plasmids that contain the nucleotide sequence for bovine corticotropin-beta-lipoprotein precursor. *Proc Natl Acad Sci USA*. 1978; 75: 6021-6025.

DNA CLONING STARTS IN OTHER LABS

Beginning the Distribution of the pSC101 Plasmid

Hughes: Well, let's go back to the plasmids. Talk, please, about the distribution method. When did you start sending out plasmids?

Cohen: Well the pSC101 plasmid was first given to others in late 1973, and the first recipient was David Hogness. David, who as I've mentioned is a professor of biochemistry at Stanford, was interested in studying *Drosophila* genes and had been trying to develop bacteriophage lambda as a DNA cloning system to isolate them. In earlier work, he and Dale Kaiser had made fundamentally important contributions to an understanding of bacteriophage lambda biology, but his lab had shifted to work with *Drosophila*. Once our experiments were done with *Xenopus* DNA, everyone knew that it had become possible to clone eukaryotic DNA in bacteria. There were several scientists at Stanford who were interested in isolating eukaryotic genes and Dave was one of them.

Although Morrow had finished his work as a biochemistry graduate student and all of the experiments he was doing on *Xenopus* DNA cloning were being done in my lab, he had not vacated his lab bench in the biochemistry department and saw people in that department every day. So, almost everyone in Biochemistry at Stanford knew about our results on an almost daily basis. A few days after we found that *Xenopus* DNA fragments could be propagated in *E. coli*, Dave asked me for the pSC101 plasmid, which was the only vector at the time known to be suitable for these experiments, so that he could use it to clone *Drosophila* DNA. At that time, we hadn't produced even an outline for a paper on the *Xenopus* work, were relatively early in the Staph work, and were still a few months away from publication of our *E. coli* plasmid DNA cloning experiments because of the *PNAS* delay that I've mentioned. I wasn't sure how to respond to his request. It's customary, and even required by standard protocol, to provide research materials to other scientists from the time of publication. But, our *Xenopus* DNA cloning experiments had been done only a few days earlier, and we had not yet even confirmed the result. On one hand, Dave was a friend and had been quite generous to me personally in letting me work in his lab when I first came to Stanford. I appreciated that and had expressed my gratitude to him many times, and so I was initially inclined to give pSC101 to him. But when I discussed this with Herb, his remark was, "Are you crazy? Do you really want to do that at this point?"

I started thinking about it a little more, and a day or so later, feeling very uncomfortable, went upstairs to see Dave. I said, "Dave, I have a lot of personal torment about this because I appreciate the help that I've received from you. Of course you can have the plasmid the day that our first paper about it and its use for DNA cloning is published. But, the DNA cloning work is probably going to be the most important research that I will ever do. I'm a junior scientist working in the Department of Medicine with clinical responsibilities, and you're an internationally known molecular biologist. If I give you the plasmid and you publish the cloning of *Drosophila* DNA about the same time as we publish our work, the cloning of eukaryotic DNA will be seen largely as your discovery. I'd like to wait a few months before giving you the plasmid."

Dave was not happy about my response. He felt that he was entitled to the plasmid right then, because he was in the same institution and we were institutional colleagues. I was told later by John Morrow that Dave subsequently took some of the plasmid DNA from Morrow's refrigerator in the Biochem Department because he felt so strongly that he was entitled to it, and that in subsequent discussions with a postdoc or graduate student who was to do these

experiments, Dave realized that he should not have taken the plasmid DNA without permission. Most people in the Biochemistry Department knew about this incident, and it was talked about for some time.

The discussions that were going on between Dave Hogness and me came to the attention of Paul Berg, who was then Chair of the Department of Biochemistry. Paul was very angry about my decision to delay providing pSC101 to Dave. Paul said that he and others in the Biochem Department had been instrumental in bringing me here to Stanford, which was of course true, and that my refusal to give Dave the plasmid immediately was so unreasonable that it had led Dave to do a foolish thing. Paul was really quite vituperative, I guess that is the word, and almost vengeful in his attitude, and I agreed to think further about my decision.

Hughes: His dissatisfaction was based on the premise that colleagues in the same institution should exchange material?

Cohen: That's right.

Hughes: That wasn't just a convention; that was an actual practice? It really was done that way?

Cohen: No, it wasn't a practice to exchange materials immediately after observations are made, even between colleagues at the same institution. Materials are sometimes given out early as part of a collaboration, and sometimes as a courtesy. Paul felt that if the shoe were on the other foot, that Dave would have given the plasmid to me.

I don't remember with certainly whether the Sgaramella matter affected my thinking about this, but I think that it did. As I mentioned, the work on complementary DNA ends produced by *Eco*RI was published concurrently by Sgaramella, and by Mertz and Davis. The Mertz and Davis publication was a better paper, although from my understanding of the situation, Sgaramella's observations on cohesive ends were made first. Although I've always given Vittorio credit for his role in the discovery of *Eco*RI cohesive ends, his contribution has been minimized or ignored in the retelling of the history by Paul and some others.

The Biochemistry Department was a scientific powerhouse at the time. They were the premier basic science department in the School [of Medicine]. The faculty were an outstanding and internationally recognized group of scientists, and they had the ability to move fast, and they were really quite aggressive in doing and publishing experiments. Although they had helped me to obtain a position at Stanford and to get my research program going here, and I am grateful for that, I think it's fair to say that many, but certainly not all, of the Biochemistry department faculty did not view me as a scientific equal: I was seen as a medical doctor trying to do research that involved biochemistry and genetics.

Biochemistry was also a very "clubby" department. The elitist attitude was, to a significant extent, fostered by the founder of the department here at Stanford, Arthur Kornberg. Arthur is a very smart and really quite extraordinary scientist, but he views most other people as not being capable of making decisions as correct as the ones he makes. Arthur is so smart, that this is often the case. But sometimes it isn't.

Hughes: You were not in the Department of Biochemistry and you were also untenured faculty—

Cohen: In the Department of Medicine.

Hughes: Which did not raise your status in their eyes, I imagine.

Cohen: Right.

Hughes: The request had come from Hogness, who was a full professor?

Cohen: Yes.

Hughes: And you've said he was a scientist of international repute. Wasn't this also perhaps in their thinking: "How could you, a young faculty who was in their minds somewhat indebted to their

assistance in the past and also wasn't even in the department, have the temerity to turn down the request of somebody with the status of Hogness?"

Cohen: Well, I think that was part of it. But quite honestly, the Department of Biochemistry at Stanford was the most powerful department in the school and usually got what they wanted. My plasmid was something one of them wanted, and they saw me as someone who was so ungrateful as to refuse. It was a period of torment for me because I recognized that my decision would alienate many of the biochemistry faculty. And it did not make me feel very good to have Paul angrily telling me—well, I won't use the words here—but telling me just how unappreciative I was.

I had also seen what had happened with Peter Lobban, and Peter's discoveries in Dale Kaiser's lab. I knew, as most everyone else at Stanford knew, that Lobban had worked out the key steps that led to the joining of dA-T [deoxyadenosine-thymidine]-tailed pieces of DNA. Berg's lab used the methods that Lobban had developed and Lobban's contribution has largely been lost in the scientific history. Though initially I was inclined to give the plasmid to Hogness, it was Boyer's comment to me, "Stan, are you crazy?" that led me to rethink it. That was a key turning point in my relationship to the Department of Biochemistry. There are people in that department who frankly have never forgiven me.

Hughes: Has it affected the course of your research?

Cohen: I'm not sure I know what you mean.

Hughes: Well, the work you were doing and are doing could lead to collaborations with the Department of Biochemistry.

Cohen: Well, yes. In fact, Hogness could have proposed a collaboration in which my lab would have done some of the work and his lab would have done other parts of the work. My contributions to the collaboration could have included work with the still-unpublished DNA cloning methods he wanted to use, doing transformation experiments, and doing some of the DNA analyses. I raised that possibility during our discussion, but Dave wasn't interested in a collaboration. He felt he was entitled to the plasmid, and that was it. And Paul also felt that Dave was entitled to it, but I just didn't see things that way.

The bottom line is that the pressure became enormous, so that even though our first DNA cloning paper wasn't published until November, I agreed to give Dave the plasmid a month or so before the paper appeared, after having held off earlier. The atmosphere around here was quite charged, and it was clear that a lot of the biochemistry faculty harbored ill feelings. So I felt enormous pressure and ended up giving Dave the plasmid in late October or maybe early November—I've forgotten the exact date—but, it was at least several weeks before the paper was published. He proceeded with the experiments he planned and, in fact, Dave's cloning of *Drosophila* genes was published in late 1974, the same year as our work with *Xenopus*. Dave's paper barely squeezed into 1974, I think, in the December 1974 issue of *Cell*. And he probably made it into 1974 because I had given him the plasmid a few weeks prior to our November publication date.

Hughes: You gave him a jump.

Cohen: I think it was something of a jump, although not a huge one.

By the way, I want to state clearly for the record that Dave later told me that he regretted his actions. I think that discussion took place at the time when I did give him the plasmid. Dave and I have since had a collegial and cordial relationship for the past twenty years. But I don't think Paul has ever forgiven me.

Restrictions on Recipient Use of pSC101

Hughes: Well, what about plasmid distribution to others? Did that not begin until after publication?

Cohen: After publication, I began to receive requests from other laboratories. These started in late 1973 or early 1974. At that time, pSC101 was the only vector known to work for DNA cloning. But later in 1974, people who had been trying to modify bacteriophage lambda to enable its use as a vector were successful. Lambda *dv* wasn't suitable, so they deleted some of the restriction enzyme cleavage sites from the normal lambda genome. This approach was published first by Alain Rambach and Pierre Tiollais[79] and by Noreen and Ken Murray,[80] and soon afterwards by the Ron Davis lab.[81] So lambda also became available as a cloning vector by the end of 1974. But plasmids had some important advantages as vectors, and I continued to get many requests for pSC101.

Cohen: I sent out the plasmid to scientists who requested it, as is common practice. But biohazard concerns were there in the background, and I also requested that the vector not be used for constructing antibiotic resistance combinations that didn't already exist in *E. coli*. When Annie Chang and I did the staphylococcal gene cloning experiments, we were mindful of the fact that genes encoding resistance to penicillins were already present in *E. coli*, so we wouldn't be introducing a new resistance capability. But there were some resistance traits that normally were not expressed by *E. coli*, and the potential for creating new combinations of resistance genes was a matter of concern to me.

Hughes: So your concern was not prompted by the growing biohazard controversy?

Cohen: It certainly preceded the Gordon conference and Singer-Söll letter. Antibiotic resistance was the focus of my research, and I was concerned about its spread before the biohazards controversy. The penicillin resistance gene from *Staphylococcus* was specifically chosen for our interspecies DNA cloning experiments because penicillin resistance was not new to *E. coli*. I'm glad you raised the point, because it is something that should be in the record.

Hughes: Was there a form or an agreement that had to be signed by plasmid recipients?

Cohen: No, I didn't require that they sign a form, and wasn't thinking about having a legally binding document. I certainly would not have brought legal action if a recipient of the plasmid violated the conditions. But I sent plasmids out with a letter saying that the plasmid was being sent with the understanding that it will not be used for introducing antibiotic resistance combinations that don't exist in nature, and also that it will not be distributed to other laboratories without my permission. I felt that there was enough mutual respect among the community that by accepting the plasmid scientific colleagues would use it under the specified conditions. And so I didn't require a signature. But I kept a record of persons I had sent the plasmid to. I wanted to be able to keep track of just who had received it.[82]

Hughes: Why?

Cohen: Well, most labs keep records of whom they send materials to, whatever those materials are. But also, concerns were being raised about any use of antibiotic resistance plasmids for DNA cloning, even if no new antibiotic resistance combinations were made. When a modified

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[79] Rambach, A, Tiollais, P. Bacteriophage lambda having EcoRI endonuclease sites only in the nonessential region of the genome. *Proc Natl Acad Sci USA*. 1974 October; 71 (10): 3927-30.

[80] Murray, NE, Murray, K. Manipulation of restriction targets in phage lambda to form receptor chromosomes for DNA fragments. *Nature*. 1974 October 11; 251 (5475): 476-81.

[81] Thomas, M, Cameron, J, Davis, R. Viable molecular hybrids of bacteriophage lambda and eukaryotic DNA. *Proc Natl Acad Sci USA*. 1974; 71: 4579-4583.

[82] For a list of those who received the pSC101 plasmid as of September 1974, see Cohen to Dick Roblin, September 5, 1974. (Cohen correspondence, Cohen's office, Department of Genetics, Stanford, folder: MIT #3. Hereafter, Cohen correspondence.)

version of lambda was first reported to be suitable for use as vector in 1974, there was even an editorial in *Nature* which said something like, "Great, now DNA cloning can be done safely without using these [terrible] antibiotic resistance plasmids." I wanted to be able to notify recipients in the event of a problem.

EXPANSION OF THE BIOHAZARD CONTROVERSY

Raising Concern in the Draft Version of the *Xenopus* DNA Cloning Paper About Potential Biohazards

Cohen: The *Xenopus* DNA cloning paper was completed late in 1973, and I asked Josh Lederberg to communicate it to the *PNAS* for publication. That was about six months after Herb Boyer had presented a report of our work at the 1973 Gordon conference on Nucleic Acids, and about five months after publication of the letter of concern written by at Maxine Singer and Dieter Söll following the realization that hybrid DNA molecules can be cloned. So there was by then a lot of discussion about possible biohazards, even before we found that even eukaryotic DNA can be propagated in bacteria by the procedures we had used for plasmid DNA fragments.

We said in the last paragraph of the *Xenopus* DNA cloning manuscript:

"The procedure reported here offers a general approach for the cloning of DNA molecules from various sources, provided that both molecular species have cohesive termini made by restriction endonuclease, and that insertion of a DNA segment at the cleavage site of the plasmid does not interfere with expression of genes essential for its replication and selection." But, I was beginning to distribute plasmids to multiple other labs, and in the originally submitted manuscript, this statement was followed by:

"However, the implications and potential biohazards of experiments employing this approach should be carefully considered, since the biological role of molecular chimeras containing both prokaryotic and eukaryotic genes is unknown."

I didn't know that it was Don Brown who was the referee for the paper until he told me some years later. His comment on his referee's report was:

"The cautionary paragraph at the end is ridiculous. If they have something to contribute about the morals and ethics of this kind of experiment they should say it. Whatever they have in mind hasn't stopped them from doing these experiments, and I don't think that it should have. I cannot see any benefit to ending such nice work with a vague ominous warning about the hazards of these experiments. If there is social responsibility involved, this doesn't fulfill their responsibility."[83]

I thought about the reviewer's comment and thought that he had a valid point, and I discussed it with Josh Lederberg. I decided, and Herb agreed, that we would remove the cautionary statement and it was not included in our final published paper.

The Committee on Recombinant DNA Molecules, National Academy of Sciences—The "Berg *et al.*" Committee

[83] Don Brown. Request for opinion on manuscript by J.F. Morrow, *et al*., *Proc Natl Acad Sci USA.* February 1974. (Cohen correspondence, folder: MIT #3.)

Cohen: Pollack's concern about the advisability of joining SV40 DNA to DNA from bacteria had prompted the initial discussions about possible biohazardous consequences of using DNA joining methods. The publication in November, 1972 of the Jackson, Symons, and Berg paper showing that ends of DNA molecules taken from different species can be joined biochemically when they are held together by base pairing and treated with DNA ligase wasn't too surprising, given what was then known about ability of ligase to form phosphodiester bonds between the ends of DNA chains held together in that way. But at that point, it was only possible to speculate about whether foreign DNA could survive in bacteria. However, when Boyer talked about our results at the Nucleic Acids Gordon conference in June 1973, there was the realization that unnatural combinations of biochemically joined DNA fragments from *E. coli* were viable. That realization immediately prompted the Singer-Söll letter, and discussions of possible biohazardous effects of chimeric DNA molecules began in earnest.

My own concern that the methods Boyer and I had devised might be used to bring together novel combinations of antibiotic resistance genes or toxins began when our successful initial results were obtained in March 1973 and, as I've said already, when I designed the staphyloccous DNA transplantation experiments a couple of months later, I was very aware of that issue.

After the Singer-Söll letter, the tempo of discussions about biohazards picked up rapidly. As you can see from my inclusion of the cautionary statement in the original version of the *Xenopus* DNA cloning paper, by February 1974, I was feeling substantial pressure to say something publicly. Phil Handler, who was president of the National Academy of Sciences, had asked Paul Berg to advise the academy on how to respond to the biohazard concerns that had been raised after the Gordon conference. Paul brought together a group of scientists to prepare recommendations, and that group subsequently met at MIT in the spring of 1974. Later, the group was designated as an official committee of the NAS.

Quite coincidentally, I happened to be giving a seminar at MIT the day after the committee meeting. My host at that seminar was David Botstein who currently is Chairman of Genetics at Stanford. In the elevator I ran into David Baltimore whom I had known through Jerry Hurwitz; David and I both had been postdocs in Jerry's lab. David was one of the people that Paul had invited to serve on the advisory group, and he told me who the group's other members were. He also said that the committee had identified two specific types of experiments that should not be done. One type was making DNA hybrids that contain tumor virus genes. Additionally, a moratorium was proposed on the use of any antibiotic resistance genes or plasmids in any DNA cloning experiments. I said that I didn't understand the scientific basis for the proposed moratorium on the use of resistance genes and plasmids. He said, "Well, you know, we're worried about this resistance stuff." I pointed out that there was no one on the advisory group who had experience working with bacterial antibiotic resistance or plasmids and suggested that the group limit its recommendations to tumor viruses, where they had ample expertise. I said that I certainly agreed that novel combinations of resistance genes shouldn't be made using these methods, and reminded him that I had set that up as a condition for receiving pSC101—but also told him my opinion that there would not be any hazard in using an antibiotic resistance gene or a plasmid as tool in DNA cloning if the resistance gene is not novel to the host bacterium. It seemed to me that scientifically flawed judgments were being made by some really outstanding scientists, and I felt that the process that had been set in motion was leading to irrational recommendations about the experimental use of plasmids.

Hughes: That was news to you?

Cohen: Well, I knew from Paul that the committee had been formed, and had suggested to him that he appoint some additional committee members with expertise in microbiology. He said that his concerns were about tumor viruses, SV40 and others, and he had chosen experts in that area.

He didn't see any point in having plasmid biologists on the committee. But my short discussion with David Baltimore indicated that the group had expanded its mission substantially. Norton Zinder was a member of the committee, and he certainly has a solid background in bacteriology and bacteriophage biology. But even he had not worked with antibiotic resistance genes or plasmids.

Berg Expands Committee Membership

Cohen: I phoned Herb Boyer when I returned to Stanford after my encounter with Baltimore. I said, "Look, Herb, there was a meeting of this Berg committee and they are planning to propose a moratorium on exactly the type of experiment that we've done and published. I think there's no scientific basis for that proposal and it makes it seem like our work is being censured."

Hughes: It was also going to stop your research.

Cohen: Yes, it was going to stop not only our research, but would also affect the research of other scientists trying to find a solution to the problem of bacterial drug resistance. And I felt that the proposal was misguided. So I started to draft a letter that was intended to be a published statement from Herb and me indicating our feelings about the importance of continuing the study of antibiotic resistance genes and plasmids and their use in DNA cloning, but also expressing our concern about making new antibiotic resistance combinations. I asked Stanley Falkow and some other people who were knowledgeable in the field of resistance plasmids to review what I had written, and they agreed that it made sense.

Paul learned about my intended letter. I've forgotten just how that happened, but there were no secrets at Stanford; everyone knew what was going on. He called me and said, "Well, this is a little bit silly; we shouldn't be sending separate letters. Why don't you and Herb just add your signatures to our letter?" I agreed that it made sense to do this, but only if I could agree with the committee's recommendations.

After several iterations that resulted in significant modification of the section on antibiotic resistance genes and plasmids, we came up with a statement that everyone could live with. Paul decided that since the list of signers had expanded to include Boyer and me, he would also invite two other colleagues from Stanford who were doing recombinant DNA research to be signatories: Ron Davis, who at that point was developing a lambda phage that could be used for DNA cloning, and David Hogness. So the final letter was published in the *PNAS*, *Science*, and *Nature* as "Potential Biohazards of Recombinant DNA Molecules"[84] and was signed by the original group that had met at MIT, plus myself and Herb, and Ron Davis and Dave Hogness. It became known as the Berg *et al.* letter.

Hughes: This was a very Stanford-centered activity. From the science I suppose that is logical, but in terms of the politics, was that wise?

Cohen: I'm not sure I know what you mean. Are you asking about the institutional affiliation of the signers?

Hughes: Well, I'm actually thinking more widely than that. Stanford played an extremely prominent role in the science and in the political controversies that arose from it. There were accusations, and I don't know if I ever heard exactly where they came from, that Stanford just wanted to control things so it could continue its research.

Cohen: Oh, I see.

[84] Berg, P, Baltimore, D, Boyer, HW, Cohen, SN, Davis, RW, Hogness, DS, Nathans, D, Roblin, R, Watson, JD, Weissman, S, Zinder, ND. Potential biohazards of recombinant DNA molecules. *Science*. 1974; 185 (148): 303.

Hughes: So from a standpoint of diplomacy one could argue that it might have been wiser to get a broader representation.

Cohen: Well, you know, I wasn't involved in choosing the members of the committee. The original ones were Dick Roblin, who was I think at Harvard at the time, and Norton Zinder, who was at Rockefeller, and David Baltimore, who was at MIT, and Berg of course was at Stanford. Sherman Weissman is at Yale, Dan Nathans at Johns Hopkins, and Watson at Cold Spring Harbor. Initially, Paul was the only committee member from Stanford. The other Stanford signers came because of the circumstances I've just described.[87]

I think the way Paul viewed things was that he wanted the scientists who were in a position to do these experiments to indicate that they were in agreement with the moratorium. There weren't a lot of people who could do these experiments at the time. There was only one vector, pSC101, and it was given out only by me to scientists who requested it. Ron Davis was developing lambda phage as a vector, and there were groups in the U.K. and France that also were doing this, but the lambda vectors weren't ready for use at that point.

Hughes: Yes, I recognize that nobody could avoid the political ramifications, but I suspect in the beginning the hope was to confine the debate to strictly scientific issues.

The Press Conference Announcing the Berg *et al.* Letter

Cohen: Well, that's right. As a matter of fact, my first awareness that the impact of the committee's letter would extend far beyond the scientific community came from a brief conversation I had with Paul in one of the medical school corridors a day or two before the letter was released. He told me about the planned press conference. And I said, "Press conference? Why in the world do you want a press conference for this?" And he said Phil Handler had wanted it; that Phil felt that it was important scientifically. And I told him that I thought the decision was a mistake. As a group of scientists we were urging our colleagues to consider possible biohazardous consequences of certain types of DNA cloning experiments they may be thinking about doing. But holding a press conference to announce this proposed moratorium may lead the public to think that we feel there is imminent and clear danger in this kind of research. And he said, "Well, it's already planned. Dave Baltimore is flying out, and Dick Roblin's coming out, and we're having a press conference." And so they did.

That led to headlines in most major newspapers, "Scientists Call Halt to Genetic Experimentation."[85] And in my opinion, the way the moratorium was announced to the public was an important factor in creating the perception that recombinant DNA research must be much more dangerous than anything else that was being done in the biological sciences. Scientists were working regularly and cautiously with known pathogens, but weren't calling press conferences to talk about a halt to such experiments. Microbes were involved in pollution and other environmental problems, and we weren't saying anything about that in press conferences. But here was a press conference being called to stress the fact that scientists were voluntarily calling a halt to experiments that use this new research tool. In fact, the recommendations concerned only two very specific kinds of experiments, but that fact was lost on the media. Some of the headlines were about halting genetic experimentation altogether. And as I wrote some years later in my *Science* article, "Recombinant DNA: Fact and Fiction,"[86] I think that the way the moratorium was announced fostered the perception that the technique itself was dangerous.

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[85] For examples of other headlines, see Watson and Tooze, p. 12.

[86] Cohen, SN. Recombinant DNA: Fact and Fiction. *Science*. 1977; 195: 654-657.

Change in Public Perceptions After the Berg *et al*. Letter

Hughes: I believe you received media attention in connection with the *Xenopus* experiments. Am I right?

Cohen: Yes. That story involves David Baltimore, a friend of Victor McElheny who, at that time, was a science writer or science editor of the *New York Times*. About the time of publication of our work, McElheny called Baltimore and said, "Well, what's new and important in your scientific field?" Baltimore told him about the *Xenopus* work and McElheny phoned me and wanted to do a story about it. I agreed to that, and he talked with me and with Josh Lederberg, who had communicated the paper to the *PNAS*, and with other people, and he wrote his article.[87] But what was interesting, and I'm glad you asked about this, Sally, is that the *Xenopus* paper was published in May 1974, just a few weeks before the Berg *et al*. letter on biohazard concerns came out. And the reaction to recombinant DNA in the press was very positive at that time.

Hughes: It [the Berg *et al*. letter] was published in July, but I believe it was circulating before then.

Cohen: Yes, it was among scientists, but the press conference about biohazard issues didn't occur until at the time of publication of the letter. In May, McElheny had written a very upbeat article in the *New York Times* saying that this technology would likely lead to important new drugs and help deal with pollution and energy problems. I don't think there was a word in that article about biohazard concerns, and yet all of the scientific information that was available at the time of the press conference was there in May. But, after the Berg *et al*. letter was published, the whole climate changed and people were fearful about the research. So instead of viewing the ability to transplant animal cell genes to bacteria as a positive scientific advance, the press started reporting it as something to be feared.

Hughes: I assume that the intention of the Berg committee was to calm fears. I guess where the mistake was made was to assume that there were fears to be calmed. It wanted to demonstrate that science was taking over and everything was under control.

Cohen: That scientists were policing themselves.

Hughes: Yes, exactly.

Cohen: I think to this day, Paul feels that the moratorium was the correct thing to do, that it showed that scientists were able to police themselves, and that science is better off because of it.

Hughes: There was criticism at the time—and in the secondary literature that has since been written on this issue—that the scientists narrowed the discussion to biohazards and purposely did not deal with the broader issues raised by recombinant DNA technology.[88]

Cohen: What was, in retrospect, distressing is that we [the signers of the Berg *et al*. letter] were a group of scientists focusing on hazards that were entirely conjectural. None of the signers of the letter would have considered publishing a scientific paper based on such conjecture. But, the self policing of scientific research that began with Robert Pollack's concern that hybrid DNA molecules containing SV40 DNA might be constructed by the Berg lab was now being driven largely by Paul, who felt that self-policing needed to be extended to other kinds of experiments that might be done by other scientists who were less wise. But all of us felt at the time that we were doing a good thing.

Hughes: As you pointed out, the situation had moved beyond science. Perhaps there were earlier

[87] McElheny, V. Animal gene shifted to bacteria: Aid seen to medicine and farm. *New York Times*. May 20, 1974; 1.
[88] See, for example: News and Comment: Wade, N. Genetics: Conference sets strict controls to replace moratorium. *Science* 1975 March 14, 187 (4180): 931-934.

instances, but when Handler requested of Berg that a press conference be held, it was no longer just a scientific issue.

Cohen: I don't know whether the idea of a press conference originated with the Academy or with Paul. But, the Academy planned it, and Paul was certainly in favor of it.

Hughes: My point is that, for that reason and probably other instances, the problem was no longer within the realm of science.

Cohen: I agree with that. Not only was the discussion no longer just among scientists, but the view of recombinant DNA research by the non-scientific community was dramatically altered. A favorable view of what this scientific approach might accomplish had been put forth in McElheny's article in May, but fearful headlines appeared after the letter and press conference just a short while later.

Hughes: Well, it's a wonderful illustration of the interpenetration of science and society.

Cohen: Right.

Interview 6: March 7, 1995

THE PLASMID NOMENCLATURE WORKING GROUP

Need for a Uniform Nomenclature for Plasmids

Hughes: Today seems as good a time as any to discuss plasmid nomenclature.

Cohen: Okay. Up until the mid-1970s, there really wasn't any standard nomenclature for plasmids. Someone would isolate a plasmid and give it whatever name seemed suitable. Usually the name was related in some way to the function of genes carried by the plasmid. For example, the F factor, which was then later changed to "F plasmid," was named "F" for "fertility." It promoted conjugal mating in bacteria. Colicinogenic plasmids made substances that killed *E. coli*; these plasmids were initially called "colicinogenic factors." We've discussed the R factors, which carry resistance genes.

Scientists in different parts of the world isolated R factors and then gave them names that sometimes did not take into account the naming of R factors by others. For example, two investigators might isolate new resistance factors in their separate labs, and then independently name each of these as "R1," and the two R1s could be totally different plasmids. Also, there were other terms in common use that were confusing. For example, some scientists used the word "plasmid" for an extrachromosomal element, while others called these elements "episomes" because they were genetically separate from the chromosome, and some used "episome" to describe just the extrachromosomal state of plasmids that moved in and out of chromosomes. There were words like "transmissible" and "nontransmissible" being used sometimes, rather than "conjugative" and "nonconjugative," to indicate whether a plasmid has the ability to pass between bacterial cells. But some plasmids can transfer not only themselves but can also enable the transfer of other plasmids that are currently present. The nomenclature used by workers in the field became complicated.

Formation of the Plasmid Nomenclature Working Group

Cohen: There is a general nomenclature for genetics which goes back to the days of [Milislav] Demerec in the '40s or '50s, and a classic paper that set forth a standard way of referring to bacterial genes and to genotypes and phenotypes.[89] Scientists working with plasmids began to realize that a standardized nomenclature was also needed for plasmids. At the Honolulu meeting on plasmids in November 1972, which we've already talked about, a working group assigned the task of preparing a preparing a proposal on plasmid nomenclature was formed. The person who took a leadership role in moving this forward was Richard Novick, and Richard was able to raise some money to support the undertaking. The people asked to participate on the committee were Roy Clowes who was an old-time plasmid worker and had done some of the early work with colicinogenic plasmids, Stanley Falkow, Naomi Datta, who was a plasmid biologist from the U.K. who had done important early work on resistance plasmids, and me.

Hughes: Curtiss?

Cohen: Yes, and Roy Curtiss. Richard also asked Don Helinski but Don was heavily occupied with other things at the time and declined to serve as a member of the committee.

The nomenclature committee worked hard and was really quite productive. We went into issues in great detail and discussed the fine points of how to name and define plasmids. We had a lot of correspondence on this and met initially in March 1973 to prepare draft proposal that was distributed to about 200 scientists for comments and suggestions. We met again in January 1974 and had other multi-day meetings in late 1974 and early 1975 to modify the proposal in response to comments that were received and to come up with a suitable nomenclature.[90] All of us recognized that the ability to construct plasmids *de novo* from fragments of other plasmids made the need for a uniform nomenclature especially urgent. Unless there was a uniform way of describing and naming plasmids, we'd have a mess as more and more plasmids were constructed.

Devising the Nomenclature

Hughes: Did you base your nomenclature on genetic tradition?

Cohen: Yes, it was based on the genetic tradition of Demerec and others who wrote the earlier nomenclature paper for bacterial genetics. We decided that extrachromosomal elements would be designated "p" for the plasmid, followed by the initials of the person whose lab it had been isolated in, or constructed in. At that time, we didn't envision every graduate student and postdoc constructing a plasmid carrying his own initials. We thought that using a two-letter, or even three-letter alphabetical identifier for the lab, plus a numerical identifier for different plasmid from that lab, it would be possible to cover a large number of plasmids. So, for example, pSC101 is the plasmid of Stan Cohen 101. I chose arbitrarily to begin at 101, instead of beginning at number one. We had isolated plasmids before then but hadn't assigned pSC-based identifiers.

Hughes: You are saying that every new plasmid constructed or isolated…

Cohen: …would have its own designation. And the original description of each plasmid would provide information about its isolation or construction, essentially its lineage, and the information would indicate what fragments of which plasmids had been joined together to generate that

[89] Demerec, M, Adelberg, EA, Clark, AJ, Hartman, PE. A proposal for a uniform nomenclature in bacterial genetics. *Genetics*: 1966; 34: 61-76.

[90] Novick, RP, Clowes, RC, Cohen, SN, Curtiss, R, Datta, N, Falkow, S. Uniform nomenclature for bacterial plasmids: a proposal. *Bacteriol Rev*. 1976 March; 40 (1): 168–189. p. 168.

particular construct.

Hughes: And is that indeed done?

Cohen: Well, it's done by geneticists, but the nomenclature plan hasn't been followed uniformly by everyone using plasmids. Plasmids are widespread tools in multiple areas of biology, and some people have deviated very substantially from the recommended nomenclature.

Hughes: You intended that anybody working in a laboratory would use the initials of the head of the lab, in the case of yours, SC?

Cohen: Right.

Hughes: But that has not happened, has it?

Cohen: Right. At this point, not in my laboratory either. For a while after the nomenclature report, we did name plasmids in my lab using my initials, but later used the initials of the person who actually constructed the plasmid. By 1977, Annie Chang had constructed a series of plasmids based on a cryptic replicon that didn't carry any resistance markers on it, a plasmid replicon that originally was called p15A. We named them pACYC177, pACYC 184, *et cetera*.[91] Annie was eager to have the plasmids carry her initials and that was fine with me. At that point AC had been used for Al Chakrabarty, and so we chose a four-letter designation, pACYC for Annie C. Y. Chang. Since that time, my lab has not followed the original plan to identify our plasmids by the pSC designation.

These days, people add various types of genes to plasmids and some give the constructs names intended to indicate the genetic or phenotypic properties of the plasmid. In our initial nomenclature discussion, we recognized that such names would become cumbersome because one can't conveniently carry along lengthy genetic or phenotypic descriptions in plasmid names or use lengthy identifiers in the text of a publication. So we proposed a more simple method of identifying plasmids.

A Nomenclature for Transposons

The general approaches the Working Group used for plasmid nomenclature were subsequently adopted for bacterial transposable elements, the Tn elements. The first bacterial transposons, Tn1 through Tn10, were discovered prior to use of a uniform transposon nomenclature and were named retrospectively according to the date of publication of the paper originally reporting their discovery. Tn1 was the first bacterial transposon. It was discovered by Bob Hedges and Allen Jacob who, so far as I know, also coined the name "transposon."[92]

Tn2 and Tn3 were also ampicillin transposons that Falkow's lab and mine identified a little later, about the same time. The three ampicillin resistance transposons which had all been named tnA for ampicillin resistance were identified initially on different plasmids and we didn't know then whether their DNA sequences would turn out to be similar or different.

In order to help avoid duplication when new transposons were discovered concurrently in different laboratories, a mechanism was established for assigning transposon identifiers. I requested and was assigned a certain number of Tn element slots, starting with Tn21, and the Tn elements we discovered after Tn3 are in that numerical group. Similarly, when someone named a new plasmid, that information was deposited in a plasmid repository that Esther

[91] Chang, ACY, Cohen, SN. Construction and characterization of amplifiable, multicopy DNA cloning vehicles derived from the p15A cryptic miniplasmid. *Journal of Bacteriology* 1978, 134:1141-1156.

[92] Hedges, RW, Jacob, AE. Transposition of ampicillin resistance from RP4 to other replicons. *Mol Gen Genet*. 1974; 132 (1): 31-40.

Lederberg ran for several years at Stanford with National Science Foundation support. And that approach helped to avoid what I think could have been considerable confusion in the scientific literature. Although as I've said, the original nomenclature plan has not been followed rigorously, it was more or less followed. There's no longer a plasmid repository, but the overall approach for naming plasmids has stuck.

THE PLASMID NOMENCLATURE GROUP'S ROLE IN THE ASILOMAR CONFERENCE

Establishing the Plasmid Working Group for the Asilomar Conference on Recombinant DNA

Cohen: Now, some of this discussion is not only relevant to plasmid nomenclature but is also relevant to the biohazard controversy. The Berg *et al*. letter recommended that a conference be convened to more fully consider biohazard concerns associated with recombinant DNA research, and working committees were appointed by Berg in preparation for that conference, which was scheduled to be held at Asilomar in February 1975. The group of scientists that had worked together on the plasmid nomenclature committee was asked to make recommendations about how potential biohazards regarding plasmids might be mitigated. We compiled a lengthy report that eventually was presented to the Asilomar participants.[93] A group had been formed in England to consider biohazards and had just issued its own report as "Her Majesty's Working Party on the Experimental Manipulation of the Genetic Composition of Microorganisms"[94] [The Ashby Committee]. Prior to Asilomar, we facetiously named our group, "The Working Party on Potential Biohazards Associated With Experimentation Involving Genetically Altered Microorganisms, With Special Reference to Bacterial Plasmids and Phages."

Developing a Protocol for Defining Potential Hazards

Cohen: The discussions that we had were very Talmudic; we were discussing hazards that weren't known to exist, and the lack of evidence for actual hazard necessitated a lot of assumptions and speculation. For example, we thought that working with larger volumes of potentially biohazardous bacteria would create a greater opportunity for dissemination than working with smaller volumes. So, we tried to define what was a "larger amount" versus a "smaller amount."

Well, we said, in the course of an experiment, during use of a continuous-flow centrifuge, there would be greater opportunity for aerosol production than during the use of a standard centrifuge containing bacteria in tightly capped bottles. And the largest rotor then available for standard centrifuges could hold up to six capped 500-milliliter containers. To avoid leakage, the containers shouldn't be completely filled. Thus, a single centrifuge run could sediment about 2500 milliliters of cultured bacteria. In a practical sense, one could do maybe three or four centrifuge runs a day. Therefore we came up with the notion that working with cultures of bacteria greater than 10 liters in volume would involve a higher level of risk. And that 10-liter

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[93] Working Party on potential biohazards associated with experimentation involving genetically altered microorganisms, with special reference to bacterial plasmids and phages to the Committee on Recombinant DNA Molecules. National Academy of Sciences, February 24, 1975. (Cohen correspondence, folder: Asilomar.) Hereafter, Plasmid Working Group Report.

[94] Report of the Working Party on the Practice of Genetic Manipulation. Presented to Parliament by the Secretary of State for Education and Science by Command of Her Majesty. London: 1976 August.

figure, which was arrived at with these kinds of considerations, was later written into the NIH Guidelines and then used for other guidelines.

Later, the 10-liter figure was codified into regulations that were passed. Unless bacterial culture volumes of greater than 10 liters were handled under high-risk conditions, the scientist doing the work was in violation of the rules, as if the 10-liter cut off were some magical figure derived from great wisdom. But it was derived just as I've described, by speculation and guessing.

Hughes: Were there other instances where people used the best guess and it later became codified?

Cohen: Yes, and that was one of the things that some of us were concerned about. If we pulled up the report we prepared for Asilomar, I think that I would remember other instances just by leafing through the document. We were a group of scientists knowledgeable about plasmids, but there was no indication that the research could create any actual hazard at all, and we were making "best guess" recommendations on the basis of perceived potential for hazard.

Group Dynamics Among Committee Members

Hughes: Stan, I'm interested in the interaction of the group itself, both internally and with other groups. What difference did it make that by the time of the Asilomar conference [February 1975], this particular group was quite accustomed to working together?

Cohen: Well, we worked very closely together, but there certainly was not uniformity of opinion. There were members of the group—and I suppose that I was one of them—who felt that because there was no actual evidence of hazard that that we had no scientific basis for our classifications, and that we should be very explicit in saying so. Yet, we all had concerns because no one knew, and the unknown is scary. Roy Curtiss tended to be at the other end of the spectrum, and Roy Clowes was kind of in the middle and a very thoughtful mediator. Naomi Datta worked hard on the nomenclature report, but didn't want to be part of the successor group preparing a report for Asilomar

If you look through my files, as you have, you can see some of the correspondence; we got into great detail on issues. We were also concerned about how our positions would be viewed by scientists who had no experience working with plasmids. We expected that our recommendations would be viewed negatively by some and favorably by others. But that didn't cause us to alter what we said.

We had a lot of respect for each other, and we worked well as a group. On some occasions the discussions got loud and argumentative, but we worked through our differences in opinion. I suppose that's the way that committees should operate.

Hughes: When you are a member of a rather long-standing committee, such as this, that met on a number of occasions, are there scientific repercussions as well?

Cohen: I'm not sure what you mean by scientific repercussions.

Hughes: Committee members have a chance for scientific exchange, and I would think that there would be opportunities to set up research collaborations above and beyond the actual work of the committee.

Cohen: Well, yes, we did exchange data openly as to what was going on in our respective labs.

Hughes: More so than you would have if the committee had not been called?

Cohen: Well, we were in close communication—I mean phone calls multiple times a week, correspondence back and forth, but there were no collaborations that actually developed.

Hughes: Was everybody on the committee doing recombinant research?

Cohen: I think at that point, yes. Stanley Falkow was interested in pathogenesis, as was Roy Curtiss to some extent, although with a different system. Roy Clowes was doing work with transposons, which was somewhat different from the work that my lab was doing with them. Richard Novick was working with gram-positive bacteria, staphylococci. There were no specific collaborations that I can recall that grew out of those meetings, but there were many scientific discussions. I think what we should do is pull that report, so why don't we stop for a moment.[Interruption]

Devising a Classification for Experiments According to Perceived Potential for Hazard

Cohen: Looking through the document again now reminds me about the approach we took in preparing for Asilomar. I think that an actual system of classification of experiments according to the perceived hazard came first from our group. We started by saying that working with hazardous microorganisms is nothing new. That had been done for many years with natural pathogens. We felt that the background of information that had been accumulated during work with known pathogens was applicable to our overall goal, and defined the factors that might affect biohazard potential, such as whether the organism the introduced genes had come from was itself pathogenic, the potential for dissemination, the potential for the alteration of ecology, the potential for persistence in the environment, whether or not the foreign genes were likely to be expressed, and the purity of the DNA. Also relevant was the extent of information available about the donor and recipient of the DNA. We attempted to classify different types of experiments according to these criteria.

Then we set forth a series of containment procedures that were graded relative to the perceived potential for hazard. That approach was consistent with practices used in working with known pathogenic microorganisms, and it was readily accepted at Asilomar. We were the only reporting group that had gone into containment issues in any detail, [95] and our classifications provided a framework for the ones used in the NIH guidelines. We didn't realize that the very act of writing down our speculative assessments in a document would give credibility and a sense of reality to the speculations. Classifying experiments according to how hazardous we thought they might be made the hazards real in people's minds. We had moved from questioning whether or not there was any scientific basis at all for thinking there might be a hazard, to categorizing hazards and discussing how we were going to protect against them; that fostered the view that assumptions about hazards were valid.

Hughes: Now were you aware before the Asilomar conference of the tenuous nature of your assumptions?

Cohen: Yes, we were aware of it. it. But we were the only Asilomar committee that had significant experience working with bacteria. Most scientists on the other working groups were eukaryotic cell biologists or virologists who were concerned about the potential for creating cancer-forming bacteria, and they had very little knowledge of drug resistance or standard bacterial containment procedures, issues that we were dealing with every day.

Hughes: You knew, obviously, that the other groups were working on different aspects of the biohazards problem, but you didn't know that they were not going to take it to the deep level that your committee had?

[95] The three groups set up by the Asilomar organizing committee, chaired by Paul Berg, were the Eukaryotic DNA Working Group, the Animal Virus Working Group, and the Plasmid Working Group. (*Molecular Politics*, p. 145-147.)

Cohen: That's correct.

Hughes: I understand that the reports that came out of the other two committees were very short.

Cohen: They were short reports. As I've said, the plasmid group's report became the basis for the subsequent principles that were adopted at Asilomar and the [NIH recombinant DNA] guidelines.

Hughes: Which you did not know while you were formulating them?

Cohen: Well, we didn't know this, but we had concerns about what the outcome of Asilomar would be. The whole mood in the lay press was one of increasing fearfulness. Publicity about the research had generated fear in the minds of the public. I think that all of us knew that Asilomar was going to be difficult in terms of the discussions and decisions, and no one really knew what would happen. As I was leaving for Asilomar, I went back to get antacid tablets from my office drawer. I had problems with gastric reflux at the time and had a lot of heartburn. The night before Asilomar we had stayed up most of the night completing our report and collating it, and the documents were carted out to our car from my office at Stanford. As we left the parking lot, I said, "Wait a minute, I have to go back and get my antacids."

I'll talk more about Asilomar another time, but I just want to say now that I found the experience one of the most depressing that I can remember. Despite the qualifications of the scientists in attendance, decisions were being made on the basis of fearful speculation and on how our actions would be viewed by non-scientists, rather than on scientific evidence. I felt that there was a lot of political posturing throughout the meeting. It was kind of a circus atmosphere where the press had been invited in the interest of having an open meeting. That was fine, but the press, as the press usually does, was looking to write interesting articles that would appear on the front pages of newspapers and would be widely read. Reporters followed us around and tried to get on-the-spot interviews, and scientists were giving them. We read our statements, taken out of context, in the next day's newspaper, and it was stressful.

Interview 7: March 22, 1995

MORE ON THE PLASMID COMMITTEE FORMED PRIOR TO THE ASILOMAR MEETING

Hughes: Dr. Cohen, we talked a little last time about Asilomar and the formation of the Plasmid Committee that morphed into the Asilomar sub-committee on plasmids. Today, I think we should go into it in a bit more depth. I understand from having read Richard Novick's MIT oral history that there were two separate but related problems that the [Asilomar] committee was to address. One of them was the molecular studies of plasmids and the other one was the epidemiological problem if they escaped. Do you remember that?

Cohen: Yes, sure.

Hughes: And who decided that it should be?

Cohen: I don't know where that mission came from. Maybe from Paul Berg, who initially contacted Richard. As a matter of fact, I should say something more about this. As I've mentioned, I had been suggesting to Paul from the time I first learned about his plans to pull together a group to advise the NAS on the biohazard concerns raised after the 1973 Gordon conference that there was a need to involve experts in plasmid biology. I raised the issue again in connection with the planned conference at Asilomar. I told him about the Plasmid Nomenclature Committee and

suggested that he consider having this already-existing group serve as a subcommittee of plasmid experts for the Asilomar meeting. All of the members of the plasmid nomenclature group had backgrounds in bacteriology and most had worked with bacterial viruses as well as plasmids. I said that Novick, who had chaired the Nomenclature Committee, would be an excellent chair for the Asilomar subcommittee. Paul thought that was a good idea and contacted Richard.

The reasons for needing plasmid expertise were pretty clear. Although some people thought that the future of DNA cloning was with phages, I expected plasmids would continue to have a major role.

Basis of Recommendations of Plasmid Committee for Asilomar

So what were the principles that governed our recommendations for the Asilomar meeting? We knew that it was not going to be practical to contain every bacterial cell containing a cloned gene, so the level of containment should be matched with perceived risk. Other considerations were the potential for alteration of the ecology and the potential for persistence in the environment—and the potential for phenotypic expression of the foreign genes. We thought that a gene that was unlikely to be expressed in bacteria would be less likely to alter the properties of the new host. Still another factor was the extent of genetic information available about the organism that the cloned gene came from. We felt that DNA from organisms that were well characterized and known not to have any pathogenic effects would have a lower potential to be hazardous. We also felt that the purity and characterization of the DNA used in forming the recombinant molecules was important as well. So on the basis of those considerations, we came up with six classes of experiments for which we proposed levels of increasingly stringent containment that we imagined would match the level of perceived risk. In actuality, most of our recommendations were based on conjecture, but they nevertheless served as the basis for the RAC [Recombinant DNA Advisory Committee] guidelines. The guidelines developed in most other nations were also based on the same considerations.

Hughes: Did those classifications come out of the air? Or, you must have been looking at something to give you some guidance in how to begin to partition those problems.

Cohen: Oh yes. I think I've mentioned that the classifications were influenced by the practices used in work with microorganisms that are known to be hazardous. Research labs had of course worked for some years with pathogenic microorganisms that produce diseases such as anthrax, diphtheria, and others. In general, the more pathogenic the organism, the tighter the conditions used to prevent its escape from the laboratory. When the extent of risk is known, it is straightforward to determine the level of containment that should be used. But we were trying to match containment with hypothetical risks, and we had no way to know whether our guesses about how risky particular experiments might be were accurate. There was no evidence of actual risk in any of the experiments we were discussing. But we got very involved with trying to match containment with perceived risk, and during this mental exercise the possibility of hazard took on a sense of reality. That was more so for some members of the group than for others. As I've mentioned to you, I personally felt that we should base our assessment of possible risk more on what was known about the organism the gene had come from than on speculative considerations. There were some microorganisms that contained genes that we knew encoded hazardous products and I thought that a scientist cloning DNA from those organisms should follow the same precautions that would be used for working with the pathogenic organism itself.

Ironically, the levels of containment that were eventually adopted by the RAC for recombinant DNA molecules lacking any known potential for hazard were greater than were required for working with microorganisms that were known to be hazardous.

Hughes: How did that come to be?

Cohen: Well, I think that was a result of the general climate of fear that existed during that period. After Asilomar, DNA cloning itself was viewed by many as being dangerous and that worry led to responses that were excessive. But there was also another factor that generated valid concerns. Many scientists who started DNA cloning experiments in bacteria had little microbiological training; they were engineers and chemists and biochemists and were not experienced in working with living organisms that have the ability to reproduce. Stanley Falkow, in particular, was concerned about scientists who routinely tossed bacterial cultures down the sink drain, and he was vocal about these concerns during the discussions of the Plasmid Committee. I was concerned about people pipetting cultures of bacteria by mouth, which was a common practice in many biochemistry labs, and about possible hazards associated with eating and drinking and smoking in labs. It was a bacteriology issue not uniquely associated with recombinant DNA experiments.

Use of Standard Microbiological Practices

The Plasmid Committee recommended that the precautions that were standard in microbiology labs be required for DNA cloning work. Even if microbes containing cloned genes were not uniquely dangerous, good microbiological practices needed to be implemented. That has been one of the beneficial outcomes of our committee's recommendations. For example, the practice of pipetting bacteria by mouth has been virtually abandoned for all microbiology experiments. The technology to accurately pipette small amounts of liquids automatically was there before, but there wasn't a demand for it. Scientists would routinely transfer liquids by suctioning them up into pipettes inserted into their mouths, and if they were transferring bacterial cultures, they would insert cotton plugs into the pipette to reduce the chance of accidentally swallowing some of the culture. If they were working with a hazardous microorganism, they would use a rubber bulb to create the suction. But there weren't the accurate battery-driven mechanical pipetters that were developed in response to the biohazard concerns raised about recombinant DNA.

Hughes: Well, you bring up an issue that I believe underlies this entire debate, namely the tension that I sense exists between those with a strictly molecular background and those that come out of, or at least have been exposed to, microbiology. My perception is that the American Society of Microbiology does not appear, in the documents anyway, to be playing a very prominent role in this whole issue, and is that all related? To put it crassly, had the molecular biologists taken over and those with a microbiological background been forced to take a back seat?

Cohen: Well, I think that's one way of viewing it, at least during the early days of the Berg *et al*. committee. Initially most of the people on the committee were animal virologists or biochemists, and there was not a lot of attention to microbiology. I should point out that for at least several years prior to our development of methods for cloning DNA, there was a move away from work with bacteria. Some scientists were still working with bacterial viruses, and some were studying plasmids and antibiotic resistance. But many of the hot shots in molecular biology felt that the golden years of bacterial and bacteriophage genetics were over. And…

Hughes: Because eukaryotic work was now more feasible?

Cohen: No, this was before recombinant DNA.

Hughes: Oh. But why did it happen?

Cohen: Well, I think there were certain scientists in the field that led that exodus. One of these was Sydney Brenner, who had made a number of major contributions in genetics working with *E. coli* and is a highly respected molecular biologist. Sidney started working with worms because he concluded that more complex model systems offered greater opportunity for significant discovery. Sidney did, in fact, lead the founding of a new field, which has contributed enormously to an understanding of genetics. And a number of other leading scientists also moved away from work with bacteria and bacteriophages. For example, David Hogness, who had made important contributions working with lambda, moved to work with *Drosophila*. Paul Berg, who had worked on the biochemistry of tRNA [transfer RNA] enzymes and RNA polymerase of *E. coli* and a number of other bacterial enzymes, began to work with the mammalian cell virus SV40. Those of us who were still studying bacterial systems were viewed by some as being perhaps a little passé.

However, in the years following the DNA cloning work that Boyer and I published, there was a resurgence of interest in bacterial studies. And bacteria continue to provide useful model systems for asking important biological questions. For example, though DNA transposition had been discovered years earlier by [Barbara] McClintock in maize, bacterial transposons provided a model for work that was being done with transposons in mammalian and plant cells. There previously had not been adequate systems for studying the molecular nature of transposons until they could be studied in bacteria.

Differences of Opinion Among Committee Members

Hughes: You mentioned the diversity, or implied the diversity of opinion within the Plasmid Committee itself. I'm wondering if you have any comment to make about why that should be when you are a group of five who have been working together for some years now, beginning with the nomenclature work, and you're roughly working in the same areas. Why was there polarity in perspective, or diversity, maybe not polarity?

Cohen: Well, I think polarity really isn't the right word. We were friends, and overall our individual perspectives had a lot in common. But we were five different individuals who had different experiences and diverse backgrounds. We had different points of view on some of the biohazard issues and there were gradations of opinion about others.

Hughes: Is some of it just a matter of philosophy? For example, you could divide the whole issue into those who felt that scientists were perfectly capable of policing their own affairs, you know, t sort of a laissez faire approach to the whole problem; and others who thought no, this was too complicated an issue for scientists on their own to handle, that they needed society at large to step in.

Cohen: The points of difference weren't related to that issue.

Hughes: No?

Cohen: Our discussions weren't about policing or enforcement. We weren't talking about whether society should police or scientists should police. We were discussing ways to assess potential hazards of experiments and were talking mostly about the validity of conjecture about the perceived hazard.

Hughes: But you were talking about guidelines.

Cohen: I suppose that depends on what you mean by guidelines. We discussed the factors that scientists should consider in evaluating the potential hazard of a particular experiment.

Hughes: I see.

Cohen: It's true that one of our objectives was to provide a framework that others might use as a guide in determining the risk associated with an experiment. But there was no consideration of how or whether our suggestions, which were "guidelines" in a literal sense—not regulations—would be enforced. But a point that Josh Lederberg did, in fact, make at Asilomar was that guidelines would get codified and that it would be very difficult to change them.

Hughes: And he was right and wrong in a sense. They did become codified but they were quickly relaxed.

Cohen: Well…

Hughes: I mean they were changed.

Cohen: They were changed, but considerable effort was expended to modify provisions when it was realized that conjectural hazards being addressed by regulations had no valid scientific basis.

Hughes: Well, stepping back though, before RAC became a reality, I mean while you were still in the Plasmid Committee debates, am I understanding you right that the mindset was that these guidelines set up by people who knew the field were to be adopted by those doing this particular kind of research on a voluntary basis? And the scientist in charge would regulate his or her own research, without the idea that there was going to be this external body which was the RAC?

Cohen: Well, I think the answer to your question is, "Yes." Although enforcement was not discussed explicitly, the notion that it would be voluntary was implicit in the way that we proceeded. Self-policing had been used in research with pathogenic organisms. Scientists who were carrying out experiments with microbes that could cause human disease worked according to guidelines that were established by the U.S. Center for Disease Control. The precautions were followed by scientists to protect themselves, others in the lab, and the broader community. I wasn't aware of any policing mechanism to ensure that the CDC recommendations were followed, although it had been the practice for a couple of decades to restrict studies of highly contagious diseases of livestock to an island [Plum Island] located off of Long Island in New York. In any case, my recollection is that the policing issue was not raised in our committee discussions. We wanted simply to provide guidance for scientists trying to make decisions about how to do recombinant DNA experiments, in much the same way that guidance already existed for work with known pathogens.

At same point, we prepared—I've forgotten whether it was included in the final report or part of an appendix—a list of examples of containment conditions that we recommended for different types of experiments. After preparing this list, we tested ourselves with a little questionnaire that asked how each of us would independently rate particular experiments that were not mentioned in our list of examples. We had discussed the basis for classification at great length, and we wanted to know how much agreement there would be when the principles we had set forth were applied. We were surprised about how much agreement there was about the containment level. For most experiments, the categorization was straightforward, and we felt that our recommendations would be useful to other scientists.

Hughes: Well, returning to the issue of the degree to which scientists before Asilomar thought they were in control of what was going on: I'm thinking that the Berg letter, which was published in July 1974, called for—it's not called the Recombinant DNA Advisory Committee, but that's what it is talking about—establishment of an objective agency to oversee recombinant research.

Cohen: I think that the published Berg *et al*. letter asked for a voluntary deferment of two kinds of experiments, those two types were: creating antibiotic resistance combinations that didn't exist in nature, and putting tumor virus genes in bacteria. And it also called for the exercise of caution in experiments that introduce other mammalian genes into bacteria. It's correct that the letter also asked the NIH director to consider establishing an advisory committee to oversee a

program that evaluates potential hazards and develops guidelines for scientists to use in minimizing risk, but I didn't view this as a call for establishment of a regulatory agency. From my perspective, the letter was intended primarily to call the attention of other scientists to the issues we had raised.

Hughes: Well then, is the RAC a creation of Asilomar itself?

Cohen: Plans to create a group to advise the NIH on recombinant DNA research certainly predated Asilomar. The Berg *et al*. letter requested that. But classification of recombinant DNA experiments according to perceived hazard was to a significant extent an outgrowth of Asilomar. Of course, a key question is who does the classifying? Well, the Plasmid Committee had for certain experiments, but we had not gotten into the classification of perceived risk associated with the cloning of different types of non-bacterial DNA. And there were some new issues that were raised at Asilomar about developing biological approaches to containment. This idea was initially raised by Sydney Brenner, I believe, and then followed with a proposal by Curtiss for developing chi1776.

Hughes: Why would that be something that Brenner would bring up particularly?

Cohen: I think I have notes on that session at Asilomar and I will look at them, but I don't remember the circumstances that prompted him to raise the issue.

Hughes: Was biological containment a rather unusual approach?

Cohen: Not totally. The recommendations presented by the Plasmid Committee also considered the use of bacterial hosts that reduce the potential for risk, for example using *recA* mutant bacteria to decrease the chance that foreign genes would be inserted into the chromosomes of bacteria used for DNA cloning. We also talked about choosing bacterial hosts that would have a decreased potential for persisting in the environment. But we did not have the idea of specifically designing bacterial strains that do not survive outside of labs. That approach was especially attractive to non-microbiologists who didn't find it appealing to have to use physical containment procedures for their experiments. The notion was, if it can't survive or grow outside of the lab, physical containment would be less important.

Hughes: But not a natural avenue in microbiology. I'm not meaning that it was never thought of, because obviously it had been thought of.

Cohen: Sure.

Hughes: But the usual procedure was to control experiments through physical containment.

Cohen: Well, previously, the microbes that needed containment were naturally occurring pathogens. Non-virulent or less virulent strains were being used to study some of these bacteria, but the only way to study the virulent ones safely was to contain them physically.

Guidelines versus Regulations

Hughes: Right, I see that. Well, again getting back to RAC, a letter that you wrote to the remainder of the plasmid group in October of 1974 at least indirectly reaffirms what you were saying previously: namely, that the RAC was certainly not in your mind as you were meeting with the Plasmid Committee, because you apparently suggested that institutions receiving federal funds for recombinant research should establish an institutional biohazards repository file, as you called it. And, you were opposed to establishing biohazards committees, which certainly leads me to believe that if the RAC had been a reality at that point, you would have had some words to say about the RAC, presumably to oppose it.

Cohen: I don't remember that particular letter. It would be useful to look at it. [Recording stops and restarts after Cohen reviews the letter.] Yes, thank you for raising this. I see now that I was pointing out that in many areas of science, the extent of risk in an experiment cannot be quantified before the experiment is actually done. But scientists don't ordinarily try to protect against all of the possible consequences they can imagine. They try to assess risk on the basis of what is known, and in working with pathogens, for example, they use a level of protection that is commensurate with the information available. I said that I thought the same considerations should apply to recombinant DNA research. But you're right, I did say I was uncomfortable about having local biosafety committees making judgments about the level of containment required for individual experiments using general guidelines, and I thought that different standards would likely be used at different institutions. I also said that we needed to do a better job of informing the public that the use of DNA cloning methods wasn't *per se* hazardous. The hazard, if there was one, would result from properties of a manipulated gene, not from the method used to do the manipulation.

Hughes: Both today and last time you expressed some dismay, if that's the correct word, about the fact that some of the criteria that you had set up were not based on hard scientific evidence and yet were taken and codified as we've discussed by the RAC. What would you have liked to have had happen?

Cohen: That's a good question. I would have liked the recommendations to have been viewed as guidelines, rather than as regulations. Regulations tend to have a kind of perpetuating force all their own, as Lederberg noted at Asilomar, and scientists in violation of a guideline stood the risk of losing grant support as a penalty. I believe that establishing a group such as the RAC to consider and evaluate new information, to make modifications in guidelines, and to disseminate updated information was important. But the RAC also became a body for the review and approval of experiments.

For example, to get ahead a bit in the story, I think I have mentioned previously the experiments that my lab carried out with Bob Schimke's laboratory, which resulted in the first instance of expression of a biologically functional eukaryotic protein in bacteria. That experiment was delayed for probably a year and a half because the RAC was concerned that it involved putting the gene into an *E. coli* chi1776 variant that produces the natural metabolite thymine. The modification of chi1776 was viewed by most scientists as a trivial change. But it was a change that technically had to be approved by the RAC and there was much discussion and much correspondence prior to the approval, even though there was no scientific basis for thinking that the change would be hazardous. At that point, we had gotten into the regulation mode; the "guidelines" had regulatory force and deviating just slightly from the largely arbitrarily conceived genotype of chi1776 required evaluation by the RAC and approval by a RAC subcommittee. The situation had shifted from a simple scientific determination of whether or not it was reasonable to do an experiment in a particular way to whether the experiment fully complied with all regulations. It was not ever suggested that the specific experiment was potentially hazardous and thus required the use of chi1776. But at that time all experiments involving introduction of any mammalian gene into bacteria were determined, rather arbitrarily, to be subject to this requirement.

Hughes: So the guidelines, at least in some instances, were impeding research?

Cohen: Yes, there's no question in my mind about that. In fact your question raises another interesting issue: following Asilomar and the establishment of the U.S. guidelines, many countries around the world adopted modified versions of the U.S. and/or British guidelines. Some adopted the U.S. guidelines exactly. Some countries developed their own guidelines, and there were countries where the guidelines or regulations used were much less stringent than those in the U.S. And during that period, biotechnology companies were being established in the U.S. and

elsewhere. Some of these companies wanted to introduce genes encoding insulin, interferon, and other medically important drugs into bacteria. There were also academic labs that wanted to do this. But these cloning experiments involved mammalian DNA and they couldn't be done in the U.S. under the guidelines that were in effect here at the time. But restrictions were much less stringent in France for example and in certain other countries in Europe, so the DNA cloning experiments could be done there by U.S. scientists. This led to what people jokingly referred to as the ice bucket brigade, where a U.S. scientist would make transatlantic flights with his enzymes and DNA and do experiments in other countries that could not be done legally in the U.S.

Hughes: How did you feel about that?

Cohen: Well, I felt that…are you talking about in a moral sense or in a biological sense?

Hughes: Either.

Cohen: Well, in a biological sense I think it pointed out the silliness of some of the actions that had been taken. Microorganisms don't respect national boundaries and it didn't make sense to have different rules in different countries. If a microbe should escape from a lab in France, it would quickly get to the U.S., so there was no point in having controls here that were more stringent. They wouldn't provide us with any greater protection. But I also felt that the "bucket brigade scientists" were not playing on a level field and I didn't like that, even though I knew that the U.S. guidelines—which then had become rules—had a weak scientific basis and that the bucket brigade people weren't doing anything illegal. But in taking advantage of the system, their actions were highlighting the absurdity of differences in levels of control in different countries.

Biohazard Likelihood as Viewed from Different Perspectives

Hughes: Was one of the—at least underlying—purposes of the Plasmid Committee to ensure that the guidelines that were imposed on research related to phage and plasmids were commensurate with the guidelines that were placed on other types of recombinant research?

Cohen: I'm sorry. I'm not following your question.

Hughes: Well, I have caught indirectly that one of the concerns, and I think you more or less stated it, was that as the Berg committee was initially constituted, there was nobody, in a sense, advocating for the case of the plasmids.

Cohen: Right.

Hughes: And there was a worry, I felt, and I want your comments on that, that because there was nobody who really understood what research with plasmids involved, that the guidelines—and there was evidence already—were more stringent on that type of research then on the research that the makers of these guidelines were themselves doing.

Cohen: I think that's true. A number of us felt that way. But I don't think there was malevolence by anyone involved. It was simply that people didn't view their own experiments as being hazardous, because they had greater knowledge about the system that they were working with, and there wasn't a scientific basis for imagining the existence of a hazard for that system. But it was easier for them to conceive of possible hazards for systems that they didn't know a whole lot about.

Hughes: As somebody said, the line is always drawn north of you.

Cohen: That's right. And so, as I've mentioned, in the original discussions of the Berg *et al*. group, there was the notion that just any work with antibiotic resistance genes would be hazardous because no one in that group knew very much about antibiotic resistance. And I think that this

general situation applied later on as well. Scientists wanting to put mammalian genes into bacteria had worked with DNA from mammalian cells and they argued that putting mammalian DNA into bacteria is unlikely to be hazardous; "It's those people that want to put genes from one bacterial species into another one that are doing the hazardous experiments." It was all conjecture in either case, but I think that what you're saying is basically correct.

Hughes: There apparently was talk of publishing the plasmid report. Do you remember the background for that?

Cohen: Yes, the issue was the following. At the final session at Asilomar there was an affirmation of principles by the attendees; there was not an actual report and I was uncomfortable about agreeing to a statement that hadn't been written yet. Although it was argued that we were just being asked to affirm principles, I felt that the details of how the principles would be implemented were important. The details of the final recommendations ultimately would be viewed as the output of Asilomar. Some members of the plasmid group felt that since the final Asilomar report wouldn't be completed for several months, that we publish the Plasmid Committee's document. I've forgotten the details as to why that was not done. Richard Novick probably would remember.

Hughes: What was the reaction to the plasmid group report when it was circulated at Asilomar?

Cohen: Well, I think that it contained more information in it than many people at the meeting wished to see. It was 35 pages long. The other committees had dealt with their areas in a much more cursory way, and had submitted reports consisting of two pages in one instance and four pages in the other. They really hadn't analyzed the issues in any depth.

Hughes: Why was that, Stan?

Cohen: I don't know.

The steering group running the meeting were mostly biochemists and animal virologists. But Novick, as chair of the plasmid group, was also a member of the committee that wrote the summary statement of the Asilomar meeting, if I remember correctly. But I think that the plasmid biologists were not viewed by some of the people that had been involved in organizing the meeting as being in the mainstream of what was going on. At least I felt that way, and I think thatat other members of the plasmid committee had similar feelings.

Hughes: Which is really ironic, isn't it, considering that the research that made this issue come to the fore was done by you, a plasmologist, in plasmids.

Cohen: Well, that's right. Most people there didn't think plasmids themselves were really very interesting. They were viewed simply as tools that everyone wanted to use to clone their favorite gene, but I don't think most scientists at the meeting felt that plasmids were something important to study *per se*.

THE ASILOMAR CONFERENCE, PACIFIC GROVE, CALIFORNIA FEBRUARY 1975

Participants

Hughes: Well, let's see. Let's go to Asilomar *per se*.

Cohen: Okay.

Hughes: I don't think we've talked about on what grounds people were invited. I know one thing: you sent Roblin a list of scientists you sent your plasmid to, and I believe he used that list of names… Well, he invited those people or submitted them to Berg; I don't know how it went.

Cohen: I'm not really sure. I know that list was an important part of that because it was the best information available as to who was interested in doing, or was doing, this work, and no one else had that information. So I did provide that list and it was, I believe, used by Paul and by Dick Roblin for doing the inviting.

Hughes: But was that sufficient?

Cohen: No.

Hughes: But there were many others who turned up.

Cohen: Right, yes. I don't know the basis for inviting other people [beyond the list of pSC101 recipients]. They were scientific leaders in countries around the world. They were senior and influential scientists who were viewed as being able to affect policies in their individual countries. I really don't have the details of that, Sally.

Expectations for Asilomar

Hughes: Was it clear from the outset what Asilomar was intended to accomplish?

Cohen: Well, that depends. Clear to whom?

Hughes: Right. Clear to you.

Cohen: Okay. Yes and no. Up until that meeting, I think that most or all scientists doing recombinant DNA research were following the guidelines of the Berg *et al*. letter. But that was just a very short letter. Its recommendations were brief, and as we've already discussed, they were developed on the basis of limited discussion, limited consideration of issues, and limited epidemiological experience. It was clear that something was needed to succeed the Berg *et al*. letter, which had been prepared almost a year prior to the Asilomar meeting. And I saw Asilomar as a meeting where we would discuss the issues more deeply and consider the reports that had been prepared by the various working groups: the plasmid group, the mammalian gene group, and the animal virus group—and we would agree on a statement that would supplement and supplant the recommendations of the Berg *et al*. letter. I expected that we would also be brought up to date on how the science had progressed in the labs actively working in the field.

Hughes: Did you in a more concrete sense also anticipate that the moratorium would be lifted?

Cohen: Well, what had existed was a voluntary moratorium on just two kinds of experiments…

Hughes: Right.

Cohen: …and I certainly did not favor doing experiments that created novel combinations of antibiotic resistance genes that didn't exist in nature, or introduced a resistance trait into a bacterial species where that type of resistance didn't exist. I expected that any recommendations that would come out of the meeting would continue to say that such experiments should not be done. But I had never considered the cloning of mammalian genes in bacteria to be risky, as some did. And although my knowledge about animal cell viruses was limited, I felt that introducing a single gene or a few genes would not create a bacterium capable of producing cancer. I thought that tumorigenesis was likely to be much more complicated than that.

Hughes: Yes.

Cohen: But I didn't feel strongly about the tumor virus issue. I didn't have the expertise and thought that the experts in that field should hash that one out. But it seemed illogical to me that

introducing mammalian DNA should be considered risky while DNA from *Drosophila* and other lower eukaryotes would be O.K. However, no moratorium on cloning of mammalian cell genes had been recommended in the Berg *et al*. letter. Scientists had been urged simply to "exercise caution."

I expected that some of the discussions at Asilomar would be difficult and had brought my package of antacids for the heartburn I expected to have. But what I did not expect was the almost religious fervor that existed there. Some of the organizers viewed this as not so much as a meeting called because of the need to address an issue important to both the conduct of science and the public, but rather—I'm not really sure just how to say this—as an emotionally uplifting event. The mood among some of the organizers was self-congratulatory.

Public Nature of the Discussions

Reporters from wire services and from most major newspapers and some small ones had come to the meeting. There was great public interest in this research, and it was appropriate for the press to be invited. It would have been awkward, at the least, to have excluded them. On the other hand, the fact that so many reporters were there created a circus environment. Scientists were followed around by reporters asking questions about anything and everything. Most of us were not accustomed to dealing with the press at that level, and as I've mentioned, sometimes we made comments that we would read in the next day's paper, and what we had said was often used out of context. It was a sobering experience.

Because of the fervor that pervaded the meeting, I saw this as the beginning of a very difficult period. When I returned home after Asilomar, I was drained emotionally, and my wife Joan looked at me and said she had never seen me looking so pale and tired. The way that I viewed Asilomar was that things had gotten out of hand, and an issue that had been raised because of scientific concerns had become a political football. It had been taken out of the hands of scientists and had turned into a sort of "witch hunt," and I was concerned about that.

Hughes: Now, was it the taking out of the hands of the scientists that most concerned you?

Cohen: No, it was not that taking the issue out of the hands of scientists *per se* concerned me, but the fact that decisions were being made not on the basis of scientific evidence, but rather on the basis of how they would appear to others. Issues of "appearance" had become paramount, and, in fact, this point was stated explicitly at the meeting.

Hughes: I see.

Fearfulness at Asilomar

Cohen: Other scientists at Asilomar had similar concerns, but were very private about them. And that was the issue that I was most uncomfortable about: people who had contrary positions were afraid to say so. It was the first time I had encountered a situation where scientists were fearful of speaking their mind about scientific issues. There was an attempt by the organizers to promote the view that there was a consensus on the actions they were proposing, but it was clear from the discussion that there was much disagreement. There was an attempt to ram through the recommendations of the organizers without a vote, but a number of participants resisted this. When there finally was a vote on accepting on faith an organizing committee consensus statement that was yet to be prepared, I saw the hands of only two other people who voted negatively. This morning, I was looking through a newspaper article about the meeting and…

Hughes: Which one is this?

Cohen: An article from the *Washington Post* by Stuart Auerbach, dated March 9, 1975, pretty well summarized my feelings about the session: "…Stanley Cohen of Stanford, felt that no matter what the conference approved, ultimately it's the responsibility of the individual investigator to be responsible for what he does. 'Short of having a policeman in a lab 24 hours a day, there's no way we can control a scientist's actions.'" And so I felt that some of the things that were being proposed were unrealistic. At another point, Auerbach says, "Stanley Cohen pointed out that the report would be considered by the public and most scientists as coming from the conference as a whole, despite the disclaimers from the organizing committee. He asked for a vote on the report, something Berg had hoped to avoid, since he feared there might be great opposition. 'The group,' said Cohen, 'should determine whether this should be issued to the media and our colleagues as the consensus of the group.'" There was a discussion about that and ultimately there were only a few negative votes. Others privately expressed concerns about the steamroller that seemed to be gathering speed, but said they were afraid to speak up publicly. And that's what unsettled me the most.

Opposition to the Consensus Statement

Even though I was quite vocal in stating my positions at Asilomar, I was also edgy because of the fear. One of the reporters, I think for *Rolling Stone*, in describing the atmosphere at Asilomar, caught this feeling and wrote about it. There were newspaper people taking photographs everywhere, poking cameras in our faces. The reporter cited an instance where one "young scientist" when confronted with a photographer's camera, put a hat over his face "in the style of a newly busted member of the Mafioso." He didn't mention me by name, but I was the person that he referred to in the article. I didn't want to talk to the press or be photographed, although there were some people who did want this. But what was dismaying to me is that views contrary to the notion that we were on a wondrous mission at Asilomar were conspicuously absent from discussions that took place publicly; people were afraid to have them reported. On one hand, it was good for the meeting to be an open public event. On the other hand, it was disconcerting to scientists not accustomed to interacting in a political arena to find their comments reported in the *Wall Street Journal* or the *New York Times* the next day. No one wanted to be seen as being insensitive to public safety concerns, and this limited discussion about whether there was a valid scientific basis for the concerns.

Hughes: What about the ideology, almost, that you referred to earlier which I think stemmed primarily from Berg, that this event was being put forward to the public as a sterling example of scientists taking responsibility for their research, and so if that…

Cohen: Yes, some people almost seemed to be pinning a medal on themselves…

Hughes: Exactly. I can imagine that if indeed that feeling was fairly widespread, that this indeed was the intent of those who were organizing it, that was going to be somewhat of a deterrent, I would suspect, to speaking up and saying, "Well, I just don't go along with the report, the consensus statement." Could there have been an unstated pressure to conform to present science as unified on this one issue?

Cohen: Yes, that's right.

Hughes: Well, that would be pretty intimidating, wouldn't it? If the prestigious figures in your field of science are trying to present a certain image, it's going to take a certain amount of courage to speak up in opposition to that.

Cohen: Well, opposition did take some boldness, or a certain level of immunity to the consequences of

saying things contrary to the image that the organizers were trying to create. As Nobel laureates, both Watson and Lederberg had that immunity. And, as Auerbach pointed out in the newspaper article I'm holding in my hand, "Lederberg insisted that any controls put on experiments will add to scientists' paperwork, thwart research and hurt science in general. He said that far more dangerous experiments have been done for years without this kind of public hue and cry. But genetic engineering, he said, "captures headlines and public attention," and he suggested that raising the issue publicly may have been part of an effort to raise the social consciousness of scientists about the implications of their work. "'There is nothing worse than a moratorium,' said Lederberg, 'to over-dramatize a problem.'" And Lederberg also said, "Recombining genes is analogous to crossing beans and corn in a field to get succotash."

Anyway, Watson's and Lederberg's views were quoted widely in the press, and this resulted in friction between Lederberg and Berg at the meeting. Berg was clearly annoyed about Lederberg's public statements; you could tell that from some of his responses to Lederberg's comments. I think that Josh was uncomfortable about the strained interactions. Watson was always viewed as someone who was going to say whatever he wanted to say, in any case. There's a wonderful quote in Auerbach's article of one of Watson's comments: "Josh is so bright and articulate," he said, "that everybody pays attention to him. I stopped paying attention to him in 1951." But that was exactly the point. People viewed Lederberg as a very thoughtful and very senior scientist who was quite cautious in his statements, whereas everyone knew that Watson would readily say whatever was on his mind, regardless of the consequences. And I think that's why Paul was particularly annoyed by Josh's statements, whereas from Watson, he probably thought, "Well, what do you expect?"

Hughes: Exactly. You could dismiss it.

Cohen: And I don't really know how Paul felt about my public comments, except I was relatively junior and there wasn't much prospect of my influencing the overall process.

Towards the end of Asilomar, I began to wonder where this all was leading, because the mood was one of a scientific witch hunt, in a sense. But I also felt that if the collective wisdom of the Asilomar attendees didn't result in recommendations, they would come from other groups that were less qualified, and I said this at one of the sessions.

At the final session of the meeting, there was an acrimonious discussion. I felt that a steamroller had taken over events. The night before, an executive committee of meeting organizers prepared a provisional statement of general principles that should be used in going forward. But during the discussion, there were differences in opinion about the definition of "hazard," the actual risk of particular experiments, and the level of containment required—and multiple revisions to the provisional statement were proposed. Ultimately, we were asked to approve a document that the organizers said they would be writing afterwards to address some these issues. My own feeling was that I could not support a statement that I had not seen and voted against giving the organizers the authority to prepare posthoc a statement that would be presented as the "consensus" of the group at Asilomar. In the vote against the final recommendations, up until the moment that I put up my hand as a "no," I wasn't sure that I would be bold enough to do that. But when I did, I looked around and saw the hands of only two other people. They were Watson's and Lederberg's.

The fact that I was one of the three people who voted against accepting unseen provisions was reported in the press. In one report, I was depicted as exactly the kind of person that these guidelines were intended to control. I had concern about how all of this would affect my scientific career, my ability to do research, the grant support I was receiving from the NIH, *et cetera*. A number of colleagues and friends confided that even though they felt the same way that I did, they decided that it was foolhardy to buck the trend.

Hughes: Lederberg and Watson objected for what reason?

Cohen: I think it was more or less the same reason. Watson's objections were a little more broadly stated, something like, "Well, what are we doing? What is the basis for these guidelines?" I think there are quotes from him in the transcript of the meeting. During Asilomar, Jim began to feel that this whole thing was a lot of nonsense, and he was very vocal in stating these feelings. If he thought that something was nonsense, he would say this openly, and he had the stature to do that. I had to be more circumspect in my comments.

Interview 8: March 29, 1995

THE BIOHAZARD CONTROVERSY POST-ASILOMAR

Early Days of the RAC

Hughes: Well, Dr. Cohen, you know we talked at some length last time about the lead in to Asilomar through the Plasmid Committee, and I'd like to concentrate this time on the guidelines and their evolution. I was wondering, to begin with, how closely you were following what the RAC was doing, and how—through what means—were you doing that?

Cohen: Okay. Well, the RAC was formed after the Asilomar conference.

Hughes: The first meeting was actually right after Asilomar and I think it was in San Francisco.

Cohen: I think that may have been just a very brief discussion of what their mission would be. I think the first substantive meeting, at least the first one that I'm aware of, was in San Diego, I don't remember when, but a short time later. Is that consistent with what you have?

Hughes: Yes.

Cohen: I wasn't a member of the RAC but was invited to attend, and I did. But I remember very little about it. Don Helinski was a RAC member. He was a good friend and a plasmid biologist. You probably remember that Don and I, with Watanabe, had arranged the meeting in Honolulu at which Boyer and I met. I continued to have close scientific interactions with Don and I was very comfortable about his expertise in plasmid biology and his ability to represent the plasmid area on the RAC. And I think another person who was one of the original members of the RAC, was Waclaw Szybalski.

Hughes: That's right.

Cohen: Waclaw had a background working with phage, and his views were very much akin to mine. I was happy that these views would be represented on the RAC.

Hughes: Do you know how the membership was chosen?

Cohen: Well, the person who was managing this at the NIH was Bill…

Hughes: Gartland.

Cohen: Yes, and I've forgotten who were the other RAC members that Bill selected.

Hughes: Do you want me to read who was on the original committee?

Cohen: Yes, please.

Hughes: It was Edward Adelberg…

Cohen: Yes.

Hughes: Ernest Chu, Roy Curtiss, Stanley Falkow, Donald Helinski, David Hogness, Jane Setlow and how did you pronounce his name?

Cohen: Waclaw Szybalski.

Hughes: And William Gartland, of course.

Cohen: Right.

Hughes: Head of the show.

Cohen: Yes. And of course Falkow and Curtiss were also experts in plasmid biology. And very soon the original membership expanded…

Hughes: Right, I think maybe even by the next meeting.

Cohen: …to represent, or at least to include, non-scientists.

Hughes: There's a letter in your files; I think it's probably the second or third meeting of the RAC, and apparently there was some sort of complaint that the fields of epidemiology and animal virology were not represented then.

Cohen: I think that Falkow was one of the people making that argument, and I agreed with it.

Hughes: Were you ever approached?

Cohen: No. I think that my views were seen as being too polarized.

Hughes: Well, of those names that I just read off, there are only two whose connections I didn't know.

Cohen: Adelberg is a well-known bacterial geneticist.

Hughes: Yes, I know that from talking to Dr. Boyer.

Cohen: That's right, of course; Herb had worked with Ed.

Hughes: Who's Ernest Chu?

Cohen: Good question. I think that Ernest Chu was appointed as someone from industry.

Hughes: Well, I can find that out. And the only woman was Jane Setlow.

Cohen: Jane Setlow is a person who's an expert in the area of DNA repair. She's very outspoken and direct, and she probably livened up the RAC meetings.

Hughes: All right. How were you following what was going on?

Cohen: Well, RAC actions were public information.

Hughes: You mean in written form or conversation?

Cohen: Minutes from RAC meetings were made available publicly by the Office of Recombinant DNA Activities. I also was interacting scientifically with some of the RAC members and talked with them periodically by phone. The views of the plasmid biologists on the RAC were not too disparate.

Hughes: I can understand that. There apparently was some trouble within the committee after Asilomar. To tell you the truth, I can't remember the specifics, but I think it was along the lines of some squabbling over documents being submitted without the full committee's consent. Does this ring any bells?

Cohen: Vaguely. I sort of remember that vaguely but I really don't remember the details at this point.

Hughes: I do have a letter, and I believe this was right before you were off to England on sabbatical in which you sound pretty fed up. I'll show it to you if you like.

Cohen: Okay.

Hughes: You are concerned that the guidelines for prokaryote research were stricter than for eukaryote. You say a little bit later in the letter, "I've had it."I don't think that you were referring to what

was going on within the Plasmid Committee but in the larger context of the post-Asilomar controversy.

Cohen: I think the issue was that, if I remember correctly, a…[end of tape]

Hughes: Does the letter bring back any…

Cohen: Yes, I'm reading the letter now. It reminds me that what I objected to was that the proposed regulations, alias guidelines, didn't make sense to me. They would, in a practical sense, prevent the transfer of genes between different harmless bacterial species, while still allowing tumor virus DNA, which was the basis of the initial concerns of Berg *et al*., to be introduced. And in my letter, I pointed out several examples of such incongruity. The reason I wrote to Don Helinski, if I remember correctly, was that Don had been appointed as one of the initial members of the RAC and he would be involved in formulating RAC guidelines. I sent copies of the letter to the members of the plasmid group. In the letter I said: "We now appear to have recommendations designed to meet the specific experimental needs of animal virologists (the explicit reduction of containment level required for 'demonstrably non-transforming regions of oncogenic viral DNA')." It was almost funny. How could one say that a DNA region was "demonstrably non-transforming"? All that anyone could reasonably state was that neoplastic transformation had not been observed under the particular conditions used for the test. Even the terminology seemed self-serving. There were a number of other points I made in the letter, but I don't want to read the entire thing here. I noted that I "had spent a major amount of time attempting to contribute to the development of credible, internally consistent and appropriate guidelines that would ensure safety of experimentation in genetic manipulation." But I was disappointed by logical inconsistencies in the summary statement that the Asilomar organizing committee had prepared after the meeting as the "consensus view."

And after Asilomar, there was greater emphasis on biological containment. Curtiss became very focused on this and was almost evangelical in his zeal.

Hughes: In what regard?

Biological Containment for Recombinant DNA

Cohen: Roy seemed to view biological containment as the ultimate solution to virtually all of the concerns that people had about the safety of recombinant DNA; he felt that developing a strain that couldn't survive outside of the lab would address most or all of the problems. And I think that Roy's focus diverted him from questioning whether the biohazards were real or imaginary. In a sense, one can appreciate how this happened, because if the biohazards were less serious than some people thought, there wouldn't be as urgent a need for a containment strain.

Hughes: I see.

Cohen: So Roy came away from Asilomar with the mission of constructing a bacterial strain that wouldn't survive if it escaped from a lab. The press was especially intrigued by this approach. Newspaper accounts about his mission emphasized its importance.

Hughes: The minutes, of course, may reflect Curtiss' viewpoint, but very early on, the development of safe vectors is an emphasis of the committee, and with funding recommended as well.

Cohen: Well, it's certainly true that vectors that have desirable features were being designed in other labs.

Hughes: And certainly in yours as well. But, was this a widespread activity?

Cohen: The development of additional vectors and safe bacteria strains were somewhat separate issues. Roy's goal was to produce an *E. coli* strain that could survive and grow only under special

conditions maintained in a lab. But it also seemed desirable from a safety perspective to work with nonconjugative plasmid vectors, which could not be transferred as easily among bacterial cells. And it would be better to use plasmids that have a narrow host range, rather than a broad one. These considerations were pretty obvious, and were discussed even in the recommendations that the plasmid group prepared for Asilomar.

Hughes: Because the biohazards issue is so much in people's minds, is science getting deflected towards such things as development of safe vectors and feebler strains of *E. coli,* where if this were not an issue, perhaps that energy would have gone into a different application of the science? Not that that wasn't going on, but would there have been such a heavy emphasis on providing the means to do these experiments safely?

Cohen: Well, it's likely that the development of strains and vectors to address biohazard concerns did divert some scientific efforts from other projects.

Hughes: Presumably scientists want to do things efficiently so there would have been a certain amount of effort in developing more efficient cloning vehicles, for example, regardless of the biohazard issue.

Cohen: Yes, there were various reasons for developing additional cloning vehicles. For example, pSC101, the plasmid vector we used in our initial DNA cloning experiments, has a relatively low copy number. Don Helinski and Herb Boyer, and then Stan Falkow and Boyer, collaborated the next year to develop higher copy number replicons as vectors to make it easier to isolate large amounts of plasmid DNA. One of these vectors, pBR322, has become very popular. It uses the replication mechanism of ColE, and that allows the plasmid copy number to be amplified several fold. My lab also constructed new vectors, but my interest was in making vectors that might be more effective in expressing genes that we were cloning rather than in producing higher copy number plasmids. Vector construction was a natural outgrowth of the need to address different types of technical issues. But I think that biohazard concerns probably accelerated the development of both bacterial strains and cloning vehicles.

Hughes: Well, one of the points that the opponents to recombinant technology made is the danger of using *E. coli*, a natural inhabitant of the human gut. Was it ever a serious consideration to use something other than *E. coli*?

Doomsday Scenarios Involving Conjectural Biohazards

Cohen: Well, yes. Sure. That issue certainly was raised. Everyone knew that human intestines are filled with *E. coli* and there was concern that if *E. coli* cells that were engineered to produce insulin somehow made their way into the intestines of humans, the bacteria would make people hypoglycemic. But this concern had no scientific basis. The *E. coli* K12 strain, which is what was being used for the experiments, wasn't the type of *E. coli* found in the gut and it can't compete well with other *E. coli* in natural habitats. There are billions upon billions of bacteria already in human intestines and it was not scientifically reasonable to propose that ingestion of a few bacteria would overcome all of the *E. coli* naturally present there, and produce an active hormone that would be secreted, would be insensitive to proteolytic digestion by enzymes in the intestine, and be absorbed into the blood stream. The concerns were based on science fiction scenarios that the opponents of the research started talking about and writing about, and the press eagerly picked up the scenarios and reported them as genuine possibilities. Another doomsday scenario was that if someone engineered an oil-eating *E. coli*, it might escape from the lab, enter the fuel tank of a transoceanic airliner, and eat up all of the plane's fuel somewhere over an ocean—plunging the plane and its inhabitants into the sea. These scenarios

were pure science-fiction, and talking about them 20 years later, I can smile and think they're kind of funny, but they were seriously believed by much of the public at the time.

A factor that many of the opponents of the research ignored is that there is an important element of biological selection working during natural evolution. They assumed that an *E. coli* cell containing an insulin gene would replace other *E. coli* in the ecosystem. However, bacteria have evolved over billions of years and, as I pointed out in my "Fact and Fiction" *Science* article a few years later,[96] the reason that *E. coli* don't naturally contain insulin genes is not likely to be a lack of any prior encounter with insulin-encoding DNA; cells from intestinal walls slough off regularly into the intestinal lumen, and the DNA from countless quadrillions of animal cells must get into intestines. Under certain natural conditions, bacteria in the intestine might take up some of the DNA, and perhaps there might be an insulin gene expressed in one of them. But unless having and expressing the insulin gene provides a biological advantage to the microbe, that microbe does not overcome the native bacterial population. These issues were not considered by the writers of doomsday scenarios and there was a need for experts in epidemiology and evolutionary biology on the RAC to address them.

Hughes: Now, another reason for immediately dismissing the idea of discounting *E. coli* as an experimental tool would have been, well to put it simplistically: Wasn't *E. coli* the *Drosophila* of experimental bacteriology?

Cohen: Well, the other way of looking at it is that *Drosophila* was the *E. coli* of experimental eukaryotes. But, yes, there's been a long history of experimentation with *E. coli*.

Hughes: Right.

Cohen: And that was one of the reasons why a number of people, including myself, felt that it was a good organism to work with in studying genes from other species. So much was known about it genetically, and the experimental tools were there to work with. But there were also reasons for developing other bacterial systems for cloning DNA, and I expected that scientists wouldn't forever be restricted to cloning genes in just *E. coli*. There were reasons to clone and study genes in *Bacillus subtilis*, for example, and someone who wanted to investigate antibiotic production and design new antibiotics might want to clone genes in an antibiotic-producing microbe, such as one of the *Streptomyces* species. By the late 1970s, researchers were also cloning genes in plant cells and animal cells. And so, there were efforts, sparked mostly by scientific motives rather than by biohazard issues, to look beyond *E. coli*. But biohazard concerns did underlie some of the work on vector development and much of the work on further enfeebling *E. coli* K12.

Hughes: And there was ample funding to go along with these lines of research?

Cohen: I think that funding such research was a priority for the NIH. My work on vector modification and DNA cloning in other bacteria was carried out mainly to pursue the scientific questions I was interested in, and I didn't apply for funds specifically for those experiments.

Hughes: Now presumably you worked with a K12 strain?

Cohen: Yes.

Hughes: What was the effect on your research?

Cohen: I'm not sure I understand the question.

Hughes: Well, maybe it's my scientific ignorance, but I'm speculating that if one is working with an enfeebled strain like K12 that there are sorts of things that are more difficult to do.

Cohen: Well, *E. coli* K12 is less robust than the *E. coli* strains found in peoples' intestines, but it grows readily in the laboratory in most culture media.

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[96] Cohen, S.N. Recombinant DNA: Fact and fiction. S*cience*. 1977; 195: 654-657.

The RAC in Operation: Getting Permission for Production of a Functional Mammalian Protein in *E. coli*

Cohen: None of the early DNA cloning experiments was successful in producing a functional mammalian cell protein in bacteria. But in 1976, I thought of a possible way to do this, and thought that having such information would be useful in expressing other mammalian proteins in *E. coli*. I think that I mentioned this work earlier. The idea was to use DNA encoding the mammalian enzyme dehydrofolate reductase (DHFR), which encodes an enzyme that I expected would result in bacteria resistance to the antibiotic trimethoprim. Bob Schimke, a faculty colleague at Stanford in the Departments of Pharmacology and Biology, had been studying DHFR; his lab had isolated DHFR messenger RNA from mouse cells, and we collaborated with Schimke to use this mRNA, together with the enzyme reverse transcriptase, to synthesize a DNA segment that encodes that mouse DHFR enzyme. Trimethoprim was being used clinically and there were some *E. coli* that were already resistant to this drug, so expressing the mammalian DNA into *E. coli* wouldn't create any novel resistance capabilities, and I didn't have safety concerns about the experiment. The plan was to introduce the mammalian DHFR-encoding DNA into trimethoprim-sensitive isolates of *E. coli*, and then select for any bacterial clones that became resistant to the drug. We could then study the DNA sequence upstream from the mammalian DNA in trimethoprim-resistant bacteria and identify the features that allowed the mammalian cell enzyme to be produced.

We were all set up to do the experiment and when the study was finally approved by the RAC in mid 1978 and we proceeded with the work, within two months we had shown that *E. coli* could express a functioning mammalian cell protein. But as you've seen from looking at the correspondence in my files, there was well over a year and a half of discussion between me and the RAC and RAC subcommittees, prior to the work being done. According to the guidelines, the experiment required use of chi1776, and growth of this strain necessitated addition of the nucleic acid base thymine to the media. And for technical reasons that I won't get into here, it was necessary for us to use a bacterial host that didn't require growth media containing thymine, and so I…

[Tape change]

Cohen: ...slightly modified chi1776 to eliminate the requirement for adding it. Finally, permission was granted to use the modified strain, but it took multiple discussions by the RAC and its subcommittees before that happened.

Hughes: What was the hang up?

Cohen: Well, the question was whether the modification would sufficiently un-enfeeble chi1776 and prevent adequate containment of the strain. There was speculation that the modified strain might be slightly less feeble if it escaped from the laboratory. I argued that the short DNA segment that we wanted to introduce would create no conceivable biohazard, and the RAC eventually agreed that for this particular DNA, modifying chi1776 wouldn't be an issue of concern. When the permission was granted, use of the chi1776 variant was authorized for only this experiment.

When the experiment was finally done, it gave us some exciting results. It was the first instance of phenotypic expression of a mammalian gene in bacteria, and this showed that it is practical to use *E. coli* as a protein factory that makes mammalian enzymes. The discovery could have

been made a year and a half earlier if not for concerns about modifying a minor property of chi1776. But the experiment was eventually done, and we prepared a paper and submitted it to *Nature*. The paper was quickly accepted and was published three or four weeks after we sent it in.

Hughes: At almost the same time, I believe, the Genentech group was working on somatostatin. Wasn't this an instance in which a mammalian gene had actually been expressed for the first time?

Cohen: Yes, but there was an important difference. In the Genentech experiment, somatostatin was attached to beta galactosidase to make a fusion protein, and because somatostatin didn't have a methionine in it—it was a very short peptide—it was possible to separate somatostatin from the beta galactosidase component of the composite protein. But in most cases, cutting out the desired peptide from a fusion protein isn't practical. So, it was important to be able to make mammalian hormones and enzymes as discrete proteins.

Hughes: I see.

Cohen: Prior to publication of the DHFR work, Wally Gilbert and other scientists were synthesizing insulin in *E. coli* as part of fusion proteins. But once we found that it is possible to get expression of discrete mammalian proteins in bacteria, essentially by putting an additional ribosome-binding site into a complex transcript and using that ribosome-binding site to initiate the eukaryotic protein at its own translation start codon, people quickly switched from making mammalian peptides in *E. coli* as fusion proteins. Except for special experimental purposes.

Hughes: Well, am I understanding you right that your experiment showed that, yes indeed, mammalian proteins could be expressed in *E. coli*?

Cohen: Yes, and that we could make a mammalian protein in bacteria that was biologically functional.

The functionality of mammalian proteins made in *E. coli* raises another point. In some ways our findings increased biohazard concerns because they showed that *E. coli* cells could be phenotypically altered by expressing a mammalian protein in them.

FEDERAL AND STATE LEGISLATION AIMED AT REGULATION OF RECOMBINANT DNA RESEARCH

Views of Stanford Faculty and Administration

Hughes: Well, let's go back a bit, because I'm interested in the social structure of this all, first at Stanford and then on a larger basis. Stanford seems to me to have been at the very heart of the controversy. This was a high-stakes issue for the Stanford group on several levels, not only in regard to science, but also in regard to social standing, the limits of science, and public responsibility to the public, and many other issues. How was this Stanford group operating?

Cohen: There wasn't a "Stanford group." We were individual scientists who had our own views…

Hughes: Well, was it really that loose?

Cohen: We had communication with each other, but certainly…

Hughes: One thing that made me think about this was, there are some memos back and forth between Robert Rosenzweig—what term does he use?—"DNA fans."

Cohen: Yes?

Hughes: At one stage, he gave me the impression that he was serving as a focal point for organizing the effort to slow down the move towards federal legislation. Am I reading in too much? Was there some form of organization, loose as it might have been, here at Stanford?

Cohen: I don't think that Rosenzweig or anyone else at Stanford tried to organize such an effort. In fact, Rosenzweig, who was Vice-President for Public Affairs here, was in favor of the move toward legislation.

But there were organized steps taken to implement RAC policies institutionally. For example, in accordance with RAC stipulations, a local Biohazards Committee was established here to evaluate the safety of proposed projects, and the local committee became a focal point where scientists involved in DNA cloning research interacted on a regular basis. We saw each other at committee meetings, and we talked about biosafety issues. But there were also non-scientists on the committee, and some of these committee members contributed very significantly to the process. There was a person on the committee, John Kaplan, who was professor of law here at Stanford. He is no longer alive. John was a very wise man, and I had a lot of affection and respect for him. He was appointed to the committee by the president of the university to provide a legal point of view, but he quickly became knowledgeable about the science, or at least sufficiently knowledgeable to ask penetrating questions about evidence for assertions that were being made. He was continually challenging claims, and his views had an important influence on committee deliberations.

Although we don't plan to talk at any length about the [Cohen-Boyer] patent today, your mentioning Rosenzweig's name reminds me that Rosenzweig was also involved in discussions that Stanford had with the NIH to allow Stanford to proceed with patenting the technology that Boyer and I had invented. There was an agreement between Stanford and the federal government whereby technologies developed under NIH grants would be owned by the university, and that was also the case with most other universities. Interestingly, Rosenzweig and some others in the university administration viewed the patent as a way of controlling the industrial community to ensure that industry observed the same biosafety standards as academic researchers. The NIH guidelines pertained only to institutions receiving federal funds, and questions arose about how the use of this technology by industry could be controlled. How could anyone be certain that a company wouldn't go out and do experiments that were not permitted under the NIH guidelines? I've already told you about the ice bucket brigade. One of the arguments made by Rosenzweig was that by obtaining a license from Stanford [on the Cohen-Boyer patent] to use the methods, industry would have to agree to work according to the standards that were being applied in academic institutions. A patent was viewed not only as a means of creating income for the university, but also as a way of enforcing the use of biosafety procedures by scientists who might otherwise not be required to use them.

Hughes: Well, I want to, of course, pursue the patent issue at much greater depth…

Cohen: Yes, we can get back into that.

Hughes: Well, getting back to Rosenzweig, when he's sending out these memos to, as I'm sure he facetiously says, DNA fans, are the DNA fans the panel?

Cohen: Yes, I believe so. You would have to show me the particular memo where he uses that term.

Hughes: This is all I have.

Cohen: Well, okay. But this discussion raises another point. There were two opposing views at Stanford and elsewhere about state and federal legislation that was being proposed for control of this research. Up until that time, the penalty for not observing a RAC guideline was the possible loss of NIH grant support. But proposals were being put forth for legislation that prescribed penalties involving severe fines and the imprisonment of violators. That made it seem that this area of research was so dangerous that draconian steps had to be taken. One view was that even if legislative action on this issue is not warranted on scientific grounds, Stanford and scientific societies should support legislation in order to have a voice in drafting the provisions of the proposed laws. The notion was that by doing this, it would be possible to

exercise some control over the process and the specific terms. The fear of people who argued for this position was that if we took the view that laws are not necessary or warranted, we would end up having legislation passed anyway, and the experimental procedures used in our laboratories would be patrolled—as a bill submitted to the California legislature had proposed—by the same agency that inspected the establishments of barbers and beauticians for cleanliness. According to that view, if we didn't support the passage of so-called "good" legislation, we might end up with onerous legislation.

Norton Zinder and James Watson and I were actively arguing strongly and publicly for the opposing view, that it does not make sense for scientists to support a scientifically unsound position for political reasons. We thought that supporting so-called weak legislation would give credibility to the fiction that this area of research presents a hazard so severe that it needs to be addressed by the passage of laws. We felt that we were likely to lose control of the process if it seemed to the public that even the scientific community believes it is necessary to control this research by passing laws.

Bob Rosenzweig argued that proposals for legislation should be supported. Another person who was very strongly in favor of passing laws was Harlan Halvorson, who was president of the ASM [American Society for Microbiology] at the time. Harlan persuaded the society to support the passage of legislation that the ASM concluded was not excessively restrictive. I felt that it was intellectually and scientifically dishonest to do this, and as you see from the correspondence I had with Harlan, we disagreed strongly.

Lobbying in the U.S. House of Representatives

And this issue came to a head in a discussion that I later had with Congressman Paul Rogers. Mr. Rogers was then head of a subcommittee of the House that was considering legislative action on the research. I had been invited to testify before a U.S. Senate committee earlier that day and although I had decided against testifying at the Senate hearing, I was able to arrange a meeting in the afternoon with Congressman Rogers. I've forgotten the reason why I decided not to accept the Senate invitation, but John Lear, in his book, criticizes me roundly for doing that while meeting privately later in the day with Congressman Rogers. During the meeting with Congressman Rogers, I explained my views and the scientific arguments underlying my position in some detail. After listening thoughtfully to the points I was making, Mr. Rogers said, "Well, you know Dr. Cohen, you make some convincing arguments, but what I don't understand is that if what you say is true, why are some of your colleagues like Harlan Halvorson and the ASM pushing for the passage of my legislation." I knew from my discussions with Harlan that he viewed the Rogers legislation as being far less restrictive than the other laws that were being considered and suggested to Rogers that this might be a factor. I offered my opinion that Harlan may have made a political decision to support a law that he considered to be less onerous. And Rogers looked hard at me and said, "Look, Dr. Cohen, you worry about the science and let us worry about the politics." And that statement neatly summarized the situation that had developed. Here was the scientific community mucking around in the arena of political tactics.

I felt that Mr. Rogers went away from that meeting uncertain about whom to believe, and I didn't know how he would be proceeding. Some years later, Paul Rogers and I became members of the Board of Trustees of the University of Pennsylvania Medical Center, after his retirement from Congress, and at one of the Trustee dinners we talked about the biohazards controversy and the discussion we had in his office in Washington. He told me that he had remained concerned about the possible effects of political expediency on expert opinions offered by reputable scientists.

Hughes: I've seen some of the correspondence with Halvorson. One of the points that you were making is that it is a mistake to work towards a bill that includes "preemption." Apparently there was great fear—almost more fear—that local and state legislation would make recombinant research almost impossible.

Cohen: Right.

Hughes: As somebody said, there would be a patchwork of laws.

Cohen: Yes.

Hughes: Hence it was better to get a federal law that would preempt state and local laws.

Cohen: Exactly. And that was the view of some people on the other side of that argument.

Hughes: Right.

Cohen: The point I made in this memo to Bob Rosenzweig is that the tide appeared to have turned. I wrote: "In recent weeks there's been a ground swell of scientific and political opposition to any recombinant DNA legislation at the national level." I pointed out that the position taken by the administration and faculty of universities in New York State, including Rockefeller University and the University of Rochester, had convinced the New York governor to veto the recombinant DNA act passed by that state's legislature. Norton Zinder, who is a professor at Rockefeller, had the key role in the New York State battle. And I thought that we were beginning to see the effects of being scientifically forthright about the issue, instead of making political judgments based on perceived expediency. The New York governor said he vetoed the bill because he believed legislation that unnecessarily interferes with free scientific inquiry is undesirable, not because he thought impending national legislation made state legislation unnecessary.

But some scientists still felt that national legislation was needed to preempt the possibility that a patchwork of differing state and local laws would be passed. I hoped that states and communities would ultimately realize that people and animals cross state lines and travel between communities and that local laws restricting the research wouldn't have any practical value. Maybe that hope was a little naïve. Laws were enacted by the city of Cambridge, Massachusetts for a period of time, and activists opposing the research were advising the California state legislature to also pass laws here controlling the research.

But going back to the Rogers legislation in the U.S. House of Representatives, there's another story that I'd like to relate. The bill that Paul Rogers' subcommittee was considering didn't proceed to the floor of the House, but probably for reasons unrelated to my discussion with Mr. Rogers. A few days after my meeting in Rogers' office, I telephoned Congressman Harley Staggers, who was the Chairman of the House Committee on Science and Technology, which was the parent of Mr. Rogers' subcommittee. I was surprised when he answered the phone himself. Staggers previously had spoken with Norton Zinder and was willing to discuss the biohazard issues with me. We talked by phone for 10 or 15 minutes, and I made the same points I had made to Rogers. I was delighted when Staggers said that he had decided that he would not let Mr. Rogers' bill get to the floor of his Committee. I thought that the points I made in our phone discussion had influenced his decision and I felt pretty good about this. But I learned much later that Congressmen Staggers and Rogers didn't get along especially well and that my arguments may have simply provided a rationale for Staggers decision to reject the Rogers bill.

Hughes: Yes.

Cohen: At Penn, Rogers told me that he thought this might have been a factor, but there was no way to know for certain. So ultimately, the politics of congressional committees and personal feelings among members of Congress may have played a decisive role; but in any case, the House legislation did not proceed at that time. The legislation in the U.S. Senate was stopped for a

different reason, and I'll tell you about that and my interactions with the Senate. Let's stop for just a second.

Hughes: We inadvertently plunged into the middle of the federal legislation.

Cohen: Sorry.

Scientists and Others Supporting Control of Recombinant DNA Research

Hughes: That's all right. But let's go back now and establish the players. We've got the scientists on one side who are very much in favor of recombinant technology and, on the other side, some scientists as well and some other forces. Could you just sketch those other forces, so we know?

Cohen: Yes, thank you for raising this point. As you've said, there was not uniformity of opinion among scientists about this issue. Some argued that recombinant DNA research itself was inherently hazardous. And among these people were some very distinguished scientists, including Bob Sinsheimer, Erwin Chargaff and Jon Beckwith. There also was a scientist named Liebe Cavaliere who wrote an article for the *New York Times Magazine* that was very instrumental in exciting public fear of this area of research: it was called "New strains of life—or death."[97] And there were the professional journalists churning out article after article saying essentially that while this research might offer the possibility of creating new therapies and diagnostics, the research also carries great risk. The risks were portrayed in a way that led the public to believe that they were much more than conjectural: the people who read the articles were convinced that there was actual evidence that the research was risky. And the proposal to use different levels of precaution for different categories of experiments fostered the notion that the risks were quantifiable and, therefore real.

[Tape changed and portion of interview was not recorded]

Cohen: Yes, there was an organization in Cambridge called Science for the People, and Jon Beckwith was heavily involved with this group. Jon is an outstanding scientist who also was a political activist in his early days, and he was very conscious of the obligations of individuals towards society. Jon was a leading opponent of the research during the Cambridge controversy. And in Cambridge there were also George Wald, who won the Nobel Prize for his work with vision, and his wife Ruth Hubbard, who were both actively involved with the group. I had an interesting discussion with Wald at the time of the National Academy of Sciences conference on recombinant DNA in 1977. At that point Wald had written many articles about biohazard concerns and had became a very vocal spokesman among the people opposing this research. During a break in the meeting on one of the afternoons, Paul Berg and I were invited to talk about the scientific and societal issues on radio with Wald and, I can't think of his name just now, a scientist from M.I.T. [Jonathan King]. Wald and I shared a taxi going to the radio studio. And I said to him, "Well, George, this whole area seems to be somewhat distant from your primary scientific focus. How did you ever become so involved in this controversy?" And he was very candid about it and he said, "Well, you know, Stan, Ruth and I were very much involved in the opposition to the Vietnam War and as the Vietnam War began to wind down, we were looking for another cause…

Hughes: And you provided one.

[97] Cavalieri, L. New strains of life—or death. *New York Times Magazine*. 1976, August 22.

Cohen: Well, that's what he said. "…For another cause that was really worth our attention. And we were convinced that this was one. It's an area where we thought we had an opportunity to have important input as scientists." George Wald had been active in political protests for many years, and he previously had found a number of causes to be worthy of his efforts. I think that some of the other scientists actively involved in opposing the research also had moved their activist efforts from other causes to this one. For example, prior to the recombinant DNA controversy, Beckwith was vocal in expressing concerns about the use of genetic typing methods and the dangers of categorizing persons on the basis of the information obtained. The cells of males normally contain only one Y chromosome, but here had been a publication suggesting that males carrying multiple Y chromosomes have an innately increased tendency for aggression because they make more testosterone. That article claimed that prison populations disproportionately include persons with that genetic abnormality. For ethical reasons, Beckwith and Science for the People objected to using genetic knowledge to type people's personalities or to attempt to predict what might be their behavior. So Jon was concerned about the use of genetic knowledge in general. The concern was that society might not be able to deal with such knowledge.

And, in fact, at this same 1977 meeting at the National Academy, Jon made the point that if genetic typing was used to identify persons having more than one Y chromosome, recombinant DNA methods might be employed to alter genes in those persons against their will, in order to diminish possible aggressive tendencies. That was analogous to some of the things we had all read about in *Brave New World* and *1984*. I pointed out that one didn't need to use recombinant DNA to unethically affect testosterone production. If society were allowed to reduce testosterone levels in males involuntarily, there was currently a more simple method of doing it: castration. But society doesn't permit involuntary castration. I viewed the issue of how society uses genetic knowledge as being very important. But from my perspective, the more valid concern is about the use of knowledge, not its acquisition. And so, these kinds of discussions were going on at ethical levels as well as at scientific levels.

Jonathan King is the person whose name I couldn't think of a few minutes ago

Hughes: Ah yes, I should have known that.

Cohen: And, by the way, like Jon Beckwith and others opposing the research at the time, Jonathan King subsequently changed his views dramatically about the science. Of course, both now use the methods in their own labs. In a discussion I later had with Jonathan King, it seemed to me—although he didn't say so outright—that he was not really too concerned about possible biohazards per se. The issue that bothered him most was the "industrial-university axis" and research by both industry and academia that might yield knowledge that could be used to control political dissent. This was a Science for the People notion. But different motives were driving different people to be opposed to recombinant DNA research. There were some, like Beckwith and King and Wald, whose motives seemed to me to be largely political, and then there were people like Sinsheimer. Bob is a thoughtful but "hand wringing" person who agonizes personally about issues and Bob was truly troubled by biohazards concerns about the research.

Hughes: Specifically it was breaching the species barrier?

Cohen: Right, that issue troubled Bob enormously. We had many discussions about this. I tried to convince him that the notion that species barriers had been created in nature to prevent genetic mixing didn't make sense scientifically. That lateral transfer of DNA among species was likely to occur normally, and that we don't see more "human" genes in bacterial populations largely because these genes don't provide a selective advantage. Ultimately, Bob came to agree with this view.

Erwin Chargaff

Another vocal opponent of the research, Erwin Chargaff, was widely known for promoting antiscience views on multiple issues, although he himself had made very important scientific contributions. I didn't know Chargaff well, but many believed that his antiscience views were prompted in part by some bitterness in not having shared the Nobel Prize that Watson and Crick received. It was Chargaff who found that the frequency of As equals the frequency of Ts in DNA, and that Gs and Cs are equal. This information was a key element in the insight that Watson and Crick had about nucleotide base pairing in their model of the double helix.

Hughes: I believe too with Chargaff that there was a semi-philosophical issue in that he perceived molecular biology as being a very reductionist approach and inimical with his ideas that biology is an extremely complicated subject. This is pure speculation on my part, that there was also an element of sour grapes, perhaps stemming from the fact that he saw his idea of the base pairing neglected.

Cohen: It wasn't really neglect. Almost everyone knew of the importance of Chargaff's work, and the equal percentages of As and Ts and of Gs and Cs in DNA was known as "Chargaff's Rule." But it is evident from his writings that Chargaff felt that his discoveries should have been recognized by the Prize. On the other hand, the insight that put together the findings of Chargaff, of Rosalind Franklin, and of others—including Maurice Wilkins who shared the Prize with Watson and Crick—was ultimately Watson's and Crick's. Chargaff had some crucial data, but he hadn't made the connection to base pairing that Watson and Crick did.

Hughes: Right.

Cohen: And I've been told by people who know Chargaff far better than I did that he resented the fact that Watson and Crick hadn't done experimental work beyond model building, and had utilized information that he and others had obtained in coming up with their insight.

Hughes: Yes.

Cohen: Another point that has been made about Chargaff is that he was a World War II refugee and that he had negative feelings about genetic research because of the experiments done by the Nazis on humans. From what I've read, and from the public debates, he is a very complicated man.

Hughes: Do you know his essay in which there was an imaginary dialogue between a biochemist, whom I think is Chargaff, and a young molecular biologist?

Cohen: No. But I'd love to see it.

Hughes: I'll bring it to you. His feelings against molecular biology are pretty strong.

Cohen: That's very interesting. It doesn't surprise me.

Hughes: Well, getting back…

Cohen: To the players…

Hughes: I read Zimmerman's…

Cohen: Burke Zimmerman?

Hughes: …MIT oral history…

Cohen: Burke Zimmerman was an assistant to Paul Rogers at the time.

Hughes: Right.

Cohen: And he was very much involved in shepherding the Rogers legislation. He may have been the person who actually drafted the proposed law. He was absolutely furious when that legislation

didn't proceed, and I was a person that he blamed for this. I'd be happy to take credit for stopping passage of the bill, but as I've mentioned, other factors were also at work. But Burke was very bitter at the time because he had spent much time on the issue and was building his career around it.

Opponents to Legislation Restricting Recombinant DNA Research

Hughes: Right, well, that's interesting. He places the center of militarism against legislation at Stanford. In fact he says, "Most of the West Coast militants are from Stanford." Now is that the way you perceived yourselves? Did you think of the group at Stanford as the leaders of the pack, so to speak?

Cohen: No.

Hughes: No?

Cohen: Well, I didn't think of it that way. But Paul Berg was also opposed to legislation, as was Dave Hogness. Dave was on the RAC, and he had expressed his views in that venue. Paul also stated his views openly, although he was more circumspect in what he said. I think that most people would agree that Paul is a very politically astute person, and I don't suppose that many people would view me as being politically astute.

But I think that Burke regarded me as the militant partly because I was working actively at multiple levels. As I look back at it, it's hard to imagine how I got any science done during that period.

Hughes: Yes right. Were Berg and Hogness and the others at Stanford not quite as involved?

Cohen: Paul's opposition to legislation was very important, but I don't think he was as actively involved in discussions with lawmakers. Hogness was also vocally opposed to legislation. All three of us had similar views on this issue.

Hughes: What about the San Francisco group? Do Boyer and [William J.] Rutter and any of those people figure in?

Cohen: Well, that question is quite interesting. So far as I am aware, Herb Boyer wasn't heavily involved. Boyer was in a somewhat exposed position as the co-founder of Genentech, and he had been criticized for supposedly doing experiments that were not allowed under the guidelines. And also for doing experiments at UCSF to benefit Genentech. One of the reasons, and we can go back to this later, that I didn't start a company at that time was that I was so heavily involved in trying to prevent the passage of anti-recombinant DNA laws. I felt that my ability to affect legislation would be compromised if I had founded a company that could benefit from the research. That issue often came up in discussions I had with legislators or their aides, who would say: "I suppose you've started some company that's going to benefit from all this work." My response was "No, but I am a consultant to Cetus and have received some Cetus stock options." But being a scientific advisor and being compensated for this was O.K., whereas starting a company had some taint associated with it in the minds of lawmakers.

I don't really remember, Sally, how active Rutter was in the political controversy. As I remember, he opposed legislation, but wasn't especially active in this opposition. I was probably the scientist most actively involved in the legislation battle on the West Coast, and Norton Zinder, was probably the most actively involved on the East Coast. Mark Ptashne and some others at Harvard were also vocal in their opposition.

Hughes: You spoke last time, and I am of course paraphrasing, about Asilomar being perceived—how to put this?—as sort of dignifying concerns which might not have been as heightened if there

hadn't been an Asilomar. I'm meaning that the very fact that scientists were meeting could have been interpreted by the public as indicating, well, yes, they wouldn't be meeting unless there was a problem here.

Cohen: Right.

Explaining the Berg *et al*. Letter Retrospectively

Hughes: The biohazards. Could you look at the Berg letter in the same light, and was it used as a tool against you key scientists, you ten people who were very much engaged in this work and signed a letter that was asking for the formation of a committee and expressing concern about the potential dangers of this [recombinant DNA research] activity? Was that ever used as a tool against you?

Cohen: It was. And my response was that the Berg *et al*. letter reflected the signers' belief that there was a need to consider potential hazards before experiments using the newly developed methods were widely performed. I pointed out that our earlier concerns related only to certain types of experiments, but that the press and public had interpreted our letter as an indication of concern about the techniques themselves. And when the potential for hazard was considered more fully, as the Berg *et al*. letter proposed, and after quadrillions or quintillions of bacteria containing foreign genes from various sources had been grown, we concluded that there wasn't a valid scientific basis for continued concern. When data don't agree with a hypothesis, it's necessary to change the hypothesis and that's what the scientific community had done. But it wasn't so simple for the public to lose the fears that had been generated by scientists' caution.

Hughes: Did you hesitate to sign that letter?

Cohen: Well, I think I told you how my decision to sign the letter was made. The original group that prepared the letter did not include me.

Hughes: Right. I remember that.

Cohen: And it was only after Boyer and I started to prepare our own letter that we were invited to participate. By joining the group of signers, I was able to propose modifications. The part that I had key input in changing related to the used of antibiotic resistance plasmids. And although I didn't agree entirely with the final wording, it was something that I could live with. And I think that if I had not been involved, the letter might have come out recommending that no experiments with any antibiotic resistance genes be carried out, as had been originally proposed. So, did I hesitate to sign the letter? I thought that making some sort of statement was appropriate, but wasn't totally happy about the wording and would have preferred a letter that included a sentence stating explicitly that the concerns were entirely conjectural.

Hughes: Do you remember suggesting that?

Cohen: Yes, but the final wording was a compromise.

Early Interactions with Larry Horowitzand Senator Edward Kennedy

Hughes: Well, I know you want to talk about Larry Horowitz, but I believe Larry Horowitz is related to the 1977 issues, and we haven't talked about your appearance before the Senate in 1975. I wondered how that came about.

Cohen: Larry Horowitz was also relevant to the Senate hearing.

Hughes: All right, talk about Larry Horowitz.

Cohen: Well, Larry was one of Senator Edward Kennedy's principal aides. Larry is an extremely smart guy, who was quite young at the time. He had graduated from medical school at Yale and decided not to practice medicine. He is very astute politically and extremely articulate. I'm not sure how he started working for Senator Kennedy, but when I first met Larry he had advanced to a position where he had the trust of the Senator and was the Senator's "point man" on issues related to science or medicine. Recombinant DNA was such an issue. Larry had interests and concerns about the delivery of health care and societal aspects of medicine, and as part of his responsibilities to Kennedy had come to Stanford to spend part of his time working with Hal Holman.. Hal was still Chairman of Medicine at that time, and had become highly interested in developing new ways to improve the delivery of health care. And Larry came out to Stanford with Hal to expand his knowledge about health care delivery issues.

[Tape change]

Cohen: Quite coincidentally, the office assigned to Larry was just across the corridor from my laboratory. We got to know each other and spent a lot of time talking together. Obviously recombinant DNA was a politically charged issue, and Larry and I spent many hours discussing this. Although our views certainly were not congruent, I think we developed mutual respect for each other's perspective. But it was also clear that Larry was ambitious, and the controversy offered an opportunity for Larry to both help himself and involve Senator Kennedy in an issue that mattered to the public. At least that was the way I perceived it.

Hal Holman had political views that were in many ways similar to those of Science for the People, a group I talked about earlier. Hal felt that scientists needed to be controlled. Hal is also an extremely articulate person who can be very persuasive, and he liked to debate the merits of opposing positions. Hal's office was right next to Larry's, so the three of us spent many hours in discussions about science policy issues, and particularly about the role of science in society and vice versa. Hal's wife, Barbara, also had strong political views. These were very much at the left of the political spectrum, and she felt that the academic industrial complex was out to destroy the rights of individuals. Although I considered myself politically to be a "liberal," I didn't share her views. An interesting side story to relate here is that at the time that I originally came to Stanford for my interview as a young postdoc seeking a job as an assistant professor, Hal and Barbara invited me to their home for dinner. And after very pleasant dinner, Hal suggested that we go outside onto his patio to talk further. They had a glass door between their dining room and the patio. The glass was so transparent and I was so involved in the discussion, I didn't see that the door was closed. I banged into it with my forehead and it shattered. Fortunately I wasn't hurt, but I was really quite embarrassed to have broken the door of the Chairman of Medicine as he was taking me out onto his patio for a discussion about a possible job offer. The Holmans were very gracious, "Oh, it's okay, don't worry," and so on. But some years later when Barbara and I were in the heat of a political discussion about recombinant DNA issues, she said, "Stan Cohen, you've always been a young man in such a hurry. I still remember when you broke my door. You have to look where you're going scientifically as well as otherwise." And I thought about that comment for a long time

The U.S. Senate Hearing

Larry and Kennedy decided that there should be a Senate hearing on whether there was a need for the passage of laws to regulate this area of research, and both Hal and I were invited to testify. Some time around that hearing, I was asked by Larry to give a seminar at the U.S.

Senate on the scientific aspects of the technology. A few senators were there briefly while I tried to explain, in very lay terms, the nature of the research, but the talk was well attended by legislative aides. This reminds me that I also was asked to give a similar seminar at the Patent Office, which anticipated that lots of patent applications would be received in this area of technology and had examiners who had very limited knowledge about the science.

In any case, Don Brown, who held a view that was similar to mine, was also invited to testify at the Senate hearing, and on the other side of the argument were Hal Holman and Gaylin—I've forgotten his first name, but you probably have it. He was from an organization called something like "Institute for Ethics in the Sciences"…

Hughes: Oh, I didn't catch that.

Cohen: …in Hastings-on-Hudson in upstate New York. Anyway, each of us gave our presentations.

Hughes: Had you been assigned a part of the question to discuss, or was it completely left to you?

Cohen: It was left to each of us to decide what to say in opening statements, and then we were asked questions.

Hughes: Did you coordinate your statements before you appeared on the floor?

Cohen: Don and I had talked about what we would say and Hal and I also had spoken in general about our planned comments. Hal and I had opposing views, and of course we knew in advance of the hearing that we would be disagreeing publicly. But we tried to persuade each other to soften our positions and made arguments to each other aimed at encouraging the softening of positions: "Well, you have to consider this and you have to consider that," and so forth. "Coordinate" isn't the right word, but we knew what our respective positions were and we prepared accordingly.

Hughes: What was your reception?

Cohen: Do you mean the reception that the Senate…

Hughes: Yes, how did they receive each of the arguments?

Cohen: Well, the arguments made by each of us were predictable, and I think fairly presented, and Senator Kennedy's questioning was interactive and thoughtful. But at a later point he asked a question that caught me completely off guard. The Senator had of course been prompted by Larry prior to the hearing. During his stay at Stanford, Larry had heard that pSC101 plasmid DNA had been taken from a test tube in John Morrow's refrigerator. Senator Kennedy asked me about whether there had been any accidents or problems in controlling plasmids and whether there had been any material that was "unaccounted for." I hadn't anticipated this question, so my response was awkward. It wouldn't be correct to state that material was unaccounted for, and my information about the removal of plasmid DNA from John's refrigerator was not based on first-hand knowledge. I told the Senator that I was not aware of any violation of the NIH guidelines, and so far as I knew, there had been strict compliance. And that statement was true, but it was clear that Kennedy was not completely satisfied with the answer to that question.

On the other hand, I thought that Don Brown and I were given an opportunity to amply express our views, and I was happy about that. In reading the transcript of the hearing later, I felt that I could have said things a bit differently and could have presented my arguments more strongly. Don Brown was very articulate and did an extremely nice job of representing our side.

Hughes: Well, the *Science* article that covered this testimony said that you came from the hearing feeling somewhat abused, because you felt your views had not been accepted or totally accepted.

Cohen: Abused? I don't think I felt that way at all.

Hughes: I think the words were, "the Senate didn't find your arguments very persuasive," was how *Science* reported it anyway.

Cohen: Well, as I've said, I felt that my response to one of the Senator's questions was awkward, and I was not happy that the question was posed, but "abused" is not a correct description. But I did think that the Senator reacted more favorably to the arguments of the other side.

Hughes: How persuasive was Holman?

Cohen: Well, Hal is a gifted and persuasive debater, but the hazards he portrayed as likely were totally hypothetical. And many of the points Hal raised were "straw man" issues; for example, he argued that scientists should not be allowed to do whatever they want for "idle curiosity"—but no one was proposing that. My position and Don's was that legal impediments to research should be based on more than conjecture.

Interview 9: April 5, 1995

FURTHER DISCUSSION OF THE PERIOD FROM 1975 THROUGH 1985

Effects of the NIH Guidelines on Research in the Cohen Lab

Hughes: Dr. Cohen, last time we talked about the guidelines and legislation, and there are just a few more things that I want to hear about the guidelines before we move on. You mentioned one instance in which the guidelines had definitely affected your research. Could you comment on how much of a problem, if indeed that's the case, they were in pursuing the research you wanted to do, and in the field in general? I mean, what effect did the guidelines have on the momentum of recombinant research?

Cohen: I can't comment on how much they affected the research of others. The only instance where they directly affected my research is the one I've mentioned, where the experiment was delayed for more than a year because approval for use of a chi1776 variant was needed. But, the guidelines and biohazards controversy generated anxiety in other ways. I'll tell you about a situation that I haven't previously discussed or written about.

At one point during the height of the controversy, I sensed that a postdoctoral fellow in my lab was being secretive about some of his experiments. I had the feeling that he wasn't being entirely open with me when we discussed his project and results. One day I received a telephone call from a senior scientist at another university. My postdoc had contacted him and had asked for certain bacterial strains that were known to be pathogenic to plants. Under the guidelines, the cloning of the genes from these strains required the use of very stringent containment conditions. The senior scientist said that he had told my postdoc, "Well, if Dr. Cohen wants these strains, I need to hear from him directly." Then, after thinking about this further, this senior scientist decided to telephone me. And the bottom line was that I wasn't aware that the postdoc had requested the strains, and I had no plans to have my lab do any work at all with them. I confronted my postdoctoral fellow about the situation, and he admitted that he had been "thinking about" cloning genes from the pathogen, but didn't want me to know. When I said that he would have to leave my lab, he made a veiled threat, telling me that he had a very extensive gun collection and knew how to use it. He didn't directly threaten me, but the implication of what he was saying was clear.

Hughes: Right.

Cohen: He argued that the guidelines were not rational or meaningful, so it didn't make any difference whether they were followed. And I told him that whatever his personal views were about particular provisions of the guidelines, my lab was committed to following them. I insisted that he leave my lab, and he did.

But there was another important question: should the incident be made public? I discussed this with several colleagues, and a couple of them felt that I should inform the NIH in order to protect myself from possible criticism. What would happen if the press got hold of the story and wrote it up as attempted violation of the guidelines by my lab? As the P.I. [Principal Investigator], I am responsible for what goes on in my lab. Ultimately, I decided that notifying the NIH about the incident would be gratuitous because there was no actual violation of any guideline, and the plant pathogens were never sent to my lab. But I documented the incident in great detail in writing in case the matter ever came up again. Anyway, this story may give you some insight about the mood and fears of the time.

Hughes: Yes. The actual, you know, day-to-day enforcement of the guidelines, I suppose, was the responsibility of the lab director.

Cohen: That's exactly right.

Hughes: I know from Janet [Hobson's] article in the *Smithsonian* magazine—which came out in 1977, I think it was—certainly there was a variation in attitude towards abiding by the guidelines. I think she was particularly referring to the younger scientists, the postdocs. But were you aware that there was a variation in the seriousness with which people in different laboratories took the guidelines?

Cohen: There was a difference in opinion about the scientific soundness of certain guideline provisions, but observance was nevertheless taken very seriously. Because of my active opposition to legislation, I was an especially visible target for criticism. I made every effort to be scrupulously clean in following the guidelines. And doing this was difficult at times because, as I've discussed, I knew that some of the required practices had been determined arbitrarily and were not based on scientific information. Nevertheless, those were the requirements, and while I was doing my best to have the practices changed, I felt that I would lose whatever effectiveness I might have as an opponent to legislation if my credibility was questioned. As I've mentioned, that was also one of the reasons why I opted not to start a company.

Consulting for Cetus

Hughes: Would you and others have been aware of some of the repercussions of forming a company if you had not had Genentech as a rather sore example?

Cohen: Well, as we've discussed, it had been claimed that Herb did experiments at UCSF to benefit Genentech, and his Genentech affiliation had been criticized. I thought it was best to limit my commercial activities to being a consultant.

Hughes: Well, what was the context for this? How au courant was it at that time, 1975, for biological scientists at Stanford to be consultants?

Cohen: There certainly were other scientists involved as consultants to pharmaceutical companies. It was a common practice, particularly for chemists.

Hughes: Yes. That has quite a long history.

Cohen: Yes, and there was also a history of computer scientists and engineers serving as consultants, so…

Hughes: But it was a relatively new phenomenon in biology?

Cohen: In biology it was. Prior to the growth of the biotechnology industry, there wasn't much interest in hiring basic scientists to provide advice about genetics or biochemistry.

Hughes: The applications were not clear.

Cohen: That's right.

Hughes: Were you asked to be a consultant to Cetus specifically because of your expertise in recombinant technology?

Cohen: I think so. Lederberg knew about my work and clearly saw its practical applications. He introduced me to Cetus.

Hughes: Is it simplistic to say that Lederberg was initially responsible for convincing Ron Cape that Cetus should look at recombinant technology?

Cohen: Well, I'm sure that Lederberg had a central role, but I think that other people in Cetus recognized the potential as well. Cape has a Ph.D. degree in chemistry, and he's a smart man and probably saw the potential on his own, as did a number of other people associated with Cetus. Certainly Don Glaser did. But Lederberg was their premier biologist [consultant], and my guess is that he was initially responsible.

Hughes: When you became a consultant, were they engaged in research using recombinant technology?

Cohen: No, their business strategy was to use a device that Don Glaser had invented to screen for identifying new antimicrobials. Cetus saw potential opportunities in recombinant DNA technology, but the company's management had difficulty making decisions about what projects should be pursued. They had an excellent group of scientific advisors, and I enjoyed meetings of the S.A.B. [Scientific Advisory Board] enormously because I learned at least as much as I taught. We proposed a variety of recombinant DNA projects to Cetus and suggested that the company try to express genes for human hormones in bacteria. I remember trying early on to persuade Peter Farley, who was the President and Chief Operating Officer, and Ron Cape, the Cetus CEO, to use recombinant DNA methods try to produce human growth hormone, which is a relatively small single-chain polypeptide. They felt that there were relatively few pituitary dwarfs needing human growth hormone and didn't want to pursue development of a drug unless it had a potential market that was very large. Cetus didn't pay me for business advice, and ordinarily I didn't give it to them, but I was convinced that it was important for Cetus to have an accomplishment that established the company's credibility in the area of recombinant DNA. Cetus decided against proceeding with the project, but of course that was exactly what Genentech did later, and it helped to attract first-rate scientists to that company.

Hughes: Despite the aura of having two Nobel Prize winners associated with Cetus? That wasn't enough of a magnet?

Cohen: You mean in terms of attracting scientists?

Hughes: Attracting good scientists.

Cohen: Well, there were some excellent scientists at Cetus, Tom White, David Gelfand, Henry Erlich, and others. But I think that one of the reasons that Genentech was successful in attracting Peter Seeburg and Axel Ullrich, for example, was the fact that Genentech actually made progress towards developing commercial products. And those two scientists were very important, as was David Goeddel, in helping Genentech to develop in its early days, as I suspect Herb Boyer has told you. Cetus had some excellent scientists working at the company, but the indecisiveness of management was an ongoing frustration. I don't know whether this is the time to talk further about Cetus, but if you'd like me to, I can go on and say some other things.

Hughes: Well, I think it's pertinent.

Cetus' Missed Opportunity

Cohen: Okay. Although I had avoided starting a company during the height of the biohazards controversy, by the late 1970s, there was no longer a concern that legislation would be enacted to regulate recombinant DNA research. In 1981 two groups of scientific advisors formed separate Cetus subsidiaries: Cetus Immune and Cetus Palo Alto. I was one of the Cetus Palo Alto founders, along with Stanley Falkow, Gary Schoolnik and Jack Remington. Later, Cetus Palo Alto was acquired by a larger company in Maryland, BRL-Gibco, which became Life Technologies (LTI), and we continued to be scientific advisors to LTI—some of us for about 10 years.

But, I'm getting a little ahead in the story. Peter Farley left Cetus in 1982, and the company hired Bob Fildes as president and CEO. Bob had an interesting history. He previously had been the president of Biogen, and it was generally known that while there, he had serious problems interacting with scientists, especially Wally Gilbert, who was one of Biogen's founders. Bob, who also has a Ph.D., is someone who believes that he knows best about almost everything and doesn't readily accept advice. It seemed to me that Bob viewed scientists as though they were centrifuges: if a company needs to buy another ten centrifuges, it goes out and buys them, and he acted like he thought the same thing was true for scientists. I don't think that Bob had any notion of the importance of scientific creativity to a company. With Bob at the helm, most of the members of the S.A.B. soon ended our relationship with Cetus.

In the early 1980s, Kary Mullis at Cetus, invented PCR. And, Henry Ehrlich and some others there made it work. As a scientific advisor, I had learned about the PCR idea before the relationship between Cetus and me ended, and I thought that it was a great invention. But when Bob Fildes took over at Cetus, a decision was made to devote almost all of the company's resources to developing interleukin-2 as an anti-cancer drug, and Cetus ignored PCR. In fact, if I remember correctly, Cetus had thrown in rights to sell a thermocycler for PCR with rights to a little automatic pipetter that Perkin-Elmer was interested in marketing. The scientists I knew at Cetus felt that PCR was a major asset and were frustrated by this.

One of Ron Cape's daughters was being married about that time. Ron and I had remained friends when I stopped consulting for Cetus, and I was invited to his daughter's wedding. At the wedding reception, some of the Cetus scientists there brought me up to date on PCR and Bob Fildes' continuing lack of interest in it. I telephoned Bob the following Monday and said, "Bob, we really don't get along, and I'm no longer a Cetus scientific advisor, but I do have some Cetus stock and care about the company's future. If you'll take me to lunch, I'll give you some free advice." And he decided to take me up on that and we had a lengthy mid-day discussion.

Fildes was more open then than I had ever seen. I told him my continuing feelings about the potential for PCR and suggested that Cetus consider using the same approach that Stanford had used in licensing the recombinant DNA invention, because like recombinant DNA, PCR was a widely applicable technology and could provide major royalty income to Cetus. I said that I thought it was an enormous opportunity. But Bob said that it was too late; he had proceeded too far along another route to reverse the direction. Bob continued to run Cetus into the ground until, eventually, he was fired by the board. And as you know, PCR was sold to Roche for something like 300 million dollars and the rest of Cetus was acquired by Chiron in a stock exchange. And that was the end of Cetus. The outcome was disappointing because Cetus was the first company to enter the biotechnology field, and Cetus had some outstanding in-house science and good external advisors. And they had lots of money in the bank from investors. Cetus could have been the premier biotechnology company, but missed the opportunity.

Hughes: It's an interesting history, isn't it?

Cohen: It is. I understand that several books are being written about that history.

On Consulting Relationships With Industry

Hughes: Well, I want to pursue the theme of university-industry interactions, but maybe let's put that off until we talk about patents because it seems to me to fit in better there.

Cohen: Sure.

Hughes: Just one question on this subject, though. In 1975, when you were first becoming a consultant to Cetus, you'd never had a formal relationship with industry prior to that time?

Cohen: No. I had given seminars at companies, and as I've mentioned to you, some companies had provided funds to help start up my lab and helped to support some of my research. But the research-support funds were gifts given by the companies to the university and I didn't have a consulting relationship with the companies.

Hughes: But the Pharmacology Division, as I remember, was supported by Wellcome?

Cohen: Well, that was the Burroughs Wellcome Fund, which is a charitable fund. The Burroughs Wellcome Fund gave me an award that helped to establish the Clinical Pharmacology Division.

Hughes: I see.

Cohen: And I became a Burroughs Wellcome Scholar as a result of that award, but was not a consultant to Burroughs Wellcome.

Hughes: Were you aware of any feeling amongst your colleagues here or elsewhere that having a consulting relationship with industry was inappropriate for a faculty member?

Cohen: Well, that's an interesting point. As I've said, in the early days, by early I mean 1974 or '75, for example at the time of the Miles Symposium in Boston, patents were viewed in a negative way and consulting relationships were also viewed somewhat negatively, but during the next couple of years, there was a dramatic change in the way that the scientific community viewed industrial connections. During all of that period I had an arm's length relationship with industry as a scientific consultant. But many scientists who in 1975 expressed concerns about applying for patents on basic scientific advances, which Stanford had done, had founded companies two years later.

Hughes: But before that happened, were you aware of any feeling that such relationships were inappropriate?

Cohen: Well, there was a long history of consulting relationships between university scientists and companies. But there had been the view that the founding of companies by scientists was different. Too close a relationship with industry was seen as problematic. If a scientist's laboratory at the university was engaged in the same type of research as his company, there was the concern that results obtained by postdocs and students in a university lab might simply be passed off to the company. Or even if that didn't happen, one of the concerns was there might be "two classes" of students in a university professor's lab; some would be individuals working on a project of economic interest to the professor, and there was concern that these projects and students might be favored. I felt that both concerns were legitimate.

Hughes: And such things did indeed occur?

Cohen: My guess is that they probably did, but if you asked me for specific examples, I couldn't give you any.

Hughes: Well, we can talk about these issues in greater detail, maybe even in the next session, but let's get back to the guidelines and legislation issues.

Cohen: Okay.

The NAS Forum, Washington, D.C., March 1977

Hughes: I wondered how salient the NAS forum, that took place in March of 1977, was in the controversy in general? Specifically, I would like to hear about the presentation that you and Dr. Boyer gave at that symposium. I understand there was a lot of tension between the activists on the other side and the scientists. Could you give me a flavor of what that particular forum was like.

Cohen: Yes, there was a lot of tension. As a National Academy forum, it was a public event and there were many non-scientist attendees. The invited participants were in the lower part of the Academy auditorium, and then there was the public gallery in the upper part. Jeremy Rifkin and others used that occasion to try to advance the argument that very strict laws were needed to control this research.

My "Recombinant DNA: Fact and Fiction" article, which I would like to think was a factor in turning around the views of some people, or at least causing them to think more extensively about the issues rather than just automatically accepting the notion that this research was hazardous, had been published a short while before the symposium. That article had its origin in comments that I had written earlier for the *Stanford Magazine*, which you and I looked at the last time we met. And then I expanded on those comments in testimony I gave before the California Division of the American Medical Association, which was asked by state legislators to evaluate the need for legal action on this issue. The material I prepared for the medical association testimony included "benefit scenarios." "Scare scenarios" were previously written by critics of the research who claimed that the experiments would generate some horrible science fiction creature or an "Andromeda Strain" that would destroy the world. The scare scenarios were total fantasy, and I thought that it was just as valid to suggest "benefit" scenarios, which at that time were also fiction. But they made the point that conjecture can be in either direction. An example of a benefit scenario was how in the nick of time, recombinant DNA methods produced a remedy for a disease that otherwise could have produced a world-wide epidemic. I wrote several of them.

My testimony, minus the scenarios, was developed into the "Recombinant DNA: Fact and Fiction" article. The article was published in *Science* and I think was widely read.

Hughes: Widely read by all sides, do you believe?

Cohen: Yes. There was evidence of this at the NAS symposium, where Chargaff again asked, "Do we have the right to counteract four billion years of evolution for the ambition and greed of a few scientists?"[98] I challenged his statement and pointed out, as I had in my article, that virtually all of biomedical science was aimed at counteracting what evolution had provided. Evolution had given us typhoid and cancer and diabetes and so forth, and we were trying, as biomedical scientists, to deal with problems that were a consequence of natural evolution. Biomedical science is a continuing assault on what we were handed by evolution. And Chargaff responded, "Yes, yes, yes, I know all of those things, I've read Dr. Cohen's article, and it's enough to have to deal with nature's afflictions, but do I have to deal with Dr. Cohen's?" So it was clear that he had read the article, and in his use of the words "nature's afflictions" and in the discussion that took place after that, he had conceded that all of the consequences of evolution weren't necessarily beneficial.

[98] Research with Recombinant DNA: An Academy Forum March 7-9, 1977. National Academy of Sciences. Washington DC: 1977, p. 56-57.

But Chargaff and some others still had the notion that there was some master plan of finely tuned evolution and that the scientists doing this research were about to screw up that plan. My response was that humans had been altering the evolutionary process from the time that mankind first domesticated animals and planted crops, and had already provided an advantage to certain biological forms. This had been done for thousands and thousands of years, and transplanting genes by recombinant DNA methods in order to learn about how genes work and develop treatments for diseases was not conceptually different, except that the genes being cloned were much smaller in number. There was probably less hazard in transplanting genes by recombinant DNA than in making genetic crosses that transplanted and combined large numbers of genes, most of them unknown.

Hughes: I see. Well, talk about the general atmosphere. You had presumably been to other events sponsored by the NAS. How did this compare?

Cohen: Well, no, I hadn't been to any previous NAS-sponsored event. At that time, I wasn't yet a member of the Academy, and the meeting was my first visit to the NAS building.

In some ways the meeting was a little like the one at Asilomar; there were reporters all around us and there were daily articles in the press about the meeting. But Asilomar had been a relatively small meeting of scientists, and this was a large, publicly attended meeting, with public protests in the back section of the auditorium. You've probably seen the picture of Jeremy Rifkin and his supporters unfurling a banner, "We shall create a perfect race," or something like that, which was cited as a quote coming from Adolf Hitler. The scientists doing the research were portrayed as supporters of eugenics—human gene modification—and as persons who wanted to do genetic engineering to "perfect the human race." When in fact, the research that we were talking about involved transplanting genes into bacteria.

But it was also true that the ability to clone genes and study them might eventually enable therapeutic use of genes in humans afflicted with some genetic diseases. The effects of a genetic mutation that leads to deficient immune responsiveness, for example, might be treated by adding back a normal gene that expresses genetic information missing from cells of the afflicted person. That's the premise of what has since become known as gene therapy. And even in 1977, the scientists and protestors both said that that the potential to do that wasn't many years away. There were, and still are, legitimate questions that should be raised about gene therapy: how it should be carried out, who is to decide what traits of cells should be altered and what traits should not be altered? But there is a big difference between the treatment of a disease in an individual and making genetic changes that can be inherited.

On the Responsibilities of Scientists Doing Basic Research

Unfortunately, many of the demonstrations that took place at the meeting produced confusion and the blurring of lines between issues related to the safety of recombinant DNA experiments and the longer-term societal issues related to genetic engineering. In my view, whether DNA cloning in bacteria should be done was a matter related to safety, not ethics. My feeling was that safety issues could best be addressed by scientific data, and that once the information was obtained, whether the actual risk, if any, was worth taking relative to the benefits was a societal decision. There was no one on our side who argued, as Chargaff and some other the opponents of the research had claimed, that scientists should be able to just go ahead and do any research they wanted to regardless of the consequences. But we felt that judgments about whether the experiments were safe should be made on the basis of data, not conjecture. And, the data had to be evaluated in the same way that other scientific data are evaluated.

Whether gene therapy would help to treat human afflictions is also a valid question, both scientifically and medically, but my opinion was that some of the issues related to gene therapy were largely ethical. And the people who tried to associate DNA cloning research with genetic engineering of humans were not making a distinction between somatic cell gene therapy, which would involve treating a genetic affliction in a particular individual, and germline modification, which would involve altering the genetic properties of the treated person's offspring.

Hughes: Well, we're talking, it seems to me, about where a scientist's responsibility begins and ends for the social implications of his research. Do you have feelings on that issue and have they changed because of the experience that we're talking about—namely, the recombinant experience? Did that affect the way you thought about your responsibilities as a scientist?

Cohen: My feeling is that most scientists do think about the consequences of what they're doing and their responsibilities as a scientist. And especially in the area of biological research, scientists commonly choose a project not only because it's intellectually stimulating to them and they're passionate about the scientific questions they're asking, but also because they hope that what they're doing will be beneficial to the public, and more generally to the world. My own research has been supported by taxpayer dollars through the National Institutes of Health and, in the past, by contributions given by people to the American Cancer Society. I'd like to think that the taxpayers and contributors are getting something useful for their money or will receive something useful eventually. That's one way of looking at responsibilities as a scientist. Another way is that we all like to feel that the work we're doing in life has some practical value, apart from who is supporting the research.

It has been argued that all research supported by public funds should be relevant to public needs. The problem is that it's easy to talk about practical relevance, but when someone is doing basic research, it's not so simple to determine in advance whether a particular avenue of research will yield findings that will be useful to society. I think that the work that Herb Boyer and I did is a good example of that. Yes, I was interested in studying antibiotic resistance plasmids, which I felt were certainly relevant to the clinical problem of antibiotic resistance. But I was interested in studying them at a molecular level and understanding how they had evolved. If someone had asked whether my work was likely to produce information that would be used by clinicians treating patients with antibiotics, I would have had answered, "Not in the near future." And Herb was studying restriction enzymes, and it was difficult to argue that there was some practical relevance to that. Yet, the DNA cloning methods we developed have produced a lot of information relevant to the diagnosis and treatment of human disease, and have resulted in pharmaceuticals that have benefited thousands of patients.

I once heard talk by the Director of UNESCO. He was quoting Bernardo Hussay, the eminent Argentinean physiologist, who said, "There is no applied science if there is no science to apply." When you come right down to it, I believe that advancing scientific knowledge almost always benefits the public if individual rights aren't infringed and obtaining the knowledge can be done safely. But ethical, political, economic, and scientific considerations should affect how knowledge is used by the public. A strategy of the opponents of the research at the NAS symposium was to try to blur the distinction between the creation of knowledge and its use.

Hughes: Then it's the public's responsibility for the use of that knowledge?

Cohen: Ultimately, yes. The public should seek information from persons who have professional expertise in the field, but ultimately, I believe that it is society's responsibility to determine how knowledge is applied. I know that this opinion runs counter to the view that scientists should take responsibility for the use of knowledge coming out from their research, but as I've said, in basic research it's often not possible to know in advance how the information obtained might be used. It is certainly not possible to predict all of the ways.

Hughes: Well, returning to the NAS forum, you and Dr. Boyer gave a workshop called "The Benefits and the Risks of Prokaryote Gene Exchange." Is there anything in particular to be said about that? Was this unusual for the two of you to be presenters?

Cohen: No, it wasn't unusual.

Hughes: Did you and Dr. Boyer together do a number of these sessions? I mean, not specifically there, but anywhere in the country?

Cohen: No, Herb and I did not have a lot of contact after our collaboration ended. That basically reflects the fact that we've gone in different scientific directions. We've seen each other occasionally at scientific meetings and I was at the party held when Herb retired from the UCSF faculty. We've received a number of joint awards over the years and have enjoyed getting together on those occasions. We've arranged to see each other socially a few other times and have talked about my going up with Herb in his plane or his coming sailing with me, but it just hasn't worked out.

Scientific Findings Leading to Withdrawal of Proposed Legislation by Senator Kennedy

Hughes: Well, let's return to the subject of legislation, in this case the federal legislation, because in September of 1977 you sent a copy of a manuscript which was currently in press at *PNAS* to Donald Fredrickson, the director of NIH. Would you like to tell me about that?

Cohen: I had argued for some time that the experiments being done in laboratories using the methods that Herb and I had developed were akin to biological processes that can occur in nature. Restriction enzymes are biological products made in bacteria. Of course, plasmids are also natural products of evolution. And somewhere along the way I decided—I've forgotten what started me thinking about this—to try to determine experimentally whether something akin to the DNA cleavage and joining that was being done in test tubes in laboratories, occurs also in nature in bacterial cells. If so, making recombinant DNA wouldn't be "unnatural" after all.

Our earliest DNA cloning experiments had shown that ligation of *Eco*RI-cleaved DNA molecules can occur *in vivo*. As restriction enzymes also work *in vivo* to cut DNA—that was the underlying basis for the restriction phenomenon—I thought that bacteria might produce constructs akin to DNA molecules constructed outside of cells. In addition to being of scientific interest, I thought that a demonstration that complementary DNA ends can be generated by restriction enzyme cleavage *in vivo* and that these ends can be joined in living cells might address some of the concerns about biohazards related to the novelty of recombinant DNA procedures. But if such cutting and joining occurred, I expected that it would be a rare event in bacterial populations, and the challenge was to detect it. I designed an experiment to do this. My idea was to determine whether a DNA fragment that contains half of an antibiotic resistance gene could be cut out of a plasmid *in vivo* cells and then re-inserted again in the opposite direction to form a full-length functional resistance gene. Any bacterial cells made resistant to the antibiotic by the gene-flipping event could be isolated and the structure of the plasmid confirmed. And I persuaded Shing Chang, who was a postdoctoral fellow in my lab, to undertake the experiment.

Anyway, the findings turned out exactly as I had hoped, and the results enabled us to say in the final paragraph of the paper, [reading from reprint] "In the continuing process of gene exchange among different bacterial species in nature, plasmids can be passed through a series of microorganisms that potentially are producers of different restriction endonucleases. Thus, plasmids may be subjected to a series of site-specific recombinational events that bring about structural reorganization of their genes. It seems reasonable to speculate from our findings that

restriction endonucleases may play a major role in the natural evolution of plasmid and perhaps chromosomal, genomes."

The work supported the arguments that a number of us had been trying to make to legislators. I showed the manuscript to Josh Lederberg and asked him to consider communicating it to the *PNAS*. Josh thought the experiments were interesting and scientifically important, and agreed to send it out to reviewers for further evaluation. So he sent it out for scientific review and the referees concluded that the data were sound and that the interpretations were valid.

At that time, drafts for legislation to regulate recombinant DNA research were proceeding along in the U.S. Senate and in the House of Representatives. Don Fredrickson was asked to comment on the bill proposed by the Senate or House, I've forgotten which, and I decided to send Don a copy of the paper prior to publication. I would have liked to wait until the paper had been published, but things were moving very quickly. My paper had passed peer review and had been communicated to the *PNAS* by Lederberg in early August, but it wasn't scheduled to appear in print until November, and I wanted Don to have the information it contained. And so I sent an advance copy to Don, also a copy to Larry Horowitz. Larry solicited the opinions of several other scientists, who agreed that the findings were persuasive, and this had a role in a decision by Larry to suggest that Senator Kennedy withdraw his proposed legislation.

When the Senator announced the withdrawal of his proposed bill, he said, "New evidence from the laboratory of Dr. Stanley Cohen at Stanford has led us to reconsider…." That evidence, of course, hadn't been published yet, and I was immediately besieged by questions from reporters who wanted to know just what the new evidence was. My feeling has always been that initial disclosure of scientific findings should be in scientific journals or at scientific meetings rather than by public announcements in the media, and I hadn't realized that Larry and the Senator would be acting so quickly or would be publicly mentioning my research findings. I made a foolish decision and said, "Well, my paper is [in press]. [Wait a] couple of months for me to discuss the results with you." The news media roundly criticized me for that position, and in retrospect, the way I proceeded was pretty stupid. I had sent my paper to Fredrickson and to Horowitz and should have anticipated what would happen. I had released the data to them, and I should have given the data to the news media.

Hughes: You mean it would serve as preprint?

Cohen: Yes, what was given to Fredrickson and Horowitz was a preprint of our paper…

Hughes: So that in your eyes, where you erred was not in giving it to Fredrickson or Horowitz.

Cohen: Where I erred was in not also giving the preprint to the news media when they requested it, after it had been sent privately to Fredrickson and Horowitz.

Hughes: Ah.

Cohen: Another reason I didn't give it to the media was because I didn't want the conclusions from my paper published by the press and then picked apart for political reasons by opponents of the research who had not seen the actual data. I had mixed feelings at the time. And as I've said, I erred in not realizing how the situation would unfold.

Hughes: Yes.

Cohen: And I should have played by the political rules rather than the scientific ones.

Hughes: Did you have any anticipation of the stir that this would cause?

Cohen: Well, I expected that the results would cause something of a stir. The published paper quickly received a lot of attention. That had been anticipated because of the articles in the press, and Kennedy's comment. And when the paper was published, *Nature* ran a commentary discussing the findings and pointing out their scientific importance. In any case, publication of the research was one of a series of events that began to change perceptions about biohazards of recombinant

DNA. And the experiments specifically had an important role in persuading Kennedy to withdraw his legislation.

Hughes: Did he actually say that?

Cohen: Oh yes.

Hughes: Yes?

Cohen: Sure. He said that explicitly at his press conference.

Hughes: Well, as you well know, you were criticized by your scientific colleagues, and I'm not…

Cohen: I think that the criticism came from the press, and later from Lear and others who have written about these events.

Hughes: Yes, but also there were scientists who thought that the way the experiment… Richard Novick, for example, thought that the circumstances of the experiments that you did were forced, that these were not natural circumstances. For one thing, they were occurring *in vitro*.

Cohen: No, they were occurring in living cells. That was the point of the experiment. The experiments were "forced" in the sense that we had set up an assay able to detect very rare events, but that's the nature of many genetic experiments. The same thing could be said of the assays set up to detect bacteria containing recombinant DNA in the original experiments that Boyer and I did.

Hughes: Right.

Cohen: I was not claiming that the joining of DNA ends generated by restriction enzymes *in vivo* is a common occurrence, but just that it can occur in nature.

Hughes: Yes.

Cohen: It was necessary to devise a strategy for detecting such rare events, but we didn't force the event to occur.

Hughes: I see. Well, Diana Dutton quotes Richard Novick, and I can't now remember if she gave more context. Anyway, what she quoted him as saying is, "Richard Novick contends that 'the conditions under which this interpretation took place are so extremely artificial that there is essentially no chance of their occurring in nature.'"

Cohen: I don't know whether the quote by Dutton is correct, but the data were unambiguous and I would be surprised if Richard actually said there was essentially no chance of restriction enzyme cleavage and subsequent ligation of the resulting DNA fragments to occur in nature.

Hughes: Well, we'll see how Dutton cites it. Is there more that we should say here on the subject of federal legislation?

Change in Public Perceptions About Recombinant DNA Research

Cohen: Well, perhaps just a couple of additional things. After the Kennedy bill was withdrawn, and when the bills in the House also did not proceed, the air seemed to come out of the balloon that was being floated for legislation. In a remarkably short period of time, the views of the public began to change. We were continuing to see benefits of the research without seeing any evidence of hazard. There was the prospect of making clinically useful amounts of human insulin in bacteria, and the production of other useful medications by recombinant DNA seemed closer. My article had appeared in *Science* and an article by Watson was published making similar points—I've forgotten where he published it. It was called "An Imaginary Monster."

Hughes: Yes, right.

Cohen: The mood changed, as there was more experience with bacteria containing recombinant DNA molecules. Rather than making bacteria more robust, most foreign genes being introduced into bacteria were found to make them less robust, and this made the bacteria less able to survive—contrary to the science fiction biohazard scenarios that had been passed around the year before. There were also actual data from epidemiologists and geneticists indicating that genetically modified bacteria don't take over populations unless they are specifically cultured under conditions that give those bacteria a selective advantage. The press also got tired of writing about the same old issues, and I think that this also contributed to the change in public perceptions.

And as described in the Watson and Tooze book, *The DNA Story*, there was an attempt to produce a second Berg *et al*. letter.[99] Paul recognized that the mood had changed, and Watson and I especially were pushing for, if not a retraction, a public statement that concerns that we had raised three years previously had turned out to be unwarranted as new information had become available. We went through several drafts of a proposed statement, as you may have seen in the Watson and Tooze book. They published photos of some of the drafts and also some of the correspondence relating to them. But the group couldn't agree on a text that all of us were comfortable with. And the effort fell apart, and therefore a second Berg *et al*. letter was not published.

Hughes: Simply because there was not consensus.

Cohen: There was consensus that we should say something, but not a consensus on what should be said. An important issue was that there was not agreement about making an explicit statement on whether the initial concerns were valid.

Hughes: There was mellowing of the climate surrounding the recombinant issue and, of course, the guidelines were reflecting this mellowing?

Cohen: Right.

Hughes: Over time, there was a relaxation of the guidelines. In April of 1981, you wrote to William Gartland offering, and I quote you, "strong support for the proposal to convert the NIH guidelines into a non-regulatory code of standard practice and to reduce the recommended containment level for some experiments." Now, does that mean that the guidelines indeed had been mandatory, and you were now suggesting they be reduced to a recommended status.

Cohen: Yes, and as I've said previously, I think that the term "guidelines" was a misnomer, or it was at least for scientists receiving research support from the NIH, and also from many non-governmental sources. Organizations like the cancer society also required grant recipients to observe the NIH-mandated practices as a condition for support. Even though legislation controlling the research had not been enacted, in practice, the guidelines were really regulations, and it was necessary for local biohazard committees established at universities to evaluate and approve memoranda of understanding before experiments could be carried out.

So a federal mechanism for regulating recombinant DNA research had been established, and university mechanisms for doing this were also put in place. Yet, for known bacterial and viral pathogens, the responsibility for doing experiments safely was left to the investigator. Underlying that letter to Gartland was my feeling was that if leaving the responsibility for carrying out research safely to the investigator was considered to be sufficient for microorganisms known to be hazardous, it certainly should be sufficient for microorganisms for which there was no evidence at all of actual hazard.

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[99] Watson, JD, Tooze, J. *The DNA Story: A Documentary History of Gene Cloning*. San Francisco: WH Freeman, 1981.

Hughes: Was there any particular reason that you were writing at that juncture? Was there a reason that you were writing a letter to Gartland in April 1981, suggesting a relaxation of the guidelines.

Cohen: I don't remember whether there was a precipitating event that led me to write at that particular time. I suspect that there probably was, but I don't remember. Did I say anything in the letter about…?

Hughes: I don't have it in front of me. We can look that up. I'll bring it next time and we'll see.

Cohen: Okay.

Hughes: In reference to the *Stanford M.D.* article, you wanted to say something about peer review in relationship to the recombinant story. Is there time to talk?

The Issue of Public Control of Scientific Research

Cohen: Okay. The issue was how the public should exercise its right to control the use of public funds that support scientific research. The question is sort of related to one you asked a few minutes ago. As I've mentioned, I felt that the testimony that Holman gave before the Kennedy committee in 1975 raised a "straw man" issue by saying that some scientists think that the public shouldn't oversee their work. That was not my position, nor was it the position of anyone I interacted with on the recombinant DNA issue. It certainly is the public's right to be assured that scientific experiments are carried out safely. And it's also the public's right to determine how knowledge acquired through public support of basic scientific research should be applied. But there was a crucial difference between Holman's position and mine: Holman argued that the public should also make decisions about the scientific merits of a particular line of research. My view was that it is the public's prerogative to specify how the resources it provides will be used, but that public control of research is best accomplished by delegating the responsibility for evaluating the scientific merit of a particular line of research to a system of peer review. I don't think that it is in the public interest to micromanage basic scientific research, either legislatively or through any other federal bureaucratic mechanism.

COGENE (Committee on Genetic Experimentation)

Cohen: I should probably say something here about COGENE. COGENE is an international committee. It's an acronym for *Co*mmittee on *Gen*etic *E*xperimentation. It's a subcommittee of ICSU, the International Council of Scientific Unions. COGENE was relevant to the implementation of recombinant DNA guidelines and the issue of different standards for recombinant DNA research in different countries.

In 1977, when COGENE was formed, I was asked to be one of its initial members, and agreed. One of the tasks of COGENE that I became involved in was the review of guidelines of various nations and the reconciliation of differences in guidelines. We issued a report in 1979, calling ourselves the "Working Group on Recombinant DNA Guidelines," in which we had done a survey of guidelines in different countries and pointed out the disparities. At a later point, our report was instrumental in causing the NIH to reexamine our practices in the U.S. and to relax some of the NIH procedures and regulations, which had been more stringent than those existing in many parts of the world.

Hughes: I read—and this was in the secondary literature, so I'm not sure of its accuracy—that COGENE in 1979 urged the NIH to eliminate the guidelines. Do you know of that?

Cohen: No, I don't remember that at all. That secondary literature reference was probably referring to the report of the working group, which I had chaired. The statement that COGENE urged elimination of the guidelines certainly is not true. I'm not aware of any proposal that it eliminate…

Hughes: This was in a book by Diana Dutton.

Cohen: Diana Dutton was never known for accuracy.

Hughes: Well, that's why I'm checking with you, because it struck me as rather an extreme recommendation.

Cohen: No, our report noted that the level of precaution required for identical experiments in different nations is strikingly different, and then we pointed out the level of containment required in the U.S. for work with recombinant DNA that includes genetic material from known pathogens is often greater than the containment level recommended for the pathogen itself. We also expressed the opinion that recombinant DNA experiments could in at least some instances allow the study of genes from medically important disease-producing microbes at reduced risk, and that our analysis of guidelines has revealed no scientific justification for requiring a higher containment for DNA from an organism than for the organism itself. There was no statement anywhere, nor was it the intent of anyone that I knew on COGENE, to recommend elimination of the guidelines. But by pointing out the disparities in the guidelines of different nations, I think that COGENE did have a role in causing relaxation in certain provisions of the U.S. guidelines, as I've mentioned.

Hughes: Well, since we're on COGENE, let's continue. Whose idea was it?

Cohen: I think the idea for COGENE came originally from Bill Whalen, who was chairman of the Department of Biochemistry at the University of Miami in Florida, and Bill invited me to be a COGENE member at the Miami Winter Symposium that I attended in January 1977. I had been invited there to give a talk, and Bill had just gotten approval from ICSU to form COGENE. Paul Berg was also there. Paul was also invited to be one of the original members. It was a multinational group; the initial membership included Berg and me, Giorgio Bernardi from France, Anne Skalka from the U.S., John Tooze who was subsequently secretary of EMBO, Bill Whalen who was chairman of COGENE, Eli Wolman from France, and other scientists from India, the U.K., the U.S.S.R., and other nations. Alexander Bayev was, and is, a very senior Russian microbiologist who was director of the Microbiological Institute in Moscow. I guess at this point he's nearing 90, but I believe he's still alive. COGENE still exists, but only three members of the original group remain: Giorgio Bernardi, who is the current chairman, Ann Skalka, and myself.

At one of the early COGENE meetings held in Paris, I was discussing, during one of the breaks, the fact that certain experiments that were not permitted in the U.S. were allowed in other nations, and indicated my view that it was illogical to have different rules in different countries. Bayev suggested that I let the regulators in the U.S. know that scientists in the Soviet Union were putting mammalian genes into *E. coli*. He thought that would encourage them to allow the same kinds of experiments to be done in the U.S. Soon after I returned to the U.S. from that meeting I had a visit by someone from the CIA who knew that I had a discussion with Bayev in Paris at the COGENE meeting and wanted to know what I had learned about recombinant DNA experiments in the Soviet Union. And as Bayev had suggested, I told him that the Soviets were putting mammalian genes into *E. coli.*

Hughes: Now was the CIA monitoring this sort of thing because of the military?

Cohen: Well, one of the issues that had been raised was the possibility that these methods could be used for biological warfare.

Hughes: You bring that up in the Plasmid Committee report, I believe.

Cohen: Yes, we certainly were aware of that potential.

Hughes: I mean, just sort of in passing.

Cohen: Yes. And, in fact, in the book by Susan Wright that you were kind enough to loan to me,[100] she quotes that part of the report. She mentions that the Plasmid Committee had that concern. Of course, we understood that there was now a method of intentionally constructing microorganisms with new kinds of capabilities, although it was also my feeling and the feeling of other members of the committee, that it would be difficult and cumbersome to intentionally construct a biohazardous organism. We felt that if a nation wanted to engage in biological warfare, it would be a lot simpler to use one of the already-available natural pathogens. In principle, one could imagine that the methods could be used to intentionally construct hazardous microbes, but there were much easier ways to carry out biological warfare than by using recombinant DNA methods.

Interview 10: April 14, 1995

Additional Discussion About Efforts Toward Legislation in California and About Opposition to Recombinant DNA Research by Environmentalist Organizations

Hughes: Dr. Cohen, last time we talked about legislation at the federal level. Today I'd like to shift to the state, where you also had a role. I believe you were in touch with Marc Lappé who was, I believe, a sociologist at the state Department of Health and who was largely responsible for drafting one of the bills. There were, as I understand it, two bills. There was one that came out of Barry Keene's committee, and there was one I can't remember.

Cohen: Yes, Keene was in the State Senate, and the key person in the Assembly may have been Art Torres.

Hughes: Well, that could be, but the name I had is Charles Warren for the Assembly. Charles Warren wrote to the president of Stanford and also to the chancellor of UCSF saying that the hearings were upcoming in early 1977 and asking for certain information from both universities. I don't know what his relationship was to the issue.

Cohen: I don't remember interacting with Mr. Warren, but I do remember Barry Keene and, of course, Marc Lappé. One thing I learned while interacting with elected officials during the recombinant DNA controversy is the extent to which lawmakers' positions are molded by the staff that they hire. When an energetic legislative staff member gets onto an issue such as this one, the staff person drives the lawmaker's pursuit of the issue. For example, at the federal level, the key person in the Paul Rogers effort was Burke Zimmerman, who later wrote a book on the subject. And of course, in the U.S. Senate there was Larry Horowitz., who was Edward Kennedy's aide. Staff members generally are young people beholden to the political objectives of their lawmaker bosses, but they are ambitious and also have their own career objectives. I felt that Marc was using the recombinant DNA controversy as a vehicle for advancing himself personally in the field of public control of science.

Position of Environmentalist Organizations

[100] Wright, S. *Molecular Politics: Developing American and British Regulatory Policy for Genetic Engineering, 1972-1982.* Chicago: University of Chicago Press. 1994, p. 71.

I think that analogous self-interest also motivated some people in the environmentalist movement to oppose the research. I've always considered myself as a champion of environmental causes and over the years have contributed both time and money to promoting these causes. During the controversy, the Sierra Club and Friends of the Earth, two organizations that I had strongly supported, took positions that I viewed as uninformed and anti-environmental. In my opinion, recombinant DNA research offered the prospect of producing insecticides that are not toxic to the environment, of modifying plants so that they wouldn't require environmentally harmful fertilizers, and of providing better ways to clean up toxic spills. But activists in these organizations persuaded the governing boards that recombinant DNA research was dangerous to the environment, and the organizations vocally opposed the research. The boards took that position without seeking much information, and that made me wonder whether other positions taken by the environmentalist organizations I've always supported were equally uninformed.

But there were some environmentally concerned organizations that tried to make informed decisions. Shortly after the NAS symposium, I received a telephone call from the president of the Wilderness Society. He invited me to be a speaker at a Smithsonian Institution symposium celebrating the Wilderness Act, which had been enacted a decade earlier largely through the efforts of the society. I had been disappointed by the position taken by Friends of the Earth and my first reaction to his phone call was that the Wilderness Society was looking for a scientist to bash at their symposium. He said he was sincerely interested in hearing my views and told me that another scientist who had agreed to speak at the symposium was Peter Raven, who was then Director of the Missouri Botanical Gardens and subsequently became Home Secretary of the National Academy. Peter was very active in environmentalist causes and was known as a very thoughtful person. The Wilderness Society convinced me that they were genuinely looking for information and I decided to accept their invitation. I was almost ecstatic about the reception that my talk received. After the talk and the open discussion that followed it, I was surrounded by attendees who thanked me for explaining the issues to them, and they conceded that they had almost automatically accepted the views of Jeremy Rifkin and others that the research was dangerous without really spending the time to look into the matter. And I felt that the event was useful in increasing the dialogue between the environmentalist movement and the scientists involved in the research.

Hughes: Did you have any dealings with the Sierra Club or the Friends of the Earth?

Cohen: I had correspondence with Francine Simering, who was the Friends of the Earth member that led opposition to the research, but was not invited to meet with anyone in that organization. I felt that their position was: "Don't confuse me with the facts; we've made up our mind that this is a bad thing."

Hughes: Yes. And as far as you know, nobody else who represented your viewpoint was allowed a forum?

Cohen: Not so far as I know.

Hughes: Well, tell me…

Cohen: Anyway, back to Marc.

Hughes: Yes, back to Marc.

Cohen: Marc Lappé was one person in California that was working actively to oppose the research. Diana Dutton was another. Marc's job enabled him to influence the opinions of state lawmakers. You may have some correspondence that might further refresh my memory about Marc. I think he was involved in preparing some of the earlier scare scenarios that were being circulated.

Hughes: I have. [tape switched off and on]

Cohen: Thank you for giving me this material;[101] it does jog my memory.

Marc tried to position the controversy as a battle between people defending public interests and scientists who didn't want to be controlled. According to him, scientists wanted the freedom to do any experiments they wished. Marc, Diana Dutton, and Hal Holman all wanted to debate these issues, rather than whether there was any validity to the notion that the research was hazardous. They tried to shift the battleground from the issue of public safety to the question of public control of science in a broader sense. Anyway, in the letter you're holding in your hand, I pointed out to Marc that data obtained since the 1974 Berg *et al*. letter had convinced me that the conjectural concerns that others and I had raised earlier had no scientific basis. I said that I viewed the situation differently in 1977. By 1977, Roy Curtiss, who in the year or so following the Asilomar meeting spent a lot of time developing chi1776, and others also, indicated that they had become increasingly distressed by the degeneration of the debate: opinions were being stated as fact, and claims presented as facts were in conflict with published data. Roy felt that that there had been an unwillingness to adhere to the principles of scientific objectivity and noted that he had "painfully and reluctantly" arrived at the conclusion that the concerns were overblown. He said, "It is contrary to my past feelings about the biohazards of recombinant DNA research."

As scientists, Curtiss and I and others could say that we had changed our opinions. When the data don't support a hypothesis, you modify the hypothesis. But the steamroller that began moving because of the initial concerns of scientists was difficult to stop. The perceptions of the public continued to be that the research was a threat to public safety, and this perception provided a rationale for legislative attempts to control the research. I concluded that it was going to be difficult to stop the steamroller in California. I felt that the best that the scientific community could do in this state was to limit action to the appointment of a study commission to look into the issues further, and hopefully to return at a later time with a report that would refute the assertions being made by Lappé and Dutton and others.

Hughes: Wasn't your tactic also to urge state legislators to wait until the federal legislation was decided or in place?

Cohen: Yes. That's right. I argued that microbes don't recognize state boundaries, and that it did not make sense for California to have rules different from those in Nevada, for example, or Oregon. If the hazard was real enough to require legislation—and I indicated clearly that I believed it was not—then regulations should be implemented at the national level. If the federal government concluded that no new laws were required, actions should not be taken by State legislators, who were likely to be less well advised about the technical issues than the Congress in Washington.

Hughes: Now you could have taken the harder line, and I wonder if some people did, namely, "I don't, we don't, want state legislation under any circumstances, regardless of what the federal government does."

Federal Laws versus State or Local Laws

Cohen: But if there had been national legislation, then it would have been applicable to California.

Hughes: Would it?

Cohen: I think there would have been national standards that applied to all states. But as we've already

[101] Referencing letter to Marc Lappé from Cohen's files.

discussed, by 1977 the mood was changing at the national level, and there was increasing likelihood that sense would prevail in the debate in Washington. But state legislators and local councils were lagging behind in the mood change, and there was the chance that states and communities might still enact laws, leaving the nation with a patchwork of different standards in different places. And that didn't make sense. I argued, I believe legitimately, that if this really was an issue to be concerned about, we should deal with it as a nation. And if there proved to be no need to address the issue by enacting federal laws, the state should not do this either.

Hughes: Well, we know that the pending federal legislation was withdrawn, but communities do enact local legislation or regulation, perhaps Cambridge being the most salient example. And didn't Emeryville?

Cohen: I think so, yes.

Hughes: Emeryville and Berkeley.

Cohen: I think Emeryville and Cambridge did. I think that Emeryville acted because Cetus was there.

Hughes: Yes.

Cohen: It just didn't make scientific sense that experiments carried out in Emeryville required different experimental conditions than those applied a few miles away in Fremont, for example. But the elected local officials felt that they had to do something.

Hughes: Was this a real concern to Cetus?

Cohen: Well, no. I think that companies were happy to use whatever containment conditions were required, so long as their work could proceed. Industry had considerably more resources than university-based scientists, so a company could build a high security lab to do experiments that couldn't, according to the regulations, be carried out at an open bench. There was some extra expense, but a lot of money was being poured into the biotechnology industry at that time and companies wanted to get beyond the uncertainty about whether and how they could proceed. In fact I think that some companies were taking the same pro-legislation position as the ASM [American Society for Microbiology] at the time. Industry is regulated in multiple ways, and I think that most companies felt that if the passage of laws controlling the research would make the legislators and public happy, that would be fine.

Hughes: Well, let's go back to the state legislation.

Cohen: Okay.

Hughes: Would you like to tell me how you came to testify?

Cohen: I don't really remember. I think it probably was because I had been a very visible proponent of a particular point of view, had written about the subject for the public, and had appeared before the Senate committee in Washington. By that time my "Fact and Fiction" article had received a lot of attention, and the article had been reprinted in the *Western Journal of Medicine* and either the *Ladies' Home Journal* or *Woman's Day*, I can't remember which. Parts of it were reprinted in the *Los Angeles Times* and in many other newspapers. I think that this article was the first widely circulated argument that was contrary to what most of the public believed. I was living and working in California, so I was invited to testify.

Hughes: Do you remember how the testimony was received?

Cohen: Actually, I don't. I don't remember the details of my interactions with California legislators. I do remember my discussions with Senator Keene, but I had forgotten most of the discussions I had by phone or in person with Marc Lappé, until you showed me this material. I remember going to Sacramento and appearing before the committee, but I don't remember the details.

Hughes: It seems to me that you were writing letters at this time to drum up support for your position,

and one of the people that you wrote to was the head of the Committee on the Environment; it probably had a slightly different name, but that's what it was. One thing that you said, and I wonder if you will remember what you were thinking, is, "I urge that your committee and the CMA oppose what seems to be a politically motivated move to promote the interests of some California legislators."

Cohen: Yes, thanks. I do remember that. What I meant by that statement was that I felt after my discussions with some legislators, that they didn't have enough information to have a meaningful position one way or the other. They were being guided by their aides. And if their position was not based on an understanding of the issues or any real conviction, maybe it was based on political considerations.

Hughes: Right.

Cohen: Some years later, I happened to be invited to a summertime picnic held at Fetzer Vineyards in Mendocino County because I had been the president of an organization called Medical Friends of Wine. At that picnic at the Fetzer winery, which was a private invitation event, there was Senator Barry Keene. This was, oh, I guess in the early 1980s or mid 1980s. We started talking and he was very cordial. He remembered me and our discussion in Sacramento about laws to control recombinant DNA research. He said that he had been wrong, and perhaps had been carried away by some of the comments and advice that he was receiving at the time. I felt that it was a sincere statement.

Hughes: At the federal level, as we've discussed, people were becoming aware of the practical applications, the beneficial applications of this technology. We discussed Phillip Handler's announcement of the somatostatin work on the floor of the Senate, etcetera. Does that play a role in the California debate as well?

Cohen: Well, I think it did because California is one of the centers of biotechnology, and the biotechnology industry was beginning to grow here at the time. And the point being made to California legislators by entrepreneurs was that if California implemented uniquely restrictive laws, then other states would be favored as sites for company start-ups.

Additional Scientists Voicing Biohazard Concerns

Hughes: Well, another person to whom you wrote at this time was Sinsheimer. He not only was prominent because of his position in science but he was also prominent as a scientist who was, at best, doubtful about the wisdom of going ahead with recombinant technology. Your argument to him was, "Let's at least wait to see what happens at the federal level." And he did indeed write a letter saying precisely that. Do you remember?

Cohen: Yes, I remember interacting with Bob on this. Bob Sinsheimer is someone who I've always had enormous respect for as a scientist. I think I've said in one of the earlier interviews that there were basically two groups of scientists that actively opposed the research. There were people like Bob who, in my view, were genuinely concerned about safety. These were introspective and thoughtful people who had concerns that I felt were not justified by the scientific data. In a sense I found that a little surprising, because some of them were first-rate scientists, and I felt that they were letting their emotional feelings cloud their scientific judgment. But I could easily understand how this could occur: the signers of the Berg *et al*. letter had done the same thing. Nevertheless, I felt that Sinsheimer was motivated by sincere concerns about safety.

Hughes: Who else would you put in that category?

Cohen: Who else? Richard Novick was in that category.

Hughes: Holman?

Cohen: Holman really wasn't scientifically familiar with this area. Certainly, as Chairman of Medicine at Stanford, he had been influential in American medicine, but he wasn't working in genetics or molecular biology. Hal was also a political activist, and he had a broader agenda. If you read what he wrote at the time, his concerns didn't really address the issue of biohazards; he acknowledged that he didn't have the scientific background to evaluate that issue. But he had underlying concerns about the need for society to control science, and that was what he wrote about and argued for repeatedly. I think he saw the recombinant DNA controversy as a vehicle for dealing with the broader issue, in much the same way that Jon Beckwith and Jonathan King did, and George Wald and Ruth Hubbard did.

So among the protagonists in the controversy were scientists who had sincere concerns about safety and scientists that were using the controversy to address a political agenda. There were the young legislative staff people who had their own personal agendas, and there were the business agendas of companies. And of course, one could argue that the scientists opposing the passage of laws had our own agendas too.

Hughes: Yes.

Cohen: If I didn't say that you probably would have. But I think what motivated scientists to work actively to try to prevent the passage of legislation was the sincere belief that the biohazard concerns were overblown and that the proposed laws were not in the public interest. It certainly was not, as Chargaff suggested, to satisfy curiosity and ambition. We believed that this was a tempest in a teapot.

Hughes: I see. Well, what became of these two bills?

Cohen: The California bills died in the legislature. I don't remember the details, but don't think that an actual vote was taken on either bill. The outcome was bitterly resented by Marc Lappé and others who had labored hard and long to promote the state legislation.

Hughes: Well, is there any more to say about the state legislation?

Cohen: No, I think that's probably enough. But, I'd like to look through this file for a moment. Something else may jog my memory. [Pause]

Hughes: Lear, in his book, says that you received hate mail.

Cohen: Yes, I did receive hate mail, and that was quite distressing. I received letters, sometimes signed, most often unsigned, from people who believed that the experiments that Boyer and I had done were contrary to the will of God and that I would be punished by God and by people that would carry out God's Will. At that point in time, I decided to have my telephone number de-listed, and to this day, my home address isn't printed in the phone book.

Importance of Terminology

Hughes: I also know, and it's from that *Stanford M.D.* magazine, that you objected, and presumably still object, to the term "genetic engineering."

Cohen: Yes. I'm glad that you've raised this. There are several terminology issues worth mentioning. But perhaps this is nit-picking to the point of pedantry.

At Asilomar, I preferred the term "vehicle" for the plasmids that were being used as gene carriers, but Brenner and some of the other Asilomar participants preferred the term "vector." The reason I didn't especially like "vector" was that the term was applied to agents that were carriers of infectious diseases; for example, mosquitoes are vectors for the spread of malaria.

Hughes: Right.

Cohen: I thought that "vector" is a "loaded" word and I didn't like the connotation. "Vehicle" was more neutral. Obviously, my view didn't prevail, and "vector" is now the generally-used term. In retrospect, I think that "vector" is viewed more neutrally than I feared at the time.

Hughes: Well, that somewhat is discipline contingent, wouldn't you say?

Cohen: It probably is.

Hughes: You had a microbiological background, whereas a lot of people were coming out of biochemistry or molecular biology, and that probably was a new word…

Cohen: That's right.

Hughes: …for them, and it didn't have these connotations.

Cohen: I think that's probably true. Another point involves the terms "recombinant DNA" and "gene splicing." I think that the term "recombinant DNA molecules" was first used in the Mertz and Davis paper[102] to refer to molecules generated *in vitro* by the ligation of two separate DNA fragments, and in my early DNA cloning papers, I used the term in that way. The collaborative experiments described in the Gordon conference talk by Herb Boyer that led Maxine Singer and Dieter Söll to write to *Science*[103] reported that DNA molecules constructed *in vitro* could be propagated and cloned in bacteria, and the Berg *et al*. letter[104] soon afterwards referred to the "construction of biologically active recombinant DNA molecules." But after a short time the term "recombinant DNA" was being used to indicate not just DNA molecules put together in test tubes, but also the entire process of cloning DNA. And then when the term "gene splicing" became popular, that term was also used for not just the joining of DNA fragments *in vitro*, but additionally to refer to the entire DNA cloning process. Ultimately, this use of terminology has led to a blurring of the distinction between the ability to biochemically join DNA fragments and propagation and cloning of DNA in living cells.

Hughes: Did you present another term?

Cohen: Well, the event that prompted the Berg *et al*. letter was the discovery that recombinant DNA molecules put together *in vitro* could be propagated and cloned in cells, rather than simply DNA fragment joining, and I thought that the DNA cloning term was more accurate.

Some people refer to the DNA cloning process as "genetic engineering," but I felt that this was an unfortunate term because it raised the notion of gene modification in humans and had connotations that brought to mind the discredited field of eugenics and gene modification in humans. That was confusing, and the association in some people's minds was highlighted by the protests at the 1977 NAS meeting in Washington. Books in print at the time, books such as *1984* and *Brave New World* and other science fiction novels, had used genetic engineering in that context. But we were talking about putting genes into bacteria, and that is quite different.

Hughes: Yes.

Cohen: But terms like "DNA cloning" or "gene cloning" are a bit much for the public to readily understand, whereas it's simpler for newspapers to write about "genetic engineering." Anyway, it's clear that I haven't had much success with my terminology preferences.

Hughes: No, but I see your points.

Cohen: It's the terms used by the lay press that create the images that persist in the public mind.

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[102] Mertz, JE, Davis, RW. Cleavage of DNA by R 1 restriction endonuclease generates cohesive ends. *Proc Natl Acad Sci USA*. 1972 November; 69 (11): 3370-4.

[103] Singer, M, Söll, D. Guidelines for hybrid DNA molecules. *Science*. 1973, September 21; 181: 1114.

[104] Berg, P, Baltimore, D, Boyer, HW, Cohen, SN, Davis, RW, Hogness, DS, Nathans, D, Roblin, R, Watson, JD, Weissman, S, Zinder, ND. Potential biohazards of recombinant DNA molecules. *Science*. 1974; 185 (148): 303.

Awarding of the Nobel Prize to Paul Berg

Hughes: Well, Dr. Cohen, as we were discussing terminology, it ran through my mind in your definition of recombinant DNA, or your objections to it, the question of the Nobel Prize. As you well know, in 1980, it was awarded to three individuals, presumably for the technology and the actual joining of pieces of DNA, in the more narrow sense, than what you were meaning. True?

Cohen: No. I think that the 1980 Nobel Prize in chemistry was given to Paul Berg, for his role in recombinant DNA, and that the two other people, Fred Sanger and Wally Gilbert, shared the other half for developing methods of DNA sequencing. Those were separate technologies.

Hughes: Well, what Dr. Berg had done is related as we've discussed to what you and Dr. Boyer subsequently did. Was there some feeling on your part that it would have been well to have been included?

Cohen: Well, that's an interesting question, Sally. What you're asking about is my feeling about that Nobel Prize decision.

Hughes: Yes.

Cohen: Let me start out by saying that I have never really understood how the selection was made for that prize, although I understand the overall process because I've nominated scientists for Nobel Prizes. And in fact one of my nominees, Barbara McClintock, was awarded the prize. I nominated Barbara for both that prize and for the Lasker Award. Without answering your question directly for a moment, but just getting to the issue of prizes more generally, I want to say that I think that most scientists don't do their work with the hope of receiving any prize. For me, what I wanted as a young scientist was to do research that would increase knowledge about a natural phenomenon, antibiotic resistance, and to learn things about resistance plasmids that ultimately might also be useful in a practical sense. On the other hand, when a scientist makes a discovery like the one that Herb Boyer and I made, colleagues start saying, "Well, you're going to win a Nobel Prize for this." I responded to such comments by saying, really quite truthfully that one never knows what motivates award committees.

I was a member of the Lasker jury for eight or nine years after I received the Lasker Award. Our assignment was to pick awardees of "Nobel caliber," and the Lasker Foundation booklets specifically listed Lasker Award recipients that later received the Nobel Prize. I had been on other selection committees previously, but as a member of the Lasker jury, I gained some insight about the process of selecting recipients of a really major award. Some jury members were directly knowledgeable about the actual contributions of nominees who were said to have made an important discovery and knew just which scientist did what. But during the years that I served on the jury, I found that other members of the jury were less directly knowledgeable, and their decisions were strongly influenced by who the nominator was and what the nominator had written. And I found that sometimes material presented in a nomination was not factually correct. A task of the jury was to determine which claims were true. Who really had contributed what? But it takes an enormous amount of time and diligence to get into the nitty gritty details of a discovery that is not exactly in your field of expertise, and it's a lot easier to accept as fact the statements of a nominator who is an expert in the field, especially if the nominator is a very distinguished scientist.

When colleagues said to me, well, you're going to win a Nobel Prize for this discovery, I took that with a "grain of salt." Nobel Prizes are few, and there are many important discoveries. Besides, my work had led to a serious controversy about possible biohazards, and it wasn't clear that it would be politically acceptable to award a Nobel Prize for DNA cloning. Nevertheless, I did feel that if a Nobel Prize was given in this area, the discovery that genes

could be transplanted to and propagated and cloned in a foreign host was the key discovery; and that discovery came from the work that my lab and Boyer's had done. Berg was also being talked about as a possible Nobel Prize recipient. But I felt that the joining of DNA ends had been done by others prior to Berg's work, and that once it was known that DNA ends—synthetic or natural—held together by base pairing of nucleotides could be ligated *in vitro*, there was not something special about being able to ligate the ends of DNA molecules that were taken from different species. DNA ends are DNA ends, regardless of the source. But DNAs from different sources have differences in overall sequence and nucleotide composition, and the key question had been whether the sequence of nucleotides in a DNA molecule taken from a different species would allow its propagation in a foreign host.

But, as I explained earlier, "recombinant DNA" had been equated throughout the biohazards controversy with simply the splicing together of different DNAs, rather than with replication of the resulting DNA in a living cell. The splicing of SV40 DNA to lambda *dv* DNA by Berg's lab was widely known, and Berg had been regarded as being wise and heroic for not trying to propagate the spliced DNA molecule. I thought that he might share in any Nobel Prize that might be given in this area, but on the other hand, I also felt that our discovery that novel DNA molecules constructed *in vitro* could be propagated and cloned didn't depend in any way on Berg's earlier work or use any information his lab had produced. If his work had not been done, there would not have been a difference in the development of the field, and it was hard for me to imagine that Berg would receive a prize for an experiment that he did not do. But anyway, I didn't think that a Nobel Prize would be given to anyone for work in this area, at least not for a great while. But in 1980, the year that the Lasker Award was shared by Berg, Boyer, Kaiser, and me, Paul did receive a Nobel Prize that cited his work on recombinant DNA, and he was named the sole recipient of the prize.

Hughes: It was the same year, yes.

Cohen: As I've said, Peter Lobban and his mentor Dale Kaiser had developed the enzymology that, according to the Jackson, Symons, and Berg paper, had allowed Berg's experiments to be carried out. In fact, the strategy for the dA-T joining approach was described by Peter in his 1969 proposal; Berg states that he independently thought of this strategy. The ligation of DNA ends *in vitro* using base pairing of complementary nucleotides had been shown even earlier. But if a prize was to be given for the use of the dA-T method for the joining of DNA molecules, the work of Lobban and Kaiser had been central to making this approach work, as Berg's own paper on SV40/lambda *dv* hybrids acknowledges. Although Berg had received most of the recognition for the dA-T joining method, I thought that the Lasker jury had really done their homework when they included Kaiser along with Berg as a recipient of the 1980 Lasker Award.

In any case, let's see if I can go back to your question. Despite the Lasker jury's decision, I didn't anticipate that a Nobel Prize would be awarded in 1980 for recombinant DNA. The biohazard controversy was just then in the process of cooling down. The Nobel Prize in [Physiology and] Medicine that year was given for an immunological accomplishment, and it never occurred to me that a prize for recombinant DNA would be considered and given by another Nobel committee—in chemistry. So far as I was concerned, the awarding of the 1980 Prize in Medicine meant that any prize for recombinant DNA would not be given that year.

I was surprised a few days later to receive a morning call at home from a reporter asking me what I thought about the fact that Paul Berg had just won the Nobel Prize. "Really?" I said, "The Nobel Prize in what?" Honestly, my first thought was that it was possibly the Peace Prize for Paul's role in encouraging scientific self-control in an important area of biomedical research. I asked, "What contributions were cited by the Nobel committee?" And I was told that the prize would be given in chemistry for Paul's "contributions to the biochemistry of nucleic

acids with special regard to recombinant DNA." Paul had made a number of earlier important contributions to nucleic acid chemistry, but I didn't understand what was meant by the vague statement "with special regard to recombinant DNA," and I didn't know what specific achievement was being referred to by that statement. I said, "I have no comments to make," and just left it at that. But I was very, very surprised. Later that morning, to get ahead in the story, is when I learned that the contribution being recognized was not DNA propagation or cloning, but rather simply the biochemical process of joining together DNA ends. But recombinant DNA technology was much more than end-to-end joining of DNA molecules; it was the propagation of DNA in a foreign host, and that was not done by Berg.

Obviously Stanford was abuzz with the news when I came into work that morning. The Biochemistry Department announced that there was going to be a noontime party to celebrate Paul's Nobel Prize. I was invited to attend, as were other colleagues here at Stanford. Around 11 in the morning, Spyros Andreopoulos, who was the media contact at Stanford Medical School came to talk to me about all of this and wanted to know what I thought. We ran into each other in the corridor just outside my lab. I said, "Well, Spyros, I never understood why Avery didn't win the Nobel Prize, and I guess I also don't understand what specific achievement Paul was given the Nobel Prize for." Spyros repeated the wording of the announcement by the Nobel committee, but as I've said, that was a little vague. Just then, Arthur Kornberg, whose laboratory was located just down the hall from mine, appeared. Arthur was clearly very pleased about the awarding of the prize to Berg, and indicated to Spyros and me that he had had an important role in the Nobel selection process. He said he was very proud of that, and he thought Paul deserved the prize for his great work. And then Spyros said: "You know, Arthur, the wording of the announcement is," and he read the exact wording of the award citation indicating that the prize was given particularly for Paul's contributions to recombinant DNA. That wording seemed to stun Arthur. He said, "But that's not what it's supposed to be for, that's not what his contribution was." And then Arthur at that point was a little embarrassed, and it was very unusual to ever see Arthur embarrassed about anything. Arthur and Spyros probably remember that conversation that the three of us had, but in any case, beyond relating these events to you, what does one say?

I think that award committees are human like everyone else, and they make decisions based on the information they have. The prize wasn't awarded in medicine by the Karolinska Institute, as usually happens with Nobel Prizes related to biological discoveries, and I have to assume that the Nobel committee that selected Paul for the chemistry prize—a different group of scientists working in the area of chemistry—believed that his contribution was the seminal one.

Reaction of Watson and Others to Nobel Prize Decision

Over the years, I've listened to many, many comments from colleagues who have stated their feelings that the decision to award the Nobel Prize to Paul for this work was not a correct decision, but how does one respond to such a comment? Jim Watson's opinion was stated publicly at a symposium that I attended in Paris a couple of years ago. That occasion was a symposium celebrating the fortieth anniversary of the discovery of the structure of the DNA helix by Watson and Crick, and the proceedings were published in the journal *Gene*. [Pause while Cohen retrieves a book from a shelf in his office.] Jim gave a talk on DNA sequencing in which he said, "The vast powers of new DNA sequencing procedures would remain underutilized, however, if it were not for the simultaneous opening of a fifth major phase of genetics." He outlined the major phases of genetics, and then said, "Here the crucial step was taken in 1973 by Stanley Cohen and Herb Boyer in their respective nearby labs at Stanford and the University of California." And then he describes the work we did and said, "Their clever

use of small bacterial plasmids as cloning vectors ushered in a recombination DNA revolution that has made possible not only the isolation of specific genes from cellular chromosomes but also provided the means to move them from one organism to another. Before recombinant DNA procedures came into play, there was no general way to study cellular DNA at the molecular level, and there were increasing predictions as the 1960s ended that the glory days of genetics were ending. But such worries vanished as the powers of recombinant DNA came into play in the late 1970s." He then said, "Here I should note the recombinant DNA age is now itself twenty years old. I'm not aware of any big meeting honoring it. But then there was no celebration for twenty years of the double helix. Part of the reason may be the powerful jealousy that naturally arises when unknown individuals through single discoveries suddenly acquire more fame than their contemporaries can ever, themselves, have reason to anticipate. I saw this reaction when the majority of Cambridge biochemists took pleasure in calling the double helix the 'W.C. structure,' so I am not surprised that the big Boyer and Cohen discovery has not yet received reverence commensurate with its enormous impact. Hopefully by its twenty fifth anniversary we can celebrate their success for what is the most momentous practical procedure yet devised to harness genetic knowledge for the good of humanity." So, you know, there are people out there who feel that perhaps Herb and I should have received that recognition, but that's now ancient history.

Hughes: Does the fact that a Nobel has been awarded in a field preempt the awarding of other Nobels in that field for a time?

Cohen: I don't really know enough about the history of Nobel Prize awards, Sally, to be able to answer that. But in an earlier celebration for the double helix at a meeting in England, Watson had raised the Nobel Prize matter even more specifically. [Pause to locate item]. In his statement, which was quoted in *Nature*, he asked, "Why haven't Boyer and Cohen won the Nobel Prize for their work?" In any case, my feeling has been that it's now a dead issue; even if another Nobel committee were to conclude there was a mistake, I don't think that there is a process of correcting mistakes. And you know, the Nobel Prize issue bothered me for a long while, to be honest about it, but it's something I've gotten over. The fact is, not winning the Nobel Prize hasn't affected my ability to carry out my research, which is really what a scientific career is all about in the most important sense. And, there has been a lot of other recognition that Herb and I have received.

Hughes: Have you talked to Dr. Boyer on this subject?

Cohen: Yes, I have.

Hughes: Does he hold similar views or?

Cohen: Well, I suppose you should ask him his views directly. I really don't want to discuss them here, but I can state my feeling is that he holds similar views.

Hughes: Well, do we have time to start a new subject?

Cohen: Probably not..

Hughes: All right.

Interview 11: April 18, 1995

ADDITIONAL DIUSCUSSION OF THE BIOHAZARD CONTROVERSY

<u>More About the Asilomar Meeting</u>

Hughes: Dr. Cohen, you found two items related to some of the discussions we've had about your speaking out at the end of the Asilomar meetings. I'd like to hear your comments.

Cohen: Okay. Well, one was an article by James Watson published in *Clinical Research* and reprinted in Watson's book with John Tooze, *The DNA Story*. In this article, Jim wrote that any regulations by necessity had to be capricious, and that in the absence of even the slightest reason for being afraid, we were very silly to be making all the fuss. And he also said something in his article that was quite similar to what I told you earlier about the mood of fearfulness that prevailed among Asilomar participants. His statement was, "Only Joshua Lederberg and Stanley Cohen were publicly on my side, and those who privately agreed with me thought I was risking my hide for appearing so indifferent to the general good." And this was James Watson saying this! I mentioned earlier that there was a kind of missionary zeal among those who were running the meeting, and it's remarkable that even someone of James Watson's stature was thought to be risking his hide by speaking out in opposition to the positions of the organizers. There was a steamroller that could not be stopped. And friends advised us not to try.

The other item I came across is the text of a discussion that followed the presentation of a paper by Dr. Charles Weiner of MIT at a meeting organized by COGENE and the Royal Society at Wye College in the U.K. a few years after Asilomar. The discussions were printed verbatim in the book *Recombinant DNA and Genetic Experimentation*, which Bill Whelan and Jane Morgan edited.[105] Weiner and several of the other speakers at the meeting expressed the view that the signing of the Berg letter was a responsible act and that the scientific community should not allow the subsequent events to discourage us and future scientists from showing such responsibility in the future.

I responded by saying that I felt it is important to distinguish between the perception of responsibility and actual responsibility. I agreed that that the seven scientists that met at MIT and the four of us who contributed to the later versions of the original Berg *et al*. letter believed we were acting responsibly, and our action was considered by the overall scientific community as being highly responsible. But I said that in retrospect I felt that our actions weren't really responsible at all. There had been no valid scientific basis for anticipating a hazard, and simply because we could not be certain that there wasn't a hazard, and without any epidemiological input, a public statement that incited widespread fear had been released. Because of the collective scientific credentials of the signers, the recommendations of the letter were given credence out of proportion to their intrinsic merit. The recommendations were published by what was designated as an official committee of the National Academy of Sciences [Committee on Recombinant DNA Molecules, Assembly of Life Sciences, National Research Council, National Academy of Sciences, Washington, D.C. 20418], and this gave further weight to what the letter stated was "serious concern." And the press conference held to announce publication of the letter increased public fearfulness. Yes, of course I agreed that future scientists should not be deterred from going public with ethical or scientific concerns, but I said that I hoped that they would do this more responsibly than we did.

Hughes: In the transcripts of the discussion after Weiner's paper, there's no indication of a response to your comments about the Berg letter. Was there privately or in any form?

Cohen: Well, I think there was. Let me just stop for a moment while I look again at the text of the

[105] Morgan, J, Whelan, WJ. *Recombinant DNA and genetic experimentation: Proceedings of a conference on recombinant DNA* jointly organised by the Committee on Genetic Experimentation (COGENE) and the Royal Society of London, held at Wye College, Kent, UK, *1-4 April 1979*, Oxford: Pergamon Press, 1979, p. 296.

discussion. [pause] At a later point in that discussion, Charles Weiner said, "I am very much interested to hear Stanley Cohen's view and I'm not trying to argue with him. I was giving an interpretation from views of several of the people who, like Stanley Cohen, had been involved in the signing of the Berg letter and I'd like to hear from others that are here. I think though, that several of the people who participated at that early stage have stated that based on the information they had, not to have acted would have been irresponsible, and secondly, they were talking about the possibility of hazards." Anyway, Weiner and I disagreed. But James Watson had spoken earlier at that meeting and had also stated his feelings that he and the other Berg *et al*. signers had acted foolishly in the absence of any scientific data.

Formation of the Stanford Biosafety Committee

Hughes: All right. Shall we move on to the Stanford Recombinant DNA Panel?

Cohen: Okay, sure.

Hughes: There was, I'm gathering, a predecessor to the actual panel. I found a letter, which is dated sometime in 1974, from a committee which calls itself the Biohazard Control Committee, which met early in 1974, and in it Berg suggested that a committee be formed to decide on the value and risk of recombinant DNA research. Do you remember anything about that?

Cohen: I do remember a little about that, but not a whole lot. Paul Berg was actively involved at a national level in discussions of biohazard issues, and I believe he felt that we at Stanford had a special responsibility to keep our own house "in order." And a committee to ensure this was formed. It was a small committee and I think that you're right: it was the predecessor of the university panel that reviewed the memoranda of understanding that scientists were later required to submit to show that proposed experiments would be done in accordance with the NIH guidelines.

Hughes: Is that a standard lag time? This committee that we're now referring to met in October of 1974, and yet the actual Recombinant DNA Committee Panel, I guess Stanford called it, was not set up until mid 1976.

Cohen: Local recombinant DNA committees weren't established until official guidelines were formulated and released by the RAC, and that took a while. In October 1974, only a relatively few labs were doing DNA cloning experiments. But as more scientists started using these methods, the NIH required a mechanism for formal review of experiments at the local level. And a point of discussion at the time was whether the cloning of DNAs that were considered to be "safe" according the RAC guidelines could be approved by local committees, or whether review of such experiments by the RAC would also be necessary. Some had wanted each and every DNA cloning experiment, no matter what kind of DNA was involved, to pass through a federal review process. But it was decided that safety measures could be implemented and monitored locally if the RAC had clearly specified the level of biological and physical containment required for experiments of that type. RAC review of specific experiments was required only when an exception from the guidelines was requested.

Hughes: I see.

Cohen: The local committees included non-scientists. However, opponents of the research felt that scientists shouldn't be monitoring themselves and should be excluded from the monitoring process. Chargaff said that having scientists involved in monitoring was akin to assigning the fox to take care of the chicken coop.

Workings of the Stanford Committee

Hughes: What was the nature of the review? Did the committee members actually read through the entire research proposal?

Cohen: Not the entire research proposal. A scientist proposing a particular experiment submitted a "Memorandum of Understanding" [MUA]. This described the goals of the experiment, the vectors to be used, the type of foreign DNA that was to be introduced, the species it had come from, any known functions of the gene the investigator was trying to isolate, the level of containment specified in the RAC guidelines, and a description of the procedures the investigator would use to ensure compliance. And several members of the committee were assigned the task of reviewing each submitted MUA. Obviously scientists on the committees weren't asked to review their own MUAs. The reviewers reported on their findings and made a recommendation, and after questions and discussion, a decision was made about whether the experiment could proceed as proposed. At Stanford, there were two principal reviewers for each MUA who reviewed the proposal in detail. If there was disagreement between the reviewers, usually action was deferred and another member of the panel was assigned to do a separate detailed review. Sometimes the committee decided that more information from the investigator was needed before a decision could be made.

As I've said, there were not just scientists on the panel, but there were faculty from other academic disciplines, a professor of law, a sociologist, etc., as well as non-faculty university staff. While these members of the committee didn't have expertise to evaluate the science described in MUAs, they were asked to be the "conscience" of the panel. Chargaff and some others believed that the scientists on such panels would have no conscience. The university also hired a staff person whose job was to implement safety procedures. This was analogous to what previously had been done to address radiation hazards. All of the members of the Stanford panel approached their responsibilities seriously, and the discussions were rigorous, probing, and honest. There was no "horse trading," among scientist members, which was an issue that opponents of the research were concerned about.

Hughes: Was the reviewers' only criterion in doing the review to determine if and then how the proposed research was to fit the guidelines?

Cohen: Yes, local committees weren't expected to modify the guidelines; that was being managed at the federal level by the RAC. The task of the local panel was to ensure that the scientific investigator and university were in compliance with the guidelines.

Hughes: Was it a biosafety or a biohazard committee?

Cohen: That's an interesting point. Would we refer to our group as a biohazard committee or a biosafety committee?

Hughes: You called it the Recombinant DNA Panel.

Cohen: But was its mission to control biohazards or ensure biosafety? I proposed use of the "biosafety" terminology.

Hughes: I remember seeing that.

Cohen: That was in keeping with the views I mentioned earlier. I felt that it wasn't appropriate to convey the notion that the committee had been appointed to deal with biohazards. In actuality, there was no evidence at all that the research would result in any hazard.

Hughes: Right.

Cohen: Perhaps it's a subtle difference, but words affect perceptions.

Hughes: I remember a similar debate in connection with the naming of the UCSF panel as well, hence "biosafety" rather than "biohazards." Am I to gather that these biosafety committees or their

counterparts, presumably being set up wherever recombinant research was being done in academia, are a direct fallout from the NIH guidelines, and that none of these committees preceded the formal release of the guideline?

Biosafety Approaches Prior to RAC Guidelines

Cohen: That's my recollection, Sally. Prior to release of the RAC guidelines, three steps had been taken towards biosafety in this area of research, and all involved voluntary compliance. One was the Berg *et al*. letter, but even before that letter, there was my own stipulation of conditions for receiving the pSC101 plasmid—which was then the only DNA cloning vector available. And, of course, additional recommendations had come from the Asilomar meeting. None of these steps involved a formal mechanism for enforcing compliance. After all, voluntary compliance with safety procedures had sufficed for work with known pathogens.

Hughes: Right. And was this a tradition in research dealing with pathogenic microorganisms or viruses, that when materials were exchanged there were stipulations on their usage?

Cohen: I think that is most likely the case. But my lab hasn't worked with actual pathogens, and I don't know the details of the practices followed.

Hughes: But when you deemed it necessary to put certain restrictions on the distribution of pSC101, that was something that you came to on your own; you weren't conforming to a tradition?

Cohen: I came to that decision on my own. I didn't consider pSC101 to be a disease-producing agent, but experiments that brought together antibiotic resistance genes in combinations not found on plasmids seemed clearly undesirable. And, as I've mentioned, I kept a record of scientists that I had sent the plasmid to, and asked that they not send the plasmid to others without my permission.

Hughes: Well, was it just so that you had a record? Wasn't it also so that you had control over the distribution?

Cohen: Well, it wasn't really "control." At that point, my experiments with the plasmid had been reported in the scientific literature, and I didn't deny the plasmid to anyone.

Hughes: As long, though, as they agreed to your…

Cohen: …stipulations. But if someone said that they agreed, I had no way to monitor what they did.

Hughes: Yes, right.

Cohen: I don't think that any scientist who requested the plasmid was playing games. Everyone realized that this was a sensitive and serious issue.

Hughes: I know I'm belaboring this point, but I think it is an issue: the fact that the distribution of pSC101 was centered in your laboratory did, by that very fact, exert a standard anyway. Because if the opposite had been the case, if you indeed had released pSC101 and then allowed further distribution, the different layers of distribution may or may not have had your stipulations on them. The rules that you had established for the distribution of pSC101 may or may not have been enforced by the other people.

Cohen: Well, I guess in that sense I have to agree with you, yes.

Initial Feelings About Establishing and Serving on Local Committee

Hughes: I'm interested in this representation on the committee with a deliberate purpose of having

different perspectives represented. Was that RAC-generated? Or, let me put it differently, was that something that each local committee came up with as a schema?

Cohen: I don't remember whether that was something that was handed down by RAC or something that was done locally by Stanford. Bob Rosenzweig was very involved in these discussions and I think was the person who appointed the committee, or at least had a role in choosing its members. Everyone wanted to have a broad-based committee that reflected various points of view.

Hughes: I believe in 1978, in an administrative memo—which I believe was responding to Stanford's requirement to define the composition and purpose of committees—there is a statement about what sorts of people should be on this committee. But I didn't find anything like that for the very early committee.

Cohen: I don't remember the basis for choosing the members. I was asked to serve on the committee. I had mixed feelings about taking on what I expected would be a lot of additional work, because during that period I was also involved in anti-legislation efforts both at the national and state level, and in my own research in my lab, and… There was just a lot going on in my life. But I felt I couldn't refuse to serve on the committee, given my role in the development of the methods and the extent of my involvement with the issues. So I agreed.

Hughes: You weren't particularly eager to serve, that may have been it, too. But it did strike me that you were an individual at the very core of all this, and [it was reasonable to ask you to serve] on the committee.

Cohen: I also didn't particularly want to get involved in enforcing regulations.

Hughes: According to a letter, you were opposed, at least as of 1974, to creating what you then called "biohazard committees"; that was your term in a letter that you wrote to Roy Clowes on October 31, 1974. Actually, it's to everybody on what I call the Plasmid Committee.

Cohen: Right.

Hughes: You had "biohazards committee" in quotation marks, as though even then you were questioning the propriety of the term.

Cohen: Well, as I've mentioned, I thought that these committees should be called "biosafety committees." I'm looking now at the letter. Do you have a specific question?

Hughes: Well, I just wanted to reiterate that, at that point anyway, you were opposed to setting up biosafety committees at all in any form.

Cohen: What I said in the letter reflects my opinion about that: "I do not believe that most institutions or commercial organizations could establish local committees with sufficient expertise to make appropriate evaluations of the biohazards of specific research proposals." And I also wrote: "Even at institutions where there is a sufficient amount of expertise to get an effective committee operating initially, I anticipate that within a few years, the authority to make judgments about highly specialized experiments will pass into the hands of individuals unqualified for the job. Furthermore, the lack of data will necessarily make such judgments by such individuals entirely arbitrary and based largely on emotions." Anyway, I was not in favor of having such committees.

Hughes: With the advantage of hindsight, do you think those worries of yours in 1974 actually materialized?

Cohen: No. I think this is one of the instances where I was wrong.

Hughes: I'm not trying to trip you up.

Cohen: No, that's fine, Sally. I think that's fair to say that I was overly concerned. The committees, at least the one at Stanford, worked well, and the establishment of local biosafety committees

proved to be a reasonable way of satisfying community concerns about the research. You know, at that point, I was very emotionally involved in the controversy and was weary of it. We all like to think that our views are reasonable, but that's not always the case; and this was an instance where I was wrong.

Hughes: I suspect that you were responding to the heightened emotion of that period

Cohen: Yes.

The Workings of the Stanford Committee

Hughes: And wondering if this was going to affect judgments about research proposals.

Cohen: Well, that was one of my concerns about the committees. I felt that irrational decisions would be made. I thought that many of the discussions about the research at the national and state levels were not rationally grounded, and was concerned that decisions at the local level would also be driven by conjecture. I had concerns about whether local committees could function objectively in the climate that existed at that time.

Hughes: What happened in institutions that were not doing much recombinant DNA?

Cohen: I don't know.

Hughes: Because then you would wonder if it were possible to find disinterested people. I mean by that, people who weren't directly involved in the proposed research but on the other hand had some scientific knowledge of that field.

Cohen: It's a good question, Sally, but I don't know the answer.

Hughes: What about this concept of the MUA? Is that something that predated the recombinant question?

Cohen: Well, for me, MUA was a new term. "Memorandum of Understanding" is, I guess, a quasi-legal term. I later learned that MUAs commonly were used to set down details of relationships between the government and other parties.

Hughes: So it's a government term.

Cohen: It's not a term that originated with the committee. I don't know what first prompted its use in this context.

Hughes: In the minutes of that very first meeting that predated the formal panel, there's reference to the fact that you and Dr. Clayton withdrew your requests for review of a proposal in which you were proposing to insert mice mitochondrial DNA into *E. coli*. Is that anything significant?

Cohen: I don't remember the circumstances.

Hughes: I just thought that it might have been reflective of the atmosphere.

Cohen: Well, no, we did the experiments and published the results in *Cell*, so we must have re-submitted the request for review. The studies were aimed at determining whether transcription can be initiated within eukaryotic DNA segments introduced into bacteria. The earlier cloning experiments that we had done with *Xenopus* ribosomal DNA showed that transcripts corresponding to the eukaryotic DNA sequences were being made in *E. coli*, but we didn't know whether these originated from promoters on the eukaryotic DNA or from promoters on the plasmid. Clayton and I collaborated to attach full-length mouse mitochondrial genomes to an *E. coli* plasmid at different locations and in different orientations. The rationale for these experiments was that if the transcription was being initiated within the eukaryotic DNA, the transcripts would be the same size and would originate from the same strand independently of the site of insertion of the mitochondrial DNA into the plasmid and the direction of insertion. If,

on the other hand, the transcripts originated in the plasmid, the strand transcribed would be affected by the site and orientation of attachment of the two DNAs. And we observed that the latter occurred.

Hughes: I came across a risk algorithm that Massey, what was his first name?

Cohen: William. Bill Massey.

Hughes: William Massey apparently submitted it to the panel for consideration. I don't expect you to remember that, although I have a copy of it right here. But that brings up the question of how much leeway there was between the guidelines and the actuality. I mean, if the guidelines are totally adequate, the local committee isn't going to need a risk algorithm; you're just going to be able to slot every proposal right into a category. But that obviously was not the case.

Cohen: I don't remember the risk algorithm.

Hughes: But do you remember discussions about trying to assess risk?

Cohen: Well, we all had our own views about risk, and how it should be assessed, but our personal views were irrelevant to the workings of the panel. Our responsibility was to ensure that the conditions proposed for the experiment conformed to the conditions required by the RAC. Whether any one of us felt that the recommended level of containment was appropriate or whether the RAC assessments were correct was not the point. Congruence of our personal views with RAC assessments varied from experiment to experiment. Sometimes the same panel member felt that the level of containment specified by the RAC was unnecessarily stringent for one experiment, but felt that the RAC had been insufficiently stringent in assessing the risk of another one.

Implementation of RAC Guidelines Locally

Hughes: You mean the inappropriateness was determined by the guidelines themselves?

Cohen: Yes. Our task was to ensure that the experimental conditions being proposed were what the guidelines specified.

Hughes: But was that always clear?

Cohen: Well, much of the time, but not always. Sometimes there was ambiguity about whether the investigator had made the correct determination about what containment conditions should be applied, and sometimes the conditions required for a particular experiment were not clear from the guidelines.

Hughes: I guess I'm asking how good the guidelines were in an operational way.

Cohen: Well, in some areas the guidelines were very good in anticipating various types of experiments that might be proposed, and in other areas they were not. Sometimes we had to make a judgment about just where a particular experiment fitted.

Hughes: And sometimes you indeed had to modify the guideline, right?

Cohen: No.

Hughes: Well, not you, but…

Cohen: No, we didn't and couldn't modify the guidelines. But we did have responsibility for interpreting and applying them locally.

Hughes: No, that was put wrong. I meant the guidelines, as you well know, do evolve over the years. Some of that evolution, I'm presuming, is because people out in the field were saying, there are certain aspects that are not clear and should be firmed up.

Cohen: I don't remember such a situation.

Hughes: What about…well I should ask you this: Wasn't there a gradual shift of responsibility from RAC to the local committees, the local committees assuming more importance?

Cohen: Well, I think, Sally, that perhaps you're mixing up two separate issues here. One is the responsibility for defining the experimental conditions that should be used, and that was RAC's.

Hughes: Right.

Cohen: The second was the responsibility for assuring that the conditions stipulated by the RAC were being applied appropriately by the scientist proposing a particular experiment; that responsibility was assigned to the local committee.

Hughes: I see.

Cohen: And so far as I remember, there was no shift in responsibilities with the passage of time.

Hughes: So the local committee was an enforcement committee.

Cohen: Well, it didn't formally have legal power to enforce, but in a practical sense that is what occurred.

Hughes: There is a letter dated October 1978 from President Saxon, who was president of the University of California at this time. He was writing to Donald Fredrickson, asking for a relaxation of the guidelines. He called them "costly, time consuming and complex to implement." Was this a move at the time?

Cohen: I don't remember seeing that letter or being involved in what was going on at UC.

Hughes: I thought maybe you had a role in that.

Cohen: No, I didn't. I was occupied with the legislation issue.

Hughes: That was a busy time. Have you said enough about industry and the guidelines? Last time, I believe it was, you said that industry supported the ASM's position.

Cohen: Yes. I think we've discussed that earlier.

Hughes: Do you have anything to say about the fact that industry was not covered by the guidelines?

Cohen: Proponents of legislation argued that there was a need for laws to cover industry because industry was not covered by the NIH guidelines. Technically, the guidelines covered just NIH-supported research. But I think that, in a practical sense, companies recognized that if any of their work violated the guidelines, the company would be at risk. Residents of the community might take legal action against the company for violating commonly accepted safety standards.

AFFILIATION WITH THE STANFORD DEPARTMENT OF GENETICS[106]

Sabbatical Leave in Norwich, U.K., July-December 1975

Hughes: Do you want to talk more now about the Department of Genetics?

Cohen: Okay, we can do that.

Hughes: When you came back from sabbatical, which was 1976, was it?

Cohen: The sabbatical was during the 1975-1976 academic year. I wanted to develop a cloning system for the antibiotic-producing bacterial genus *Streptomyces*, and arranged to take a period of

[106] Also see prior discussion of "Joining the Department of Genetics" on page 25.

sabbatical leave in David Hopwood's laboratory at the John Innes Institute in Norwich, England to learn about *Streptomyces* biology. David was the world's leading *Streptomyces* biologist, and still is. But I didn't want to be away from my lab for an extended period of time and thought that I could spend the second six months of the sabbatical year working at a bench in my own laboratory. At that time, I still reserved a lab bench that I hoped to use for experiments done with my own hands. In actuality, other activities at Stanford kept diverting me from benchtop experiments, and "Stan's bench" became a sort of lab joke, and the second six months ended up not being a "sabbatical" at all.

In mid 1975, my family and I traveled to Norwich. In Norwich, I worked at the lab bench every day doing experiments with *Streptomyces*, and I enjoyed that. But I was also in very close contact with my own lab back at Stanford. There was a teletype machine in the basement of the institute, and it was possible for me to dial up a phone number in London and be connected with a network of computers linked together by ARPA [Advanced Research Products Agency], a research arm of the U.S. Department of Defense. Josh Lederberg had been involved in establishing this network and there was also a teletype at Stanford that could connect to the network.

I used ARPA to send and receive messages and data from Stanford, and so I communicated with my lab on a daily basis.

I enjoyed working in David's lab but ended the sabbatical leave a little earlier than I had intended, in mid-December. My wife's father became ill late in the fall of 1975 and died the week before Christmas. My wife, Joan, and I traveled back to the U.S. several times during the last few months of 1975. We all moved back to California in mid-December.

Decision to Reduce Clinical Involvement

After my return to Stanford for the second half of my sabbatical year, I started thinking increasingly about my future career plans. It had become clear that teaching clinical medicine, which I had done all along from the time I arrived at Stanford in 1968, was an increasingly difficult task. Although some of my research involved computer-based projects related to clinical pharmacology, most of my research was lab-based. Each year I had made teaching rounds on patients admitted to the medical wards and also made consultation rounds as a clinical pharmacologist. And I had administrative responsibilities as head of the Division of Clinical Pharmacology.

In the months prior to my sabbatical leave, the pace of my laboratory research and my involvement in the biohazard controversy were intense, and I did little reading of literature related to clinical medicine. My reading was in molecular biology, microbiology, genetics, and biochemistry, but I also had medical teaching responsibilities. In order to be able to teach students effectively during clinical rounds, I asked the medical housestaff to phone the evening before and let me know which patients would be presented for discussion the following day. That gave me the opportunity to refresh my memory about the diseases by reading a couple of recent clinical papers, and to teach effectively the next morning. I had a solid background in clinical medicine, and this strategy worked out reasonably well. My training in basic science also helped in my analysis of clinical cases, and I think that I contributed meaningfully to the clinical teaching program.

But it became increasingly difficult to work in what had become a very competitive area of basic research and continue to make clinical rounds regularly on patients. After I ended my year of sabbatical leave, I talked with Dan Federman, who was Chairman of Medicine, and asked whether I could be excused for a couple of years from my general attending rounds in Medicine

so that I could spend more time with my research. I knew that some of the other younger faculty in the department wanted to be more involved clinically and that getting coverage for the department's general teaching responsibilities wouldn't be a problem. I proposed that I continue with my teaching activities in Clinical Pharmacology as before. Dan said that he expected all of the Department of Medicine faculty to make general attending and teaching rounds and insisted that I continue to do this if I wanted to be based in the Medicine department.

Joshua Lederberg, who had communicated some of my manuscripts to the *PNAS*, and I talked regularly about science, and to some extent about the politics of the recombinant DNA controversy, and I told him of my discussion with Dan. Josh offered me the opportunity of a joint appointment in Genetics. At that point, Josh had begun to consider the presidency of Rockefeller University, although I didn't know about it at the time. I learned afterwards that he had me in mind as a possible successor to him as chairman of Genetics.

Chairmanship of the Department of Genetics

In 1978, my primary departmental affiliation was switched to Genetics, and when Josh moved to Rockefeller, I was asked to become the Genetics chair. That wasn't a job that I sought or was eager to have, but there were not many resources that the School of Medicine could use to attract a Genetics chairperson from the outside. And there were some faculty in the Biochemistry Department who argued that so much genetic research was being done in the Biochemistry Department, and other departments in the School of Medicine, that continuing to have a separate Department of Genetics wasn't necessary. I certainly did not agree with the view that the Department of Genetics was redundant and should be disbanded. Genetics is a distinct and exciting scientific discipline, not just a provider of tools for biochemical experiments. Besides, the continued existence of a Department of Genetics at Stanford was important to me personally. I agreed to assume the chairmanship.

Hughes: What do you think of when you say resources?

Cohen: Well, ordinarily when a new chairman comes on the scene, it's customary for a school, especially when attracting someone from the outside, to make commitments for new space and to provide other resources to make the job appealing enough for someone to move to a new institution. That's customary in academia. But there were other priorities at the School of Medicine at the time of Josh's departure, and there were problems in the Department of Medicine, which was the largest and single most important department in the school. Dan Federman had decided to leave Stanford and an acting chair had been appointed in Medicine; and, there were chairmanship positions that were unfilled in other clinical departments. There were major institutional needs, and I knew that the school wasn't likely to be able to provide a package appealing enough to attract the right kind of outside person as chair of the Department of Genetics. And so I agreed to serve as chair for a period of five years. I viewed this as temporary, and felt that during these five years the other needs of the School would be addressed, and then we could recruit a genetics chair from the outside. But I ended up doing it for six, seven, and finally…

Hughes: Largely for that reason? Because the resources…

Cohen: Well, like most other people, deans and other university officials pay most attention to the shoe that pinches. When the five years had elapsed, Genetics was moving along on a more or less even keel, and recruiting an outside chair still wasn't a priority for the school administration. Important clinical department chairs remained empty.

Hughes: Do you think Lederberg had arranged your appointment with the idea that you would succeed

him as…

Cohen: It was in his mind. He later told me that. But I don't think his offer was made with as much Machiavellian motivation as your question suggests. In any case, after being Genetics chair for a couple of years longer than the five year period that I had originally agreed to serve, I wrote to the Dean, David Korn, saying that my chairmanship had extended longer than I had intended, and I planned to step down as chair at the end of that academic year. The school arranged for a formal review of the department and sought external advice about the future of genetics as a discipline. The report concluded that a department of genetics is vital to Stanford and that a major commitment of resources was necessary to attract a first-rate geneticist from the outside.

But no search committee to find a successor was appointed and I reminded David informally about my plans on a couple of other occasions. About two or three months before the academic year ended, I walked into David's office and said, "You know, I'm stepping down as chair in a few months. How are you planning to proceed?" David, who was a friend I had known since our time at the NIH in the mid 1960s, said, "You're not serious are you?" I said, "Yes, David I am." And the bottom line was that I did step down, and my colleague Luca Cavalli, who is a very distinguished population geneticist, agreed to serve as an interim chair during a period when the school actively searched for an outside person. To help induce Luca to do this, I agreed to continue to run the student training program and to continue with some other departmental administrative responsibilities during Luca's chairmanship.

I'm convinced that if I hadn't been firm about ending my period as chairman of Genetics, the school would not have made the commitments necessary to build the department further.

Succession of Departmental Chairmanship

A search committee was appointed to seek a successor to Luca as chair. In a practical sense, I think that the decision of the School to commit major financial resources to the further development of genetics here was helped by the fact that substantial income had begun to flow to the school, and also to the department, from my recombinant DNA patents during Luca's chairmanship. Some modest income was also being received from the licensing of an earlier invention made in the department. Although much of the School of Medicine's share of patent income was being used for the needs of other departments, I was able to make the argument to the Dean that more of the school's share should be used toward Genetics Department development. Those funds enabled the renovations necessary to attract David Botstein to Stanford as chair. During the last 9 or 10 months of Luca's chairmanship, he was on sabbatical leave and I was asked to serve as acting chair until Botstein arrived. I had done the job previously and there wasn't anyone else who could take this on, so I agreed.

Hughes: Were there any particular things that you went into the chairmanship hoping to achieve?

Cohen: Well, yes. Prior to my chairmanship, the Department of Genetics had been very much Josh's department. Josh is an intellectual and scientific giant who founded the department and recruited all of its faculty. His imprint was everywhere. Even the department library was devoted largely to housing Josh's papers, notes, and memoirs. Department faculty met infrequently. Josh made the departmental decisions, and they almost always were the correct ones.

When I succeeded Josh as chair, I wanted to have the faculty more collectively responsible for the functioning of the department. I discussed this with Josh, and he advised me not to be too democratic. I respected that advice, but it wasn't consistent with my style. In retrospect, Josh was right. I think that I would have been a better chair if I had taken it. When resources are substantial, it's okay to make collective decisions. But, during times of limited resources, which

was the case at that time, I found myself in the position of mediating issues between a School that wanted to give less and a faculty that wanted more. I didn't enjoy that role.

Hughes: I imagine too that it's not an easy thing to follow an extremely strong figure—one who has really built the department to his image.

Cohen: Well, I don't think of it quite that way. Josh certainly was an internationally known scientist and had recruited outstanding faculty, especially Luca Cavalli, Len Herzenberg, and Eric Shooter. But mainly, we were a collection of individuals who were well-regarded scientists rather than a cohesive department. I think Josh recognized that the department wasn't a cohesive group, but he was comfortable with that.

Hughes: Were you successful in some ways in bringing…

Cohen: The department had established a graduate training program under Josh; I expanded this by applying for a second NIH training grant and by getting incremental funds to increase the number of graduate students supported by our original training program grant. We began regular departmental seminars and journal clubs, and I started the practice of having annual department retreats where the faculty and the students and postdocs working in their labs, presented their latest research findings. We established a formal process that provided student rotations among different faculty labs during the first year of their training; previously, graduate students were accepted directly into faculty labs. The annual retreats held near the start of the academic year helped newly admitted graduate students identify faculty that they wanted to work with. And we recruited some additional faculty. I think that my chairmanship had its good points and bad ones. I certainly feel that during this period Genetics became more of a department, and this enabled us to proceed to the next stage. But, if you're asking whether I think that I accomplished everything that I would have liked to have done, the answer is no; but, that's true of many things in life.

Effect of Non-Scientific Activities on Research Momentum

Hughes: Once again you are somewhat reluctantly taken into an enterprise when—am I right in thinking?—that what you really wanted to do was be at the bench? I mean, you go from the recombinant DNA controversy—I know there's some overlap—to being department chair. What effect are these activities having on the momentum of your research?

Cohen: Well, that's a good question. I think that for the first few years of my chairmanship, through 1980 or 1981, my research continued to go as well as ever. But after that time, there were several years where my projects moved ahead at a slower pace, even though the recombinant DNA controversy had wound down. I think there were several reasons for that and I think that the principal one was that I was spread too thin scientifically.

Up until 1980, my lab had been able to compete successfully in multiple areas at the same time and be competitive in all of these areas. But then I saw that other laboratories increasingly were scooping us. The first time that happened was in interferon gene cloning experiments. I had become interested in interferon because of its role in host defense against virus infection and I assigned two postdoctoral fellows to work part-time on cloning the gene that encodes interferon activity. Charles Weissmann, working with a team of seven or eight scientists at the University of Zurich also set out to clone the gene and express it in *E. coli*, and Charles won that race handily in 1980.

In addition to being spread too thin, my lab wasn't organized to pursue projects in the most efficient way. The lab is run like a "mom and pop shop," as I think I've mentioned. Each person in the lab is a "generalist" who works on multiple parts of a project. It's more efficient

when different members of a team carry out specialized tasks, but that isn't optimal for training young scientists.

I continued to publish, obtain NIH grant support, and receive applications from excellent young scientists who wanted to come to my lab as students or postdocs. But there was a period when research projects proceeded more slowly than I would have liked.

Hughes: I see.

Cohen: Of course, I saw that at the time, but it's easier to talk about in retrospect.

Hughes: Yes, twelve years have gone by. In the case of the interferon, for example, when you found that for whatever reasons you didn't have the resources that your competitors had, was that the time to cut back?

Cohen: To fold up that particular tent, yes. It was clear that my lab had nothing special to offer either conceptually or experimentally. It seemed more reasonable to focus on other research where I might be able to make a novel contribution.

INTERACTIONS BETWEEN STANFORD AND INDUSTRY

Establishing the Office of Technology Licensing (OTL)

Hughes: Well, I wanted to now begin the lead up to the patent discussion by discussing some of the ties that the School of Medicine develops or has already developed with industry. I'm interested in establishing the general context of what is to go on. Is it relevant that Stanford is proximate to Silicon Valley, with a history of interactions with industry?

Cohen: Stanford was one of the few universities, maybe the only one, that by the early 1970's had already established an Office of Technology Licensing (OTL). My understanding is that the Silicon Valley connection was important in the decision of the university to establish the technology licensing program. Silicon Valley developed just south of Stanford, largely as a result of work done at Stanford in William Shockley's lab, and later by some of his students, who founded early companies in the electronics industry. I have a friend who has worked for many years in that industry and once showed me a chart that displays the genealogy of Silicon Valley. Many of the companies were founded by Shockley's students, and technology developed at the university was licensed to these companies. Certainly, there was not a biotechnology industry at that time. And it was because Stanford had an Office of Technology Licensing that the Cohen-Boyer patents exist. If OTL hadn't existed, I certainly wouldn't have thought of applying for a patent. And as I've mentioned, it was only because of a telephone call I received from Niels Reimers that a patent application was pursued.

Interactions Between Biologists and Industry

Hughes: Well, one of the manifestations of these ties occurs in 1981 when the Department of Medicine, I believe through Ken Melmon, established the Institute of Biological and Clinical Investigation. My interpretation of the material that I've read—which was simply an article in *Science* magazine, so I don't have any behind-the-scenes knowledge at all—was that the institute was to serve as a consulting firm and granting agency. Do you know about that and would you like to talk about it?

Cohen: Well, I know a little bit about it, but it's not something that I had a significant role in, although

I served on one of the committees that made decisions about the distribution of institute funds, which I think came largely from Syntex. Syntex provided the funds to Stanford in return for scientific advice provided by Ken and some other Stanford faculty in matters related to Syntex's commercial interests. The funds that the institute received were used to help younger faculty establish research programs and were an important source of support for junior faculty. I thought that the program that Ken established was a good idea and agreed to help in the selection of junior faculty to receive funds. I wasn't one of the Stanford faculty providing advice to Syntex.

Hughes: Was it controversial in any way?

Cohen: Not to my knowledge.

Hughes: By 1981, there was a lot of interaction between university and industry in the biological sciences….

Cohen: Sure, but I think that most of it was unrelated to my patent.

Hughes: Well, the institute is an illustration, though, of the direct ties between industry and the university, which certainly had been less common in previous history.

Cohen: Well, previously, there were pretty extensive ties between the university and industry in engineering and electronics, and also in the chemical sciences.

Hughes: Well, true. I'm thinking of the biological sciences in general.

Cohen: In the biological sciences, certainly, there had not been extensive ties. Don Kennedy who was president of Stanford during that period encouraged only arm's length relationships between faculty and industry. Don was concerned about the potential for corruption of academic standards by the involvement of faculty with industry. I think that message was heard clearly throughout the university.

Considering Conflict of Interest Issues at Stanford

Cohen: As we've discussed, I was eager to avoid any appearance of conflict of interest while I was lobbying against federal and state legislation to regulate DNA cloning research, and kept my consulting activities separate from my lab research.

Hughes: Sort of a reification of some of these concerns, I believe, is the conference that occurs in 1982 at Pajaro Dunes. Do you remember that? The presidents of five universities that were heavily involved in recombinant research—Caltech, MIT, Stanford, UC, and I'm missing one [Harvard], which I can easily find—and also representatives from industry met for a three-day conference.

Cohen: I know of the conference, but wasn't there.

Hughes: I didn't see any sign that you were, but I was wondering…

Cohen: I just don't know a whole lot about it.

Hughes: And Engenics is another thing that comes up at about this time too.

Cohen: Yes. Right.

Hughes: Do you want to talk about that?

Cohen: Yes, I can say something about it.

I had been collaborating scientifically with Alan Michaels and Channing Robertson in the Engineering Department at Stanford. We published several papers together on the use of hollow fiber membranes to grow mammalian cells in large quantities for the potential isolation of

products made using recombinant DNA methods. Students and postdocs from all three labs worked on the project and were on the thesis committees of some of the Engineering Ph.D. students. Channing concluded that the development of improved methods for isolating and processing gene products made in microbes offered a commercial opportunity and started a company to do that. I think that Arthur Kornberg and maybe Charles Yanofsky joined him in that venture, which he called "Engenics." I was not involved in Engenics at all, but knew about it because the company licensed technology that Alan, Channing, and I had developed at Stanford. Channing obtained financial support for the company from outside investors, and I think that Stanford also invested money or provided start-up space. But for some reason that company didn't get off the ground.

Hughes: Well, it seemed to me another form of relationship between industry and the university. The way I understand it, Stanford and UC and six corporations set up a not-for-profit research center.

Cohen: I think that's correct, but I wasn't involved and don't remember much about it.

Hughes: I'm trying to show that this is a period in history when universities are trying a lot of different ways of making connections to industry and this was one.

Cohen: That's right. And one way was for universities to hold stock in companies that they granted licenses to.

Hughes: This was, at least on this scale, a relatively new thing for biology?

Cohen: I think that's true.

Hughes: Well, there were some repercussions from what I'm gathering.

Cohen: With Engenics?

Hughes: No, in general for this move at Stanford. The Stanford Graduate Association becomes involved, for one thing, not always negatively, although there is some of that. I've seen a poster, for example, which unfortunately I don't have with me, advertising for an ombudsperson who would consult with Stanford graduate students who felt that they were being unfairly used for industry purposes. It's more or less what you were describing before. I have no indication of how widespread that sentiment was, whether anybody took up on it at all. But the very fact that there was a mechanism set up I think is indicative. Anyway, in the spring of 1982, the Stanford Graduate Association sponsored a symposium on university entrepreneurship and graduate education. Do you remember any of that going on?

Cohen: Very vaguely.

Hughes: It went on for quite a time, nine days or something, and they brought in speakers from a variety of different institutions. If you can just bear with me, one more question. When do you remember Stanford requiring its faculty to report on outside relationships?

Stanford's Consulting Policies

Cohen: That's an interesting question. As I've mentioned, Don Kennedy was concerned about issues and perceptions of conflict of interest and wanted to ensure that private, for-profit activities of faculty were not carried out in their university labs. In the mid 1980s, a faculty committee was formed—I think it was a medical school committee—that was assigned responsibility for reviewing conflict-of-interest issues and making recommendations that would help the university and its faculty avoid conflicts. At that point, faculty consulting for biotechnology companies had become commonplace. The committee included clinicians as well as Paul Berg and me, and I think a couple of other faculty from basic science departments.

The Stanford consulting policy is very liberal towards faculty; the university allows consulting for up to one day a week. My own consulting activities didn't even approach this limit, and I think that relatively few basic science faculty spent 13 days per quarter in outside consulting. But the supplemental income received by faculty was substantial for even a few days of consulting, relative to faculty salaries in the basic sciences. Clinicians with full-time faculty appointments who spent any of their time practicing medicine outside of the university medical system were required to return their private consulting income to the university, and they wanted consulting income received by basic scientists to be similarly returned. The basic scientists on the committee thought that doing this would be appropriate if faculty salaries were comparable in different medical school departments. But they are not; a professor of radiology, for example, was paid a Stanford salary that was two to three times the salary of a professor of biochemistry, microbiology, or genetics.

Hughes: Right.

Cohen: The committee's analysis confirmed that there was a significant marketplace for expertise in the basic biological sciences. The suggestion that any outside consulting income received by basic scientists be confiscated by the university was dropped, but out of our committee discussions came recommendations for addressing actual or perceived conflicts of interest, including the requirement that faculty report the extent and nature of private consulting activities to their departmental chairperson each year. Chairpersons reported any of their own consultation activities directly to the Dean.

I don't know whether Stanford was the first university to address conflict of interest issues stemming from outside consulting activities and other commercial involvement of faculty, but practices to prevent or mitigate conflict have now become standard. Of course the NIH and other federal agencies are now also heavily involved with conflict of interest issues.

Hughes: Well, shall we stop there?

Cohen: Yes, let's do that.

Interview 12: May 5, 1995

THE COHEN-BOYER PATENTS[107]

The *New York Times* Article

Hughes: Dr. Cohen, can you tell me how the issue of patenting was first presented to you?

Cohen: Well, the initiating event was an article by Victor McElheny in *The New York Times* about the work that Herb and I had published. McElheny was a science writer at the *Times* and a friend of David Baltimore, and was alerted to the research by David. His article appeared in May of 1974.[108] He wrote mostly about some of the potential practical applications of the gene cloning methods we had developed.

[107] Cohen Boyer patent. Patent # 4,237,224. December 2, 1980.

[108] Victor K. McElheny. "Animal gene shifted to bacteria; aid seen to medicine and farm." *New York Times*, May 20, 1974, p.1.

Cohen's Initial Reluctance and Being Persuaded to Proceed with Patent

Cohen: McElheny's article was seen by Niels Reimers, who was head of OTL at Stanford.[109] Niels telephoned me and said that he wanted to discuss patenting of my invention. I knew nothing about patenting, and my first reaction was to question whether one could patent basic research findings. I agreed that the discovery that DNA fragments can be propagated in bacteria by linking them to plasmids was likely to have practical value, but no commercial application had yet been shown. And the second point I raised was that our discoveries were dependent partly on the earlier discovery of DNA ligase and on years of basic research with plasmids, so I wondered whether the technology could be termed an "invention."

Niels said that Stanford viewed the licensing of technology developed at the university as a way to provide funds for scientific research, and that commercial users receive a "free ride" if there is no patent. He persuaded me that no invention is made in a vacuum and that every invention is of course dependent on knowledge and information that precedes it. The issue that determines an invention, he explained, was whether the advance made by the invention was obvious to one "skilled in the art." I told him that the findings had not been obvious, and that there were multiple reasons for thinking that the procedure would not work. Based on the information I had provided, Niels said he wanted to apply for a patent together with the University of California. I told him that I would think about his proposal and discuss it with Herb. I did both, and eventually Herb and I agreed to proceed.

Bert Rowland, who was an attorney that Stanford hired to prepare the patent application, met with me over a period of a few months to obtain information for the application. The application that Bert prepared covered both the process of making recombinant DNA molecules and products that would come from that process.

Determining the Inventors

Patent applications list the inventors, and Stanford and the University of California had to determine inventorship for this technology. There were co-authors on the scientific papers reporting the DNA cloning research, and some of the co-authors felt that they also were "inventors."[110] Bert indicated that incorrect exclusion or inclusion of inventors can invalidate a patent, and that authorship and inventorship are separate issues. He said that inventorship is a legal determination, and he concluded that the co-authors were not inventors. I was told that the decision was easy for Bert to make because the invention of DNA cloning had occurred during discussions that Herb Boyer and I had in Hawaii, and Annie Chang and Bob Helling, who were the co-authors of the paper describing the invention, were not present. There were witnesses to the Hawaii discussion, including Stanley Falkow, who provided confirmation. So Bert determined that Herb and I were the sole inventors and prepared a patent application indicating this. Herb and I didn't have anything to do with that decision.

When the other individual co-authors were asked by Stanford to sign disclaimers of inventorship, some were not willing to do this. Bob Helling challenged the inventorship

[109] See the oral history with Reimers in the Bancroft Library biotechnology series at the University of California, Berkeley.

[110] Robert Helling and John Morrow were co-authors claiming inventorship.

determination, and then John Morrow did as well. I didn't want to be involved in the dispute with colleagues over inventorship and wasn't.

Hughes: Did you ever discuss it with them?

Cohen: No. My role in the patent application was to provide scientific information for Bert to prepare the application and then to review and make corrections, and I stayed totally out of any discussions about inventorship. The patent rights had been assigned to Stanford, which then negotiated an agreement with the University of California to administer the patent on behalf of both institutions and to share the patent income.

Cohen Provides Scientific Information for the Patent Application

Cohen: Having worked later with various attorneys on patent applications and learning something about the patenting process, I can say that the job done by Bert Rowland on the Cohen-Boyer patent application was truly remarkable. This was an important patent in an area where there had been no real precedent, and Bert had to prepare the application with assistance from a university professor who had little or no understanding of patent law at the time. I wanted only to give him the information he needed, get the application out of the way, and go back to my research.

Hughes: Was Boyer participating?

Cohen: He participated to some extent in providing information, but most of the information came from me. Herb was much less involved.

Hughes: Do you think that was somewhat because of geography?

Cohen: I don't know.

Boyer's and Cohen's Relationships with Genentech and Cetus, Respectively

Hughes: Did Genentech have an influence?

Cohen: Oh, this was before Genentech.[111]

Hughes: Yes, these early discussions were, but Genentech becomes an issue as the patent process winds on.

Cohen: Yes. As you know, three patent applications eventually were filed, and it was the third one that was issued as a patent in 1980. The first discussions about patenting occurred as early as 1974. So pursuit of a patent by Stanford was a six-year process and during that period, Genentech was founded by Boyer and Bob Swanson. And I became associated with Cetus.

Cohen: As a consultant.

Hughes: That's right. These corporate connections became an issue, particularly in the period when the university was negotiating with both companies, when it still was a question of an exclusive license.

Cohen: Yes. You have a letter you said you wanted to show me.

Hughes: Yes. [Pause while Cohen reads letter.]

Cohen: In this letter to Niels Reimers dated June 14, 1976,[112] I wrote that I didn't want to be

[111] Boyer and Robert Swanson founded Genentech in April 1976.

involved in Stanford's business discussions with potential licensees. I knew that the university was having these discussions, but wasn't a participant and didn't know the status of them.

The Miles Symposium, June 8-10, 1976

But about the time of this letter to Reimers, I attended a scientific meeting, the Miles Symposium in Boston. At that symposium, the question of the patent was raised during a discussion period. A participant said that she had heard a rumor that someone was patenting DNA cloning methods, and she was concerned about this. David Baltimore, who was chairing the session said, "Now that you mention it, I know something about it. I think it's a dead issue, but if Stanley Cohen is here he might like to comment."

Well, I was standing in the back of the auditorium, and it was clear that I had to say something. I explained the status of the patent application and said concerns that a Stanford patent would restrict use of the methods by scientists from other universities were not consistent with my understanding of Stanford's aims.

Hughes: Were comments made to that effect at the Miles Symposium?

Cohen: Yes, I think there was the notion that Stanford was trying to control DNA cloning research by applying for a patent. I explained that Stanford's OTL had indicated that if a patent is issued, royalties would be paid by only commercial users and that the aim of a patent was to provide funds for the support of scientific research at the university. I also said that Stanford planned to require any industrial licensees to agree to subscribe to the NIH guidelines, and that some university officials saw this as helping to ensure the biosafety of research in this area.

Hughes: In June of 1976 you wrote to Reimers saying that you wished to reverse your original request to remain uninformed about the university's patenting activities because of your connection with Cetus.[113] Do you remember what your thinking was there?

Cohen: [Reading letter.] Well, yes. It's not that I wished to reverse my earlier request because of my connection with Cetus; the point made in the letter was that my original request to remain uninformed was made because I had become an advisor to Cetus, and I was eager to avoid any appearance of conflict of interest. However, by June 1976, I came to realize that being informed was more important, and in this memo to Niels I indicated the reasons why. I was being asked questions about the patent; it didn't make sense for me to remain uninformed about it. In fact this memo was written after I returned here from the Miles Symposium.

Hughes: I think it was right after it.[114]

Cohen: It became clear to me that whatever position the university took, it was not realistic for me to simply say, "Well, I have nothing to do with it, " which is what Bob Rosenzweig had suggested my position should be. I would be affected by fallout from the university's decisions about the patent, and I felt that I needed to know what was going on. That is what I stated in this memo to Niels.

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[112] OTL, S74-43, correspondence 1974-1976.

[113] Cohen to Reimers, June 14, 1976 (OTL, S74-43, correspondence 1974-1979).

[114] The Miles symposium occurred in early June 1975.

The Issue of Exclusive Licensing

The discussion at the Miles Symposium made me realize that I couldn't distance myself from Stanford's actions in licensing the patent, and that it was foolish to try to be uninvolved. I returned from the meeting and learned—I've forgotten how but probably from Niels Reimers—that Stanford was considering giving an exclusive license to one of the companies OTL was talking with. I felt that an exclusive license was a bad idea for several reasons. It would encourage the view that the university was being restrictive. I also felt that this was such broadly useful technology that an exclusive license would encourage violation of the patent because non-licensed companies would try to use the published technology anyway. I felt that this was a broadly useful invention, and it should be licensed broadly.

Hughes: Had Stanford usually given exclusive licenses?

Cohen: Yes.

Hughes: Or was it naiveté about the scientific and financial potential of this patent?

Cohen: Well, I think it was both. The reason that exclusive licenses were given, according to Niels, was that companies must invest substantial amounts in the further development of licensed technology, and they weren't likely to do that unless they had exclusivity. But the university hadn't previously licensed an invention of such broad applicability and this technology was different from what OTL was accustomed to.

I certainly wasn't knowledgeable about how to best proceed to grant licenses, but I expected that different companies would want to use DNA cloning methods for developing different products. It was reasonable to grant non-exclusive licenses to multiple companies or maybe a series of exclusive licenses for particular application, but not an exclusive license for the whole technology.

Hughes: Well, there was evidence for OTL fairly early on that indeed this was a significant patent. In 1975, an individual associated with the Stanford School of Business was given an assignment to research the potential for this patent. There are several glowing memos in the documents at OTL mentioning not only the scientific significance, but also the potential profit involved.[115] It was not just another patent.

Cohen: That's true. But OTL nevertheless initially wanted to license it exclusively. After the Miles Symposium episode, Niels and I had many discussions about this. Eventually, Stanford decided to grant multiple licenses on a nonexclusive basis.

Hughes: Who was responsible for changing the approach to a nonexclusive license?

Cohen: Well, I think that ultimately it was Niels' decision to do that. But I also think that the discussions I had with him about this influenced his thinking.

Hughes: Well, there's evidence in the files that there was pressure from Genentech, mainly through Swanson who apparently was in touch with OTL, urging an exclusive license.

Cohen: Well, you know that Stanford has often granted exclusive licenses to companies that inventors have founded. The university's view is that such companies are often in a particularly good position to develop the technology further. Swanson's request for an exclusive license was not unreasonable, seeing as Boyer had been a founder of Genentech and he was an inventor.

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[115] For example, see: Ken Imatani to Niels Reimers, August 13, 1975. (OTL, S74-43, correspondence 1974-1979); Niels Reimers to Rodney Adams, April 8, 1976. (OTL, S74-43, correspondence 1974-1979.)

Hughes: An exclusive license was pro forma at that time?

Cohen: I'm not sure that I could say it was pro forma, but it was what was usually done. In fact, I think that somewhere it was stated in writing that OTL "looks favorably" on granting licenses to companies having an affiliation with the inventor.

Hughes: Is that just loyalty to one's own, or is there more to it than that?

Cohen: Well, I think there's more to it than loyalty. It probably indicates a notion that companies associated with the inventor have a particular advantage in being able to exploit the invention to its fullest capacity.

By the way, I'm looking again at this memo to Niels in June of '76.[116]

"Our recent discussion in which you indicated that Stanford has been considering an exclusive short-term licensing agreement with one particular company—and that was Genentech—provides an example of the basis for my concern. The question of exclusivity is perhaps the most sensitive issue associated with this patent so far as the scientific community is concerned. If Stanford were to proceed with an exclusive agreement, I believe that both the University's image and my personal image as a scientist would be affected. Until our recent telephone discussion, I had no information about these plans which have a potential for being detrimental to me."

The telephone call referred to in the memo is the one I mentioned to you a few minutes ago. It was made soon after my return from the Miles Symposium. And when I learned from Niels that the university was considering an exclusive license, it increased my concern.

Office of Technology Licensing, Patenting Strategy

Cohen: Stanford also pushed hard to get the patent issued as soon as possible. Although this seemed to OTL to be a reasonable goal at the time, I suspect that subsequently OTL may have had regrets, because the patent will expire in just a few years from now, in 1997. The products coming to market as a result of this technology are currently increasing rapidly in number, and if the patent had a life span of five or so more years, Stanford would likely have had a manifold increase in income.

Hughes: Were you in on the decision to split the patent application into separate processes and product applications?

Cohen: No, that was entirely a legal decision.

But another issue that came up during Stanford's pursuit of intellectual property rights on this invention was whether the patent(s) would apply to eukaryotic genes put into bacteria. The methods underlying he claims of the original application were described in the first DNA cloning paper, and that didn't involve eukaryotic genes. I've forgotten the details, but there was some legal issue about the breadth of coverage that the patent office would allow because of this. Ultimately, three separate patents were granted.

Hughes: Well, there was also a public relations aspect. It was felt, at the time, that there was more controversy associated with trying to license a eukaryotic product than a prokaryotic.

Cohen: I don't remember that.

Commercialism in Academia

[116] Cohen to Reimers, June 14, 1976 (OTL, S74-43, correspondence 1974-1979).

Hughes: The general climate in regard to commercialization of biological science was more benign by the mid-1980s than it was during the first patent application process.

Cohen: Yes. Some of the scientists raising concerns at the Miles Symposium about Stanford's approach in patenting subsequently went on, during the next couple of years, to start companies and apply for patents. Berg had opposed Stanford's efforts to seek a patent, but a few years later, he and Arthur Kornberg joined Alex Zafaroni in founding DNAX. The attitude of the scientific community about commercialism clearly had changed.

Cohen Declines the Chance to Join Boyer and Swanson in Starting Genentech

Hughes: Dr. Boyer said in his oral history[117] that he approached you—I think it was in the context that he had learned, or you had told him, that you were considering becoming a scientific advisor to Cetus—to switch your loyalties to Genentech.

Cohen: Right. There are a lot of people who believe that I am one of the founders of Genentech because Boyer is. In fact, just last week when I was in Taiwan there was someone whom I met who still thinks that. Herb's discussion with me about joining Genentech occurred at a time when I had just begun my association with Cetus as a scientific advisor. As I mentioned to you in an earlier discussion, I was heavily involved in opposing legislative control of recombinant DNA research and felt that being a scientific advisor was an arm's-length relationship that would not affect my credibility in the discussions I was having during my lobbying activities, but that being a principal in a company would. Herb proposed that both of us work together to get Genentech going. I decided not to do that and the decision probably was a foolish one.

Hughes: So Genentech wasn't off the ground yet?

Cohen: There had been discussions between Herb and Bob Swanson, and maybe even some written agreement at that point. As I recall, Bob had initially visited Niels Reimers at Stanford to discuss starting a company and to seek a license to the technology. Reimers talked to me about this, but I don't remember having any direct conversation with Swanson during his visit. Swanson later talked with Boyer, and they decided to proceed with Genentech. After that, Herb met with me to discuss the possibility of me joining them. And for the reasons that I've mentioned, I didn't.

More Details on the Co-inventorship Issue

Hughes: I have a letter that you wrote to Bert Rowland in January of 1975. I'll read you a bit of it:

"From the start, I have indicated my belief that scientific advances such as the one we have been involved in are in fact the result of multiple discoveries carried out by many individuals over a long period of time. The procedure for the construction of biologically functional DNA chimeras has resulted from the efforts of many, and I agreed to allow the university to proceed with the patent on the understanding that it would be made perfectly clear to all concerned that the matter was pursued at the initiative of the university and that I would receive no personal

[117] Transcripts of interviews with Boyer conducted by the present interviewer are currently being reviewed by Dr. Boyer.

gain from the patent." [118]

You may have been reacting to a stir that was created when Rowland sent out letters—how many I don't know—to scientists whom he thought could be associated with this work, asking them to sign a disclaimer [to inventorship]. Is that what precipitated your letter?

Cohen: I don't remember. Let me see if there is other information in the letter that jogs my memory. [Pause]. I guess from what I've said here: "Unfortunately, the ambiguity in your letter has resulted in some misunderstanding among my colleagues about my role in all of this, and about how a patent might affect scientific use of these procedures. I would appreciate your clarifying the situation in a letter to them."

You're right, from what I said in the letter, I think I might have been reacting to wording in Bert's letter that implied that Boyer and I were filing the patent application and had requested the disclaimers.

Hughes: Rowland was asking them to sign off in regards to any claim to the inventorship?

Cohen: Well, that's the implication, but I don't know without seeing his letter. [Hughes hands Cohen another letter; pause while Cohen reads it.]

This letter from Niels to Bill Massey says:

"Our patent attorney had innocently touched off the above concerns by sending out a letter to a number of Dr. Cohen's and Dr. Boyer's colleagues asking them to sign affidavits that they were not co-inventors. The letter gave the mistaken impression that Drs. Cohen and Boyer were personally filing the patent application and would receive material benefit. Hurry-up phone calls, letters, and telegrams were sent out but not before the situation was known to quite a number of people. We seem to have been able to diffuse the situation with respect to Drs. Cohen and Boyer."[119]

So that was the event behind the follow-up to the letter that I wrote. So you're probably right; my letter to Bert Rowland in January seems to be in response to wording that I was unhappy about.

Hughes: Can you remember if Rowland's letter was the first alert to your colleagues that the patenting process was beginning?

Cohen: I don't remember, but I think it probably wasn't.

It was necessary for the university to file the patent application prior to November 1974, which was the one-year anniversary of publication of the initial DNA cloning paper. And that deadline was at least a couple of months prior to the Rowland letter that I was responding to. Certainly, Annie Chang was aware of the patent application because she was working in my lab.

I don't know whether Helling was aware of the patent application before receiving Bert's letter. Bob had gone back to Michigan by then and communication was between Boyer and Helling. I had very little contact with Bob. In fact, I think I've only seen Bob once in the past twenty-some years after the *Xenopus* DNA paper was submitted, and that was at a scientific meeting where both of us were invited as speakers.

Hughes: There was no residue of tension from the patenting issue?

Cohen: Do you mean when we later saw each other? I don't remember any, but that encounter with him was maybe fifteen years ago and at this point I don't remember a whole lot about it.

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[118] Cohen to Bertram I. Rowland, January 22, 1975. (OTL, S74-43, correspondence 1974-1979.)

[119] Reimers to William Massy, February 19, 1975 (OTL, S74-43, correspondence 1974-1979).

Faculty Concerns About the Implications of Patenting

Hughes: Let's go to a meeting that occurred here in May of 1976 in which some of the tensions amongst the Stanford faculty come out. The purpose of the meeting, and I can show you the document, was to discuss patenting biomedical discoveries in general, but the specific issue was the Boyer-Cohen patent. One of the concerns, and this was expressed by Lederberg, was that patenting would interfere with scientific communication.

Cohen: Well, I think it's an important point. I developed similar concerns as the biotechnology industry appeared on the scene. At that point, companies had begun to increase their support of research in university labs. Herb Boyer had been criticized for having his lab at UCSF carry out experiments that were said to be beneficial to Genentech, and questions had been raised also about company support of research in the labs of some Stanford faculty. There was the concern that postdocs working on a company-supported project might feel uneasy about exchanging information about the project with people working on other projects. This was seen as a potential problem not only within a lab, but also between labs. If lab X was receiving funds from company A and lab Y down the hall was getting from company B, and companies A and B were competing, potentially this might limit free communication between the labs. These were theoretical concerns, and I can't say that I have ever seen any actual problems of this kind at Stanford.

Another issue was whether scientific publications or the presentation of findings at scientific meetings would be delayed in order to enable the university to file a patent application before the data were made public. My opinion was that scientific priority is sufficiently important that most scientists wouldn't intentionally delay publication of an important discovery. I personally would certainly not be willing to delay publication to enable filing of a patent application, and the university has never asked that I do that.

Paul Berg's Contentions

Hughes: Paul Berg was playing a leading role in the recombinant DNA debate, and he brought up the issue of Stanford's credibility if it was taking a prominent role in the debate and at the same time pursuing the patent. Do you remember? You were at this meeting.

Cohen: I don't remember the details of that particular meeting, which was some twenty years ago. But yes, Paul expressed his opinion about this on more than one occasion.

Initially, I was hesitant about going ahead with a patent application, as we've discussed, but was convinced by Niels Reimers to proceed, and am quite happy about that decision. As Niels has pointed out to me a number of times, a patent helps to clarify just whose scientific contributions underlie an invention, and the issuing of a patent, especially one that withstands challenges about inventorship, legally establishes the priority of a discovery.

Hughes: Berg's second point was: Why were you and Dr. Boyer the only inventors when he felt that others had played a significant role?

Cohen: Well, as I've said, that was a legal determination, not my decision or Boyer's. Because of the inventorship challenges by Helling and Morrow, and public assertions, particularly by Berg, about the priority of discoveries that underlie the invention, there was an especially extensive analysis of the scientific history before a patent listing Boyer and me as inventors was issued. Clearly, prior research had provided a foundation for the invention, as I told Reimers when he first approached me about applying for a patent. The invention used a

restriction endonuclease that had been reported on previously, and was dependent on earlier published work from my lab on plasmid transformation, and on work from multiple labs showing that DNA ends could be joined by DNA ligase. I talked earlier about the history of those discoveries. Whether, the experimental contributions of Helling, or Chang, or Morrow constituted inventorship was a question that was raised, and the lawyers representing Stanford and UCSF, and the patent office, concluded that they did not.

Since you've raised this point, I also want to mention that the invention of DNA cloning was not dependent at all on the work of Berg or on enzymological advances made by Arthur Kornberg—notwithstanding claims that I've heard and read from both scientists. If Berg had not done his work with the dA-T joining of DNA ends, and Kornberg had not done any enzymology, the invention would have occurred anyway.

The Faculty's Accommodation to Patenting

Hughes: Did the patenting issue divide the faculty?

Cohen: Well, we've already talked about Paul's position on this patent, and there was also a range of opinions among the faculty about whether discoveries in academic labs should be patented, but that was twenty years ago. Fellowships funded by royalties that the Cohen-Boyer patent has produced go to all departments in the biological sciences. And, additional funds that have come out of the share of the Office of Technology Licensing have been used to provide grants for faculty research more generally to the university. Patenting isn't the divisive issue it once was, and it hasn't been for at least fifteen years.

Inter-Departmental Interactions at Stanford

Hughes: How did the Stanford context compare with UCSF's?

Cohen: Which is what?

Hughes: UCSF portrays itself as having a collaborative, multidisciplinary, coordinated approach to basic biomedical research. Nonetheless, there have been some incredible controversies that split departments—Genentech being a prime example.

Cohen: Right. Also, later problems between Boyer and Goodman as well as the whole insulin story. They've had their share of conflicts.

Hughes: Do you want to talk about what effect the strong departmental hierarchy at Stanford—if you agree that that indeed is true—has meant to the pursuit of biomedical research here?

Cohen: Well, that's a complicated issue, Sally. Until relatively recently, I think there was a legacy of insular basic science departments at the Stanford Medical School. With a new generation of younger faculty, things here have changed significantly. But it's clear that a lot of great science has come out of Stanford in the past thirty years. Major, major discoveries have been made here in many areas and my colleagues in both the basic biomedical sciences and clinical departments are highly respected throughout the world for their accomplishments. So certainly, whatever might be claimed about collegiality, or about the alleged lack of it, at Stanford, the "proof is in the pudding." The environment at Stanford has been very successful in producing first-rate science. Having said that, one can always ask whether the contributions would have been even greater if there had been more interaction and collegiality.

Coolness Between the Stanford Departments of Biochemistry and Genetics

As we've discussed, Paul Berg, Dale Kaiser, and others in the Biochemistry department were instrumental in bringing me here to the Department of Medicine. Some of the biochemistry faculty were friends as well as colleagues, and they still are. I was studying plasmid DNA, and my research was relevant to work going on in Biochemistry. In a more collegial environment, my intellectual and scientific interactions with the Department of Biochemistry might have been formalized by a secondary appointment in Biochemistry.

In a discussion that I had recently with Josh Lederberg about this point, I asked for his assessment on whether the existing coolness between the Genetics and Biochemistry departments had resulted from some of the issues of contention in the recombinant DNA area that you and I have talked about or whether it preceded these tensions. Josh told me that when he came to Stanford, had hoped to receive a joint appointment in Biochemistry, but that even for someone of Josh's stature, the biochemists weren't inclined to do that. A coolness existed between the Genetics and Biochemistry Departments long before the issues related to recombinant DNA.

Concerns of an Anonymous Reviewer of Stanford's Patent Application

Hughes: Well, another issue that Berg brought up was the breadth of the patent being considered. I'd like to read the comments of an anonymous patent reviewer:

"A more serious drawback to the patent in my view is that it represents the development of a very basic process in molecular biology; a process that has great implications with respect to basic research. One can, with some justification, argue that this basic process should be left in the public and scientific community domain and not be patented. Furthermore, another important consideration is that other individuals have been involved in the important publications relating to the process, i.e. the earlier work of Mertz, Davis, and Berg."[120]

And then he goes on to say:

"I am concerned that given the fundamental nature of the work and the number of scientists involved, either directly or indirectly, that this patent will not reflect favorably on the public service ideals of the university."

And that's dated July 1, 1975. What we haven't discussed is the breadth of the application.

Cohen: The claims in the patent application prepared by Bert Rowland were very broad. I've been told that it's standard practice for attorneys writing patent applications to claim everything that they possibly can. The patent office ultimately denies some claims and approves others. Not all of the claims in the application prepared by Bert Rowland were granted, but the fundamental claims were.

The comments you've just read are almost identical to what Paul Berg has stated was his view of the matter. However, the fact is that there was no earlier work by Paul that contributed at all to the invention of DNA cloning. On the other hand, the discovery by Sgaramella, by Mertz and Davis, and by Joe Hedgepeth in Boyer's lab, that *Eco*RI creates complementary ends when it cleaves DNA was certainly relevant to the invention, and that

[120] "In confidence. Re: Process and composition for biologically functional DNA chimeras - Cohen-Boyer" (OTL, S74-43, correspondence 1974-1979).

was one of the points I made initially to Reimers. His response to me was yes, that research provided important information, but did not "teach" the invention.

It's interesting that the anonymous reviewer mentions the work by Mertz and Davis, but not the results published in the same issue of the PNAS by Sgaramella and by Hedgepeth *et al*.

Hughes: Is Sgaramella still around?

Cohen: Yes, he is. I've been planning to contact him to resolve some points for a book I've started to write.[121]

The Patent and University Public Service

Hughes: What about the point made at the end that "this patent will not reflect favorably on the public service ideals of the University"?

Cohen: Well, in essence, that was one of the questions that I had raised initially to Reimers—whether one can and should patent the fruits of basic research carried out at a university. Reimers argued that Stanford could and should patent the invention, and that the public service ideals of the university would be better served if Stanford used income from the invention to support education and research instead of allowing profit-making enterprises to have free access to the technology. I felt that this was a valid point and decided that it was reasonable for the university to proceed with patent application, and I aided the Office of Technology in doing that. The scientific community had a range of views about the issue at the time. But there are now probably thousands of patents providing income for universities, and patenting university research discoveries is no longer perceived as the diminishing of public service ideals by universities.

Hughes: Part of the public service aspect of the university, I would think, would be the transfer of knowledge to the public sector.

Cohen: My understanding is that the patent system was established to do just that. The intent was to eliminate any motivation for secrecy. An inventor's intellectual property would be protected even after the invention was publicly disclosed.

Hughes: There was also the fear that research, even the nature of the problem chosen to work on, could be influenced by the type of commercial funding that was available. In other words, people would choose research problems that they knew industry would support; and that was a corruption of the freedom of basic scientific inquiry.

Cohen: Well, I think that is a reasonable point to raise.

Some scientists probably did choose to pursue particular research projects because of the availability of industry support. On the other hand, the ability to obtain research support is a factor that most scientists have to consider anyway in choosing projects. Research supported by the NIH must be relevant, at least in a general way, to the mission of the Institute supporting the research, and sometimes the NIH earmarks funds for a particular programmatic purpose. The NIH decision to commit support to certain areas of research, such as HIV, is intended to encourage researchers to move into that area. So, yes, the availability of funds does influence the research projects that scientists choose to pursue.

Hughes: That's nothing new.

Cohen: That's the reality.

[121] Dr. Cohen is writing a history of the invention of recombinant DNA technology.

Hughes: It seems to me that it changes things when the endpoint is a product that is going to be placed on the market, rather than research strictly to further scientific knowledge.

Cohen: Well, yes, but I think that you're setting up a "straw man." If you're asking whether university laboratories should be developing marketable products for companies, the answer is no, of course not. Is it appropriate for university laboratories to carry out research in areas that are important to public welfare? Yes. But companies try to develop products that they can sell, and scientific knowledge obtained in university labs may advance industry's ability to do this. For example, I think the work that Boyer and I carried out wasn't intended to create a technology that would be commercially useful, but that has turned out to be one outcome of the research.

Niels Reimers' Contributions

Hughes: You mentioned that Rowland wrote the patent application. So what exactly was Reimers' role in the patent application itself?

Cohen: Niels is the person that first recognized that the discoveries that Herb and I had made had resulted in an invention that should be patented. Without Niels there would not have been a patent application, but he had no role in preparing the application itself; that was done by Bert Rowland. Niels later had a key role in deciding how the invention would be exploited for the benefit of the university, in setting forth a licensing policy, and in implementing it as head of the Office of Technology Licensing.

Hughes: What about the role of OTL in the various discussions that went on before the final patent application? As you remember, there were several applications. Did OTL initiate those meetings?

Cohen: I don't know.

Stanford's Patent Agreement with NIH

Hughes: In those notes that I showed you of that meeting in which Stanford scientists and administrators met, including you, there was mention of taking the patenting issue to the NIH, which indeed happened. I suppose through Reimers? There's some correspondence indicating that NIH approved Stanford's plan to license the technology. And that's the part I didn't understand.

Cohen: There was a general agreement that Stanford had with the NIH about the licensing of technology developed at the university. I think that because of the biosafety issues and Stanford's concern about its image, the university wanted additional approval from the NIH to proceed. But I don't know that for a fact. I wasn't involved in those negotiations or discussions.

Hughes: It wasn't just Stanford that had this agreement with NIH.

Cohen: No, I believe that other universities also had agreements.

Hughes: NIH had made a policy allowing universities to patent inventions based on NIH-funded research.

Opening the Patent File to the Public

Hughes: It was Reimers' idea to open the patent file to the public for examination. Potential licensees examined it in some detail. Reimers was also very active in soliciting opinions from various parties. Was some of the motivation to show the public that Stanford was trying to handle the patenting process in a democratic fashion?

Cohen: I don't know whether that was a factor. My recollection is that Niels' hoped that by opening the file to the public, that any challenges made would come early during the process and there would be the opportunity to address them at that time rather than later. I don't actually know whether Niels, Bert, or someone else made this decision. Stanford could use this strategy because the science and technology were publicly known and there was nothing to be lost by opening up the patent file.

Hughes: Good point.

Cohen: Although at a later point in the prosecution, I think Stanford decided to close the file.

Hughes: It did, in 1983.

Cohen: And the reason for that, I was told, was that there were articles in *Nature* about the patent prosecution almost every other week. It was a kind of blow-by-blow description and analysis of Stanford's pursuit of the patent. I had no role in either the decision to open the file or the decision to close it, and don't know the details.

The Patent in Comparison to Other University Patents

Hughes: Was there an awareness on the part of the people involved in the patenting and licensing processes that they might be setting precedents for patenting in biology?

Cohen: Well, I certainly was not thinking about the setting of precedents, but other basic technologies invented at universities have since been licensed non-exclusively and broadly. I suspect that Stanford's success with this strategy may have influenced others to proceed in this way. I think that the Cohen-Boyer patent now has about 400 licensees. I don't know of other university patents that have that number of licensees.

Hughes: Or the income?

Cohen: I think that there are other university patents that have generated considerably greater income. It has to do in part with the royalty rate.

The Low Royalty Rate

Hughes: You apparently were one of the proponents of having a low royalty rate. Why? I can show you the memo.[122] [Pause while Cohen reads memo.]

Cohen: I guess the memo to Don Kennedy speaks for itself. I suggested a low royalty rate because I felt that recombinant DNA technology would be broadly used, that the patent shouldn't be a barrier to its use, and that Stanford would, in principle, be able to obtain hundreds of licenses if the rates were reasonable and licenses were given on a non-exclusive basis. There was also the issue of not making the royalty rate so high that it would force companies to work with the technology in labs outside of the U.S. Because a patent application was not submitted prior to publication of our paper describing the technology, only a U.S. patent could be obtained, the intent was to avoid a prohibitively high royalty

[122] Cohen to Donald Kennedy, December 19, 1980 (OTL, S74-43, correspondence 1980-1982.)

rate that would encourage companies to evade the patent by working abroad.

Reimers' Reserve Fund

Hughes: Another thing that apparently caused some controversy was that in 1981 Reimers set aside $200,000 for a reserve fund to cover potential litigation. Do you remember anything about that?

Cohen: Vaguely, yes.

Hughes: Was the patent ever challenged?

Cohen: Well, we've discussed the inventorship challenges, but I don't think there was ever litigation. There also have been companies that used the technology but initially refused to take out licenses. But as far as I know, all of them eventually became licensees.

Hughes: The patent office indicated that it was going to issue the product patent in the summer of 1982, and then it didn't. Week after week there were articles in *Nature* and in other journals, and there were several criticisms. One of them was that apparently you and Dr. Boyer didn't deposit pSC101 in the type culture collection until six months after the original 1980 process patent was issued.

Cohen: I do remember that issue; I think it was raised by companies that didn't want to take out licenses and tried to prevent issuance of the patent. Ultimately, the patent office decided that the lack of deposit of the plasmid at the ATTC [American Type Culture Collection] was a non-issue, since I had records of having distributed pSC101 to dozens of laboratories, and many publications had appeared describing research using it. I think that this challenge was based on a technical issue relating to patent law, but I don't remember the details.

Hughes: It was argued that pSC101 was part of the enabling process, and if scientists did not have access to pSC101, then the patent wasn't enabling.

Cohen: Right. Thank you for reminding me. The university's position was that the plasmid was made widely available by me, which gave other scientists access to it and made the patent enabling.

The Ziff Article

Hughes: Another issue involved Edward Ziff's article on your method in the *New Scientist*, and whether that was enabling.

Cohen: Yes, that was an interesting situation. The issue was that Herb Boyer's presentation at the Gordon conference [June 1973] was reported in an article published in *New Scientist* by Ed Ziff. Gordon conferences are supposed to be private meetings and everyone signs an agreement that information presented will not be made public. But Ziff's article described the approach, though in general terms, and the issue was whether his article contained information that was enabling or whether it enabled a scientist to go ahead and perform the procedure described in the patent application. If so, a patent couldn't legally be issued, as the patent application was submitted more than a year after publication of the Ziff article.

Hughes: Right.

Cohen: The patent office concluded that because Gordon conferences are private meetings, Herb's presentation wasn't a public disclosure, and that Ziff's published statements were not enabling.

Some people challenging the patent claimed that the publication of DNA end joining methods by Sgaramella, Mertz, Davis, Jackson, Symons, Berg, Lobban, and Kaiser were also "prior art," and that publication of that work more than a year prior to submission of the Cohen-Boyer patent precluded issuance of the patent. But the patent office concluded that those publications did not teach that or how DNA could be cloned.

In the discussion I had with Lederberg just a few days ago, he mentioned that he had also been thinking about trying to clone and isolate individual genes and even had written that this would be a desirable scientific goal. But, he pointed out that it's one thing to think about doing something, or even to try to do something, and it's another to actually make it happen. So the question is whether intent constitutes inventorship.

Hughes: Ziff's article didn't provide sufficient information to reproduce?

Cohen: To enable a skilled scientist to clone DNA.

Hughes: I would think that would also have applied to Dr. Boyer's remarks, in addition to the fact that it was also a private meeting.

Cohen: Well, maybe Boyer didn't provide enough information for Ziff to have written an enabling article.

Revised Origin of the pSC101 Plasmid

Hughes: One last point and then I'll stop.

Cohen: No, you don't have to stop. We're here to get these points on the record.

Hughes: Another issue was the debate over the actual origins of pSC101. Your paper had already come out, indicating that the origin was probably different than you had originally thought.[123]

Cohen: That's correct.

Hughes: How did the lawyers tie that in?

Cohen: The challenge was raised because the origin of pSC101 wasn't described correctly in the patent application.

Hughes: Was it just a question of accuracy?

Cohen: Well, my earlier conclusion about how the plasmid originated was wrong. In the fall of 1973 when the application was submitted, we thought that pSC101 had been derived from a larger plasmid, but additional experiments showed that this interpretation was not correct. The patent office decided that the issue was not relevant to the use of pSC101 as a vector.

Hughes: Critics tried to make it relevant by saying that if you gave the wrong information about the origin of the plasmid then that was disabling.

Cohen: Well, we gave the best information that was available to us at the time, and we supplemented and corrected that information in print when additional data became available.

Royalty Distribution at Stanford

[123] Cohen, SN, Chang, ACY. Revised interpretation of the origin of the pSC101 plasmid. *Journal of Bacteriology*. 1977; 132: 734-737.

Hughes: Why don't you describe how royalties are distributed at Stanford?

Cohen: In general, or for this particular patent?

Hughes: Both.

Cohen: In general, the Stanford policy is that the Office of Technology Licensing receives a share of the royalties received, which I think is 15%, plus out of pocket expenses that OTL incurs during pursuit of a patent or a licensing of a technology. And since Stanford was acting on behalf of UCSF for the Cohen-Boyer patent, OTL collected 15% of total royalties, not just on Stanford's half. And after deducting its expenses, Stanford then split the remaining amount evenly with UCSF. The Stanford share of patent income is then distributed in three ways: one-third to the university or school, one-third to the department, and one-third to the inventor.

In earlier days of OTL, what is now the medical school's share of patent income went into the university's budget. When Don Kennedy was President of Stanford, I suggested that this portion go instead to the medical school. I pointed out that the university was already receiving the OTL share, and he agreed to make the change. As a result of that discussion, the income received by the School of Medicine has been substantial.

Hughes: It can be used any way?

Cohen: In any way that the Dean chooses to use it. The question you've asked is interesting because of my feeling that the medical school could have better used its share of the patent income to create programs of long-range value. The School of Medicine has used its share of Cohen-Boyer patent income to pay expenses for day-to-day administrative needs, but the institution largely hasn't created anything incremental or enduring with the funds it has received. Some of the Department's share of the patent income was allocated to renovate Department of Genetics space to attract our current chairman, and that's been very useful, and the Dean of the School also contributed some funds for this purpose. But overall, little of long-range value has been done with the medical school's share.

The departmental share of Cohen-Boyer patent income originally went entirely to the Department of Medicine, since I held a faculty position in that department at the time that the technology was invented. Later, as Chair of Genetics, I asked the Chairman of Medicine at that time, Ken Melmon, to split future income from the Department of Medicine share with the Department of Genetics, as I had then held appointments in both departments. Ken generously agreed to do this. The Department of Medicine still receives income from patent royalties, as does the Department of Genetics.

Interview 13: June 7, 1995

OTHER POST-RECOMBINANT DNA RESEARCH IN COHEN'S LAB

Early Post-*Xenopus* Experiments Involving Eukaryotic DNA

Hughes: Dr. Cohen, I was wondering since, you know, we've talked a lot about recombinant technology, but I'm wondering what effect, if any, it had on the success of your research, the fact that you were one of the inventors? I guess putting it succinctly, did that give you a jump-start on the rest of the field?

Cohen: Well, I think we talked about this a little during an earlier discussion, but the answer is yes, for several years. But it was straightforward for others to learn to use the technology and it was inevitable that the initial advantage wouldn't last for long.

But during those several years my lab could proceed in scientific areas I was interested in without a lot of competition. My lab used DNA cloning methods to study the modes of replication of bacterial plasmids; Ken Timmis and Felipe Cabello, who were postdocs in my lab, did most of those experiments. Peter Kretchmer, Annie Chang, and Dean Taylor used DNA cloning methods to study other aspects of plasmid biology. On the eukaryotic side, one of the questions stemming from the *Xenopus* DNA cloning experiments was whether eukaryotic transcription initiation signals would work in bacteria and, as we've discussed, the mitochondrial DNA collaboration between my lab and David Clayton's showed that they do not.[124] In other experiments, I collaborated with Larry Kedes to investigate the use of DNA cloning methods as a tool for the study of eukaryotic gene organization. We cloned sea urchin histone genes, which was the first time protein-coding eukaryotic genes were propagated in bacteria, and the experiments showed the power of the DNA cloning approach for genome organization studies.[125]

Cloning of Histone Genes

Hughes: Now, why histone genes?

Cohen: Histone gene messenger RNA from sea urchins in the Kedes lab was well characterized and we could isolate large amounts of the RNA and use it as a probe to detect bacterial cells that contained the histone DNA sequence. We did these experiments using a procedure called "sub-culture cloning." That was before Mike Grunstein and Dave Hogness here at Stanford developed a colony hybridization technique to pick out bacterial colonies containing cloned DNA segments. I don't know whether this is the appropriate place to go into the details of sub-culture cloning.

Hughes: Yes, it's not an issue.

Cohen: The idea is the following: If the bacterial clone containing a desired gene is present in the population at a frequency of one in a thousand, in principle, one could clone each of a thousand cells by putting individual cells in different flasks and growing up clonal populations. You could then use some kind of probe to identify the flask containing the clonal population that has the gene you want. Alternatively, you could take a thousand bacterial cells and put a hundred cells into each of ten flasks. One of the ten flasks should contain the clone you are seeking, along with a lot of clones that you don't want, but the frequency of the desired clone in the cell population in that flask has now been enriched from one in a thousand to one in a hundred. And you can repeat the procedure to enrich to one in 10 and then to eventually obtain a pure population of the clone you are seeking. Sub-culture cloning is a well-established genetic strategy, and this is what we used to isolate clones containing histone genes.

But a major goal of multiple labs was to express eukaryotic proteins in bacteria. Several groups were trying to produce insulin-containing fusion proteins in *E. coli*, and Boyer and

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[124] Chang, ACY, Lansman, RA, Clayton, DA, Cohen, SN. Studies of mouse mitochondrial DNA in *Escherichia coli*: Structure and function of the eucaryotic-procaryotic chimeric plasmids. *Cell*. 1975; 6: 231-244.

[125] Kedes, LH, Cohn, RH, Lowry, JC, Chang, ACY, Cohen, SN. The organization of sea urchin histone genes. *Cell*. 1975; 6: 359-369.

Genentech worked out a way to produce somatostatin, which is a small single chain polypeptide. Somatostatin was made as fusion protein and then separated later from the bacterial segment of the protein. But the procedure worked only with short peptides that lacked methionine.

Using DNA Cloning to Discover Hormones

I previously described the strategy that Bob Schimke and I used to express the first eukaryotic protein that was functional in bacterial cells, the mouse dihydrofolate reductase, and the subsequent work on proopiomelanocortin that Schimke and I did collaboratively with Shosaka Numa and Shigitada Nakanishi. I think that my lab still had an advantage during that that period. But by 1979, there were hundreds, and perhaps thousands, of labs using the technology, and many of these labs had much greater financial and human resources than my lab did. I think I've mentioned that the first time we were really badly scooped by someone else using DNA cloning methods was with interferon, the work of Charles Weissmann in Switzerland.[126] Yes, we initially had skills that didn't exist in other labs, but in a few years, that no longer made a difference.

Constructing a P3 Facility and Work with Hepatitis B Virus

Hughes: I also read that you were involved in the design of a P3 facility.

Cohen: Yes.

Hughes: Is there any comment to be made there?

Cohen: Well, at that time, P3 facilities were required for certain experiments, and my lab had begun collaborative experiments with Bill Robinson, who has studying the hepatitis B virus. And even though P3 facilities weren't required for working with the hepatitis virus itself…

Hughes: Is that so?

Cohen: …they were required for any cloning experiments done with hepatitis B virus DNA. There was only one P3 facility at Stanford: the one that Paul Berg had constructed in the biochemistry department to work with tumor viruses. I persuaded Joshua Lederberg to assign some space for conversion to a P3 facility for use in other departments in the School of Medicine. The NIH guidelines specified P3 level containment for a wide variety of experiments, and Josh and I felt that institutionally, another P3 facility was needed at the medical center. Whether or not a P3 lab was really necessary to assure biosafety was a different issue. And so one of the engineers at the medical center and I designed a P3 lab, which required a system of negative pressure, double-paned windows, special containment devices, and a feed-through autoclave that could be entered from either the P3 lab or the vestibule.

Hughes: And was it broadly used?

Cohen: No, not broadly, but it was used by several faculty. When the containment requirements for DNA cloning were relaxed, fewer and fewer experiments required a P3 lab. The room is now being used for routine tissue culture experiments, and no P3 level work is done in it.

Hughes: Is now the time to talk about the hepatitis research?

[126] Nagata, S, Taira, H, Hall, A, Johnsrud, L, Streuli, M, Ecsödi, J, Boll, W, Cantell, K, Weissmann, C. Synthesis in *E. coli* of a polypeptide with human leukocyte interferon activity. *Nature*. 1980 March 27; 284 (5754): 316-20.

Cohen: If you'd like.

Hughes: Well, there are several ways we could go.

Cohen: Okay. My lab had begun a collaboration with Bill Robinson, a colleague in the Department of Medicine, who had a long-standing interest in the hepatitis B virus. Working together, our labs were the first or second group to clone the hepatitis B virus genome. We reported this in a *Nature* paper.[127]

Hughes: Is he a virologist?

Cohen: Yes. And, at a scientific meeting, I think in 1977, in the Loire region of France, I talked about the hepatitis work, which was still incomplete, with some colleagues; and across the table was Bill Rutter. I said that I thought that the work we were trying to do with the hepatitis B virus might have practical utility and could eventually lead to production of a hepatitis B vaccine. Bill seemed especially attentive to that discussion but didn't say anything. I've never asked whether he had already begun his work on the hepatitis B surface antigen protein by that time or whether the discussion helped to stimulate his interest.

Hughes: That was 1977?

Cohen: I think so. It was a CNRS meeting, I think in late September or early October. I remember the meeting well because the weather was quite cold and it rained every day. We were housed in unheated rooms and I hadn't brought along enough warm clothes. But scientifically it was a very good meeting.

Hughes: And it was on hepatitis?

Cohen: No, it was on genetics and genetic engineering.

Hughes: Right. How did you feel about being up against a large operation, a large and moneyed operation?

Cohen: Well, I've mentioned that, in general, I've tried to work in scientific areas that are less competitive. I chose plasmid biology as an initial research area partly for this reason; that was long before the area had become popular. The head start I had with DNA cloning methods led me to jump into the competition of trying to get functional expression of eukaryotic proteins in bacteria, and we succeeded in winning that race. And then with the cloning of the POMC gene, we reported the first instance where a cloned gene predicted the existence of a hormone. So I was a bit giddy at the time I entered the interferon race and was naïve to think that I could do this with only a couple of people working on the project part time.

Hughes: Do you remember when this was occurring?

Cohen: Weissmann's paper was published in late 1980.

At that time, my lab was also working in prokaryotic areas that had become competitive. We were studying bacterial transposons. And, we were continuing to investigate plasmid DNA structure and mechanisms controlling distribution of plasmids when bacterial cells divide; Peter Meacock, in my lab, had just identified specific sequences on the pSC101 plasmid that are important to the partitioning process.[128] We were studying genetic control signals in bacteria using reporter gene methods that Malcolm Casadaban, a postdoc in my lab, had developed. And after my sabbatical leave in Hopwood's lab, my lab also started

[127] Sninsky, JJ, Siddiqui, A, Robinson, WS, Cohen, SN. Cloning and endonuclease mapping of the hepatitis B viral genome. *Nature*. 1979; 279: 346-348.

[128] Meacock, PA, Cohen, SN. Partitioning of bacterial plasmids during cell division: a cis-acting locus that accomplishes stable plasmid inheritance. C*ell*. 1980 June; 20 (2): 529-42.

working on *Streptomyces* biology and established the first DNA cloning system in *Streptomyces*. I enjoyed the scientific and intellectual challenge of this diversity, but my lab was relatively small and we were spread very thin. I viewed the interferon event as a warning that I couldn't continue to try to work on so many diverse projects.

Hughes: But, isn't this somewhat of a new thing now, because the early '80s are the boom years of the creation of new biotech companies? I believe more were formed in 1981 than any other year, which has a lot to do with the economic climate, of course, which we don't need to get into. But, I guess what you're saying rings a chord with something that Dr. Rutter said to me about a meeting that he attended which was in connection with diabetes—I can't for the life of me remember what specifically it was—and he came to a similar realization that this was a field where he, with his relatively small academic group at UCSF, could in no way compete with these big players who had industry support. And I think it's that influx of industry support that really is beginning to change the face of biological research; it is more difficult in these commercially viable fields for the small academic laboratory team to really get ahead. I mean, the resources just aren't there.

Cohen: Yes, I think that's true for projects that are seen as having a commercial payoff. And interferon gene expression was one of those projects.

Hughes: But that wasn't a setback as far as you were concerned?

Development of Reporter Gene Systems

Cohen: Well, it was disappointing but I didn't really think of it as a "setback." It was an indication that I needed to focus on areas where my lab had a special ability to contribute. Two of these areas were *Streptomyces* biology and DNA transposition, and another was the development of "reporter gene" methods for identifying and studying gene control signals. Reporter genes are now so widely used by both academic and industrial scientists that people tend to forget that the approach had to have been invented by someone, and that someone was Malcolm Casadaban. There had been some earlier work on gene fusions, but the concept of using reporter genes to study the expression of other genes came from Malcolm, who was a graduate student in Jon Beckwith's lab at Harvard. Malcolm used bacteriophage lambda and genetic recombination to place the *E. coli lacZ* gene under control of the promoter for another gene *E. coli* gene, *ara*. This allowed him to use *lacZ* expression to measure the activity of the *ara* promoter. Malcolm came to my lab for postdoctoral training, and the project we decided on was to design and test plasmid vectors and bacterial strains that could be used to clone DNA fragments containing gene control signals upstream from *lacZ*. The approach was successful and *lacZ* probably still is the most widely used reporter gene, although other reporters have since been developed. Malcolm and I used the reporter gene approach to study signals that regulate DNA transposition in bacteria. Should I say something briefly about that?

Hughes: Yes.

Studies of Bacterial Transposons

Cohen: The EM [electron microscopy] work that I did collaboratively with Phil Sharp and Norman Davidson in the early 1970s[129] made clear that antibiotic resistance plasmids had evolved in

[129] Sharp, PA, Cohen, SN, Davidson, N. Electron microscope heteroduplex studies of sequence relations

a modular way. There were segments of different plasmids that showed high homology, and these were interspersed with blocks of sequences unique to a particular plasmid. Large segments that seemed identical by EM heteroduplex analysis were present on multiple plasmids. During this work, we observed that when a small plasmid was introduced into a bacterial cell containing a larger plasmid and the resulting cell was grown for a period in culture, some small plasmid DNA molecules increased in size. In repeated experiments, the size increase was always the same. It was almost as though a particular segment of DNA was being acquired by the small plasmid on multiple occasions. And the increase in size was accompanied by acquisition by the small plasmid of the ability to confer resistance to ampicillin.

Dennis Kopecko, who was a postdoc in my lab and I began to study this phenomenon. Dennis found, by heteroduplex analysis, that the segment being added to the small plasmid had inverted repeat segments at both of its ends; inverted repeats are DNA segments containing a nucleotide sequence that has been duplicated, but in the opposite orientation, so that stretches of DNA on the same strand are complementary. When the DNA is denatured by heating and the DNA is then cooled slowly, the complementary nucleotides on the same DNA strand interact with each other by base pairing. We also found that the segment of DNA acquired by the small plasmid was homologous with a segment present on the larger plasmid.

These observations were made at a time when my lab was developing DNA cloning methods. I was also involved in teaching clinical medicine and was designing a computer system for detecting drug interactions in patients. Before Dennis and I had established the molecular basis for what we had observed, a paper reporting the transposition of an ampicillin resistance gene from one plasmid to another was published in *Molecular Genetics and Genomics* by Bob Hedges and Alan Jacob,[130] in early 1974. And, in fact, Hedges and Jacob coined the name transposon in that paper. They thought that transposition involved ordinary recombination, but Dennis had observed that movement of the DNA segments he was observing occurred in bacteria that are defective in a gene essential for normal recombination. And Dennis also found that the transposable DNA segment had inverted repeat segments at its ends. We published our work on this in April 1975. In a period of ten months during that year, six other bacterial transposons were identified and reported by Fred Heffron and Stanley Falkow,[131] Doug Berg,[132] Nancy Kleckner and David Botstein,[133] and others,[134 135] and these genetic elements contained a variety of antibiotic resistance genes.

Hughes: What is the—excuse me for interrupting—but what is the significance of the inverted repeats?

Cohen: The inverted repeats contain sequences recognized by enzymes that cut out the transposon site-specifically and reinserted it at another DNA location.

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among plasmids of *Escherichia coli*. II. Structure of drug resistance (R) factors and F factors. *J Mol Biol.* 1973; 75: 235-255.

[130] Hedges, RW, Jacob, AE. Transposition of ampicillin resistance from RP4 to other replicons. *Mol Gen Genet*. 1974; 132 (1): 31-40.

[131] Heffron, F, Sublett, R, Hedges, RW, Jacob, A, Falkow, S. Origin of the TEM-beta-lactamase gene found on plasmids. *J Bacteriol*. 1975 April; 122 (1): 250-256.

[132] Berg, DE, Davies, J, Allet, B, Rochaix, JD. Transposition of R factor genes to bacteriophage lambda. *Proc Natl Acad Sci USA*. 1975 September 1; 72 (9): 3628-3632.

[133] Kleckner, N, Chan, RK, Tye, BK, Botstein, D. Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition. *J Mol Biol*. 1975 October 5; 97 (4): 561-75.

[134] Foster TJ, Howe TG, Richmond KM. Translocation of the tetracycline resistance determinant from R100-1 to the Escherichia coli K-12 chromosome. *J Bacteriol*. 1975 December; 124 (3): 1153-1158.

[135] Gottesman, MM, Rosner, JL. Acquisition of a determinant for chloramphenicol resistance by coliphage lambda. *Proc Natl Acad Sci USA*. 1975 December; 72 (12): 5041-5045.

Hughes: I see.

Cohen: Transposition is a site-specific recombination process that is independent of the mechanisms that carry out "ordinary" or "general" genetic recombination in bacterial cells. Anyway, Dennis, and then Perry Nisen and Jean Brevet in my lab, went on to show that antibiotic resistance plasmids had evolved by a series of insertions of transposons and that inverted repeats are present also at sites of dissociation and reassociation of segments of large R-factors.[136] [137] Another postdoctoral fellow, David Tu identified the specific DNA sequences that determine where transposons insert;[138] transposition has turned out to be an important factor in plasmid evolution. Then, using reporter gene methods, Malcolm and others in the lab went on to identify gene control signals within transposons and to study how they work.

By that time, bacterial transposons had become a hot area of investigation and there were multiple groups studying them.

Hughes: Was this general area, of which transposons were a part, namely what I believe was a new concept, that indeed the genome was not a stable entity, that there were these bits and pieces of DNA from various sources jumping around in what appeared, at first I guess, to be an unrelated fashion, and how controversial was that as an idea?

Cohen: Well, jumping genes wasn't an idea that originated with me.

Hughes: No, I know, but you're a part of the wave, though.

Barbara McClintock and DNA Transposition

Cohen: That's true. But the idea of DNA transposition came originally from Barbara McClintock and her studies of maize in the 1940s. And there were also genetic elements called IS [insertion sequence] elements, which were found in the late 1960s in bacteria. Later, these were recognized as being mini-transposons. Previously, there had been genetic evidence of transposition and perhaps a little of molecular evidence, but with the ability to clone segments of plasmid DNA, it became possible to analyze IS elements and antibiotic resistance transposons in great detail, and that was being done by many labs. So, the notion of fluidity of the genome wasn't a novel idea at that point. However, the role of these elements in the evolution of antibiotic resistance plasmids was a novel discovery, and the study of transposons that carry resistance genes produced fundamental insights about the mechanisms of transposition and the enzymes and DNA sites involved.

Hughes: Now would you consider that McClintock's work was the deciding factor in convincing the world, the scientific world, about the fluidity of the genome?

Cohen: Well, McClintock's work in maize was certainly the seminal work, but its implications were not fully appreciated by a significant part of the scientific community for many years. And it was, I think, the work done in bacteria on IS elements and resistance gene transposons in the mid and late 1970s that brought the McClintock discoveries into sharp focus again and provided detailed molecular information about the nature of transposable elements. But the

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[136] Kopecko, DJ, Brevet J, Cohen, SN. Involvement of multiple translocating DNA segments and recombinational hotspots in the structural evolution of bacterial plasmids. *J Mol Biol.* 1976; December; 108 (2): 333-60.

[137] Nisen, PD, Kopecko, DJ, Chou, J, Cohen, SN. Site-specific DNA deletions occurring adjacent to the termini of a transposable ampicillin resistance element (Tn3). *J Mol Biol.* 1977; December 25; 117 (4): 975-8.

[138] Tu, CP, Cohen, SN. Translocation specificity of the Tn3 element: characterization of sites of multiple insertions. *Cell*. 1980; January; 19 (1): 151-60.

original conceptual insights were McClintock's. She was one of the giants of genetics, but as I've mentioned, even at the time she was being considered for the Lasker Award, it took some explaining to make clear the importance of her discoveries.

In 1981, MClintock and I were jointly awarded the Wolf Prize. I had met her only once or twice before then. During the few days after the award ceremony, we were taken on a tour of parts of Israel, and even though Barbara was then almost 80, she walked vigorously with the group along the rim of the Masada mesa. And her discussions about science were absolutely clear.

Hughes: Now was this, maybe not initial resistance, but just not knowing very much about her work, did that have a conceptual basis or was it because she was working in maize, which was not a mainstream subject to be working on?

Cohen: I think, well, no, maize was a mainstream area of investigation for geneticists.

But part of the problem may be that McClintock's papers are difficult to read and follow. The science is very clear. But they are densely packed with information. In any case, I think it is correct that the work of McClintock was not fully appreciated outside of her field of maize genetics before the IS and bacterial transposon work.

Hughes: Well that, you see, is interesting in itself; I mean, that it was largely maize geneticists. It wasn't people in the general field of genetics who were leaping onto her work as sort of a breakthrough that should be pursued.

Cohen: I guess I should ask Josh Lederberg what he thought, in the 1950s, about the work of McClintock

Hughes: Yeah, you should ask him. It'd be interesting. Okay. Should we stop or do you want to try to get through the science your lab has done?

Cohen: Well, let's see. We can go a little bit further.

Hughes: Okay.

***Streptomyces* Biology**

Cohen: We've talked about transposition and we've talked about reporter genes and the use of reporter genes to study transposition. My studies of *Streptomyces* biology started because I was interested in the relationship between antibiotic resistance genes and antibiotic production in these bacteria. And in order to study this, it was necessary to establish a DNA cloning system in *Streptomyces*. That was done by two postdocs: Mervyn Bibb, who was a graduate student in Hopwood's lab and subsequently came to my lab as a postdoc, and Janet Shottel.[139]

Streptomyces species were considered to be a possible source of some of the antibiotic resistance genes that had moved to other species of bacteria. There has been the notion that resistance genes may have evolved initially in *Streptomyces* to protect these antibiotic-producing organisms from the antibiotics they make. Like *E. coli* and many other bacterial species, *Streptomyces* can exchange genes by mating. We found that segments of *Streptomyces* chromosomes can excise and form plasmids when the bacteria mate. These DNA segments, which we named "plasmidogenic," or plasmid forming, move in and out of attachment sites on the chromosome. Charles Omer and others in my lab worked for some years to understand how this process is regulated.

Other studies that my lab has done in *Streptomyces* also began with objectives related to

[139] Bibb, M, Schottel, JL, Cohen, SN. A DNA cloning system for interspecies gene transfer in antibiotic-producing *Streptomyces*. *Nature*. 1980 April 10; 284 (5756): 526-31.

understanding the spread of antibiotic resistance. When we started studying *Streptomyces* plasmids, I expected that the DNA molecules would be circular, as are the plasmids of most bacteria. And *Streptomyces* do have circular plasmids, but some species also contain plasmids that consist of linear DNA, and some of these plasmids contain as many as 800,000 nucleotide base pairs. Proteins were found by others to bind to the ends of linear plasmids, which are called telomeres, and it was thought that these proteins initiated plasmid replication at the ends of the linear DNA molecules. But P.C. Chang, a postdoc in my lab, found that these plasmids replicate bidirectionally from an internal origin of replication.

Hughes: Is this unique to *Streptomyces*?

Cohen: I suspect it isn't, but so far, the linear plasmids of *Streptomyces* are the only linear replicons that reproduce by this mechanism.

Hughes: And is it related to the linearity?

Cohen: Yes, because…well, this gets into complicated issues relating to DNA replication. After a linear DNA segment is replicated, there needs to be a mechanism for making the lengths of the two DNA strands equal. Why doesn't replication itself do this? I don't really want to go into that level of detail, but linear replicons have different mechanisms to even up the ends of the replicon. Maybe we should stop.

Hughes: Okay.

Interview 14: June 23, 1995

Starting Investigations of Messenger RNA Stability

Hughes: Last time we began to talk about your more recent research—meaning by that, after the critical recombinant DNA work when you were working out the methodology— and today I'd like to start with messenger RNA stability.

Cohen: Okay.

Hughes: And maybe you can say how you got into that field?

Cohen: Okay. My interest in mRNA stability was stimulated partly by my work with *Streptomyces*, which proceed through a developmentally complex life cycle. They start growing from spores. And, out of the spores come hairlike projections called "mycelia," which penetrate into the media or, if the *Streptomyces* are in their natural environment, into the soil. And when nutrients become depleted, out of this tangled web come some mycelia that extend outwards or upwards. Eventually partitions form in these structures and spores develop from the compartments. And the cycle is repeated.

I thought it was possible that expression of certain *Streptomyces* genes would occur throughout the life cycle, while other genes would be expressed only at particular stages. Potentially, this could occur by regulation of promoter activity, but another possibility was that RNAs encoded by different genes could have different stabilities. There wasn't much information available about messenger RNA decay at that time. It wasn't even known whether degradation of RNA was subject to genetic control.

Hughes: Now was that a novel idea?

Cohen: Well, it was known that cells contain stable RNAs, ribosomal RNA for example, and

unstable messenger RNA. But there wasn't a lot of consideration of possible mechanisms that might regulate RNA decay.

Hughes: Yeah. So there wasn't a strict dogma that control was at the transcriptional level?

Cohen: No, there certainly wasn't any dogma that regulation of messenger RNA abundance occurred only transcriptionally. But most scientists studying gene expression at the molecular level were focused on transcriptional or translational control. Everyone knew that specific signals determine the sites of initiation of mRNA synthesis and of mRNA translation, but degradation of mRNA wasn't generally viewed as a regulated process.

Other observations that contributed to my interest in studying RNA decay occurred during my lab's studies of DHFR gene expression. We had observed that DHFR constructs synthesizing mRNAs of different lengths showed different levels of phenotypic expression in *E. coli*, even though the transcripts were all initiated at the same promoter, and should for reasons I won't go into here, also have been translated at the same efficiency. And the question was whether differences in mRNA stability might underlie the differences in gene expression.

Anyway, we started experiments to isolate messenger RNAs from *Streptomyces* that were especially stable, and the work didn't proceed very well, largely for technical reasons; mRNA in *Streptomyces* has a high G+C content, as do the ribosomal RNAs of most organisms, and the amount of stable ribosomal RNA present in the bacterial cells was overwhelming. We kept isolating only ribosomal RNAs and I decided that it would be better to use *E. coli* as a model system for studies of bacterial mRNA stability.

I was interested in identifying possible signals in the mRNA that affected its stability and Alex von Gabain, who had worked on the interferon experiments in my lab, was interested in working on the project. The initial question was whether decay of mRNA in *E. coli* is initiated at one of the RNA ends and the first step was to develop an assay that would allow us to look at segmental aspects of RNA decay. Alex, together with Joel Belasco and others in my lab, showed that there were dramatic differences in the stability of different *E. coli* messenger RNAs, and that degradation of mRNA has directionality.[140] The methods that Alex and Joel developed enabled us to show later that signals located at specific sites on RNA molecules can accelerate or inhibit decay. We made fusions between stable and unstable RNAs, identified the RNA sites responsible for stabilizing or destabilizing the composite transcripts, and identified specific determinants that affect RNA stability characteristics.

Expansion of Investigations of RNA Decay

My lab's work on RNA decay quickly expanded. And, later experiments we did collaborative with Alex after he started his own lab, and with Gisella Nilsson, a graduate student in his lab, showed that the stability of certain messages was influenced by cell growth rate. There had been some previous suggestions of physiological control of mRNA decay, but I think that Gisella's paper[141] may have been the first definitive demonstration of that. And then a little later, in *Rhodobacter capsulatus*, a photosynthetic bacterial organism we were studying, Joel,

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[140] von Gabain, A, Belasco, JG, Schottel, JL, Chang, AC, Cohen, SN. Decay of mRNA in *Escherichia coli*: investigation of the fate of specific segments of transcripts. *Proc Natl Acad Sci USA.* 1983 February; 80 (3): 653-357.

[141] Nilsson, G, Belasco, JG, Cohen, SN, von Gabain, A. Growth-rate dependent regulation of mRNA stability in *Escherichia coli*. *Nature*. 1984 Nov 1-7; 312 (5989): 75-7.

Alex, Tom Beatty, another postdoc, and Cam Adams, who was graduate student, identified structural signals that lead to differential rates of decay of different segments of transcripts in that organism.[142]

More recently, Sue Lin-Chao, a postdoctoral fellow who has now gone back to start her own laboratory in Taiwan, showed that decay of RNAI, an antisense regulator of replication of col E-1 type plasmids, is a key factor in controlling plasmid copy number.[143] The *E. coli* enzyme that regulates this is RNase E and those experiments have led my lab to continue to study RNase E, its specificity, and how it works, and also to continue to use RNAI as a model substrate for the study of RNA decay. A couple of years ago, Feng-Feng Xu, as postdoc in my lab, made the surprising discovery, using RNAI, that polyadenylation of RNA in bacteria influences RNA decay.

Role of Polyadenylation in Bacterial RNA Decay

The history of RNA polyadenylation is interesting. An enzyme that adds multiple adenine nucleotides to 3' ends of RNA molecules was discovered in 1962 in the laboratory of my former postdoctoral mentor, Jerry Hurwitz, by a scientist that Jerry was collaborating with, Tom August.[144] The biological role of that enzyme, which Tom isolated from *E. coli*, wasn't known. Experiments done a few years ago showed that messenger RNA occurs normally in eukaryotic cells, where it affects both the rate of mRNA decay of mRNA and translation of mRNA. Evidence for polyadenylation of RNA in prokaryotes was looked for, but not found. And, it was thought that the addition of polyA tails to RNA occurs only in eukaryotic cells. Later, reports of RNA polyadenylation in bacteria began to appear, but the experiments were criticized and the results were attributed to artifacts. The scientific community continued to doubt that bacterial RNA is polyadenylated, and biology textbooks published before the last year or so continued to state that polyadenylation is a distinguishing feature of eukaryotic mRNA.

A few years ago, while investigating the decay of RNAI in *E. coli*, Feng discovered that RNAI is polyadenylated, and his experiments showed this unambiguously. He also identified the bacterial gene that encodes the enzyme that polyadenylates *E. coli* RNA and showed that mutations in this gene prevent polyadenylation and dramatically slow the rate of RNA decay. His experiments showing a biological role for polyadenylation in bacteria, and two recent papers from other labs that have confirmed and extended his work have attracted other scientists and it's no longer a quiet little area.

Hughes: This seems to be a theme, Stan.

Cohen: Well, many other labs have also added knowledge about RNA decay in bacteria during the 15 years or so that I've been working in the area, but I'm happy about my lab's contributions to the field.

Hughes: Mm-hmm. Well, may I divert you for a moment into the very interesting notion of the acceptance of the scientific idea or fact?

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[142] Belasco, JG, Beatty, JT, Adams, CW, von Gabain, A, Cohen, SN. Differential expression of photosynthesis genes in *R. capsulata* results from segmental differences in stability within the polycistronic rxcA transcript. *Cell*. 1985 January; 40(1): 171-81.

[143] Lin-Chao, S, Cohen, SN. The rate of processing and degradation of antisense RNAI regulates the replication of ColE1-type plasmids in vivo. *Cell*. 1991 June 28; 65 (7): 1233-42.

[144] August, JT, Ortiz, PJ, Hurwitz, J. Ribonucleic acid-dependent ribonucleotide incorporation. I. Purification and properties of the enzyme. *J. Biol. Chem.* 1962 December; 237: 3786-93.

Cohen: I suspected you might find that interesting.

Hughes: Because I, you know, as you were talking, I could think of all kinds of reasons why a given fact would fall on fallow ground or not, but I'd like to hear your ideas—maybe in general, but then in specific—about why this polyadenylation and the association with eukaryotes stuck, you know, even though the facts indicated otherwise. People were disregarding them?

Cohen: Well, largely yes, Sally. If you ask why, I don't know with certainty. But I think it was a combination of a very early publication that looked for and didn't find polyA tails on bacterial RNA and the absence of any demonstration of a biological role for RNA polyadenylation in bacteria. Despite multiple papers to the contrary, primarily from Nilima Sarker's lab, the notion that only eukaryotic RNA has polyA tails somehow stuck.

Hughes: I see.

Cohen: After Feng's data showing that polyA tails have a profound effect on RNA stability were published, RNA polyadenylation became part of the overall framework of knowledge in the area. Although our experiments provided incremental and undeniable evidence that RNA polyadenylation occurs in bacteria, the earlier work, largely by Nalima Sarkar and her collaborators, should not have been overlooked. They had found polyadenylation. They just didn't know what it was doing.

Hughes: Well, it was ignored on one front, but from what you were saying it was not ignored on another, namely as a means of distinguishing prokaryotes from eukaryotes. Well, I'm on shaky ground here in speculation but I'm also wondering about humankind's tendency to categorize. I mean, it makes living in this world easier, and is there a tendency in science, for example, in reference to this prokaryote/eukaryote distinction—again, I'm sounding very deterministic—to try to keep the categories very clear and separate, where in actual fact, they are not?

Cohen: Well…

Hughes: And so it's upsetting to find something that doesn't hold anymore?

Cohen: Well, there are differences between the processing of eukaryotic and prokaryotic RNAs. And without going into a lot of detail, let me say that polyadenylation of eukaryotic RNA occurs after the entire transcript has been made. The mRNA is cleaved in the 3' untranslated region at a specific site and this signals the addition of polyA to the RNA. A series of processing events occur. Prokaryotic RNA isn't known to be processed in the same way. Termination is accomplished differently, and commonly the 3' untranslated region is rather short, certainly in comparison with eukaryotic 3' untranslated regions.

Hughes: Yeah. Well, it sounds reasonable to me. Do we move on?

Cohen: Yes.

Plasmid Partitioning Studies

Hughes: To plasmid partitioning?

Cohen: Okay. Yes.

Hughes: Is that a good leap?

Cohen: Yes. Sure. Plasmid partitioning. As I've discussed at earlier sessions, we've been interested in the biology of bacterial plasmids beginning with my interest in antibiotic resistance plasmids in the late 1970s. Some of my interests related to understanding the mechanisms

underlying plasmid replication and the inability of closely related plasmids to co-exist in the same cell. In the mid and late 1970s, Ken Timmis and Felipe Cabello, who were postdoctoral fellows in my lab, published several papers on these topics. And one could go through some calculations, which Richard Novick and others in the field had done, to come to the conclusion that there was probably an active mechanism to ensure that plasmids would be stably inherited in cell populations—at least for plasmids that are maintained in bacteria in only a few copies per cell. If a plasmid's copy number control mechanism keep that particular plasmid's copy number high, then when the bacterial cell divides there is a high probability—on a random basis—of each daughter cell receiving at least one copy of the plasmid. If a cell receives only one or two copies of the plasmid, the mechanism controlling plasmid DNA replication would bring the plasmid copy number in that cell up to the normal level, so by the time of the next cell division, the copy number per cell would again be high. If, on the other hand, a particular plasmid is normally maintained at a low copy number per cell, and there is not an active mechanism for ensuring that each of the daughter cells receives at least one copy of the plasmid, some of the cells generated by bacterial cell division lack plasmids. Consequently, in the absence of selection for cells containing plasmids, for example, by the addition of an antibiotic that ensures that only cells carrying plasmids will survive, the frequency of plasmid-containing cells in the population would diminish over time. Because this does not occur, there was likely to be a mechanism that could actively partition at least low copy number plasmids to daughter cells when bacteria divide. It was known that in eukaryotic cells, there are structures called the centromeres that go to opposite poles of the cells and drag chromosomes to different sides of the plane of cell division. It was reasonable to think that low copy number plasmids might have an analogous mechanism that does this.

And then in my lab in the late 1970s, David Tu and Peter Meacock, two postdoctoral fellows that were working with different DNA fragments containing the replication region of the pSC101 plasmid, made an interesting discovery. The larger fragment of pSC101 that David was working with was stably inherited in the absence of selection for the antibiotic resistance genes that had been joined to that fragment, whereas the smaller plasmid that Peter was working with showed unstable inheritance. This suggested that there were sequences on the larger piece that might be partitioning the plasmid to daughter cells. And Peter Meacock dissected the plasmid using DNA cloning methods, and identified the critical region. He found that removing the partition region destabilized plasmid inheritance and that putting the sequence of nucleotides back on stabilized the plasmid again. It was possible to use the sequence element—which we named "par," for partitioning—to stabilize the inheritance of even unrelated plasmids. About the same time, partitioning loci were found by others on two other plasmids. A par element was found on plasmid RI by Kurt Nordstrom who was then working in Odense, Denmark. And, a par element was also found by Bob Rownd and his group on plasmid NRI, which they called the "stb," for stability, element. We published our paper a short while before the other two, but all three discoveries or partitioning elements were published the same year and the discoveries were made in all three labs independently. Other labs quickly began to study plasmid partitioning, and very soon this area of plasmid biology was relatively crowded.

[Tape change]

Cohen: I thought initially that in four or five years we would thoroughly understand the mechanism of action of par elements. But now it's been more than 15 years, and there are still things about partitioning that haven't been elucidated. Initially we thought that the pSC101 par

locus, which was a cis-acting site, might work by attaching the plasmid to sites on the cell membrane and that would allow the plasmid to be dragged to daughter cells. But our efforts to find evidence of this were not successful, although we had some data indicating that par did affect membrane binding, and other labs had some similar data. But, the progress of the project was much slower than I had hoped. Experiments that Don Biek, a postdoc in my lab in the late 1980s, had carried out started us thinking about the effects of par on DNA tertiary structure. In 1990, Chris Miller, in my lab, found that the par locus of pSC101 alters the superhelical density of the plasmid, the amount of coiling of the plasmid DNA.[145] And this was totally unexpected. After this observation, we began to think about it a little differently.

Growth Control in Mammalian Cells

Hughes: Very good. All right. Another area you've worked in is growth control in mammalian cells.

Cohen: Okay. I'll say something about that briefly. I've mentioned reporter genes, Sally, and reporter genes have had an important role in understanding gene control in bacteria, not just in our work, but in a lot of work by others. The *E. coli lacZ* gene had also been fused to mammalian promoters and its expression was used as an indicator of the activity of the fused promoter. For some years, I had thought that reporter genes could find even wider use in mammalian cells if a way could be found to use them to identify promoters that were turned on during important cellular events, like movement through various stages of the cell cycle. I persuaded Daniel Brenner and Sue Lin-Chao, who were postdocs in my lab in the late 1980s, to try such experiments, and the system they established enabled us to identify genes that were turned on when mammalian cells deprived of nutrients entered a stage of arrested growth. Almost concurrently, other labs were developing similar systems. They called them promoter trap, which I always have felt was a misnomer because they didn't really trap promoters. The system trapped the reporter gene next to the promoter. But in any case, this is the term that has stuck. And in a paper we published in 1989, we identified two chromosomal promoters that were turned on when mammalian cell growth is arrested. Ordinarily, when you deprive mammalian cells of nutrients, they go into a state of growth arrest and they stop cycling through what is the cell division cycle. Similarly, when cells grow to a high density in culture so that they are touching each other, they also go into a state of growth arrest. Subsequently, the genes attached to those promoters have been cloned and sequenced and they are still being studied in my laboratory and in Sue's in Taiwan.

Hughes: Yeah. All right. Well, does that cover the main areas?

Cohen: Well, probably most of them. For a while, David Gilbert and Kelly Ten Hagen, and Britt Ravnan, who were graduate students in my lab in the mid-1980s studied bovine papilloma virus, which is a small circular replicon in eukaryotic cells. I've been at Stanford for almost 30 years and there are also some other things that we haven't talked about, but maybe we've covered enough.

One positive effect of the diversity of research in my lab has been that postdocs and students coming to the lab for training have had exposure to multiple areas of molecular genetics while they've been here. On the other hand, it has been increasingly difficult to manage a diverse lab as fields that were non-existent when we started—like reporter genes

[145] Miller, CA, Beaucage, SL, Cohen, SN. Role of DNA superhelicity in partitioning of the pSC101 plasmid. *Cell*. 1990 July 13; 62 (1): 127-3.

and plasmid partitioning—have burgeoned.

Hughes: Well, I want to hear more about that…

Cohen: Okay.

Factors in Decisions to Initiate or End a Research Project

Hughes: But in the meantime, could you say something about what factors you consider when you are deciding that a particular line of research should be dropped? You talked about having done that because you realized you were spread out too thin.

Cohen: A key factor is whether I feel that I have unique insights to contribute or my lab has something special to offer, either conceptually or experimentally. There is no point in being a "me too" lab.

Hughes: Another thing that I've noticed as you've been talking is the adoption of different organisms which become the substrates for different lines of research. And perhaps one of the most noticeable, to me anyway, was when you did decided to work with *Streptomyces,* which I believe was after you had visited Hopwood's lab?

Cohen: Right. The reason I decided to go to Hopwood's lab was that *Streptomyces* biology was an area I was considering working in.

Hughes: Yeah. And why? Strictly on scientific grounds or is it more complicated than that?

Cohen: Initially, I was interested in the possible relationship between antibiotic resistance and antibiotic production. I wanted to know more about antibiotic biosynthesis pathways and about *Streptomyces* plasmids. Over the years, my interest in *Streptomyces* biology has grown. My initial studies of RNA degradation were started in *Streptomyces* for reasons I've already mentioned. But I found that I couldn't effectively address the questions I wanted to answer in *Streptomyces,* and moved to *E. coli* as a model system for this research.

Hughes: When you do take on a new organism, talk about what that really means in a practical, everyday sense.

Cohen: Well, I don't really think of it as "taking on" a new organism. A few years ago I shared a taxi with a young scientist from a university in the Boston area, and we got to talking about each other's scientific interests. She said that she knew that I "worked on" several different organisms, and asked why that was the case. I pointed out that I don't really "work on organisms," I work on questions. And I think that's an important difference, because there are many scientists who do "work on" organisms.

Hughes: Yes.

Cohen: Some scientists want to understand everything there is to know about a particular organism, and may spend an entire scientific career trying to fully understand the biology of that organism. But that hasn't been the shape of my own scientific career. I've chosen systems that I've felt are particularly suitable to address scientific questions that I've been interested in answering. A particular system may be suitable for asking certain questions but not suitable for others.

Hughes: All right. I guess what I was trying to get at and it probably will seem very, very prosaic to you, but what—at the level of the lab—does it mean when a new organism becomes a target of research? I mean you had been working with *E. coli,* which is a bacterium and now you are taking *Streptomyces*. For one thing, the culture, I mean, the media are different? You know, how do you prevent cross contamination? How do you train your personnel? Do you bring people in? I mean, you mentioned Mervin Bibb.

Cohen: Well, let's take this example. I went to David Hopwood's lab on sabbatical partly to learn to work at the lab bench with *Streptomyces* in a practical sense. And I came back to my own lab and I had a new postdoctoral fellow, Janet Shottel, to whom I taught some of the *Streptomyces* techniques that I had learned, and she began working in my lab to try to set up a DNA cloning system in *Streptomyces*.

Janet made some progress on the project, but a key factor in advancing the work was Mervyn Bibb's arrival in my lab. Mervyn had trained in Hopwood's lab as a graduate student, and decided to come to my lab as a postdoc. Unlike Janet, he had prior hands-on experience working with *Streptomyces* systems. Merv and Janet formed a great team and things began to move along well. Now what usually happens when a lab starts publishing meaningful contributions in a particular area is that young scientists interested in that area apply to the lab, many of whom have relevant prior benchtop experience. So, after the first few *Streptomyces* papers were published from my lab, I started getting inquiries from potential postdocs who wanted to come to my laboratory to work on *Streptomyces* projects. Some of these young scientists had prior hands-on experience working with *Streptomyces*, so they didn't need basic training with the organism. On the other hand, others did not have an initial interest in working on *Streptomyces* biology or prior experience with the organism, but by then the methods were being used routinely by others in my lab, so it was easy for them to take on a *Streptomyces* project.

Transfer of Scientific Knowledge

Cohen: Generally, there's transfer of knowledge in a lab from one generation of students and postdocs to the next. And part of running a lab is managing things so that transfer of knowledge is efficient. The diversity of science in my lab has made knowledge transfer challenging. Often, in laboratories working for many years in the same area, there is a longtime employee that provides continuity of information, but this isn't really practical for a lab working in multiple areas. So it has been important to have overlap between incoming trainees and postdocs and students who are about to move on to the next stage of their careers.

Hughes: I see. Yeah.

Cohen: But Chris Miller has worked on plasmid partitioning and replication in my lab for many, many years and she has provided continuity in that area.

Hughes: Aren't there also written protocols?

Cohen: Yes. In fact some years ago, my lab prepared what we called the *Cohen Lab Manual*; there's a copy up there on my shelf. It was a collection of protocols we were using for the various types of experiments we were carrying out in the lab. I gave a copy to each entering postdoc or student. We went through a number of editions, but there are now commercially available manuals of lab protocols and we no longer maintain our own procedures manual. But some people prefer the protocols in our old manual.

Hughes: This also is a real interest in the history of science, summarized as tacit knowledge because there is an understanding, probably among scientists too, that you can just get so much down in writing that when you come down to it, in most cases, individuals who have been doing a given procedure for any length of time have a style about doing it that is very difficult to exactly convey unless you're right there watching.

Cohen: Well, sometimes that occurs, but not in most cases.

Hughes: No?

Cohen: I think that what you describe does happen, but scientific outcomes shouldn't be affected by the style of the experimenter. It should be possible to transmit in writing information that enables someone else to reproduce a result, although obviously in the real world it doesn't always work that way. Usually, the reason is that the person writing the protocol wasn't aware of some of factors relevant to the outcome. I don't remember whether I've told you about an incident that occurred in my lab some years ago when I was still working at the lab bench. I had worked out a protocol for getting a high yield of RNA from the mammalian cells that we were using in our experiments, and then I asked Annie Chang, who was then a research assistant in my lab, to follow that protocol and prepare more RNA. She did the experiment according to the protocol I gave her, but it didn't work. And I purified RNA using the same protocol and got a good yield. She tried it again, and it still didn't work. We went though the protocol in detail, and so far as we knew, we were doing the same things. But having gone back and forth on this a few times, we decided to carry out the experiments side by side. There had to be a valid scientific reason for the different outcome. And we discovered the reason why my experiment worked and hers didn't was that the way I had done the experiment wasn't precisely as indicated in the protocol, even though the steps were the same. I had been interrupted by phone calls during my experiments and couldn't complete both the cell isolation and RNA purification in a single day. So I froze the cells overnight and continued with the RNA purification the next day after thawing them. The freezing and thawing wasn't formally a step in the procedure I had designed, but it had resulted in better cell lysis, and a much higher RNA yield. Although I had written down the steps of the procedure accurately, I hadn't mentioned overnight storage of cells in a frozen state. My postdoctoral advisor, Jerry Hurwitz, often said, if you're purifying an enzyme and at a particular point in the procedure, you sneeze accidentally into the prep, and the enzyme purification works out well, make certain that you sneeze into the prep at the same stage of subsequent purifications. Of course that was said in jest, but the point that he was making is valid. Factors that one might not ordinarily consider having an effect on the outcome of an experiment can be important.

Hughes: Yes.

Cohen: Anyway, I think that there shouldn't be anything mystical about the transfer of scientific information.

Intuition in Scientific Research

Hughes: Have you though, in dealing with people in your lab or elsewhere, encountered the phenomenon—I mean the belief that there is an element of mystique—that a procedure has worked by doing it a certain way and somebody else comes along and says, "Well, you really don't need step B and C?"

Cohen: Well, yes. A procedure that works often can be simplified.

Hughes: Yeah. But some people I understand would resist that.

Cohen: Not most scientists. There's almost always room for improvement.

Hughes: Yeah, no, I wasn't meaning so much you, but as much as you would like to make this a totally rational process, at least in some people's minds it is not totally rational.

Cohen: Well, maybe there are some points to be made here, Sally.

Hughes: Yeah.

Cohen: Sometimes it's said that a person has "good hands" in the lab. That means that they have the ability to make experiments work. On the other hand, some people just can't get

repeatable experimental results. And why? Well, I think there are basically two reasons. One is that the researcher may not be following the steps of a protocol rigorously enough. There's not an intent to modify the protocol, but a lack of care in following the prescription. In other instances, a procedure may be intentionally modified by the experimenter and the modification may be detrimental. There are places where a step in a previously-developed protocol can be eliminated and other places where shortcuts can't be taken. Knowing which is which usually is a matter of experience, but also some people are better at deciding what modifications can be made without messing up the outcome. And some scientists are not inclined to make any modification.

Hughes: And you're mainly ascribing that to meticulousness?

Cohen: To some extent, but also there's a certain amount of intuition as well. We can get into a philosophical discussion about what intuition is, but I think that intuition is largely inference that is based on prior experience. Some people are better than others at this.

Hughes: And isn't there an element of just plain manual dexterity as well?

Cohen: For certain things. For example, when I was working at the lab bench in Taiwan a few years ago, I learned how to inject into the nuclei of single cells, but I couldn't do that nearly as well as the person who was teaching me the procedure. That person had a lot of experience doing nuclear injections, but I suspect that even with years of experience, I could not inject nuclei nearly so well. But most experiments in genetics and molecular biology don't require much manual dexterity.

Hughes: Am I correct, though, in inferring that when you really want to learn a *Streptomyces* technique, the best way of doing it is to go to Hopwood's lab, for example, rather than sit down at the library and read a protocol?

Cohen: Well, my decision to go to Hopwood's lab involved more than just learning lab protocols. The principal reason was to learn how David and people in his lab thought about the biology of the organism. Most techniques can be learned from a lab manual, just as someone can learn how to prepare decent-tasting bouillabaisse from a cookbook. But being able to read and follow a recipe doesn't make someone a great chef. When postdoc applicants come for an interview, I ask what they hope to learn from working in my lab. Sometimes they tell me that they'd like to master a particular technique. But most techniques can be learned in a few weeks or months. What a student or postdoc should really be learning from his mentor is how to identify the important scientific questions and how to design meaningful experiments. I did go to Hopwood's lab partly to learn how to work with *Streptomyces* at the bench, but I went mainly because I wanted to better understand what a leader in the field considered to be the most important scientific questions, and to learn what issues needed to be addressed in answering these questions. You can get a certain amount of information from the literature, but how to think about a particular subject is better learned on-site.

On Developing a DNA Cloning System for a New Host

Hughes: Well, another thing that you probably take for granted that I'd like a little bit more explanation about is the cloning system [you developed for *Streptomyces*]. I'm assuming that, not only the word, but the system, is something that developed out of recombinant DNA technology and was not a concept before.

Cohen: But "recombinant DNA technology" is DNA cloning, although as we've discussed, the term hasn't always been used that way. Well, what does it really mean when I say that we developed a DNA cloning system for *Streptomyces*?

Hughes: Yes, exactly.

Cohen: Okay. What that means is that we wanted to use *Streptomyces* as a bacterial host for propagating and making multiple copies of individual genes. To do this, it was necessary to identify a replicon that works as a gene carrier in *Streptomyces*. We needed to have a way of joining other DNA to the *Streptomyces* replicon, and the procedures we had developed to clone genes in *E. coli* were directly applicable. And, we needed some way of causing the *Streptomyces* cells to take up the DNA molecules we wanted to introduce. We also needed a way to identify cells in the population that acquired DNA molecules we constructed. The joining together of pieces of DNA is the least challenging part of establishing a new DNA cloning system. More challenging is finding a vector that replicates in a particular organism and working out a way of getting DNA into that organism.

When I went to Hopwood's laboratory in 1974, I thought that it would be easy to establish a DNA cloning system for *Streptomyces*. Hopwood had genetic evidence that a plasmid, called [SCP1] for *Streptomyces coelicolor* plasmid, was present in the bacteria we were working with. And I tried to isolate that plasmid DNA. My plan was to map its restriction endonuclease cleavage sites and attach other genes to the plasmid. Well, the plasmid DNA isolation methods I tried didn't work. Much later, it was discovered that SCP1 is a linear plasmid, and the methods I was using were aimed at isolating circular DNA from *Streptomyces* cells. Linear plasmids weren't known to exist in the mid-1970s.

There also wasn't a method for introducing DNA into *Streptomyces.* While I was in Hopwood's lab, Hopwood, Mervyn Bibb, Helen Wright, and I tried ways to alter the surface of *Streptomyces* cells to cause them to fuse. It turned out that some of those methods were also suitable for introducing plasmid DNA into *Streptomyces* cells and when Mervyn later came to my lab as a postdoctoral fellow, he and Janet identified another plasmid vector that we could use.

Hughes: Now when you're searching for a vector, is that a very deliberate search? I mean, you have certain parameters in mind?

Cohen: Yes.

Hughes: It's not a random, "We'll try this and see if it works"?

Cohen: There are specific ways to approach the problem. For example, if a replicon indigenous to bacterial species intended as a host can't be identified, it's reasonable to test whether a plasmid replicon from a related organism can be propagated in that microbe.

Hughes: Well, is there anything in particular to say about *B. subtilis* which also was a system developed relatively early on?

Cohen: Yes. That was the cloning system that was the first to follow *E. coli*. There were *B. subtilis* plasmids that could be used as vectors.

Hughes: Now is that why that was the second?

Cohen: Well, the plasmids in *B. subtilis* were part of the reason. And also genetic transformation by DNA had been shown for *B. subtilis*, even before it was demonstrated in *E. coli* actually. *B. subtilis* cells become naturally competent for uptake of chromosomal DNA, so treatment of bacteria with calcium chloride isn't necessary. But, it turned out that the plasmids are not taken up very efficiently by naturally competent *B. subtilis*. Shing Chang, who was a postdoc in my lab in the late 1970s, and I reported a method for getting plasmid DNA taken up with great efficiency in *B. subtilis*.[146]

[146] Chang, S, Cohen, SN. High frequency transformation of *Bacillus subtilis* protoplasts by plasmid DNA. *Mol Gen Genet*. 1979 January 5; 168 (1): 111-5.

Hughes: All right. And that's a cloning system, right?

Cohen: Well, yes. It enabled one.

Hughes: Do you want to stop?

Cohen: I'm happy to continue if you'd like to.

Filter Affinity Transfer (Western Blotting)

Hughes: Western blotting seems to me a fairly important technique. You've mentioned it just in passing [during a non-recorded conversation] and I know you called it something else, filter affinity—what was it—transfer?

Cohen: Oh, okay, that story is interesting. Western blotting is a widely used technique that uses antibodies to detect proteins separated by electrophoresis in gels. I think that the first paper to report this procedure was the one published in 1978 in the journal *Cell*, with Henry Ehrlich, Hugh McDevitt and myself as the authors.[147] But it wasn't called "Western blotting."

Henry was a postdoc in Hugh's lab and the three of us wanted to detect proteins encoded by specific cloned DNA fragments. Henry developed a procedure that we called "filter affinity transfer". He used antibodies to detect protein bands transferred to filter paper from polyacrylamide gels. The procedure didn't receive much attention, possibly because at that time there weren't many scientists who wanted to detect a protein product expressed from a cloned gene.

Prior to our publication, Ed Southern had developed a procedure that transferred DNA fragments from agarose gels onto filter paper, and detected DNA fragments that contain sequences complementary to a DNA probe.[148] The procedure met an important need, and it was quickly and widely adopted. It became known as "Southern blotting." Soon afterwards, a method of detecting bands of RNA bands separated in gels using a similar approach was reported,[149] and I think that was developed largely by Jim Alwine in George Stark's lab here at Stanford. And to distinguish it from Southern blotting, the RNA detection procedure was called "Northern blotting." And a couple of months later, a similar procedure was reported by Wally Gilbert.[150] The following year, protein-blotting procedures were described by others[151] [152] and later called "Western blotting." I think the person who gave it that name was Neal Burnette in Seattle.[153]

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[147] Erlich, HA, Cohen, SN, McDevitt, HO. A sensitive radioimmunoassay for detecting products translated from cloned DNA fragments. *Cell*. 1978 April; 13 (4): 681-9.

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[149] Alwine, JC, Kemp, DJ, Stark, GR. Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl-paper and hybridization with DNA probes. *Proc. Natl. Acad. Sci. USA*. 1977; 74 (12): 5350-4.

[150] Broome, S, Gilbert, W. Immunological screening methods to detect specific translation products. *Proc Natl Acad Sci USA*. 1978 June; 75: 2746-2749.

[151] Towbin, H, Staehelin, T, Gordon, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA*. 1979; 76 (9): 4350-4354.

[152] Renart, J, Reiser, J, Stark, GR. Transfer of proteins from gels to diazobenzyloxymethyl-paper and detection with antisera: a method for studying antibody specificity and antigen structure. *Proc Natl Acad Sci USA*. 1979; 76 (7): 3116-3120.

[153] Burnette, WN. 'Western blotting': electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and

Cohen's View of His Own Research

Hughes: Mm-hmm. All right. This is probably an unfair question, but I'll ask it nonetheless.

Cohen: Okay.

Hughes: I was wondering if you can somehow weigh your experimental work in relationship to what you will inevitably go down in history for, namely the development of recombinant DNA technology—or whatever you wish to call it, how about calling it recombinant DNA science? Do you like that better?

Cohen: That's probably a better term. Well, as I think I've mentioned earlier, when I began my scientific career, my goal was to do work that was intellectually stimulating and enjoyable and that would also contribute to biological and medical knowledge. If my research that produced the discovery of recombinant DNA science had not happened, I would still feel very comfortable about the other contributions that have come from my lab. In a sense, it has been a little unfortunate that other research from my lab has been overshadowed by the recombinant DNA work. I certainly know which scientific contribution is generally viewed as the most important, but I feel that there are other important ones too. I think it's almost like asking which of your children do you love the most?

Hughes: I know, well, I told you it was a bad, an impossible question.

Evolution of Practices for Distribution of Research Materials

Hughes: All right. Back to a prosaic question. We talked at some length about plasmid distribution in the very early days.

Cohen: Yes.

Hughes: And, the fact that you had documented to whom you'd sent them. But my question now relates to the possibility that there was an evolution in the protocol for plasmid distribution and perhaps for any of the materials needed for recombinant DNA. What's in the back of my mind is the relationship, if any, to the development of the biotechnology industry. I'm just wondering if the requirements got stiffer before you would release, say, a plasmid, to a fellow scientist because of what was happening both within and without science?

Cohen: Evolution of the protocol for plasmid distribution?

Hughes: Well, I guess it comes from watching some things happen in Dr. [Rutter's] correspondence, and of course I haven't actually talked to him about it, but it seemed to me that in the early days, there were very simple requirements for giving out a plasmid but as time went on they got [more complicated].

Cohen: Well, maybe in some respects, but I think that the fundamental principles that govern the distribution of scientific materials have remained the same. Inherent in the nature of science is the need to have confirmation of results, and for experiments to be repeated and results confirmed; the materials used in those experiments must be available to others. This much is pretty standard. But as time progressed, it became clear that some DNA constructs had significant commercial value, and these constructs commonly were designated as "TRP," or tangible research property. After publication, a scientist is expected to give out even a commercially valuable construct to another scientist for experiments aimed at confirming

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radioiodinated protein A. *Analytical Biochemistry*. United States: Academic Press, 1981.

results, but not necessarily for other purposes. Cloning a particular gene might have required a couple of years of work, and when a construct containing the gene is sent out without restriction, the recipient immediately has access to the fruits of these efforts and might be able to do, if he wanted to, the very same experiments that the person constructing the plasmid had spent years setting up for. So in sending out such property, a scientist and his university might attach restrictions on what could be done with it, or ask for a share of income generated from commercially valuable discoveries made during its use. Is that what you are referring to?

Hughes: Yeah.

Interview 15: July 5, 1995

ANSWERING SOME GENERAL QUESTIONS

Day-to-Day Management of Laboratory Research Activities

Hughes: Stan, one of the themes of these interviews has been the eclectic nature of your research and I was wondering what that characteristic does to your style of lab management, or the other way around. When you are trying to deal with a great range of different research projects, what sort of management of the laboratory have you evolved?

Cohen: Well, I'm not really sure at what level you'd like me to answer that.

Hughes: I think I mean in a very practical, sort of day-to-day way. How do you make sure that projects progress in a way close to what you would wish?

Cohen: Okay, let's talk for a minute about interactions between me and the people working in my lab.

Hughes: Right.

Cohen: There are graduate students and postdocs working in my lab, as you know, and a research assistant and a research associate. I interact with various people in different ways depending on their abilities and the stage of their training. Some young scientists starting work in the lab can work more independently from the time they arrive, and others need more supervision. And, of course, scientific independence usually increases as the person gains more experience in the lab, and that's an important goal of the training. There are multiple channels of communication that help me manage my lab and train students and postdocs. For example, every week there's a 90-minute general lab meeting that everyone attends, and each week a different person presents experimental results. With between 15 and 20 scientists working in my lab, each gives a presentation two or three times a year. These talks are for the whole lab to listen to and discuss.

I also meet regularly with people in my lab individually, usually for an hour or 90 minutes each time to go over experimental results. These meetings are arranged on an *ad hoc* basis, and the frequency depends on the stage of the project, the stage of training of the student or postdoc, and whether there is a particular experimental issue to discuss or new data to interpret. I sometimes stop at a lab bench to talk informally with a student or postdoc about the outcome of an experiment and they pop their heads through my office door to discuss a result or problem. Sometimes these brief discussions lead me to set up an appointment for a longer meeting. Some students readily come to see me when they are having a problem

with their experiments, while others prefer trying to first come up with a solution independently. Both styles are fine, but if I haven't interacted with someone over a period of several weeks, even in a casual way, one of my favorite questions is, "Is there anything I should know?" That question often initiates discussion of a problem. And prior to lab meeting presentations, some students like to stop in for a brief discussion that helps them prepare for the lab meeting.

Writing scientific papers also is done differently with different people in the lab. Usually an initial manuscript draft is written by the student or postdoc, and if the draft is a good one, I usually make modifications in pencil, raise questions as needed either on the draft itself or at a meeting, and give the manuscript back to them to prepare the next version. Usually a paper goes through at least three or four revisions before it is submitted for publication, sometimes more. Some students write more clearly than others, and some think more logically than others. Sometimes a manuscript needs to be totally rewritten from scratch, and I'll just do that on my computer and give the re-written manuscript back. In fact, I'm going through one of these total re-writes right now. Although the experiments are clearly described, I feel that the "story line" and the sequence of presentation of the data should be modified. So I'm totally rewriting most of the manuscript.

Funding of Lab Research

Part of lab management involves obtaining funds to support the research. New research proposals or renewal applications that require peer review generally involve requests for 4 or 5 years of support, and it's necessary to think in some detail about the long-range goals and to describe experiments that I plan to carry out several years in the future. Although I don't enjoy preparing grant applications, having to write them imposes the discipline of having to sit down and think not only about the next series of experiments and about the experiments just beyond them, but also to plan much further ahead. Normally, I tend to think about where the work will be going during the next three or six months, or maybe even during the next one or two years, but preparing grant applications prompts longer-range thinking.

When I offer someone a postdoc position in the lab, I have been able to guarantee stipend support for at least two years, but I generally also ask the person to submit an application for funds from the NIH or another outside organization. More often than not, postdoc applicants receive these individual fellowship awards, and that allows me to recycle funds that I've reserved for them. It also helps a postdoc's career to have successfully competed for an individual fellowship award. Research proposals prepared are usually submitted before actual arrival of the postdoc in the lab, and prior to the submission, I sit down with the applicant either in person or during a long phone call and discuss potential projects. Usually, I try to suggest a project that relates not only to a project going on in my lab, but also relates to some extent to the postdoc's prior background, and this helps in the preparation of a solid research proposal. But frequently, the project described in the proposal is not the project they work on when they get here. Postdoc arrangements in my lab usually are made 12 months or longer in advance, and sometimes the arrival of a new postdoc is delayed. During the interval, discoveries made in my lab or elsewhere, can lead to a change in focus of the proposal or to modification of the experimental approach. But even when that occurs, I think it's useful for a postdoc to have prepared the research proposal.

Sometimes a student or a postdoc will be interested in working on a project that hasn't been included among my list of suggestions. Some of the most creative postdocs coming into the

lab have done this. And we sit down and discuss the scientific questions the person would like to ask. If I think that the idea they have is sound and it is related to my overall scientific interests, they can proceed with the project.

Occasionally a student or a postdoc will come up with an idea that's not related to ongoing work in my lab, but is nevertheless very interesting, and I've suggested that the student pursue this as a "second project." So the answer to your question is that there's no prescription for lab management. It varies very much on the circumstances and the people.

Interactions with Students and Postdocs

Hughes: At the lab group meetings, is the focus on many levels and, I mean, not only on the scientific content but also on the methodology?

Cohen: The lab group meetings are intended to be progress reports. The meetings help postdoctoral and graduate students learn to critically evaluate data, and in the discussions, students often come up with suggestions that are useful to others in the lab. Not infrequently, a suggestion is one that I hadn't thought of. It's a very useful mechanism of scientific exchange.

Sometimes I receive a manuscript to review, and if my schedule doesn't allow me to do a timely review, I contact the editor and ask whether the task can be assigned to a postdoc in the lab. Almost always, the editor agrees to this. Before the review is submitted, I will sit down with the postdoc and go over the points covered by the evaluation. I read the manuscript, but don't spend the amount of time with the manuscript that would have been needed for me to do a primary review; this enables me to discuss the review with the postdoc. Learning to critically review and evaluate papers is also part of the training that young scientists should receive.

When someone is nearing the end of a period of training in my lab, prior to job interviews or Ph.D. thesis dissertation defenses, I usually have practice sessions with postdoctoral and graduate students, as I think I've mentioned to you. Some students need more practice than others. Most of the practice sessions are attended by other students and postdocs in the lab. And comments and suggestions come from multiple sources. Most of the time there's been very good feeling of camaraderie among the people I've had working in my lab, and they genuinely enjoy helping each other.

But despite the generally good feelings that people in the lab have about each other, small problems come up regularly. After the scientific presentation part of the lab meeting, and after questions and discussion about the science, we spend a few minutes every week on what we call "business matters." We briefly discuss issues that arise in the day-to-day operation of the lab, such as whether or not a new supplier of a reagent has worked out well or problems that may come up in the use of common supplies and equipment. Sometimes someone leaves a mess after using common equipment or consumes the last amount of a reagent and doesn't re-order. When you have 15 to 20 people working together in the same lab on separate projects, and each of them is intensely interested in his or her own experiments, people sometimes forget about the problems and needs of colleagues in day-to-day interactions. That's sort of incongruous, because the same people care enough to spend time going to each other's practice sessions for job interviews and thesis defenses and to help them solve scientific problems they are encountering. Anyway, does all of this give you a picture?

Hughes: Yes, that does give me a picture. In terms of your interaction with your lab group, where do you suppose you fall relative—I know this is an impossible question—to the operation of other lab groups that you're familiar with?

Cohen: Well, I've actually thought about that, Sally. I often feel that I am under considerable time pressure and can't do everything I'd like to in running my lab, but I know that there are colleagues who successfully manage much larger laboratories and yet seem to have much more time for themselves and feel less pressure. I've concluded that I am probably more "hands on." P.I.s with smaller labs are probably involved as much as I am in the day-to-day workings of the lab, but I think my level of involvement is unusual for labs the size of mine. For example, I spend a lot of time on manuscripts, and virtually no manuscript goes out without my having rewritten a major part of it. But one of the reasons that I spend so much time on manuscripts is that I enjoy the scientific discussions I have with students and postdocs about them.

The same is true about knowing the details of experiments that researchers in the lab are doing. Some scientists that have large laboratories work through intermediaries, who meet with the P.I. and then direct the research of less senior people in the lab. But that has not been the way I've chosen to run my lab. One of my excellent former students, David Gilbert, went as a postdoc to the laboratory of Pierre Chambon, who's a first rate and very well known French scientist. Pierre has a laboratory of almost a hundred people, and David, who is extremely independent in his scientific thinking and scientific approach and who knew about the size and style of Pierre's laboratory before he went there, was nevertheless somewhat disconcerted by the fact that he had talked with Chambon himself only three times during the two years that he spent in his laboratory. He met with Chambon when he arrived, once about a year later, and once before he left. At other times, David was mentored by someone more senior to him, but who was an intermediary. But that system works well for Pierre, whose lab has made absolutely major scientific contributions, and also worked well for David, who did a very nice piece of research in Chambon's lab and published several excellent papers. Many labs, especially large laboratories, work that way. That is probably more common in Europe than in the U.S., but even in laboratories the size of mine, I think that most P.I. are not as much involved in day-to-day interactions over the science as I have been.

Hughes: In the end, do you think that laboratory management is a reflection of personality?

Cohen: Yes, I think that personality is an important determinant in lab management, but I think that lab management also varies with the stage of the P.I.'s career. I think I told you during an earlier discussion that when I had a small laboratory, I would go out and buy ice cream on Friday afternoons for the three or four people in the laboratory and it was a much more intimate style of lab management. That style is not practical with the number of people that I have. I guess I could get someone to help me to carry buckets of ice cream, but it's more complicated than that.

Over the years the views of students and postdocs about their scientific careers have also changed to some extent. The people in my lab are still excited by science and are very motivated, but I think that they, as a group, are probably more rounded individuals than the postdocs and students I had in my lab 15 or 20 years ago. A larger percentage of them are married and have families, and whether they are men or women, they are balancing their scientific careers with family life, and that may be part of it. Overall, I think that the scientific atmosphere is less intense than it was when I first started the lab. That isn't a situation unique to my lab. I've heard this feeling expressed by others who are my contemporaries.

Hughes: Considering that you, like so many scientists at your level, I mean your seniority, are no longer doing bench work or only sporadically doing bench work, what difference, if any, does that make in your grasp of the details of the science itself?

Cohen: That's a very good question. Initially, all of the procedures done in my lab were ones that I

had carried out previously with my own hands.

Hughes: Right.

Cohen: And when I discussed an experimental procedure with a student or postdoc in the lab, I had the perspective of someone who had personally done that experiment or that type of experiment in the relatively recent past. Today, there are many procedures being used in my lab that I have not personally performed with my own hands. But, I have been surprised by the extent to which it's possible to anticipate technical problems occurring in procedures that I've not actually done myself, and to ask the right questions necessary to identify these problems. As I've mentioned, a few years ago when I was spending time working in Taiwan, I went back to working at the bench and I was also surprised to discover just how much satisfaction that gave me, and to find that I can still do good experiments.

Hughes: Makes one feel good.

Cohen: That's right.

Perceptions About Other Labs

Hughes: How do you think your lab is perceived by outsiders?

Cohen: I don't think that there's a general answer to that. I suppose it would depend on which outsider you ask. I'm not being flippant, but….

Hughes: No, no, no, I know you're not.

Cohen: …I just don't know how to answer that.

Hughes: But I'm presuming that like a class in college, that lab groups have characteristics that somehow persist over time. I mean that the Cohen lab means something to those in the know, I mean, some images float before the mind. Am I right?

Cohen: Well, I guess so. I imagine that's the case, but I also think that the images that float to mind depend on whose mind they are floating in.

Hughes: Right. Yeah.

Cohen: I hope that my lab is perceived as one that has made meaningful contributions in diverse areas of science.

The principal factor that creates impressions about a lab is the research coming from it. But perceptions about a lab are also influenced by the perceptions of trainees that have been there, as well as by what these people accomplish during their own scientific careers. Each trainee who has been through a lab has a snapshot view of the lab as it existed at that time, and these snapshots collectively also contribute to the overall picture that the outside world has of a lab. Outside scientists who interact with people who have actually worked in a lab get a perspective that may be different from the perspective of those who haven't had that interaction, but have only read the scientific papers coming from a lab. I think that there's likely to be a whole spectrum of perceptions, so I don't know that there's a simple answer to your question.

Hughes: What about characterizing the atmosphere?

Cohen: Again, I'm not sure that there is a general answer to that question. I don't think about the atmosphere of laboratories that I haven't worked in. I certainly have an opinion about the quality and creativity of research from other labs. But any information about the day-to-day workings of a lab usually comes through people who have spent time there.

Hughes: Right.

Cohen: And it's not a primary impression, it's secondary information.

Hughes: Well, yeah, although I'm suspecting that there are certain characteristics that those at Stanford, for example, would have. And I'm thinking in, you know, in broad outlines; I mean, the fact that you have described a very interactive style of lab direction, in that you do participate at many levels in what actually is going on in the lab, would tell people something about what it would be like to be in your lab.

Cohen: Yes, but I don't think that other faculty would know that.

Hughes: Oh, they wouldn't?

Cohen: Well, not directly. But certainly there are things that one hears about other labs because students and postdocs from different labs talk to each other. Sometimes information about labs filters from students and postdocs to their advisors.

I also expect that someone who's considering coming to my lab as a student or postdoc would speak with the students and postdocs already here. In fact, when I invite candidates for interviews, I routinely schedule time for them to meet with people currently in the lab to get a feeling for the lab. And, of course, I seek the opinions of my current people about the candidate. It's the practice of my department at Stanford and of most others in the basic sciences to have graduate students spend a period rotating in a laboratory before deciding on a laboratory for a thesis project.

Hughes: Right. Yeah.

Cohen: But it's true that over time, faculty acquire reputations for being good or not so good mentors, and there's usually some basis for the reputation.

A formal opportunity for assessing, although indirectly, the quality of mentorship in other labs, and I suppose to some extent the atmosphere of the lab, is provided by service on advisory committees for students mentored by other faculty. We serve on thesis committees for other faculty members' students and this provides information that leads to impressions about other labs. Review of a student's thesis work certainly results in impressions about the lab, as well as in assessment of the intellectual ability and accomplishments of the student. And sometimes I see a student who seems not to have extraordinary ability but has come up with an extraordinary piece of work. And in that case I think that the student has probably been mentored extremely well. I can imagine that the advisor has put in a lot of work with that student. Sometimes the advisor seems to have had only very limited interaction with a student and seems almost detached from the work. So one gets those kinds of impressions.

Hughes: Yeah, I see.

Cohen: And over the course of many years on a faculty, there is interaction with enough students on enough thesis advisory committees to draw some inferences about the mentorship style and mentorship abilities of faculty colleagues.

About Ethical Behavior in the Lab

Hughes: Well, we've talked a little about intellectual property, I think mainly in terms of order of authors on papers, but is there more to say in reference to the lab group per se? Again, I guess I was prompted by one of the articles in the most recent issue of *Science*, in which one individual was credited with actually keeping track, or attempting to keep track, of who initiated ideas in his lab group.

Cohen: Mm-hmm.

Hughes: Is this something that you are concerned with in directing the lab?

Cohen: In terms of initiating ideas?

Hughes: Yeah, or just making sure that people get credit where credit is due.

Cohen: Oh sure. If there are multiple students or postdocs working on a project, it is especially important to see that they get appropriate credit for their respective contributions. When a student or postdoc arrives in the lab, I'd say about 80 to 90% of time, I either assign a project to them or give them an option of several projects, and they focus on one or two. And most often students and postdocs work on multiple projects, and some of these are in collaboration with others in the lab. And if the collaboration leads to publishable fundings, eventually the matter of how the authors should be listed comes up. Usually, it's clear who has been carrying the ball in a collaborative project, but sometimes it's less certain. It's not always the person who initiated the work who is the first author; sometimes a student or postdoc who joins a project team later may devise a new approach, make crucial observations, or provide a major insight.

Hughes: Do you consider it your responsibility to instill, in whatever way, ethical standards in science?

Cohen: To instill?

Hughes: I didn't want to put it quite that forcibly. But is there an aspect of your directorship of the lab which involves, either directly or indirectly, consideration of ethical means of conducting one's self in science?

Cohen: Yes, there is certainly a means of conducting oneself ethically in science, and I try to help my students and postdocs understand what is ethical behavior and what is not. When questions come up about how to proceed on a matter involving ethical issues, we discuss what is appropriate. Sometimes a person has every intention of acting ethically, but doesn't know what standards to apply and needs more information to know what the ethical course of action is. One way I try to do what you're referring to as "instilling ethical behavior" is by example. Students working in a lab observe and think about how their mentor behaves.

And ethical issues come up in various ways. For example, in considering intellectual property and credit, one part of behaving ethically in science is the appropriate citation of prior research contributions. I take that seriously in writing papers and in evaluating papers by others, and I believe that I'm a stickler for accuracy in assigning credit for scientific discoveries. I guess at this point we don't have to go into the reasons.

Ethical issues arise commonly in day-to-day activities of a lab. Some have to do with the exchange of information. Let me give you an example: There was an incident several years ago where I mentioned newly obtained observations from my laboratory during a private discussion at a scientific meeting. It was clear from the discussion and from subsequent correspondence that the scientist I was speaking with hadn't known about the phenomenon I revealed to her and was not at all familiar with the approach I described. A few months later, she sent me a friendly note that contained photos taken at the meeting, and casually asked how things were going and whether I had written up my findings yet. I sent back a note thanking her for the photos, and said no, not yet. Some three or four months later, I was asked by the editor of a scientific journal to review a manuscript that the other scientist had submitted. When I read the manuscript, I saw that the experiments and discovery that she was reporting were similar to what I had described to her six months previously. The findings were presented entirely as her own. Well, okay, so what to do? Her manuscript was hastily put together and proper controls were not done for many of the experiments. I felt that it was a bad paper scientifically, and if I were to formally evaluate it, I could not recommend publication. But under the circumstances, my objectivity could reasonably be

questioned, so I decided not to serve as a reviewer. I sent it back to the editor promptly, indicating that I had a conflict of interest and could not do the review.

Hughes: So modeling is a major part of teaching ethical behavior?

Cohen: Yes, but for me, teaching ethical behavior hasn't involved an intentional effort to provide a good model. I guess it may sound funny, but I think that one of the reasons why I try to behave ethically is that I think it's personally demeaning to do otherwise.

Hughes: Yes. Yeah.

Cohen: …in the sense that it is demeaning to have to live with the thought that it's necessary to stoop to being unethical in order to be successful. At a symposium planned by my students and postdocs to celebrate my 60th birthday, one of my former students [David Stein], who was making after-dinner comments, said that one of the most important things he had learned from being in my lab is that one can behave ethically in the very competitive world of science and still be successful.

Hughes: That's very nice.

Cohen: Yes it was. But beyond the philosophical and moral and, for some, religious reasons to behave ethically, I was deeply moved to be told that my personal views about ethical behavior had been well communicated.

Hughes: Well, what you describe seems to me to be summarized in the fact that your ethics are a natural extension of your life. But the reason I belabor this point is…

Cohen: Because the subject is interesting?

Hughes: Well, that too. But also it has been much in the public eye and you know, one ramification of that being the current focus in *Science*. But another sign of that, I think, is the fact that, well, my understanding is that since 1992 Stanford University indeed has had a course on scientific ethics. Is that an obligatory thing for students to attend?

Cohen: I don't think so.

Hughes: No. Do members of your lab group attend?

Cohen: Some of them do. Courses that teach students the parameters and standards of ethical behavior are useful, but they don't substitute for teaching ethical behavior during everyday activities, and I feel that this can best be done by a student's scientific mentor.

On Holding an M.D. Degree and Doing Basic Scientific Research

Hughes: Would you comment on what effect, if any, it has had on the way you do your science to be located in a medical school and the holder of an M.D. as opposed to a Ph.D.?

Cohen: Well, the answer to that is a little complicated. As you know, when I first came to Stanford, my primary appointment was in the Department of Medicine. My activities were split between basic research and clinical responsibilities. I didn't want to be in the situation where my basic science colleagues would say, "Well, his science is not so great but the science is okay for a physician trying to do doing basic research." Or where my clinician colleagues would say, "Well, he's not such a great clinician but he's working in the laboratory so we really can't expect him to be a first rate clinician." Eventually, I decided that I couldn't remain clinically involved and still be competitive in my area of basic research.

At this point, the type of doctoral degree I hold is not really relevant to how my scientific contributions are viewed. And even going back to when Lederberg offered me an

appointment in the Department of Genetics, he didn't consider the type of degree. I think that my training as a physician has been useful to me scientifically in the sense that it has given me a broader perspective about biology.

Interactions with Colleagues at Other Institutions

Hughes: All right. Earlier in our discussions we talked of the plasmid group and I'm wondering if an entity that identifies itself, at least loosely, in that fashion still exists. And, if so, is there a system that goes along with it that involves sharing materials and ideas and a certain work ethic?

Cohen: Well, I think that in most areas of science, there are groups of individuals that become friends and feel a certain affinity to each other and have similar ideas and views about the science. This is certainly the case in the scientific areas that have been a focus of my research over the long term: plasmid biology, *Streptomyces* biology, and RNA decay. I've formed both personal and professional relationships in each of these areas.

Hughes: Maybe I'm searching for too much definition, but I'm wondering, again like lab groups, do each of these groups have a certain understanding of how things should work that might differ from how things would be understood in another group?

Cohen: Well yes. People who view science similarly often develop more affinity to each other. But, one can also make friends with scientists in the field of research who interpret things differently.

For a few years, I used photosynthetic bacteria as a model system for studying some aspects of RNA decay, as I've mentioned. I had become interested in the role of RNA decay in controlling expression of genes that encode the photosynthetic apparatus in *R. capsulatus*. And when I, as a newcomer to the field, attended scientific meetings on photosynthetic bacteria, I discovered a surprising amount of animosity among leaders in the field. The basis for this animosity was disagreement about the interpretation of scientific results. And the animosity made it unpleasant to do research in the field at that time. That wasn't the principal reason why I stopped working with photosynthetic bacteria, but it was a contributing factor. It just wasn't fun.

On the other hand, in the research area of *Streptomyces* biology, there has been friendly interaction and genuine cooperation between scientific competitors. Most workers in the field have, at one point or another, been through David Hopwood's lab in Norwich. David is thought of as the father of modern *Streptomyces* biology and he's always been open in freely communicating information and sharing materials. That has set the overall tone in the field. In the area of plasmid biology, there are groups of people that have worked in the area for so long, and have worked together in so many different frameworks that we've become good friends and care about each other personally and try to help each other in every way possible. There's a lot of mutual good feeling. In the area of RNA stability, the situation is mixed. There are some scientists that have a feeling of competitiveness that overwhelms the general feeling of good will among workers in the field, but even so, there is civility—unlike what I had observed in the photosynthetic bacteria field. So, yes, each area has its own "personality" and there are different dynamics among scientists working in different fields.

How Cohen Views His Professional Identity

Hughes: How do you identify yourself in a professional setting?

Cohen: I suppose primarily as a geneticist or a geneticist-microbiologist—at least in a professional setting. At a cocktail party or some other social event unrelated to any professional activity, when someone says, "What do you do?" I say that I'm a geneticist. Most non-scientists know what a geneticist is, at least at some level.

Hughes: But you're unlikely to identify yourself as a physician?

Cohen: That's right. I've been trained as a physician, but I haven't been involved in clinical medicine for twenty years and my daily activities are not the activities of a physician. What I do every day is a combination of genetics, microbiology, and molecular biology.

Hughes: How do you define molecular biology? Do you want me to say more than that or is that enough to go on?

Cohen: Well, I think that historically it's an outgrowth of the traditional scientific disciplines of biochemistry and genetics and it has elements of both disciplines.

Hughes: But I get confused, well, let me step back. Could you some years ago, maybe you're not old enough, but say you were at that cocktail party in the 1960s, would there have been any tendency for you or somebody like you to identify yourself as a molecular biologist?

Cohen: Probably not at a cocktail party because most people wouldn't have understood what a molecular biologist was.

Hughes: Yeah. All right. Well, pick another setting then.

Cohen: Well, I don't really know. At some universities, there are Departments of Molecular Biology, so molecular biology has become recognized as a distinct academic discipline. There's a division of FASEB [Federation of Associated Societies of Biology and Experimental Biology] that used to be called the Biochemistry Division but now it's called the Molecular Biology and Biochemistry Division.

I suppose that departmental affiliation has a role in determining how people identify themselves. When I was elected to the National Academy of Sciences in 1979, it was necessary for me to decide on which NAS section I wanted to affiliate with. Often a scientist's interests fit well with multiple NAS sections, and several sections of the academy may recruit newly elected members. I was invited to affiliate with the Biochemistry and Genetics Sections, and also with the Microbiology Section. There is no molecular biology section. I chose Genetics, and learned afterwards that that section had, in fact, been the one that proposed me for election to the NAS. I suppose that this gives you an additional indication of how I identify myself professionally. And of course, my primary faculty appointment is in the Department of Genetics.

Hughes: Okay. Yeah. Since molecular biology is so pervasive, I'm wondering, to scientists themselves—or biological scientists themselves—is it, to them, namely a discipline or is it a methodology or is it a conceptual approach or…

Cohen: I think that the term molecular biology may have originally come from writings of François Jacob. It's a discipline aimed at describing and defining biological events in terms of the molecules that are involved. And in the late 1950's and early 1960's, that notion became popular. Prior to that time, there wasn't much knowledge about the molecular transactions that underlie biological processes. One could describe consequences of genetic matings and follow the inheritance of traits without understanding the molecular basis for those events.

How New Projects Are Started

Hughes: Can you say something about how you go about thinking of a scientific project? I mean, what actual processes do you use? For example, is it a very visual thing with you or how do you start?

Cohen: Well, there's no simple answer to that. It varies. Often, new projects relate to results obtained during the course of our experiments, and they're extensions of current work. A novel observation made during the course of a study sometimes opens up a new area, and this involves following your nose and instincts. Sometimes reading an observation reported in the literature or listening to a comment made in a seminar I attend causes me to think about one of my own ongoing projects differently and alters its direction.

On Scientific Insight

Hughes: Mm-hmm. Well, that gives me an idea. In your experience, is there an intuitional or even irrational component to doing science? Ever? I mean, not as a steady thing.

Cohen: Well, I think I've previously mentioned my opinion that "intuition" results from a collection of impressions from prior experiences. It involves using experience to make judgments. I often have "gut feelings," and that's the term that I usually use rather than intuition, about whether a particular approach or idea is likely to pan out. It's usually difficult to define the factors underlying that kind of feeling, but sometimes I can. Sometimes I'll say, "Well, my gut feeling is…" and then ask myself why I have that gut feeling. Usually, I can identify at least some of the reasons. I don't view intuition as being something spiritual.

Hughes: Have there been times that you can recall when you've had a sudden insight and the pieces have fallen into place?

Cohen: Yes. Some of the insights I've had have occurred just as I've been about to doze off to sleep, and sometimes I wake up from sleep with an insight in the middle of the night. So, that's a very interesting phenomenon in the sense of how the mind works. Of course, the brain is active during sleep and I suppose that when it is relieved of whatever constraints are imposed by the state of wakefulness, the resulting thought processes can produce an insight. My guess is that someone probably has investigated this scientifically.

I keep paper and a pen nearby so that I can write down thoughts that occur during the night. I've found that if I don't write down the thought or insight, I have great difficulty falling back to sleep because I continue to think about the issue. But after writing down the thought, I can stop thinking and can fall back to sleep.

Hughes: How often roughly does this occur?

Cohen: Well, it has varied, but in recent years, I'd say about two or three times a year, or maybe four times a year.

Hughes: Is it your belief that biological processes can be explained adequately in molecular terms?

Cohen: Yes.

Hughes: Would you care to elaborate?

Cohen: No. Neurobiology is not an area where I have expertise. But in a general sense my opinion about this stems from the view that all biological processes can be explained in molecular terms.

Hughes: But, adequately? I mean that, in other words, that's all there is?

Cohen: Yes. Am I a mystic? The answer is no.

Hughes: Yeah, well, mystic is maybe pushing it a bit far, but even scientists sometimes talk about emergent phenomena, things that are more than the sum of the parts.

Cohen: That's true. But I think they may have that impression because they don't understand everything about the workings of the parts. I think that when biological phenomena are understood more thoroughly, there are logical scientific explanations. But there may be external factors such as cosmic rays that influence molecular functions or interactions.

Hughes: So you're in no way a vitalist?

Cohen: That's correct.

What Science Is

Hughes: I could have guessed. Would you care to define what you consider to be science?

Cohen: Well, the goal of science is to obtain knowledge and understanding. I think that the key to good science is being able to provide such knowledge. This involves defining the important questions to ask, and in the experimental sciences, designing experiments that definitively answer them. Or if this is not possible, designing experiments where the answer helps to provide a direction for future experiments. Basically, good science comes from knowing the appropriate questions to ask at multiple stages of the project and interpreting the results correctly. This includes doing the right controls to ensure that the interpretations are unambiguous.

And I think that some science is done by people who design experiments that don't answer questions or test hypotheses. My students have learned not to say, "I'd like to do this experiment and see what happens." Certainly, sometimes it is necessary to simply accumulate information before a testable hypothesis can be formulated, but ultimately, I think that definitive answers to most scientific questions involve hypothesis-driven research. The public often views scientists as simply doing experiments "to see what happens." Some scientists do this, but in my opinion, not the good ones.

Cohen's Life Outside of Science

Hughes: We've talked almost exclusively about science and its ramifications. Give me a little sketch of what you do that is not science, and you also may want to say a word about what role your wife has played in your life?

Cohen: Okay. What I do that is not science? You mean other activities that I…

Hughes: What are your nonscientific interests? Maybe you could start by saying how much of your life roughly is spent not doing science.

Cohen: Well, I don't know that I would want to put a percent on it, but an important focus of my life has been my children. I've spent a lot of my life on issues related to my children. They've brought me much joy, but there also have been medical problems in my family that have been emotionally demanding and time consuming. For fun, I play the banjo, I ski, and I enjoy sailing, though I haven't been out in my sailboat probably since last November, and here it is July. I read, not novels at any frequency, but I enjoy reading non-fiction and I read newspapers. I read through much of the *New York Times* most every day, and have done this for almost 50 years. I consult for companies. I spend time enjoying my family and

friends—the kinds of things that most people do in their lives. I don't know whether that's the level of detail that you had in mind.

Hughes: No, that's right.

Cohen: And I've been married, since 1961, to one person. We have two children, Anne and Geoff. My wife, Joan, is a graduate of the college that you also attended, Bryn Mawr. She has worked for a number of years as an editor for the *Annual Reviews* publisher. She's not a scientist, but tries to understand my work, and she enjoys having me try to explain something that I'm excited about scientifically.

Hughes: All right. The last question: What do you consider to be your greatest contribution?

Cohen: To science?

Hughes: Whatever.

Cohen: That's certainly not an easy question to answer. I think that most people would say my greatest scientific contribution has been the invention of recombinant DNA, DNA cloning. Although I've been very happy about many other things I've done in my scientific career, if you're asking about what contribution I think has had the greatest overall impact on biology and on the world, I suppose that would have to be it. On the other hand, it may turn out that my greatest scientific contribution will be the legacy of scientists that I've trained, who have gone on to train additional young scientists. But probably the most important thing I've done during my lifetime, at least the most important to me, has been to be able to help my daughter who was born with serious medical problems, to live a life that is now working out very well for her.

Hughes: A nice note to end. But I've asked a lot of questions and I'm sure I've left out lots of things. Is there something, or things, that you want to talk about?

Cohen: Not at this point, Sally. What I think we should do is see what the transcripts look like and there probably will be some additional things that come up at that time and we can try to fill in if it's appropriate.

Hughes: Okay.

[End of Interview]

Dates of Interviews and Corresponding Tapes[154]

January 11, 1995
Tapes 1-2

January 18, 1995
Tapes 3-4

February 1, 1995
Tapes 5-6

February 7, 1995
Tapes 7-8

March 1, 1995
Tapes 9-10

March 7, 1995
Tapes 11-12

March 22, 1995
Tapes 13-14

March 29, 1995
Tapes 15-17

April 5, 1995
Tapes 18-19

April 14, 1995
Tapes 20-21

April 18, 1995
Tapes 22-23

May 5, 1995
Tapes 24-25

June 7, 1995
Tapes 26-27

June 23, 1995
Tapes 28-29

July 5, 1995
Tapes 30-31

[154] Portions from interviews have been moved for better continuity, as indicated in the transcript. This is a guide to the tapes in accordance with their interview date.

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APPENDIX: TABLE OF CONTENTS

Plasmid Conf

October 24, 1972

Dr. Herb Boyer
Department of Microbiology
University of California Medical Center
San Francisco, California

Dear Herb:

As a followup to our telephone conversation, Don Hellinski and I would like to formally invite you to attend the joint U.S.-Japan Conference on Bacterial Plasmids to be held in Honolulu, Hawaii on November 13, 14 and 15, 1972. The Conference is being sponsored by the NSF and its counterpart Japanese agency to enable the free exchange of recent developments and ideas between U.S. and Japanese investigators in the field of bacterial plasmids.

The guidelines of the sponsoring agencies allow a limited number of invited discussants from this country, and it is a pleasure to invite you to attend this meeting to take part in the Conference discussions and to present data on your research. A list of investigators who are planning to attend the meeting is enclosed. Unfortunately, no funds are available from the NSF Cooperative Science Program for your support, but on the basis of our telephone conversation, I anticipate that you will be able to obtain funds from another source. Your participation will contribute in a major way to the success of the meeting.

We have notified the NSF about this invitation, and have indicated your acceptance. I expect that you will be receiving information from Mrs. Laurie Trent of the NSF about the meeting, but I suggest that you proceed to make your own hotel and travel arrangements as soon as possible. Some of the investigators attending the meeting will be staying at the Kaimana Beach Hotel, but I understand that a number of people will be using other hotels. All sessions will be at the East-West center.

We are delighted with your verbal acceptance of our invitation, and look forward to seeing you at the meeting. With best regards,

Sincerely yours,

Stan Cohen

SC:no

Enclosure

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Construction of Biologically Functional Bacterial Plasmids *In Vitro*

(R factor/restriction enzyme/transformation/endonuclease/antibiotic resistance)

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ABSTRACT **The construction of new plasmid DNA species by *in vitro* joining of restriction endonuclease-generated fragments of separate plasmids is described. Newly constructed plasmids that are inserted into *Escherichia coli* by transformation are shown to be biologically functional replicons that possess genetic properties and nucleotide base sequences from both of the parent DNA molecules. Functional plasmids can be obtained by reassociation of endonuclease-generated fragments of larger replicons, as well as by joining of plasmid DNA molecules of entirely different origins.**

Controlled shearing of antibiotic resistance (R) factor DNA leads to formation of plasmid DNA segments that can be taken up by appropriately treated *Escherichia coli* cells and that recircularize to form new, autonomously replicating plasmids (1). One such plasmid that is formed after transformation of *E. coli* by a fragment of sheared R6-5 DNA, pSC101 (previously referred to as Tc6-5), has a molecular weight of 5.8×10^6, which represents about 10% of the genome of the parent R factor. This plasmid carries genetic information necessary for its own replication and for expression of resistance to tetracycline, but lacks the other drug resistance determinants and the fertility functions carried by R6-5 (1).

Two recently described restriction endonucleases, *Eco*RI and *Eco*RII, cleave double-stranded DNA so as to produce short overlapping single-stranded ends. The nucleotide sequences cleaved are unique and self-complementary (2–6) so that DNA fragments produced by one of these enzymes can associate by hydrogen-bonding with other fragments produced by the same enzyme. After hydrogen-bonding, the 3′-hydroxyl and 5′-phosphate ends can be joined by DNA ligase (6). Thus, these restriction endonucleases appeared to have great potential value for the construction of new plasmid species by joining DNA molecules from different sources. The *Eco*RI endonuclease seemed especially useful for this purpose, because on a random basis the sequence cleaved is expected to occur only about once for every 4,000 to 16,000 nucleotide pairs (2); thus, most *Eco*RI-generated DNA fragments should contain one or more intact genes.

We describe here the construction of new plasmid DNA species by *in vitro* association of the *Eco*RI-derived DNA fragments from separate plasmids. In one instance a new plasmid has been constructed from two DNA species of entirely different origin, while in another, a plasmid which has itself been derived from *Eco*RI-generated DNA fragments of a larger parent plasmid genome has been joined to another replicon derived independently from the same parent plasmid. Plasmids that have been constructed by the *in vitro* joining of *Eco*RI-generated fragments have been inserted into appropriately-treated *E. coli* by transformation (7) and have been shown to form biologically functional replicons that possess genetic properties and nucleotide base sequences of both parent DNA species.

MATERIALS AND METHODS

E. coli strain W1485 containing the RSF1010 plasmid, which carries resistance to streptomycin and sulfonamide, was obtained from S. Falkow. Other bacterial strains and R factors and procedures for DNA isolation, electron microscopy, and transformation of *E. coli* by plasmid DNA have been described (1, 7, 8). Purification and use of the *Eco*RI restriction endonuclease have been described (5). Plasmid heteroduplex studies were performed as previously described (9, 10). *E. coli* DNA ligase was a gift from P. Modrich and R. L. Lehman and was used as described (11). The detailed procedures for gel electrophoresis of DNA will be described elsewhere (Helling, Goodman, and Boyer, in preparation); in brief, duplex DNA was subjected to electrophoresis in a tube-type apparatus (Hoefer Scientific Instrument) (0.6 × 15-cm gel) at about 20° in 0.7% agarose at 22.5 V with 40 mM Tris–acetate buffer (pH 8.05) containing 20 mM sodium acetate, 2 mM EDTA, and 18 mM sodium chloride. The gels were then soaked in ethidium bromide (5 μg/ml) and the DNA was visualized by fluorescence under long wavelength ultraviolet light ("black light"). The molecular weight of each fragment in the range of 1 to 200×10^5 was determined from its mobility relative to the mobilities of DNA standards of known molecular weight included in the same gel (Helling, Goodman, and Boyer, in preparation).

RESULTS

R6-5 and pSC101 plasmid DNA preparations were treated with the *Eco*RI restriction endonuclease, and the resulting DNA products were analyzed by electrophoresis in agarose gels. Photographs of the fluorescing DNA bands derived from these plasmids are presented in Fig. 1*b* and *c*. Only one band is observed after *Eco*RI endonucleolytic digestion of pSC101 DNA (Fig. 1*c*), suggesting that this plasmid has a single site susceptible to cleavage by the enzyme. In addition, endonuclease-treated pSC101 DNA is located at the position in the gel that would be expected if the covalently closed circular plasmid is cleaved once to form noncircular DNA of the same molecular weight. The molecular weight of the linear fragment estimated from its mobility in the gel is 5.8×10^6, in agreement with independent measurements of the size of the intact molecule (1). Because pSC101 has a single *Eco*RI cleavage site and is derived from R6-5, the equivalent DNA sequences of

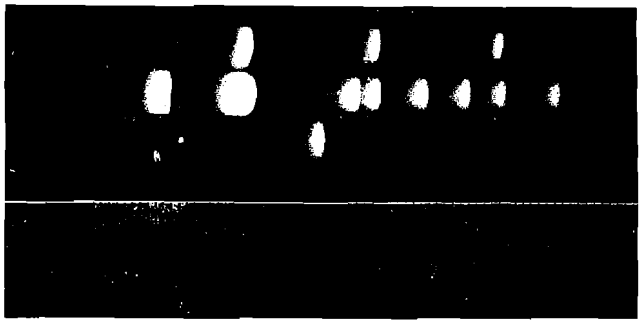


FIG. 1. Agarose-gel electrophoresis of *Eco*RI digests. (*a*) pSC102. The three fragments derived from the plasmid correspond to fragments III, V, and VIII of R6-5 (Fig. 1*b* below) as shown here and as confirmed by electrophoresis in other gels (see *text*). (*b*) R6-5. The molecular weights calculated for the fragments, as indicated in *Methods*, are (from *left* to *right*) I, 17.0; II & III (double band), 9.6 and 9.1; IV, 5.2; V, 4.9; VI, 4.3; VII, 3.8; VIII, 3.4; IX, 2.9. All molecular weight values have been multiplied by 10^{-6}. (*c*) pSC101. The calculated molecular weight of the single fragment is 5.8×10^6. Migration in all gels was from *left* (cathode) to *right;* samples were subjected to electrophoresis for 19 hr and 50 min.

the parent plasmid must be distributed in two separate *Eco*RI fragments.

The *Eco*RI endonuclease products of R6-5 plasmid DNA were separated into 12 distinct bands, eight of which are seen in the gel shown in Fig. 1*b;* the largest fragment has a molecular weight of 17×10^6, while three fragments (not shown in Fig. 1*b*) have molecular weights of less than 1×10^6, as determined by their relative mobilities in agarose gels. As seen in the figure, an increased intensity of fluorescence, of the second band suggests that this band contains two or more DNA fragments of almost equal size; when smaller amounts of *Eco*RI-treated R6-5 DNA are subjected to electrophoresis for a longer period of time, resolution of the two fragments (i.e., II and III) is narrowly attainable. Because 12 different *Eco*RI-generated DNA fragments can be identified after endonuclease treatment of covalently closed circular R6-5, there must be at least 12 substrate sites for *Eco*RI endonuclease present on this plasmid, or an average of one site for every 8000 nucleotide pairs. The molecular weight for each fragment shown is given in the caption to Fig. 1. The sum of the molecular weights of the *Eco*RI fragments of R6-5 DNA is 61.5×10^6, which is in close agreement with independent estimates for the molecular weight of the intact plasmid (7, 10).

The results of separate transformations of *E. coli* C600 by endonuclease-treated pSC101 or R6-5 DNA are shown in Table 1. As seen in the table, cleaved pSC101 DNA transforms *E. coli* C600 with a frequency about 10-fold lower than was observed with covalently closed or nicked circular (1) molecules of the same plasmid. The ability of cleaved pSC-101 DNA to function in transformation suggests that plasmid DNA fragments with short cohesive endonuclease-generated termini can recircularize in *E. coli* and be ligated *in vivo;* since the denaturing temperature (T_m) for the termini generated by the *Eco*RI endonuclease is 5–6° (6) and the transformation procedure includes a 42° incubation step (7), it is unlikely that the plasmid DNA molecules enter bacterial cells with their termini already hydrogen-bonded. A corresponding observation has been made with *Eco*RI endonuclease-cleaved

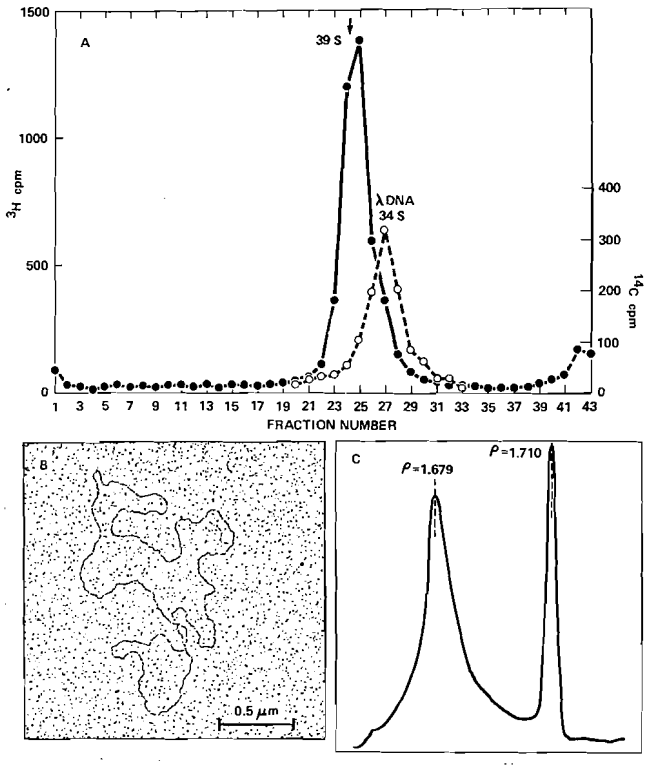


FIG. 2. Physical properties of the pSC102 plasmid derived from *Eco*RI fragments of R6–5. (*A*) Sucrose gradient centrifugation analysis (1, 8) of covalently closed circular plasmid DNA (●——●) isolated from an *E. coli* transformant clone as described in text. 34 S linear [^{14}C]DNA from λ was used as a standard (○- - -○). (*B*) Electron photomicrograph of nicked (7) pSC102 DNA. The length of this molecule is approximately 8.7 μm. (*C*) Densitometer tracing of analytical ultracentrifugation (8) photograph of pSC102 plasmid DNA. Centrifugation in CsCl (ρ = 1.710 g/cm^3) was carried out in the presence of d(A-T)$_n$·d(A-T)$_n$ density marker (ρ = 1.679 g/cm^3).

SV40 DNA, which forms covalently closed circular DNA molecules in mammalian cells *in vivo* (6).

Transformation for each of the antibiotic resistance markers present on the R6-5 plasmid was also reduced after treatment of this DNA with *Eco*RI endonuclease (Table 1). Since the pSC101 (tetracycline-resistance) plasmid was derived from R6-5 by controlled shearing of R6-5 DNA (1), and no tetracycline-resistant clone was recovered after transformation by the *Eco*RI endonuclease products of R6-5, [whereas tetracycline-resistant clones are recovered after transformation with intact R6-5 DNA (1)], an *Eco*RI restriction site may separate the tetracycline resistance gene of R6-5 from its replicator locus. Our finding that the linear fragment produced by treatment of pSC101 DNA with *Eco*RI endonuclease does not correspond to any of the *Eco*RI-generated fragments of R6-5 (Fig. 1) is consistent with this interpretation.

A single clone that had been selected for resistance to kanamycin and which was found also to carry resistance to neomycin and sulfonamide, but not to tetracycline, chloramphenicol, or streptomycin after transformation of *E. coli* by *Eco*RI-generated DNA fragments of R6-5, was examined further. Closed circular DNA obtained from this isolate (plasmid designation pSC102) by CsCl–ethidium bromide gradient

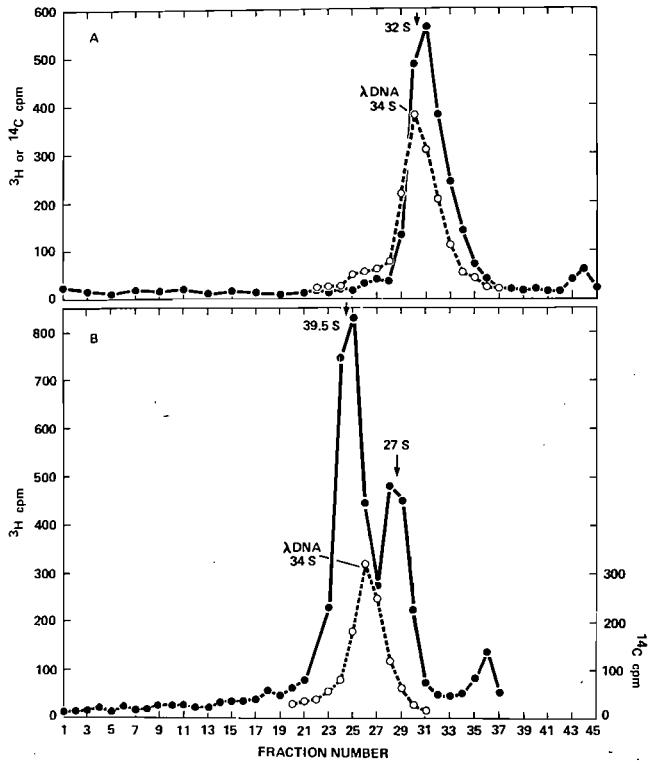


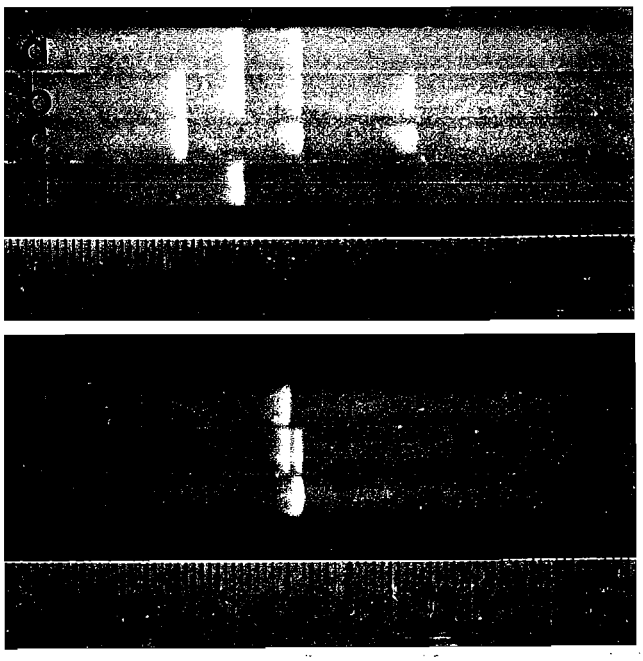
FIG. 3. Sucrose gradient centrifugation of DNA isolated from *E. coli* clones transformed for both tetracycline and kanamycin resistance by a mixture of pSC101 and pSC102 DNA. (*A*) The DNA mixture was treated with *Eco*RI endonuclease and was ligated prior to use in the transformation procedure. Covalently closed circular DNA isolated (7, 8) from a transformant clone carrying resistance to both tetracycline and kanamycin was examined by sedimentation in a neutral 5–20% sucrose gradient (8). (*B*) Sucrose sedimentation pattern of covalently closed circular DNA isolated from a tetracycline and kanamycin resistant clone transformed with an *untreated* mixture of pSC101 and pSC102 plasmid DNA.

centrifugation has an S value of 39.5 in neutral sucrose gradients (Fig. 2*A*) and a contour length of 8.7 μm when nicked (Fig. 2*B*). These data indicate a molecular weight about 17 $\times$ 10^6. Isopycnic centrifugation in cesium chloride of this non-self-transmissible plasmid indicated it has a buoyant density of 1.710 g/cm^3 (Fig. 2*C*). Since the nucleotide base composition of the antibiotic resistance determinant (R-determinant) segment of the parent R factor is 1.718 g/cm^3 (8), the various component regions of the resistance unit must have widely different base compositions, and the pSC102 plasmid must lack a part of this unit that is rich in high buoyant density G+C nucleotide pairs. The existence of such a high buoyant density *Eco*RI fragment of R6-5 DNA was confirmed by centrifugation of *Eco*RI-treated R6-5 DNA in neutral cesium chloride gradients (Cohen and Chang, unpublished data).

TABLE 1. *Transformation by covalently closed circular and EcoRI-treated plasmid DNA*

Plasmid DNA species		Transformants per μg DNA: Tetracycline	Kanamycin (neomycin)	Chloramphenicol
pSC101	covalently closed circle	3×10^5	—	—
	*Eco*RI-treated	2.8×10^4	—	—
R6-5	covalently closed circle	—	1.3×10^4	1.3×10^4
	*Eco*RI-treated	<5	1×10^2	4×10^1

Transformation of *E. coli* strain C600 by plasmid DNA was carried out as indicated in *Methods*. The kanamycin resistance determinant of R6-5 codes also for resistance to neomycin (15). Antibiotics used for selection were tetracycline (10 μg/ml), kanamycin (25 μg/ml) or chloramphenicol (25 μg/ml).



FIGS. 4 and 5. Agarose-gel electrophoresis of *Eco*RI digests of newly constructed plasmid species. Conditions were as described in *Methods*.

FIG. 4. (*top*) Gels were subjected to electrophoresis for 19 hr and 10 min. (*a*) pSC105 DNA. (*b*) Mixture of pSC101 and pSC102 DNA. (*c*) pSC102 DNA. (*d*) pSC101 DNA.

FIG. 5. (*bottom*) Gels were subjected to electrophoresis for 18 hr and 30 min. (*a*) pSC101 DNA. (*b*) pSC109 DNA. (*c*) RSF1010 DNA. Evidence that the single band observed in this gel represents a linear fragment of cleaved RSF1010 DNA was obtained by comparing the relative mobilities of *Eco*RI-treated DNA and untreated (covalently closed circular and nicked circular) RSF1010 DNA in gels. The molecular weight of RSF-1010 calculated from its mobility in gels is 5.5 $\times$ 10^6.

Treatment of pSC102 plasmid DNA with *Eco*RI restriction endonuclease results in formation of three fragments that are separable by electrophoresis is agarose gels (Fig. 1*a*); the estimated molecular weights of these fragments determined by gel mobility total 17.4 $\times$ 10^6, which is in close agreement with the molecular weight of the intact pSC102 plasmid determined by sucrose gradient centrifugation and electron microscopy (Fig. 2). Comparison with the *Eco*RI-generated fragments of R6-5 indicates that the pSC102 fragments correspond to fragments III (as determined by long-term electrophoresis in gels containing smaller amounts of DNA), V, and VIII of the parent plasmid (Fig. 1*b*). These results suggest that *E. coli* cells transformed with *Eco*RI-generated DNA fragments of R6-5

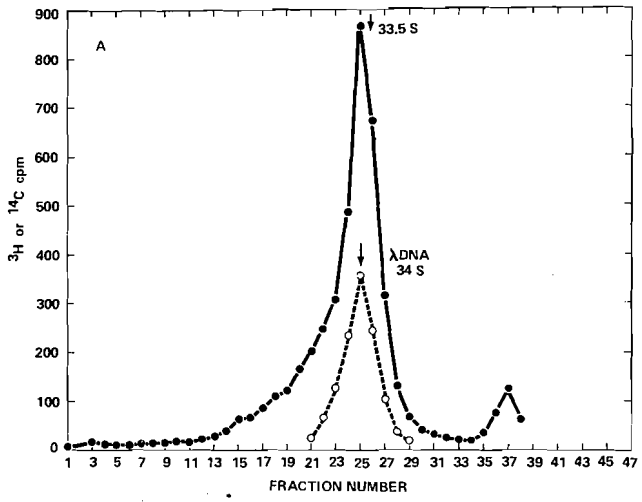


FIG. 6. Sucrose gradient sedimentation of covalently closed circular DNA representing the pSC109 plasmid derived from RSF1010 and pSC101.

can ligate reassociated DNA fragments *in vivo*, and that reassociated molecules carrying antibiotic resistance genes and capable of replication can circularize and can be recovered as functional plasmids by appropriate selection.

A mixture of pSC101 and pSC102 plasmid DNA species, which had been separately purified by dye–buoyant density centrifugation, was treated with the *Eco*RI endonuclease, and then was either used directly to transform *E. coli* or was ligated prior to use in the transformation procedure (Table 2). In a control experiment, a plasmid DNA mixture that had not been subjected to endonuclease digestion was employed for transformation. As seen in this table, transformants carrying resistance to both tetracycline and kanamycin were isolated in all three instances. Cotransformation of tetracycline and kanamycin resistance by the untreated DNA mixture occurred at a 500- to 1000-fold lower frequency than transformation for the individual markers. Examination of three different transformant clones derived from this DNA mixture indicated that each contained two separate covalently closed circular DNA species having the sedimentation characteristics of the pSC101 and pSC102 plasmids (Fig. 3*B*). The ability of two plasmids derived from the same parental plasmid (i.e., R6-5) to exist stably as separate replicons (12) in a single

TABLE 2. *Transformation of E. coli C600 by a mixture of pSC101 and pSC102 DNA*

Treatment of DNA	Transformation frequency for antibiotic resistance markers: Tetracycline	Kanamycin	Tetracycline + kanamycin
None	2×10^5	1×10^5	2×10^2
*Eco*RI	1×10^4	1.1×10^3	7×10^1
*Eco*RI + DNA ligase	1.2×10^4	1.3×10^3	5.7×10^2

Transformation frequency is shown in transformants per μg of DNA of each plasmid species in the mixture. Antibiotic concentrations are indicated in legend of Table 1.

bacterial host cell suggests that the parent plasmid may contain at least two distinct replicator sites. This interpretation is consistent with earlier observations which indicate that the R6 plasmid dissociates into two separate compatible replicons in *Proteus mirabilis* (8). Cotransformation of tetracycline and kanamycin resistance by the *Eco*RI treated DNA mixture was 10- to 100-fold lower than transformation of either tetracycline or kanamycin resistance alone, and was increased about 8-fold by treatment of the endonuclease digest with DNA ligase (Table 2). Each of four studied clones derived by transformation with the endonuclease-treated and/or ligated DNA mixture contained only a single 32S covalently closed circular DNA species (Fig. 3*A*) that carries resistance to both tetracycline and kanamycin, and which can transform *E. coli* for resistance to both antibiotics. One of the clones derived from the ligase-treated mixture was selected for further study, and this plasmid was designated pSC105.

When the plasmid DNA of pSC105 was digested by the *Eco*RI endonuclease and analyzed by electrophoresis in agarose gels, two component fragments were identified (Fig. 4); the larger fragment was indistinguishable from endonuclease-treated pSC101 DNA (Fig. 4*d*) while the smaller fragment corresponded to the 4.9×10^6 dalton fragment of pSC102 plasmid DNA (Fig. 4*c*). Two endonuclease fragments of pSC102 were lacking in the pSC105 plasmid; presumably the sulfonamide resistance determinant of pSC102 is located on one of these fragments, since pSC105 does not specify re-

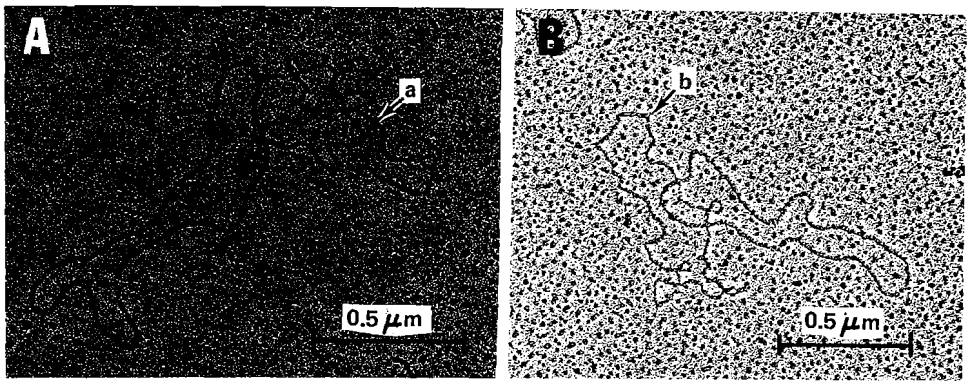


FIG. 7. (*A*) Heteroduplex of pSC101/pSC109. The single-stranded DNA loop marked by *a* represents the contribution of RSF1010 to the pSC109 plasmid. (*B*) Heteroduplex of RSF1010/pSC109. The single-stranded DNA loop marked by *b* represents the contribution of pSC101 to the pSC109 plasmid. pSC101 and RSF1010 homoduplexes served as internal standards for DNA length measurements. The scale is indicated by the bar on each electron photomicrograph.

sistance to this antibiotic. Since kanamycin resistance *is* expressed by pSC105, we conclude that this resistance gene resides on the 4.9×10^6 dalton fragment of pSC102 (fragment V of its parent, R6-5). The molecular weight of the pSC105 plasmid is estimated to be 10.5×10^6 by addition of the molecular weights of its two component fragments; this value is consistent with the molecular weight determined for this recombinant plasmid by sucrose gradient centrifugation (Fig. 3*A*) and electron microscopy. The recovery of a biologically functional plasmid (i.e., pSC105) that was formed by insertion of a fragment of another plasmid fragment into pSC101 indicates that the *Eco*RI restriction site on pSC101 does not interrupt the genetic continuity of either the tetracycline resistance gene or the replicating element of this plasmid.

We also constructed new biologically functional plasmids *in vitro* by joining cohesive-ended plasmid DNA molecules of entirely different origin. RSF1010 is a streptomycin and sulfonamide resistance plasmid which has a 55% G+C nucleotide base composition (13) and which was isolated originally from *Salmonella typhimurium* (14). Like pSC101, this nonself-transmissible plasmid is cleaved at a single site by the *Eco*RI endonuclease (Fig. 5*c*). A mixture of covalently closed circular DNA containing the RSF1010 and pSC101 plasmids was treated with the *Eco*RI endonuclease, ligated, and used for transformation. A transformant clone resistant to both tetracycline and streptomycin was selected, and covalently closed circular DNA (plasmid designation pSC109) isolated from this clone by dye–buoyant density centrifugation was shown to contain a single molecular species sedimenting at 33.5 S, corresponding to an approximate molecular weight of 11.5×10^6 (Fig. 6). Analysis of this DNA by agarose gel electrophoresis after *Eco*RI digestion (Fig. 5*b*) indicates that it consists of two separate DNA fragments that are indistinguishable from the *Eco*RI-treated RSF1010 and pSC101 plasmids (Fig. 5*a* and *c*).

Heteroduplexes shown in Fig. 7*A* and *B* demonstrate the existence of DNA nucleotide sequence homology between pSC109 and each of its component plasmids. As seen in this figure, the heteroduplex pSC101/pSC109 shows a double-stranded region about 3 μm in length and a slightly shorter single-stranded loop, which represents the contribution of RSF1010 to the recombinant plasmid. The heteroduplex formed between RSF1010 and pSC109 shows both a duplex region and a region of nonhomology, which contains the DNA contribution of pSC101 to pSC109.

SUMMARY AND DISCUSSION

These experiments indicate that bacterial antibiotic resistance plasmids that are constructed *in vitro* by the joining of *Eco*RI-treated plasmids or plasmid DNA fragments are biologically functional when inserted into *E. coli* by transformation. The recombinant plasmids possess genetic properties and DNA nucleotide base sequences of both parent molecular species. Although ligation of reassociated *Eco*RI-treated fragments increases the efficiency of new plasmid formation, recombinant plasmids are also formed after transformation by *unligated Eco*RI-treated fragments.

The general procedure described here is potentially useful for insertion of specific sequences from prokaryotic or eukaryotic chromosomes or extrachromosomal DNA into independently replicating bacterial plasmids. The antibiotic resistance plasmid pSC101 constitutes a replicon of considerable potential usefulness for the selection of such constructed molecules, since its replication machinery and its tetracycline resistance gene are left intact after cleavage by the *Eco*RI endonuclease.

We thank P. A. Sharp and J. Sambrooke for suggesting use of ethidium bromide for staining DNA fragments in agarose gels. These studies were supported by Grants AI08619 and GM14378 from the National Institutes of Health and by Grant GB-30581 from the National Science Foundation. S.N.C. is the recipient of a USPHS Career Development Award. R.B.H. is a USPHS Special Fellow of the Institute of General Medical Sciences on leave from the Department of Botany, University of Michigan.

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Potential Biohazards of Recombinant DNA Molecules

Recent advances in techniques for the isolation and rejoining of segments of DNA now permit construction of biologically active recombinant DNA molecules in vitro. For example, DNA restriction endonucleases, which generate DNA fragments containing cohesive ends especially suitable for rejoining, have been used to create new types of biologically functional bacterial plasmids carrying antibiotic resistance markers (*1*) and to link *Xenopus laevis* ribosomal DNA to DNA from a bacterial plasmid. This latter recombinant plasmid has been shown to replicate stably in *Escherichia coli* where it synthesizes RNA that is complementary to *X. laevis* ribsomal DNA (*2*). Similarly, segments of *Drosophila* chromosomal DNA have been incorporated into both plasmid and bacteriophage DNA's to yield hybrid molecules that can infect and replicate in *E. coli* (*3*).

Several groups of scientists are now planning to use this technology to create recombinant DNA's from a variety of other viral, animal, and bacterial sources. Although such experiments are likely to facilitate the solution of important theoretical and practical biological problems, they would also result in the creation of novel types of infectious DNA elements whose biological properties cannot be completely predicted in advance.

There is serious concern that some of these artificial recombinant DNA molecules could prove biologically hazardous. One potential hazard in current experiments derives from the need to use a bacterium like *E. coli* to clone the recombinant DNA molecules and to amplify their number. Strains of *E. coli* commonly reside in the human intestinal tract, and they are capable of exchanging genetic information with other types of bacteria, some of which are pathogenic to man. Thus, new DNA elements introduced into *E. coli* might possibly become widely disseminated among human, bacterial, plant, or animal populations with unpredictable effects.

Concern for these emerging capabilities was raised by scientists attending the 1973 Gordon Research Conference on Nucleic Acids (*4*), who requested that the National Academy of Sciences give consideration to these matters. The undersigned members of a committee, acting on behalf of and with the endorsement of the Assembly of Life Sciences of the National Research Council on this matter, propose the following recommendations.

First, and most important, that until the potential hazards of such recombinant DNA molecules have been better evaluated or until adequate methods are developed for preventing their spread, scientists throughout the world join with the members of this committee in voluntarily deferring the following types of experiments.

► *Type 1*: Construction of new, autonomously replicating bacterial plasmids that might result in the introduction of genetic determinants for antibiotic resistance or bacterial toxin formation into bacterial strains that do not at present carry such determinants; or construction of new bacterial plasmids containing combinations of resistance to clinically useful antibiotics unless plasmids containing such combinations of antibiotic resistance determinants already exist in nature.

► *Type 2*: Linkage of all or segments of the DNA's from oncogenic or other animal viruses to autonomously replicating DNA elements such as bacterial plasmids or other viral DNA's. Such recombinant DNA molecules might be more easily disseminated to bacterial populations in humans and other species, and thus possibly increase the incidence of cancer or other diseases.

Second, plans to link fragments of animal DNA's to bacterial plasmid DNA or bacteriophage DNA should be carefully weighed in light of the fact that many types of animal cell DNA's contain sequences common to RNA tumor viruses. Since joining of any foreign DNA to a DNA replication system creates new recombinant DNA molecules whose biological properties cannot be predicted with certainty, such experiments should not be undertaken lightly.

Third, the director of the National Institutes of Health is requested to give immediate consideration to establishing an advisory committee charged with (i) overseeing an experimental program to evaluate the potential biological and ecological hazards of the above types of recombinant DNA molecules; (ii) developing procedures which will minimize the spread of such molecules within human and other populations; and (iii) devising guidelines to be followed by investigators working with potentially hazardous recombinant DNA molecules.

Fourth, an international meeting of involved scientists from all over the world should be convened early in the coming year to review scientific progress in this area and to further discuss appropriate ways to deal with the potential biohazards of recombinant DNA molecules.

The above recommendations are made with the realization (*i*) that our concern is based on judgments of potential rather than demonstrated risk since there are few available experimental data on the hazards of such DNA molecules and (*ii*) that adherence to our major recommendations will entail postponement or possibly abandonment of certain types of scientifically worthwhile experiments. Moreover, we are aware of many theoretical and practical difficulties involved in evaluating the human hazards of such recombinant DNA molecules. Nonetheless, our concern for the possible unfortunate consequences of indiscriminate application of these techniques motivates us to urge all scientists working in this area to join us in agreeing not to initiate experiments of types 1 and 2 above until attempts have been made to evaluate the hazards and some resolution of the outstanding questions has been achieved.

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Committee on Recombinant DNA Molecules Assembly of Life Sciences, National Research Council, National Academy of Sciences, Washington, D.C. 20418

References and Notes

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February 24, 1975

TO: The Committee on Recombinant DNA Molecules
Assembly of Life Sciences
National Research Council
National Academy of Sciences
Washington, D.C., U.S.A.
Paul Berg, Chairman

FROM: Working Party on potential biohazards associated with experimentation involving genetically altered microorganisms, with special reference to bacterial plasmids and phages

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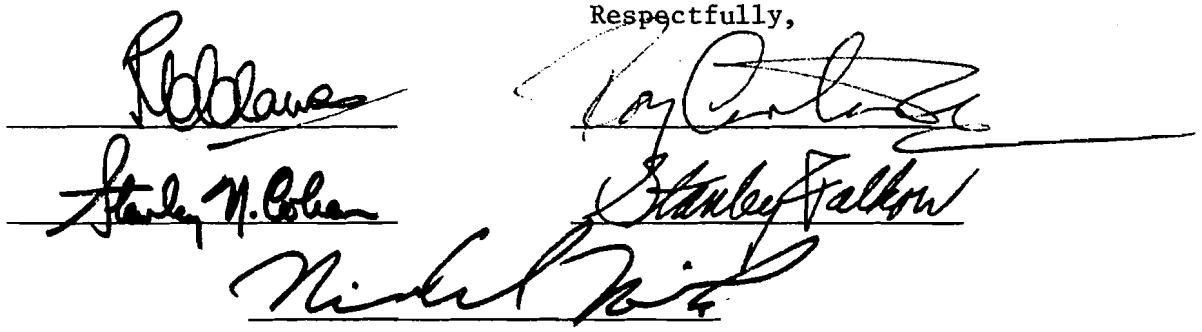
Sirs:

We have the pleasure to transmit herewith for your consideration proposed Guidelines on Potential Biohazards Associated with Experiments Involving Genetically Altered Microorganisms. This report was written in final form during two meetings, the first held in New York City, November 7 - 10, 1974, the second in Palo Alto, California, February 20 - 23, 1975.

Respectfully,



PROPOSED GUIDELINES ON POTENTIAL BIOHAZARDS ASSOCIATED WITH EXPERIMENTS INVOLVING GENETICALLY ALTERED MICROORGANISMS

Prepared by: Working Party on potential biohazards associated with experimentation involving gentically altered microorganisms, with special reference to bacterial plasmids and phages

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February 24, 1975

PROPOSED GUIDELINES ON POTENTIAL BIOHAZARDS ASSOCIATED WITH EXPERIMENTS INVOLVING GENETICALLY ALTERED MICROORGANISMS

Contents

I. INTRODUCTION

A. Scope and Purpose

1. Scope. These guidelines cover the modification of prokaryotic microorganisms by the introduction of foreign genetic information. Although this document has been prepared in response to a recommendation by the Committee on Recombinant DNA Molecules (Berg et. al., Proc. Nat. Acad. Sci., Wash. 71, 2593, 1974) that guidelines be devised for experiments involving "potentially hazardous recombinant DNA molecules", it is our view that there are certain other types of genetic manipulation and reconstruction that have so strong a logical kinship to the above that it would be artificial to omit them. At its broadest, then, this document will deal with all genetic manipulations involving the introduction into a prokaryotic species of genetic material that may or may not be native to that species and may be unlikely to be acquired by it in the natural environment.

For the purpose of this discussion, we will refer to a microorganism whose genome has been artificially modified by the addition of genetic information that is foreign to the species and unlikely to be acquired by it in nature as a novel recombinant biotype (or microorganisms). As current technology involves primarily the use of bacterial and phage genomes as carriers of foreign DNA, this term refers primarily to bacteria carrying foreign phages or plasmids or to native phages or plasmids that have had segments of foreign DNA added in vitro. While it includes, also, microorganisms with foreign DNA carried chromosomally, it excludes organisms produced from pre-existing ones by simple mutation.

The limitation of our recommendations to prokaryotic organisms is a practical one that is dictated by current limits of technology and of available information. These guidelines can and should be extended to eukaryotic microorganisms if and when those modifications along similar lines become feasible.

2. Purpose. The purpose of this document is two-fold: first to explore and detail the potential biohazards posed by a wide variety of classes of experiments involving recombinant microorganisms so as to raise the general level of awareness of these biohazards; and second, to make available suggestions for dealing with potential biohazards so that the individual need not rely entirely upon his or her own judgment.

Thus, it is hoped that the principle will be established that an open evaluation of biohazard potential and the adoption of an appropriate biohazard minimization procedure will be an integral part of experiments dealing with genetically altered microorganisms. Once this principle is accepted, a set of guidelines developed by an open, collective process that has taken into consideration the gamut of potentially conflicting interests will serve to enhance the safety and effectiveness of this line of research rather than to interfere with freedom of scientific inquiry, as has been feared.

B. Background

Recent developments in DNA biochemistry and microbial genetics have made it possible to join in vitro segments of genetically active DNA from diverse sources, thus creating biologically active novel gene combinations that are exceedingly unlikely to occur naturally. Thus far, such recombinant chimeras

have involved the attachment of a DNA segment to a functional extrachromosomal replicon of bacterial origin (a plasmid or a bacteriophage genome) and the introduction of the recombinant molecule into a suitable bacterial host cell where it replicates autonomously, serving to clone the added DNA segment. It is already certain that DNA from eukaryotic as well as from prokaryotic sources can thus be replicated and transcribed in bacterial hosts. Although it is not yet known whether or not eukaryotic DNA can be faithfully translated in bacteria, the consensus is that any barriers to translation could be bypassed by relatively straightforward manipulations.

This new technology thus constitutes a major breakthrough in molecular biology and gives rise to the possibility of important advances in at least four areas: (1) fundamental knowledge of gene structure, organization, and function; (2) genotypic modification of plants or animals to improve their usefulness to man (e.g., the development of nitrogen-fixing non-leguminous plants); (3) construction of bacteria or other such organisms able to produce rare and medically valuable biological substances such as insulin, growth hormone, etc.; and (4) genetic restitution of human hereditary diseases.

As with other major technological and scientific advances, gene grafting entails (along with its great potential benefits) at least the potential of serious and often unpredictable adverse consequences. Among these are biohazards that might result from the intentional or unintentional release into the environment of microorganisms carrying novel combinations of genes that have never existed before and are very unlikely to arise in the course of natural evolution. These biohazards would result, basically, from modification of the relationship between the organism and its environment - the genetically modified organism might be able to occupy new ecological niches or to function in a novel way within its normal environment, or both. One important subclass of these biohazards would involve an increase in the ability of a microorganism to cause human disease, including enhanced pathogenicity as well as increased resistance to eradication or treatment.

These possibilities have given rise to a significant level of concern among the general public as well as within the scientific community as there is ample precedent for the fear that the accidental introduction of organisms into new environments may have uncontrollable and sometimes dramatic untoward consequences. As examples of this, one might point to fire ants, killer bees, mudfish, snails, Xenopus toads and to Chestnut blight and Dutch elm disease. More germane, perhaps. to the present document is the serious biohazard inherent in the astonishing spread in the space of a mere 30 years of bacterial plasmids carrying resistance to antibiotics consequent to the vast overuse and misuse of these valuable therapeutic agents.* The recent de novo appearance of such plasmids in Hemophilus influenzae and Streptococcus species suggests that their spread may by now have encompassed bacterial species to which they were never native before the present era.

The worry over possibilities such as these is not new; it has been expressed through legislation to prevent the transportation of certain plant and

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* For documentation see, for example, the Report of the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine (Chairman: Sir M. M. Swann) HMSO London, 1969

animal species between countries and between certain states in the U.S., and it has been expressed in the elaborate decontamination procedures to which leaving and re-entering space vehicles have been subjected. However, there has been little more than anguished hand-wringing over the antibiotic-induced spread of resistance plasmids. Perhaps the actions recommended in these pages to minimize the potential hazards of novel recombinant microorganisms will serve to stimulate similar actions to control the existant serious problem of antibiotic induced plasmid spread.

Concern over potential biohazards of novel microorganisms produced by *in vitro* genetic reconstruction was first articulated publicly in a report by a group of distinguished scientists, the Committee on Recombinant DNA molecules, published in the Proceedings of the National Acad. of Sci. U.S. (*71*:2593, 1974). in the summer of 1974. In this report, the Committee urged that a set of guidelines be developed to aid individual scientists to perform safely experiments involving the production and study of novel recombinant microorganisms. These guidelines would help in the assessment of the degree of danger involved and would recommend commensurate precautions. As a preliminary move, the Committee recommended a voluntary termporary deferral for two types of experiments and recommended that a third be performed with caution, until the appropriate guidelines were developed.

It appears that this deferral was largely successful and that the letter had the intended effect of setting in motion a number of independent inquiries to deal with the problem. One of these has already come to fruition in the form of a report, dated Dec. 13, 1974, to the British Parliament by a "working party on the experimental manipulation of the genetic composition of microorganisms" under the chairmanship of Lord Ashby. This report contains a very thoughtful analysis of the potential benefits and hazards attendant upon gene grafting research and outlines very briefly a set of broad recommendations.

The present document is in agreement with the philosophical position of the British report and is offered as a somewhat more detailed analysis of experimental systems intending to provide an explicit set of working guidelines for experimentation in this field. The two documents will thus be seen as complementary to one another, and their joint effect will be to replace the moratorium with specific recommendations as urged in the NAS Committee letter.

C. Principles

The philosophical position underlying this proposal and its contents is best expressed in the form of a set of basic principles, some of which are clearly established as facts, while others may be regarded as assumptions:

1. Since man has some measure of control over his actions, there is an operational dichotomy between the activities of man and the processes of the natural world. The distinction between "man-made" and "natural" is therefore meaningful and control of the former is both worthwhile and possible.

2. It is possible to modify profoundly the genome of a (micro) organism by artificial means involving the *in vitro* joining of unrelated DNA segments. Such modifications may find expression in the organism's phenotype as well as in its genetic constitution.

3. Modified (micro) organisms may behave in an unpredictable manner with

respect to the expression of foreign genes, and to the effect of this expression upon their ecological potential (including pathogenicity).

4. The genetic effects of these manipulations may be different from anything that ordinarily occurs during the natural process of evolution.

5. Historically unforeseen ecological effects of technological developments have been more often than not detrimental to man and his environment.

6. The release of a self-replicating entity into the environment will prove to be irreversible should that entity prove viable in the natural environment.

D. Experimental systems and their safety

In view of the foregoing, a set of basic questions may be posed, which this proposal is a rather elaborate attempt to answer: Is it or is it not possible to evaluate a potential biohazard? i.e., How likely is it in any particular case that foreseeable or unforseeable adverse consequences will follow the release of a novel recombinant organism into the environment? Or, alternatively, granting the possibility of adverse consequences, how likely is it that a potentially hazardous but scientifically useful experimental system can be contained?

In general terms, the view to be developed here is that (a) it is often possible to evaluate to a greater or lesser extent (but rarely, if ever, fully) the potential biohazard associated with any novel biotype; (b) it is never possible to ensure absolute containment; but (c) it is often possible to reduce a potential biohazard to an acceptable level of risk without seriously compromising an experimental system.

Consequently, our recommendations will be based upon the following considerations:

(a) While it is not possible to ensure absolute containment, it is possible to develop containment procedures that are effective at various levels of stringency.

(b) Therefore, where it is judged that the escape of even a small number of experimental organisms would constitute a serious biohazard, the experiment should not be attempted.

(c) Where (b) is not the case, then containment procedures should be adopted whose stringency is based upon the best available evaluation of the biohazard potential as expressed as a permissible escape frequency for the novel recombinant organism - since escape frequency is really the only parameter involved in containment systems.

(d) Where possible, especially where evaluation of biohazard potential is difficult or impossible, the undesirable alternative of simply accepting the best available guess and acting accordingly should be circumvented by developing an experimental organism with very low potential for survival or transfer of its genetic material upon escape (see appendix C). Thus, a central consideration that will be dealt with here is the evaluation of normally used laboratory strains of bacteria with respect to their ecological potential and to the various possible ways of modifying them genetically so as to reduce their ecological potential and their ability to transfer DNA to other organisms.

(e) Finally, it must be stressed that while this set of guidelines is designed to help the investigator perform responsibly and with confidence those experiments deemed sufficiently important to justify whatever risk may be involved. These guidelines are not intended as a license to do unrestricted experimentation in this area. Experiments involving the construction of potentially hazardous novel recombinant biotypes should not be undertaken casually even within the containment framework appropriate for the level of risk involved.

II. CLASSIFICATION OF EXPERIMENTS

A. Considerations for the Assessment of Potential Biohazards

1. Introduction

After deciding to construct a genetically altered microorganism, an investigator should consider each of the following points in deciding on an appropriate classification for the experiment to determine the type of containment necessary.

2. Specific Considerations

a. Potential for Alteration of Pathogenicity.

For our purposes, pathogenicity and virulence are defined similarly as the "capacity to cause disease". How great is the known pathogenicity of the organisms involved? Will the genetic manipulation contemplated cause an increase in pathogenicity? If genetic information specifying traits that contribute to pathogenicity is used to construct a recombinant DNA molecule, then it is pertinent to ask:

i) Is the ecology or reservoir of the virulence genes being changed?
ii) Do these virulence genes occur naturally in the donor and recipient species in the general environment, in the local environment or in both?
iii) What is the potential for the transmission of these virulence genes from the modified organism to other microorganisms?

b. Potential for Dissemination.

If the genetically altered microorganism is pathogenic, can growth be controlled by antibiotics customarily used against the recipient strain? If antibiotic resistance is specified by the recombinant DNA, is this resistance to a drug of choice for treatment of infections by the microorganism? Is it a drug for which resistance is commonly expressed by the recipient organism? Is this drug resistance phenotype common locally among microorganisms of this type? Do the donor and recipient species naturally exchange genetic information? What is the potential for intercellular spread of the DNA chimera? When using plasmid DNA to construct recombinant molecules, do plasmids specify conjugal gene transfer? Are the recombinant DNA molecules normally restricted to an intracellular existence (as with plasmids) or do they normally persist extracellularly as encapsulated phage particles? Is the recipient lysogenic? Does the recipient possess plasmids (cryptic, conjugative or non-conjugative, autonomous or integrated)? Are the chimeric DNA molecules likely to recombine by natural means with other genetic material present in the recipient species? Is the recombinant DNA likely to undergo genetic alteration in its new host that may affect its biological potential?

c. Potential for Alteration of Ecology.

For our purposes, ecological potential is defined as the ability to occupy ecological habitats and the ability to alter the local ecosystem. Do the donor and recipient organisms share a common habitat? Does the donor organism possess phenotypic properties which, if expressed in the recipient, might substantially alter the ecological potential of the recipient? Will the genetically altered microorganism possess any unique metabolic properties that will alter the local ecosystem? Is it likely that the normal ecological habitat of the recipient will

be a factor affecting the biohazard potential when new metabolic capabilities are introduced?

d. Potential for Persistence in the Environment.

Would the recombinant molecules be expected to offer a biological advantage to the recipient organism which might affect its ecological potential? Does the genetically altered microorganism have a reduced susceptibility to disinfection or sterilization (e.g. resistance to ultraviolet irradiation, resistance to mercury-containing disinfectants, increased capacity for spore formation, etc.)?

e. Phenotypic Expression of Foreign Genes.

Are the phenotypic traits specified by the foreign DNA known to be expressed by strains of the recipient species? What is the likelihood of accurate transcription, translation and phenotypic expression of the foreign DNA in the recipient? What biological consequences are likely to result from their phenotypic expression in the recipient?

f. Availability of Genetic Information About Organisms Involved.

How well characterized are the organisms? Have they been isolated recently or are they well-studied laboratory strains?

g. Purity and Characterization of DNA Used in Forming Recombinant Molecules.

Are the DNA molecules used in the experiment derived from plasmid or phage species having well-characterized genetic and molecular properties? Does the DNA sample represent a single molecular species or does it contain a random assortment of molecules or fragments?

3. General Considerations

a. When an investigator is in doubt, the experiment should be placed in the higher of two classes being considered.

b. Since there is a corresponding increase in potential biohazard when large numbers of microorganisms are used, investigators should classify large-scale experiments as more hazardous than those in which the new microorganism was initially constructed which involved relatively small numbers of cells.

c. It should be recognized that mutagenesis may alter the host range of bacteriophages and plasmids used as cloning vehicles. It is therefore prudent following recent mutagenesis of either genetically altered microorganisms or cloning vehicles to place the experiment in the next higher containment class until it has been determined that the host range has been unaltered.

B. Classes of Experiments

Experiments on the construction of genetically altered microorganisms have been categorized into six classes in terms of severity of the known or potential biohazards as follows:

1. Class I Experiment: Class I includes experiments in which the biohazard can be assessed and is known to be insignificant. More specifically, all of the following conditions must be fulfilled:

a. The pathogenicity of the donor and recipient organisms is minimal and is known to be unchanged by the procedure in question, and
b. It is known that dissemination of the organisms involved is fully and easily controllable, and
c. All DNA species involved are well characterized and their genetic properties are well understood, and
d. The experiment does not alter the ecological potential of the recipient compared to other strains of the same species, and
e. The genotypic and phenotypic properties under study occur naturally in the recipient species or can be readily transmitted to strains of the recipient species.

Examples of Class I Experiment: Gene transfer or genetic recombination between laboratory strains of Escherichia coli such as K-12, B, C and 15. This includes conjugal transfer by F^+, F'-containing and Hfr donors. See Appendix B for additional examples.

2. Class II experiment: Class II includes experiments in which the biohazards can be reasonably assessed and from what is known about them one can expect them to be minimal. More specifically, all of the following conditions must be fulfilled:

a. The species used to construct the genetically altered microorganism have either low or moderate pathogenicity similar to that expressed by Salmonella typhimurium, Staphylococcus aureus or Haemophilus influenzae, and
b. The genetic material used to construct the altered microorganism is derived from organisms known to be capable of transmitting genetic information to the recipient, and
c. The genetically altered microorganism should not have ecological potentials greater than can be conferred as a consequence of normally occurring genetic exchange processes, and
d. The genetically altered microorganism does not contain genetic information that would prevent effective treatment of infections caused by it.

It should be noted that in some instances an organism serving as a DNA donor may have a greater potential either to exhibit pathogenicity or to occupy unique ecological habitats than the recipient organisms and hence poses a greater potential biohazard than the recipient. In this event it is the potential biohazards associated with the donor of the DNA that determines the classification of the experiment.

Examples of Class II Experiment: The construction of recombinant molecules either in vitro or in vivo between R and F' plasmids, between Col and F' plasmids, between Col and F' plasmids or between bacteriophage λ and a Col or R plasmid when introduced into E. coli. See Appendix B for additional examples.

Classes III, IV and V Experiments include:

(i) all constructions of genetically altered microorganisms which use donor and recipient organisms that ordinarily do not exchange genetic information and

(ii) some constructions of genetically altered microorganisms which use organisms which ordinarily do exchange genetic information.

3. Class III Experiment: Class III includes experiments in which the biohazards usually cannot be totally predicted. However, on the basis of all available information, it is considered likely that:

a. The recombinant DNA will not contribute significantly increased pathogenicity to the recipient, nor significantly alter its ecological potential, and
b. Pathogenicity of the genetically altered microorganism or its parents is minimal (e.g., B. subtilis), low (e.g., E. coli) or moderate (e.g., S. typhimurium), but not severe (e.g., Y. pestis), and
c. The genetically altered microorganism does not contain information that would prevent effective treatment of infections caused by it.

Examples of Class III Experiment: Construction of a hybrid plasmid or phage that includes an antibiotic resistance gene derived from S. aureus when introduced into E. coli, so long as genes conferring resistance to that antibiotic are found in E. coli. Construction of a hybrid plasmid or phage that includes ribosomal genes from Xenopus laevis or random fragments of Drosophila melangaster DNA when introduced into E. coli. See Appendix B for additional examples.

4. Class IV Experiment: Class IV, like Class III, includes experiments in which the biohazards are usually unknown, and cannot be accurately assessed, but because of the known genotypic and/or phenotypic properties of the DNA and/or organisms used to construct the genetically altered microorganism, they are judged to be potentially significant in affecting either the ecologic potential or pathogenicity of the recipient organism.

Examples of Class IV Experiment: Construction of a hybrid between random DNA fragments from S. pyogenes and an F'lac plasmid and its introduction into E. coli. Construction of hybrids between random DNA fragments from normal human fibroblasts and an E. coli plasmid or phage when introduced into E. coli. Construction of a hybrid between either λ or plasmid DNA and the genes specifying synthesis of cellulase and/or ligninase from Polyporus annosus and its introduction into E. coli. See Appendix B for additional examples.

5. Class V Experiment: Class V also includes experiments in which the biohazards are usually unknown, but because of the known genotypic and/or phenotypic properties of the DNA and/or the organisms used in the construction of the genetically altered microorganism, they are judged to be severe in affecting either the ecological potential or pathogenicity of the recipient organism.

Examples of Class V Experiment: The construction of a recombinant DNA molecule between the plasmid from S. aureus determining exfoliative toxin and an R plasmid or λ and its introduction into E. coli. Construction of hybrids between E. coli phage or plasmid DNA, and unknown genes from Y. pestis, B. anthracis, or B. abortus, when the hybrid is introduced into E. coli. See Appendix B for additional examples.

6. Class VI Experiment: Class VI includes experiments in which the biohazards are judged to be of such great potential severity as to preclude performance of the experiment at the present time under any circumstances, and regardless of containment conditions.

Example of Class VI Experiment: The introduction by any means of the genes for botulinum toxin biosynthesis into E. coli. See Appendix B for additional examples.

C. Summary of Classification

We have categorized experiments involving the construction of genetically altered microorganisms into six classes. The assignment of experiments to Classes I and II involves little difficulty, since genetic exchange between the organisms used occurs normally. Classes III, IV and V experiments, however, primarily include the construction of genetically altered microorganisms which use donor and recipients that ordinarily do not exchange genetic material. We recognize that in many specific instances the distinction between a Class III and a Class IV or between a Class IV and Class V experiment will be difficult to make since these classes include experiments in which the potential biohazards cannot be totally assessed. Ultimately, the distinction will depend upon the ecology of the recipient bacterial species, the nature of the cloning vehicle and the likelihood of phenotypic changes resulting from introduction of the recombinant DNA.

A natural tendency is to consider changes in pathogenicity as the primary biohazard concern since these come to mind most readily when considering microorganisms; other changes which may affect the fundamental ecological potential, adaptability, metabolism, etc. of a recipient organism may be more subtle and much more difficult to assess than pathogenicity. However, these alterations may potentially present an equal or greater biohazard. We can offer only a relatively few guidelines to help an investigator in determining the class assignment of an experiment in Classes III, IV or V; perhaps the most critical is the extent of characterization of the genetic material being employed in the experiment since we believe that the potential biohazards of a purified and well-characterized donor DNA species are more easily assessed than the biohazards inherent in the introduction of a random assortment of DNA fragments.

III. CONTAINMENT PRINCIPLES AND PROCEDURES

A. Introduction and General Recommendations

Biological safety and environmental control programs for dealing with pathogenic bacteria have been implemented in clinical and biomedical research laboratories for many years (refs. 1-12). Once a potential biohazard has been defined and the risk has been assessed, the major thrust of the procedures employed to minimize the biohazard involves steps to limit risk to the laboratory worker and to prevent the escape of potentially hazardous biological material.

Many of the basic problems of containment that face an investigator studying recombinant DNA in a microbial species are similar to those faced in every medical microbiology laboratory. A clinical specimen received for microbiological analysis may contain an etiologic agent ranging from those of ordinary potential hazard to those which may require the most stringent conditions for their containment. One cannot be certain until the etiologic agent is isolated and its known pathogenicity (i.e. its potential hazard) assessed. By the same token, an investigator who employs a random assortment of DNA molecules for construction of recombinant DNA molecules could, at least in theory, isolate a variety of novel transformant bacterial clones which range in their potential biohazard. The following safety considerations are applicable to all procedures involving etiological agents in the clinical laboratory. As such they may be considered as prudent standard procedures for those working with bacteria containing recombinant DNA molecules. Obviously, those investigators working with animal or plant viruses will need to satisfy the special containment problems inherent in the laboratory manipulation of these agents.

The procedures listed below are a reiteration of long-standing microbiological practices and simply reenforce the concept that microbiological safety is a matter of good working habits. All of the general recommendations listed below are desirable for all classes of experiments, although we recognize that they are not specifically needed for the safe handling or containment of all agents.

1. Consequently, our primary recommendation for containment of potential biohazards is that all individuals planning research with recombinant DNA molecules in bacteria receive adequate training in microbiology. Such training should not be construed to mean that one needs to learn only aseptic techniques or the procedures for handling potentially infectious material. Rather, investigators cannot afford to ignore the basic biology of the microorganism -- its ecology, innate pathogenicity, physiology, growth requirements, etc. In short, an investigator must try to think in microbiological terms before initiating experiments that could potentially affect the basic ecology and/or pathogenic potential of an organism that serves as a carrier for a recombinant DNA molecule. The microorganism is not simply a "warm body" to house a recombinant DNA molecule of interest.

It is axiomatic that no safety facilities or equipment (no matter now sophisticated) can take the place of an investigator's responsibility. In terms of biological safety, the principal investigator cannot delegate, reassign, abandon or ignore his or her responsibility that adequate safety training be given to all laboratory personnel. We have appended a list of books and other publications which deal with the general topics of laboratory safety, biohazards in biological research and the handling of specific bacterial agents which may prove useful as a source of specific information.

2. As a general principle, doors to laboratories in which potentially biohazardous material is handled should be kept closed.

3. Eating, drinking or smoking in the laboratory is undesirable and in areas in which potentially biohazardous material is handled should be specifically forbidden.

4. The most frequent causes of laboratory acquired infections are accidental oral aspiration of infectious material through a pipette, accidental inoculation with syringe needles and animal bites (10,11). A further important cause of both laboratory acquired infections and contamination of the environment is aerosols from centrifugation, blending, loose needles on syringes and even the improper flame sterilization of contaminated inoculating loops and needles. (see chapters by Dimmick et.al., ref. 1) As minimal recommendations, handwashing by laboratory personnel should be encouraged and direct mouth pipetting should be discouraged. The use of cotton plugged pipettes may be acceptable for agents of low or moderate hazard but a mechanical pipetting device is preferable. Special aerosol precautions are generally not required for most bacterial species, but their use deserves careful consideration.

5. Bacterial cultures and potentially hazardous DNA should be disinfected or sterilized by autoclaving. The laboratory should be cleaned, work surfaces decontaminated and all contaminated material placed in discard pans (preferably covered) containing a suitable disinfectant or autoclaved at the end of the day. The use of specific disinfectants cannot be recommended here, since they will vary from bacterial species to bacterial species and, additionally, must be capable of rendering nucleic acid solutions "non-infectious". One should not accept manufacturer's claims for disinfectant effectiveness -- there is no substitute for a use-test evaluation performed against the microorganism and nucleic acid solutions processed in the laboratory.

6. Any research group working with agents with a known or potential biohazard should have an emergency plan, including a clean-up procedure to follow if an accident contaminates personnel or environment. Here again, the principal investigator must insure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan.

7. If a research group is working with a known bacterial pathogen for which a vaccine is available, all workers should be vaccinated. Immunization is not, however, a license for procedural short-cuts nor a substitute for safe laboratory practice.

B. Levels of Containment

The containment procedures proposed are designed to match the previously defined classes of experiments involving novel recombinant bacteria.

Since containment cannot be absolute, the rationale underlying these containment recommendations is that the greater the potential biohazard, the more stringent should be the containment. In our judgment, each level of containment implies an acceptable level of protection for laboratory workers and an acceptably low probability of escape for the organisms involved.

Class I Experiments: Requires no special containment other than practice of standard aseptic technique (i.e. use of procedures to maintain pure cultures and disinfection of discarded materials).

Class II Experiments: The basic criteria for this category are those minimal operating procedures employed in a clinical microbiology laboratory. These are:

1. Eating, drinking and smoking are forbidden in the laboratory.
2. Laboratory coats are required during handling of biohazardous material. These should not be worn outside the work area.

3. Cotton-plugged pipettes or mechanical pipetting devices are required. The latter are preferable.
4. Routine disinfection of work surfaces and prompt disinfection or sterilization of all contaminated material should be carried out.
5. Immunization of personnel is required for experimenting with S. typhi, V. cholerae, C. diphtheriae and C. tetani.
6. Specific aerosol precautions are required (see below, III, 3) when large volumes (6 or more liters) of biohazardous materials are centrifuged.

Class III Experiments: The same minimal standards described for Class II are applicable with the added provisions that:

1. No mouth pipetting of potentially biohazardous material is permitted. Mechanical pipetting devices are required.
2. The experiments are performed in laboratories that are under controlled access. This does not require a separate room in which no other work is concurrently being conducted. Rather, the intent of this containment feature is to exclude extraneous persons from the area and, hence, reduce the number of exposed individuals should a laboratory spill or other accident occur. Appropriate biohazard signs will be posted on the doors of laboratories during biohazardous experimentation as well as on the doors of storage areas or cabinets containing potentially hazardous materials. Visitors to these work areas are prohibited unless they have permission from the investigator in charge who is responsible for the visitors while they are in the area.
3. Specific aerosol precautions are mandatory (see for example, R.L. Dimmick, W.F. Voge and M.A. Chatigny. Potential for accidental Microbial Aerosol Transmission in the Biological Laboratory In Biohazards in Biological Research ed A. Hillman, M.N. Oxman and R. Pollack. Cold Spring Harbor Laboratory, 1973, pp. 246-266). Thus, syringes to which the needle is firmly fixed (e.g. Luer-Lok) should be used. Screw-capped safety cups on centrifuge tubes are required when centrifuging biohazardous materials. Operation of centrifuges in hoods or other enclosed areas is desirable. Safety equipment to prevent the dissemination of aerosols generated by blending, sonication, centrifugation, etc. is commercially available (1).

Class IV Experiments: The same minimal standards required for Class III experiments are applicable with the added provisions that:

1. At the minimum, a partial containment cabinet (see W.E. Barkly, ref. 1) or its equivalent should be used for experiments in this category. This is a local exhaust ventilation hood with a limited front opening in which air entering through is subjected to high efficiency particulate air (Hepa) filtration or incinerated before being exhausted from the area.
2. Special aerosol precautions are mandatory for experiments in this class. Centrifuges, blenders and other equipment capable of creating aerosols should be operated in separate isolation rooms or hoods (see Dimmick, et.al. and Bonn, ref. 1). The standard biological hazard sign used for highly infectious agents (op. cit. p. I-22) will be posted on cabinets, freezers, refrigerators, and/or work area where biohazardous materials are kept or are being used. Only personnel who work in the laboratory may enter the

area when this sign is posted.

Class V Experiments: The potential severity of risk entailed in Class V experiments dictates that they be carried out in specially constructed facilities used to contain highly infectious microbiological agents. In such facilities, personnel enter through a change room, shower, put on special protective clothing (i.e. disposable gloves, gowns, and foot covers), walk through a disinfecting foot bath, and enter an enclosed laboratory area that contains an "absolute containment cabinet" (Class III see Barkly, op. cit.). These cabinets are provided with ultrafilters that can be sterilized, and the hood must be capable of being fumigated. The room in which the hood is located should be completely sealed, with vapor locks around the door, light fixtures sealed into the ceiling, and all air coming out of the room must pass through appropriate Hepa filters. A double door autoclave should be mounted in the wall of the room so that after sterilization, materials can be taken out of the autoclave into another room. Upon completion of experimental procedures, the personnel must walk through a foot bath, dispose of clothing which would be sterilized, shower, go back into an entrance room, and put on their normal street clothes.

Class VI Experiments: No acceptable level of containment compatible with potential biohazard.

C. Pertinent References

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IV. RECOMMENDATIONS FOR THE IMPLEMENTATION OF GUIDELINES

A. Committees specifically dealing with potential biohazards associated with recombinant DNA should be established at every academic institution and commercial organization where experiments with potential or known biohazards are proposed.

B. All investigators wishing to carry out experiments involving possible biohazards would be required to submit a proposal to the institutional committee, indicating the purpose of the experiment, the explicit benefits to be derived, and an assessment of the potential biohazards and precautions for containment that are proposed.

C. The responsibilities of the committee would be to familiarize themselves with the extent of potential biohazards and the necessary measures for their minimization and containment. It should ensure that no experiments of this nature are carried out unless the investigator had submitted such a proposal. It would ensure that the investigator was familiar with appropriate guidelines and that a thorough review and assessment of the biohazards and their containment had been carried out. It would then evaluate the proposal and any supporting evidence and would make its recommendation on the proposed research.

D. The submitted proposal and the committee's review would be filed as public documents in a biohazards repository at the institution. This file would be submitted with all grant proposals and applications related to the experiments. Any subsequent modifications to the research program which materially affected the extent of the biohazards would require a new proposal and a further review. Progress reports would be required at yearly intervals to ensure that the proposed experiments, precautions and containment were adhered to. A complete file of all approval programs under study would also be kept in a federal repository and would be available for publication. The file of documents on each proposal would be made available by the investigator to those journals where publication policy required appropriate documentation.

E. Since the types of experiments under discussion usually require only minimal equipment, of the type generally available at most academic institutions, it is recognized that in the absence of continual supervision or monitoring, the responsibility to pursue such a program of research rests finally with the investigator. We believe therefore that the Principal Investigator must shoulder the ultimate responsibility for the experiments. Thus, responsibility for proceeding with an experiment should not be shifted from the PI to a local committee, absolving the PI from responsibilities. The local committee should make recommendations and provide advice but cannot approve a program. Thus, even in face of a favorable review by the committee, an investigator would need to ensure that a program satisfied the requirements of the guidelines. In the event that the investigator decided to ignore recommendations of the local committee, supporting evidence for proceeding with the experiments should be obtained from outside the institution which would justify the ultimate course of action.

[We believe that a combination of scientific integrity combined with peer pressure generated in the face of public availability and scrutiny of the documents mentioned above, will result in strict adherence to the guidelines, while at the same time avoiding the extremes of the approval of hazardous series of experiments by a poorly informed local committee or a veto by a local committee of experiments which would be generally accepted as valid and worthwhile under the conditions of containment that have been proposed.]

F. No potentially ecologically hazardous microorganism would be released into the environment intentionally without the approval of an international body which would be duly constituted to make judgements on such release.

G. All individuals embarking upon experiments categorized as Class II to V, should receive training in the handling of potential or infectious material and must be familiar with the NIH and ASM guidelines (See refs 2 and 3; also 9, 10 and 11) of experimental use of such materials.

[An experimenter who has been well trained in working with pathogenic microorganisms and who is familiar with the ASM Handbook of Clinical Microbiology Guidelines should have sufficient expertise to be able to make appropriate judgements regarding the classification of individual experiments in the laboratory situation. Familiarity with this information should enable him to prescribe appropriate containment procedures for that particular type of experiment and will also enable him to make correct judgement about the type of training required for technical personnel that may participate in the experiment.]

H. In those countries where experiments of the type referred to in this proposal are being carried out, it would seem necessary that national bodies sould be constituted to establish, monitor and promulgate guidelines. An international body should also be established

1. to consult with and advise national organizations on the development and implementation of guidelines;
2. to encourage the maintainance of uniform standards throughout the world;
3. to coordinate and periodically review the efficiency and applicability of international guidelines; and
4. to authorize any dissemination into the environment of new recombinant types that are likely to produce significant ecological effects.

V. CONCLUSIONS

We believe that considerable benefits are likely to result from experiments involving the genetic alteration of microorganisms. The range of possible benefits extends from the use of these techniques to add to our knowledge of basic biological phenomena, to possible practical applications in the areas of agriculture and medicine.

We believe also that a scale of risks exists in the construction of genetically altered microorganisms, and we are uncomfortable about our inability to assess precisely the extent of such risks for many types of experiments. However, we believe that the containment procedures described in this proposal will reduce any risk to laboratory workers and to the environment to a level that is acceptably low and which will allow investigators to carry out research in this area. We believe that certain experiments should presently not be carried out under any circumstances (i.e. Class VI), but that most experiments can be done if containment facilities appropriate to the risk are utilized.

We recommend that specific steps be taken as soon as possible to develop cloning vehicle-host systems which will further reduce biohazard potential, will minimize the necessity of elaborate containment facilities, and will obviate judgements which must necessarily be based on little or no data at the present time. Specifically, we recommend that special sponsored programs be instituted immediately for the development and testing of such systems. We recommend also the prompt establishment of experimental programs intended to evaluate more fully the potential hazards that may be involved in the genetic alteration of microorganisms.

We believe that perhaps the greatest potential for biohazards involving genetic alteration of microorganisms relates to possible military applications. We believe strongly that construction of genetically altered microorganisms for any military purpose should be expressly prohibited by international treaty, and we urge that such prohibition be agreed upon as expeditiously as possible.

Other recommendations for implementation of the guidelines proposed in this report are contained in Section IV.

APPENDIX A

THE ECOLOGY OF PLASMIDS AND BACTERIOPHAGES

A. Plasmid Ecology

The term R plasmid came into use in 1959 with the discovery in Japan that multiple antibiotic resistance could be transmitted by conjugation from strains of resistant epidemic Shigella to drug sensitive Escherichia coli. The significance of this observation became clear over the next four years when it became apparent that the problem of transmissible antibiotic resistance was a worldwide problem with broad implications to medicine. It is now quite clear that when a clinical isolate of an enteric organism displays multiple antibiotic resistance, the resistance is most often mediated by an R plasmid. Moreover, recent surveys of the resident flora of asymptomatic individuals in the community-at-large and the bacteria in the community environment have shown that the incidence of drug resistance has been increasing at a remarkable rate. The increase in the incidence of R plasmids in human populations is, of course, directly linked to the use of antimicrobial agents in medicine. Domestic animals also have shown a parallel emergence of resistant strains. However, the entire problem of bacterial drug resistance in animals is complicated by the fact that most classes of animals grown for food are fed diets containing antibiotic supplements for the stimulation of growth. There has, therefore, been an enormous selection for microorganisms containing plasmids because of a massive alteration in the environment.

The microbial geneticist was attracted to the study of R plasmids not only from the standpoint of their similarity to the classical F transfer system, but also from the standpoint of public health, and the unique opportunity to monitor the extent of change and the genetic basis of change in natural bacterial populations. The increased attention to natural bacterial populations has led to a broad view of the ecology of bacterial plasmids. For example, fully one-third of Escherichia coli from asymptomatic human and domestic animal populations possess at least one self-transmissible (conjugative) plasmid that confers few or no known phenotypic traits other than conjugal fertility. Bacterial plasmids confer a far greater diversity of phenotypic traits upon the bacteria that possess them than 'simply' antibiotic resistance or genes (such as enterotoxin biosynthesis) that contribute to bacterial pathogenicity. Plasmids have been identified in a variety of bacterial genera and associated with such diverse functions as the control of lactose fermentation in Streptococcus lactis, sporulation in Bacillus pumilus, and camphor degradation in species of Pseudomonas. There has been a growing appreciation of the fact that the genes for antibiotic resistance, toxin biosynthesis and other genes such as lactose fermentation, which are of 'transient' evolutionary advantage may be carried by virtually identical molecular vehicles. Thus, it is possible to isolate conjugative plasmids which are identical in over 80 percent of their molecular lengths but which carry on the one hand antibiotic resistance genes, on the other hand genes for the biosynthesis of enterotoxin and, in yet another instance, genes which control the utilization of lactose; there are numerous other examples to suggest that the same plasmid wearing different phenotypic garb is often isolated independently in several laboratories. To a great extent it appears that the genetic information which controls essential plasmid functions such as replication, the distribution of progeny replicas and, to a somewhat lesser degree, transfer functions is conserved; indeed, plasmids, regardless of phenotype, can be 'speciated' by genetic and molecular studies.

However, the origin of and relationships among plasmid genes which determine antibiotic resistance and other properties is the subject of considerable speculation. In some instances, for example certain types of ampicillin and tetracycline resistance, it now appears that the genes in question reside upon a segment of DNA which is readily translocated from replicon to replicon and that this event can occur in recombination deficient (rec$^-$) bacteria. More recent studies have shown that this 'rec' independent translocation of genes is not restricted to antibiotic resistance determinants but affects other plasmid and host genes as well.

B. Plasmid Transmission in vivo

1. Introduction

Given a conjugative R plasmid residing within an enteric organism inhabiting the bowel, the immediate thought might be that R plasmid transfer could occur quite readily to other enterobacterial strains. This conclusion would seem justified by the laboratory observation that R plasmid transfer from one strain to another can usually be demonstrated without difficulty even when it occurs at a low rate. The bowel of an animal is a far cry from a test tube, however, and from a practical point of view it is important to ask to what extent transfer occurs in vivo. The available evidence indicates that transfer does not occur on so grand a scale in vivo as it does in vitro. The following sections which deal specifically with the strain E. coli K-12 outline the general parameters of in vivo genetic transmission and will, hopefully, help interested investigators to evaluate the steps that must be taken to prevent the dissemination of recombinant plasmid DNA.

2. The "Infectivity of E. coli K-12"

Thus far, the 'cloning' of recombinant DNA molecules has been restricted to substrains of Escherichia coli K-12, B or genetic hybrids of the two. Both E. coli K-12 and B are long established laboratory strains which were initially isolated from man. One of the first questions to be asked, therefore, is how commonly these E. coli substrains can colonize the human or animal intestine. Although this precise question has not been studied extensively, it has been shown that E. coli K-12 is a very poor colonizer of the normal bowel. For example, after feeding of between 5×10^{11} to 1×10^{12} E. coli K-12 cells to calves, only about 10^7 cells can be recovered per gram of feces in 24 hours and by 72 hours cannot be identified at all (< 10 cells) (3). Similarly in man, ingestion of 10^9 cells does not normally lead to colonization, indeed, the detection of more than 100 K-12 cells/gm after 24 hours is rare. Consequently, it appears that E. coli K-12 has very little inherent capacity to colonize man.

There are, however, exceptions to this general rule. If the normal flora of man or animals is disrupted, for example, by therapeutic levels of antibiotics, the ingestion of E. coli K-12 bearing the resistance determinants to these antibiotics leads to colonization at easily detectable levels (about 10^5 per gm of feces). Similarly, individuals who have had surgical treatment for stomach or bowel disorders are far more easily colonized by all enteric species (including E. coli K-12). Finally any substance which 'protects' an ingested organism from the acidity of the stomach leads often to a higher level of K-12 excretion (although subsequent colonization of the normal bowel does not occur, the length of time of excretion may be increased by a few days). Therefore, a few simple rules appear to be prudent with regard to handling E. coli K-12, particularly when they contain either recombinant DNA molecules or naturally occurring plasmids for that matter:

a. The usual laboratory procedures employed in dealing with enteric pathogens should be followed as described above.
b. Individuals who are receiving antibiotic therapy should not work with the strains during the period they are receiving therapy and for seven days after the cessation of therapy.

c. Individuals who have functional intestinal disorders and those who have had surgical removal of part of the stomach or bowel should not work with these strains.
d. Individuals who take large amounts of antacids should be aware that they are more readily colonized by ingested bacteria. Obviously, the usual laboratory precaution of no eating in the laboratory should be followed.

3. Gene Transfer in the Gut

Although E. coli K-12 and B derivatives do not usually actively multiply and colonize the normal animal bowel, the organisms that survive the acidity of the stomach and other natural host defenses, remain viable and can act as genetic recipients or genetic donors under the proper circumstances. In so far as we are aware all of the recombinant DNA molecules that have been prepared thus far are nonconjugative, that is, they do not inherently have the ability to initiate transfer of DNA. Nonetheless, these nonconjugative plasmids can be mobilized by a transfer plasmid (such as the classical F plasmid) residing in the same cell. A possible scenario for extension of the reservoir of a recombinant DNA molecule could be as follows: A research worker ingests E. coli K-12 containing a recombinant DNA plasmid. The surviving cells while in the gut, engaged in conjugation with a member of the normal flora containing a transfer plasmid. (Note: about 38% of all E. coli strains from asymptomatic animals and man harbor at least one transfer plasmid.) The converted K-12 organism containing both the transfer plasmid and the recombinant plasmid mates with a member of the normal gut flora and the recombinant plasmid is transferred. The latter strain is fully capable of survival in the gut and can, in turn, mate with other strains.

This hypothetical sequence of events has a certain probability that can be calculated on the basis of laboratory experiments at 1 in 10^{-6} - 1 in 10^{-8} per bacterial cell. Experiments of this nature suggest, however, that the probability of this occurrence in the normal gut is on the order of 1 in 10^{-12} to 1 x 10^{-14}. This differential between laboratory and gut illustrates the concept that the best defense against R plasmid and other gene transfer is a normal gut and gut flora. Conditions in the bowel such as Eh, pH, fatty acid concentration, etc. are simply not optimal for genetic transfer. Indeed these same physiological conditions of the normal bowel provide us with one of the major natural defense mechanisms against infection by enteric pathogens. A major exception is, again, instances in which the normal flora has been modified by antibiotic treatment or if there is a functional or pathological bowel disorder. Under these circumstances, the probability of in vivo transfer increases to an average of 1 x 10^{-6} to 1 x 10^{-9}. Thus, the parameters which affect the colonization of E. coli K-12 likewise affect the probability of genetic transmission and the guidelines listed above apply to the prevention of in vivo genetic transmission. Of course, the probability of gene transfer by an ingested K-12 is exceedingly low particularly after the first 24 hours of ingestion. In our judgement, gene transfer from E. coli K-12 is not a significant hazard so long as normal precautions of the bacteriology laboratory and the containment guidelines listed earlier are followed.

4. Gene Transfer Outside the Gut

There is one situation in which gene transfer might contribute to the dissemination of recombinant plasmid species. This situation could result from an

unfortunately common practice in some laboratories, namely the discarding of culture supernatants and even viable cultures of E. coli K-12 and other "non-pathogenic" bacterial species into the laboratory sink which empties into the community sewer system. On the face of the matter it might be imagined that virtually any form of sewage treatment would effectively destroy the bacteria. This assumption is totally unfounded, however. For example, in Washington, D.C., during periods of heavy water use or during a period of heavy rainfall, it is quite possible that a high proportion of organisms disposed of down a drain would reach the Potomac River where E. coli counts in excess of 10^7/100 ml are not uncommon. (Note that this situation is found, of course, in most urban areas). There is relatively little data available concerning the frequency of genetic exchange in water. However, it is known that fecal E. coli harboring R plasmids have a very good survival potential in sewage and in river water. At any rate, it should be reemphasized that it is not a good practice to dispose of any viable bacterial culture into the community sewage disposal system. This is, of course, particularly critical with respect to cultures containing recombinant plasmid species or naturally occurring R plasmids for that matter. All such strains should be considered to have at least some minimal degree of hazard and treated with the common sense experimental practices detailed in the section on containment. Similarly, one does not know the potential hazards of gene transfer on bench tops, etc. which may be contaminated by spills. Again, one needs to reemphasize the basic methodology that is taught to every beginning student of microbiology.

Roughly 10-15% of normal, asymptomatic individuals harbor E. coli and other coloiform organisms in their nasopharynx. It is not known with any degree of certainty to what extent well-established laboratory strains of E. coli such as K-12 may colonize this anatomical region. This possibility should be investigated.

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C. Bacteriophage Ecology

The literature on bacteriophage is enormous and it would be obviously futile to attempt to summarize all that is known about their distribution in nature. Virulent bacteriophages are capable of only a productive life cycle in bacteria so that their propagation invariably leads to death and lysis of the bacterial host. Temperate phages on the other hand, as exemplified by the phage λ of E. coli K-12, lead a sort of Jekyll-Hyde existence in bacteria. They are capable of productive growth (lysis) or may become inserted into the bacterial chromosome and so assume a relatively passive role (lysogeny). The decision to lyse or insert is under the control of a complex system of genetically controlled biochemical 'switches' and it is possible for the inserted bacteriophage chromosome (called a prophage) to become induced to a productive

growth cycle after peacefully coexisting with the bacterial host for many generations. Other temperate phages such as P1, have prophages that do not integrate into the bacterial chromosome but rather replicate while attached to the bacterial inner cell membrane. As such, these prophages are plasmids.

One need only examine filtrates of fecal suspensions, raw sewage, soil, water, unpasteurized diary products or even diseased tissue to learn that both virulent and temperate phages are very common in nature. The systematic search of bacterial species for the presence of a carried temperate phage is so often successful that some writers have been moved to remark that it is difficult to believe that there are many bacterial cells that are not carrying at least one temperate phage! This certainly seems to be the case, for example, when speaking of staphylococci but for other bacterial species the reported incidence of carried phage varies from 2% to 94%. Since for the major purpose of this document we are primarily interested in the strain E. coli K-12 and the bacteriophage λ and its derivatives, it is probably best to simply focus on how often E. coli species of natural origin carry phages which can also infect E. coli K-12 and how many of these phages are 'lambdoid'.

Apparently phages resembling λ are not uncommon in wild-type E. coli. For example some 20 years ago Jacob and Wollman found that 32 or 500 fecal E. coli carried temperate phages capable of propagation on an E. coli K-12 derivative. Among these 32 phages, 3 were apparently identical to λ and at least six others could recombine with λ. All of the other phages could be effectively carried by E. coli K-12 but were not related to λ. More recent unpublished observations from several laboratories have confirmed these findings and it is probably fair to say that some 8% to 10% of all fecal E. coli harbor at least one phage capable of infecting E. coli K-12 and that from 1% to 2% of fecal E. coli carry a phage that is closely related to λ.

Some temperate phages alter profoundly the properties of bacteria that become lysogenized. This process has been termed phage conversion and is responsible for the synthesis of a number of clinically important bacterial products such as diphtheria toxin, (C. diphtheriae), fibrinolysin (S. aureus), erythrogenic toxin (S. pyogenes), tetanus toxin (C. tetani), botulinum toxin (C. botulinum), and for the serological specificity of the somatic antigens (endotoxins) of Salmonella species and enteropathogenic E. coli. In each case, the bacteriophage genome encodes the genetic information for the synthesis of the specific protein product.

Phages are capable of transduction (phage-mediated gene transfer) and this is probably true for all temperate phages as well as some virulent phages. Transducing phages can pick up DNA from prophages and/or plasmids in donor strains as well as chromosomal DNA and introduce it into appropriate recipient strains. Transduction has been demonstrated to occur in mice by using lysogenic donor and non-lysogenic recipient strains for both S. aureus and E. coli. Transducing phages or their DNA are also taken up by mammalian cells in culture where they persist and/or replicate and in at least one instance express functional gene products.

In closing, it should be noted that there has been increasing evidence over the years to suggest specific relationships between temperate phages and plasmids. Mutant derivatives of λ have been found that fail to integrate into the chromosome but replicate and persist in bacterial cells as extrachromosomal DNA or plasmids. The generalized transducing phage pf16 of Pseudomonas putida, in picking up the genes for degradation of mandelate, was found to acquire the ability to act as a conjugative plasmid and to promote transfer of both

chromosomal genes and genes for mandelate degradation to recipient strains. The discovery that inheritance of donor genetic markers in intergeneric matings between E. coli donors and S. typhi recipients and between Klebsiella pneumoniae donors and E. coli recipients often results in the formation of new plasmids, raises the question as to the origin of the genes to permit autonomous replication of these elements. The ubiquity of both defective and non-defective prophages in lysogenic bacteria that should contain such information leads us to believe that such defective and/or non-defective integrated prophages might contribute the necessary information for the formation and replication of donor DNA fragments as autonomously replicating circular plasmid molecules in recipient strains as a consequence of intergeneric matings.

APPENDIX B

ILLUSTRATIVE EXAMPLES OF EXPERIMENTS IN EACH CLASS

The examples given below are mainly for illustrative purposes. Some of the experiments might not be possible, and there is little or no justification for the performance of certain others.

A. Examples of Class I Experiment:

1. Transductional gene transfer to Escherichia coli using phages P1 or λ from E. coli K-12.
2. Transformation of E. coli K-12 with E. coli K-12 chromosomal, F plasmid or ϕ80 DNA.
3. Transformation, transduction, or transfection of Bacillus subtilis 168 with B. subtilis 168 chromosomal DNA or PBS1 phage.
4. Transformation of a well-established laboratory strain of Neisseria catarrhalis by DNA derived from the same strain.

B. Examples of Class II Experiment:

1. Conjugal gene transfer between Hfr and F^- strains of Salmonella typhimurium LT2.
2. Conjugal gene transfer between Hfr and F^- enteropathogenic E. coli strains.
3. Formation of a recombinant plasmid between the pSC101 (tetracycline resistance) and RSF1010 (streptomycin and sulfonamode resistance) plasmids when introduced into E. coli strain K-12.

4. Formation of a recombinant replicon between phage λ and the ColE1 plasmid when introduced into E. coli K-12.
5. Integration of the plasmid R64 into the chromosome of S. typhimurium LT2, and its excision to isolate an R' plasmid.
6. A survey of the host range of R plasmids found in S. typhi strains isolated from nature when introduced into E. coli K-12, S. typhimurium LT2 and Shigella dysenteriae SH.
7. Construction of a recombinant between phage P1 and an ampicillin resistance (Ap) plasmid, and the introduction of the recombinant P1-Ap molecule into E. coli K-12.
8. Construction of a recombinant between bacteriophage Mu and the R plasmid R1drd19 and its introduction into E. coli K-12.
9. Construction of recombinant molecules between phage ϕ80 and the Col trp (Fredericq) plasmid when introduced into E. coli. (It should be noted that a colicin V gene identical or similar to that on the Fredericq plasmid has been identified in a high proportion of bacterial strains involved in extra-intestinal infection.)
10. Construction of a recombinant DNA molecule involving the plasmid of B. pumilus (carrying genetic information for the inhibition of sporulation) and a temperate phage from B. subtilis when introduced into B. subtilis.
11. Intrageneric transformation of chromosomal DNA in avirulent strains of Streptococci.
12. Intrageneric transformation of chromosomal DNA in Bacillus species except B. anthracis.

13. The introduction of bacteriophage λ into S. typhimurium.

14. Intrageneric chromosome transfer between E. coli K-12 donor and either S. typhimurium, Proteus mirabilis, of Klebsiella aerogenes recipients.

15. Introduction of the genes for nitrogen fixation of the Nif plasmid of K. pneumoneae into recipient strains of E. coli K-12.

C. Examples of Class III Experiment:

1. Construction of a recombinant DNA molecule between the cryptic plasmid from S. typhimurium LT2 and the Staphylococcus aureus plasmid pI258 and its introduction into S. aureus.

2. The introduction of a phage from S. aureus that leads to production of fibrinolysin into a S. albus strain.

3. Construction of recombinant DNA molecules between sea urchin histone genes and a plasmid or bacteriophage replicon from E. coli, and their introduction into E. coli.

4. Construction of recombinant DNA molecules between the Cm plasmid (specifies chloramphenicol resistance) from S. pneumoniae and ColE1, and their introduction into E. coli.

5. Construction of a recombinant DNA molecule between λ or pSC101 and a plasmid derived from Streptomyces coelicolor and its introduction into E. coli.

6. Construction of recombinant DNA molecules between E. coli genes involved in histidine biosynthesis and a B. pumilus plasmid, and their introduction into B. subtilis.

7. Construction of a recombinant plasmid or phage that includes fibroin genes from Bombyx mori, when introduced into E. coli.

8. Construction of a recombinant DNA molecule between the chicken ovalbumin gene and ColE1 and its introduction into E. coli.

9. Construction of a recombinant molecule between the OCT plasmid of Pseudomonas putida and either phage λ or the RSF1010 plasmid, and its introduction into E. coli.

10. Construction of a DNA chimera between mouse mitochondrial DNA and phage λ or the pSC101 plasmid when introduced into E. coli K-12.

D. Examples of Class IV Experiment:

1. Construction of recombinant DNA molecules containing DNA from a phage of S. aureus that codes for the production of fibrinolysin and either E. coli plasmid or phage DNA, and their introduction into E. coli.

2. Construction of recombinant molecules between genes for photosynthesis, derived from any prokaryotic or eukaryotic organism, and E. coli phage or plasmid DNA and their introduction into E. coli.

3. Construction of a recombinant DNA molecule between plasmid DNA (specifying the synthesis of kanamycin) from Streptomyces kanamyceticus and E. coli plasmid or bacteriophage DNA, and its introduction into E. coli.

4. Construction of a recombinant between an S. mutans cariogenic plasmid and an E. coli plasmid and its introduction into E. coli.

5. Construction of a chimeric DNA molecule containing a single purified DNA fragment derived from cucumber mosaic virus and ColE1 and its introduction into E. coli.

6. Construction of a recombinant between the gene coding for the synthesis of human growth hormone and the pSC101 plasmid, and its introduction into E. coli.

E. Examples of Class V Experiment:

1. Construction of a recombinant between the S. aureus plasmid that specifies exfoliative toxin production and an E. coli phage or plasmid, and its introduction into E. coli.

2. Construction of recombinant DNA molecules between cryptic plasmid DNA from microorganisms such as Yersinia pestis, B. anthracis, or Brucella abortus and any other carrier molecule and their introduction into E. coli.

3. Construction of a chimeric DNA molecule which includes the DNA of 'Dane' particles of the hepatitis B virus and bacteriophage λ or plasmid DNA, and its introduction into E. coli.

F. Examples of Class VI Experiment:

1. Construction of a recombinant between the β phage of Corynebacterium diphtheriae that specifies toxin production and a phage or plasmid from E. coli and its introduction into E. coli.

2. Construction of a recombinant containing genetic information for toxin production from strains of Clostridium botulinum or C. tetani and E. coli phage or plasmid DNA and its introduction into E. coli.

APPENDIX C

GUIDELINES FOR MINIMIZING BIOHAZARDS

A. Introduction

Investigators wishing to construct genetically altered microorganisms should select both the DNA cloning vehicle and the recipient strain with the intent of achieving the greatest possible reduction of known and potential biohazards consistent with the aims of the particular experiment. Whenever possible, the investigators should utilize a recipient-chimera system designed to (1) minimize possible pathogenicity of the genetically altered microorganism; and (2) reduce the likelihood of its dissemination.

These goals may be accomplished by selection of appropriate naturally occurring cloning vehicles and recipient hosts, and by specific genetic manipulation of these vehicles and hosts. The following suggestions may assist in design of experiments, and may permit assignment of a particular experiment to a classification having less stringent levels of containment than might otherwise be possible. We stress that these ideas are offered as guidelines, and not as requirements, since the dictates of any given experiment will determine to a large extent which, if any, of these procedures can be utilized.

B. General Guidance Principles Regarding the Choice of Vehicles for DNA Cloning Experiments

1. By selecting and/or genetically manipulating vehicles used in cloning foreign DNA, investigators may minimize the possible biohazards involved in the construction of genetically altered microorganisms without sacrificing the objectives of the experiment. In general, non-conjugative plasmids are preferable to conjugative plasmids as cloning vehicles.

2. Cloning vehicles which do not offer any biological advantage to recipient bacteria are preferable to vehicles which may offer such an advantage.

3. Cloning vehicles which ordinarily have an intracellular existence are preferable to those existing as encapsulated extracellular particles.

4. Cloning vehicles that express genotypic or phenotypic properties that are already common in the recipient bacterial species are preferable to those expressing less common properties.

5. A vehicle which has not been subjected to experimental procedures, such as mutagenesis, which may alter its biological host range, is preferable to a vehicle which has been subjected to such procedures.

6. Cloning vehicles carrying genetic defects which may restrict their propagation are preferable to wild-type cloning vehicles.

7. Cloning vehicles that have been well characterized with regard to their genetic and molecular properties are preferable to those which have not been as well studied.

C. General Principles for Use of Antibiotic Resistance Plasmids as Cloning Vehicles

1. The cloning vehicle selected must not result in introduction of an antibiotic resistance phenotype to a medically important bacterial species in which the resistance phenotype is not found, especially tic is a drug of choice for the clinical control of the species (e.g., introduction of penicillin resistance into Streptococcus pyogenes or Streptococcus pneumoneae.)

2. The use of plasmids which carry antibiotic resistance genes that are normally rare in extrachromosomal gene pools (e.g. resistance to trimethoprim and fusidic acid) should be avoided.

3. Certain antibiotic resistance genes are preferable to others for use as selective agents in DNA cloning experiments; hence, tetracycline, sulfonamide, and streptomycin resistance are preferable for use because they occur naturally at high frequency among microorganisms present in both human and domestic animal populations.

D. Guidelines for Selection of Bacteria as DNA Donors and Recipients

1. Hosts that possess conjugative plasmids or prophages, which may facilitate dissemination of genetic material to other hosts, should be avoided if consistent with the objectives of the experiment.

2. When little is known about the genetic, metabolic, and/or ecological properties of a donor or recipient strain, such strains should be avoided for construction of genetically altered microorganisms.

3. Spore-forming microorganisms should not be used as donors or recipients of chimeric DNA molecules; mutant derivatives unable to form spores should be employed; restoration of sporogeny should not be a possible outcome of the experiment.

E. Suggestions for Possible Genetic Modification of Recipient Strains

Genetic modification of the recipient strains prior to introduction of recombinant DNA molecules may contribute further to reducing or eliminating possible biohazards. The use of recipient strains that possess mutations that reduce pathogenicity, ability to survive and/or establish in a diversity of ecological habitats and/or transmit genetic information is therefore desirable. Examples of genetic modifications that can be introduced into E. coli strains to accomplish the above objectives are provided below:

1. Use of a pur$^-$ mutant since purine-deficient mutants of many pathogenic microorganisms are avirulent.
2. Use of a dap$^-$ mutant since the amino acid diaminopimelic acid is not very prevalent in natural environments and its absence will result in inability to synthesize the cell wall and thus lead to cell lysis.
3. Use of a temperature-sensitive mutant that cannot grow at 37°C. This would minimize the ability of the genetically altered microorganism to colonize animal hosts.
4. Use of a cold-sensitive mutant that cannot grow at temperatures below 32°C. This would minimize the ability of the genetically altered microorganism to survive in soil, water and other natural environments.

5. Use of a strain that would be unable to ferment or utilize a diversity of carbohydrates - e.g. a pts mutant, phosphotransferase system defective. This would contribute to the inability of the genetically altered microorganism to grow in a diversity of ecological habitats.

6. Use of a mutant with mutations such as uvr, polA, etc. that would confer increased sensitivity to ultraviolet light, since this would contribute to inability of the genetically altered microorganism to survive in natural environments.

7. Use of a rec$^-$ mutant since this might reduce the exchange of genetic information by the recipient strain.

8. Use of a bacterial mutant that is deficient as a recipient of genetic information by conjugation. This would reduce the likelihood of introduction of conjugative plasmids from other bacteria in the natural environments and thus reduce the likelihood of mobilization and transmission of the information on the recombinant DNA molecule by conjugation. Some mutations that inhibit conjugation by bacteria may also confer increased resistance to a diversity of bacteriophages, and thus might reduce the likelihood of transmission of genetic information by transduction.

9. Use of a mutant that is resistant to a multitude of potential transducing phages since this would minimize the likelihood of dissemination of genetic information from the genetically altered microorganism.

APPENDIX D

GUIDELINES FOR MONITORING AND REASSESSMENT OF BIOHAZARDS ASSOCIATED WITH RECOMBINANT DNA MOLECULES INTRODUCED INTO MICROORGANISMS

A. Introduction

After construction of a recombinant DNA molecule and its introduction into a microbial host, it will be important for the investigator to assess the real biohazards associated with the formation of this genetically altered microorganism. In many instances the information obtained from these studies will require reclassification of the experiment into a new class category. Reclassification might result in the experiment being designated in a class requiring less containment, although in certain circumstances the determined biohazards may be more severe than originally expected which would require the reclassification of the experiment into a class requiring a more stringent level of containment.

Certain principles should be followed in obtaining information that might be useful in assessing the real biohazards associated with any given experiment. One should initially conduct specific experiments to determine whether there are any alterations in the pathogenicity of the genetically altered microorganism and any changes in its ecological potentials. If the altered microorganism contains DNA specifying unknown gene products it will be difficult, if not impossible, to assess the biohazards associated with the distribution of this genetic information among microorganisms occupying the same ecological niches as the recipient strain. In these instances it will not be possible to reclassify the experiment to employ less stringent degrees of containment. In these evaluation experiments, the cells containing recombinant DNA should be grown under the same conditions of containment as were used in the experiments that produced them. If cell products are to be analyzed, the cells should be lysed or extracted under these same conditions and these extracts tested for sterility prior to taking the material into a general research laboratory where less containment is necessary. If the product is potentially toxic, then appropriate precautions need to be taken to protect the investigator from exposure, and special facilities should be utilized to house any animals and/or plants used for testing the product. When the genetically altered microorganisms are being evaluated for pathogenicity in animal or plant hosts, these animals or plants should be under containment facilities similar to those used for the construction of the genetically altered microorganism. Such animal or plant hosts must be disposed of in a way that will not permit dissemination of the organism being tested. Tests requiring large numbers of altered microorganisms should be avoided if possible until there has been some assessment of the biohazard. If this is not possible, then such experiments should be conducted under conditions of more stringent containment.

B. Information That Will Be Helpful in Evaluating Pathogenicity

The following tests should not be considered to be all-inclusive since the particular tests to be performed will be dictated by the nature of the genetically altered microorganism, with respect to both the origin of the genetic information on the recombinant DNA molecule and the particular attributes of the recipient host species. The design and conduct of specific experiments to evaluate the real biohazards will therefore require careful evaluation by the

investigator. Some of the relevant types of experiments that can be conducted on the genetically altered microorganism include determination of its properties in the following tests:

1. Infectivity in appropriate animals or plants.
2. Colonization in the gut, oral cavity, on the skin, etc. of model animal hosts or on the roots, leaves, etc. of appropriate plants.
3. Production of keratoconjunctivitis in guinea pigs (the Sereny test) which would be an indication of the capacity of the altered microorganism to penetrate the intestinal mucosa.
4. Invasion and proliferation in macrophages and/or fibroblasts.
5. Production of such cell products as bacteriocins, hemolysins, fibrinolysins, collagenases, pectinases, etc. that might contribute to colonizing ability and/or invasiveness and toxins of various sorts and to test the potency of such toxins by using appropriate cell cultures of eukaryotic organisms, ligated intestinal loops of appropriate animal hosts or appropriate plant or animal species.
6. Production of hypersensitivity and/or necrosis by cells or extracts when injected intradermally into the skin of appropriate animal hosts.
7. Determination of the minimal inhibitory concentrations of various antimicrobial agents useful in killing and/or inhibiting growth of the altered microorganism.
8. Determination of whether or not the gene products specified by the recombinant DNA appear extracellularly, intracellularly or in the periplasmic space.

C. Information That Will Be Helpful in Evaluating Ecological Potential

The individual experiments needed to assess ecological potential of the altered microorganism will of necessity be dictated by the properties of the strains used to construct it. The following types of experiments should therefore only serve to illustrate the range of tests to determine the properties of the genetically altered microorganism:

1. Expression of the genetic traits that are specified by the recombinant DNA molecule.
2. Resistance to UV, disinfectants, etc.
3. Survival in soil, water and the dry state or in any ecological habitat likely to be occupied.
4. Ability to form spores.
5. General metabolic activities and attributes including changes in growth rate, utilizable and preferred substrates, temperature and pH optima for growth, aerobic vs. anaerobic growth, photosynthetic and N_2 fixing ability, etc.
6. Production of substances that displace or inhibit other microorganisms that normally occupy the same ecological habitats.

D. Other Information Needed to Evaluate the Severity of Biohazards

It will be extremely important to test the ability of the recombinant DNA contained in the altered microorganism to be transmitted by phage and/or

conjugative plasmids to other strains of the same species as the recipient as well as to other species of bacteria known to exchange genetic information with the recipient host species. Such tests should also be performed with other strains of the bacterial species from which DNA was obtained to construct the recombinant DNA, even when these species are not known to exchange genetic information with each other. Since some microbial species are known to excrete DNA into the medium which is sometimes biologically active, tests should also be done to determine whether the recombinant DNA is capable of being taken up and expressed in other microorganisms by transformation. Such tests for examining transmission of the recombinant DNA by transduction, transfection, conjugation, transformation and/or by encapsulation of the recombinant DNA in phage virions should be tested *in vitro* experiments and in some instances under *in vivo* conditions with appropriate animal and/or plant hosts.

E. Summary

If one performs any or all of the above experimental tests to evaluate potential biohazards of genetically altered microorganisms, it will be necessary to include as controls the organisms used as donors of the genetic information to form the recombinant DNA molecule as well as the recipient host strain.

May 28, 1975

Dr. Donald R. Helinski
Department of Biology
University of California
P.O. Box 109
La Jolla, California 92037

Dear Don:

I have had the opportunity to read over the summary statement of the organizing committee for the Asilomar Conference, and I share your concern and dismay. According to the way the statement reads, one is free to transfer regions of oncogenic viral DNAs into E. coli with "safer" vector host system under low risk containment, but if one were to introduce metabolism genes from a sporulation deficient strain of B. subtilus into E. coli which "can confer upon the recipient organisms new metabolic properties not native to the species", one would require moderate or high risk containment --- apparently regardless of what cloning vehicles or recipient cell strains are used. Similarly, moderate or high risk containment must be used "if prokaryotic experiments involve pathogenic organisms" (how ambiguous! What does "involve" mean? Is E. coli a "pathogenic organism"?), but as soon as an animal virologist concludes that he has a "safer vector-host system", he will be able to freely introduce "characterized" segments of pathogenic animal viruses into bacteria under low risk containment.

Note that the report does not define what is an acceptably "safer" vector-host system, nor does it require any testing of the safety of the system, except for high risk experiments. Is the development of temperature sensitive ColE1 or pSC101 plasmids or a DAP-requiring E. coli mutant going to be judged sufficient to allow cloning of pathogenic viral genomes in bacteria under low-risk containment? The disparity between the level of safety required by the document for prokaryotic genes and that required for animal virus genes is astonishing.

It is also interesting to note that once cloned segments of even warm-blooded vertebrate DNA are completely characterized, they no longer need be maintained in moderate risk containment laboratories. However, no such allowance is provided for non-pathogenic prokaryotic genes once they have been characterized.

The statement at the bottom of page 4 requiring the adoption of "safer" vectors and hosts as they become available needs to be modified. It implies that existing vectors are inadequate for low-risk experiments. The use of newer vectors for low-risk experiments may be fine if all you are interested in is the foreign gene being cloned, but many low risk experiments involving prokaryotic genes utilize a particular vector as an integral part of the experiment --- and therequirement to adopt another one may change the experiment conceptually. The investigator must be given flexibility for low risk studies.

It is interesting (page 8) that capacity to introduce new metabolic activities is not even considered in the case of eukaryotic or animal viral DNA, but only pathogenicity is. However, this point is considered for prokaryotic studies. Any "shotgun" experiment from a prokaryotic organism having metabolic activities not found in E. coli has the potential of conferring these new activities on some of the transformants. The document would require moderate risk containment for such experiments. Yet, although the same concern is valid for shotgun experiments involving DNA from eukaryotic organisms (which certainly contain metabolic activities not found in E. coli), these shotgun experiments would require only low risk containment.

I wonder what evidence there is for the statement on page 10 of the document that "genetic transformation of bacteria does occur in animals". Also, page 5, line 2, "possibility" should certainly be substituted for "probability". Also, page 8, item 4, should read "Class III and higher".

My overall view of the document is that it makes light of the dangers which may result from recombinant DNA molecula experiments involving oncogenic and other pathogenic viral genomes, while posing restrictions that prevent introduction of simple metabolism genes from other species into E. coli. As I read the document, it would even make no difference if the genes could be put into E. coli from the other species by conjugation or transduction, moderate or high risk containment would be required so long as the genes are not "native" to the recipient species".

At this point, Don, I have had it. I have spent a major amount of my time for more than a year attempting to contribute whatever I could to the development of credible, internally consistent, and appropriate guidelines that would insure safety of experimentation in genetic manipulation. We now appear to have recommendations designed to meet the specific experimental needs of animal virologists (the explicit reduction of containment level required for "demonstrably non-transforming regions of oncogenic viral DNA" is almost funny!), while the consideration of prokaryotic experiments uses entirely different standards.

In this letter I have pointed out the specific items that lead to my most serious concern. I hope that these comments will be useful to you and to the other members of the NIH committee in attempting to formulate a meaningful code of practice for the conduct of experiments in this area.

I will be leaving for England in several weeks, and boy am I glad! Please telephone me at Stanford if you would like to discuss any of the specific points in this letter.

With best wishes,

Sincerely yours,

Stan Cohen

SNC:lr
c.c.: Stan Falkow, Roy Curtiss, Dick Novick, Roy Clowes

Genetic manipulation to be patented?

A patent application governing commercial uses of recombinant DNA techniques has been filed in the United States. Colin Norman reports.

WHILE the scientific community has been loudly debating the potential hazards and benefits associated with a new technique for manipulating genes in living organisms, Stanford University and the University of California have been quietly trying to patent the technique in the United States. Rumours about the patent application, which apparently has been pending for at least 18 months, surfaced at a scientific symposium held at the Massachusetts Institute of Technology earlier this month; they were confirmed by university officials last week.

Though the people concerned with the patent application are reluctant to discuss it in detail while the matter is pending, the patent is understood to be worded broadly enough to cover commercial uses of any method of transplanting genes from one organism into another. The patent, if awarded, would not affect research uses of the technique, and it would apply only in the United States.

The basis for the application is research published in 1973 and 1974 by groups led by Stanley Cohen at Stanford and Herbert Boyer at the University of California, San Francisco. Their efforts led to the first demonstration that genes can be snipped from the DNA of virtually any organism and spliced into a bacterial plasmid (a ring of DNA which reproduces inside a bacterium independent of the bacterium's chromosomes). The key part of their research showed that the modified plasmid could be reinserted into a bacterium so that the transplanted, 'foreign' genes are copied each time the bacterium reproduces. It is understood that the patent application covers the process for constructing hybrid DNA moleoules capable of self-reproduction, which means that, if awarded, it would also apply to transplanting genes into viruses and bacteriophages.

There has been speculation that the technique might, eventually, lead to such commercial uses as the insertion into bacteria of genes capable of producing pharmaceutical products, such as insulin, so that specially engineered bacteria would be capable of secreting expensive drugs. Another speculative commercial application would be to graft genes capable of fixing nitrogen into crop plants such as wheat, to produce a new variety capable of synthesising its own nitrogen fertilisers from the atmosphere. Such uses of the technique would be covered by the patent application.

Discussion of the patent application arose at a symposium on genetic manipulation held by Miles Laboratories at MIT on June 8–10. Noting that there have been persistent rumours that somebody is trying to patent the technique, a speaker asked whether any participants could shed some light on the matter. Cohen, who was present at the meeting, confirmed that Stanford and the University of California are looking into the possibility of taking out a patent, and he emphasised that neither he nor any of the other researchers involved would benefit financially if it were awarded.

A patent application can be filed in the United States up to a year after the information on which it is based has been made public. The first paper by Cohen and Boyer's groups was published in November, 1973, which indicates that the application should have been filed before November 1974. Asked last week whether that assumption is correct, a Stanford patent officer replied that "it would be reasonable to assume that we were prudent in filing our application". In some countries, such as Great Britain, a patent must be applied for before any public disclosure is made. Britain's National Research and Development Corporation initially looked into the possibility of seeking a patent on genetic manipulation techniques developed by Kenneth and Noreen Murray at Edinburgh University, but dropped the idea because of prior disclosure.

When the matter was raised at the Miles symposium, two concerns were discussed. First, some scientists were worried that the patent, if awarded, might interfere with research. And second, it was suggested that it may force some commercial concerns to seek a less safe, but patent-free, genetic manipulation technique. Neither concern seems to be valid, however, and in fact the patent, if awarded, could have some safety benefits.

As for the research implications, the patent would apply only to commercial use of the technique—it would not cover either academic or industrial research uses. Moreover, asked whether it may hold up beneficial applications of the technique, a Stanford official argued that royalties derived from the patent would be "reasonable" and would not limit its use.

As far as safety implications are concerned, the application seems to cover the key steps in the genetic manipulation process and thus, unless it is drastically narrowed by the US Patent Office, it would be difficult to see how a different patent-free process could be developed. If the patent is awarded, Cohen suggested at the Miles symposium that Stanford and the University of California could insist that commercial users of the process be required to sign an undertaking to abide by safety guidelines laid down by the National Institutes of Health (NIH).

NIH is due to issue guidelines on June 23 governing genetic manipulation research which it supports. At a meeting earlier this month, NIH Director Donald Frederickson briefed a number of industry officials on the guidelines, and though they met with general support, some drug company representatives expressed reservations about one or two provisions. The NIH guidelines, moreover, would not be binding on industry, since they would apply only to NIH-sponsored research. The patent may, therefore, provide a means of extending the coverage of the guidelines.

Cohen and Boyer's work was supported by the National Institutes of Health, but the federal government is unlikely to have a stake in the patent. Stanford has a standing agreement with the Department of Health, Education and Welfare (HEW) which gives the university patent rights on inventions produced from research supported by HEW grants, unless the project has been exempted from the agreement. According to an HEW official, the genetic manipulation studies were not exempted. Stanford patent officers have, however, been discussing the application with federal officials, particularly as regards establishing licensing arrangements if the patent is awarded. □

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

August 23, 1976

Dr. Stanley N. Cohen
Department of Medicine
Stanford University School
of Medicine
Stanford University Medical Center
Stanford, CA 94305

Attention: Diane E. Bush

Please distribute a copy to everyone in the lab. Have sign-up list for copy

Dear Dr. Cohen:

Enclosed are the NIH Guidelines for Research Involving Recombinant DNA Molecules and the Decision of the Director, NIH, as published in the Federal Register.

The next printing of the Guidelines will be in a manual style which will facilitate periodic updating. In the near future, a postcard will be sent to all concerned to determine those who wish to receive the Guidelines in this format.

If any additional information is needed, please feel free to contact me.

Sincerely yours,

William J. Gartland (sgd)

William J. Gartland, Jr., Ph.D.
Acting Director
Office of Recombinant DNA Activities
National Institute of General Medical Sciences

Enclosures

SC: S Chang
FC: F Cuatrecasas
MC: M. Goodobon
AC: A Kiho Cheng
JC: Joany Chen
CM:
PK: P. Kretschmer
PN:
D.Tu: David Tu
Dean Taylor:
CR: Chuck Kaplan
Peter Pearcell

RECOMBINANT DNA: FACT AND FICTION

Stanley N. Cohen
Stanford University School of Medicine

Statement prepared for a meeting of the Committee on Environmental Health, California Medical Association

November 18, 1976

Almost three years ago, I joined with a group of scientific colleagues in publicly calling attention to possible biohazards of certain kinds of experiments that could be carried out using newly developed techniques for linking together genes from diverse sources into biologically functionally molecules. Because of the newness and relative simplicity of these techniques, we were concerned that experiments involving certain genetic combinations that seemed to us to be hazardous might be performed before adequate consideration had been given to the potential dangers. Contrary to what was believed by many observers, our concerns pertained to a few very specific type of experiments that could be carried out using the new techniques, not to the techniques themselves.

Guidelines have long been available to protect laboratory personnel and the general public against known hazards associated with the handling of certain chemicals, radioisotopes, and pathogenic micro-organisms, but because of the newness of recombinant DNA techniques no guidelines were yet available for this research. My colleagues and I wanted to insure that the powerful new techniques wouldn't be employed, for example, for the construction of Streptococci or Pneumococci resistant to penicillin --- or for the creation of E. coli capable of synthesizing botulinum toxin or diptheria toxin. We asked that these experiments not be done, and also called for deferral of construction of recombinants containing tumor virus genes until the implications of such experiments could be given further consideration.

During the past two years, much fiction has been written about "recombinant DNA research". What began as an act of responsibility by scientists, including a number of those involved in the development of the new techniques, has become the breeding ground for a horde of publicists --- most poorly informed, some well-meaning, some self-serving. This statement represents an attempt to inject some relevant facts into the extensive public discussion of recombinant DNA research. First of all:

"Recombinant DNA research" is not a single entity, but rather is a technique that can be used for a wide variety of possible experiments. Much confusion

has resulted from a lack of understanding of this point by many who have written about the subject. Recombinant DNA techniques, like chemicals on a shelf, are neither "good" nor "bad" per se: certain experiments that can be done using these techniques are likely to be hazardous (just as certain experiments done with chemical combinations taken from the shelf will be hazardous), and there is universal agreement that such recombinant DNA experiments should not be done. Other experiments that use the very same techniques, such as taking apart a DNA molecule and putting segments of it back together again, are without conceivable hazard -- and anyone who has looked into the subject has concluded that these experiments can be done without concern.

Then, there is the area "in between": for many experiments, there is no evidence of biohazard, but there is also no certainty that there is not a hazard. For these experiments, guidelines have been developed in an attempt to match a level of containment with a degree of hypothetical risk. Perhaps the single point that has been most misunderstood in the controversy about recombinant DNA research, is that discussion of "risk" in the middle category of experiments relates entirely to hypothetical and speculative possibilities, not expected consequences or even phenomena that seem likely to occur on the basis of what is known. Unfortunately, much of the speculation has been interpreted as fact.

There is nothing novel about the principle of matching a level of containment with the level of anticipated hazard; the containment procedures used for pathogenic bacteria, toxic substances, and radioisotopes attempt to do this. However, the containment measures used in these areas address themselves only to known hazards and do not attempt to protect against the unknown. If the same principle of protecting only against known hazards were followed in recombinant DNA research, there would be no containment whatsoever except for a very few experiments. In this instance, we are asking not only that there be no evidence of hazard, but that there be positive evidence that there is no hazard. In developing guidelines for recombinant DNA research we have attempted to take precautionary steps to protect ourselves against hazards which are not known to exist -- and this unprecedented act of caution is so novel that it has been widely misinterpreted as implying the imminence or at least the likelihood of danger.

Much has been made of the fact that even if a particular recombinant DNA molecule shows no evidence of being hazardous at the present time, we are unable to say for certain that it will not devastate mankind some years hence.

Of course this statement is correct, just as we are unable to say for certain that the vaccines we are administering to millions of children do not contain genes that will produce contagious cancer some years hence, we are unable to say for certain that a huge meteor will not fall on and destroy New York City next week, we are unable to say for certain that a new virulent virus will not arise spontaneously next winter and cause a world wide fatal epidemic of a hitherto unknown disease, we are unable to say for certain that novel hybrid plants being bred around the world will not suddenly become weeds that will overcome our major food crops and cause world wide famine, etc., etc., etc.

The statement that potential hazards could result from certain experiments using recombinant DNA techniques is akin to the statement that a meteor could wipe out New York City next week, a world wide pandemic caused by a new mutant virus could destroy mankind, a vaccine injected today into millions of people could lead to cancer in twenty years or a new plant species could uncontrollably destroy the world's food supply. We have no reason to expect that any of these things will happen, but we are unable to say for certain that they will not happen. Similarly we are unable to guarantee that any of man's efforts to influence the earth's weather, explore space, modify crops, or cure disease will not carry with them the seeds for the ultimate destruction of civilization. Can we in fact point to one major area of human activity where one can say for certain that there is zero risk? Clearly, it is possible to develop plausible "scare scenarios" involving virtually any activity or process, and these would have as much (or as little) basis in fact as most of the scenarios involving recombinant DNA. We must distinguish fear of the unknown from fear that has some basis in fact; this appears to be the crux of the controversy surrounding recombinant DNA.

Unfortunately, the public has been led to believe that the biohazards described in various scenarios are likely or probable outcomes of recombinant DNA research. "If the scientists themselves are concerned enough to raise the issue", goes the fiction, "the problem is probably much worse than anyone will admit." However, the simple fact is that there is no evidence to even suggest that a bacterium carrying any recombinant DNA molecule poses a hazard beyond the hazard that can be predicted from the known properties of the components of the recombinant. And experiments involving genes from organisms carrying genes that produce toxic substances or pose other known hazards are prohibited.

Freedom of Scientific Inquiry

This issue has been raised repeatedly during discussions of recombinant DNA research. "The time has come" the critics charge, "for scientists to abandon their long-held belief that they should be free to pursue the acquisition of new knowledge regardless of the consequences". The fact is that no one has proposed that freedom of inquiry should extend to scientific experiments that endanger public safety. Yet, "freedom of scientific inquiry" is repeatedly raised as a straw man issue by critics who imply that somewhere there are those who argue that there should be no restraint on research.

Instead, the history of this issue is one of self-imposed restraint by scientists from the very start. The scientific group that first raised the question of possible hazard in some kinds of recombinant DNA experiments included most of the scientists involved in the development of the techniques -- and their concern was made public so that other investigators who had not adequately considered the possibility of hazard could exercise appropriate restraint. While most scientists would defend their right to freedom of scientific thought and discourse; I do not know of anyone who would propose that scientists should be free to do whatever experiments they choose regardless of the consequences.

Interference with "evolutionary wisdom"

We are asked by some critics of recombinant DNA research to believe that the process of evolution of plants and animals has remained delicately controlled for millions of years, and that the construction of recombinant DNA molecules now threatens the master plan of evolution. Such thinking, which requires a belief that nature is endowed with wisdom, intent, and foresight, is alien to most post-Darwinian biologists. Is there any evidence that the evolutionary process is presently delicately controlled by nature? To the contrary, man has long ago modified the process of evolution as it existed in the Garden of Eden, and biological evolution continues to be dominated by man. Primitive man's domestication of animals and cultivation of crops provided an "unnatural" advantage to certain biological species and a perturbation of evolution. The later creation by man of hybrid plants and animals has resulted in the propagation of new genetic combinations that are not the product of natural evolution. In the microbiological world, the use of antimicrobial agents to treat infections has interfered irreversibly with any delicate evolutionary control that may have existed previously.

In a recent letter to Science that has been widely quoted

by critics of recombinant DNA research, Professor Erwin Chargaff of Columbia University asks, "Have we the right to counteract irreversibly the evolutionary wisdom of millions of years ...?" It is this so called "evolutionary wisdom" that gave us the gene combinations for bubonic plague, smallpox, yellow fever, typhoid, polio, diabetes, and for that matter -- cancer. It is this "wisdom" that continues to give us uncontrollable diseases such as Lassa fever, Marburg virus, and very recently the Marburg-related hemorrhagic fever virus which has resulted in nearly 100% mortality in infected individuals in Zaire and the Sudan. The acquisition and use of all biological and medical knowledge constitutes an intentional and continuing assault on "evolutionary wisdom". Is this the "warfare against nature" that Professor Chargaff fears from recombinant DNA?

How about the "benefits?"

For all but a very few experiments, the "risks" of recombinant DNA research are entirely speculative and it seems appropriate to ask those who claim otherwise for evidence to support their assertions. Are the benefits equally speculative or is there some factual basis for expecting that benefits will occur from this technique? I believe that the anticipation of benefits has a substantial basis in fact, and that the benefits fall into two principal categories: (1) advancement of fundamental scientific and medical knowledge, and (2) possible practical applications.

In the short space of three and a half years the use of the recombinant DNA technology has already been of major importance in the advancement of fundamental knowledge. We need to understand the structure and function of genes and this methodology provides a way to prepare large quantities of specific segments of DNA in pure form. For example, recombinant DNA methodology has provided us with much information about the structure of plasmids that cause antibiotic resistance in bacteria, and has given us insights into how these elements propagate themselves, how they evolve, and how their genes are controlled. In the past, our inability to isolate specific genetic regions of the chromosomes of higher organisms has limited our understanding of the genes of complex cells; now with preparations of pure genes we can begin to examine the structure and function of normal and malfunctioning chromosomes, to learn for example how abnormal hemoglobins are produced in certain human disorders. Use of recombinant DNA techniques has provided important new

insights into how genes are organized into chromosomes and how gene expression is controlled. With such knowledge we can begin to learn how defects in the structure of such genes alter their function and regulation.

On a more practical level, recombinant DNA techniques potentially enable the construction of bacterial strains that can produce biologically important substances such as antibodies and hormones. Although the full expression of higher organism DNA that is necessary to accomplish such production has not yet been achieved in bacteria, the steps that need to be taken to reach this goal are defined, and we can reasonably expect that the introduction of appropriate "start" and "stop" control signals into recombinant DNA molecules will enable the expression of animal cell genes. On an even shorter time scale, we can expect recombinant DNA techniques to revolutionize the production of antibiotics, vitamins, and medically and industrially useful chemicals by eliminating the need to grow and process the often exotic bacterial and fungal strains currently used as sources for such agents. We can anticipate the construction of modified antimicrobial agents that are not destroyed by the antibiotic inactivating enzymes responsible for drug resistance in bacteria.

In the area of vaccine production, we can anticipate the construction of specific bacterial strains able to produce desired antigenic products -- eliminating the present need for immunization with killed or attenuated specimens of disease-causing viruses. One practical application of recombinant DNA technology in the area of vaccine production is already close to being realized: an *E. coli* plasmid coding for an enteric toxin fatal to livestock has been taken apart, and the toxin gene has been separated from the remainder of the plasmid. The next step is to cut away a small segment of the toxin-producing gene so that the substance produced by the resulting gene will not have toxic properties but will be immunologically active in stimulating antibody production.

Other benefits from recombinant DNA research in the areas of food and energy production are more speculative. However, even in these areas there is a scientific basis for expecting that the benefits will someday be realized. The limited availability of fertilizers and the potential hazards associated with excessive use of fertilizers presently limits certain crop yields, but agricultural experts suggest that transplantation of the nitrogenase system from the chromosomes of certain bacteria into plants or into other bacteria that live

symbiotically with food crop plants may eliminate the need for fertilizers. For many years, scientists have modified the heredity of plants by comparatively primitive techniques. Now there is an efficient means of doing this by design, rather than by chance.

Certain algae are known to produce hydrogen from water using sunlight as energy. This process potentially can yield a virtually limitless source of pollution-free energy if technical and biochemical problems indigenous to the presently known hydrogen-producing organisms are solved. Recombinant DNA techniques represent a possible means of solution of these problems.

Even if hazards are speculative and the potential benefits are significant and convincing, wouldn't it still be better to carry out recombinant DNA experiments under conditions that provide an added measure of safety -- just in case some of the conjectural hazards prove to be real?

This is exactly what is required under the NIH guidelines for recombinant DNA research:

1. These guidelines prohibit experiments in which there is some scientific basis for anticipating that a hazard will occur. In addition, they prohibit experiments in which a hazard, although it might be entirely speculative, was judged by the National Institutes of Health to be potentially serious enough to warrant prohibition of the experiment.

2. The guidelines require that a large class of other experiments be carried out in P4 high level containment facilities of the type designed for work with the most hazardous naturally-occurring microorganisms known to man (eg. Lassa fever virus, Marburg virus, Zaire hemorrhagic fever virus, etc.). It is difficult to imagine more hazardous self-propagating biological agents than such viruses, some of which lead to nearly 100% mortality in infected individuals. P4 containment requires a specially built laboratory with airlocks and filters, biological safety cabinets, clothing changes for personnel, autoclaves within the facility, etc. This level of containment is required for recombinant DNA experiments for which there presently is no evidence of hazard, but for which it is perceived that the hazard might be potentially serious if conjectural fears prove to be real. There are only four or five installations in the United States where P4 experiments can be carried out.

3. Experiments associated with a still lesser degree of hypothetical risk can be conducted in P3 containment facilities. These are also specially constructed laboratories requiring double door entrances, negative air pressure, and special air filtration devices. Facilities where P3 experiments can be

performed are limited in number, but they exist at some universities.
4. Experiments in which the hazard is problematical and is considered unlikely to be serious even if it occurs still require laboratory procedures (P2 containment level) that have for years been considered sufficient for research with pathogenic bacteria such as Salmonella typhosa, Clostridiumbotulinum, and Cholera vibrio. The federal guidelines require that P2 facilities be used for work with bacteria carrying inter-species recombinant DNA molecules that have shown no evidence of being hazardous -- and even for some recombinant DNA experiments in which there is substantial evidence of lack of hazard.
5. The P1 (lowest) level of containment can be used only for recombinant DNA molecules that potentially can be made by ordinary biological recombination procedures in bacteria. Even this lowest level of containment requires decontamination of work surfaces daily and following spills of biological materials, decontamination of liquid and solid waste leaving the laboratory, use of mechanical pipetting devices or cotton plug pipettes, and a pest control program in the laboratory.

In other areas of actual or potential biological hazard, physical containment is all that microbiologists have had to reply upon; if the Lassa fever virus were to be disseminated from a P4 facility, there would be no further barrier to prevent the propagation of this virus which is known to be deadly and for which no specific therapy exists. However, even though the hazards of recombinant DNA molecules are conjectural, the NIH guidelines have added an additional level of safety for workers and the public: this is a system of biological containment that is designed to reduce by many orders of magnitude the chance of propagation of microorganisms used as hosts for recombinant DNA molecules.

An inevitable consequence of these containment procedures is that they have made it difficult for the public to appreciate that most of the hazards under discussion are entirely conjectural. Because in the past, governmental agencies have often been slow to respond to clear and definite dangers in other areas of technology, it has been inconceivable to scientists working in other fields and to the pbulic at large that an extensive and costly federal machinery would have been established to provide protection in this area of research unless severe hazards were known to exist. The fact that recombinant DNA research has prompted international meetings, extensive coverage in the news media, and governmental intervention at the federal level has been perceived by the

public as prima facie evidence that this research "must be more dangerous than all the rest". The scientific community's response the been to establish increasingly elaborate procedures to police itself -- but these very acts of scientific caution and responsibility have only served to perpetuate and strengthen the general belief that the hazards under discussion must be clear-cut and imminent in order for such steps to be necessary.

It is worth pointing out that despite the scenarios that predict imminent disaster from recombinant DNA experiments, the fact remains that in the past three and a half years, many billions of bacteria containing a wide variety of recombinant DNA molecules have been grown and propagated in the United States and abroad, incorporating DNA from viruses, protozoa, insects, sea urchins, frogs, yeast, mammals, and unrelated bacterial species into E. coli. The majority of these experiments were not subject to the stringent containment procedures specified in the current federal guidelines. However, in no instance has any of these recombinants been found to confer a novel hazardous property upon its host E. coli, and in no instance has any adverse consequence been observed.

Despite the absence of detectable hazards in experiments done thus far, it will always be possible to validly argue that recombinant DNA molecules that seem safe today may prove hazardous tomorrow: one can no more prove the safety of a particular genetic combination under all imaginable circumstances than one can prove for certain that the swine flu vaccine presently being used does not contain an unknown and undetected substance that leads to death in certain individuals, or that an undetected substance capable of producing future cancer is present in currently administered batches of measles vaccine. No matter what evidence is collected to document the safety of a new therapeutic agent, a vaccine, a process, or a particular kind of recombinant DNA molecule, one can always conjure up the possibility of future hazards which it will not be possible to disprove. When one deals with conjecture, the number of possible hazards is unlimited; the experiments that can be done to establish the absence of hazard are finite in number.

Those who argue that we should not use recombinant DNA techniques until or unless we are "absolutely certain" that there is zero risk fail to recognize that no one will ever be able to guarantee total freedom from risk in any significant human activity. All that we can reasonably expect is a mechanism for dealing responsibly with hazards that are known to exist or which appear likely on the basis of information that is known. Beyond this, we can and should

exercise caution in any activity that carries us into previously uncharted territory, whether it is recombinant DNA research, creation of a new drug or vaccine, or bringing a space ship back to earth from the moon. Just as one can speculate about possible disasters as an outcome of recombinant DNA experiments, one can conjure up similarly frightening scenarios as possible outcomes of virtually all of mankind's scientific and intellectual endeavors.

Today, as in the past, there are those who would like to think that there is freedom from risk in the status quo. However, humanity continues to be buffeted by ancient and new diseases, and by malnutrition and pollution; recombinant DNA techniques offer a reasonable expectation for a partial solution to some of these problems. Thus, we must ask whether we can afford to allow preoccupation with and conjecture about hazards that are not known to exist, limit our ability to deal with hazards that do exist? We must ask whether there is any rational basis for predicting the dire consequences of recombinant DNA research portrayed in the scenarios proposed by some. We must then examine the "benefit" side of the ledger and weigh the already realized benefits and the reasonable expectation of additional benefits, against the vague fear of the unknown that has in my opinion been the focal point of this controversy.

Addendum

During recent months, a number of hypothetical scenarios of disastrous outcomes of "recombinant DNA research" have been widely circulated by opponents of such research. The following scenarios, although hypothetical, are based on actual events, and are provided simply to illustrate that conjecture can be applied as easily to support the need for continued research with recombinant DNA techniques, as to support a proposal to ban such research.

Scenario I

Fact: In late 1976, a previously unknown viral disease characterized by high fevers, severe bleeding, and death occurring in more than 90% of infected individuals appeared in Central Africa. The disease was caused by a virus thought to be related to the "green monkey" virus that appeared in Europe in 1967 and killed persons at West Germany's Marburg University who had handled green monekys in Africa. During a 6 week epidemic, the hemorrhagic fever disease claimed more than 300 victims in Zaire and the Sudan before its spread was drastically cut in late November by the use of stringent isolation procedures. Dr. H.T. Mahler, the Director-General of the World Health Organization, while recognizing that no specific therapy for the disease exists and doctors do not know how the infection spreads, nevertheless did not believe it was "a very imminent ... danger to the rest of humanity". However, Dr. Mahler warned, infection can spread easily "in the kind of world in which we live, with highways criss-crossing Africa

Scenario: In September 1983, a deadly disease resembling the hemorrhagic fever outbreak of 1976 appeared in East Africa. As in the case of the earlier Zaire-Sudan epidemic, mortality was nearly 100% in victims of the disease. Infection spread quickly to medical and nursing personnel from two patients brought to a small village hospital, and then to additional individuals within a 150 square mile area. Despite attempts of the government to cordon off the involved region, infective foci appeared in other parts of the country and in a matter of six weeks more than 140,000 persons had died from the disease.

Infection was spread to Europe by a passenger on one of the last plane-loads of passengers permitted to leave Africa. In order to avoid similar importation of the dread disease to the United States, the North American Continent was sealed off from physical communication with the rest of the world and the U.S. Navy and Air Force were instructed to use whatever measures are

necessary to prevent landing of foreign boats or planes on U.S. shores. Scientists on several continents working in tightly sealed laboratories sought desperately but unsuccessfully to prepare amounts of the virus sufficient to attempt vaccine production. As a last resort, investigators from several American universities who had been called to the Public Health Service Communicable Disease Center in Atlanta were granted permission to begin working again with recombinant DNA techniques that had been outlawed by Congress in mid-1978; their goal was to construct a bacterial strain able to synthesize the immunologically reactive protein coat of the hemorrhagic fever virus, and to use these bacteria for the production of a vaccine against the deadly disease.

Scenario II

Fact: In the Fall of 1976, the Royal Swedish Academy of Sciences, at a Nobel Symposium on the subject of nitrogen balance, warned about the danger of progressive depletion of the ozone content of the atmosphere as a result of too heavy use of nitrogen fertilizers. In order to make nitrogen available for growing plants, fertilizer puts unnaturally large amounts of fixed nitrogen into the soil; the Academy indicated that this condition favors denitrofication, which releases nitrous oxide into the air and leads to the consequent destruction of ozone. The Academy also warned that large amounts of nitrogen fertilizer inhibit the natural processes of nitrogen fixation carried out by legumes such as peas and beans. When nitrogen fertilizers are used to increase the productivity of an open agricultural system, the Academy said, the average yield per acre usually becomes progressively smaller and the effect on the environment progressively greater as the rate of application increases.

The Academy also warned that nitrogenous pollutants in the air may react with amines found in the body to form nitrosamines, which have been shown to cause cancer in most organs of experimental animals, and to also be a cause of genetic alterations.

Scenario: By 1986, the use of fertilizers had destroyed atmospheric ozone to the point where the World Health Organization had declared an international crisis. Crop yields had become increasingly smaller in response to fertilizer applied. Yet, increasing use of fertilizer was required in order to attempt to feed the hungry of the world, and an international plea by some nations to ban the use of nitrogen fertilizers was unsuccessful. An alarming increase in the rate of cancer and fetal abnormalities was evident in those countries that were the heaviest fertilizer users.

Although recombinant DNA experimentation had been banned for almost ten years in many countries, scientists in an eastern European nation that had continued to support such investigations announced their success in developing a recombinant DNA technique for the introduction of nitrogen fixing genes directly into wheat and corn plants, thus eliminating the need for the use of fertilizer in the growth of these important food crops. Because of the vast political and economic implications of this discovery, the United States Congress began support of a crash effort in recombinant DNA research in an attempt to develop experimental expertise and fundamental knowledge in this area.

Scenario III

Fact: In February 1976, the General Electric Company was found to be in violation of New York State's water policy standards because of its discharge of polychlorinated biphenyls into the Hudson River. The danger was brought to public attention in late 1975 when high levels of the extremely stable chemical were found in fish taken from the Hudson River, and this discovery eventually led to closing of the river to most kinds of commercial fishing. PCB was shown to be a cause of a variety of illnesses among GE workers, and to produce cancer in laboratory animals; in Japan, more than 1,000 people were stricken with liver disease and skin problems as a result of PCB's that had leaked into cooking oil. General Electric admitted having dumped PCB's into the Hudson River for more than 25 years, and it is believed that by 1976, the river contained about 500,000 pounds of the toxic chemical. While Congress moved towards a complete ban on PCB's, scientists sought a mechanism for getting rid of the toxic chemical that contaminated the Hudson River.

Scenario: The enzymes necessary to carry out part of the degradation of polychlorinated biphenols were discovered in late 1977 to be present in separate strains of *Pseudomonas putida*. Because of a nationwide ban on any research involving the joining of genes in a test tube, scientists were required to attempt the use of *in vivo* recombination procedures for the construction of a *Pseudomonas* organism able to rid the river of the PCB pollutants. Microbiological organizations tried in vain to convince the New York State Attorney General's office that although the needed gene combination eventually might be made by means of the earlier "trial and error" *in vivo* genetic recombination procedures, the use of recombinant DNA techniques could provide a faster, safer, and more precise method of achieving the same goal.

STANFORD UNIVERSITY MEDICAL CENTER
STANFORD, CALIFORNIA 94305

STANFORD UNIVERSITY SCHOOL OF MEDICINE
Department of Medicine

April 6, 1977

Hewlett Lee, M.D.
Chairman, Committee on Environmental Health
California Medical Association
Palo Alto Medical Clinic
Palo Alto, CA 94301

Dear Huey:

I am writing in connection with Assembly Bill #757 (copy enclosed) which has been introduced into the California Legislature. Hearings on the bill will proceed during the next three weeks, and it is my understanding that the bill will be reported to the Assembly by the Committee on Health on April 25. I've been advised by sources close to the legislature that this bill, or a modification of it, has an excellent chance of being adopted by the State Assembly. I believe that passage of the bill would have consequences of a most serious nature for medical research and clinical care in California, and I am writing to ask the Committee on Environmental Health and the California Medical Association to oppose the bill.

So far as the specific wording is concerned, the current definition of "hazardous biological research" in AB 757 would cover clinical studies involving items such as throat swabs, sputum cultures (TB and other microbes), administration and testing of viral vaccines, etc., -- since all of these things involve "organisms or infectious agents that are capable or can be rendered capable of causing wide-spread serious harm, directly or indirectly, to the health of a substantial population of humans or to the natural environment". The Bill would also cover the creation of hybrid plants, ordinary genetic recombination that takes place naturally in living cells, and presumably human procreation -- since this also involves "organisms...which are capable, or can be rendered capable, of causing wide-spread serious harm, etc.". Moreover, since there is no evidence that the bacterial organisms constructed in most types of recombinant DNA research are in fact capable or can be rendered capable of causing wide-spread harm, it is not clear that this research would be covered.

The definition for recombinant DNA research contained in the bill has been lifted from the NIH guidelines. However, the definition in the guidelines is about to be changed; the current definition involves regulation of the joining of two genes derived from the <u>same</u> organism if such joining is done in a cell-free system -- but not when the joining of the same two genes is done within cells. Since the end products are identical in both instances, the current definition doesn't make sense -- and this has led to a number of administrative problems. Similarly, the definition in AB 757 would cover experiments which everyone agrees would have no opportunity to produce novel biotypes or "new forms of life".

The bill also has a number of other very serious problems. It does not specify the criteria to be used for determining whether or not the research being regulated is being conducted in compliance with the law, and it makes violation of these unspecified criteria a felony offense. When it was pointed out to the State Commissioner of Health that the proposed legislation would have broad implications for all kinds of biological research, he was quoted in the press as stating that the legislation would need to be modified to exclude those organisms that cause common infectious diseases. Apparently, the Commissioner would eliminate control over those organisms that are known to be hazardous, and would cover only those organisms for which no evidence of hazard exists.

At the time Assembly Bill 757 was proposed, no federal legislation was in progress. However, there are now several bills pending before the US Senate and House of Representatives, and enactment of one of these is expected within eight weeks with the legislation to go into effect by July 1. While certain members of the California Assembly may find it politically advantageous to pursue separate state legislation, it seems reasonable to ask whether there are specific needs for the protection of California citizens that are not met by the Federal legislation. If so, what are they? What is the documentation for these needs? To what extent would state legislation duplicate what is being done at the federal level?

Even if the bill were a good one (and I believe that it is not), it seems reasonable to ask whether it makes any sense to have standards that vary among different states and communities. Even strong proponents of stringent regulations, such as Sinsheimer, are opposed to standards that vary from state to state and from community to community. Unlike automobile emission controls and certain other areas of regulation where it makes sense to modify standards according to local conditions, it does not appear to be reasonable in this area; microbes do not respect state boundaries, and interstate travel is a fact of life.

Even at present there are estimated to be about 60 separate laboratories in California involving recombinant DNA research, alone; the proposed bill covers many other areas of research also. If the bill were passed, how many inspectors would be needed to carry out the functions required? How often would they visit the laboratories? What equipment and facilities would they need to monitor the research? Where would they be housed? What level of training would they require, and who would train them? What standards of expertise for inspectors would be established? Who would hire and supervise these inspectors, and who would assure the competence of the bureaucracy that would be established to monitor this very specialized research? What advantage would all of this costly statewide apparatus provide beyond what is provided in the currently proposed federal legislation?

The proposed federal legislation has a built-in mechanism for altering standards as new scientific information about hazards, or about the absence of hazards, is acquired. This involves a national commission in which the majority of members are non-scientists, plus a consultant group of non-scientists and distinguished scientists from various areas of research. The technical input at a high level is provided by the NIH, and the NIH budget provides for experimental verification of hazard or lack of hazard in particular kinds of experiments. What

sort of mechanism would there be to assure technical competence at the state level, and how would it operate? Would the state use federally supplied information, and if so, would state decisions using Federal information be any sounder than Federal decisions using the same information.

At the November 18 meeting of the Committee of Environmental Health, I discussed my views on the recombinant DNA controversy. The statement I prepared at that time led me to write the enclosed article, "Recombinant DNA: Fact and Fiction", which was published in the February 18 issue of Science, and will be reprinted in the May issue of the Western Journal of Medicine. As noted in the article, despite the conjectural nature of the hazards involved in all but a few types of recombinant DNA research, most experiments will be permitted only under physical containment conditions at least as strict as those used for work with bacteria that cause typhoid fever and Asiatic cholera. Other experiments require containment facilities of the type designated for the most dangerous natural viruses known (eg., Lassa fever, Marburg virus, etc.). Such conditions are required for recombinant DNA experiments in which the evidence of danger is nonexistent, and the possibility of hazard is remote. Moreover, a system of biological containment is designed to reduce even further the chance of propagation of recombinant DNA molecules outside of the laboratory.

I believe that State legislation would be a serious mistake unless the legislature can identify specific areas where it feels that Federal legislation is inadequate to protect the citizens of California, unless it can show that California legislation would increase the safety of the citizens of this state, and unless it can justify the bureaucracy and cost that would be involved in establishing and maintaining a state-wide regulatory system.

It is worthwhile pointing out that the containment procedures specified in the NIH guidelines, and in the proposed Federal legislation, have made it difficult for the public to appreciate that most of the hazards under discussion are conjectural. Many billions of bacteria containing a wide variety of recombinant DNA molecules have been propagated in the United States and abroad without known hazard. In contrast, work is continuing throughout California and throughout the world with infectious disease organisms and with plant pathogens that are known to be capable of causing serious harm. In addition to the known hazards presented by such organisms, there are also unknown risks associated with organisms that are known to cause disease. Yet, as noted above, the State Commissioner of Health proposes to set up a bureaucracy for controlling organisms for which no evidence of hazard exists.

I'm sorry that it has been necessary to write such a lengthy letter, but I thought it important to put before you the various issues I am concerned about in connection with the proposed state legislation. The bill, in its current form, would be a disaster. Even in some other form, its principal value would seem to be to bolster the political careers of some of its proponents --- and the cost of establishing, training, and maintaining a huge state-wide bureaucracy to duplicate

what will be done Federally, would be enormous. There is not a great deal of time to act to defeat this bill. I urgently ask your support and the support of the Committee to accomplish this.

I'd be happy to discuss this with you in person or by telephone, if you wish. With best wishes,

Sincerely yours,

Stanley N. Cohen, M.D.
Professor of Medicine

SNC:db/nm
Enclosures

AMENDED IN ASSEMBLY APRIL 19, 1977

CALIFORNIA LEGISLATURE—1977-78 REGULAR SESSION

ASSEMBLY BILL No. 757

Introduced by the Committee on Health, (Assemblyman Keene, Chairman, Assemblymen Agnos, Mangers, Rosenthal, Torres, ~~and Tucker),~~ *Tucker, Bates, and Lanterman),* and the Committee on Resources, Land Use and Energy (Assemblyman Calvo, Chairman, Assemblymen Ellis, Goggin, Hayden, Kapiloff, Wornum, ~~and Wray)~~ *Wray, and Bates)*

March 3, 1977

REFERRED TO COMMITTEE ON HEALTH

An act to add Chapter 10 (commencing with Section 1780) to Division 2 of the Health and Safety Code, relating to biological research, and making an appropriation therefor.

LEGISLATIVE COUNSEL'S DIGEST

AB 757, as amended, Keene (Health). ~~Biological~~ *Recombinant DNA* research: California ~~Biological~~ *Recombinant DNA* Research Safety Commission.

There is no existing state law governing ~~hazardous biological~~ *recombinant DNA* research. National Institutes of Health recombinant DNA research guidelines apply to ~~biological~~ *recombinant DNA* research in some instances.

This bill would enact the California ~~Biological~~ *Recombinant DNA* Research Safety Act. The bill would create the California ~~Biological~~ *Recombinant DNA* Research Safety Commission as an independent commission in state government. The commission would be composed of ~~17~~ *11* members, with ~~11~~ *7*

April 25, 1977

STATEMENT OF STANLEY N. COHEN, M.D.

PREPARED FOR THE COMMITTEE ON HEALTH

CALIFORNIA STATE ASSEMBLY

I am Stanley Cohen, a physician and Professor of Medicine at Stanford University -- and a molecular geneticist who has played a role in the development of methods for recombinant DNA research.

During the past several years we have witnessed an extraordinary sequence of events involving the interaction of scientists, the news media, and the community at large. It began when biologists learned to duplicate in a test tube the genetic recombination process that normally is carried on by all living cells. As a result, it became possible to manipulate hereditary material (DNA), with much greater precision than had been done previously in such activities as plant and animal breeding, and to combine in a test tube segments of DNA taken from different cells.

Shortly after the discovery of these techniques, I joined with a group of scientific colleagues in calling public attention to the possibility that some gene combinations that could be made using the new techniques might prove to be hazardous. Although there was no scientific basis for anticipating a hazard, because of the newness and the relative simplicity of recombinant DNA methods, it seemed reasonable and appropriate to proceed with caution. Further information was needed to enable us to assess more fully the implications of the research, and to determine whether there was in fact any risk. In other areas of scientific and technological development, scientists and government often had not proceeded as cautiously as they might have, only to find at a later time that greater caution had been warranted. Here

was an opportunity in this new research area, we thought, to exercise care at the onset; if in the light of additional experience it subsequently turned out that our concerns had been exaggerated, then the precautions could be relaxed.

Our action was voluntary, and was taken at the initiative of scientists and in the absence of legislation or government rulings. Unfortunately, our unprecedented attempt to guard against hazards that were not known to exist was so novel that it was widely misinterpreted as implying that we thought danger was likely. It has been inconceivable to scientists working in other fields and to the public at large that we would have taken such action, or that an extensive and costly Federal response to our initiative would have occured, unless severe hazards were known.

The names of the scientists who were the leaders in raising the recombinant DNA issue to public consciousness are known to members of this Committee, and some of them have appeared before you at previous Hearings. However, these scientists have come here not as proponents of stricter controls, but as opponents to the legislation proposed by the Committee. Scientists such as Maxine Singer, Paul Berg, Herbert Boyer, James Watson, Ronald Davis, David Hogness, Roy Curtiss, Norton Zinder and myself --- people who were among the first to express concerns about the possibility that some experiments carried out using these techniques might be hazardous have come to believe that our initial concerns were in fact greatly exaggerated. Why has this change occurred? Is it because we scientists are less interested now in safety of the public and in protection of the environment than we were just a few short years ago? Is it likely that this group of scientists have abandoned their earlier principles in order to simply satisfy their "ambition" and "curiosity" as has been suggested by some critics? Or is there another reason why the scientists who took the initiative in being cautious have since become the strongest proponents of the research, and the opponents of efforts to implement increasingly tighter controls?

As scientists, we deal with data, and data are what have led us to view the situation differently from before. At the time this issue was first raised, recombinant DNA techniques were new. Many other kinds of genetic manipulation, such as the creation of hybrid plants and hybrid animals, and modification of the bacterial and fungal strains that make the antibiotics we use to treat infections, the bread we eat, and the beer we drink, have been carried out for a long time. There was little experience in gene modification using recombinant DNA techniques at the time we voiced our initial concerns. But while the public fears have been fanned by publicists and a few scientists, the work has proceeded without adverse consequences in dozens of laboratories in California and the rest of the world. During the past four years almost 200 scientific investigations of recombinant DNA have been published, and literally hundreds of billions of E. coli bacteria containing a wide variety of recombinant DNA molecules have been grown in the United States and abroad with no harm to humans or to the environment. Because the life cycle of a bacterial cell lasts only 30 minutes, it has been possible to study many thousands of generations of organisms containing recombinant DNA molecules. What has been found in these studies has persuaded us that the conjectural fears that led to most, if not all, of our earlier concerns are without basis in fact.

Dr. Roy Curtiss is a distinguished microbiologist who because of his deeply held concerns has spent much of the past three years designing and developing fail-safe bacterial strains for genetic experiments. In the course of these studies, Dr. Curtiss has accumulated much information that has modified his own thinking, and which has contributed to altering the thinking of the rest of the scientific community. Dr. Curtiss, who from the beginning has been one of the most cautious scientists in this controversy, has recently written a 13-page letter to Dr. Donald Fredrickson, Director of the National Institutes of Health. His letter states: "In view of all the accumulating information...I have gradually come to the realization that the introduction of foreign DNA sequences into EK1 and EK2 host-vectors

offers no danger whatsoever to any human being with the exception already mentioned of an extremely careless worker who might under a unique situation cause harm to him or herself". A similar conclusion was reached regarding the absence of hazard to the biosphere. "The arrival of this conclusion has been somewhat painful," Dr. Curtiss states, "and with reluctance, since it is contrary to my past 'feelings' about the biohazards of recombinant DNA research."

Along with the rest of us, Dr. Curtiss initially feared the spread of bacteria containing recombinant DNA and the possible conversion of harmless to harmful bacteria. However, much experience and data have shown us that the introduction of recombinant DNA molecules into already weakened laboratory strains of E. coli weakens them even further, so that they are unable to compete successfully in nature and are at a disadvantage outside the special protective conditions of the laboratory. Moreover, extensive studies have shown that even genes known to code for disease traits fail to make disease-producing bugs out of laboratory strains of E. coli.

Dr. Curtiss goes on to say that he has "become increasingly distressed by degeneration of the debate. Opinions have often been stated as factual certainty, statements of 'fact' have often been put forth that are in conflict with published data and there has often been an unwillingness to adhere to the principles of scientific objectivity. I have never heard or read any factual information in the debate that would contradict the conclusion about the safety of the E. coli K12 host-vector system that I have just reached. It is thus my considered belief that we are about to embark on excessive regulation of an important area of biomedical research based almost solely on fear, ignorance, and misinformation".

Mr. Chairman, what is proposed by this Committee is akin to establishing legislative restrictions to prevent the continued use of a drug or vaccine that has already been used for several years without any adverse consequences, and which according to all experimental evidence is not associated with any risk whatsoever

-- simply because there are some who have unsupportable anxiety that a still unknown hazard might possibly occur some time hence.

There are a few scientists who continue, for almost mystical reasons, to fear hazards even where experience has told us there are none. However, the views of the rest of the scientific community have shifted as data have gradually become available to allow us to replace speculative concern with fact. Careful examination of the issues by the public has led them to a parallel change in perspective. The Cambridge, Massachusetts Citizens Committee has concluded that "many of the fears held by the citizenry result from a lack of understanding about the nature of the research and the manner in which it is conducted". When the facts were examined critically by the Cambridge committee, as Time Magazine recently reported, the "citizens patiently ignored political demagoguery, perceived the false notes in the voices of doom, mastered the complex issues, and then passed their votes for continuation -- with reasonable restraints -- of free scientific inquiry". The unanimous position of the Cambridge committee, which resulted from seven months of intensive study, indicates that when non-scientists spend the time to examine carefully the issues, they reach the same inevitable conclusion as most scientists -- namely that predictions of disaster as a result of continuation of recombinant DNA research are without any reasonable basis in fact.

Unfortunately, the perception of most of the public on this issue has lagged behind the scientific data. It is ironic that even as evidence has accumulated to support the view that there are no novel hazards associated with the research, the fears of the citizenry have increased, and most of the public now believes that extensive legislative control mechanisms are necessary. Hearings were held on this issue by the U.S. Senate in April, 1975 and in the fall of 1976, and by the House of Representatives last month. When this Committee of the California Assembly began to investigate the issue, no Federal legislation was in progress. However, at this point, the Federal government is about to provide the public with an "insur-

ance policy" in the form of a Federal law on recombinant DNA, and more than half a dozen bills are now pending before the U.S. Senate and House of Representatives.

Two weeks ago, in revising AB 757, the Assembly Committee on Health deleted the parts of its earlier bill that would have supplemented the proposed Federal legislation, and has now developed a bill that simply duplicates what is being done at the Federal level. While I realize that California has the right to establish its own laws in this area, I seriously question the usefulness of doing so.

First of all, microbes do not respect state boundaries. People enter and leave California every day, and if there is any risk from recombinant DNA work, the citizens of California can be no safer than the citizens of other states regardless of what standards are legislated in Sacramento. Even the most vocal proponents of stringent controls such as Professor Robert Sinsheimer, have come out strongly for Federally administered regulatory procedures, rather than for state or local legislation.

As someone familiar with this area of research, I can assure the Committee that extensive facilities and personnel would be needed to carry out any monitoring activities established by state legislation. Where would the people and equipment be housed? Who would train the inspectors and where would they come from? The proposed Federal legislation has a built-in mechanism for modifying standards as new scientific information is accumulated. This involves a Federal Commission in which the majority of members are non-scientists; the Commission would use the NIH as a resource and repository of scientific data and technical expertise. What sort of mechanism would the State establish to assure technical competence? Would California use Federally supplied information, and if so, would State decisions using this Federal information be any sounder than Federal decisions using the same information? What, if anything, would be gained by setting up a liscensing, monitoring, and enforcement system in California to duplicate the Federal system?

In the absence of any foreseeable benefit to Californians or any demonstrable

need for State legislation, the band-wagon nevertheless rolls on. We have been told that the process can always be halted later on if Federal laws prove to be satisfactory. However, past experience suggests that once a mechanism for a regulatory apparatus is established, it will be difficult to pull back -- even though Federal laws will make a Statewide apparatus unnecessary and undesirable. Doesn't it seem preferable to determine whether there is a need for a California legislative solution before proceeding, rather than to proceed hastily with the expectation that the time and effort invested will not have been useful?

A reasonable alternative to the present version of AB 757 is the establishment of a study commission -- rather than a regulatory commission -- to evaluate calmly and responsibly the data that have become available on this subject, to sort out polemic from fact, and to analyze the claims made on both sides to see what arguments can really be substantiated.

Time constraints prevented the City Council of Cambridge from carrying out the kind of in-depth analysis of the issues made by that city's citizens committee -- and I suggest that similar time constraints have prevented an in-depth analysis by this legislative committee -- even with staff assistance. Examination of the hastily prepared text of the current version of the bill seems to support this view. Does California deserve a less deliberate and thoughtful process than Cambridge? Can this committee carry out the in-depth analysis of the Federal legislation needed to determine the adequacy of the extensive and complex Federal regulations?

Careful study is required to determine whether there are any needs for the protection of citizens of California against biological hazards that are not already provided for in the Federal law. If so, a study commission could identify these needs, whether in recombinant DNA research per se or in other areas of biological experimentation and could then recommend appropriate legislative action. If current laws and the pending Federal regulations are found to be adequate, nothing would be gained by establishing a separate regulatory and licensing system in this State.

Recombinant DNA research was born in California, and scientists in this State were the leaders in raising this issue to public awareness. This Committee of the Assembly now has the opportunity to continue the pattern of California leadership in this field, not by following a "me-too" approach for duplication of what is being done on the Federal level, not by rushing ahead with hastily drafted and even more hastily modified legislation on an issue of such major importance, but rather by adopting a creative, deliberate, and responsible legislative approach. I believe that such an approach involves careful study of the issues and of the pending Federal legislation, rather than approval of AB 757.

Penalties only prospective

Legislative fee

COMPARATIVE ANALYSIS OF H.R. 7897 (ROGERS), S. 1217 (KENNEDY, AS REPORTED), AND S. 1217 AMENDMENT (NELSON)

RELATED TO THE RECOMBINANT DNA RESEARCH ISSUE

95TH CONGRESS
1ST SESSION

H. R. 7897

IN THE HOUSE OF REPRESENTATIVES

JUNE 20, 1977

Mr. ROGERS (for himself, Mr. PREYER, Mr. SCHEUER, Mr. WAXMAN, Mr. FLORIO, Mr. MAGUIRE, Mr. MARKEY, Mr. OTTINGER, Mr. WALGREN, Mr. CARTER, Mr. MADIGAN, and Mr. SKUBITZ) introduced the following bill; which was referred to the Committee on Interstate and Foreign Commerce

A BILL

To amend the Public Health Service Act to regulate activities involving recombinant DNA, and for other purposes.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

SHORT TITLE

SECTION 1. This Act may be cited as the "Recombinant DNA Act".

Calendar No. 334

95TH CONGRESS
1ST SESSION

S. 1217

[Report No. 95-359]

IN THE SENATE OF THE UNITED STATES

APRIL 1 (legislative day, FEBRUARY 21), 1977

Mr. KENNEDY introduced the following bill; which was read twice and referred to the Committee on Human Resources

JULY 22 (legislative day, JULY 19), 1977

Reported by Mr. KENNEDY, with an amendment

[Strike out all after the enacting clause and insert the part printed in italic]

A BILL

To regulate activities involving recombinant deoxyribonucleic acid.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

SHORT TITLE

SECTION 1. This Act may be cited as the "Recombinant DNA Safety Regulation Act".

September 6, 1977

Donald Fredrickson, M.D.
Director
National Institutes of Health
Bethesda, MD 20014

Dear Don:

I have enclosed a copy of a manuscript that is now in press in the Proceedings of the National Academy of Sciences. I have taken the unusual step of sending it to you prior to publication because I believe the findings have policy, as well as scientific, importance with regard to the regulation of recombinant DNA.

The experiments reported in the paper demonstrate that:

(1) EcoRI restriction endonuclease occurring within normally growing bacterial cells promotes genetic recombination in vivo at precisely the same sites that are involved in in vitro recombinant DNA experiments using this enzyme, and

(2) Free fragments of eukaryotic DNA can be taken up by E. coli and joined to plasmid DNA molecules within the bacteria. The fesulting hybrid eukaryotic-prokaryotic DNA molecules (which have been made intracellularly by naturally-occurring biological processes) can be propagated in bacterial cells by the plasmid replication system.

These experiments and others have led us to conclude that an important biological function (perhaps the major function) of the so-called "restriction" enzymes may be site-specific recombination of DNA, and that eukaryotic DNA fragments formed biologically by restriction enzyme cleavage can link to prokaryotic DNA ~~in the test~~ in vitro recombinant DNA techniques. Our data provide compelling evidence to support the view that recombinant DNA molecules constructed in vitro using the EcoRI enzyme simply represent selected instances of a process that occurs by natural means.

In the past I believed that the in vitro joining of different segments of DNA at restriction endonuclease cleavage sites resulted in the formation of genetic combinations that could not be made otherwise; for this reason it seemed important to call attention to possible biohazards that might be associated with certain kinds of novel gene combinations. However, along with virtually all of the other scientists who first raised these questions, I have since come to believe that our initial concerns were greatly overstated. Some of the important new information that has led to this changed perception

has been summarized in Roy Curtis' recent letter to you and in the Falmouth report. The data described in the enclosed manuscript attest to the naturalness of site-specific genetic recombination mediated by restriction endonucleases and intracellular ligases, and add still another perspective to the controversy.

I would be happy to answer any specific questions that you may have about this work.

With best wishes,

Sincerely yours,

Stanley N. Cohen, M.D.
Professor of Medicine and
Professor of Genetics

SNC:seh

Enclosure

DATE: 10 July 1978

TO : Josh Lederberg

FROM : Stan Cohen

SUBJECT: Our discussions about the origins of the "recombinant DNA technique"

Dear Josh:

Nicholas Wade's inquiry about "inventorship of the recombinant DNA technique" has prompted me to pull together and set down on paper my thoughts about the scientific contributions in this area. This letter provides you with these views, as you have requested.

Since scientific knowledge is obviously a continuum, and since each discovery is dependent upon others that have gone before, Wade's inquiry about scientific "inventorship" when considered in a broad sense, raises a variety of philosophical and ethical issues. Some of the general concepts involving recombinant DNA depend on the work by Avery, MacLeod and McCarty, on Watson and Crick, on your own work, and on others; these advances depended in turn on the preceding contributions of others. However, in a more narrow sense, the answer to Wade's question about inventorship of the recombinant DNA technique depends in part on what one regards as "the recombinant DNA technique".

Most observers consider "recombinant DNA" to be conceptually equivalent to gene cloning rather than gene splicing--although they may not have thought about this distinction explicitly, and recombinant DNA has been referred to popularly as "gene splicing". The conceptual and experimental elements of gene splicing *per se* can be found in the work of Khorana and his collaborators, who in the late 1960's showed that short segments of synthetic DNA could be spliced together by the addition of overlapping complementary single-strand tails (summarized in Agarwal et al., Nature 227, 27, 1970). The use of terminal transferase to add homopolymeric dA and dT tails to the DNA segments was first reported by Jensen et al., 1971 (Biochem. Biophys. Res. Comm. 43, 389, 1971). In these conceptually sound, but only partially successful experiments, separate DNA molecules were linked together by dA-dT tails to form catenanes; however, Jensen et al. did not achieve the final step of ligation that was necessary to accomplish DNA splicing. The paper by Jackson, Symons and Berg (PNAS 69, 2904, 1972) which was the first to report success with the dA-dT method of joining, credits Lobban and Kaiser with initially making the observation that exonuclease III was needed to accomplish what Jensen et al. had failed to do: namely, the covalent joining of DNA molecules that have homopolymeric extensions of dA and dT at their ends. The Lobban and Kaiser work was published in mid-1973 (J. Mol. Biol. 78, 453, 1973).

As Paul Berg has indicated, concern about possible biohazards related to the SV40 component of the λdv-SV40 molecule that Jackson et al. had constructed, led him to decide not to try to clone the molecule in E. coli. However, there is no report of success in the cloning of analogous molecules that contain any other fragment of DNA inserted at the λdv site used in the Jackson et al. experiments. Apparently the reason for this is that the EcoRI cleavage site in λdv is located within the O gene (Helling et al., J. Virol. 14, 1235, 1974; Streek and Hobom, Eur. J. Biochem. 57, 595, 1975; Mukai et al., Mol. gen. Genet. 146, 269, 1976) which is essential for replication. Interruption of the continuity of this gene by an inserted DNA fragment prevents λdv from functioning as a replicon.

Later investigators have succeeded in using EcoRI-cleaved λdv as a cloning vector by constructing molecules that contain λdv dimers (Mukai et al., Mol. gen. Genet. 146, 269, 1976) plus the fragment to be cloned. In this case, one of the two copies of λdv provides an intact O gene and the molecule is thus able to replicate when introduced into bacterial cells. However, intermolecular linkage of DNA molecules of the same species to form dimers is prevented when the dA-dT joining method is employed (Jackson et al., PNAS 69, 2904, 1972).

As I wrote in Scientific American (July, 1975), I believe that the gene cloning technique depends directly on discoveries made in a number of different laboratories in the late 1960's and early 1970's. The component that involves the splicing together of DNA segments by means of added cohesive termini traces its conceptual and experimental origins to Khorana's work, as noted above, and the joining of separate DNA molecules by means of restriction endonuclease-generated cohesive termini was reported simultaneously by Mertz and Davis (PNAS 69, 3370, 1972) and by Sgaramella (PNAS 69, 3389, 1972). The discovery that restriction endonucleases can recognize and cleave DNA at specific nucleotide sequences was made by Kelly and Smith (J. Mol. Biol. 51, 393, 1970) and the first use of these enzymes for restructuring DNA molecules by cleaving them into fragments and joining the resulting segments together in a different arrangement was reported by Cohen et al. (PNAS 70, 3240, 1973).

The discovery and purification of DNA ligase by Gellert (PNAS 57, 148, 1967) and others was important in enabling covalent linkage of separate DNA molecules in vitro. However, linkage of physically separate restriction endonuclease-generated DNA fragments can also be accomplished in vivo by the DNA ligase (Cohen et al., PNAS 70, 3240, 1973). In fact, restructuring of DNA molecules by the combined intracellular actions of restriction endonuclease and DNA ligase can be done in vivo as well as in vitro (Chang and Cohen, PNAS 74, 4811, 1977). Cohesive termini are not essential for the linkage of DNA segments; the work of Sgaramella et al. reported in 1970

(PNAS 67, 1468, 1970) showed that even blunt-ended DNA fragments can be joined together by use of the bacteriophage T4 ligase.

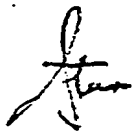
In addition to a method for splicing together different DNA segments at specific sites, the in vitro construction of biologically functional DNA molecules (Cohen et al., PNAS 70, 3240, 1973) is dependent on the concept of using a vector to introduce DNA into a recipient cell and the development of methods for accomplishing introduction of the vector experimentally. Several different systems for introducing bacteriophage DNA into E. coli were described in the 1960's. Mandel and Higa (J. Mol. Biol. 53, 159, 1970) first reported the use of calcium chloride to accomplish uptake of bacteriophage DNA into E. coli K12, and the production of viable phage particles (i.e., transfection). However, these investigators reported that they were unable to generate bacterial transformant clones. Such transformation, and the propagation of clones of E. coli containing replicas of introduced DNA molecules was first reported by Cohen, Chang and Hsu (PNAS 69, 2110, 1972), using plasmids.

The recombinant DNA technique also depends on a means of selecting from a large population of recipient cells those individuals that have received either chimeric or restructured DNA molecules, and upon the discovery that foreign DNA can be propagated in cells using a replicon indigenous to the recipient (Chang and Cohen, PNAS 71, 1030, 1974; Morrow et al., PNAS 71, 1743, 1974). This last point was not a foregone conclusion--since the genetic and structural stability of in vitro constructed DNA molecules and their capacity for biological function were not certain before the experiments were actually carried out. In fact, some DNA chimeras are not stable or biologically viable--and to this day certain DNA combinations cannot be cloned.

I appreciate your taking the time to talk about this matter with me.

With best wishes,

Sincerely yours,



STANFORD UNIVERSITY

APPLICATION FOR APPROVAL OF RESEARCH PROJECT INVOLVING RECOMBINANT DNA

Identification No. of this application
SC-101

Name and Title of Principal Investigator:

Stanley N. Cohen, Professor

Department: Telephone No. (415) 497-5315

Genetics

Title of Grant:

Gene Expression in Heterospecific Environments

Research Support (Agency and Grant No.): New ☒ Renewal ☐ Continuation ☐
(Specify to whom institutional approval should be sent)

Division of Research Grants
National Institutes of Health
Bethesda, Maryland 20205

Central Processing Section
National Science Foundation
Washington, D.C. 20550

A. **Description of Project:**

1. Description of Experiment: (Indicate whether experiment involves use of already constructed DNA molecules, organisms already containing recombinant DNAs or whether each of the above is to be constructed).

The experiments will involve further work with cDNA sequences for mouse dihydrofolate reductase. An MUA covering this work has been approved previously; the present MUA is a request for reduction of containment level, since the plasmids previously constructed have now been rigorously purified by cloning, and the DHFR cDNA segment has been sequenced. The conditions specified in footnote 3 of the NIH guidelines of December 22, 1978 have been met, since the segment cloned consists entirely of DHFR cDNA as determined by DNA sequence analysis. Lowering of containment for these very same clones has already been allowed for another lab at Stanford.

The proposed experiments will involve the introduction of segments of the sequenced cDNA into *E. coli* plasmids that contain characterized transcriptional and translational control signals, and the study of expression of the DHFR enzyme in such clones. The DNA itself will be analyzed by restriction endonuclease mapping and DNA sequencing procedures employing gel electrophoresis.

2. Source of DNA to be Cloned: (Indicate species organ or tissue, chromosomal, extrachromosomal or organelle).

AT-3000 mouse cells

3. Purity of DNA to be Cloned (e.g. comment on whether experiment involves shotgun cloning of total DNA, prior purification of organelle, purification of CCC DNA by cesium choride-ethidium bromide centrifugation cDNA from RNA, etc.):

Previously cloned and sequenced.

4. Criteria for Purity of DNA to be Cloned (if relevant to the containment level proposed):

DNA sequence has been determined for already cloned DNA species

5. Vector(s):

pSC101 and pBR322 (EK2 approved). pACYC184 (EK1).
Also other EK1 vectors derived from these plasmid replicons.

6. Hosts (and strain if relevant; e.g. E. coli, C600, X1776, B. subtilis, etc.):

E. coli K12 strains.

B. Levels of Physical and Biological Containment:

1. Levels of Containment recommended by NIH Guidelines, RELEVANT SECTIONS MUST BE CITED.

Section III-A-1-a(2) Specifies P2 + EK2
III-A-3 and III-A-3-b(1) Reduction of containment level for purified DNA that has been rigorously characterized (to P2 + EK1)

2. Level of Physical Containment to be Used, identify location (building, room number, city and state) and specific procedures used to provide required levels of containment:

P2 - S-141, S-175, Medical Sciences Building
also L-314 after lab moves in April, 1979. Rooms are all in Stanford, CA.
The containment procedures for P2 experimentation as specified in the NIH guidelines of December 22, 1978 will be used.

EK1

C. Project Personnel (list all personnel involved in the conduct of these experiments):

1. Names, Titles and Responsibilities:

S. N. Cohen, Principal Investigator, Professor of Genetics
A.C.Y. Chang, Life Science Research Assistant and Graduate Student

2. State of training of laboratory personnel working on project regarding appropriate containment procedures (For projects requiring containment conditions higher than P2 EK1, describe what training the personnel have received):

Extensive experience (5 years) working with recombinant DNA and containment.

3. Familiarity of professional personnel with the NIH Guidelines:

Have read and understood guidelines

4. Information on Health Surveillance:

Not applicable

D. Additional Comments:

I agree to comply with the NIH requirements pertaining to shipment and transfer of recombinant DNA materials. I am familiar with and agree to abide by the provisions of the current NIH Guidelines and other specific NIH instructions pertaining to the proposed project. The information above is accurate and complete.

March 12, 1979
Date

Principal Investigator

I assure that the Administrative Recombinant DNA Panel has reviewed on _____ the proposed project and the plans for facilities proposed or under construction or renovation. Recombinant DNA experimentation will not occur until the completed facilities have been reviewed by the Panel and a MUA with certification has been submitted to NIH.

The Panel as delegated by the Institution agrees to accept responsibility for the training of all laboratory workers involved in the project. The Panel will monitor throughout the duration of the project the facilities, procedures, and the training and expertise of the personnel involved in the recombinant DNA activity.

Date

Chairman
Administrative Recombinant DNA Panel

Date

Institutional Official

Date

Institutional Official*

*Additional performance sites, if applicable.

1/79

I agree to comply with the NIH requirements pertaining to shipment and transfer of recombinant DNA materials. I am familiar with and agree to abide by the provisions of the current NIH Guidelines and other specific NIH instructions pertaining to the proposed project. The information above is accurate and complete.

March 13, 1979
Date

Principal Investigator

I certify that the Administrative Recombinant DNA Panel has reviewed on _____ the proposed project for recombinant DNA experiments and has found it to be in compliance with the NIH Guidelines and other specific NIH instructions pertaining to the proposed project.

The Panel as delegated by the Institution agrees to accept responsibility for the training of all laboratory workers involved in the project. The Panel will monitor throughout the duration of the project the facilities, procedures, and the training and expertise of the personnel involved in the recombinant DNA activity.

Date

Chairman
Administrative Recombinant DNA Panel

Date

Institutional Official

Date

Institutional Official*

*Additional performance sites, if applicable.

1/79

For scientists (graduate students, post-doctoral fellow, visiting scientists, etc.) engaged in experiments using recombinant DNA techniques.

I am familiar with the containment procedures described in "The NIH Guidelines for Research Involving Recombinant DNA Molecules" that are appropriate for my work.

I agree to abide by the provisions of the NIH Guidelines in all experiments I perform at Stanford University with such molecules.

NAME		DATE
A.C.Y. Chang Signature	ANNIE C.Y. CHANG Print	

1/79

I agree to abide by the provisions of the NIH Guidelines for Recombinant DNA Molecules and that the Recombinant DNA Molecules being used will not be transferred to other investigators or institutions unless they have provided written assurance that their facilities are adequate and their procedures will be carried out in accordance with the NIH Guidelines and other NIH instructions, an approved MUA is on file and a copy of the request has been filed with their committee. Prior to shipment of recombinant DNA materials to a foreign country, I shall obtain from the requesting laboratory a statement that the research involving recombinant DNA molecules will be conducted in accordance with the containment standards of the NIH Guidelines, or under applicable national guidelines.

The sending investigator shall maintain a record of all shipments of recombinant DNA materials.

March 12, 1979
Date

Principal Investigator

NOTE: A) For projects requiring Panel review include this complete MUA signed by the Principal Investigator with copies as follows:

1. 17 copies of pages 1, 2, and 3 and any supporting documentation if necessary.

2. For new projects include one copy of the Grant Proposal.

B) The MUA, copies, and grant proposals should be sent to Jack Sidlow at 71 Encina Hall, Ext. 7-3201.

THE TRANSPLANTATION AND MANIPULATION OF GENES IN MICROORGANISMS*

STANLEY N. COHEN

Departments of Genetics and Medicine,
Stanford University,
Stanford, California

I. INTRODUCTION

UNTIL this decade, genetics has been largely a descriptive science: our knowledge of genes and their actions has been derived mostly from observing the consequences of natural biological processes such as mutation and recombination. Certainly, the ability to introduce new genetic information into bacterial cells by the manipulative processes of transduction, transformation, or conjugation has advanced knowledge of the biology of prokaryotic organisms in major ways, and concurrent progress in biochemistry and molecular biology has enabled the structural and functional study of the individual genes and gene products of prokaryotes. However, the complexity of the chromosomes of higher organisms and the inability to isolate particular segments of these DNA molecules has until recently precluded detailed molecular analysis of eukaryotic genes.

Development of the concepts and methods of "recombinant DNA" now enables the manipulation of DNA molecules *in vitro* and the cloning of new genetic combinations in microorganisms. This has permitted the investigation of prokaryotic genes at a level that was not previously possible and has allowed for the first time the analysis of individual eukaryotic genes and study of the organization of genetic information in higher organisms. The advances that laid the foundations for genetic manipulation in microorganisms were made in a number of different laboratories in the late 1960s and early 1970s. There are four general requirements: (*a*) a replicon (cloning vehicle or vector) able to propa-

*Lecture delivered May 17, 1979.

gate itself in the recipient organism; (*b*) a method of joining another DNA segment to the cloning vector; (*c*) a procedure for introducing the composite molecule into a biologically functional recipient cell; and (*d*) a means of selecting those microorganisms that have acquired the hybrid DNA species.

II. Historical Background and the Development of DNA Cloning Methods

A. Plasmids and Plasmid DNA Transformation

The studies reviewed here grew out of experiments aimed at elucidating the molecular nature of a class of genetic elements responsible for antibiotic resistance in bacteria. It has been known for some years that many bacterial species contain autonomously replicating extrachromosomal elements called *plasmids*. Most simply, plasmids can be considered as primitive bacteriophages that carry a function that allows the unit to be replicated autonomously (the replication system), but that lack the genetic information required for a complex life cycle or existence in an extracellular state (Cohen, 1976). Circular plasmid DNA molecules (Fig. 1) are physically separate from the bacterial chromosome, and they can encode a variety of genetic traits that are not essential for growth of the host cell but that commonly provide a biological advantage to cells carrying the plasmids; antibiotic resistance is one of these properties. Examples of other traits carried by plasmids are shown in Table I.

Plasmids commonly are present in multiple copies within each cell, and plasmid DNA preparations isolated from bacterial cultures contain a heterogeneous population of DNA molecules. To employ classical genetic methods for the study of plasmid mutants, and to investigate the organization of genetic information on plasmid DNA, it was therefore necessary to establish a method for the cloning of individual plasmid DNA molecules. Procedures for transforming bacteria for chromosomally encoded traits had been developed for *Pneumococcus, Haemophilus, Bacillus,* and certain other organisms (Avery *et al.*, 1944; Hotchkiss and Gabor, 1970), but transformation had not been shown for *Escherichia coli* or the other enteric bacteria with which we were working. It was known that treatment of *E. coli* cells with calcium

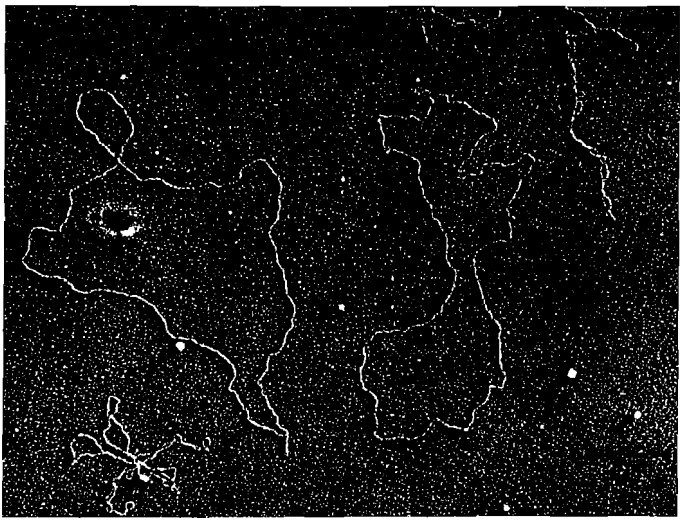


FIG. 1. Electron photomicrograph showing twisted "supercoiled" and "open" circular molecules of the antibiotic resistance plasmid, R1. From Cohen and Miller (1969).

TABLE I

SOME PROPERTIES ENCODED BY NATURALLY OCURRING PLASMIDS[a]

Antibiotic resistance
Fertility (ability to transfer genetic material by conjugation)
Production of bacteriocins
Antibiotic production
Heavy-metal resistance (Cd^{2+}, Hg^{2+})
Ultraviolet resistance
Enterotoxin
Virulence factors, hemolysin, K 88 antigen
Metabolism of camphor, octane, and other polycyclic hydrocarbons
Tumorigenicity in plants
Restriction/modification

[a] Modified from Cohen (1976).

chloride enabled them to take up DNA of the bacteriophage λ, and that viable viral particles were produced in such $CaCl_2$-treated bacteria; however, attempts to generate clones that had acquired new genetic properties from the transformed DNA had not been successful (Mandel and Higa, 1970).

In 1972, my colleagues and I found, using a modification of the previously described $CaCl_2$ procedure, that *E. coli* could take up circular plasmid DNA molecules (Fig. 2), and that a line of transformed cells that phenotypically express genetic information carried by the incoming plasmid DNA could be produced (Cohen *et al.*, 1972). While this was an inefficient process (approximately one in 10^6 cells were transformed), transformants could readily be identified and selected by utilizing the antibiotic resistance genes carried by the plasmids we were studying. Plasmid-transformed cells reproduced themselves normally, and acquired a DNA species having the same genetic and molecular properties as the parent plasmid. Since each cell in the resulting clone contains a replica of the single plasmid DNA molecule that was taken up

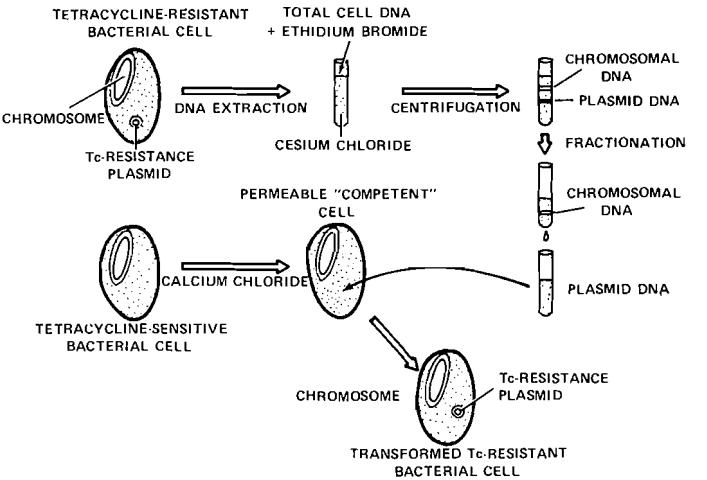


FIG. 2. Schematic presentation of plasmid DNA transformation procedure. Purified plasmid DNA separated from chromosomal DNA by cesium chloride–ethidium bromide gradient centrifugation is introduced into bacteria made permeable to DNA by treatment with calcium chloride. Antibiotic resistance genes carried by the plasmid are used in the selection of transformed bacterial cells.

by the originally transformed bacterium, the procedure made it possible to clone (and thus separate and purify biologically) genetically distinct plasmids present in a heterogeneous population. We could now apply to the study of plasmids a variety of genetic and biochemical methods that previously were restricted to bacteriophages, which could be cloned because of their plaque-forming properties.

To determine the genetic and molecular properties of specific regions of the DNA of large antibiotic resistance plasmids (R-plasmids), we began to take these plasmids apart by shearing the molecules mechanically and then introducing the resulting DNA fragments into $CaCl_2$-treated *E. coli* cells by transformation (Cohen and Chang, 1973). However, work being carried out with restriction endonucleases in other laboratories (Smith and Wilcox, 1970; Kelly and Smith, 1970; Danna and Nathans, 1971) suggested that these enzymes would be highly useful in our analysis. It had been discovered that restriction endonucleases, which are produced by many different species of bacterial cells, can recognize specific nucleotide sequences within DNA and can cleave DNA molecules at these recognition sites (Smith and Wilcox, 1970; Kelly and Smith, 1970). The cell's own DNA is protected from cleavage by modification enzymes (methylases) that add methyl groups to certain nucleotides within the recognition sequence, rendering the site resistant to cleavage by the companion endonuclease (Arber, 1965; Meselson and Yuan, 1968; Nathans and Smith, 1975). Thus, restriction endonucleases could be used to generate reproducibly a characteristic set of cleavage fragments for each plasmid; for most of our experiments, this would be preferable to generating a random series of plasmid DNA fragments by mechanical shearing. Moreover, the fragments produced by restriction enzyme cleavage could be analyzed and characterized by electrophoresis on gels; such methods had already been used effectively by Nathans and his collaborators for analysis of the SV40 animal virus genome (Danna and Nathans, 1971; Nathans and Danna, 1972).

B. The Joining of Separate DNA Fragments in Vitro

The conceptual and experimental basis for linking DNA segments by means of projecting single-strand ends having complementary nucleotides can be found in the work of Khorana and his collaborators, who in the late 1960s showed that short segments of synthetic DNA could be

joined by the addition of overlapping complementary single-stranded segments (Khorana, 1968; Agarwal *et al.*, 1970). The construction of such complementary DNA sequences by the addition of single nucleotides was laborious, however. Jensen *et al.* (1971) first reported the use of the enzyme terminal transferase to add homopolymeric stretches of deoxyadenosine (dA) or deoxythymidine (dT) to the ends of DNA fragments in an attempt to link the fragments covalently *in vitro* by (*a*) hydrogen bonding of the complentary nucleotides; (*b*) subsequent closure of the resulting single-strand breaks by DNA ligation. In these conceptually sound, but only partially successful experiments, a series of DNA molecules were joined together end-to-end by dA-dT "tails" to form catenated structures; however, Jensen *et al.* did not achieve the final step (i.e., ligation) necessary to accomplish covalent DNA linkage. It is now known that *in vitro* ligation is not required for the covalent joining of separate DNA segments that contain homopolymeric additions; ligation of such segments occurs *in vivo* when the hydrogen-bonded segments are introduced into bacterial cells by transformation (Wensink *et al.*, 1974).

The problem of *in vitro* ligation of DNA fragments that have homopolymeric extensions at their ends was solved by the discovery by Lobban and Kaiser (1973) that such covalent joining could be achieved by the use of exonuclease III, and this finding was employed by Jackson *et al.* (1972) in linking the tumor virus SV40 to DNA molecules of bacteriophage λdv. It has been of some historical interest that concern about possible biohazards related to the SV40 component of the hybrid λdv-SV40 molecule that Jackson *et al.* had constructed led Berg and his colleagues to decide not to try to clone the molecule in *E. coli* (Wade, 1974). Ironically, however, with regard to the biosafety controversy that ensued (Berg *et al.*, 1975), we can reasonably assume that no bacterial clones carrying the composite molecule would have resulted if the experiment had been tried: the λdv cleavage site at which the two DNA segments were joined is located within a gene essential for replication of λdv, and interruption of the continuity of this gene by an inserted DNA fragment prevents the bacteriophage DNA molecule from functioning as a replicon (Helling *et al.*, 1974; Streek and Hobom, 1975; Mukai *et al.*, 1976).

The subsequent discovery that restriction endonucleases could generate in one step DNA termini having projecting single-strand ends, and

that these could be linked to a complementary nucleotide sequence on another endonuclease-generated DNA fragment, made the joining of DNA segments much simpler. The nucleotide sequences that constitute the cleavage sites for several endonucleases were identified in the early 1970s; in every instance, cleavage occurred at or near an axis of bidirectional rotational symmetry: that is, the endonuclease recognition site consists of a sequence that reads the same on both DNA strands in the 5′ to 3′ direction. Often, restriction endonucleases cleave both DNA strands at precisely the same location, yielding blunt-ended DNA fragments (for review, see Nathans and Smith, 1975). Certain of these endonucleases, however (for example, the *Eco*RI enzyme), introduce breaks that are several nucleotides apart in the two DNA strands (Fig. 3). Because of the bidirectional rotational symmetry of the nucleotide sequence in the region of cleavage, cleavage of the two DNA strands at separated points within this region yields fragments that have protruding complementary nucleotide sequences at their ends. Such termini, which resemble mortise and tenon type joints, can be linked together by hydrogen bonding. Since all DNA termini generated by the enzyme are

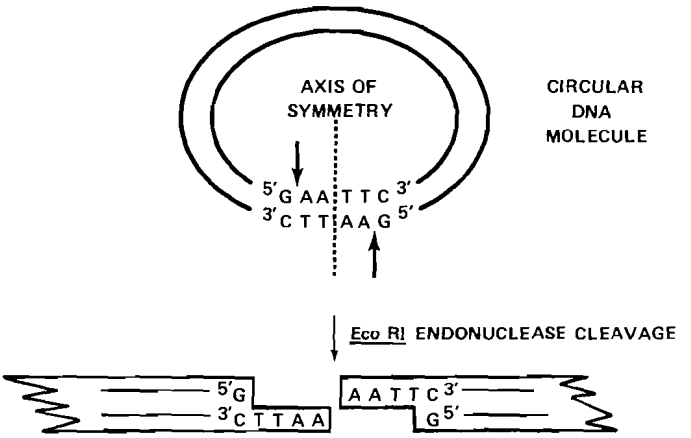


FIG. 3. The six-nucleotide-long recognition sequence cleaved by the *Eco*RI endonuclease is shown. Because of the bidirectional rotational symmetry of the nucleotide sequence in the region of the cleavage, the two DNA strands are cut at separate points, yielding fragments that have protruding complementary single-strand ends.

identical, fragments derived from different DNA molecules can be spliced together.

The finding that the DNA fragments generated by the *Eco*RI restriction endonuclease have projecting single strands at their termini was reported simultaneously in 1972 by Sgaramella (1972) and by Mertz and Davis (1972). Sgaramella found that molecules of the bacterial virus P22 cleaved with the *Eco*RI enzyme can form catenated DNA segments equal in length to two or more viral DNA molecules. Mertz and Davis observed that closed-loop SV40 DNA molecules cleaved by *Eco*RI could re-form themselves into circular molecules by hydrogen bonding and could be sealed covalently with DNA ligase; furthermore, the reconstituted molecules were infectious in animal cells growing in tissue culture. While this property of the *Eco*RI enzyme and certain other restriction endonucleases was of great importance in the development of recombinant DNA methods, it is now appreciated that cohesive DNA termini are not essential for the linkage of DNA termini. Sgaramella *et al.* (1970) had reported that even blunt-ended DNA fragments can be joined together by use of the bacteriophage T4 ligase; such blunt-ended joining has found widespread use in the linking together of DNA fragments generated by restriction endonucleases that do not yield projecting single-strand ends (Sgaramella *et al.*, 1977), and for the joining of DNA fragments that have been made blunt-ended by the S1 nuclease or DNA polymerase I (Bolivar *et al.*, 1977; Chang and Cohen, 1978).

The discovery of DNA ligases (Gellert, 1967; Weiss and Richardson, 1967; Gefter *et al.*, 1967; Olivera and Lehman, 1967; Cozzarelli *et al.*, 1967) also has had a major role in the development of recombinant DNA methods. These enzymes, which can form phosphodiester bonds between adjacent DNA nucleotides, are required for the *in vitro* joining of DNA molecules. However, as noted above it is now known that *in vitro* ligation is not necessary to join DNA fragments that are being held together by extended homopolymeric terminal additions (Wensink *et al.*, 1974). Fragments that have protruding single-strand ends generated by restriction endonucleases can also be linked together *in vivo* by the *intracellular* action of DNA ligase (Mertz and Davis, 1972; Cohen *et al.*, 1973), and such linkage can fully and accurately reconstitute the genetic continuity of the DNA sequence (Chang and Cohen, 1977).

C. Construction of Biologically Functional Bacterial Plasmids in Vitro

To determine whether large and complex plasmid DNA molecules could be reduced in size or restructured entirely by cleaving them into multiple fragments with a restriction endonuclease and joining together the resulting fragments in a different arrangement, A. C. Y. Chang, H. W. Boyer, R. B. Helling, and I studied the large antibiotic resistance plasmid R6-5 (Cohen *et al.*, 1973). We established that this plasmid (Silver and Cohen, 1972), which consists of almost 100,000 nucleotide base pairs and contains several genes encoding several different antibiotic resistances, was cleaved into 11 separate DNA fragments by the *Eco*RI endonuclease; hopefully the location of the cleavage sites would leave the replication machinery of the plasmid and one or more of its antibiotic resistance genes intact. R6-5 DNA was treated with the *Eco*RI enzyme and was introduced by transformation into $CaCl_2$-treated *E. coli* cells with or without prior ligation of the DNA. Selection was carried out for transformants that expressed one or more of the antibiotic resistance determinants located on the parent plasmid.

One such clone, which expressed kanamycin (Km) resistance but none of the other antibiotic resistances of R6–5, was identified and its plasmid DNA was isolated and characterized by *Eco*RI endonuclease digestion and agarose gel electrophoresis (Fig. 4). The digestion pattern showed that a new plasmid replicon containing only 3 of the 11 *Eco*RI fragments of R6–5 had been formed. By selecting for propagation of the Km resistance gene of R6–5, we had been able to clone a specific DNA segment carrying this gene. The Km resistance fragment, which we later showed does not have the capacity for autonomous replication, had become linked to an *Eco*RI-generated DNA fragment carrying the rep-

FIG. 4. Agarose gel electrophores of *Eco*RI digest of the pSC102 plasmid (A) containing three of the *Eco*RI-generated fragments comprising the R6–5 plasmid (B). The pSC101 plasmid is cleaved by the *Eco*RI endonuclease only once to yield a single linear DNA fragment (C). From Cohen *et al.* (1973).

lication region of R6–5, and this enabled its propagation in transformed bacteria (Cohen *et al.*, 1973). These findings demonstrated that a plasmid DNA segment carrying replication functions could serve as a cloning vehicle or "vector" for the cloning of other restriction endonuclease-generated DNA fragments. Ideally, a plasmid vector suitable for the cloning of nonreplicating *Eco*RI-generated DNA fragments would contain replication machinery plus a selectable antibiotic resistance gene on the same *Eco*RI fragment. We searched for such a vector among the antibiotic resistance plasmids we had been studying.

In our collection at Stanford was a small plasmid, 9000 base pairs in length, that carried a gene conferring resistance to the antibiotic tetracycline (Tc). When we subjected the DNA of this plasmid (pSC101) (Cohen and Chang, 1973, 1977) to cleavage by *Eco*RI endonuclease and analyzed the products by gel electrophoresis, we found that the enzyme had cut the DNA molecule at only a single location. This indicated that the pSC101 plasmid could be used as a directly selectable cloning vector if a fragment of foreign DNA could be inserted at its *Eco*RI cleavage site without interfering with either the replication functions or expression of the Tc resistance gene carried by the plasmid.

We mixed the DNA of the pSC101 plasmid with the previously constructed R6–5-derived plasmid carrying a Km resistance gene on an *Eco*RI-generated fragment, cleaved the mixture with *Eco*RI endonuclease, and treated the resulting DNA with ligase. The DNA was introduced into *E. coli* by transformation, and bacteria that expressed both the R6–5-derived Km resistance determinant and the Tc resistance gene of pSC101 were selected. A plasmid from one of the resulting clones was found to contain the entire pSC101 vector plus one of the three fragments of the Km-resistance plasmid (Fig. 5). Thus, pSC101 could at least be used to propagate a nonreplicating segment of another *Eco*RI DNA plasmid. In similar experiments, we showed that the pSC101 plasmid could be joined *in vitro* to a second *Eco*RI-cleaved replicon carrying a gene for streptomycin resistance. The procedure is summarized schematically in Fig. 6.

Chang and I proceeded to determine whether the procedure we had used to clone fragments of *E. coli* plasmids could be used to propagate and genetically express DNA from an unrelated bacterial species (Chang and Cohen, 1974). It was possible that the way genetic information was arranged on totally foreign DNA molecules or another yet unknown

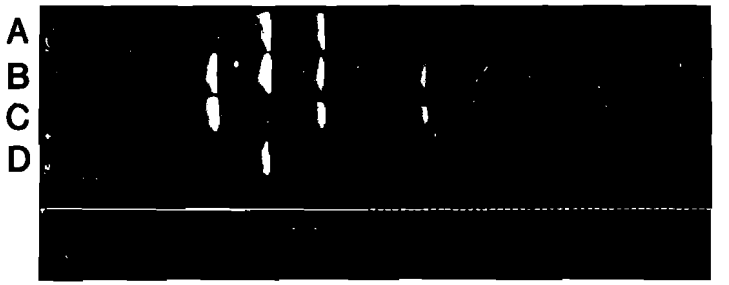


FIG. 5. Agarose gel electrophoresis of *Eco*RI digest of newly constructed plasmid DNA species. A new plasmid (A) consisting of the pSC101 vector (D) plus the kanamycin resistance (middle) fragment of pSC102. (C) has been constructed by *Eco*RI cleavage of the parental DNA molecules plus ligation and transformation. (B) shows a mixture of the *Eco*RI-cleaved plasmid DNA preparations. From Cohen *et al.* (1973).

factor might produce an aberrant situation that would prevent the survival of such hybrid molecules in a new host. [It is now known that the DNA sequence arrangement on some DNA fragments impedes their cloning or stability, or both, as part of recombinant DNA molecules (Heyneker *et al.*, 1976; Timmis *et al.*, 1978b)]. Even if DNA from a very different bacterial species, such as *Staphylococcus aureus*, could be replicated in *E. coli* by joining it to the pSC101 vector, the foreign genes might not be expressed phenotypically in a heterospecific environment. [There is now evidence that some genes derived from foreign bacterial species can be expressed phenotypically in *E. coli*, but others cannot (Chakrabarty *et al.*, 1978); we made a fortunate choice in selecting a gene that was expressed.]

*Eco*RI-cleaved pSC101 plasmid DNA and DNA from the *S. aureus* plasmid pI258, which carries a gene that encodes the enzyme β-lactamase and specifies resistance to penicillin and ampicillin (Ap), were mixed, treated with DNA ligase, and introduced into *E. coli* by transformation. Transformant cells that expressed the penicillin resistance of the *S. aureus* plasmid as well as the Tc resistance of *E. coli* were isolated; these were found to contain a new DNA species consisting of the entire pSC101 plasmid plus an *Eco*RI-generated *S. aureus* DNA fragment that contained the Ap resistance gene derived from the pI258 plasmid (Fig. 7).

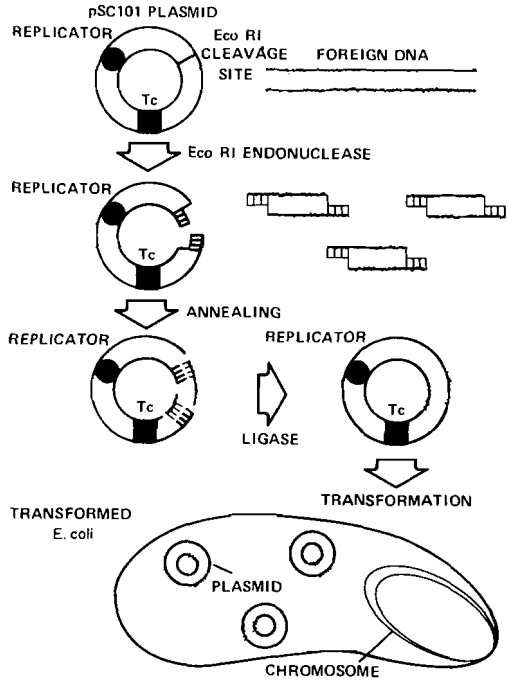


FIG. 6. Schematic representation of the procedure used in the initial DNA cloning experiments. Fragments of *Eco*RI endonuclease-cleaved DNA were joined to the similarly cleaved pSC101 plasmid vector by hydrogen bonding of protruding single strands containing complementary base sequences. After covalent joining of the fragments by DNA ligase, they were introduced by transformation into $CaCl_2$-treated bacteria. Cells resistant to tetracycline were selected, and each yielded a bacterial clone containing a plasmid identical to the plasmid DNA molecule taken up by a single transformed cell.

The replication and expression in *E. coli* of genes derived from an organism not known to exchange DNA with *E. coli* suggested that interspecies genetic combinations might be generally obtainable. We reasoned that it might be practical to use these methods to introduce into *E. coli* genes specifying metabolic and synthetic functions indigenous to other biological classes. Potentially, plasmid replicons such as pSC101 might also allow DNA derived from eukaryotic organisms to be introduced into *E. coli*, thus enabling the application of bacterial genetic and

biochemical techniques to the study of eukaryotic genes. Moreover, by fragmenting the eukaryotic chromosome and cloning segments of it on individual plasmids, it potentially would be feasible to isolate specific eukaryotic genes and to study the organization of genetic information of higher organisms in ways that were not previously possible.

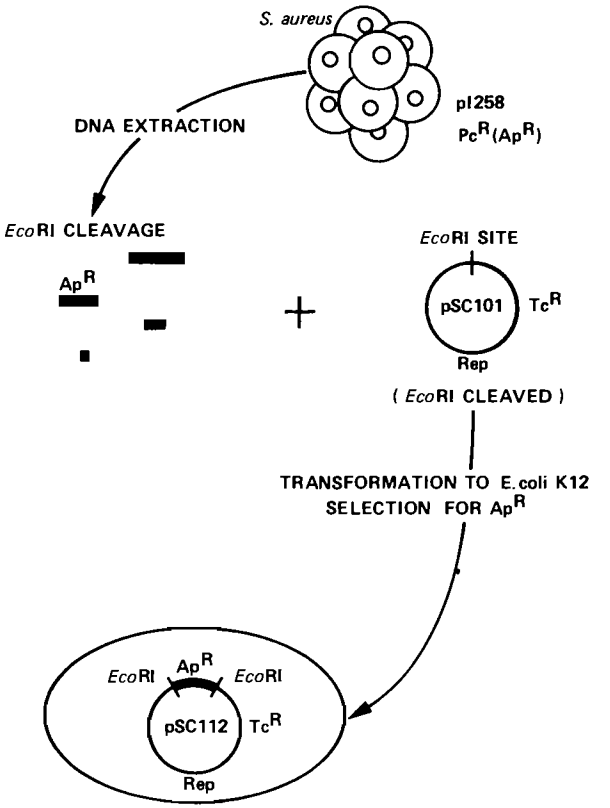


FIG. 7. Chimeric plasmids containing DNA segments derived from *Staphylococcus aureus* and *Escherichia coli* were constructed by joining an *Eco*RI-generated fragment from the *S. aureus* plasmid pI258 to the pSC101 vector and introducing the composite molecule into *E. coli*. The Ap-resistance gene carried by the *S. aureus* plasmid DNA was expressed phenotypically in the unrelated bacterial host.

D. Cloning of Eukaryotic DNA in E. coli

To determine whether eukaryotic DNA could in fact be replicated in bacteria, my colleagues and I undertook the cloning of DNA that encodes the ribosomal RNA of the frog *Xenopus laevis* (Morrow *et al.*, 1974). Although this DNA does not express traits (such as antibiotic resistance) that enable selection of bacteria carrying chimeric plasmids, *X. laevis* ribosomal DNA (rDNA) had been well characterized, and its physical properties would permit the identification of *X. laevis* DNA fragments of bacterial plasmids. The Tc resistance conferred by the pSC101 plasmid allowed us to select for transformed clones, and we could then examine the plasmid DNA isolated from such clones to determine whether any of the plasmids contained DNA fragments having molecular properties of *Xenopus* ribosomal DNA. The foreign DNA fragments being propagated in bacteria could also be tested for nucleotide sequence homology with DNA isolated directly from *X. laevis* oocytes, using electron microscope heteroduplex techniques (Davis and Davidson, 1968; Westmoreland *et al.*, 1969).

Ribosomal DNA from *X. laevis* and the pSC101 plasmid were mixed, cleaved with *Eco*RI endonuclease, and ligated using the procedures we had employed earlier. Fifty-five Tc-resistant transformants were isolated, and DNA obtained from such transformants was analyzed by gel electrophoresis, cesium chloride gradient centrifugation, and/or electron microscopy to determine the presence of an *Eco*RI-generated DNA fragment similar in size and/or buoyant density to similarly generated fragments of bona fide *X. laevis* rDNA. The results of these experiments are summarized in Table II. Fifteen of the Tc-resistance clones contained one or more *Eco*RI-generated fragments having the same size as fragments produced by cleavage of *X. laevis* rDNA. Moreover, the plasmid chimeras isolated from *E. coli* were shown to contain DNA with a buoyant density characteristic of the high G+C base composition of *X. laevis* rDNA. These experiments also produced an unexpected finding that provided an example of the type of new information that DNA cloning procedures could yield about the organization and structure of eukaryotic chromosomes. Variation in size of the *Eco*RI-generated *X. laevis* rDNA fragments present in plasmid chimeras was observed; together with the *Eco*RI cleavage pattern found in the amplified *X. laevis* rDNA isolated from frog oocytes, this finding

TABLE II
Xenopus laevis-Escherichia coli RECOMBINANT PLASMIDS[a,b]

Plasmid DNA	Molecular weight of *Eco*RI plasmid fragments estimated by gel electrophoresis ($\times 10^{-6}$)	Molecular weight from contour length ($\times 10^{-6}$)	Buoyant density of intact plasmid in CsCl (g/cm^3)
CD4	5.8, 4.2, 3.0	13.6	1.721
CD7	5.8, 4.2	—	—
CD12, CD20, CD45, CD47, CD51	5.8, 3.0	—	—
CD14	5.8,4.2,3.0	9.2	1.720
CD18	5.8, 3.9	10.0	1.719
CD35	5.8, 3.9, 3.0	—	—
CD42	5.8, 4.2	10.6	1.720
pSC101	5.8	6.0	1.710

[a] Modified from Morrow *et al.* (1974).

[b] *Eco*RI-cleaved chimeric plasmids containing *X. laevis* rDNA were characterized by buoyant density centrifugation in cesium chloride, electron microscopy, and electrophoresis in agarose gels.

suggested that the amplified repeat unit was heterogeneous in the oocytes (Morrow *et al.*, 1974).

Electron microscope analysis (Fig. 8) of a heteroduplex formed between *X. laevis* rDNA and one of the plasmid chimeras (CD42) demonstrated that this plasmid contains DNA nucleotide sequences homologous with those present in rDNA isolated directly from *X. laevis*. In some instances, segments of two separate chimeric plasmid DNA molecules were seen to form duplex regions with the single strand of *X. laevis* rDNA, consistent with the observation (Dawid *et al.*, 1970; Wensink and Brown, 1971) that the rDNA sequences of amplified *X. laevis* are tandomly repeated.

The plasmid chimeras containing both *E. coli* and *X. laevis* rDNA were found to replicate stably in bacterial hosts as part of the pSC101 plasmid replicon and could be recovered from transformed *E. coli* by procedures commonly employed for the isolation of bacterial plasmids. Tritium-labeled RNA isolated from bacteria harboring these plasmids

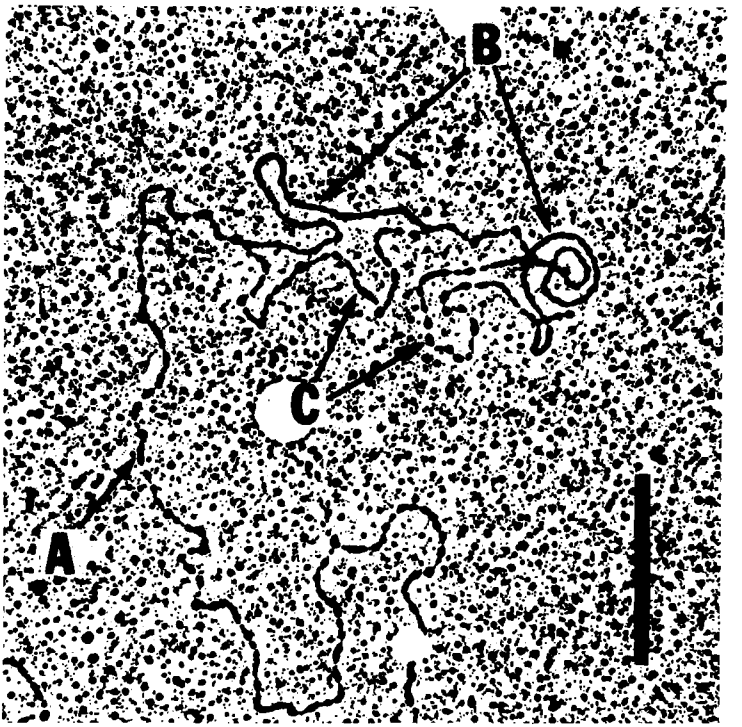


FIG. 8. Electron photomicrograph of a heteroduplex of *Xenopus laevis* ribosomal DNA and two separate molecules of a tetracycline resistance plasmid chimera (CD42) isolated from *E. coli* and containing a cloned DNA fragment derived from *X. laevis*. A, Single-strand rDNA *X. laevis;* B, double-strand regions of homology between the plasmid and *X. laevis* rDNA; C, single-strand segments corresponding in length to the DNA segment of the plasmid derived from the pSC101 plasmid vector. From Morrow *et al.* (1974).

hybridized *in vitro* to amplified *X. laevis* rDNA isolated directly from the eukaryotic organism, indicating that RNA synthesis could occur on the eukaryotic DNA transplanted into the prokaryotic host.

III. Some Subsequent Advances

Since these early DNA cloning experiments, major advances made in a number of laboratories have increased the ease and flexibility of gene

manipulation, so that segments of DNA molecules can now be taken apart and put together in a variety of different ways. Dozens of site-specific endonucleases that recognize different nucleotide sequences and thus cleave DNA at different sites have been identified and characterized (Roberts, 1976). Synthetic and natural "adaptor" fragments have been used to convert one kind of endonuclease cleavage site to another (Marians *et al.*, 1976; Heyneker *et al.*, 1976; Cohen *et al.*, 1977; Roberts, 1977; Scheller *et al.*, 1977). Additional naturally occurring plasmids suitable as vectors were identified (Hershfield *et al.*, 1974), and recombinant DNA methods have been used to modify these plasmids to yield vectors suitable for specific purposes (Armstrong *et al.*, 1977, Timmis *et al.*, 1978c; Bolivar *et al.*, 1977; Chang and Cohen, 1978). Vectors that utilize the replication and packaging systems of bacteriophage λ (Rambach and Tiollais, 1974; Murray and Murray, 1974; Thomas *et al.*, 1974; Blattner *et al.*, 1977; Leder *et al.*, 1977; Hohn and Murray, 1977) or other bacteriophages (Messing *et al.*, 1977; Hermann *et al.*, 1978). Specific messenger RNA (mRNA) species produced by certain organs or tissues has been used as template for the enzymic synthesis of double-stranded complementary DNA (cDNA) sequences corresponding to the mRNA (Ruogeon *et al.*, 1975; Rabbits, 1976; Eftratiadis *et al.*, 1976). Double-stranded DNA segments that have a nucleotide sequence corresponding to a known amino acid sequence have been synthesized chemically and have been purified and amplified by cloning them as part of a bacterial plasmid (Itakura *et al.*, 1977; Goeddel *et al.*, 1979). Novel methods of detecting plasmids that include specifically desired gene sequences have been developed using subculture cloning procedures (Kedes *et al.*, 1975) or *in situ* hybridization procedures (Grunstein and Hogness, 1975). Cotransformation procedures that enable introduction of nonselectable segments of DNA into bacteria (Kretschmer *et al.*, 1975) or mammalian cells (Wigler *et al.*, 1977) have been devised.

Although the site-specific endonucleases used for gene manipulation *in vitro* are commonly called "restriction enzymes," some of the bacterial species that encode such endonucleases show no detectable restriction of foreign DNA *in vivo*, and it has been speculated that the primary function of such enzymes may be DNA recombination (Kornberg, 1974; Nathans and Smith, 1975; Roberts, 1976). It seems highly likely that DNA cleavage by at least some restriction endonucleases also oc-

curs *in vivo:* the transforming ability of infecting phage DNA is restricted by several orders of magnitude in cells that produce the *Eco*RI enzyme (Takano *et al.*, 1968a,b), implying that most of the entering DNA molecules are cleaved *in vivo* before they can be methylated by the modification enzyme associated with the *Eco*RI restriction–modification system. There is evidence that the combined actions of the *Eco*RI endonuclease and DNA ligase can promote site-specific recombination *in vivo,* with results similar to the effects of these enzymes *in vitro* (Chang and Cohen, 1977). Moreover, "transposons," which can operate *in vivo* to join DNA segments having no ancestral relationship, can accomplish a result that is analogous to *in vitro* site-specific recombination (Cohen, 1976).

IV. Use of DNA Cloning as a Tool for the Study of Prokaryotic and Eukaryotic Biology

A. Studies of Plasmid Biology

The wish to study bacterial plasmids themselves was the motive that initially prompted our development of DNA cloning methods, and during the past 6 years my laboratory has used these methods extensively in such studies. DNA cloning has made possible elucidation of the structure and control of plasmid genes and has yielded much information about the replication of plasmid DNA. Using nonreplicating DNA fragments that contain antibiotic resistance genes as biological "probes," it has been possible to isolate and study DNA fragments carrying the replication functions of large and structurally complex plasmids (for example, Timmis *et al.*, 1975; Lovett and Helinski, 1976; Taylor and Cohen, 1979), as well as those of small plasmids (Chang and Cohen, 1978) (Fig. 9).

Using hybrid replicons formed by the fusion of two functionally different types of replication systems, we have investigated the relationship of plasmid replication and incompatibility (Timmis *et al.*, 1974; Cabello *et al.*, 1976; Meacock and Cohen, 1979) and have studied replication control in plasmids. A DNA sequence that accomplishes active partitioning of plasmids in dividing cell populations and that is functionally equivalent to the centromere of eukaryotic cells has been

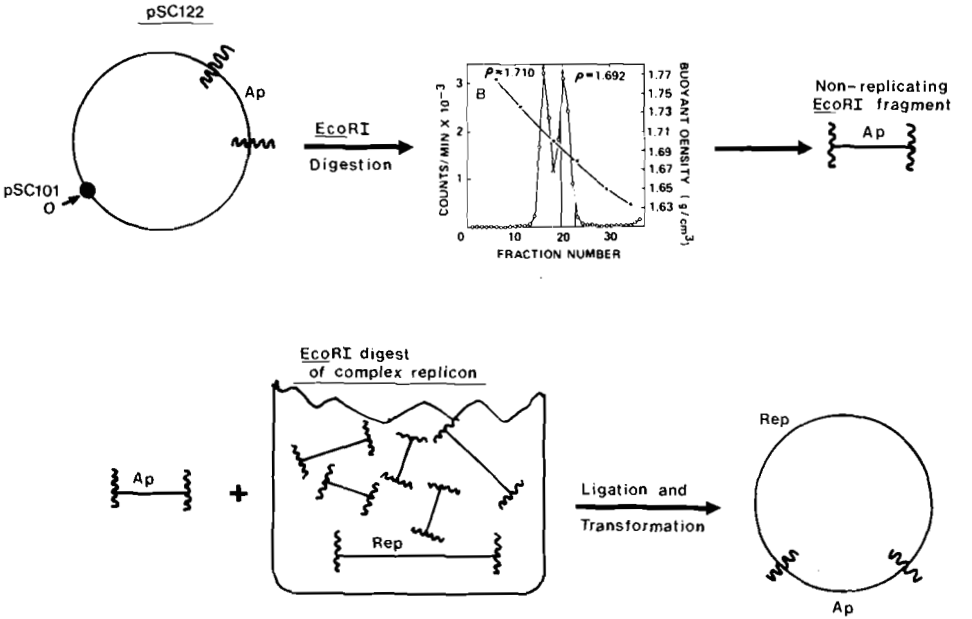


FIG. 9. Scheme for isolation of replication regions of complex plasmids. In the experiment shown, a plasmid carrying a nonreplicating Ap-resistance segment was cleaved by the *Eco*RI restriction endonuclease, and the Ap-resistance "probe" fragment was separated from its vector. The probe was then added to a mixture of DNA fragments produced by *Eco*RI cleavage of a large plasmid, and ligation and transformation were carried out. Since the probe fragment is incapable of replication, its propagation in transformants requires linkage to a DNA segment carrying replication functions. From Timmis *et al.* (1978c).

discovered and characterized using DNA cloning methods (P. Meacock and S. N. Cohen, unpublished data). The genes carried by large antibiotic resistance plasmids have been assigned to specific loci on plasmid DNA by the cloning of endonuclease-generated DNA fragments, and maps of complex plasmid genomes have been constructed (for example, see Timmis *et al.*, 1978a). Natural evolutionary variations in plasmid structure have been identified and have led to the concept that plasmid DNA is in a constant state of flux undergoing both macro- and micro-evolution (Chang *et al.*, 1975; Brutlag *et al.*, 1976; Cohen *et al.*, 1978; Timmis *et al.*, 1978b). Genes within transposable genetic elements have been studied, and their functional interactions have been elucidated.

B. Study of Organization of the Eukaryotic Genetic Sequence Encoding Pro-opiocortin

We and others have also used DNA cloning methods for the study of gene organization, evolution, and expression in eukaryotes. Of particular recent interest to my laboratory has been the genetic sequence that encodes the pituitary hormones ACTH and β-lipotropin (β-LPH). These peptide hormones are known each to include smaller peptides having distinct biological activities: α-melanotropin (α-MSH) and corticotropin-like intermediate lobe peptide (CLIP) are derived from ACTH; β-melanotropin (β-MSH), endorphins, and methionine enkephalin are included within β-LPH (Scott *et al.*, 1973; Li and Chung, 1976; Ling *et al.*, 1976; Li *et al.*, 1977) (Fig. 10). The intracellular level of the mRNA encoding the common precursor protein (pro-opiocortin) is known to be depressed by glucocorticoids, which seem to act at the transcriptional level by means of a glucocorticoid receptor (Nakanishi *et al.*, 1977; Nakamura *et al.*, 1978). The various component peptides are liberated from pro-opiocortin and secreted from pituitary cells by processing mechanisms.

Although the general positions of ACTH and β-LPH on the pro-opiocortin peptide have been known for several years, earlier studies had provided no information about the precise relationships of these peptides and the nature of the processing that the precursor molecule undergoes to yield its two major components. Moreover, ACTH and β-LPH account for only one-third to one-half of the molecular weight of

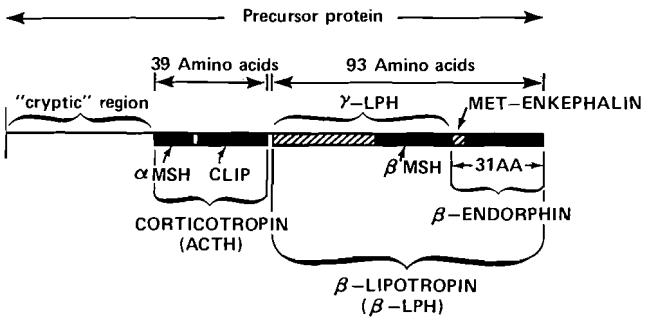


FIG. 10. Map of pro-opiocortin (corticotropin-β-lipotropin) precursor protein showing peptide components previously identified by amino acid analysis and "cryptic" region. Corticotropin (ACTH) and β-lipotropin (β-LPH) were positioned on pro-opiocortin by analysis of cloned cDNA derived from mRNA encoding the precursor protein. The length shown for β-LPH (93 amino acids) has been assigned from the nucleotide sequence of a cloned cDNA insert and differs from the commonly accepted 91 amino acid sequence for β-LPH determined by amino acid analysis (Li *et al.*, 1977).

the precursor protein; thus there has been considerable interest in, and speculation about, the primary structure and possible biological functions of the peptides encoded by the remaining "cryptic" portion. Our recent studies of the genetic sequence encoding pro-opiocortin provide an example of the application of DNA cloning methods for the investigation of gene organization in eukaryotes.

The cloning of complementary DNA (cDNA) (Nakanishi *et al.*, 1977) corresponding to the sequence encoding mRNA the ACTH-β-LPH precursor protein was carried out utilizing mRNA purified from the neurointermediate lobe of bovine pituitaries (Kita *et al.*, 1979). Avian myeloblastosis virus (AMV) reverse transcriptase was used for the sequential synthesis of the two strands of cDNA, homopolymeric dC "tails" were added, and complementary poly(dG) extensions were added to *Pst*I endonuclease cleaved-DNA of the Tc resistance plasmid vector pBR322 (Bolivar, 1977). These steps are summarized in Fig. 11. Following transformation of *E. coli* cells with the dG-dC tailed pro-opiocortin cDNA, Tc-resistant transformants were isolated, and bacterial clones that contained cDNA inserts were identified by a colony hybridization procedure (Grunstein and Hogness, 1975) using ^{32}P-labeled pituitary mRNA as a probe.

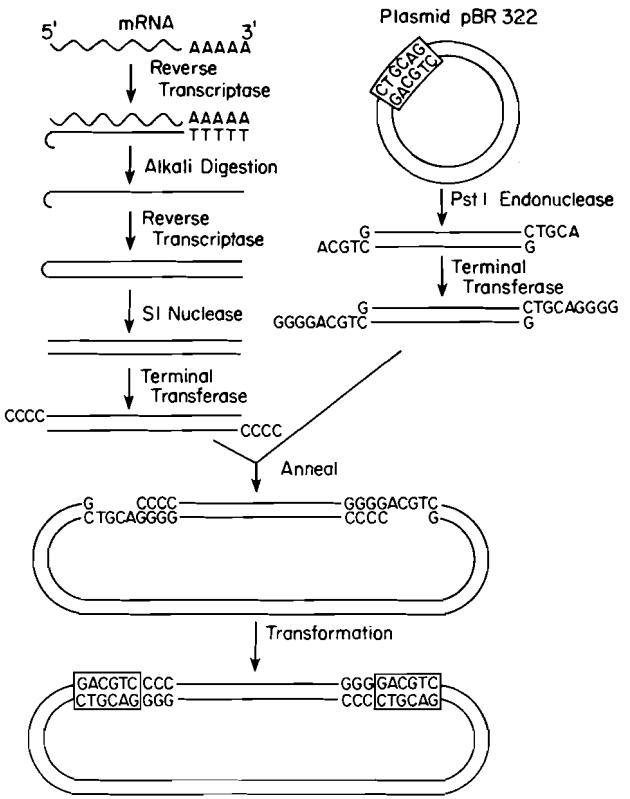


FIG. 11. Outline for protocol used for cloning of pro-opiocortin mRNA. For details, see text and Nakanishi *et al.* (1979). The Tc resistance gene on plasmid pBR322 was used for selection of transformants. As shown in the figure, the recognition sequence for *Pst*I endonuclease is regenerated at the plasmid/cDNA junction by the "tailing" procedure used.

The plasmid present in one of these clones (pSNAC20) was selected for further study. By determining the entire 1091 base pair nucleotide sequence (Maxam and Gilbert, 1978) of the cDNA insert of the pSNAC20 plasmid, we were able to infer certain important features of the protein encoded by the pro-opiocortin mRNA. Since the amino acid composition of ACTH and β-LPH are known (Scott *et al.*, 1973; Li and Chung, 1976; Ling *et al.*, 1976, Li *et al.*, 1977), the translational

reading frame of the cDNA sequence could be determined, and an amino acid sequence could thus be assigned for the previously cryptic segment of the pro-opiocortin protein. A probable translational initiation codon (AUG) for the precursor protein was identified from the translational reading frame and the previously known approximate length of pro-opiocortin. The first 20 amino acid residues following the putative initiative methionine were found to include a large proportion of hydrophobic amino acids (13 nonpolar residues, including 7 leucines), consistent with a putative role for the amino-terminal segment of pro-opiocortin as a "signal" peptide (Blobel and Dobberstein, 1975a,b) involved in secretion of the protein. This assignment has been verified recently by analysis of peptide fragments derived from the previously cryptic segment of the protein (Nakamura *et al.*, 1979; Keutmann *et al.*, 1979; E. Herbert, personal communication).

Computer analysis of amino acids assigned from the DNA sequence of the cryptic portion of the precursor protein showed that the pro-opiocortin protein contains a sequence of amino acids strikingly similar to the amino acid sequences of the previously identified hormones α-MSH and β-MSH. As in the case of α- and β-MSH, this peptide segment (which was named γ-MSH, Nakanishi *et al.*, 1979) is flanked by pairs of the basic amino acids lysine and/or arginine, suggesting that it could be liberated from pro-opiocortin by proteolytic processing. A second peptide segment located within the putative signal peptide segment of pro-opiocortin was found to have less extensive structural similarity to the MSHs; the presence of several largely homologous units within the same precursor molecule (Fig. 12) suggests that the gene for pro-opiocortin may have been formed by a series of structural duplications. The previously "cryptic" part of the pro-opiocortin molecule was also found to contain a number of amino acids in positions equivalent to those found in the hormone calcitonin, which is believed to have biological functions quite unrelated to those of the other components of molecule (Chang *et al.*, 1979).

Recently, we have isolated plasmids that include *genomic* DNA sequences encoding for *human* pro-opiocortin. Comparison of the DNA sequence of such clones with the cDNA sequence for the bovine hormone should provide information about the extent of interspecies variation within the cryptic part of the molecule and may yield data relating the ACTH and β-LPH coding sequences on the human chromosome to

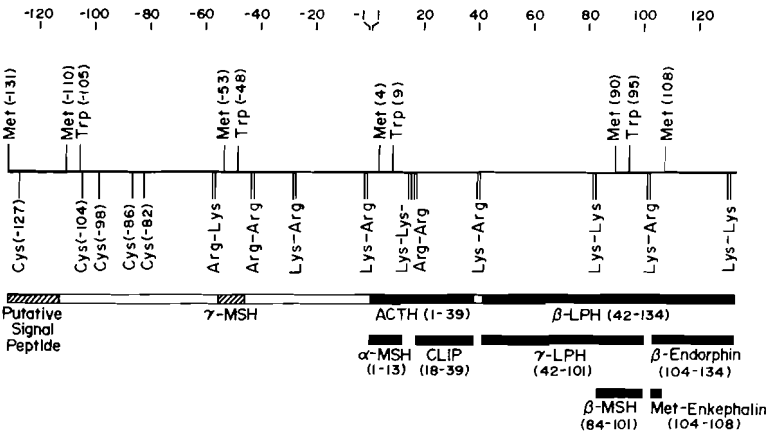


FIG. 12. Schematic representation of the structure of bovine pro-opiocortin. The numbering of the amino acid residues is as described in Nakanishi *et al.* (1979). Filled bars represent the region for which the amino acid sequence was known independently, and the open and hatched bars represent the regions for which the amino acid sequence was predicted from the nucleotide sequence of the pro-opiocortin mRNA. The Lys-Arg residues at sites of possible processing of the precursor protein into its peptide components, the positions of amino acids relevant to the MSH-like subunits of the protein, and certain other structural features are indicated. From Nakanishi *et al.* (1979).

the genes encoding calcitonin and other hormones. It should also provide insight into the relationship of intervening sequences to the protein-encoding sequences comprising the various structural and functional domains of the precursor protein.

C. Expression of Mammalian DNA Sequences in Bacterial Cells

Since the initial propagation of eukaryotic DNA in bacteria (Morrow *et al.*, 1974), several systems have been used to study expression in *E. coli* of DNA derived from higher organisms. Our early studies with cloned *X. laevis* ribosomal DNA genes indicated that the nucleotide sequences of the eukaryotic DNA could be faithfully transcribed in *E. coli* (Morrow *et al.*, 1974). However, these experiments did not show whether such RNA synthesis resulted from read-through transcription from the bacterial component of the chimeric plasmids or from initiation of RNA synthesis on the eukaryotic DNA fragment. Subsequent inves-

tigations with plasmids containing the intact mouse mitochondrial DNA genome (Chang *et al.*, 1975) indicated that the transcriptional and translational control signals located on at least this eukaryotic cell-derived DNA did not function in bacteria to yield bona fide eukaryotic proteins.

Biological activity of genes from the lower eukaryotes *Saccharomyces cerevisiae* and *Neurospora crassa* was demonstrated subsequently using phenotypic selection for functions that complement mutationally inactivated homologous bacterial genes (Struhl *et al.*, 1976; Ratzkin and Carbon, 1977; Vapnek *et al.*, 1977). Later, immunological activity with antibody against the human hormones somatostatin and insulin was shown for peptide fragments cleaved *in vitro* from hybrid "fusion" proteins encoded in part by bacterial DNA and in part by chemically synthesized somatostatin or insulin DNA sequences (Itakura *et al.*, 1977; Goeddel *et al.*, 1978). In another instance, a hybrid protein containing the amino acids of proinsulin was shown to be made by bacteria that carry a double-stranded cDNA transcript of preproinsulin mRNA (Villa-Kamaroff *et al.*, 1978). Antigenic determinants for the bacterial β-lactamase and the eukaryotic gene product were detected on fused peptides and on the peptide fragments cleaved from such fused proteins; however, biological activity of the mammalian components of such immunologically reactive hybrid proteins was not shown.

Our approach to the study of mammalian gene expression in bacteria was to generate a heterogeneous population of clones carrying a DNA sequence that encodes for a selectable mammalian gene product, and then to select directly those bacteria in the population that phenotypically express the genetic sequence (Chang *et al.*, 1978). The mammalian enzyme dihydrofolate reductase (DHFR), which catalyzes the conversion of dihydrofolic acid to tetrahydrofolic acid, was especially suitable for this purpose: the mammalian DHFR has a much lower affinity for the antimetabolic drug, trimethoprim (Tp) than does the corresponding bacterial enzyme (Burchall and Hitching, 1965). Thus, bacterial cells that biologically express mammalian DHFR activity are resistant to the levels of Tp that ordinarily would inhibit their growth. The primary DNA sequence of plasmids that showed phenotypic expression of the mammalian gene product in bacteria could then be analyzed to determine the specific sequence arrangement that accomplishes expression. Moreover, differences in the level of expression in various clones could be correlated with the primary sequence of the clone.

Figure 13 summarizes the experimental plan used in these investigations. Partially purified mRNA containing DHFR sequences from mouse cells resistant to the DHFR-inhibiting drug methotrexate (Buell *et al.*, 1978) served as a template for the preparation of double-stranded cDNA using reverse transcriptase and DNA polymerase I. As in the case of the experiments described above for the ACTH-β-LPH mRNA, homopolymeric dC "tails" were added to the unfractionated cDNA by terminal deoxynucleotidyltransferase and homopolymeric dG tails were added to the termini generated by *Pst*I endonuclease cleavage within the β-lactamase of the pBR322 plasmid. Constructed plasmids were introduced into *E. coli* by transformation, and plasmid DNA isolated from Tp-resistant colonies was isolated and subjected to fragmentation analysis by various restriction endonucleases and to DNA sequence analysis.

As shown in Table III, the nucleotide sequence in the region of the vector-cDNA junction nearest the 5′ end of the DHFR mRNA was

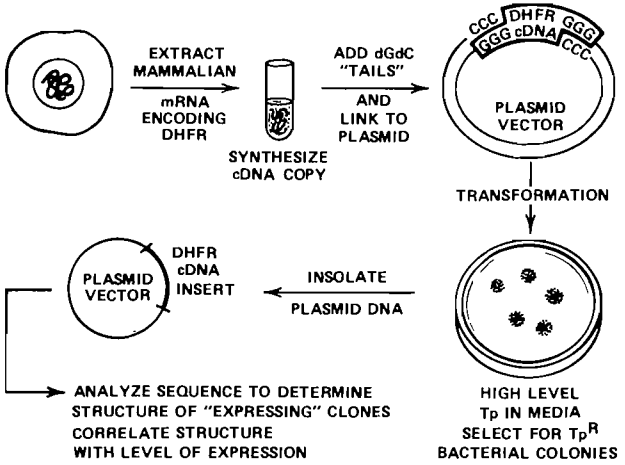


FIG. 13. Strategy used to obtain phenotypic expression of a mammalian genetic sequence in *Escherichia coli*. A heterogeneous population of clones carrying a DNA sequence that encodes for a mammalian gene product, dihydrofolate reductase (DHFR), that produces a selectable trait [high level trimethoprim resistance (Tp^R)] was generated, and those bacteria in the population that phenotypically expressed the gene were selected directly.

TABLE III

PROPERTIES OF pDHFR CHIMERIC PLASMIDS[a]

Plasmid	Nucleotide sequence[b]	Orientation of cDNA	Base pairs (bp) from A of ATG to center of 5-bp sequence	DHFR specific activity (units/mg protein)	MIC of Tp (μg/ml)	Relative DHFR activity	Reading frame of DHFR
pDHFR 7	Ala −13 −1 +1 TGCAG|GGGGGGGGGGATGGTT	A	12	2	>1000	1	+1
pDHFR 12	−28 TGC AG|GGGGGGGGGGGGGGGAGCTGCCATCATGGTT	A	10	4.2	>1000	2.1	+1
pDHRF 13	−22 TGCAG|GGGGGGGGGGGGGGCCATCATGGTT	A	11	2	>1000	1	+1
pDHFR 26	−102 −38 TGCAG|G GTGA AGGCTGGTAGGATTTTATCCCCGCTGCCATCATGGTT	A	20	ND	75	0.075	+2
pDHFR 27	−13 TGCAG|GGGGGGGGGGATGGTT	A	12	—	250	0.250	+1
pDHFR 28	−27 TGCAG|GGGGGGGGGGGGGGGGCTGCCATCATGGTT	A	11	ND	150	0.150	+2
pDHFR 29	TGC AG|GGGGGGGGGGGTCATGGTT	A	5 or 15	1	500	0.500	+1
pDHFR 23	−39 TGCAG|GGGGGG						

[a] DHFR, dihydrofolate reductase; MIC, mean inhibitory concentration; Tp, trimethoprim; ND, not detected.

[b] The black dots below nucleotides indicate homology with the nucleotide sequence at the 3′-OH terminus of 16 S rRNA (i.e., 3′-AUUCCUCCACUAGG-5′).

correlated with other properties of pDHFR chimeric plasmids, including the level of DHFR expression. In each instance, bacteria that expressed DHFR activity phenotypically were found to synthesize a protein that has the enzymic properties, immunological reactivity, and molecular size of the mouse DHFR (Chang *et al.*, 1978; Erlich *et al.*, 1979). Moreover, the DHFR cDNA segment in such clones was found to be in a different translational reading frame from the bacterial β-lactamase gene into which it had been inserted, suggesting that the biologically active DHFR being produced was not made as part of a fused protein.

Together, these findings implied that initiation of translation was occurring at the translational start codon (AUG) normally used for the synthesis of mouse DHFR in its original host. Thus, initiation of a structurally discrete and biologically functional eukaryotic peptide was occurring in bacteria on a fused (polycistronic) mRNA molecule. One structural feature important in accomplishing such translation "re-starts" is the presence of a ribosomal binding site at an appropriate distance from the translational start codon; the efficiency of expression was found to be strongly influenced by the extent of homology of this region of the mRNA with the 3′-OH end of 16 S ribosomal RNA (Shine and Dalgarno, 1974; Steitz and Steege, 1977; Steege, 1977), as well as the distance between the AUG codon and the ribosomal binding sequence of the mRNA. The sequence configuration found to accomplish phenotypic expression of the mouse DHFR genetic sequence in bacterial cells has been used for expression of other eukaryotic proteins in *E. coli,* and it seems to be generally applicable to the production of a wide variety of structurally discrete biologically functional heterospecific proteins in bacterial cells.

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United States Patent [19]

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[11] **4,237,224**

[45] **Dec. 2, 1980**

[54] **PROCESS FOR PRODUCING BIOLOGICALLY FUNCTIONAL MOLECULAR CHIMERAS**

[75] Inventors: **Stanley N. Cohen**, Portola Valley; **Herbert W. Boyer**, Mill Valley, both of Calif.

[73] Assignee: **Board of Trustees of the Leland Stanford Jr. University**, Stanford, Calif.

[21] Appl. No.: **1,021**

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[57] **ABSTRACT**

Method and compositions are provided for replication and expression of exogenous genes in microorganisms. Plasmids or virus DNA are cleaved to provide linear DNA having ligatable termini to which is inserted a gene having complementary termini, to provide a biologically functional replicon with a desired phenotypical property. The replicon is inserted into a microorganism cell by transformation. Isolation of the transformants provides cells for replication and expression of the DNA molecules present in the modified plasmid. The method provides a convenient and efficient way to introduce genetic capability into microorganisms for the production of nucleic acids and proteins, such as medically or commercially useful enzymes, which may have direct usefulness, or may find expression in the production of drugs, such as hormones, antibiotics, or the like, fixation of nitrogen, fermentation, utilization of specific feedstocks, or the like.

14 Claims, No Drawings

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