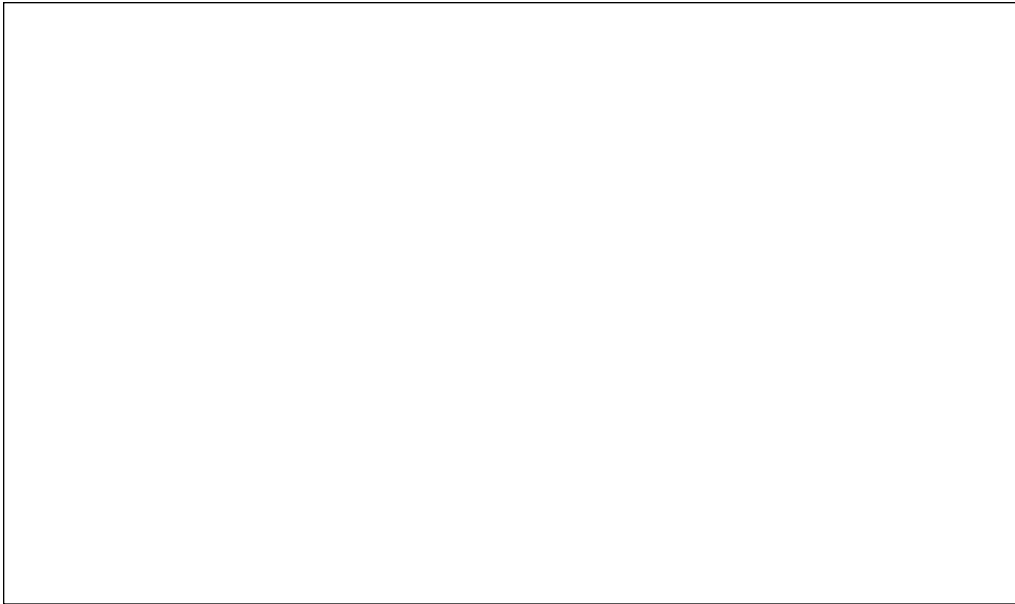**Part**

# V

*Molecular Genetics*

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# 14

# *DNA: The Genetic Material*



**FIGURE 14.1**
**DNA.** The hereditary blueprint in each cell of all living organisms is a very long, slender molecule called deoxyribonucleic acid (DNA).

## Concept Outline

The realization that patterns of heredity can be explained by the segregation of chromosomes in meiosis raised a question that occupied biologists for over 50 years: What is the exact nature of the connection between hereditary traits and chromosomes? This chapter describes the chain of experiments that have led to our current understanding of the molecular mechanisms of heredity (figure 14.1). The experiments are among the most elegant in science. Just as in a good detective story, each conclusion has led to new questions. The intellectual path taken has not always been a straight one, the best questions not always obvious. But however erratic and lurching the course of the experimental journey, our picture of heredity has become progressively clearer, the image more sharply defined.

## 14.1 What is the genetic material?

## The Hammerling Experiment: Cells Store Hereditary Information in the Nucleus

Perhaps the most basic question one can ask about hereditary information is where it is stored in the cell. To answer this question, Danish biologist Joachim Hammerling, working at the Max Plank Institute for Marine Biology in Berlin in the 1930s, cut cells into pieces and observed the pieces to see which were able to express hereditary information. For this experiment, Hammerling needed cells large enough to operate on conveniently and differentiated enough to distinguish the pieces. He chose the unicellular green alga *Acetabularia*, which grows up to 5 cm, as a **model organism** for his investigations. Just as Mendel used pea plants and Sturtevant used fruit flies as model organisms, Hammerling picked an organism that was suited to the specific experimental question he wanted to answer, assuming that what he learned could then be applied to other organisms.

Individuals of the genus *Acetabularia* have distinct foot, stalk, and cap regions; all are differentiated parts of a single cell. The nucleus is located in the foot. As a preliminary experiment, Hammerling amputated the caps of some cells and the feet of others. He found that when he amputated the cap, a new cap regenerated from the remaining portions of the cell (foot and stalk). When he amputated the foot, however, no new foot regenerated from the cap and stalk. Hammerling, therefore, hypothesized that the hereditary information resided within the foot of *Acetabularia*.

### Surgery on Single Cells

To test his hypothesis, Hammerling selected individuals from two species of the genus *Acetabularia* in which the caps look very different from one another: *A. mediterranea* has a disk-shaped cap, and *A. crenulata* has a branched, flower-like cap. Hammerling grafted a stalk from *A. crenulata* to a foot from *A. mediterranea* (figure 14.2). The cap that regenerated looked somewhat like the cap of *A. crenulata*, though not exactly the same.

Hammerling then cut off this regenerated cap and found that a disk-shaped cap exactly like that of *A. mediterranea* formed in the second regeneration and in every regeneration thereafter. This experiment supported Hammerling's hypothesis that the instructions specifying the kind of cap are stored in the foot of the cell, and that these instructions must pass from the foot through the stalk to the cap.
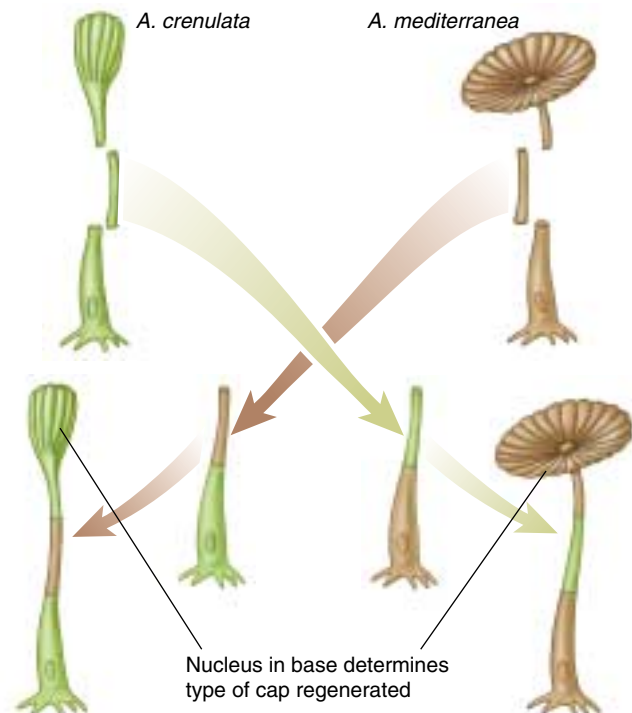


**FIGURE 14.2**
**Hammerling's *Acetabularia* reciprocal graft experiment.** Hammerling grafted a stalk of each species of *Acetabularia* onto the foot of the other species. In each case, the cap that eventually developed was dictated by the nucleus-containing foot rather than by the stalk.

In this experiment, the initial flower-shaped cap was somewhat intermediate in shape, unlike the disk-shaped caps of subsequent generations. Hammerling speculated that this initial cap, which resembled that of *A. crenulata*, was formed from instructions already present in the transplanted stalk when it was excised from the original *A. crenulata* cell. In contrast, all of the caps that regenerated subsequently used new information derived from the foot of the *A. mediterranea* cell the stalk had been grafted onto. In some unknown way, the original instructions that had been present in the stalk were eventually "used up." We now understand that genetic instructions (in the form of messenger RNA, discussed in chapter 15) pass from the nucleus in the foot upward *through the stalk* to the developing cap.

**Hereditary information in *Acetabularia* is stored in the foot of the cell, where the nucleus resides.**

## Transplantation Experiments: Each Cell Contains a Full Set of Genetic Instructions

Because the nucleus is contained in the foot of *Acetabularia*, Hammerling's experiments suggested that the nucleus is the repository of hereditary information in a cell. A direct test of this hypothesis was carried out in 1952 by American embryologists Robert Briggs and Thomas King. Using a glass pipette drawn to a fine tip and working with a microscope, Briggs and King removed the nucleus from a frog egg. Without the nucleus, the egg did not develop. However, when they replaced the nucleus with one removed from a more advanced frog embryo cell, the egg developed into an adult frog. Clearly, the nucleus was directing the egg's development (figure 14.3).

### Successfully Transplanting Nuclei

Can every nucleus in an organism direct the development of an entire adult individual? The experiment of Briggs and King did not answer this question definitively, because the nuclei they transplanted from frog embryos into eggs often caused the eggs to develop abnormally. Two experiments performed soon afterward gave a clearer answer to the question. In the first, John Gurdon, working with another species of frog at Oxford and Yale, transplanted nuclei from tadpole cells into eggs from which the nuclei had been removed. The experiments were difficult—it was necessary to synchronize the division cycles of donor and host. However, in many experiments, the eggs went on to develop normally, indicating that the nuclei of cells in later stages of development retain the genetic information necessary to direct the development of all other cells in an individual.

### Totipotency in Plants

In the second experiment, F. C. Steward at Cornell University in 1958 placed small fragments of fully developed carrot tissue (isolated from a part of the vascular system called the phloem) in a flask containing liquid growth medium. Steward observed that when individual cells broke away from the fragments, they often divided and developed into multicellular roots. When he immobilized the roots by placing them in a solid growth medium, they went on to develop normally into entire, mature plants. Steward's experiment makes it clear that, even in adult tissues, the nuclei of individual plant cells are "totipotent"—each contains a full set of hereditary instructions and can generate an entire adult individual. As you will learn in chapter 19, animal cells, like plant cells, can be totipotent, and a single adult animal cell can generate an entire adult animal.

> Hereditary information is stored in the nucleus of eukaryotic cells. Each nucleus in any eukaryotic cell contains a full set of genetic instructions.
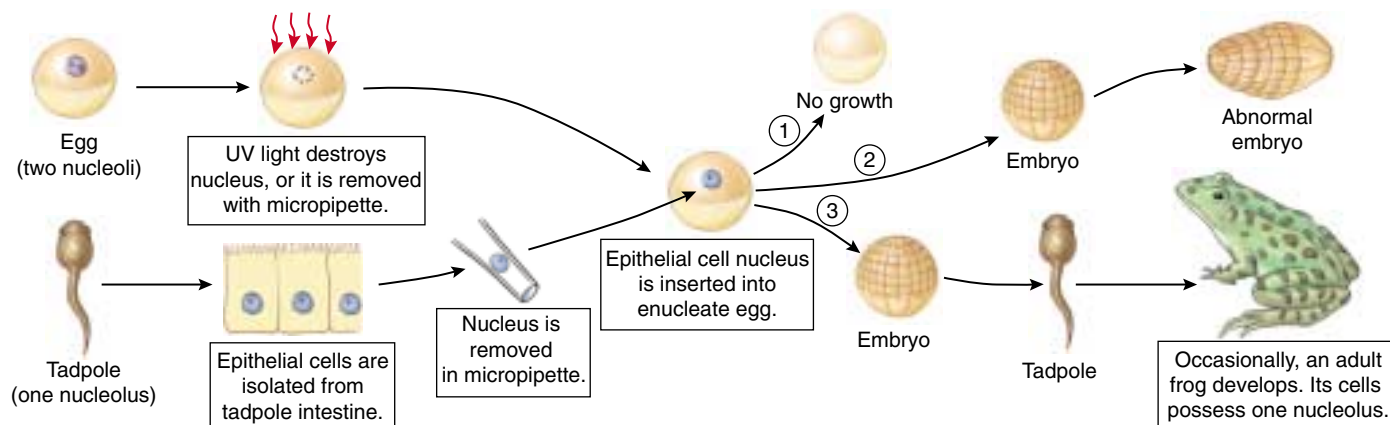


**FIGURE 14.3**

**Briggs and King's nuclear transplant experiment.** Two strains of frogs were used that differed from each other in the number of nucleoli their cells possessed. The nucleus was removed from an egg of one strain, either by sucking the egg nucleus into a micropipette or, more simply, by destroying it with ultraviolet light. A nucleus obtained from a differentiated cell of the other strain was then injected into this enucleate egg. The hybrid egg was allowed to develop. One of three results was obtained in individual experiments: (*1*) no growth occurred, perhaps reflecting damage to the egg cell during the nuclear transplant operation; (*2*) normal growth and development occurred up to an early embryo stage, but subsequent development was not normal and the embryo did not survive; and (*3*) normal growth and development occurred, eventually leading to the development of an adult frog. That frog was of the strain that contributed the nucleus and not of the strain that contributed the egg. Only a few experiments gave this third result, but they serve to clearly establish that the nucleus directs frog development.

# The Griffith Experiment: Hereditary Information Can Pass between Organisms

The identification of the nucleus as the repository of hereditary information focused attention on the chromosomes, which were already suspected to be the vehicles of Mendelian inheritance. Specifically, biologists wondered how the **genes,** the units of hereditary information studied by Mendel, were actually arranged in the chromosomes. They knew that chromosomes contained both protein and deoxyribonucleic acid (DNA). Which of these held the genes? Starting in the late 1920s and continuing for about 30 years, a series of investigations addressed this question.

In 1928, British microbiologist Frederick Griffith made a series of unexpected observations while experimenting with pathogenic (disease-causing) bacteria. When he infected mice with a virulent strain of *Streptococcus pneumoniae* bacteria (then known as *Pneumococcus*), the mice died of blood poisoning. However, when he infected similar mice with a mutant strain of *S. pneumoniae* that lacked the virulent strain's polysaccharide coat, the mice showed no ill effects. The coat was apparently necessary for virulence. The normal pathogenic form of this bacterium is referred to as the S form because it forms smooth colonies on a culture dish. The mutant form, which lacks an enzyme needed to manufacture the polysaccharide capsule, is called the R form because it forms rough colonies.

To determine whether the polysaccharide coat itself had a toxic effect, Griffith injected dead bacteria of the virulent S strain into mice; the mice remained perfectly healthy. As a control, he injected mice with a mixture containing dead S bacteria of the virulent strain and live coatless R bacteria, each of which by itself did not harm the mice (figure 14.4). Unexpectedly, the mice developed disease symptoms and many of them died. The blood of the dead mice was found to contain high levels of live, virulent *Streptococcus* type S bacteria, which had surface proteins characteristic of the live (previously R) strain. Somehow, the information specifying the polysaccharide coat had passed from the dead, virulent S bacteria to the live, coatless R bacteria in the mixture, permanently transforming the coatless R bacteria into the virulent S variety. **Transformation** is the transfer of genetic material from one cell to another and can alter the genetic makeup of the recipient cell.

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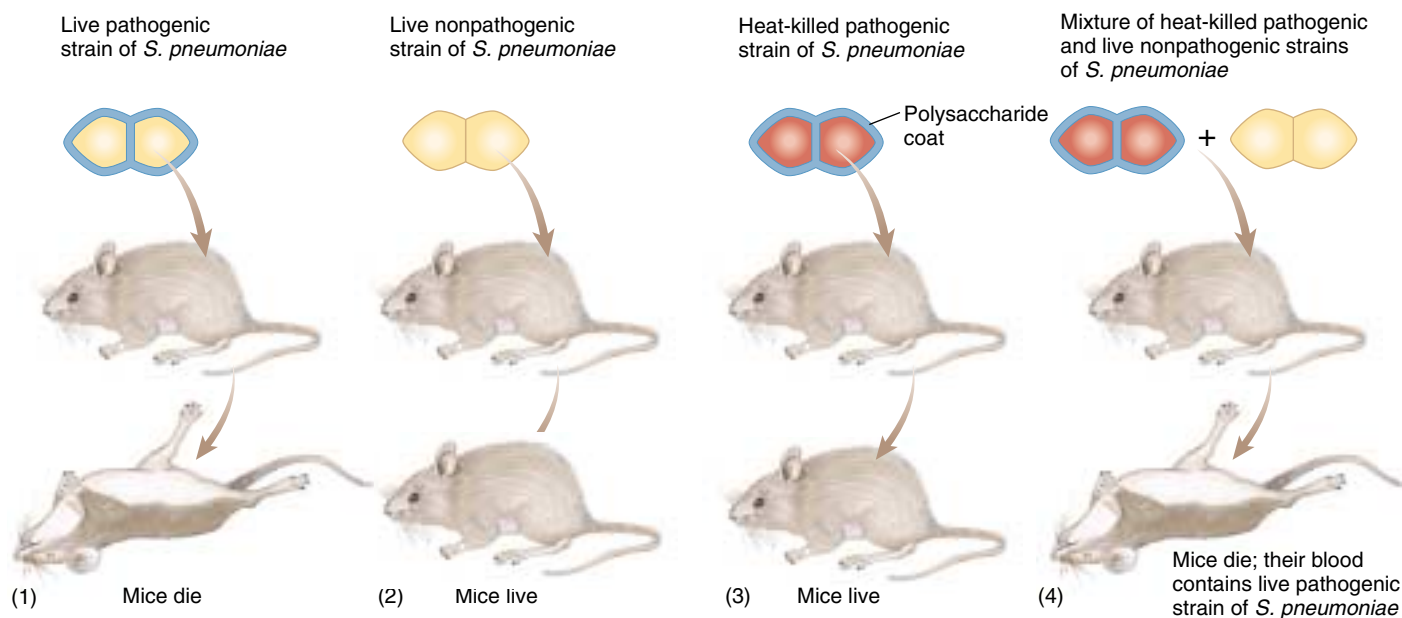**Hereditary information can pass from dead cells to living ones, transforming them.**

---



**FIGURE 14.4**

**Griffith's discovery of transformation.** (*1*) The pathogenic of the bacterium *Streptococcus pneumoniae* kills many of the mice it is injected into. The bacterial cells are covered with a polysaccharide coat, which the bacteria themselves synthesize. (*2*) Interestingly, an injection of live, coatless bacteria produced no ill effects. However, the coat itself is not the agent of disease. (*3*) When Griffith injected mice with dead bacteria that possessed polysaccharide coats, the mice were unharmed. (*4*) But when Griffith injected a mixture of dead bacteria with polysaccharide coats and live bacteria without such coats, many of the mice died, and virulent bacteria with coats were recovered. Griffith concluded that the live cells had been "transformed" by the dead ones; that is, genetic information specifying the polysaccharide coat had passed from the dead cells to the living ones.

## The Avery and Hershey-Chase Experiments: The Active Principle Is DNA

### The Avery Experiments

The agent responsible for transforming *Streptococcus* went undiscovered until 1944. In a classic series of experiments, Oswald Avery and his coworkers Colin MacLeod and Maclyn McCarty characterized what they referred to as the "transforming principle." They first prepared the mixture of dead S *Streptococcus* and live R *Streptococcus* that Griffith had used. Then Avery and his colleagues removed as much of the protein as they could from their preparation, eventually achieving 99.98% purity. Despite the removal of nearly all protein, the transforming activity was not reduced. Moreover, the properties of the transforming principle resembled those of DNA in several ways:

1. When the purified principle was analyzed chemically, the array of elements agreed closely with DNA.
2. When spun at high speeds in an ultracentrifuge, the transforming principle migrated to the same level (density) as DNA.
3. Extracting the lipid and protein from the purified transforming principle did not reduce its activity.
4. Protein-digesting enzymes did not affect the principle's activity; nor did RNA-digesting enzymes.
5. The DNA-digesting enzyme DNase destroyed all transforming activity.

The evidence was overwhelming. They concluded that "a nucleic acid of the deoxyribose type is the fundamental unit of the transforming principle of *Pneumococcus* Type III"—in essence, that DNA is the hereditary material.

### The Hershey–Chase Experiment

Avery's results were not widely accepted at first, as many biologists preferred to believe that proteins were the repository of hereditary information. Additional evidence supporting Avery's conclusion was provided in 1952 by Alfred Hershey and Martha Chase, who experimented with **bacteriophages,** viruses that attack bacteria. Viruses, described in more detail in chapter 33, consist of either DNA or RNA (ribonucleic acid) surrounded by a protein coat. When a *lytic* (potentially cell-rupturing) bacteriophage infects a bacterial cell, it first binds to the cell's outer surface and then injects its hereditary information into the cell. There, the hereditary information directs the production of thousands of new viruses within the bacterium. The bacterial cell eventually ruptures, or lyses, releasing the newly made viruses.

To identify the hereditary material injected into bacterial cells at the start of an infection, Hershey and Chase used the bacteriophage T2, which contains DNA rather than RNA. They labeled the two parts of the viruses, the DNA and the protein coat, with different radioactive isotopes
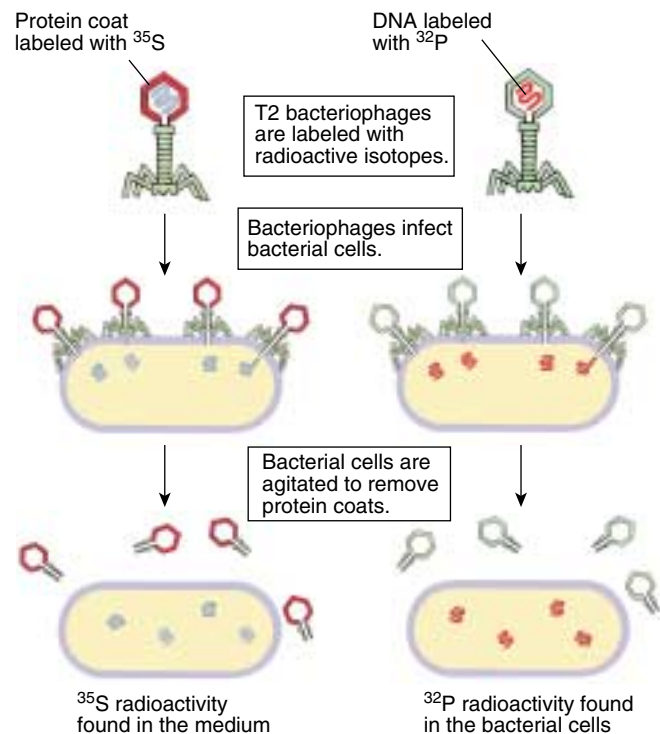


**FIGURE 14.5**
**The Hershey and Chase experiment.** Hershey and Chase found that $^{35}$S radioactivity did not enter infected bacterial cells and $^{32}$P radioactivity did. They concluded that viral DNA, not protein, was responsible for directing the production of new viruses.

that would serve as tracers. In some experiments, the viruses were grown on a medium containing an isotope of phosphorus, $^{32}$P, and the isotope was incorporated into the phosphate groups of newly synthesized DNA molecules. In other experiments, the viruses were grown on a medium containing $^{35}$S, an isotope of sulfur, which is incorporated into the amino acids of newly synthesized protein coats. The $^{32}$P and $^{35}$S isotopes are easily distinguished from each other because they emit particles with different energies when they decay.

After the labeled viruses were permitted to infect bacteria, the bacterial cells were agitated violently to remove the protein coats of the infecting viruses from the surfaces of the bacteria. This procedure removed nearly all of the $^{35}$S label (and thus nearly all of the viral protein) from the bacteria. However, the $^{32}$P label (and thus the viral DNA) had transferred to the interior of the bacteria (figure 14.5) and was found in viruses subsequently released from the infected bacteria. Hence, the hereditary information injected into the bacteria that specified the new generation of viruses was DNA and not protein.

Avery's experiments demonstrate conclusively that DNA is Griffith's transforming material. The hereditary material of bacteriophages is DNA and not protein.

# The Chemical Nature of Nucleic Acids

A German chemist, Friedrich Miescher, discovered DNA in 1869, only four years after Mendel's work was published. Miescher extracted a white substance from the nuclei of human cells and fish sperm. The proportion of nitrogen and phosphorus in the substance was different from that in any other known constituent of cells, which convinced Miescher that he had discovered a new biological substance. He called this substance "nuclein," because it seemed to be specifically associated with the nucleus.

### Levene's Analysis: DNA Is a Polymer

Because Miescher's nuclein was slightly acidic, it came to be called **nucleic acid.** For 50 years biologists did little research on the substance, because nothing was known of its function in cells. In the 1920s, the basic structure of nucleic acids was determined by the biochemist P. A. Levene, who found that DNA contains three main components (figure 14.6): (1) phosphate ($PO_4$) groups; (2) five-carbon sugars; and (3) nitrogen-containing bases called **purines** (adenine, A, and guanine, G) and **pyrimidines** (thymine, T, and cytosine, C; RNA contains uracil, U, instead of T). From the roughly equal proportions of these components, Levene concluded correctly that DNA and RNA molecules are made of repeating units of the three components. Each unit, consisting of a sugar attached to a phosphate group and a base, is called a **nucleotide.** The identity of the base distinguishes one nucleotide from another.

To identify the various chemical groups in DNA and RNA, it is customary to number the carbon atoms of the base and the sugar and then refer to any chemical group attached to a carbon atom by that number. In the sugar, four of the carbon atoms together with an oxygen atom form a five-membered ring. As illustrated in figure 14.7, the carbon atoms are numbered 1′ to 5′, proceeding clockwise from the oxygen atom; the prime symbol (′) indicates that the number refers to a carbon in a sugar rather than a base. Under this numbering scheme, the phosphate group is attached to the 5′ carbon atom of the sugar, and the base is attached to the 1′ carbon atom. In addition, a free hydroxyl (—OH) group is attached to the 3′ carbon atom.

The 5′ phosphate and 3′ hydroxyl groups allow DNA and RNA to form long chains of nucleotides, because these two groups can react chemically with each other. The reaction between the phosphate group of one nucleotide and the hydroxyl group of another is a dehydration synthesis, eliminating a water molecule and forming a covalent bond that links the two groups (figure 14.8). The linkage is called a **phosphodiester bond** because
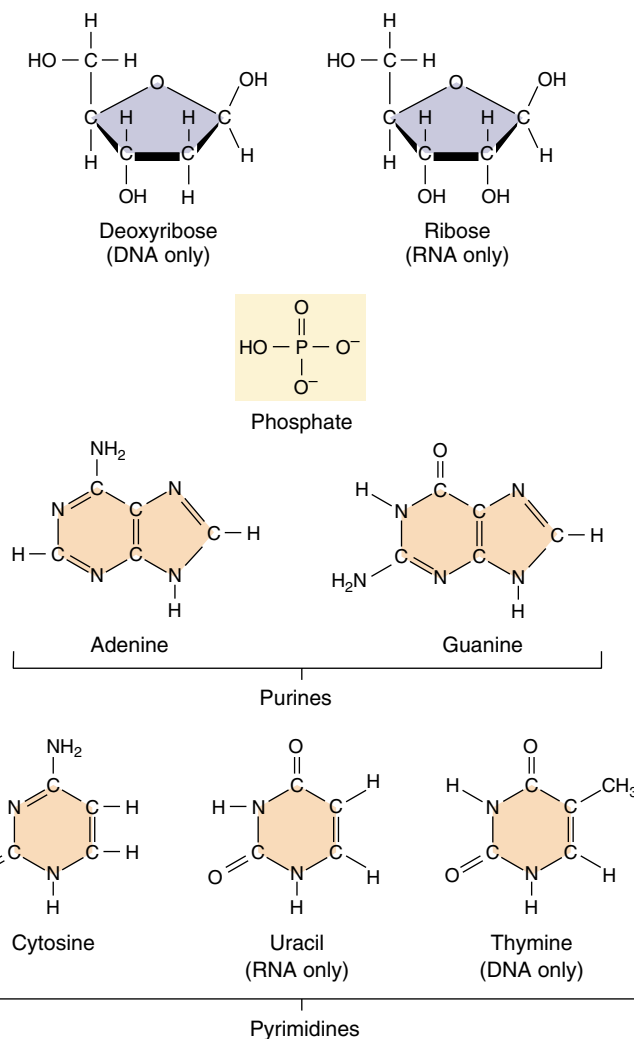


**FIGURE 14.6**
**Nucleotide subunits of DNA and RNA.** The nucleotide subunits of DNA and RNA are composed of three elements: a five-carbon sugar (deoxyribose in DNA and ribose in RNA), a phosphate group, and a nitrogenous base (either a purine or a pyrimidine).
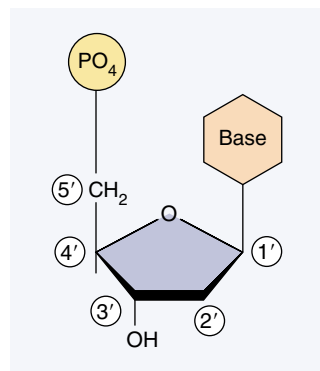


**FIGURE 14.7**
**Numbering the carbon atoms in a nucleotide.** The carbon atoms in the sugar of the nucleotide are numbered 1′ to 5′, proceeding clockwise from the oxygen atom. The "prime" symbol (′) indicates that the carbon belongs to the sugar rather than the base.

Table 14.1    Chargaff's Analysis of DNA Nucleotide Base Compositions

| Organism | Base Composition (Mole Percent) | | | |
|---|---|---|---|---|
| | A | T | G | C |
| *Escherichia coli (K12)* | 26.0 | 23.9 | 24.9 | 25.2 |
| *Mycobacterium tuberculosis* | 15.1 | 14.6 | 34.9 | 35.4 |
| Yeast | 31.3 | 32.9 | 18.7 | 17.1 |
| Herring | 27.8 | 27.5 | 22.2 | 22.6 |
| Rat | 28.6 | 28.4 | 21.4 | 21.5 |
| Human | 30.9 | 29.4 | 19.9 | 19.8 |

Source: Data from E. Chargaff and J. Davidson (editors), The Nucleic Acides, 1955, Academic Press, New York, NY.

the phosphate group is now linked to the two sugars by means of a pair of ester (P—O—C) bonds. The two-unit polymer resulting from this reaction still has a free 5′ phosphate group at one end and a free 3′ hydroxyl group at the other, so it can link to other nucleotides. In this way, many thousands of nucleotides can join together in long chains.

Linear strands of DNA or RNA, no matter how long, will almost always have a free 5′ phosphate group at one end and a free 3′ hydroxyl group at the other. Therefore, every DNA and RNA molecule has an intrinsic directionality, and we can refer unambiguously to each end of the molecule. By convention, the sequence of bases is usually expressed in the 5′-to-3′ direction. Thus, the base sequence "GTCCAT" refers to the sequence,

5′ pGpTpCpCpApT—OH 3′

where the phosphates are indicated by "p." Note that this is not the same molecule as that represented by the reverse sequence:

5′ pTpApCpCpTpG—OH 3′
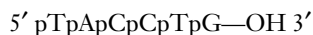
Levene's early studies indicated that all four types of DNA nucleotides were present in roughly equal amounts. This result, which later proved to be erroneous, led to the mistaken idea that DNA was a simple polymer in which the four nucleotides merely repeated (for instance, GCAT . . . GCAT . . . GCAT . . . GCAT . . .). If the sequence never varied, it was difficult to see how DNA might contain the hereditary information; this was why Avery's conclusion that DNA is the transforming principle was not readily accepted at first. It seemed more plausible that DNA was simply a structural element of the chromosomes, with proteins playing the central genetic role.
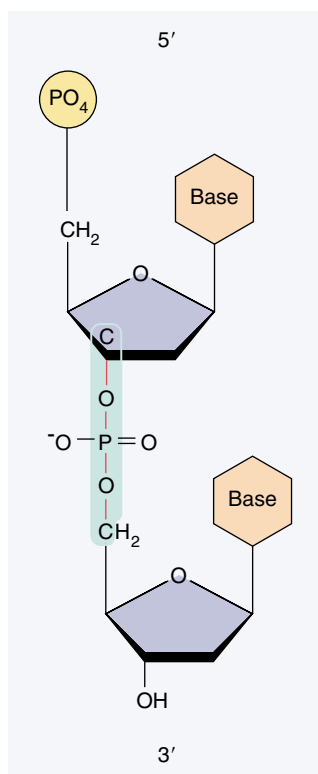


**FIGURE 14.8**
**A phosphodiester bond.**

## Chargaff's Analysis: DNA Is Not a Simple Repeating Polymer

When Levene's chemical analysis of DNA was repeated using more sensitive techniques that became available after World War II, quite a different result was obtained. The four nucleotides were *not* present in equal proportions in DNA molecules after all. A careful study carried out by Erwin Chargaff showed that the nucleotide composition of DNA molecules varied in complex ways, depending on the source of the DNA (table 14.1). This strongly suggested that DNA was not a simple repeating polymer and might have the information-encoding properties genetic material must have. Despite DNA's complexity, however, Chargaff observed an important underlying regularity in double-stranded DNA: *the amount of adenine present in DNA always equals the amount of thymine, and the amount of guanine always equals the amount of cytosine.* These findings are commonly referred to as **Chargaff's rules:**

1. The proportion of A always equals that of T, and the proportion of G always equals that of C:

A = T, and G = C.

2. It follows that there is always an equal proportion of purines (A and G) and pyrimidines (C and T).

---

**A single strand of DNA or RNA consists of a series of nucleotides joined together in a long chain. In all natural double-stranded DNA molecules, the proportion of A equals that of T, and the proportion of G equals that of C.**

# The Three-Dimensional Structure of DNA

As it became clear that DNA was the molecule that stored the hereditary information, investigators began to puzzle over how such a seemingly simple molecule could carry out such a complex function.
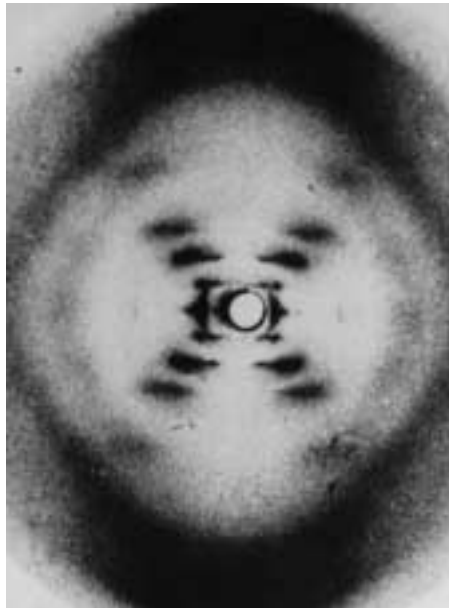
## Franklin: X-ray Diffraction Patterns of DNA

The significance of the regularities pointed out by Chargaff were not immediately obvious, but they became clear when a British chemist, Rosalind Franklin (figure 14.9*a*), carried out an X-ray diffraction analysis of DNA. In X-ray diffraction, a molecule is bombarded with a beam of X rays. When individual rays encounter atoms, their path is bent or diffracted, and the diffraction pattern is recorded on photographic film. The patterns resemble the ripples created by tossing a rock into a smooth lake (figure 14.9*b*). When carefully analyzed, they yield information about the three-dimensional structure of a molecule.

X-ray diffraction works best on substances that can be prepared as perfectly regular crystalline arrays. However, it was impossible to obtain true crystals of natural DNA at the time Franklin conducted her analysis, so she had to use DNA in the form of fibers. Franklin worked in the laboratory of British biochemist Maurice Wilkins, who was able to prepare more uniformly oriented DNA fibers than anyone had previously. Using these fibers, Franklin succeeded in obtaining crude diffraction information on natural DNA. The diffraction patterns she obtained suggested that the DNA molecule had the shape of a helix, or corkscrew, with a diameter of about 2 nanometers and a complete helical turn every 3.4 nanometers (figure 14.9*c*).

(a)

(b)

**FIGURE 14.9**
**Rosalind Franklin's X-ray diffraction patterns suggested the shape of DNA.**
(*a*) Rosalind Franklin developed techniques for taking X-ray diffraction pictures of fibers of DNA. (*b*) This is the telltale X-ray diffraction photograph of DNA fibers made in 1953 by Rosalind Franklin in the laboratory of Maurice Wilkins. (*c*) The X-ray diffraction studies of Rosalind Franklin suggested the dimensions of the double helix.
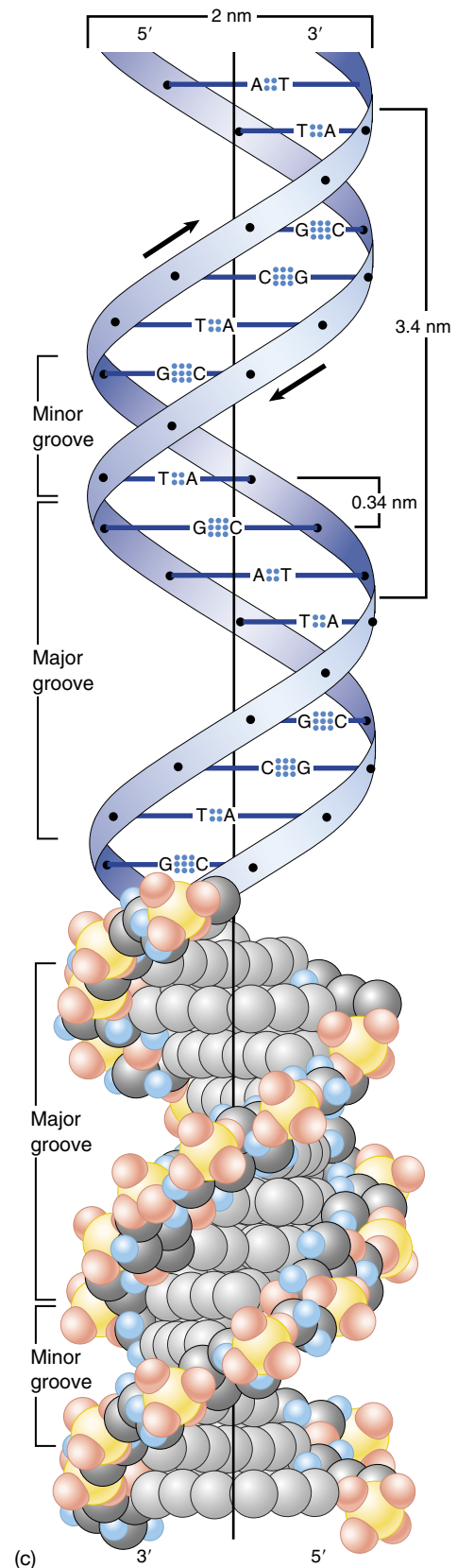
(c)

## Watson and Crick: A Model of the Double Helix

Learning informally of Franklin's results before they were published in 1953, James Watson and Francis Crick, two young investigators at Cambridge University, quickly worked out a likely structure for the DNA molecule (figure 14.10), which we now know was substantially correct. They analyzed the problem deductively, first building models of the nucleotides, and then trying to assemble the nucleotides into a molecule that matched what was known about the structure of DNA. They tried various possibilities before they finally hit on the idea that the molecule might be a simple **double helix,** with the bases of two strands pointed inward toward each other, forming **base-pairs.** In their model, base-pairs always consist of purines, which are large, pointing toward pyrimidines, which are small, keeping the diameter of the molecule a constant 2 nanometers. Because hydrogen bonds can form between the bases in a base-pair, the double helix is stabilized as a duplex DNA molecule composed of two **antiparallel strands,** one chain running 3′ to 5′ and the other 5′ to 3′. The base-pairs are planar (flat) and stack 0.34 nm apart as a result of hydrophobic interactions, contributing to the overall stability of the molecule.

The Watson–Crick model explained why Chargaff had obtained the results he had: in a double helix, adenine forms two hydrogen bonds with thymine, but it will not form hydrogen bonds properly with cytosine. Similarly, guanine forms three hydrogen bonds with cytosine, but it will not form hydrogen bonds properly with thymine. Consequently, adenine and thymine will always occur in the same proportions in any DNA molecule, as will guanine and cytosine, because of this base-pairing.

> **The DNA molecule is a double helix, the strands held together by base-pairing.**



(a)
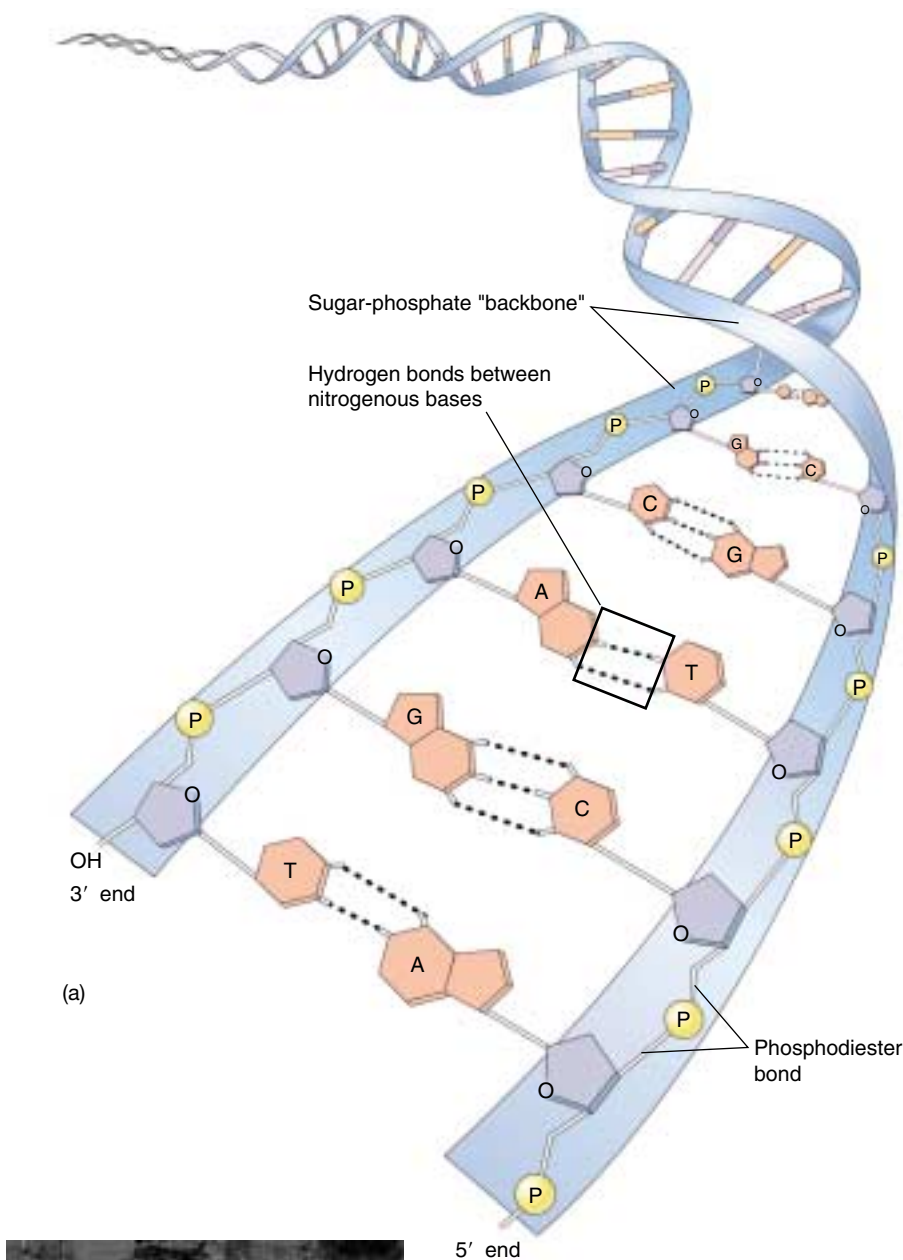
(b)

**FIGURE 14.10**
**DNA is a double helix.** (*a*) In a DNA duplex molecule, only two base-pairs are possible: adenine (A) can pair with thymine (T), and guanine (G) can pair with cytosine (C). An A-T base-pair has two hydrogen bonds, while a G-C base-pair has three. (*b*) James Watson (*far left*), and Francis Crick (*left*) deduced the structure of DNA in 1953 from Chargaff's rules and Franklin's diffraction studies.

## The Meselson–Stahl Experiment: DNA Replication Is Semiconservative

The Watson–Crick model immediately suggested that the basis for copying the genetic information is **complementarity.** One chain of the DNA molecule may have any conceivable base sequence, but this sequence completely determines the sequence of its partner in the duplex. For example, if the sequence of one chain is 5'-ATTGCAT-3', the sequence of its partner *must* be 3'-TAACGTA-5'. Thus, each chain in the duplex is a complement of the other.

The complementarity of the DNA duplex provides a ready means of accurately duplicating the molecule. If one were to "unzip" the molecule, one would need only to assemble the appropriate complementary nucleotides on the exposed single strands to form two daughter duplexes with the same sequence. This form of DNA replication is called **semiconservative,** because while the sequence of the original duplex is conserved after one round of replication, the duplex itself is not. Instead, each strand of the duplex becomes part of another duplex.

Two other hypotheses of gene replication were also proposed. The conservative model stated that the parental double helix would remain intact and generate DNA copies consisting of entirely new molecules. The dispersive model predicted that parental DNA would become dispersed throughout the new copy so that each strand of all the daughter molecules would be a mixture of old and new DNA.

The three hypotheses of DNA replication were evaluated in 1958 by Matthew Meselson and Franklin Stahl of the California Institute of Technology. These two scientists grew bacteria in a medium containing the heavy isotope of nitrogen, $^{15}$N, which became incorporated into the bases of the bacterial DNA. After several generations, the DNA of these bacteria was denser than that of bacteria grown in a medium containing the lighter isotope of nitrogen, $^{14}$N. Meselson and Stahl then transferred the bacteria from the $^{15}$N medium to the $^{14}$N medium and collected the DNA at various intervals.

By dissolving the DNA they had collected in a heavy salt called cesium chloride and then spinning the solution at very high speeds in an ultracentrifuge, Meselson and Stahl were able to separate DNA strands of different densities. The enormous centrifugal forces generated by the ultracentrifuge caused the cesium ions to migrate toward the bottom of the centrifuge tube, creating a gradient of cesium concentration, and thus of density. Each DNA strand floats or sinks in the gradient until it reaches the position where its density exactly matches the density of the cesium there. Because $^{15}$N strands are denser than $^{14}$N
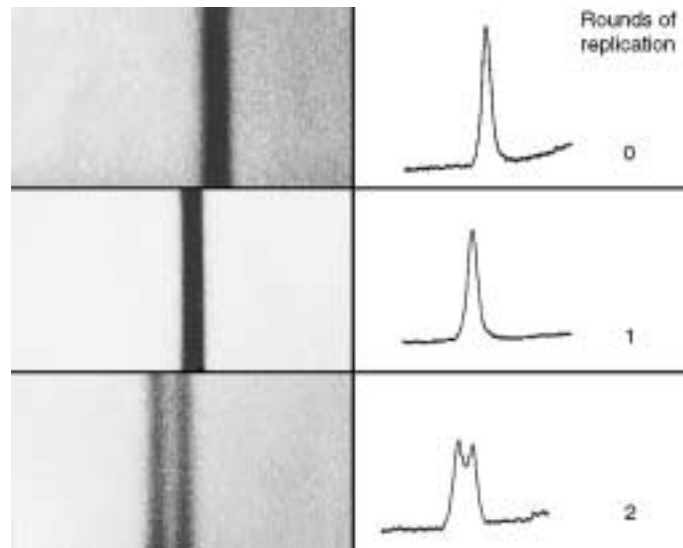


**FIGURE 14.11**
**The key result of the Meselson and Stahl experiment.** These bands of DNA, photographed on the left and scanned on the right, are from the density-gradient centrifugation experiment of Meselson and Stahl. At 0 generation, all DNA is heavy; after one replication all DNA has a hybrid density; after two replications, all DNA is hybrid or light.

strands, they migrate farther down the tube to a denser region of the cesium gradient.

The DNA collected immediately after the transfer was all dense. However, after the bacteria completed their first round of DNA replication in the $^{14}$N medium, the density of their DNA had decreased to a value intermediate between $^{14}$N-DNA and $^{15}$N-DNA. After the second round of replication, two density classes of DNA were observed, one intermediate and one equal to that of $^{14}$N-DNA (figure 14.11).

Meselson and Stahl interpreted their results as follows: after the first round of replication, each daughter DNA duplex was a hybrid possessing one of the heavy strands of the parent molecule and one light strand; when this hybrid duplex replicated, it contributed one heavy strand to form another hybrid duplex and one light strand to form a light duplex (figure 14.12). Thus, this experiment clearly confirmed the prediction of the Watson-Crick model that DNA replicates in a semiconservative manner.

**The basis for the great accuracy of DNA replication is complementarity. A DNA molecule is a duplex, containing two strands that are complementary mirror images of each other, so either one can be used as a template to reconstruct the other.**
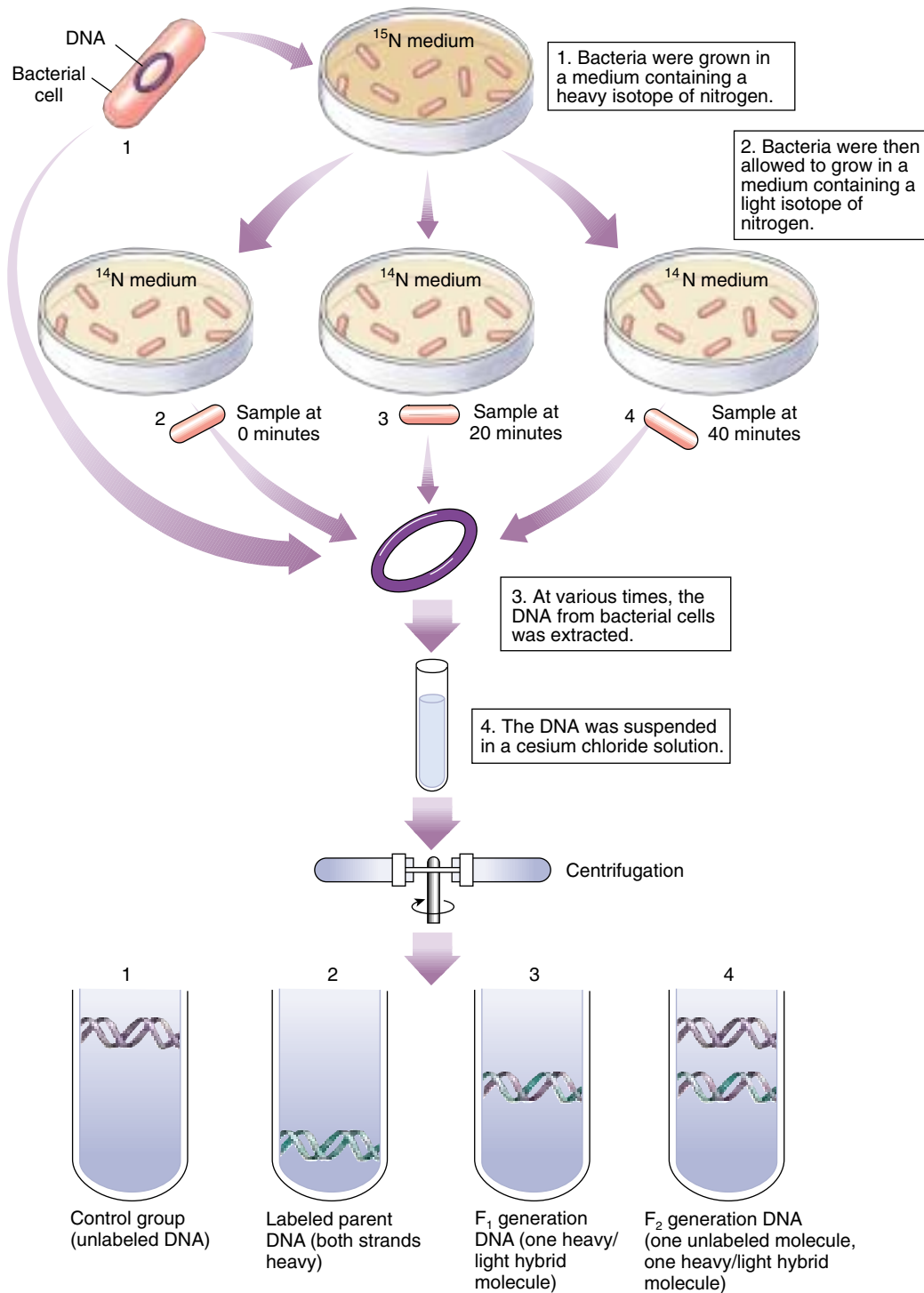
**FIGURE 14.12**

**The Meselson and Stahl experiment: evidence demonstrating semiconservative replication.** Bacterial cells were grown for several generations in a medium containing a heavy isotope of nitrogen ($^{15}N$) and then were transferred to a new medium containing the normal lighter isotope ($^{14}N$). At various times thereafter, samples of the bacteria were collected, and their DNA was dissolved in a solution of cesium chloride, which was spun rapidly in a centrifuge. Because the cesium ion is so massive, it tends to settle toward the bottom of the spinning tube, establishing a gradient of cesium density. DNA molecules sink in the gradient until they reach a place where their density equals that of the cesium; they then "float" at that position. DNA containing $^{15}N$ is denser than that containing $^{14}N$, so it sinks to a lower position in the cesium gradient. After one generation in $^{14}N$ medium, the bacteria yielded a single band of DNA with a density between that of $^{14}N$-DNA and $^{15}N$-DNA, indicating that only one strand of each duplex contained $^{15}N$. After two generations in $^{14}N$ medium, two bands were obtained; one of intermediate density (in which one of the strands contained $^{15}N$) and one of low density (in which neither strand contained $^{15}N$). Meselson and Stahl concluded that replication of the DNA duplex involves building new molecules by separating strands and assembling new partners on each of these templates.

# The Replication Process

To be effective, DNA replication must be fast and accurate. The machinery responsible has been the subject of intensive study for 40 years, and we now know a great deal about it. The replication of DNA begins at one or more sites on the DNA molecule where there is a specific sequence of nucleotides called a **replication origin** (figure 14.13). There the DNA replicating enzyme **DNA polymerase III** and other enzymes begin a complex process that catalyzes the addition of nucleotides to the growing complementary strands of DNA (figure 14.14). Table 14.2 lists the proteins involved in DNA replication in bacteria. Before considering the replication process in detail, let's take a closer look at DNA polymerase III.

## DNA Polymerase III

The first DNA polymerase enzyme to be characterized, DNA polymerase I of the bacterium *Escherichia coli*, is a relatively small enzyme that plays a key supporting role in
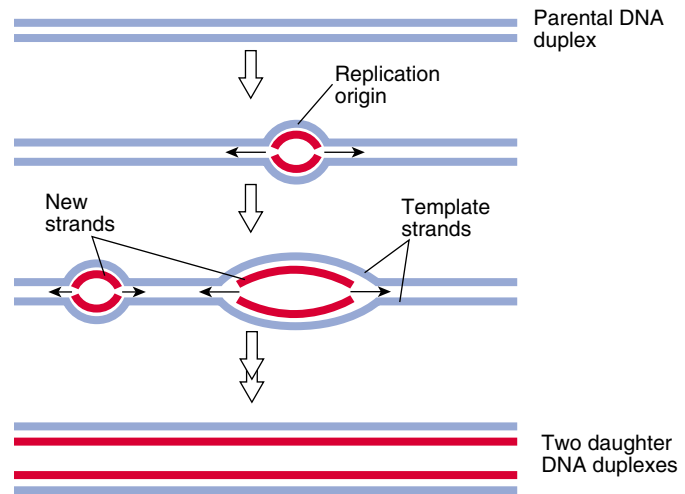


**FIGURE 14.13**
**Origins of replication.** At a site called the replication origin, the DNA duplex opens to create two separate strands, each of which can be used as a template for a new strand. Eukaryotic DNA has multiple origins of replication.
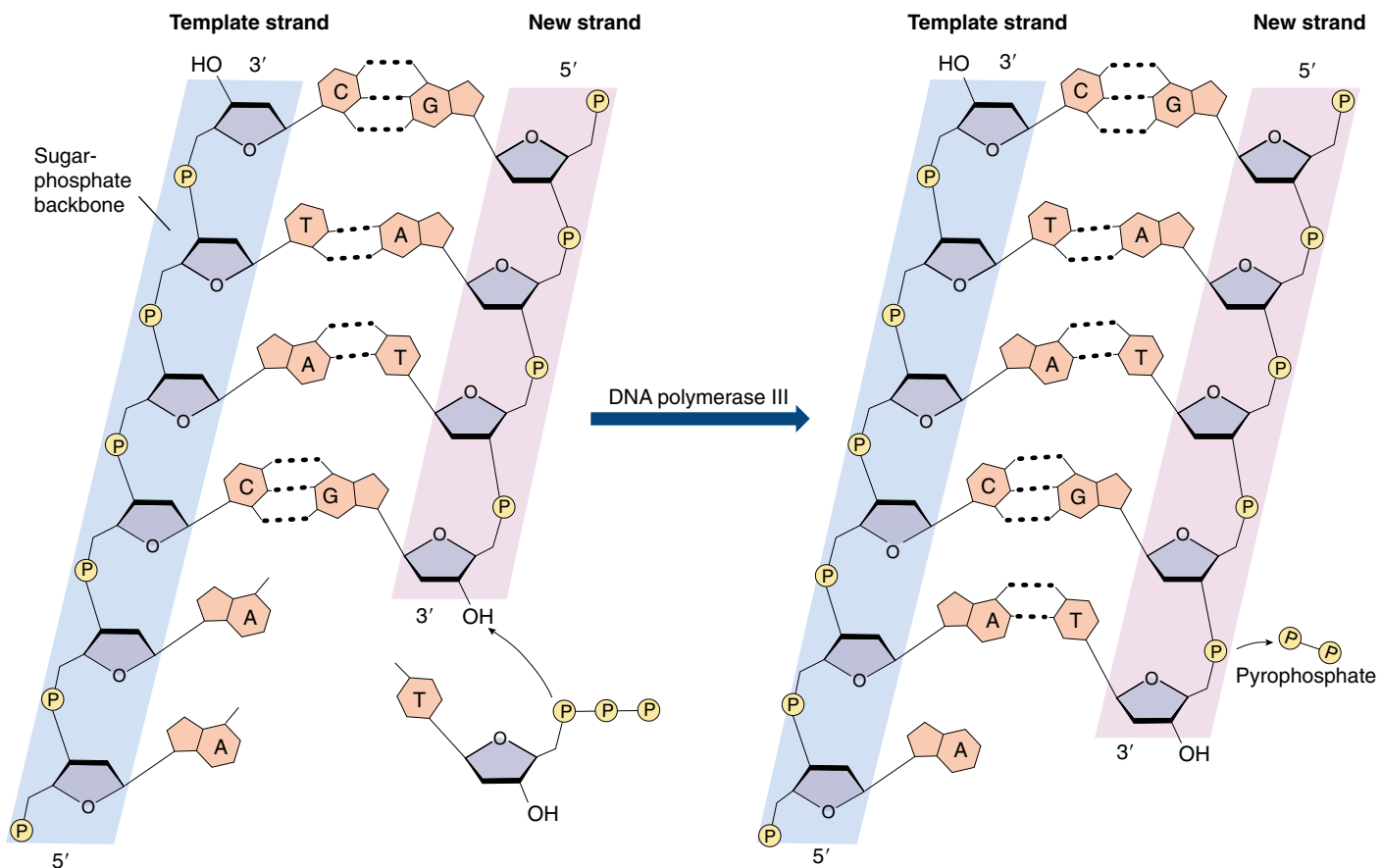


**FIGURE 14.14**
**How nucleotides are added in DNA replication.** DNA polymerase III, along with other enzymes, catalyzes the addition of nucleotides to the growing complementary strand of DNA. When a nucleotide is added, two of its phosphates are lost as pyrophosphate.

DNA replication. The true *E. coli* replicating enzyme, dubbed DNA polymerase III, is some 10 times larger and far more complex in structure. We know more about DNA polymerase III than any other organism's DNA polymerase, and so will describe it in detail here. Other DNA polymerases are thought to be broadly similar.

DNA polymerase III contains 10 different kinds of polypeptide chains, as illustrated in figure 14.15. The enzyme is a dimer, with two similar multisubunit complexes. Each complex catalyzes the replication of one DNA strand.

A variety of different proteins play key roles within each complex. The subunits include a single large catalytic $\alpha$ subunit that catalyzes 5′ to 3′ addition of nucleotides to a growing chain, a smaller $\epsilon$ subunit that proofreads 3′ to 5′ for mistakes, and a ring-shaped $\beta_2$ dimer subunit that clamps the polymerase III complex around the DNA double helix. Polymerase III progressively threads the DNA through the enzyme complex, moving it at a rapid rate, some 1000 nucleotides per second (100 full turns of the helix, 0.34 micrometers).

| Table 14.2 | DNA Replication Proteins of *E. coli* | | |
|---|---|---|---|
| **Protein** | **Role** | **Size (kd)** | **Molecules per Cell** |
| Helicase | Unwinds the double helix | 300 | 20 |
| Primase | Synthesizes RNA primers | 60 | 50 |
| Single-strand binding protein | Stabilizes single-stranded regions | 74 | 300 |
| DNA gyrase | Relieves torque | 400 | 250 |
| DNA polymerase III | Synthesizes DNA | ≈900 | 20 |
| DNA polymerase I | Erases primer and fills gaps | 103 | 300 |
| DNA ligase | Joins the ends of DNA segments | 74 | 300 |

**FIGURE 14.15**
**The DNA polymerase III complex.** (*a*) The complex contains 10 kinds of protein chains. The protein is a dimer because both strands of the DNA duplex must be replicated simultaneously. The catalytic ($\alpha$) subunits, the proofreading ($\epsilon$) subunits, and the "sliding clamp" ($\beta_2$) subunits (*yellow* and *blue*) are labeled. (*b*) The "sliding clamp" units encircle the DNA template and (*c*) move it through the catalytic subunit like a rope drawn through a ring.
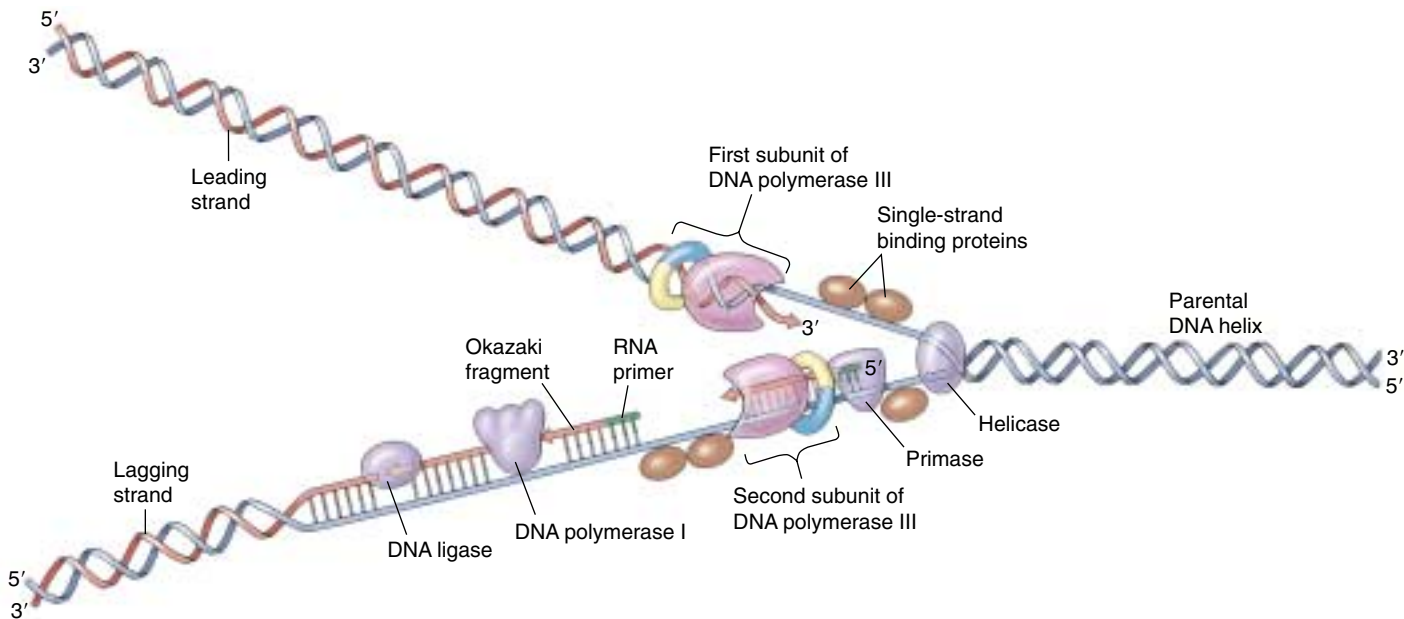
(a)

(b)

(c)
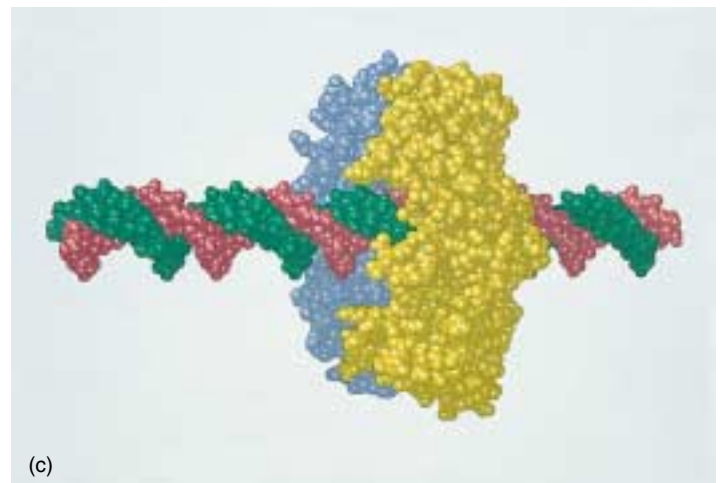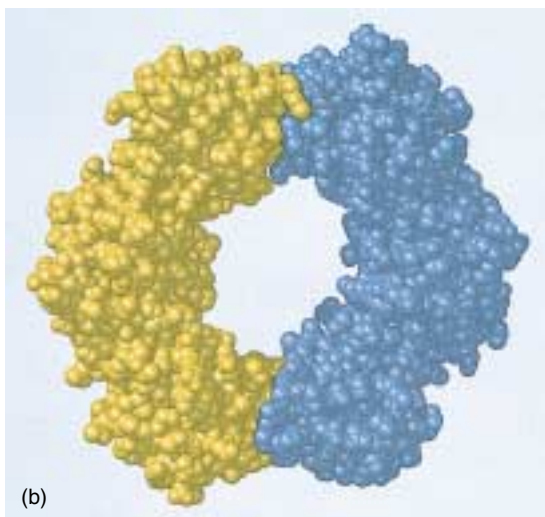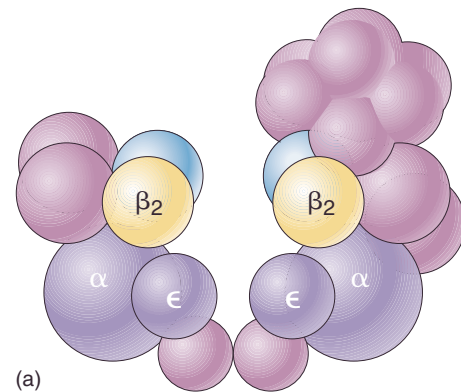
**FIGURE 14.16**
**A DNA replication fork.** Helicase enzymes separate the strands of the double helix, and single-strand binding proteins stabilize the single-stranded regions. Replication occurs by two mechanisms. (*1*) *Continuous synthesis:* After primase adds a short RNA primer, DNA polymerase III adds nucleotides to the 3′ end of the leading strand. DNA polymerase I then replaces the RNA primer with DNA nucleotides. (*2*) *Discontinuous synthesis:* Primase adds a short RNA primer (*green*) ahead of the 5′ end of the lagging strand. DNA polymerase III then adds nucleotides to the primer until the gap is filled in. DNA polymerase I replaces the primer with DNA nucleotides, and DNA ligase attaches the short segment of nucleotides to the lagging strand.

## The Need for a Primer

One of the features of DNA polymerase III is that it can add nucleotides only to a chain of nucleotides that is already paired with the parent strands. Hence, DNA polymerase cannot link the first nucleotides in a newly synthesized strand. Instead, another enzyme, an RNA polymerase called **primase,** constructs an **RNA primer,** a sequence of about 10 RNA nucleotides complementary to the parent DNA template. DNA polymerase III recognizes the primer and adds DNA nucleotides to it to construct the new DNA strands. The RNA nucleotides in the primers are then replaced by DNA nucleotides.

## The Two Strands of DNA Are Assembled in Different Ways

Another feature of DNA polymerase III is that it can add nucleotides only to the 3′ end of a DNA strand (the end with an —OH group attached to a 3′ carbon atom). This means that replication always proceeds in the 5′ → 3′ direction on a growing DNA strand. Because the two parent strands of a DNA molecule are antiparallel, *the new strands are oriented in opposite directions* along the parent templates at each replication fork (figure 14.16). Therefore, the new strands must be elongated by different mechanisms! The **leading strand,** which elongates *toward* the replication fork, is built up simply by adding nucleotides continuously

to its growing 3′ end. In contrast, the **lagging strand,** which elongates *away from* the replication fork, is synthesized discontinuously as a series of short segments that are later connected. These segments, called **Okazaki fragments,** are about 100 to 200 nucleotides long in eukaryotes and 1000 to 2000 nucleotides long in prokaryotes. Each Okazaki fragment is synthesized by DNA polymerase III in the 5′ → 3′ direction, beginning at the replication fork and moving away from it. When the polymerase reaches the 5′ end of the lagging strand, another enzyme, **DNA ligase,** attaches the fragment to the lagging strand. The DNA is further unwound, new RNA primers are constructed, and DNA polymerase III then jumps ahead 1000 to 2000 nucleotides (toward the replication fork) to begin constructing another Okazaki fragment. If one looks carefully at electron micrographs showing DNA replication in progress, one can sometimes see that one of the parent strands near the replication fork appears single-stranded over a distance of about 1000 nucleotides. Because the synthesis of the leading strand is continuous, while that of the lagging strand is discontinuous, the overall replication of DNA is said to be **semidiscontinuous.**

## The Replication Process

The replication of the DNA double helix is a complex process that has taken decades of research to understand. It takes place in five interlocking steps:
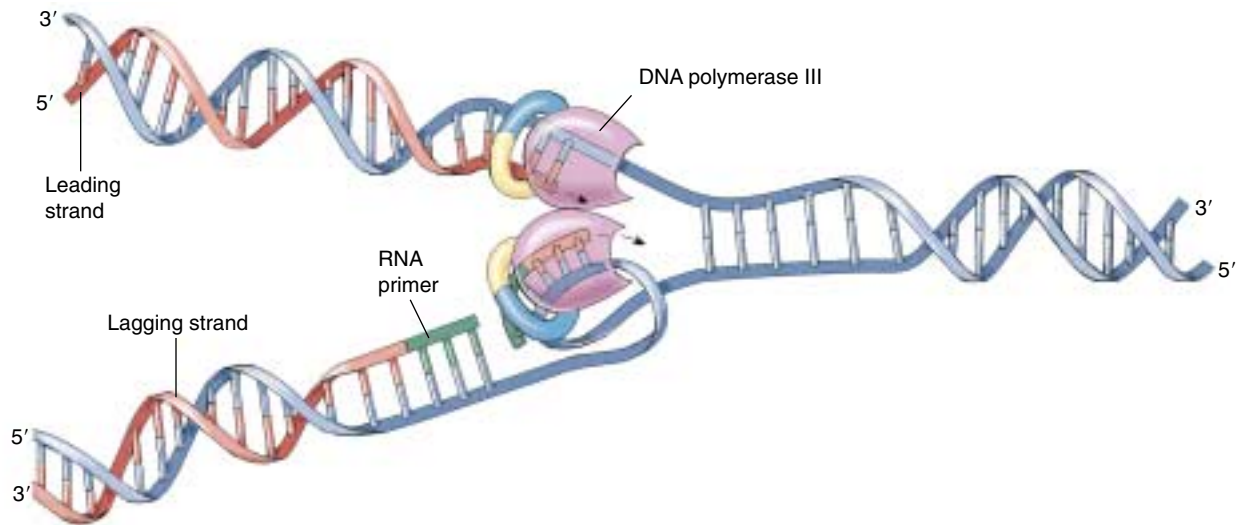
**FIGURE 14.17**
**How DNA polymerase III works.** This diagram presents a current view of how DNA polymerase III works. Note that the DNA on the lagging strand is folded to allow the dimeric DNA polymerase III molecule to replicate both strands of the parental DNA duplex simultaneously. This brings the 3′ end of each completed Okazaki fragment close to the start site for the next fragment.

1. **Opening up the DNA double helix.** The very stable DNA double helix must be opened up and its strands separated from each other for semiconservative replication to occur.

   *Stage one: Initiating replication.* The binding of **initiator proteins** to the replication origin starts an intricate series of interactions that opens the helix.

   *Stage two: Unwinding the duplex.* After initiation, "unwinding" enzymes called **helicases** bind to and move along one strand, shouldering aside the other strand as they go.

   *Stage three: Stabilizing the single strands.* The unwound portion of the DNA double helix is stabilized by **single-strand binding protein,** which binds to the exposed single strands, protecting them from cleavage and preventing them from rewinding.

   *Stage four: Relieving the torque generated by unwinding.* For replication to proceed at 1000 nucleotides per second, the parental helix ahead of the replication fork must rotate 100 revolutions per second! To relieve the resulting twisting, called torque, enzymes known as topisomerases—or, more informally, **gyrases**—cleave a strand of the helix, allow it to swivel around the intact strand, and then reseal the broken strand.

2. **Building a primer.** New DNA cannot be synthesized on the exposed templates until a primer is constructed, as DNA polymerases require 3′ primers to initiate replication. The necessary primer is a short stretch of RNA, added by a specialized RNA polymerase called *primase* in a multisubunit complex in-

formally called a *primosome.* Why an RNA primer, rather than DNA? Starting chains on exposed templates introduces many errors; RNA marks this initial stretch as "temporary," making this error-prone stretch easy to excise later.

3. **Assembling complementary strands.** Next, the dimeric DNA polymerase III then binds to the replication fork. While the leading strand complexes with one half of the polymerase dimer, the lagging strand is thought to loop around and complex with the other half of the polymerase dimer (figure 14.17). Moving in concert down the parental double helix, DNA polymerase III catalyzes the formation of complementary sequences on each of the two single strands at the same time.

4. **Removing the primer.** The enzyme DNA polymerase I now removes the RNA primer and fills in the gap, as well as any gaps between Okazaki fragments.

5. **Joining the Okazaki fragments.** After any gaps between Okazaki fragments are filled in, the enzyme DNA ligase joins the fragments to the lagging strand.

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**DNA replication involves many different proteins that open and unwind the DNA double helix, stabilize the single strands, synthesize RNA primers, assemble new complementary strands on each exposed parental strand—one of them discontinuously—remove the RNA primer, and join new discontinuous segments on the lagging strand.**
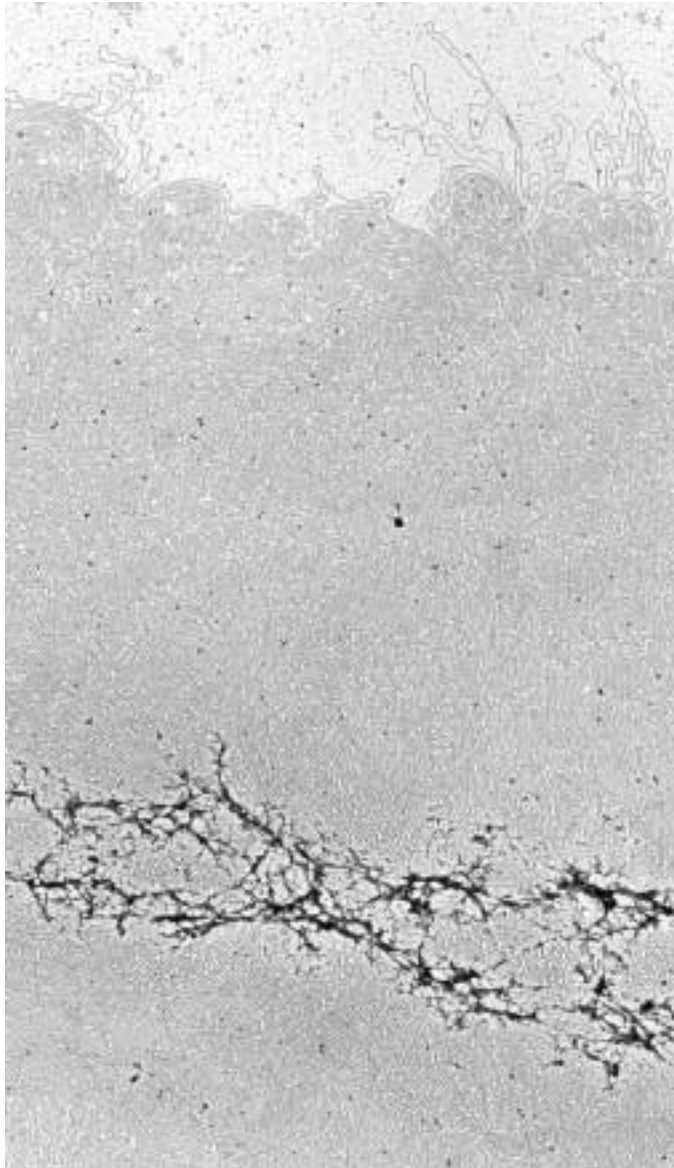
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**FIGURE 14.18**
**DNA of a single human chromosome.** This chromosome has been "exploded," or relieved, of most of its packaging proteins. The residual protein scaffolding appears as the dark material in the lower part of the micrograph.

# Eukaryotic DNA Replication

In eukaryotic cells, the DNA is packaged in nucleosomes within chromosomes (figure 14.18). Each individual zone of a chromosome replicates as a discrete section called a **replication unit,** or **replicon,** which can vary in length from 10,000 to 1 million base-pairs; most are about 100,000 base-pairs long. Each replication unit has its own origin of replication, and multiple units may be undergoing replication at any given time, as can be seen in electron micrographs of replicating chromosomes (figure 14.19). Each unit replicates in a way fundamentally similar to prokaryotic DNA replication, using similar enzymes. The advantage of having multiple origins of replication in eukaryotes is speed: replication takes approximately eight hours in humans cells, but if there were only one origin, it would take 100 times longer. Regulation of the replication process ensures that only one copy of the DNA is ultimately produced. How a cell achieves this regulation is not yet completely clear. It may involve periodic inhibitor or initiator proteins on the DNA molecule itself.

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**Eukaryotic chromosomes have multiple origins of replication.**

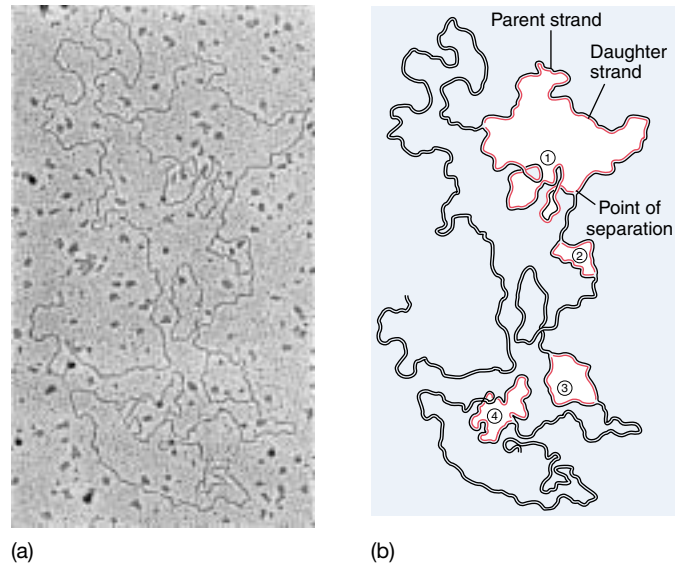---



(a)                                    (b)

**FIGURE 14.19**
**Eukaryotic chromosomes possess numerous replication forks spaced along their length.** Four replication units (each with two replication forks) are producing daughter strands (*a*) in this electron micrograph, as indicated in *red* in the (*b*) corresponding drawing.

## The One-Gene/One-Polypeptide Hypothesis

As the structure of DNA was being solved, other biologists continued to puzzle over how the genes of Mendel were related to DNA.

### Garrod: Inherited Disorders Can Involve Specific Enzymes

In 1902, a British physician, Archibald Garrod, was working with one of the early Mendelian geneticists, his countryman William Bateson, when he noted that certain diseases he encountered among his patients seemed to be more prevalent in particular families. By examining several generations of these families, he found that some of the diseases behaved as if they were the product of simple recessive alleles. Garrod concluded that these disorders were Mendelian traits and that they had resulted from changes in the hereditary information in an ancestor of the affected families.

Garrod investigated several of these disorders in detail. In alkaptonuria the patients produced urine that contained homogentisic acid (alkapton). This substance oxidized rapidly when exposed to air, turning the urine black. In normal individuals, homogentisic acid is broken down into simpler substances. With considerable insight, Garrod concluded that patients suffering from alkaptonuria lacked the enzyme necessary to catalyze this breakdown. He speculated that many other inherited diseases might also reflect enzyme deficiencies.

### Beadle and Tatum: Genes Specify Enzymes

From Garrod's finding, it took but a short leap of intuition to surmise that the information encoded within the DNA of chromosomes acts to specify particular enzymes. This point was not actually established, however, until 1941, when a series of experiments by Stanford University geneticists George Beadle and Edward Tatum provided definitive evidence on this point. Beadle and Tatum deliberately set out to create Mendelian mutations in chromosomes and then studied the effects of these mutations on the organism (figure 14.20).



**FIGURE 14.20**
**Beadle and Tatum's procedure for isolating nutritional mutants in *Neurospora*.** This fungus grows easily on an artificial medium in test tubes. In this experiment, spores were irradiated to increase the frequency of mutation; they were then placed on a "complete" medium that contained all of the nutrients necessary for growth. Once the fungal colonies were established on the complete medium, individual spores were transferred to a "minimal" medium that lacked various substances the fungus could normally manufacture. Any spore that would not grow on the minimal medium but would grow on the complete medium contained one or more mutations in genes needed to produce the missing nutrients. To determine which gene had mutated, the minimal medium was supplemented with particular substances. The mutation illustrated here produced an arginine mutant, a collection of cells that lost the ability to manufacture arginine. These cells will not grow on minimal medium but will grow on minimal medium with only arginine added.
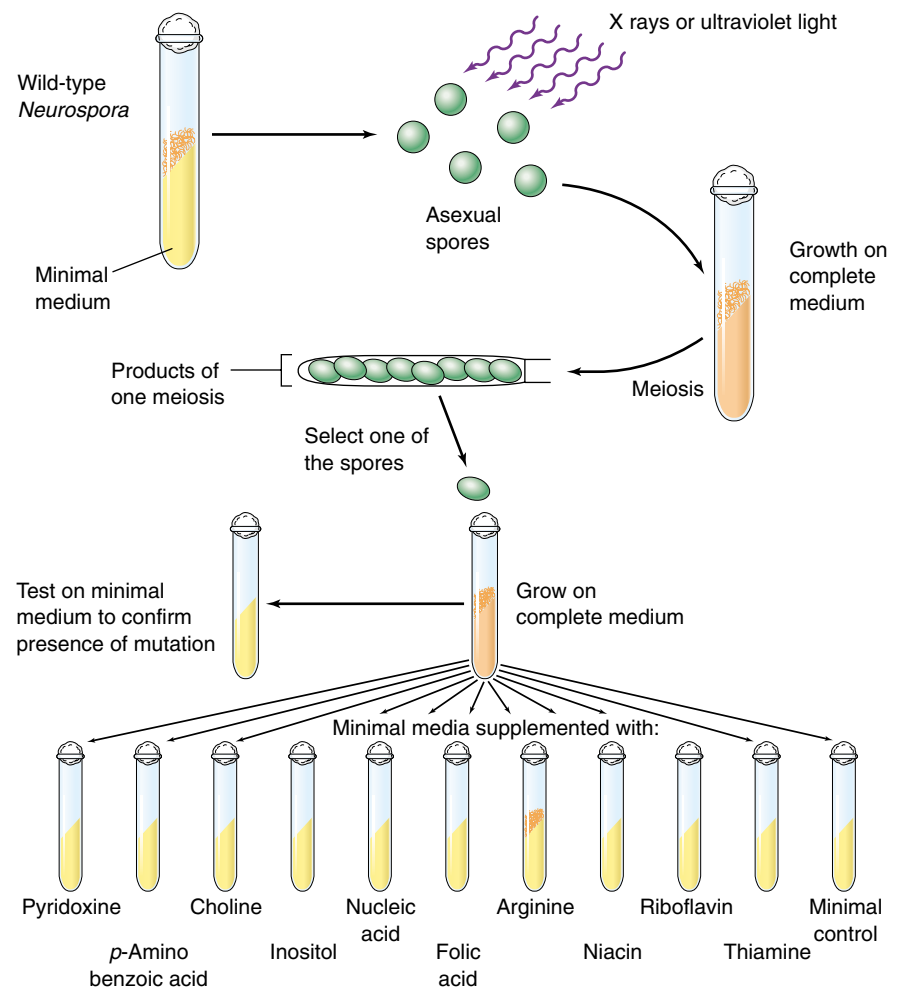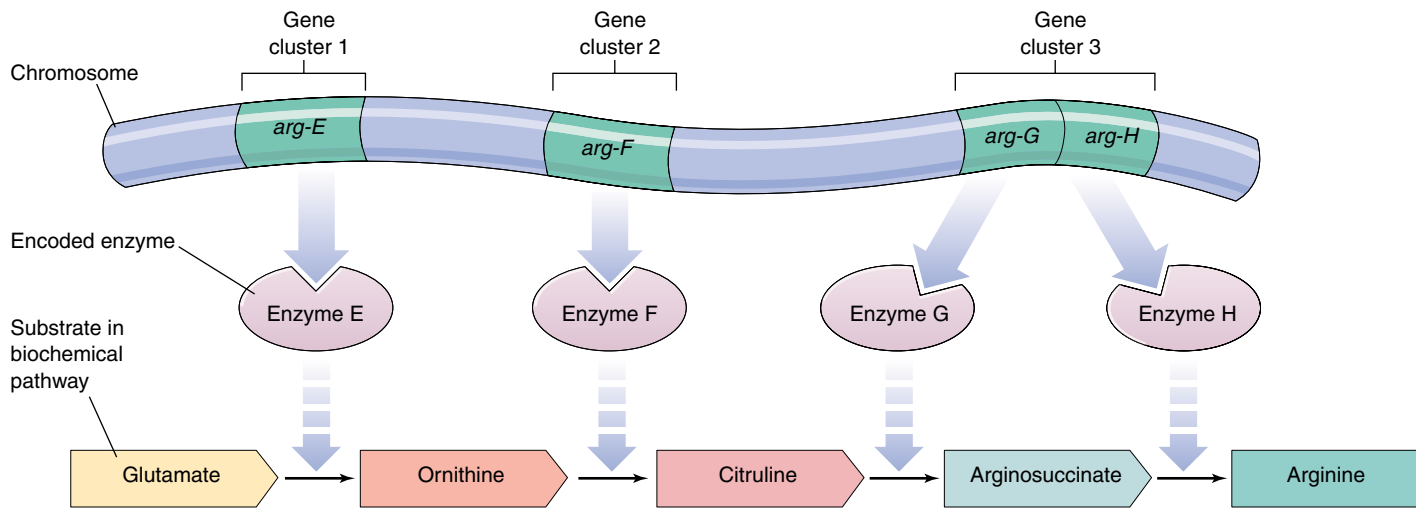
**FIGURE 14.21**

**Evidence for the "one-gene/one-polypeptide" hypothesis.** The chromosomal locations of the many arginine mutants isolated by Beadle and Tatum cluster around three locations. These locations correspond to the locations of the genes encoding the enzymes that carry out arginine biosynthesis.

**A Defined System.** One of the reasons Beadle and Tatum's experiments produced clear-cut results is that the researchers made an excellent choice of experimental organism. They chose the bread mold *Neurospora*, a fungus that can be grown readily in the laboratory on a defined medium (a medium that contains only known substances such as glucose and sodium chloride, rather than some uncharacterized mixture of substances such as ground-up yeasts). Beadle and Tatum exposed *Neurospora* spores to X rays, expecting that the DNA in some of the spores would experience damage in regions encoding the ability to make compounds needed for normal growth (see figure 14.20). DNA changes of this kind are called mutations, and organisms that have undergone such changes (in this case losing the ability to synthesize one or more compounds) are called mutants. Initially, they allowed the progeny of the irradiated spores to grow on a defined medium containing all of the nutrients necessary for growth, so that any growth-deficient mutants resulting from the irradiation would be kept alive.

**Isolating Growth-Deficient Mutants.** To determine whether any of the progeny of the irradiated spores had mutations causing metabolic deficiencies, Beadle and Tatum placed subcultures of individual fungal cells on a "minimal" medium that contained only sugar, ammonia, salts, a few vitamins, and water. Cells that had lost the ability to make other compounds necessary for growth would not survive on such a medium. Using this approach, Beadle and Tatum succeeded in identifying and isolating many growth-deficient mutants.

**Identifying the Deficiencies.** Next the researchers added various chemicals to the minimal medium in an attempt to find one that would enable a given mutant strain

to grow. This procedure allowed them to pinpoint the nature of the biochemical deficiency that strain had. The addition of arginine, for example, permitted several mutant strains, dubbed *arg* mutants, to grow. When their chromosomal positions were located, the *arg* mutations were found to cluster in three areas (figure 14.21).

## One-Gene/One-Polypeptide

For each enzyme in the arginine biosynthetic pathway, Beadle and Tatum were able to isolate a mutant strain with a defective form of that enzyme, and the mutation was always located at one of a few specific chromosomal sites. Most importantly, they found there was a different site for each enzyme. Thus, each of the mutants they examined had a defect in a single enzyme, caused by a mutation at a single site on one chromosome. Beadle and Tatum concluded that genes produce their effects by specifying the structure of enzymes and that each gene encodes the structure of one enzyme. They called this relationship the **one-gene/one-enzyme hypothesis.** Because many enzymes contain multiple protein or polypeptide subunits, each encoded by a separate gene, the relationship is today more commonly referred to as **"one-gene/one-polypeptide."**

Enzymes are responsible for catalyzing the synthesis of all the parts of an organism. They mediate the assembly of nucleic acids, proteins, carbohydrates, and lipids. Therefore, by encoding the structure of enzymes and other proteins, DNA specifies the structure of the organism itself.

Genetic traits are expressed largely as a result of the activities of enzymes. Organisms store hereditary information by encoding the structures of enzymes and other proteins in their DNA.

# How DNA Encodes Protein Structure

What kind of information must a gene encode to specify a protein? For some time, the answer to that question was not clear, as protein structure seemed impossibly complex.

## Sanger: Proteins Consist of Defined Sequences of Amino Acids

The picture changed in 1953, the same year in which Watson and Crick unraveled the structure of DNA. That year, the English biochemist Frederick Sanger, after many years of work, announced the complete sequence of amino acids in the protein insulin. Insulin, a small protein hormone, was the first protein for which the amino acid sequence was determined. Sanger's achievement was extremely significant because it demonstrated for the first time that proteins consisted of definable sequences of amino acids—for any given form of insulin, every molecule has the same amino acid sequence. Sanger's work soon led to the sequencing of many other proteins, and it became clear that all enzymes and other proteins are strings of amino acids arranged in a certain definite order. The information needed to specify a protein such as an enzyme, therefore, is an ordered list of amino acids.

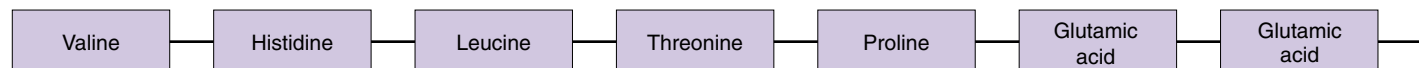## Ingram: Single Amino Acid Changes in a Protein Can Have Profound Effects

Following Sanger's pioneering work, Vernon Ingram in 1956 discovered the molecular basis of sickle cell anemia, a protein defect inherited as a Mendelian disorder. By analyzing the structures of normal and sickle cell hemoglobin, Ingram, working at Cambridge University, showed that sickle cell anemia is caused by a change from glutamic acid to valine at a single position in the protein (figure 14.22). The alleles of the gene encoding hemoglobin differed only in their specification of this one amino acid in the hemoglobin amino acid chain.

These experiments and other related ones have finally brought us to a clear understanding of the unit of heredity. The characteristics of sickle cell anemia and most other hereditary traits are defined by changes in protein structure brought about by an alteration in the sequence of amino acids that make up the protein. This sequence in turn is dictated by the order of nucleotides in a particular region of the chromosome. For example, the critical change leading to sickle cell disease is a mutation that replaces a single thymine with an adenine at the position that codes for glutamic acid, converting the position to valine. The sequence of nucleotides that determines the amino acid sequence of a protein is called a **gene**. Although most genes encode proteins or subunits of proteins, some genes are devoted to the production of special forms of RNA, many of which play important roles in protein synthesis themselves.

> A half-century of experimentation has made clear that DNA is the molecule responsible for the inheritance of traits, and that this molecule is divided into functional units called genes.
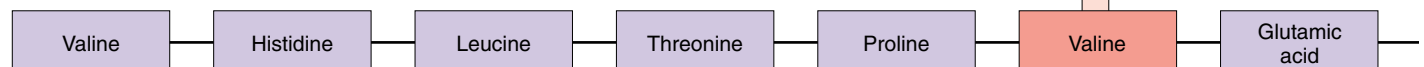


**FIGURE 14.22**
**The molecular basis of a hereditary disease.** Sickle cell anemia is produced by a recessive allele of the gene that encodes the hemoglobin β chains. It represents a change in a single amino acid, from glutamic acid to valine at the sixth position in the chains, which consequently alters the tertiary structure of the hemoglobin molecule, reducing its ability to carry oxygen.

Normal hemoglobin β chain

| Valine | Histidine | Leucine | Threonine | Proline | Glutamic acid | Glutamic acid |

Sickle cell anemia hemoglobin β chain

| Valine | Histidine | Leucine | Threonine | Proline | Valine | Glutamic acid |

| *Summary* | *Questions* | *Media Resources* |
|---|---|---|

**14.1   What is the genetic material?**

- Eukaryotic cells store hereditary information within the nucleus.

- In viruses, bacteria, and eukaryotes, the hereditary information resides in nucleic acids. The transfer of nucleic acids can lead to the transfer of hereditary traits.

- When radioactively labeled DNA viruses infect bacteria, the DNA but not the protein coat of the viruses enters the bacterial cells, indicating that the hereditary material is DNA rather than protein.

**1.** In Hammerling's experiments on *Acetabularia*, what happened when a stalk from *A. crenulata* was grafted to a foot from *A. mediterranea?*

**2.** How did Hershey and Chase determine which component of bacterial viruses contains the viruses' hereditary information?

- Experiment: Griffith/Hershey/ Chase-DNA is the Genetic Material

**14.2   What is the structure of DNA?**

- Chargaff showed that the proportion of adenine in DNA always equals that of thymine, and the proportion of guanine always equals that of cytosine.

- DNA has the structure of a double helix, consisting of two chains of nucleotides held together by hydrogen bonds between adenines and thymines, and between guanines and cytosines.

**3.** What is the three-dimensional shape of DNA, and how does this shape fit with Chargaff's observations on the proportions of purines and pyrimidines in DNA?

**4.** How did Meselson and Stahl show that DNA replication is semiconservative?

- DNA Structure
- DNA Packaging

- Nucleic Acid
- DNA Structure

- Experiment: Kornbert-Isolating DNA Poly merase

**14.3   How does DNA replicate?**

- During the S phase of the cell cycle, the hereditary message in DNA is replicated with great accuracy.

- During replication, the DNA duplex is unwound, and two new strands are assembled in opposite directions along the original strands. One strand elongates by the continuous addition of nucleotides to its growing end; the other is constructed by the addition of segments containing 100 to 2000 nucleotides, which are then joined to the end of that strand.

**5.** How is the leading strand of a DNA duplex replicated? How is the lagging strand replicated? What is the basis for the requirement that the leading and lagging strands be replicated by different mechanisms?

- DNA Replication

- DNA Replication

- Student Research: Microsatellites in Rabbits Experiment
  - Meselson-Stahl— DNA Replication is Semiconservative
  - Okazaki: DNA Synthesis is Discontinous

**14.4   What is a gene?**

- Most hereditary traits reflect the actions of enzymes.

- The traits are hereditary because the information necessary to specify the structure of the enzymes is stored within the DNA.

- Each enzyme is encoded by a specific region of the DNA called a gene.

**6.** What hypothesis did Beadle and Tatum test in their experiments on *Neurospora?* What did they do to change the DNA in individuals of this organism? How did they determine whether any of these changes affected enzymes in biosynthetic pathways?

- Scientists on Science: The Future of Molecular Biology
- Experiment: Ephrussi/Beadle/ Tatum—Genes Encode Enzymes