doi:10.1016/j.jmb.2003.11.015 J. Mol. Biol. (2004) 335, 3–26

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HISTORICAL PERSPECTIVE

The Discovery of the DNA Double Helix

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Introduction

Fifty years ago, on 25th April 1953, there appeared three papers in the journal, Nature, which changed our view of the world. The structure of the DNA double helix, with its complementary base-pairing, is one of the greatest discoveries in biology in the 20th Century. It was also most dramatic, since, quite unexpectedly, the structure itself pointed to the way in which a DNA molecule might replicate itself, and hence revealed the "secret of life". The structure was solved in the Cavendish Laboratory, Cambridge by Francis Crick and James Watson, using X-ray diffraction data from fibres of DNA obtained by Rosalind Franklin at King's College, London.

This article aims to tell the story of how this came to happen: the origin of the research on DNA, the early investigations by Maurice Wilkins at King's College, the sorting out of the two forms of DNA by Franklin, the wrong paths taken, the intervention of old rivalries from an earlier generation (Lawrence Bragg and Linus Pauling), and the final model-building by Watson and Crick to give the three dimensional structure.

I will also describe the initial, mostly hesitant, reception of the proposed structure, and its confirmation by biochemistry by Arthur Kornberg and by X-ray crystallography at King's College by Wilkins' group. Yet this remained a discovery in chemistry, until the biological principle of "semi-conservative" replication was proved by Messelson and Stahl in 1958.

The transforming principle

In 1945 The Royal Society of London awarded its highest honour, the Copley Medal, to Oswald Avery of the Rockefeller Institute of New York for "establishing the chemical nature of the transforming principle".

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The transforming principle was an extract by means of which a non-pathogenic mutant of the pneumococcus bacterium could be transformed into a pathogenic form. The President of the Society, Sir Henry Dale, commented "Here surely is a change to which, if we were dealing with higher organisms, we should accord the status of a genetic variation, and the substance inducing it—the gene, one is tempted to call it—appears to be a nucleic acid of the desoxyribose type. Whatever it be, it is something which should be capable of complete description in terms of structural chemistry". The hesitation about "gene" reflects the belief then held by some biochemists and biologists that bacteria did not possess genes.

Eight years later the President's challenge was answered: there was a complete description of the 3D structure of DNA—what a chemist would call its configuration—the double helix by Watson and Crick (Figure 1), using X-ray diffraction data from Franklin and Wilkins. This paper aims to describe how this came about. Much of the story has been told in parts, but Franklin's scientific work has never been fully described, and I have therefore drawn on her notebooks, now in the Archives of Churchill College, Cambridge, to document it.

We begin with chemical structure of DNA, that is, how the links in phosphate–deoxyribose sugar backbone are made and how the heterocyclic nitrogenous bases are connected to the sugars. This had been worked out only two years earlier by Brown and Todd in Cambridge (Figure 2).

The structure of DNA

Since the structure of DNA is so well known there is little point in keeping it to the end as a dénouement of the story.

The double helix (Figure 3) consists of two intertwined helical phosphate-sugar backbones, with the heterocyclic DNA bases projecting inwards from each of the two strands. The two chains are antiparallel, running in opposite directions, and are related by a 2-fold axis of symmetry (dyad)

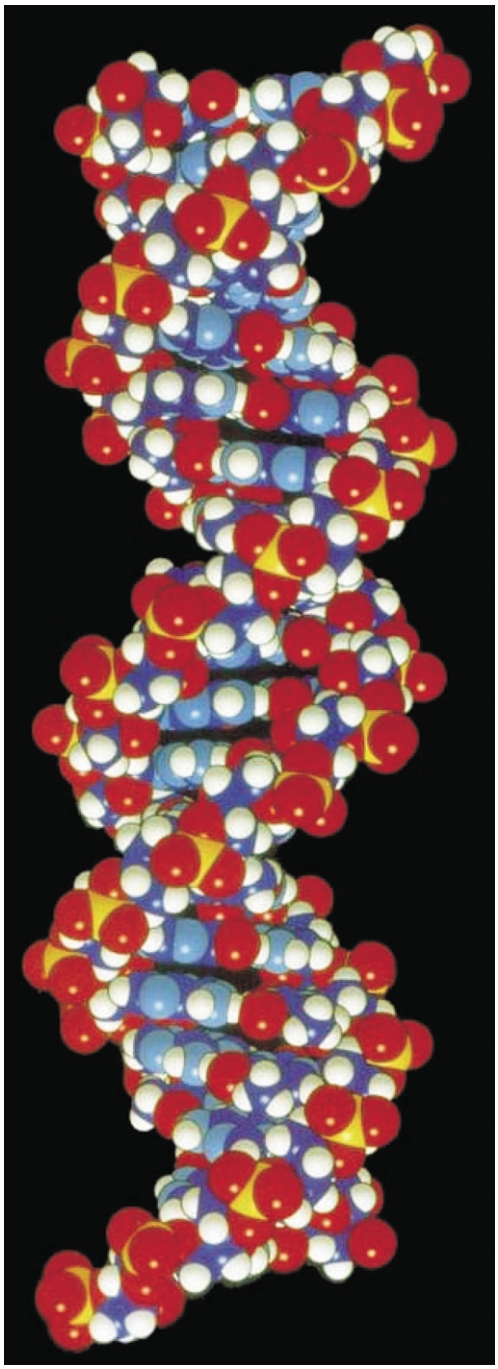


Figure 1. Space-filling atomic model of the DNA double helix. Colouring: phosphorus yellow; oxygen red; carbon dark blue; nitrogen light blue; hydrogen white.

perpendicular to the axis of the double helix. The bases are arranged in purine-pyrimidine pairs, adenine with thymine, guanine with cytosine, linked by hydrogen bonds (Figure 4), and these base-pairs are stacked on top of each other along the helix axis at a distance of 3.4 Å apart. The glycosidic bonds (the links between sugar and base) are related by the perpendicular dyad, so that they occur in identical orientations with respect to the helix axis. The two glycosidic bonds of a pair will not only be the same distance apart for all pairs, but can be fitted into the structure either way round. This feature allows all four bases to occur on both chains, and so any sequence of bases can fit into the double helix.

The two chains are said to bear a complementary relationship to each other. This means, as Crick and Watson spelt out in their second paper in Nature in May 1953, that when the two chains come apart during replication of DNA, each can be used as a template to assemble a duplicate of its former partner (Figure 5). The crucial feature of the structure of DNA is not therefore the actual double helical form of the two phosphate-sugar chains—eye-catching as it is—but the unique pairing of the bases projecting from each strand.

Structural research on DNA

In 1945, most biochemists, had doubts whether something as simple as what DNA was thought to be—repeats of the four nucleotide bases—could be the genetic substance. More complex molecules like proteins—chromosomal proteins—were thought to be more likely candidates.

There were some who did believe in DNA, in particular, the "phage group" in the USA led by Max Delbrück and Salvador Luria. This group, mostly geneticists, studied bacterial viruses, bacteriophages. A younger member of that group was James Watson, who in October 1950 went to Copenhagen to learn nucleic acid chemistry, but was converted to a structural approach by hearing Maurice Wilkins speak at a Conference on Large Molecules in Naples in May 1951. Wilkins described his X-ray diffraction studies on fibres of DNA and showed a diffraction pattern with much more detail than had been obtained by earlier workers, Astbury and Bell in 1938. Moreover, they indicated a degree of crystallinity which raised the possibility of a molecular interpretation by X-ray analysis.

Watson therefore decided to go to a laboratory where he might learn X-ray diffraction techniques, and, failing to interest Wilkins, he eventually moved his fellowship to the MRC Unit in Cambridge headed by Max Perutz. Here the structures of the proteins haemoglobin and myoglobin were being tackled. Watson arrived in September 1951 and met Francis Crick, who was working for his PhD on haemoglobin under Max Perutz, and found him like-minded about the importance of DNA.

A preamble on X-ray diffraction by crystals and by fibres

X-ray crystallography provides a way of deducing the structure of a molecule by analysing the diffraction pattern produced when a beam of X-rays falls on a crystal in which the molecules

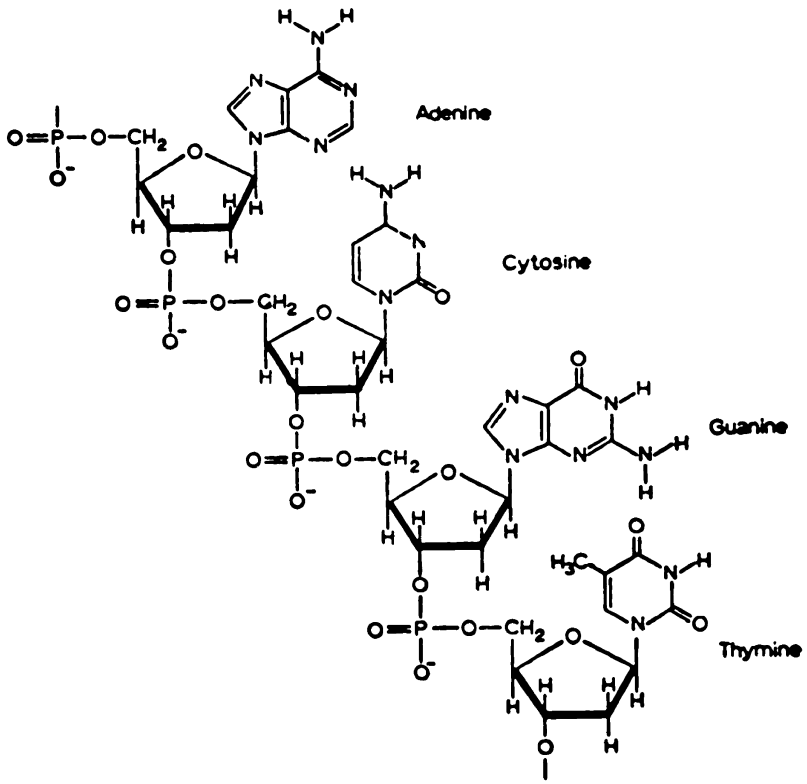
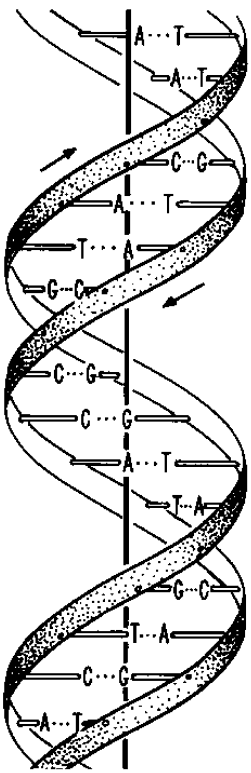


Figure 2. The chemical formula of a chain of a DNA molecule (DM Brown and AR Todd, 1952 J. Chem. Soc. p. 52). The backbone is made up of alternating sugar (2-deoxyribose) and phosphate groups. Each sugar has attached to it a side-group by a glycosidic linkage. The side groups consist of either a purine base (adenine or guanine) or a pyrimidine base (cytosine or thymine). Note that the backbone has a directionality because the phosphate group is linked differently to the sugars on either side (to the 3′ carbon atom of one sugar and to the 5′ carbon atom of the other). A phosphate-sugar linked to a base is called a nucleotide. The DNA chain is synthesised from such nucleotides in the 5′–3′ direction.



A schematic illustration of the double helix.

Figure 3. Schematic illustration of the DNA double helix as later sketched by James Watson ("The Double Helix", 1968).

are regularly arranged in three dimensions (Figure 6). The pattern is nothing like a conventional photograph: it shows a set of spots of varying intensity and inferring the structure from the pattern is not a direct process. This is because each spot corresponds to a diffracted wave from the molecules lying in a particular set of planes in the crystal. The molecular structure of the crystal could be reconstructed mathematically from a knowledge of the amplitudes and phases of the diffracted waves—amplitude means strength of the wave (which is measurable from the spot intensity); and phase means the positions of the peaks and troughs of the wave relative to some reference point, but the phase is lost in the recording. Hence arises the so called phase problem in X-ray crystallography which is to develop methods for determining indirectly these lost phases. For small molecules, analytical methods have been developed, and for large molecules like proteins the problem was solved in 1953 by Max Perutz by his implementation of the heavy atom isomorphous replacement method.

Fibrous macromolecules—polymers of small units, the monomers, regularly (or "equivalently") arranged—present a further challenge in X-ray diffraction, since in fibres, the long molecules, though roughly parallel to one another, are usually not all rotationally oriented relative to one another in a regular manner. The observed diffraction pattern then represents the rotational average of the patterns that would be given by different orientations. If the chemical structure of the monomer is known, as was, for example, the case of rubber or cellulose,

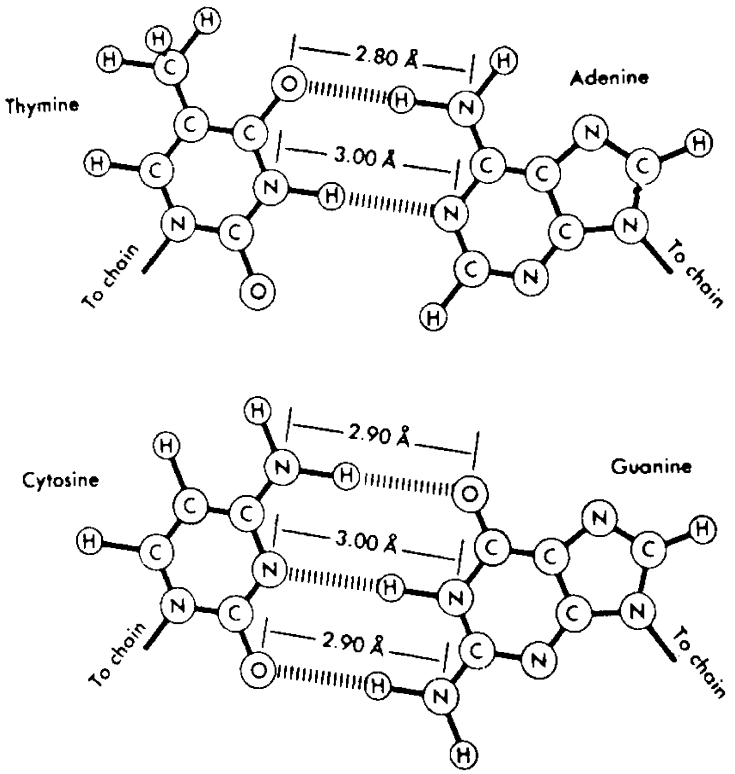


Figure 4. The pairing of bases by hydrogen bonds: adenine with thymine, guanine with cytosine (Crick and Watson, 1954 Proc., Roy. Soc. A **223** 80–96, who showed only two hydrogen bonds for the G:C pair, though tentatively suggesting a third, later confirmed by Pauling).

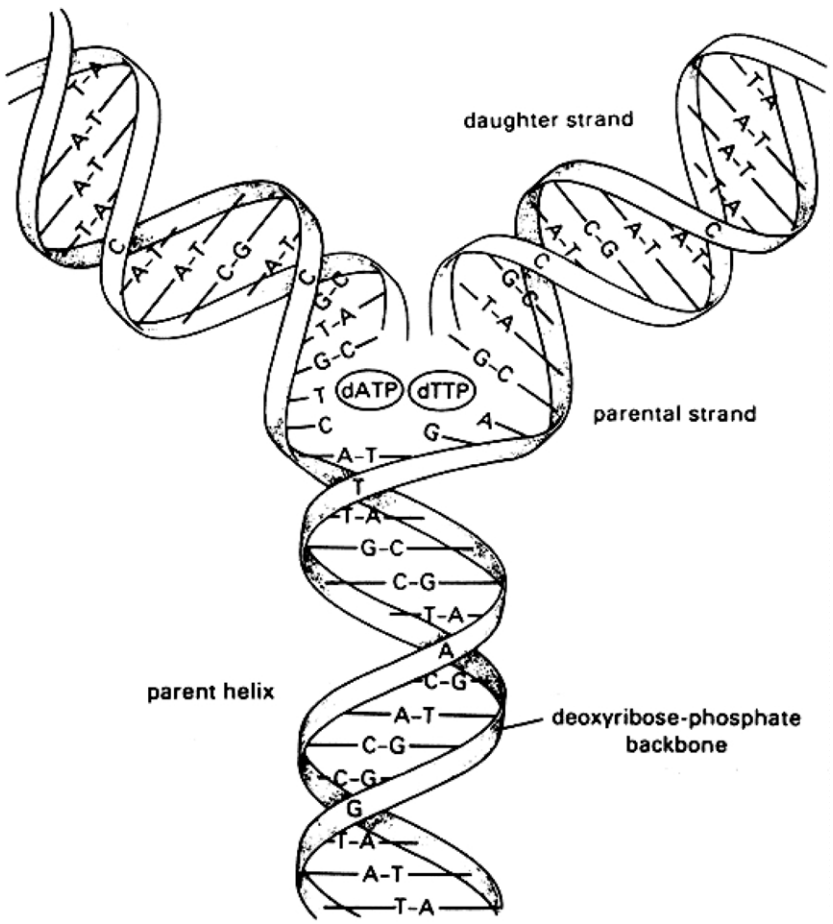


Figure 5. Principle of replication of the double helix. The helix unzips and each chain acts as a template for the synthesis of a complementary chain, thus creating two double helices, which are identical copies of the first. DATP, dTTP, adenine and thymine nucleotide triphosphates, (energy-rich) monomers being incorporated at the next step in the growing chains.

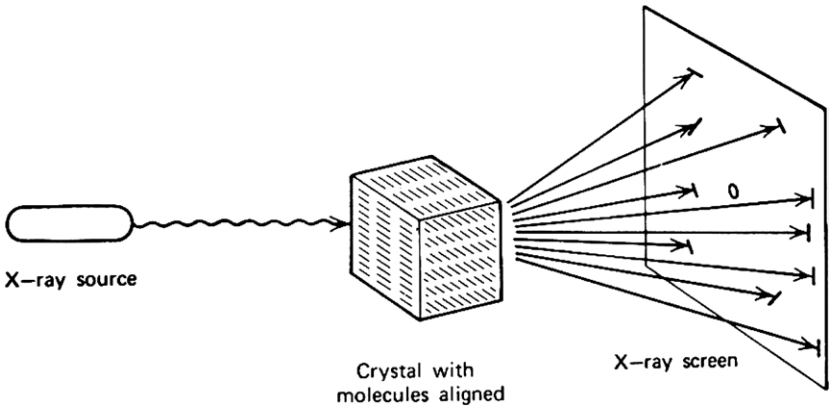


Figure 6. Schematic diagram of X-ray diffraction by a crystal containing a regular 3D arrangement of molecules. The pattern of diffracted waves depends on the particular setting of the crystal relative to the incident X-ray beam. A full 3D set of X-ray data is collected by rotating the crystal into different settings.

then the polymer structure can be solved, by building models and comparing the calculated diffraction patterns with the observed ones. This is the model building approach, which was used by Watson and Crick for DNA. The problem is that there is rotation about the single chemical bonds between monomers (and also usually within them), so other constraints must be used to fix how the monomers join head to tail.

King's College, London

Wilkins was a senior member of the MRC Biophysics Unit at King's College, London, set up by (Sir) John Randall in 1946 after the War to carry out "an interdisciplinary attack on the secrets of chromosomes and their environment". Wilkins worked to develop special microscopes, but having heard of the greatly improved methods devised by Rudolf Signer at Berne for extracting long unbroken molecules of DNA, he obtained some of the material and found a way of drawing uniform fibres from a viscous solution of DNA (Figure 7). Examination under polarized light showed them to be well ordered, characteristic of long molecules oriented parallel to one another. He enlisted the help of a graduate student in the Unit, Raymond Gosling, who was studying ram sperm by X-ray diffraction. By keeping the fibres in a wet

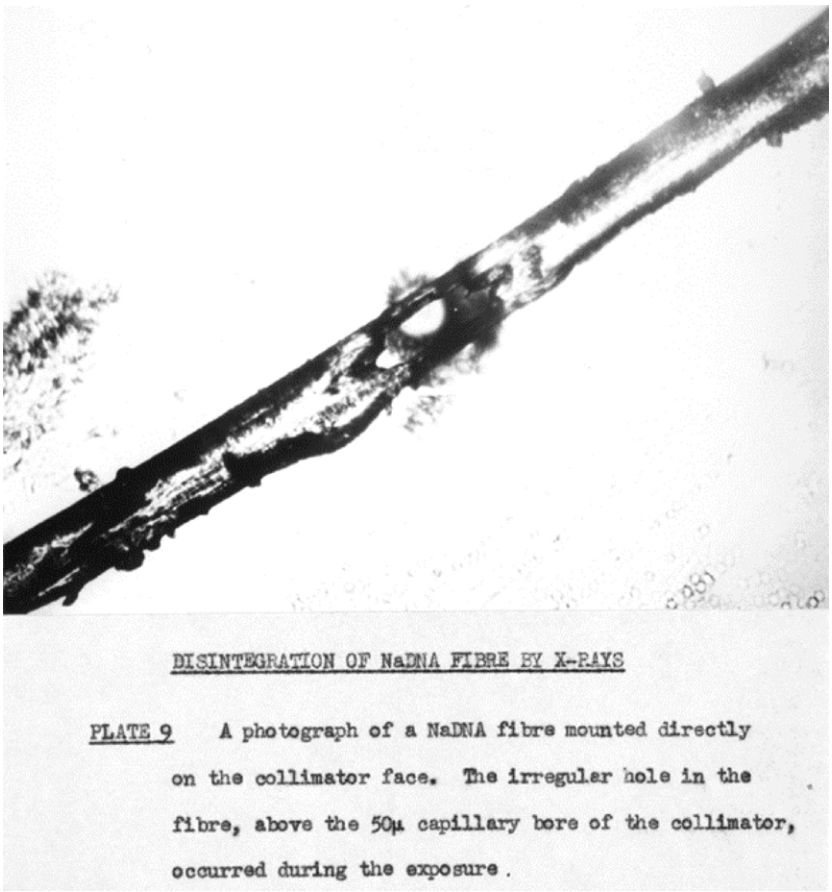
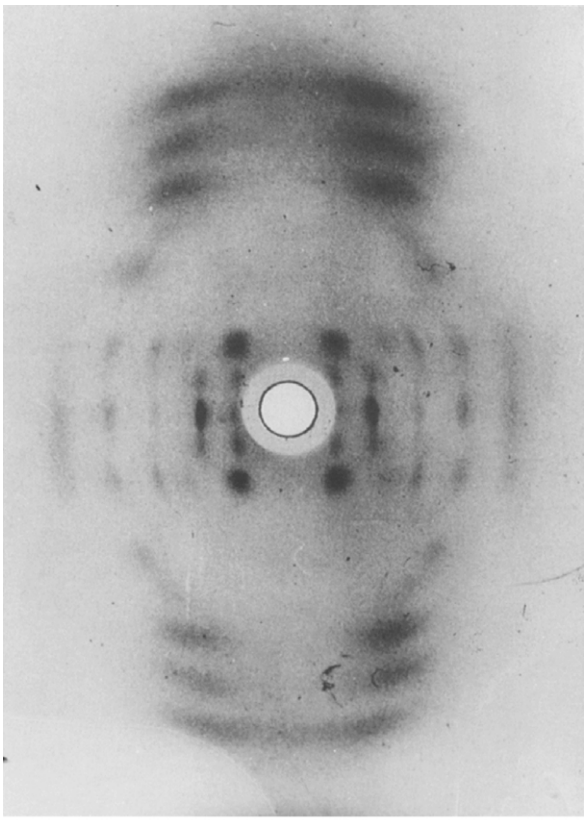


Figure 7. A fibre drawn from a DNA gel after exposure to an X-ray beam which has punched a hole in it (MHF Wilkins, WE Seeds and RG Gosling Nature 1951, **167**, 759), reproduced from Gosling's PhD thesis, King's College, London 1954.



First multifibre specimen taken on the Raymax tube
Unicam Camera, filled with hydrogen

Figure 8. The first clear crystalline pattern from a DNA fibre, King's College 1950, in what was later called the A form (from RG Gosling "Genesis of a Discovery: DNA Structure", ed S Chomet 1993). This was shown by Maurice Wilkins at the Naples conference attended by James Watson (courtesy, R G Gosling).

atmosphere, Gosling and Wilkins obtained the X-ray diffraction photograph that Wilkins later showed at Naples and which so excited Jim Watson (Figure 8). Other early diffraction photographs of various specimens (Figure 9) showed hazy patterns, later understood to be indicating helical features (Figure 10).

Rosalind Franklin

In January 1951, the King's College group was strengthened by the arrival of Rosalind Franklin (Figure 11). She was a physical chemist, who was then studying the structure of carbons (cokes and chars) using X-ray diffraction methods. She had been at a CNRS lab in Paris, where she learned, and improved, X-ray diffraction techniques for dealing quantitatively with substances of limited internal order. These presented much more difficulty than the highly ordered crystals which X-ray crystallographers were using to solve the structures of small molecules. It is important to realise in what follows that, in Paris, Franklin gained no experience of such formal X-ray crystallography.

The combination of these X-ray diffraction techniques and chemical preparatory skill attracted the attention of Randall, and Franklin was invited by him to bring her experience to London. Randall's purpose was clearly to put more professional effort into the DNA work begun by Wilkins and Gosling. Randall, however, left an unfortunate ambiguity about the respective positions of Wilkins and Franklin, which later led to dissension between them about the demarcation of the DNA research at King's. To this must be added the very different personalities of the two. A letter of Randall to Franklin in December 1950 (Figure 12) makes it clear that "on the experimental X-ray effort there would be for the moment only yourself and Gosling". Wilkins did not see this letter, and was away when Franklin arrived in January 1951 and Gosling was formally placed under her supervision. Nevertheless he still apparently thought of Franklin as a member of his team.

Wilkins handed over the Signer DNA to them, and turned to an X-ray study of sperm where DNA is complexed with proteins. It should be remembered that at the time, no one, not even Watson, had imagined that the 3D structure of DNA alone, important as that might turn out to be, would by itself indicate how the molecule replicated itself, and hence reveal "the secret of life".

Within the first year Franklin transformed the state of the field. By drawing thinner fibres she

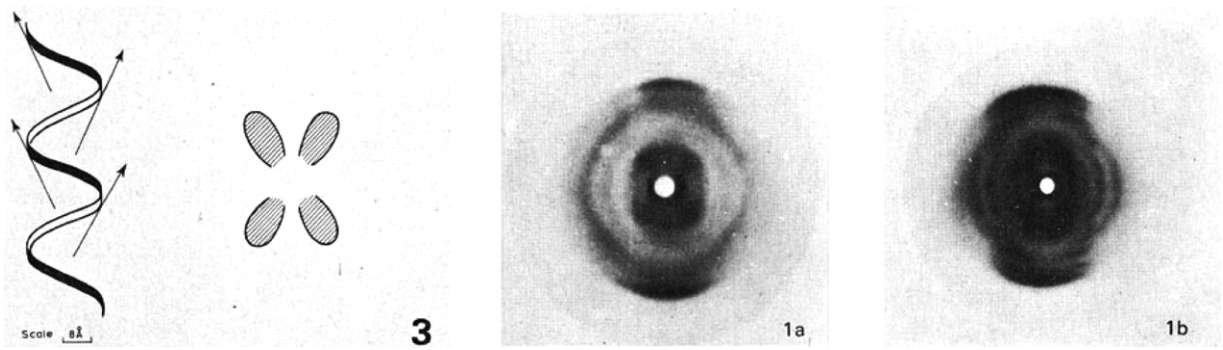


Figure 9. Early X-ray diffraction patterns obtained by the King's College group, about 1950, suggestion of a helical structure (cf. AR Stokes, in "Genesis of a Discovery: DNA Structure", ed S. Chomet, Newman Hemisphere Press 1993).

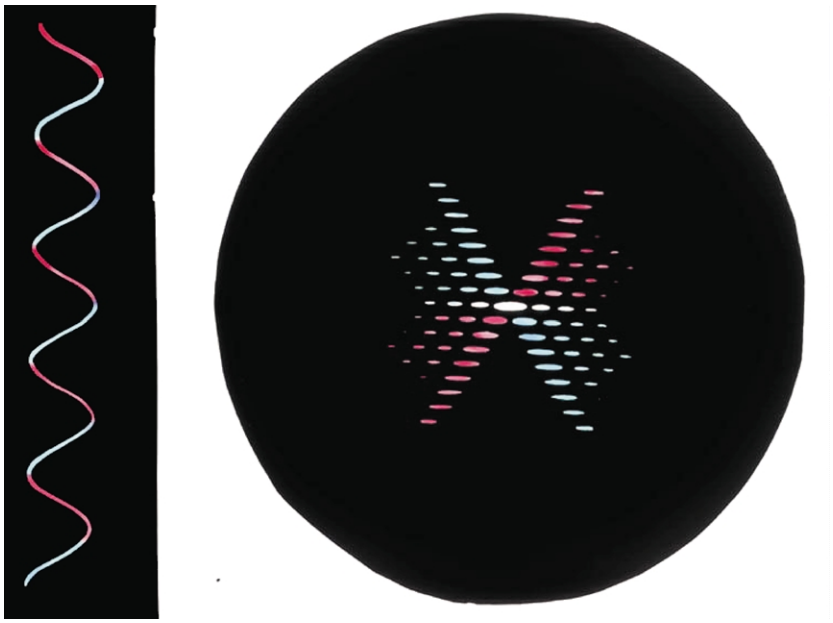


Figure 10. Diffraction pattern produced by a continuous helix: an optical analogue (from KC Holmes and DM Blow. "The Use of X-ray Diffraction in the Study of Protein and Nucleic Acid Structure", Interscience, 1966). Note the X-shaped fan of reflections emanating from the origin (at the centre).

Figure 11. Rosalind Franklin on holiday in Italy in the summer of 1950 (photo from V Luzzati, Paris).

Dr. Stokes, as I have long inferred, really wishes to concern himself almost entirely with theoretical problems in the future and these will not necessarily be confined to X-ray optics. It will probably involve microscopy in general. This means that as far as the experimental X-ray effort is concerned there will be at the moment only yourself and Gosling, together with the temporary assistance of a graduate from Syracuse, Mrs. Heller. Gosling, working in conjunction with Wilkins, has already found that fibres of desoxyribose nucleic acid derived from material provided by Professor Signer of Bern gives remarkably good fibre diagrams. The fibres are strongly negatively birefringent and become positive on stretching, and are reversible in a moist atmosphere. As you no doubt know, nucleic acid is an

Figure 12. Excerpt from a letter dated 4 December 1950 from Professor JT Randall, King's College, London, to Rosalind Franklin in Paris (Franklin papers, Churchill College Archives, Cambridge).

was able to enhance the alignment of the DNA molecules within the specimen, and these specimens, together with finer collimation of the X-ray beam generated from a microfocus X-ray tube which she and Gosling had assembled, produced sharper diffraction patterns (Figure 13). These, however, showed variable features, and it was not until Franklin made a systematic study of the fibres, that the problem was solved.

The A and B forms of DNA

In a crucial advance, Franklin controlled the relative humidity in the camera chamber by using a series of saturated salt solutions and thus was able to regulate the water content of the fibre specimens. In this way she showed that, depending on the humidity, two forms of the DNA molecule existed, which she later named A and B, and

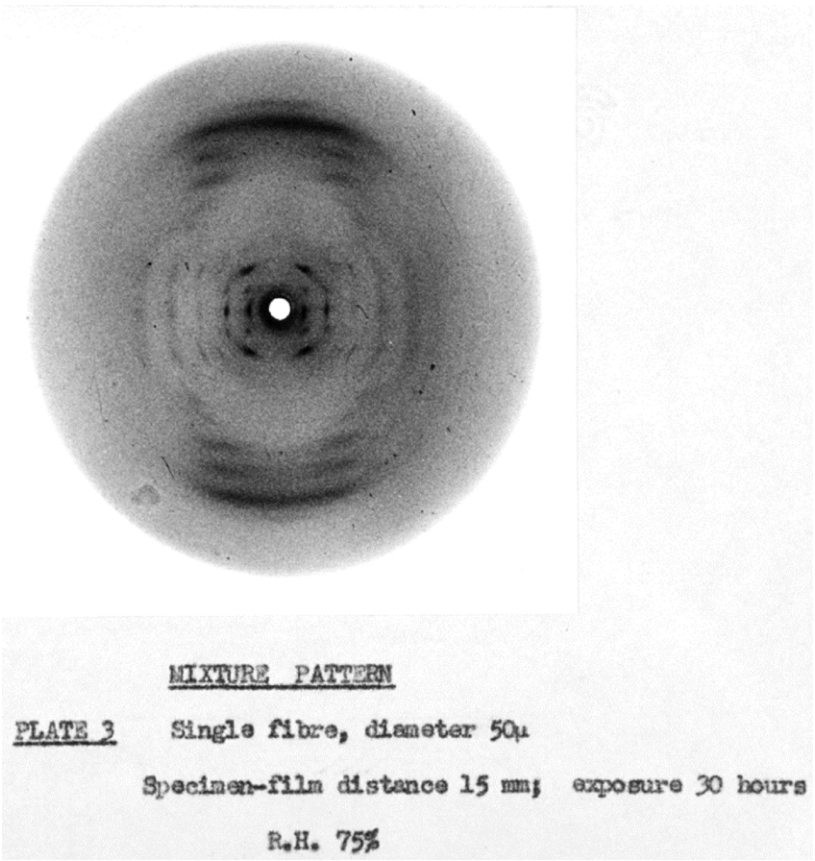


Figure 13. X-ray diffraction pattern of a DNA fibre 1951, later understood to be a "mixture" of the A and B forms of DNA (Gosling, PhD thesis, London 1954).

defined the conditions for the transition between them (Figure 14). The A form, which she first called "crystalline", is found at, and just below, 75% relative humidity. Above that point there is an abrupt transition to the B form, which she originally called "wet". The X-ray patterns of the A and B forms are shown in Figures 15 and 16, respectively.

It became clear that all previous workers had been working, unbeknown to themselves, mostly with a mixture of the two forms, or at best with poorly oriented specimens of the A form, and, in retrospect, with occasionally hazy pictures of the B form.

The B form pattern illustrated in Figure 16, is the superb picture B51, which Franklin obtained later in May 1952, and which has achieved iconic status. It was this picture that was shown by Wilkins to Watson in early 1953, and prompted the Cambridge pair into active model building. But even less striking X-ray patterns, which Franklin had obtained by September 1951 (Franklin and Gosling 1953, Acta Crystallographica, Figure 2), showed clear evidence of a helical structure. The theory of diffraction by a helix had been worked out by Alex Stokes at King's at the behest of Wilkins (unpublished, 1951), and also independently by Cochran, Crick and Vand the same year, published in early 1952. The characteristic feature of the pattern is the X-shaped pattern of streaks arranged in a set of layer lines, from which it can be deduced that the pitch of the helix in the B-form is 34 Å.

A strong X-ray reflection lies on the meridian, corresponding to a spacing of 3.4 Å: this is produced by the regular stacking of the bases on top of each other. Since the helix pitch is 34 Å, this means that the helix, whatever it is in detail, repeats after 10 (= 34/3.4) units per turn. This photograph is particularly striking in that it shows, not only the X shaped pattern of streaks in the centre, but also secondary fans emanating from the two 3.4 Å meridional reflections, top and bottom, and running obliquely to the equator. These are characteristic of a discontinuous helix (Figure 17), as is to be expected from the discrete moieties in a phosphate-sugar chain.

In the A form, the repeat of the structure is 28 Å compared with 34 Å in the B, consistent with a macroscopic shrinkage of 25% in the lengths of the fibres. The A form does not show the characteristic X, but there is a gap on the meridian of the photograph, consistent with a helical structure as Franklin recognised (and described in November 1951—see below). The A form, as later recognised by Watson, is a somewhat more tightly wound form of the B double helix, in which the bases change their tilt obliquely to the fibre axis (Figure 18), thus obscuring the characteristic X-shaped fan of reflections expected from a simple helical structure.

For, despite her discovery of the simpler B pattern, Franklin at first directed her attention mostly to the A form. Here, the molecules themselves are not in random rotational orientations, as in B, but

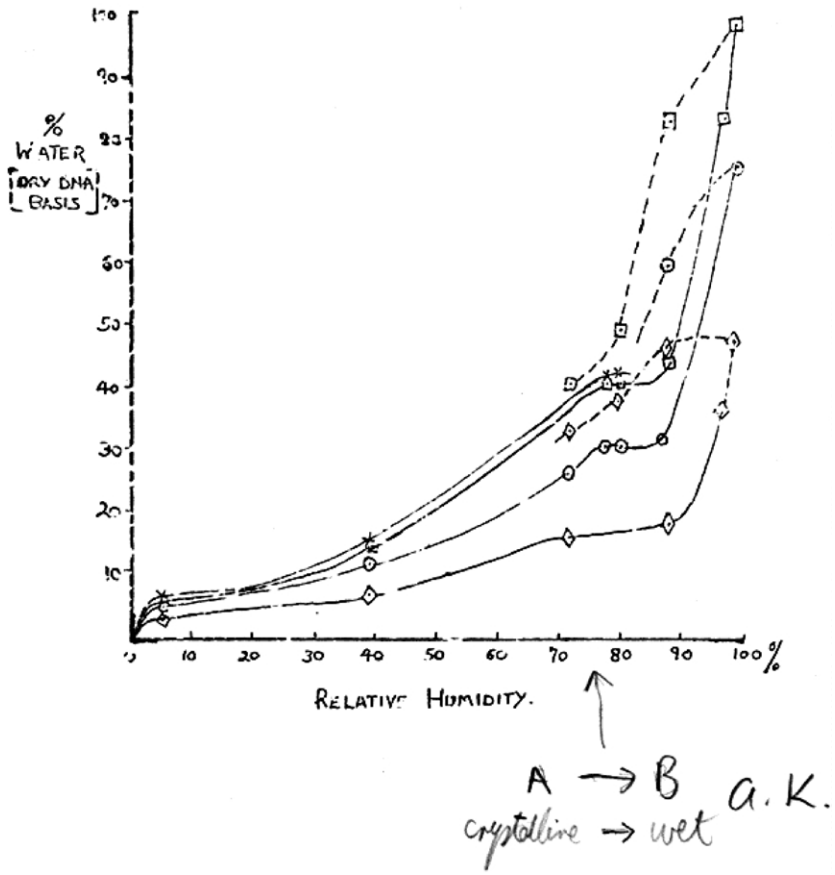


Figure 14. Franklin's measurements of water uptake by DNA fibres, made by weighing them at different ambient relative humidities, the latter controlled by the use of saturated salt solutions. (Franklin papers, Churchill College Archives, Cambridge; hitherto unpublished, described qualitatively in Franklin and Gosling, Acta Crystallog. 1953 **6**, 673). There is some hysteresis in that the curves on re-drying do not follow exactly the initial wetting curves. There is a marked transition at about 75% relative humidity, when the DNA structure changes abruptly from the "crystalline" form (later called A) to the "wet" form B. Annotation in Franklin's notebook by the author A.K.

packed regularly in small crystallites in a crystal lattice (Figure 19). However, the crystallites are randomly oriented so that the 3D X-ray data is scrambled into two dimensions on the photographic plate. The X-ray pattern nevertheless still shows sharp "spots" and offered the possibility of an objective crystallographic analysis because of the greater wealth and precision of the 3D diffraction data which could be extracted from the 2D pattern.

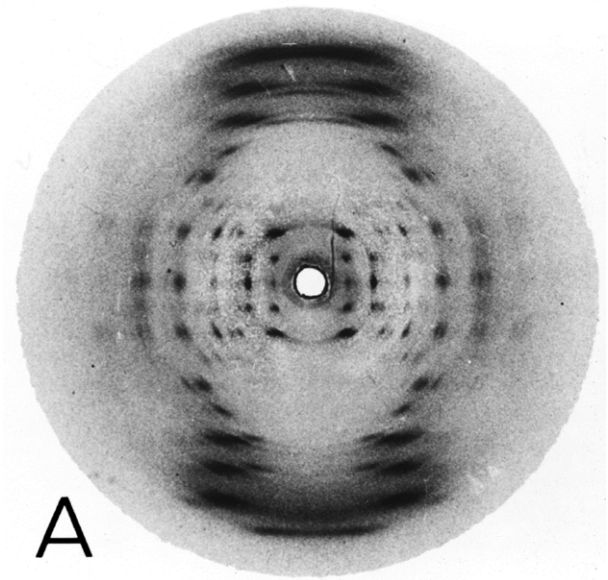


Figure 15. X-ray diffraction pattern of the A form of DNA (Franklin and Gosling, Acta Crystallog. 1953, **6**, 673, Figure 1.

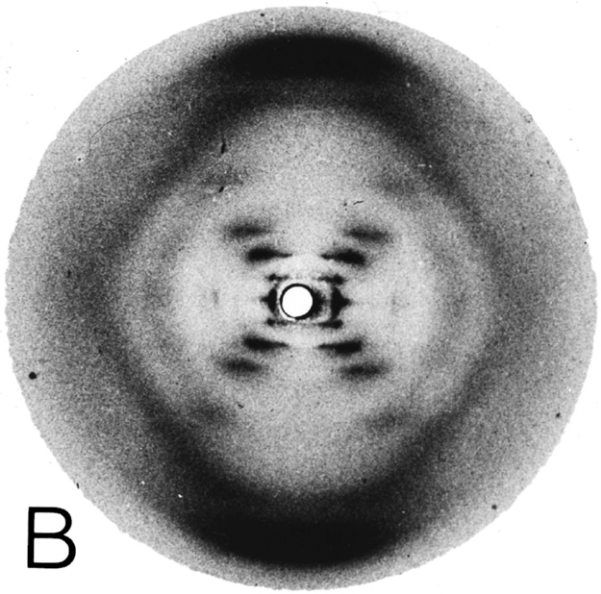


Figure 16. X-ray diffraction pattern of the B form of DNA (Franklin and Gosling, Acta Crystallog. 1953, **6**, 673, Figure 4; The B form pattern is that reproduced in Franklin and Gosling Nature 25 April 1953, **171**, 740, also known as photograph B51.

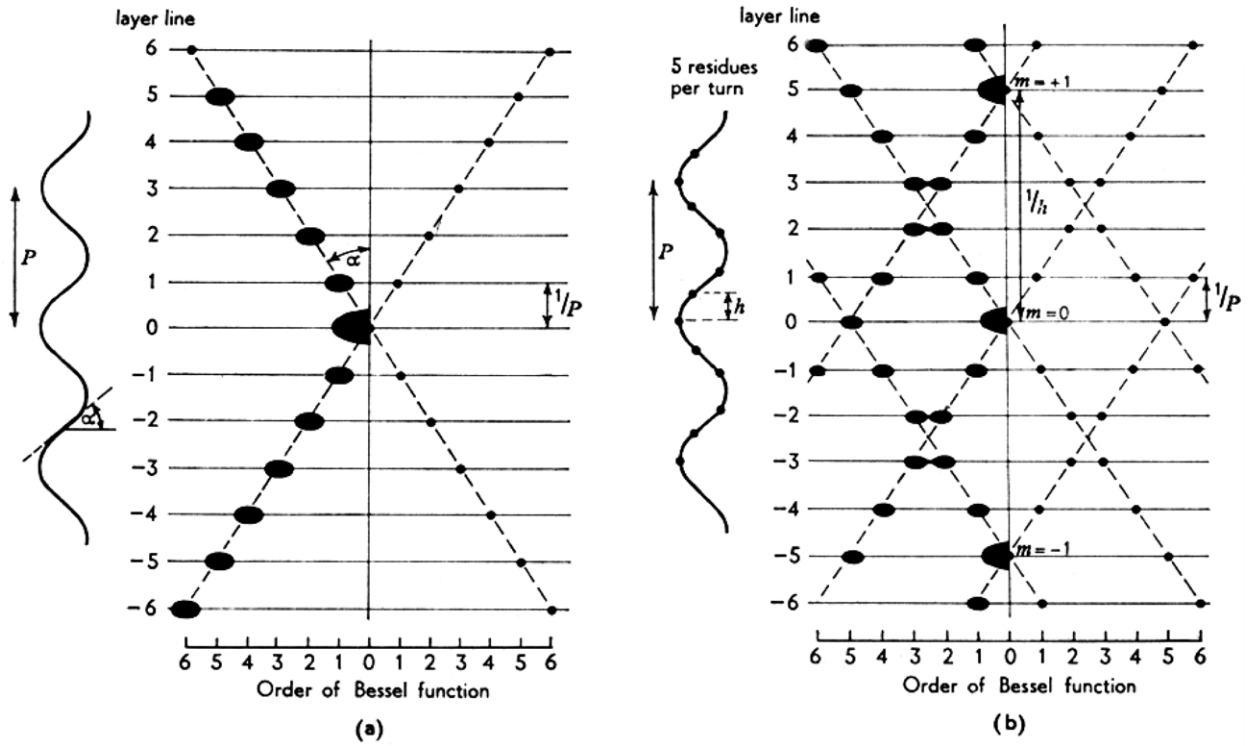


Figure 17. Diffraction pattern given by a discontinuous helix made of discrete units (right), compared with that given by a continuous helix (left, cf. Figure 10 above). In this example, there are five units per turn of the helix, giving rise to a meridional reflexion (i.e. on the axis) on the fifth layer line. There is a subsidiary X-shaped fan, emanating from the meridional reflexion, as well as the main fan emanating from the centre of the pattern, This can be seen in Franklin's X-ray pattern of the B form (Figure. 16).

In retrospect this was a misjudgement, but it was a reasonable decision at the time, because, if correctly interpreted, the A pattern would yield more precise information about the DNA molecule. She decided to use what is called Patterson function analysis on the X-ray data she had measured on the A patterns, and, as Gosling said later, let the data speak for itself. This Patterson method is an indirect method, which had been used at higher resolution to solve the structures of small molecules, but never for such large unit cells.

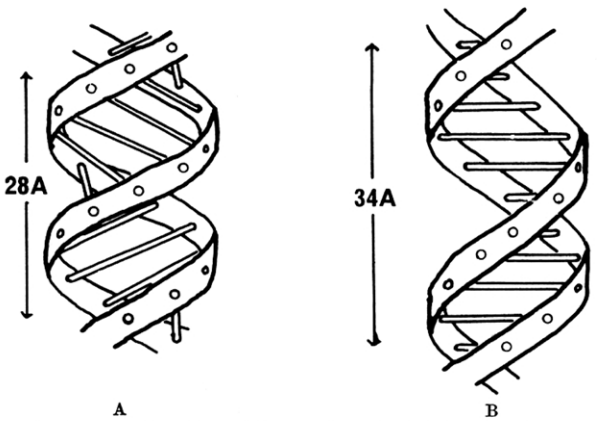


Fig. 2. Diagram (for reference) to show the principal differences between the A and B forms of DNA. The ribbons symbolize the phosphate–sugar chains, and the rods the pairs of hydrogen-bonded bases holding the chains together.

Figure 18. Early diagrams of the structures of the A and B forms of DNA (GB Sutherland and M Tsuboi, Proc. Roy. Soc. A 1957, **239**, 446, the A form after Wilkins *et al*. *Nature*, October 1953, **172**, p. 759).

Franklin's Colloquium, November 1951: Watson and Crick's first model

In November 1951 Franklin gave a colloquium on her work at King's College which Watson attended. There was much contact on and off between Wilkins and Crick, who were friends, and this led to several visits by Watson to King's.

The draft of Franklin's colloquium and her accompanying notes survive in the Archives of Churchill College, Cambridge. She describes a [very] dry form (1) and, the two forms "crystalline" (2) (later A) and "wet" (3) (later B) which is not easily re-wetted. She gives the crystal parameters, and the lattice symmetry (monoclinic space group *C*2), and also the density of A, from which she deduced that there were two or three chains of DNA per lattice point. The packing is pseudo-hexagonal, which implies that the molecules have an approximately cylindrical shape with a diameter of about 20 Å, Her notes read: "Evidence for spiral structure [we would now say helical]. Straight chain untwisted is highly improbable. Absence of reflections on meridian in xtalline form suggests

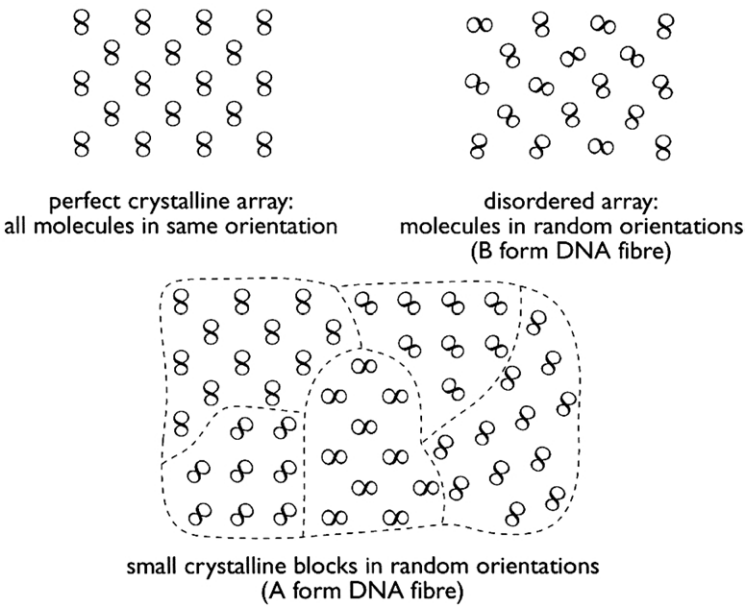


Figure 19. The packing of DNA molecules in the A and B form compared with those in a perfect crystal. The diagrams show schematic cross-sections of the arrangements.

spiral structure… Nucleotides in equivalent positions occur only at intervals of 27 Å [corresponding to] the length of turn of the spiral".

On the basis of the above, Franklin put forward her view that the molecular structure in the A form was likely to be a helical bundle of two or three chains, with the phosphate groups on the outside. The bundles are separated by weak links produced by sodium ions and water molecules (Figure 20). At the higher humidity of the B form, a water sheath disrupts the relationship between neighbouring helical bundles, and only the parallelism of their axes is preserved. (The same conclusions are found in Franklin's Fellowship Report for the year ending 1951). Watson (and others) have stated in their reminiscences that Franklin did not mention the B form, but her draft is quite explicit about the helical bundle being preserved in the transition from A to B. (Indeed, her notes read "Helical structure in the [wet] form cannot be the same as in the [crystalline] because of large increase in length".)

Watson took the news—as little, or as much, as he understood of it—back to Crick in Cambridge, and, now with some structural information to hand, they decided to build a model. They had urged this approach on the King's group, but receiving no response, now felt justified in attempting this themselves. The King's group was invited to see the result—a model built in a week. The model was of three helical chains with the phosphates on the inside, neutralised by cations, with the bases pointing outwards. Franklin asked where was the water, and received the reply that there was not any. It turned out that Watson, not understanding the relationship between a unit cell of a crystal and the asymmetric unit, had conveyed the wrong water content. After this debacle, Sir Lawrence Bragg, the head of the Cavendish Laboratory, firmly vetoed any further work on DNA

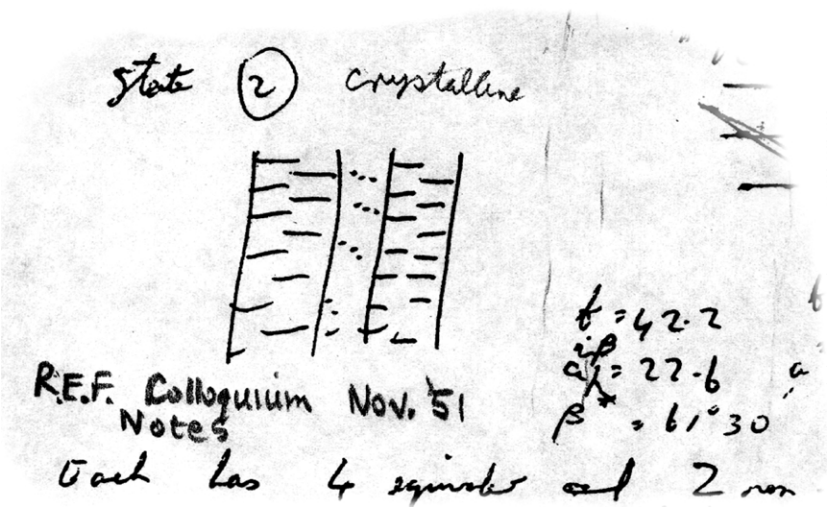


Figure 20. Diagram from Franklin's notes for the Colloquium she gave at King's College in November 1951, annotated by A.K. The DNA molecules in the A form are represented as helical bundles of two, or three, chains (here two), with the bases in the inside, the phosphates on the outside, and the individual molecules associated in the fibre through water and ionic links (dotted lines). Each molecule has six near neighbours, four equivalently related and two others approximately related. (Franklin papers, Churchill College Archives).

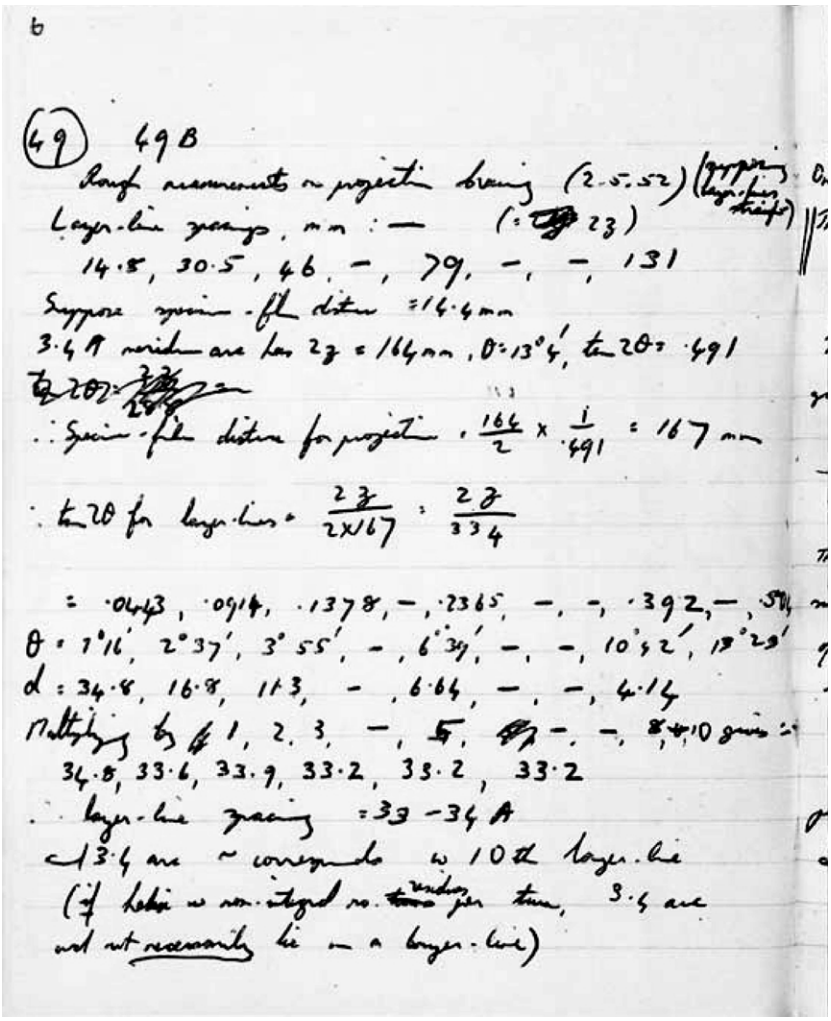


Figure 21. Excerpt from Franklin's notebook May 1952, showing an analysis of the X-ray pattern 49B (a precursor of the famous B51 picture, Figure. 16 above) in terms of helical diffraction theory (Franklin papers). This was done at a time when she was questioning whether the A form was helical.

at the MRC Unit in Cambridge. In future it would be done solely at the Unit at King's College.

Non-helical DNA?

Franklin pressed ahead with the Patterson analysis of the A Form. There is no question that all along she held the view that B form was helical (Figure 21), but could not see a way to solve it except by model building, a path she was reluctant to follow. She knew of Pauling's success in 1951 in predicting, by model building, the α-helical and β-sheet configurations of the polypeptide chains of proteins, but she equally well knew of the contemporary failure of Bragg, Kendrew and Perutz on the same problem—the "greatest fiasco of my scientific life", Bragg later called it. This last debacle of Watson and Crick would only have confirmed her decision to avoid model-building and rather to try an analytic crystallographic approach on the A form.

However, an unfortunate mechanical accident in one of the specimens led Franklin to take a wrong path. In the spring of 1952, one DNA fibre gave an X-ray pattern showing strong "double orientation", that is, the 3D crystallites in the A form were not all in random orientation about the fibre axis, but some orientations occurred move frequently than others. This suggested to her that the symmetry of the crystallite was far from cylindrical, which might rule out a helical structure in the A form. Franklin concluded that this possibility had to be considered. It is this view of hers which gave rise to her supposed "anti-helical" stance, but for her it was a question which had to be answered. Unwisely, she ignored Crick's remark to her, made in a tea-queue at a meeting, that the double orientation was an accident to be dismissed.

In fact she seems to have persuaded Wilkins, even though relations were strained between them, to the same view. Thus, ironically, while Franklin does not mention this in a Report written in late 1952 for an MRC Subcommittee on the work of the King's Unit, Wilkins does so, and accepts the possibility of a non-helical interpretation of the A form. This Report is the MRC Sub-Committee Report, which gave crucial information to Watson and Crick in February 1953 for the building of their correct model of DNA (see below).

This misjudgement on Franklin's part influenced her attempts to interpret the Patterson map of the

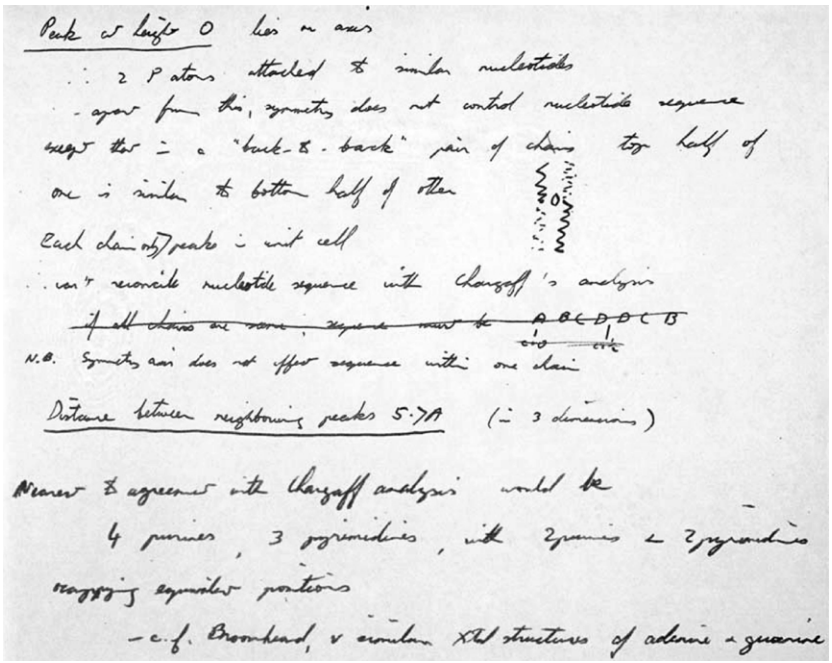


Figure 22. Excerpt from Franklin's notebook on 19 January 1953 (Franklin papers). Annotations by the author A.K. Franklin deduces by Patterson superposition analysis that the A form contains two chains related by a 2-fold axis of symmetry (the oval symbol). Also are noted Chargaff's base ratios, and Broomhead's crystal structures of adenine and guanine, which showed the correct tautomeric forms.

A form. She sought explanations in terms of rods or sheets, or a "figure of eight", all of which naturally failed. She was apparently thinking of the A form as an unwound version of the helices in the B state (rather, I imagine, like the β-sheet structure is to the α-helix in proteins and polypeptides). Presumably she thought the A-to-B transition a profound change of structure, because she notes, more than once, that, during the transition, the specimen fell off the end of the X-ray collimator to which it was attached.

One correct result which emerged in January 1953, from her application of the so-called superposition method to the Patterson map, was that the A form contained two chains, and that they ran in opposite directions (Figure 22). Had she been a crystallographer, and understood the meaning of the crystal symmetry, *C*2 face centred monoclinic, which she herself had established much earlier, she could have deduced this result at once. Of all the protagonists in the story, only Crick understood this. Moreover, *C*2 was the space group symmetry of the ox-haemoglobin crystals which he was studying for his PhD. It meant that, if the A structure was helical, it would consist of two chains, or strands, running in opposite directions, related by a 2-fold axis of symmetry perpendicular to the fibre axis, and hence to the pair of chains. (Franklin hardly ever reminisced about DNA in the years I worked with her on virus structure at Birkbeck College, but she once said that she could have kicked herself for missing the implications of the *C*2 symmetry).

Franklin's Patterson analysis ran into an impasse, and in early February, she turned to her B-form, the X-ray pattern which was clearly characteristic of some kind of helical structure (Figure 23). Her notebooks show her shuttling back and forth between the two forms. She had by now abandoned her attempts to interpret the A form in non-helical terms. On the 23rd February (Figure 24) she writes "If single-strand helix as

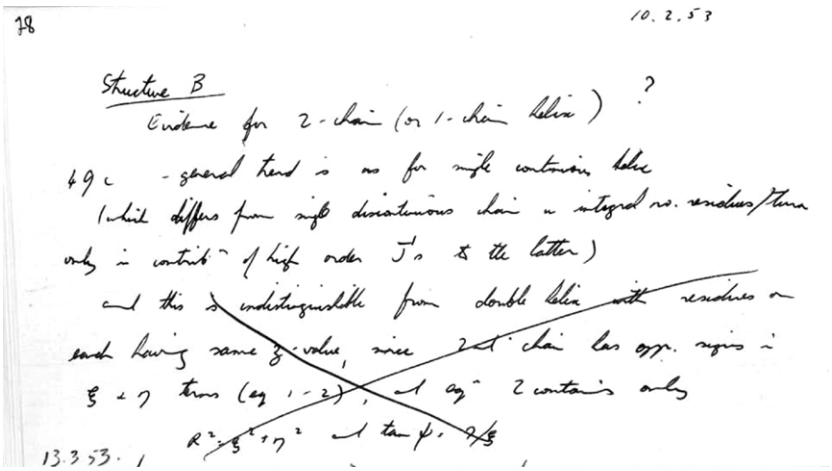


Figure 23. Excerpt from Franklin's notebook, 10 February 1953 (Franklin papers). Annotations by the author A.K. Franklin returns to the question of the number of chains in the B form.

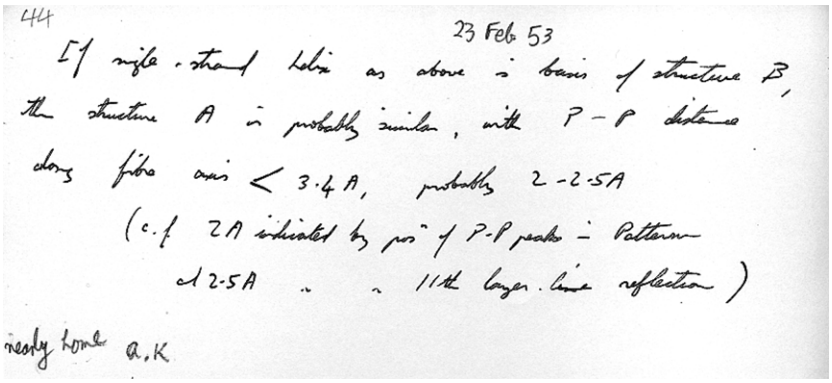


Figure 24. Excerpt from Franklin's notebook on 23 February 1953 (Franklin papers). Annotations by the author A.K. Franklin begins to consider a helical structure for the A form.

above is the basis of structure B, then Structure A is probably similar, with P–P distance along fibre axis <3.4 Å, probably 2–2.5 Å". On the 24th February (Figure 25) she is at last making the correct connection between the A and B forms—both have two chains.

Of course, she had no idea that, at that very time in Cambridge, in February 1953, Crick and Watson were now back to model building of DNA. Nor were they aware of what Franklin had been doing—Watson wrote later in his book "The Double Helix" that Franklin's instant acceptance on first seeing their model surprised him. He had then no idea how close she had come to it.

By March 1953, using helical diffraction theory, Franklin had carried the quantitative analysis of her B form patterns to the point where the paths of the backbone chains were determined. She had moved to Birkbeck College to J D Bernal's Department of Physics on14th March and there she wrote up her work in a typescript dated 17th March, that is, one day before the manuscript of Watson and Crick's structure, prepared for Nature, reached King's. Franklin's draft (Figure 26) contains all the essentials of her later paper (with Gosling) in Nature in April, which, together with one by Wilkins, Stokes and Wilson, accompanied Crick and Watson's paper announcing their model for the structure of DNA.

In Franklin's draft, it is deduced that the phosphate groups of the backbone lie, as she had long thought, on the outside of the two co-axial helical strands whose geometrical configuration is specified, with the bases arranged on the inside. The two strands are separated by 13 Å (three-eighths of the helix pitch in the axial direction). But the draft shows she had not yet grasped that the two chains in B also ran antiparallel as in the A form. Her notebooks show that for fitting the bases into the centre of a double helix, she had already formed the notion of the interchangeability of the two purine bases with each other, and also of the two pyrimidines. She also knew the correct tautomeric forms of at least three of the four bases, and was aware of Chargaff's base ratios. The step from interchangeability to the specific base-pairing postulated by Crick and Watson is a large one, but there is little doubt that Franklin was poised to make it.

What would have happened if Watson and Crick had not intervened with their great bursts of insight (Figure 27), and Franklin had been left to her own resources? It is a moot point whether she was one and a half or two steps behind, and how long it would have taken her to take them. Crick and I have discussed this several times. We agree she would have solved the structure, but the results would have come out gradually, not as a thunderbolt, in a short paper in Nature.

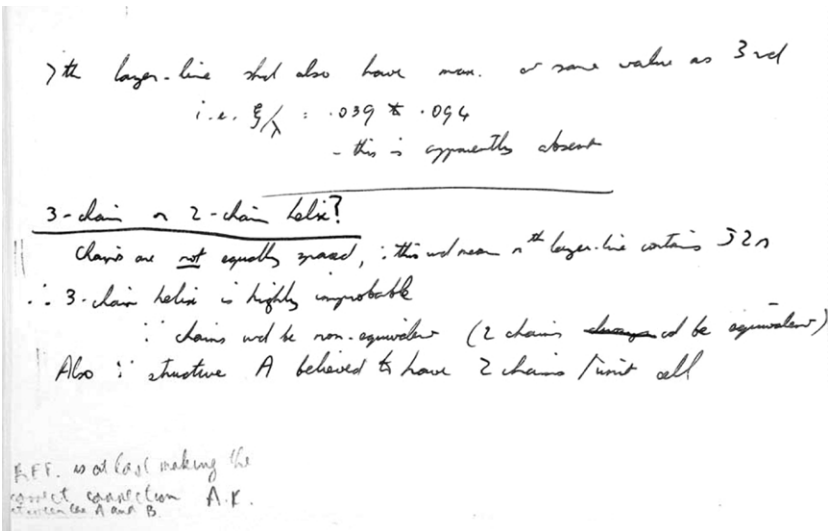


Figure 25. Excerpt from Franklin's notebook on 24 February (Franklin papers). Annotations by the author A.K. Franklin comes down in favour of two chains for the B form, making a connection with the A Form.

ROUGH DRAFT

A NOTE ON MOLECULAR CONFIGURATION IN SODIUM THYMONUCLEATE

Rosalind E. Franklin and R. G. Gosling

17/3/53.

Sodium thymonucleate fibres give two distinct types of X-ray diagram. The first, corresponding to a crystalline form obtained at about 75% relative humidity, has been described in detail elsewhere( ). At high humidities a new structure, showing a lower degree of order appears, and persists over a wide range of ambient humidity and water content. The water content of the fibres, which are crystalline at lower humidities, may vary from about 50% to several hundred per cent. of the dry weight in this structure. Other fibres which do not give crystalline structure at all, show this less ordered structure at much lower humidities. The diagram of this structure, which we have called structure B, shows in striking manner the features characteristic of helical structures( ). Although this cannot be taken as proof that the structure is helical, other considerations make the existence of a helical structure highly probable.

Figure 26. Unpublished typescript dated 17 March 1953, which is the precursor of Franklin and Gosling's paper in Nature 25 April 1953 (Churchill College Archives; see Klug, Nature 1974, **248**, p. 787).

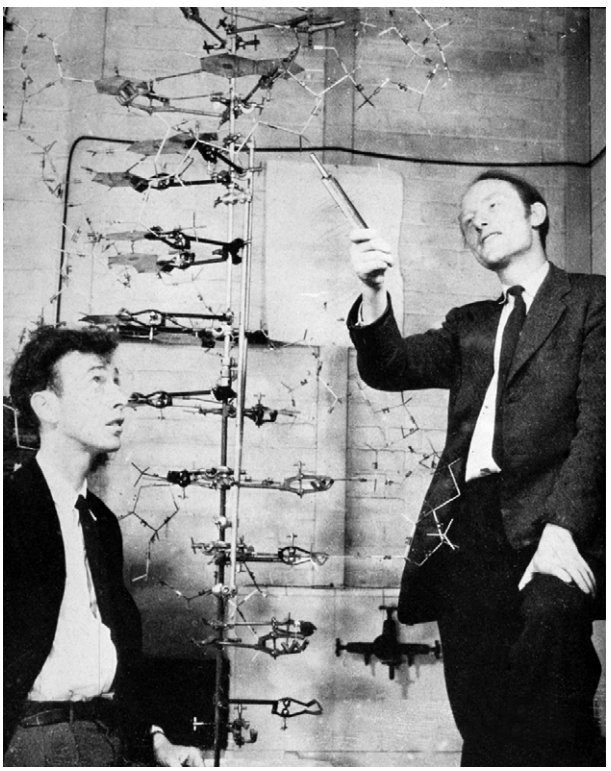


Figure 27. Francis Crick and James Watson with their model of the DNA double helix 1953 (Photo by Barrington Brown).

Pauling's entry into the field

The story now moves back to Cambridge in early 1953, when Crick and Watson re-entered the scene. News had reached them that Linus Pauling, the greatest chemist of the day, had a structure for DNA and that a manuscript was on its way. Here an old rivalry asserted itself. In the early 1930s Pauling and Bragg had been in competition about the chemical basis of silicate structures. Then later there was the chagrin Bragg felt, as described above, at having missed Pauling's α-helix.

Pauling's manuscript arrived at the Cavendish in the last week of January, and it was immediately obvious he had made a crucial chemical mistake in postulating a 3-chain structure with a central phosphate-sugar backbone, and with the phosphates unionized. It was chemically impossible, but no doubt Pauling would return, or so Watson argued to Bragg. (I doubt this—Pauling was a man with great insight, but not a magician, who could manage without data).

Watson and Crick's structure

The fact that Pauling was now in competition made for a race, and since the King's group seemed to be divided and making no progress,

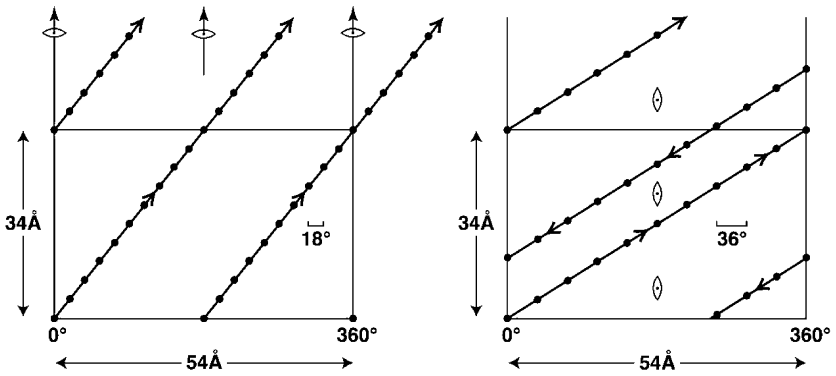


Figure 28. Cylindrical lattice diagrams of Watson and Crick's double helix structure (right) and of the double helical structure Watson was aiming to build in early February 1953 (left). The dots represent individual nucleotide (or phosphate) positions projected onto a cylindrical surface circumscribing the helices, which is then slit lengthways and unrolled flat. The oval symbols represent twofold rotation axes of symmetry.

Bragg was persuaded to unleash Crick and Watson from his earlier ban. Watson, two days earlier, had visited King's to give a copy of the Pauling manuscript to Wilkins. It was then that Wilkins showed him Franklin's striking May 1952 X-ray picture of the B form, with its clear helical features. This made a profound impression on Watson, since one could immediately count the number of layer lines leading to the 3.4 Å meridional reflection. He recounted this to Crick, along with the other parameters necessary to build a B form model: the repeat distance of 34 Å, indicating ten units per helical turn, a helix slope of 40°, the diameter of about 20 Å of the molecule, and they also remembered Franklin's arguments for the backbones being on the outside of the molecule and the bases on the inside.

The rest of the story is told in Watson's vivid account in his book, which revealed that Watson and Crick had access to details of the information in the MRC Subcommittee Report on the work at Kings.. This was given to them in the second week of February by Max Perutz, a member of that Committee. The Report confirmed much that

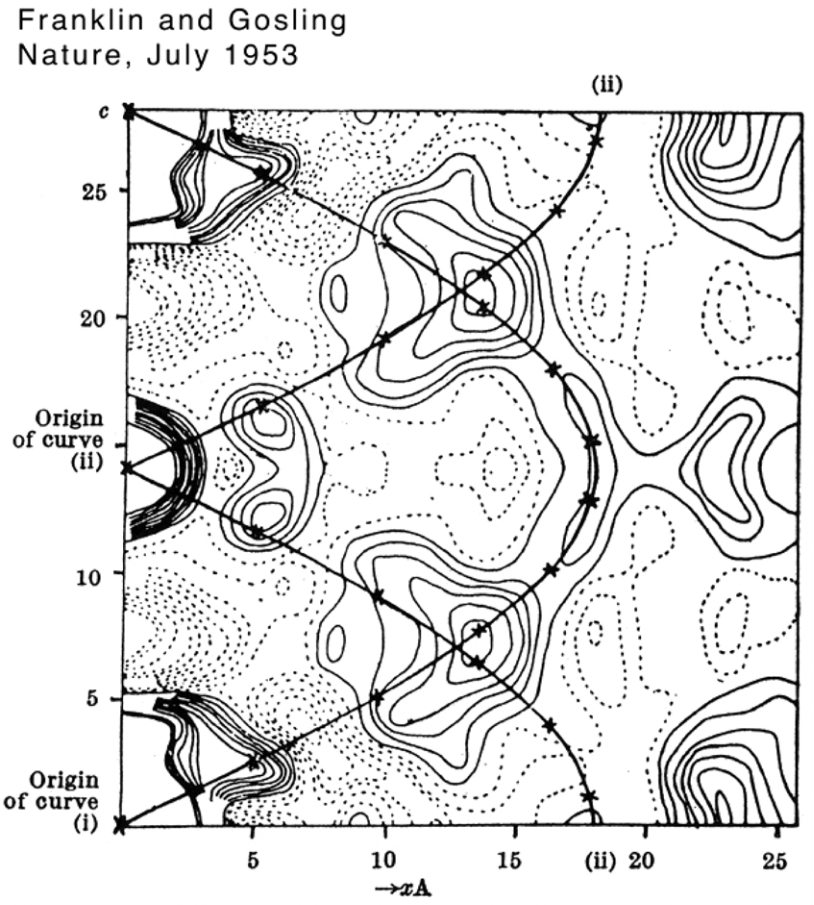


Fig. 3 (from ref. 4). Cylindrical Patterson function of crystalline sodium deoxyribonucleate, that is, the A form. The two curves (i) and (ii) show the theoretical Patterson function for two smooth coaxial helices of radius 9 Å separated by 14 Å (half their pitch) in the axial direction.

Figure 29. Analysis of Franklin and Gosling's cylindrical Patterson function map of the A form in terms of a double helix. (Nature 25 July 1953, **172**, p. 156). The curves denote the "self Pattersons" of the two helical chains, separated by half the helical pitch. The fit is improved if the "cross-Patterson" between the two chains is included (DLD Caspar, private communication 1968).

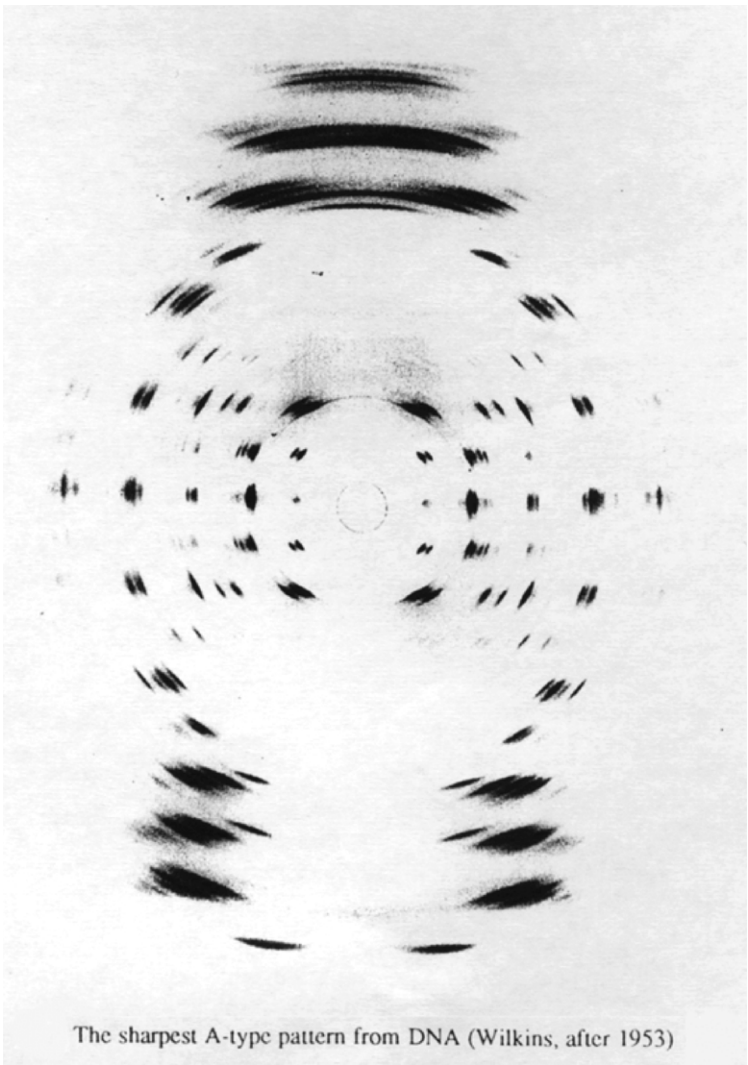


Figure 30. The sharpest, high resolution A type diffraction pattern obtained by Wilkins and the King's group, post1953. (courtesy Maurice Wilkins, in "Genesis of a Discovery", ed S Chomet, 1993).

they already knew, but the key fact was the space group symmetry *C*2 of the A form. Franklin had given this in her colloquium in November 1951, but Watson would not have understood it. Crick had heard that the crystal was monoclinic, which implied a 2-fold axis of symmetry (a dyad), but this could have been parallel or perpendicular to the fibre axis. *C*2 required it to be perpendicular to the fibre axis.

Watson had begun the building of two chain helical models with the chains running in the same direction (Figure 28, left). Each chain of pitch 68 Å would repeat after 20 nucleotides, but the two chains were to be exactly half a helical pitch apart, so that the structure would repeat after 34 Å in the axial direction. This would fit the best estimate of the number of nucleotides per lattice point (16–24, deduced from Franklin's density measurements on the A form) which could be reconciled with the tenfold repeat. The chain had a helical rotation angle of 18°(= 360°/20) between nucleotides, which brought successive sugars close together and was difficult to build. The *C*2 symmetry, however, told Crick that there were indeed two chains, that the chains ran in opposite directions and that the helical repeat of ten units per turn referred to one chain of pitch 34 Å, and so to each of the chains. Crick therefore changed the rotation angle to 36° (Figure 28, right) and Watson found the chain easier to build. This was a critical step in getting the backbone structure right.

The formal account by Crick and Watson in the Proceedings of the Royal Society (in 1954), which details their cogent reasoning in arriving at the double helix, does not mention their knowledge of the crucial fact of *C*2 symmetry which they had obtained from the MRC Report. It acknowledges information received from Wilkins and Franklin only in general terms: "We are most heavily indebted in this respect to the King's College group, and we wish to point out that without this data the formulation of our structure would have been most unlikely, if not impossible". Presumably there would have been some embarrassment about mentioning the source of their knowledge of the *C*2 symmetry. It would not have diminished their achievement to have stated it.

The next step facing Watson and Crick was to fit

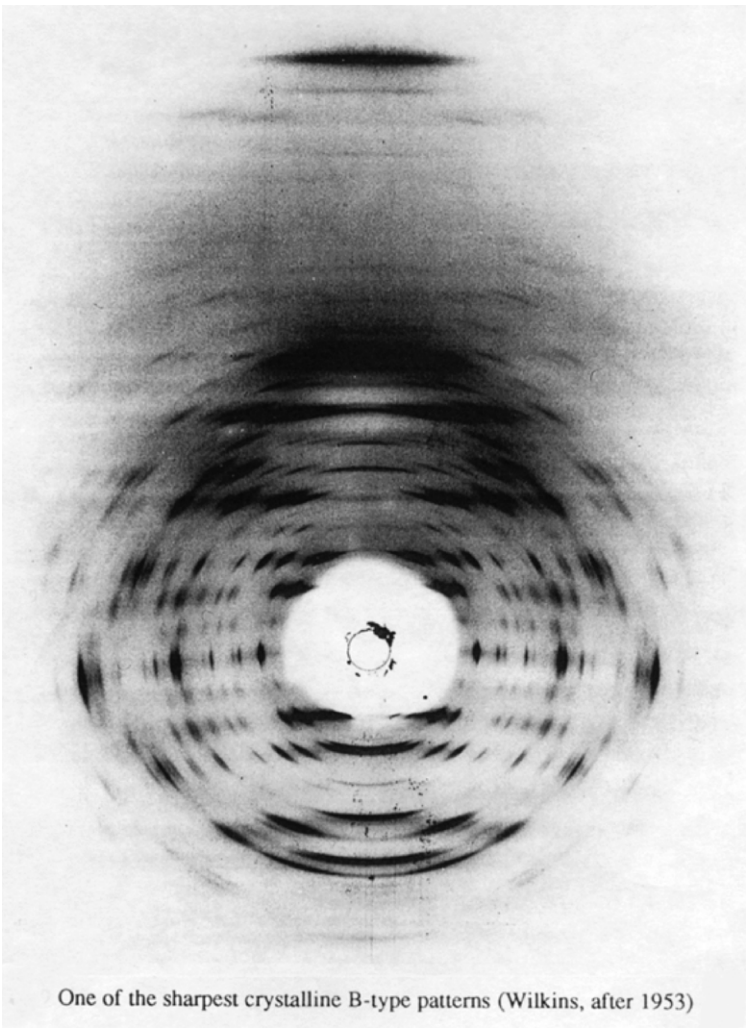


Figure 31. High resolution, sharp, X-ray diffraction pattern of a crystalline B form, post 1953, obtained from a lithium salt of DNA (Wilkins in "Genesis of a Discovery", ed. S Chomet 1993). The outermost spot corresponds to a spacing of 1.7 Å, the second order of the spacing of 3.4 Å between the bases.

the bases stacked above each other into the middle of the double helix. The bases are linked by glycosidic bonds to the sugars of the backbones. There was room for two bases in each stack and Watson had been trying different ways of making such pairs, connected by hydrogen bonds, initially pairing like with like, thus, adenine with adenine, and so on. In the last week of February, it was however, pointed out to Watson by Jerry Donohue, who shared an office with him and Crick—another chance event—that he was using the incorrect chemical formulae (tautomeric forms) for the four bases. When Watson changed these he found he could fit in adenine-thymine as a pair, and also guanine-cytosine as a pair. The geometry of each pair was almost identical!

Moreover each base-pair could fit either way round between the two chains, A with T, and T with A, and similarly for C:G and G:C. The glycosidic bonds were thus automatically related by the perpendicular dyod, thus fitting the *C*2 symmetry, although Watson had not made explicit use of the symmetry in his model building.

Remarkably, this pairing also gave an explanation of the earlier finding by Erwin Chargaff that the amount of adenine in any DNA sample equalled that of thymine, and similarly for guanine and cytosine. Chargaff's ratios thus automatically arose as a consequence of Watson's base-pairing scheme. The structure of DNA was solved!

On 28th February 1953, Crick "winged" into the Eagle pub, close to the Cavendish Laboratory, where lunch could be had for 1s 9d, and declared to anyone who cared to listen that, in the Cavendish, Watson and he had discovered "the secret of life". Wilkins came to see their model in mid March, and Franklin later at the end of the month. Her "instant acceptance amazed" Watson, but then he did not know how far she had got towards it, having heard only of her supposed "anti-helical" stance.

There was agreement between King's and Cambridge to publish separately, and three papers appeared on 25th April 1953, grouped together under the overall title "Molecular Structure of Nucleic Acids". Watson and Crick's paper contained what appeared to be the famous throwaway sentence: "It has not escaped our notice that the specific [base] pairing we have postulated immediately suggests a possible copying mechanism

for the genetic material". Crick explained later that they were not being coy, but there was a worry on Watson's part that the structure might be wrong: when they sent the first draft of the paper to King's, they had not yet seen their papers and had little idea of how strongly the King's X-ray evidence supported their structure. After seeing it they wrote their second Nature paper of May 30th entitled "Genetical Implications of the Structure of Deoxyribonucleic Acid") to spell out their postulate for the copying mechanism in DNA replication (Figure 5). This paper also contains the first clear statement on the genetic code: "The phosphate-sugar backbone of our model is completely regular, but any sequence of the pairs of bases can fit into the [double-helical] structure. It follows that in a long molecule many different permutations are possible, and it therefore seems likely that the precise sequence of bases is the code which carries the genetical information"

Proving the model

The first analytical demonstration of the general correctness of the Watson–Crick model came in July 1953 from Franklin and Gosling (Figure 29). They showed that their Patterson function map of the A form could be fitted by a helical structure with two chains.

The task of rigorously testing the model against X-ray diffraction data required more accurate intensity data from better oriented fibre specimens and this was undertaken by Wilkins and the King's College group including Herbert Wilson, Bob Langridge and Watson Fuller. It took them about seven years to carry this out. They obtained much improved diffraction patterns from several different DNA sources (Figures 30 and 31), built higher resolution X-ray cameras, introduced computers to make the calculations and used new analytic methods developed by Struther Arnot for refining models to fit X-ray fibre diffraction.

During that time there were several objections by crystallographers to the DNA model. These and other objections were finally answered by the rigorous analysis at King's, although other models appeared occasionally through the 60s and 70s. Indeed, it could be said that the formal crystallographic proof of the double helix and the base-pairing did not come until 1979, when Drew and Dickerson solved the structure of a dodecameric DNA oligonucleotide of defined sequence, by using the totally objective heavy atom method (Proc. Nat. Acad. Sci. USA **78**, 1981, 2178–83).

The reception of the double helix

It should be remembered that, in 1953, the X-ray diffraction crystallography of large biological molecules was still in its infancy and regarded as an exotic pursuit; the first protein structures of myoglobin and haemoglobin were not solved (at low resolution) until 1957 and 1959, respectively.

The double helix model was well received by geneticists and the phage group when Watson described it at the Cold Spring Harbor meeting in the summer of 1953, but there were doubts about the correctness, and indeed relevance, of the model on the part of biochemists, who, on the whole, still thought of proteins as the genetic material. The best biochemical proof that the structure was correct eventually came from Arthur Kornberg. If the "hypothetical" dyadic structure of DNA with two antiparallel chains (Figure 3) were correct, then there must also be relationships between pairs of dinucleotides, further to Chargaff's rules for individual bases. Thus the number of AG dinucleotides should equal the number of CT dinucleotides, the number of TG equal to CA, and so on. Kornberg and his colleagues measured the frequencies of dinucleotides in a variety of DNAs. The prediction was proved correct, in a most elegant way (Josse *et al.*, *J. Biol. Chem.* **236**, 1961, 804–75).

Nevertheless, the structure of the double helix, as emphasized by Todd, was still only a discovery

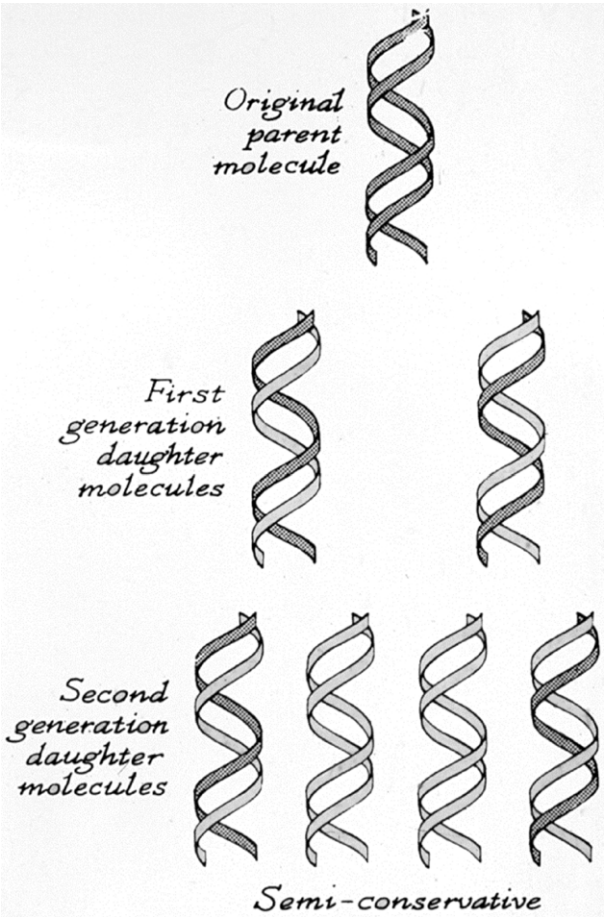


Figure 32. The interpretation of the Messelson–Stahl experiment, demonstrating semi-conservative replication (reproduced from L Stryer, Biochemistry, 4th Edition 1995, Freeman).

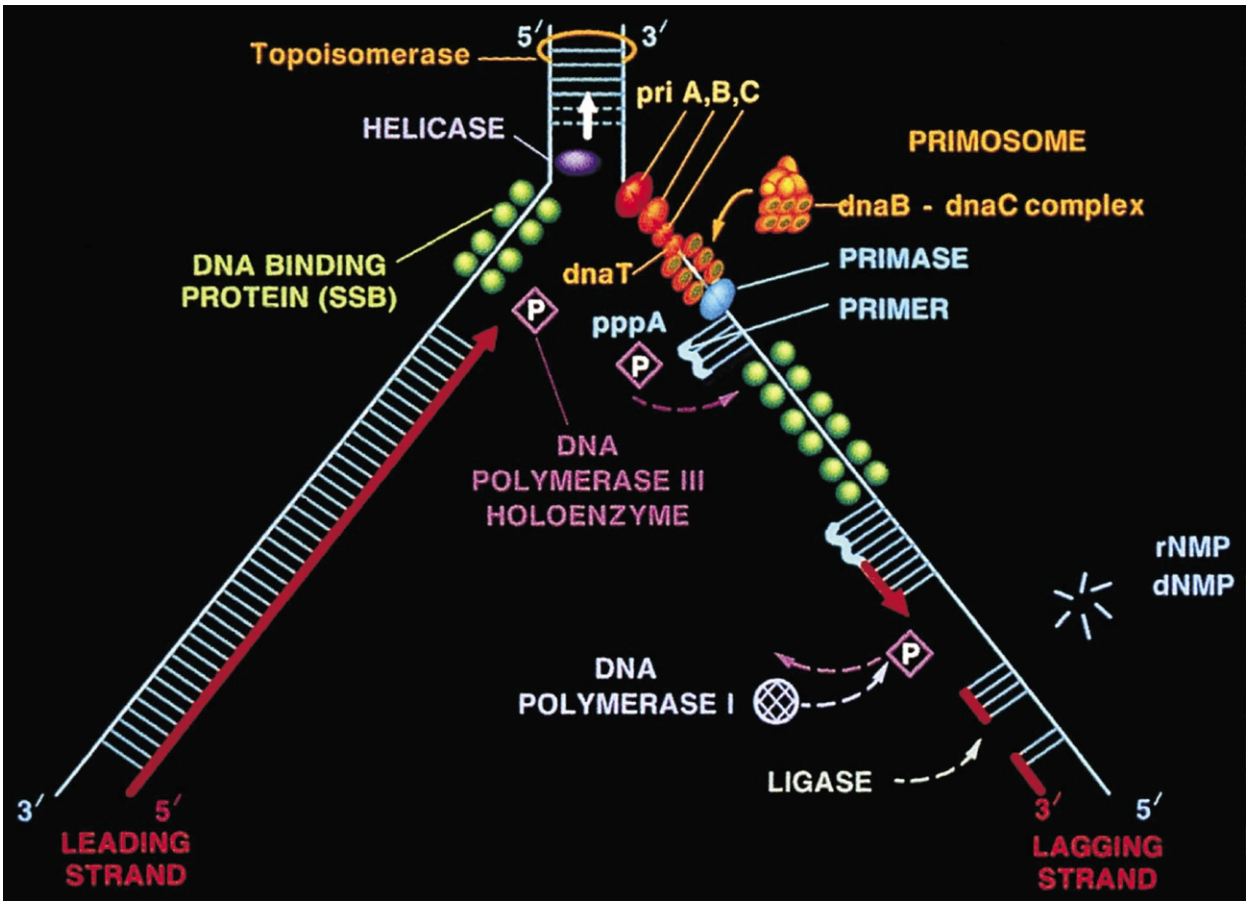


Figure 33. Scheme of DNA replication. The DNA double helix (top) is cut at the replication fork by a topoisomerase enzyme, and unwound by a helicase, the separated strands being coated with single strand DNA-binding protein (SSB). The leading strand (left) is copied into RNA (red) in straightforward way by the enzyme DNA Polymerase III (the "locomotive"). Since nucleic acids can be synthesized only in the 5′–3′ direction, the lagging strand (right) is synthesized by an elaborate mechanism, using RNA intermediates, from short DNA sequences, by DNA polymerase I (the "sewing machine"). These are then linked covalently together by ligases. (Plate 14 in "DNA Replication, Second Edition" (1992) by A. Kornberg & T. Baker, W. H. Freeman and Co., NY; courtesy of Arthur Kornberg).

in chemistry, and even if correct, the biological implications for replication ("semi-conservation replication") as postulated by Crick and Watson, persuasive as they were, did not necessarily follow. The proof came in 1958 from "the most beautiful experiment in biology" (the title of a recent book) by Messelson and Stahl (PNAS, **44**, 1958, 671–675). This demonstrated unequivocally that the complementary strands of a DNA molecule separate from one another, and that each strand then serves as the template for the synthesis of a complementary strand, duplicating its former partner, and so producing two DNA double helices (Figure 32).

Biochemists and biologists generally also began to understand that the Watson–Crick base-pairing allowed an infinite variety of irregular sequences of the four bases of DNA to be accommodated within the double helix, and so it was possible for DNA to act as carrier of genetic information based, on the four letter code A,G,C,T. In 1962, the Nobel Prize for Physiology and Medicine was awarded to Crick, Watson and Wilkins. Rosalind Franklin had died in 1958, so the Nobel Committee were spared the difficulty required by their statutes of limiting the prize to a maximum of three people. The citation reads "for the discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material." Note the word "information", a term that had never appeared in the writings of biochemists, who had been primarily concerned with the transfer of energy in chemical reactions. Indeed, the citation looks forward to the genetic code, research on which was well under way by then.

The aftermath

As is usually the case with a fundamental discovery, the discovery of the DNA structure was only a beginning of a new epoch, the beginning of

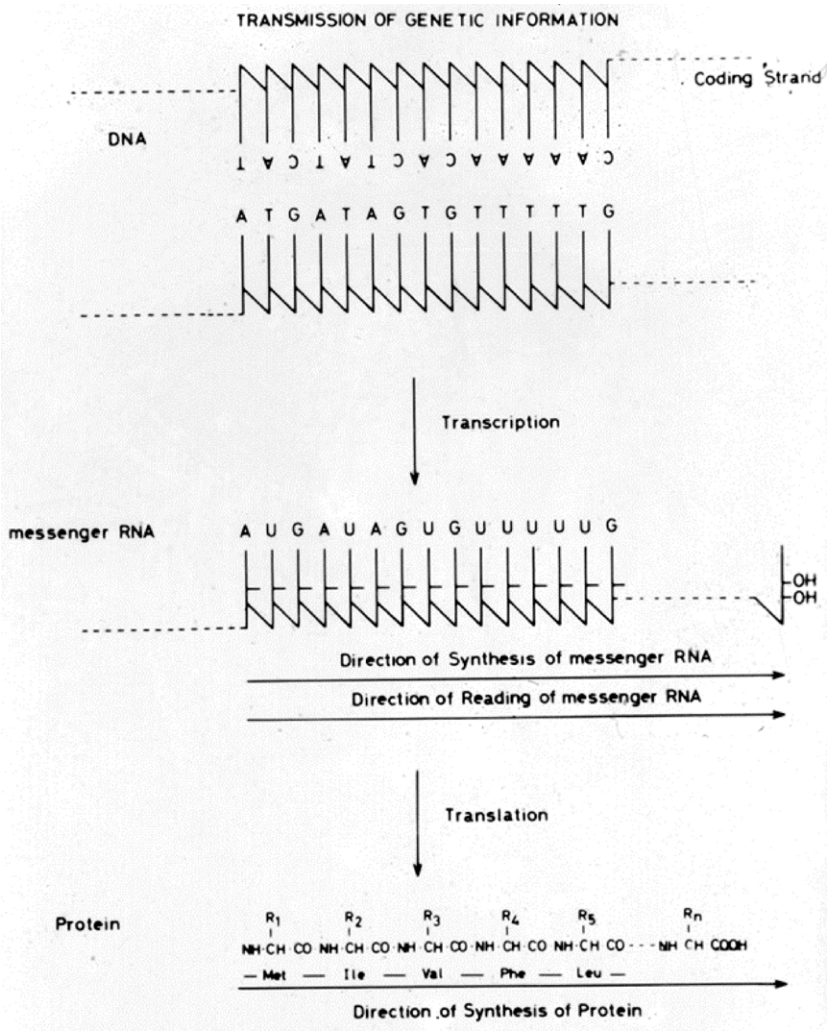


Figure 34. Transcription of the DNA double helix (represented by a two chain ladder) to make messenger RNA, the sequence of which is then translated by the protein synthesis machinery to make a sequence of amino acids, by following the genetic code.

molecular biology. Many major questions arose. I can deal here only briefly with them.

I start with the problem of the replication of DNA. The principle of semi-conservative replication suggested itself to Crick and Watson directly from the structure of the double helix and is startlingly simple. But how does the helix actually unwind, and how does the sequence of each strand get copied? The implementation is startingly complex. Nucleic acids are only synthesized in one direction (5′ to 3′): how then does the antiparallel strand get copied? Again, the work of a generation of biochemists, notably Arthur Kornberg, has shown that it takes dozens of protein complexes, each involving many proteins to accomplish this. They can be thought of as complex components of several giant molecular machines (Figure 33), which synthesize the new DNA, check it for errors, and pass it on for further interactions which package it in chromosomes.

There was a second major question. How does the information carried by the sequence of bases in a DNA molecule get finally transferred into the sequence of amino acids in a protein? The central dogma was formulated by Watson as "DNA makes RNA makes protein", and by Crick as "sequence information can only pass from nucleic acid to protein and not in reverse". This required the genetic code to be worked out (Figure 34) which was largely accomplished by 1962. It has further taken a generation of biochemists to work out the actual biochemical mechanisms involved in transcribing DNA into RNA. The enzyme responsible is RNA Polymerase, of which there are three varieties in eurkaryotes. The enzyme is another complex "molecular machine" whose structure has recently been solved by Roger Kornberg (Kornberg *fils*) (Figures 35 and 36), and this enzyme acts only after a pre-initiation complex, involving dozens of other proteins, has been set up to recruit it to the gene to be transcribed. The product RNA is then processed and passed as a messenger from the cell nucleus to the cytoplasm to ribosomes, the protein factories which synthesize proteins of defined sequence. Here the message contained in the sequence of the nucleic acid is translated into a sequence of amino acids according to the genetic code (Figure 34), and also the polypeptide chain is assembled (Figure 37).

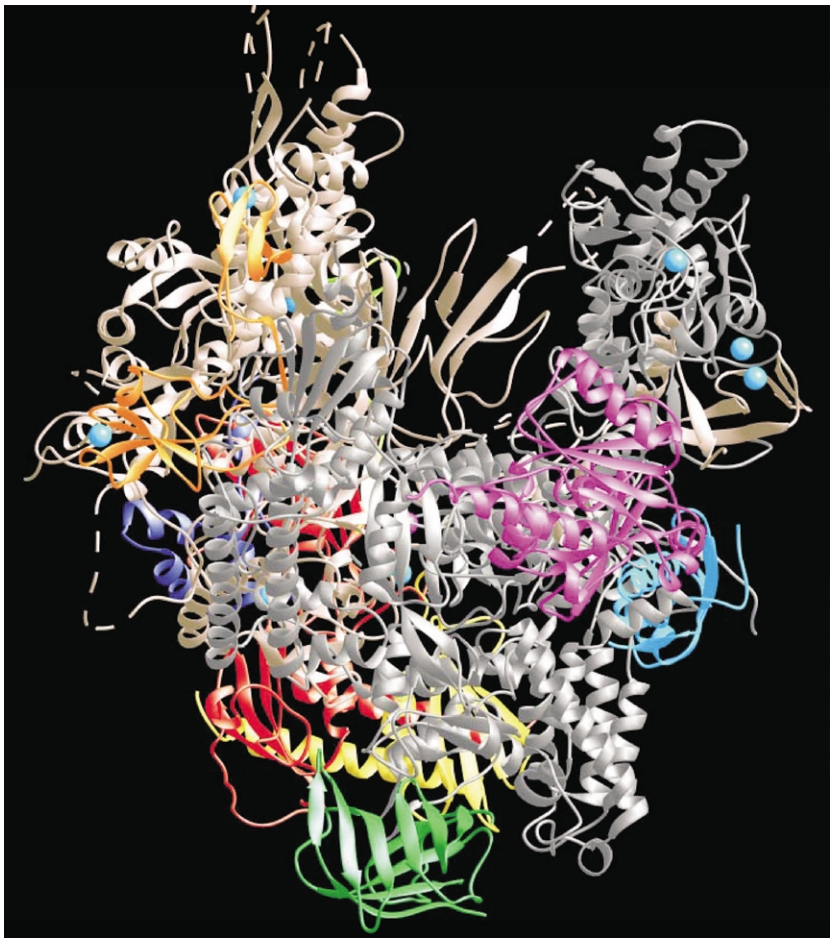


Figure 35. Structure of RNA Polymerase II, the central enzyme of DNA transcription, viewed end on to show the cleft (at the top) for locating the DNA double helix to be transcribed (Cramer *et al*. (2001) *Science* **292**, 1863; courtesy of Roger Kornberg).

Epilogue

The discovery of the double helix and the elucidation of the genetic code launched the new subjects of molecular genetics and, combined with biochemistry, the molecular biology of the gene. There also followed over the 50 years what has been called the genetic revolution in biotechnology but this did not stem directly from the new knowledge. Rather it depended on the development of tools for handling and manipulating DNA. The key methodological advances were Fred Sanger's method of sequencing DNA, and recombinant DNA technology whereby DNA molecules could be cut and pasted together in new combinations. Segments of DNA could be cloned and multiplied in bacteria, and also used to express gene products in them. To these must be added many other powerful methods, for example, the introduction of site specific mutations in DNA, and the polymerase chain reaction which has replaced cloning for many purposes.

Then there have also been great advances in understanding the regulation of gene expression, that is, the switching of genes on and off in the right place at the right time by combinations of protein transcription factors, interacting with the control regions of the gene. In higher organisms the substrate, so to speak, for the expression is not naked DNA, but chromatin in which DNA is packaged in nucleosomes, so there are complex mechanisms for making the control regions accessible to the transcription machinery. Moreover since transcription factors working on a gene are themselves the products of other genes, we really need to understand the networking of genes. This takes us on to the genome, to the human genome project and to the comparative genomes of other organisms. There is much more to find out.

Acknowledgements

I thank Francis Crick, Maurice Wilkins & Raymond Gosling for helpful discussions. This paper is based on my lecture in Cambridge on 25 April 2003 during the 50th Anniversary celebrations of the Double Helix. That lecture was an expanded version of an earlier one I gave in January at Darwin College, Cambridge. I thank Richard Henderson for useful comments on both.

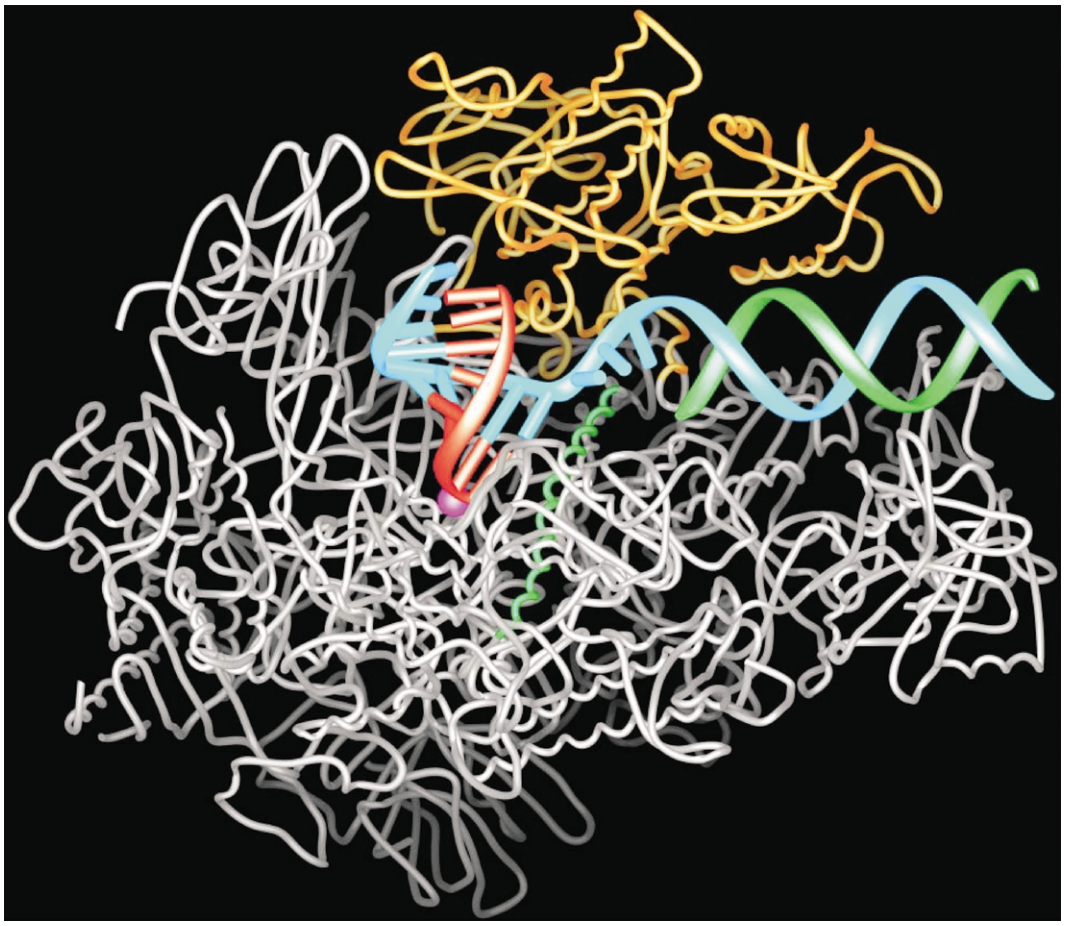


Figure 36. Cut-away side view of a complex of RNA Polymerase II, with a DNA double helix (blue) trapped in the act of transcription. The newly transcribed RNA (red) will exit from the top left-hand corner (Gnatt *et al*. (2001). *Science*, 292, 1876; courtesy of Roger Kornberg).

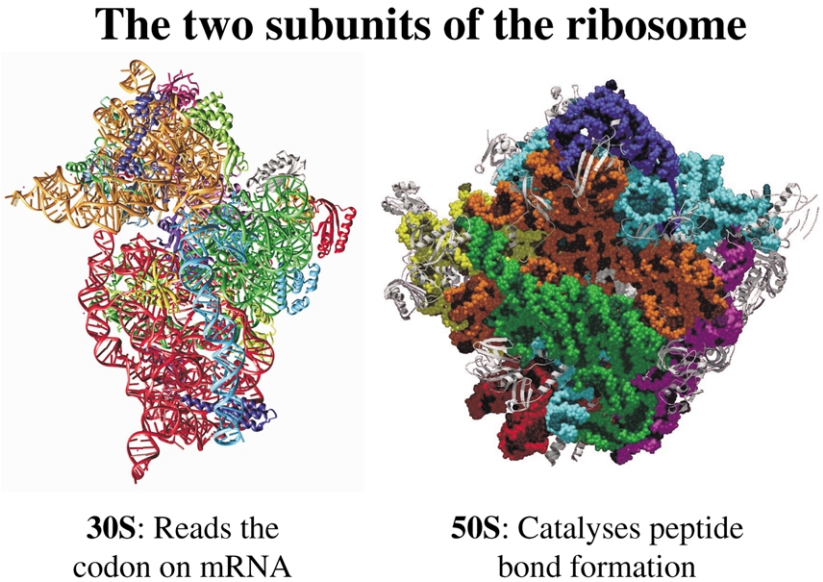


Figure 37. The two subunits of the ribosome. The 30 S subunit with the aid of tRNA, translates the sequence of the messenger RNA into a sequence of amino acids, which are successively assembled into a polypeptide chain. The two subunits are linked physically and functionally by transfer RNA which, with one end, reads the genetic code on the 30 S subunit, and, at its other end, provides an activated amino acid for peptide synthesis on the 50 S subunit. (Courtesy D. Brodersen and V. Ramakrishnan, MRC, Cambridge). From original diagrams: 30 S: Wimberly, B. T., Brodersen, D. E., Clemons, W. M., Jr, Carter, A. P., Morgan-Warren, R. J., Vonrhein, C., Hartsch, T. & Ramakrishnan, V. (2000). Structure of the 30 S ribosomal subunit. *Nature* **407**, 327–339. 50 S: Ban, N., Nissen, P., Hansen, J., Moore, P. B. & Steitz, T. A. (2000). The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* **289**, 905–920.

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(Received 10 November 2003; accepted 10 November 2003)