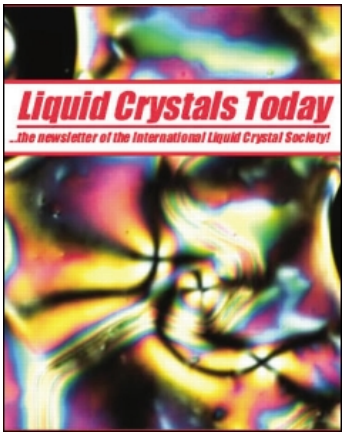

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The DNA double helix—the untold story

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This year marks the 50th anniversary of the Watson–Crick double helix structure for DNA. This year's British Liquid Crystal Society's annual conference was held at Cambridge, and since liquid crystalline phases play an important part of the story of DNA, it was a highly appropriate topic for the concluding lecture of this conference. The lecture is reproduced here.

Preface

Fifty years ago, one lunchtime in late February 1953, there was a remarkable scene in the Eagle, a public house less than a mile away from the lecture room accommodating this conference. There were echoes of the famous literary encounter at the beginning of *The Hitchhikers Guide to the Galaxy* [1] as the peaceful atmosphere was shattered by the noisy arrival of two men. One was a lanky six foot tall American in his early twenties with a shock of unruly hair, the other an Englishman ten years older, with an irritating booming laugh and of such unprepossessing appearance that he had been likened to a bookmaker's tout [2]. This unlikely couple were intimating loudly and immodestly to everyone within earshot that they had solved the secret of life. One can picture anyone there who only wanted a quiet drink before returning to work, sighing, putting down their glasses and slipping out of the back door to escape this manic couple.

The double helix

Jim Watson's book, *The Double Helix*, is the classic account of scientific discovery. It gives the reader an inside view of the way in which the greatest scientific concept of all time became apparent. It tells the story of how a particularly unlikely pair—an American bacteriologist and an English physicist—came to see what every one else had missed.

After that legendary episode in the Eagle, it took some years of patient dotting the i's and crossing the t's (mainly by Wilkins' group at the lab in King's College, London) to prove that the X-ray diffraction evidence was indeed compatible with the double helix structure—but, by then, no one was really interested. The structure had to be correct. It was too beautiful, too elegant to be wrong. It shouted the molecular mechanism of heredity "like a speak-your-weight machine". It was the Pygmalion myth reworked. As Watson and Crick said, "we were only looking for the body—but we got the soul as well".

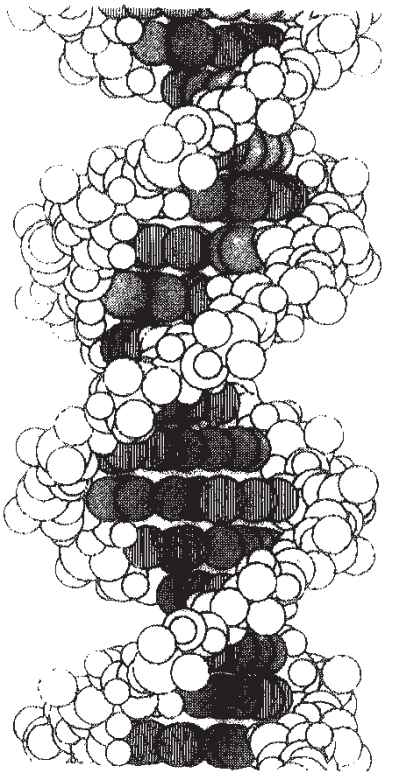

Figure 1. A typical space-filling model of the DNA double helix. (This is the most commonly built model: the B-form.) The paired bases in the centre of the helix are shown shaded and atoms of the sugar phosphate chains are shown as open circles.

The Double Helix reeks of honesty. It may not have been the truth as other players saw it—but it looks like the truth through Jim Watson's eyes—subjective if not objective. It's all there, the way they made fools of themselves with an earlier wrong model, how Erwin Chargaff thought they were incompetent amateurs

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(because they could not tell a purine from a pyrimidine), how they had overlooked the significance of the A:T and C:G ratios right up to the last minute, how Francis clung to the idea of interleaving chains with face-to-face base-pairing. Even the doubtful business of using Rosy's (Rosalind Fraklin) data is spelt out line by line. Over the years there has been plenty of criticism of the ethics of the dynamic duo over this point, but their honesty is never questioned. The benign and indulgent introduction by Sir Lawrence Bragg only adds to the picture of transparent truth, warts and all.

It comes as surprise therefore to find what Francis Crick thought about *The Double Helix*. After seeing one of the drafts he wrote an incandescently angry six-page letter to his old colleague.

"Apart from finding the book an infuriating invasion of privacy, vulgar… and a gross violation of friendship", he wrote, "should you persist in regarding your book as history, I should add that it shows such a naive and egotistical view of the subject as to be scarcely credible… Your book is misleading because it does not in fact convey the atmosphere in which the work was done. Most of the time we were engaged in complicated intellectual discussions concerning points in crystallography and biochemistry." [3]

Why was Francis Crick so angry? What had Jim misrepresented so unforgivably and what were these 'points in crystallography' that had been ignored or glossed over? Jim Watson stressed the pairing of the bases (where his major contribution had been) and virtually ignored crystallographic aspects. He must have seen this as a perfectly justifiable thing to do since a general readership would be able to grasp this point fairly easily, and it was after all, the key to genetics. Crystallographic niceties are not the stuff of best sellers.

If Francis Crick had written *his* popular account (as he was pressed to do) it would have given the story a very different slant. But *The Loose Screw* was abandoned after the first few sentences [4]. Years later he did write an autobiography (*What Mad Pursuit* [5]) but this is a more impersonal account, in which the crystallographic technicalities occupy little more than a dozen lines. You have to burrow fairly carefully through the literature to find exactly what details Francis would have spelt out.

It is a good story: every bit as good as *The Double Helix*. It starts at the beginning of structural studies of DNA, with first diffraction photographs taken in Leeds by William Astbury and Florence Bell in the last few years before the Second World War. Astbury had been one of the elder Bragg's students. His lab pioneered the use of X-ray diffraction for studying biological material. His team examined every piece of structural biological material they could find. Every fibre, tendon, skin, nail, horn or scale known to biology found its way into one of Astbury's X-ray cameras. The laboratory looked like a stage set for act I of Macbeth. And along with everything else, they looked at aligned fibres of DNA. The diffraction photographs they obtained showed a very strong axial reflection corresponding to a repeat distance of 3.4 Å along the axis of the molecules. Astbury and Bell identified this as corresponding to the stacking of the flat purine and pyrimidine bases "like a pile of pennies" along the length of the molecule. But that was as far as they got. The question is repeatedly asked why Astbury, the far-sighted visionary of molecular biology, did not push the investigation further. It would not have taken him long to realise that a single chain molecule would not fit the data. He was so nearly there, and surely the concept of two complementary chains peeling apart to replicate the genetic message is obvious enough. Astbury was always looking for the big picture. He of all people should have seen that the structure of the genetic material was of supreme importance. From our standpoint it is not easy to see why the Nobel Prize for DNA structure should not have been on the cards for that other English/American collaboration over ten years earlier.

However, things were different then. Biochemists are as guilty as any other subgroup of humanity when it comes to rewriting history—or at least not bothering to draw attention to their past errors—and in the early days there were some awful blunders. It is difficult to believe that, within living memory, it was the accepted view in most biochemical circles that the genetic material was protein. There were of course reasons for this belief which looked convincing at the time. Analysis showed that chromosomes contained both nucleic acid and protein. Virtually nothing was known about the functional roles of the nucleic acids. They were seen as unexciting materials with purely structural function, playing a supporting role as scaffolding for the important bits. Proteins on the other hand, were exciting, functional as well as structural. They were clever molecules that could do anything. They were the components of catalysts, transducers and motors—why not information blueprints also? Clearly a molecule carrying a genetic message had to be much more versatile than a linear molecule with only four different subunits.

> "Knowing what we now know from X-ray and related studies of the fibrous proteins—how they can combine so readily with nucleic acid molecules and still maintain the fibrous configuration—it is but natural to assume, as a first working hypothesis at least, that they form the long scroll on

> which is written the pattern of life. No other molecules satisfy so many requirements.''
>
> Astbury and Bell 1938

Early analyses showed that the A, T, C and G bases were present in approximately equal numbers in DNA and this was taken as indicating a polymer assembly where they occurred in a regular sequence (not dissimilar to the more or less regular sequence of amino acids in collagen). The *tetranucleotide hypothesis* of Levene took nearly three decades to play itself out, and arguably, was the main reason why the DNA structure was solved in a British rather than an American University. In spite of Oswald Avery's 1944 paper identifying the 'transforming principle' as nucleic acid, it was not until the Watson–Crick, double helix model was proposed that the whole of the biochemical world finally accepted that it was the histone proteins which had the supporting role and DNA was the star.

There was another factor which confused the issue. At the time of Astbury's and Bell's work, it was not realized that the structure of DNA depends on its state of hydration. In the 1950s Maurice Wilkins and Rosalind Franklin identified two distinct structural forms, the A and B forms. They developed the technique of keeping the specimens at constant humidity whilst recording the X-ray diffraction patterns. Astbury's and Bell's patterns were of a mixture of the two forms, and would therefore have been difficult, if not impossible, to decipher.

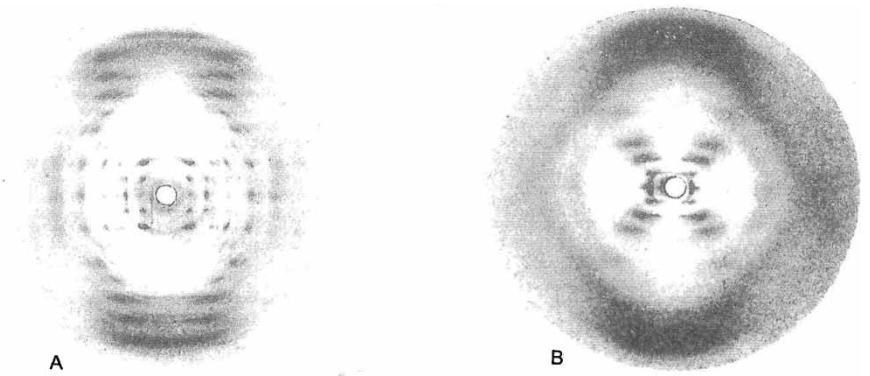

Figure 2. Typical X-ray diffraction patterns of the A and B forms of DNA.

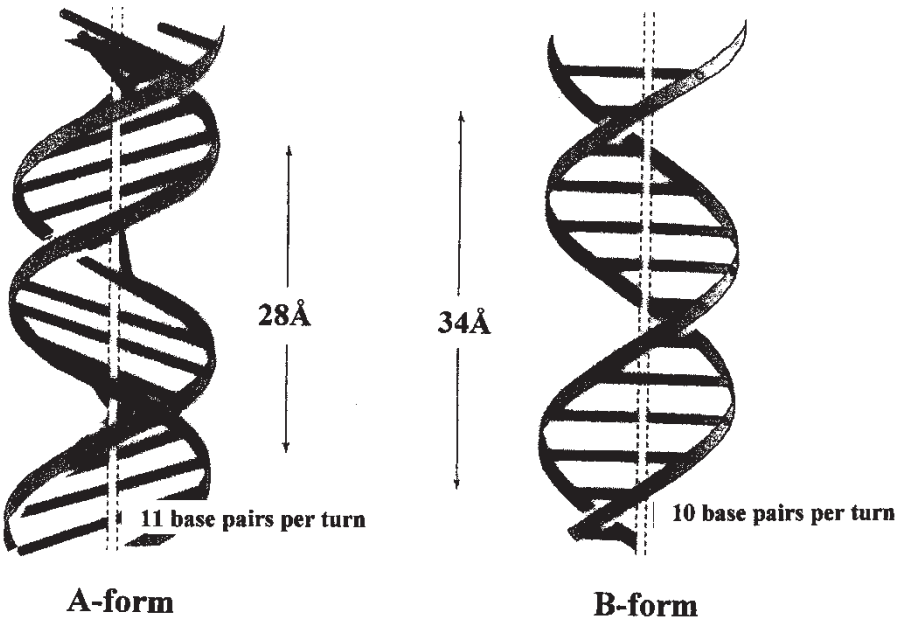

Figure 3. The geometry of the A- and B-forms of DNA. The two forms differ in terms of the helical pitch and the orientation of the bases, but the crucial difference as far as the X-ray diffraction patterns are concerned, is the ordering of the columns in the drawn fibres. In the more hydrated B-form these lie in a nematic array. In the less hydrated and more crystalline A-form, they lie in a hexagonal lattice.

There was a yet further problem. The first generation of X-ray crystallographers treated fibres as though they were composed of crystalline units. They took it as self-evident that the rules which governed how molecules pack in crystals would apply in fibres also. In particular they assumed that where helical macromolecules exist, they must have rotation axes of strict 3-, 4- or 6-fold symmetry, as would be required to fit into a crystalline lattice. It was not until the postulation of the α-helix of proteins by Linus Pauling in 1951 with its non-rational 3.6 residues per turn helix, that it was generally realized that this constraint was unjustified. Helical macromolecules in fibres do not need to have crystallographic symmetry.

Bearing in mind that the mathematical treatment of X-ray diffraction had started at the time of the First World War, an understanding of the diffraction patterns of helical structures appeared late in the day. It finally came in 1951 with the classic paper by Crick, Cochran and Vand, which laid out the Fourier transform of the helix and explained the characteristic X-shaped diffraction pattern. Before this date, even if anyone had obtained an X-ray diffraction pattern of a pure A or B-form of DNA, no one would have known how to interpret it. For Astbury and Bell the time was not yet ripe: there were too many cards stacked against them.

A particular irony is that it was one of the factors that complicated things for Astbury and Bell that made life easy for Watson and Crick at a later date. The existence of two forms of DNA, which made the early patterns difficult to interpret, helped to make the double helix structure clear. To see how this came about requires a consideration of the way in which diffraction patterns of fibres arise.

Up to the 1950s, the traditional way of explaining the diffraction patterns of semi-crystalline materials, like fibres, was to treat them as disordered crystalline solids. You start with the Bragg reflections from a single crystal of the material and modify these according to whatever rotational and positional disorder is considered appropriate. If very small crystallites are involved, add the appropriate line broadening. This approach echoed the then current process of solving structures by X-ray crystallography. You started with the lattice dimensions and angles, added the internal symmetry of the unit cell and solved the phase problem in one way or another. This gave an electron density map, and the final stage of the process was to identify the peaks on this in terms of positions of the atoms and hence the molecular structure.

There was, in principle at least, an alternative way of looking at the problem. You could start with the molecular transform (the diffraction pattern of the individual molecule) and sample this in accordance with the symmetry of the unit cell and then sample this function with reciprocal lattice. The optical transforms produced by Lipson's group at UMIST gave elegant illustrations of this concept [6].

To the eye of a professional crystallographer, the two forms of DNA give radically different diffraction patterns. The less hydrated A-form gave a much more crystalline pattern. Not only are the reflections sharper, but more significantly, they lay on clearly distinguishable layer lines and row lines. With luck they could be indexed. It was for this reason Rosalind Franklin chose to concentrate on the A-form. She determined the dimensions and symmetry of the unit cell and embarked on a routine tedious single crystal approach (of calculating the Paterson map) in an attempt at solving the structure.

One glimpse of the B-form pattern (or rather Jim

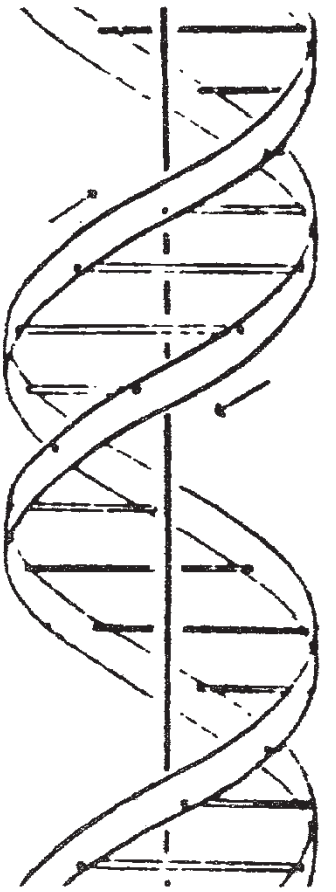

Figure 4. Odile Crick's most famous drawing. Francis Crick's wife was an artist, specialising mainly in figure drawing. This sketch of the anti-parallel 10-fold double helix for the first Watson and Crick paper in *Nature* has become the most the most famous image she created.

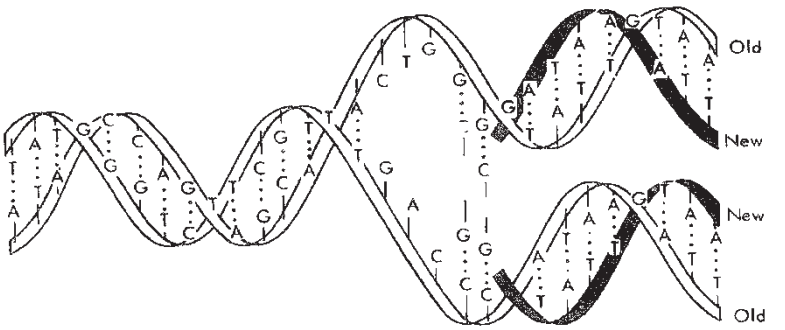

Figure 5. "It has not escaped our notice." This must have been the mental image in the minds of Watson and Crick when they wrote the immortal lines, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

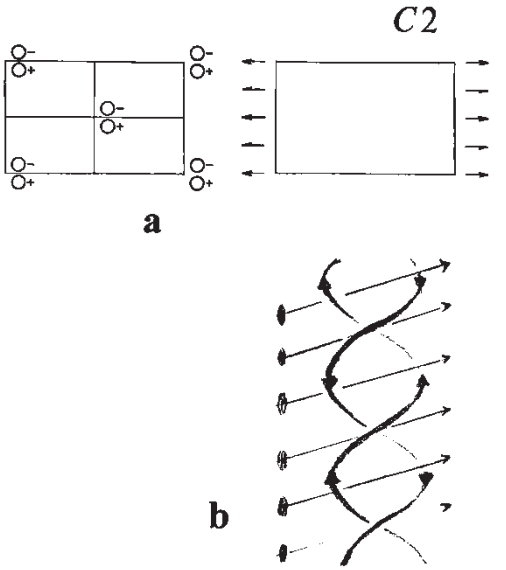

Figure 6. The space group $C2$. From the pattern of absent reflections in the diffraction photograph of A-DNA, Rosalind Franklin deduced that this form had the space group $C2$. (*a*) A typical depiction, in conventional crystallographic symbolic fashion, of the arrangement of symmetry elements in this space group. This projection corresponds to the view down the helix axis. The left-hand figure shows the arrangement of motif units in the unit cell and the right-hand side shows the arrangement of symmetry elements. The + and – symbols refer to distances above and below the plane of the page. They indicate that the motif units occur in pairs (related by the two-fold axes) with one pointing upwards and the other downwards. This is the crucial factor, which Rosalind Franklin overlooked, and Francis Crick saw the significance of. There is an array of two-fold rotation axes (indicated by the double barbed arrow symbols (→). These lie at half-cell intervals (17 Å), at right angles to the fibre axis as indicated in (*b*). Note that the repeat distance along the helix axis requires a complete 360° turn of each strand for an anti-parallel arrangement, but only a 180° turn for a parallel arrangement. When Francis Crick realised this point, he rebuilt the molecular model, doubling the twist of the sugar–phosphate chain.

Watson's description of the pattern after one glimpse) was sufficient to make Francis Crick see things differently. He knew what the molecular transform of

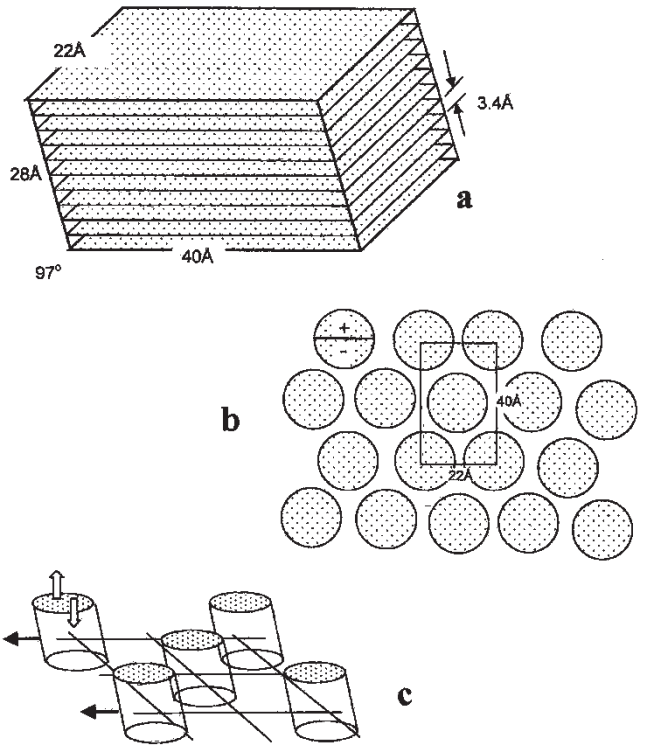

Figure 7. The unit cell of the A-form. (*a*) The dimensions of the monoclinic unit cell of as determined by Rosalind Franklin. The shaded planes indicate the pronounced axial repeat of 3.4 Å, which Astbury and Bell had previously identified with the stacking repeat of the bases. (*b*) The projection of the centred monoclinic cell with $C2$ symmetry drawn to scale and viewed down the fibre axis. Note that the molecular units lie on a lattice with near perfect hexagonal symmetry. (*c*) A perspective view of the unit cell contents, showing the way in which the anti-parallel chains are related by the two-fold rotation axes.

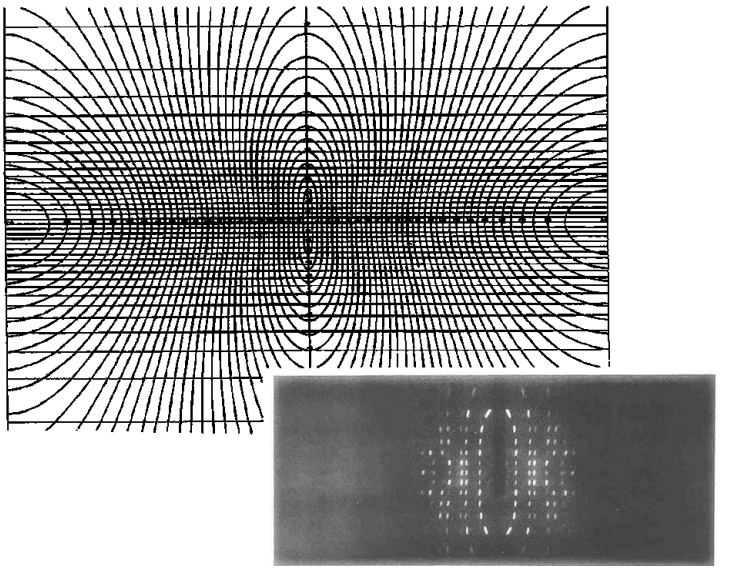

Figure 8. The interpretation of X-ray 'rotation photographs'. This diffraction photograph was obtained by the complete 360° rotation of single crystal about one of its crystallographic axes. It is superimposed on a 'Bernal chart' used in reading reciprocal lattice coordinates and hence indexing the reflections from rotation photographs. Note the distinctive horizontal 'layer lines' and the curved vertical row lines. Compare this figure with the diffraction pattern of the A-form.

a helix looked like—and here was one—handed to him on a plate. The details of the A pattern gave plenty of information, but it concerned the lattice parameters—the packing of the strands rather than the internal structure within a strand. For the B-form it was the other way round. The lateral disorder in the specimen had blurred the reciprocal lattice, leaving the transform of the molecule. Francis Crick was one of the handful of people on the planet able to recognise what the pattern was saying. It said 10-fold helix.

Up to the very last stage in the story, everyone had assumed that the component chains lay parallel, all pointing in the same direction. And no one knew how many chains there were in a single strand. The density measurements were insufficiently accurate to distinguish between two-, three- or even four-chain models (both Watson's and Crick's earlier model and Linus Pauling's model were three-chain assemblies). It was a fortuitous coincidence that led Francis Crick to realize that there were two chains and they ran in opposite directions.

Rosalind Franklin had identified the A-form as having a monoclinic cell with the space group *C*2. This was the same space group as that of oxyhaemoglobin (the material Francis Crick was supposed to be studying). He realized an implication of this symmetry that Rosalind Franklin had overlooked. There must be a two-fold rotation axis lying at right angles to the axis of the chain. This condition could only be satisfied by a chain with an even number of strands lying in an anti-parallel array. Francis Crick must have argued that, since the A- and B-forms can be interconverted simply

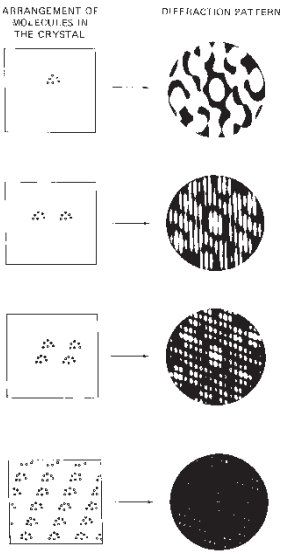

Figure 9. A series of optical diffraction masks and the corresponding diffraction patterns as a two-dimensional analogy for the diffraction of X-rays by molecules. The diffraction masks are shown on the left and the corresponding diffraction patterns on the right. Note how the positioning of the molecules on a lattice creates a diffraction pattern which is the molecular transform sampled only at grid of spots (in crystallographic jargon: the reciprocal lattice). If the crystalline array is not perfect, the reciprocal lattice becomes increasingly blurred and the diffraction pattern becomes more similar to the molecular transform. The A-form of DNA has a highly crystalline structure and the diffraction pattern clearly shows the reciprocal lattice points. In contrast the B-form is much more disordered and the diffraction pattern is virtually a pure molecular transform. (This figure is taken from ref. [6].)

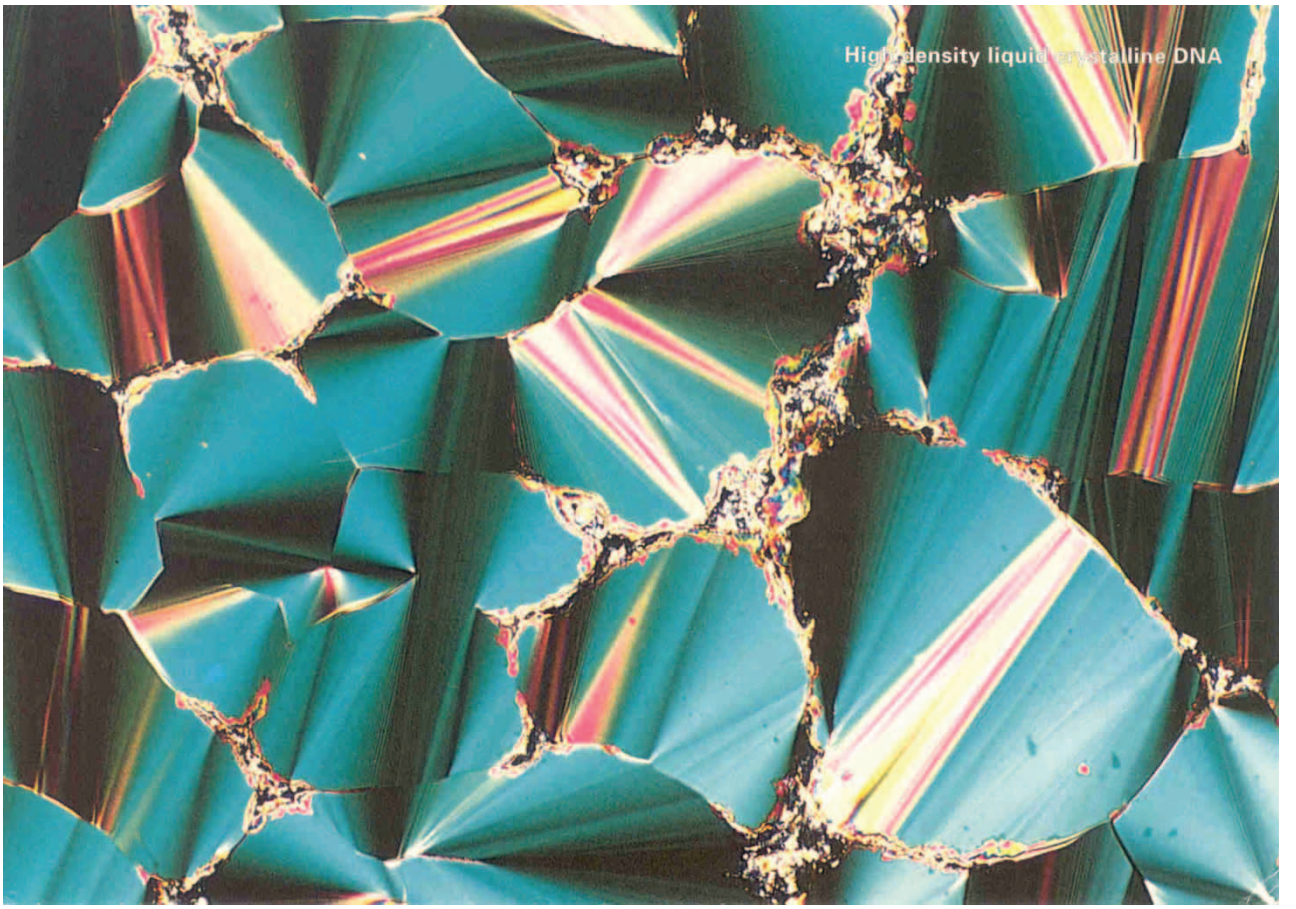

Figure 10. The optical texture of the B-DNA mesophase.

by changing the level of hydration, they must be very similar in structure. It is scarcely credible that the chains could separate and reassemble during an A↔B transformation. If the A-form is an anti-parallel double-stranded structure, the B-form must be also.

There were further important implications of the *C*2 symmetry. The chains must be twisted at twice the rate. The repeat distance for an *anti-parallel* double chain structure is a complete 360° turn of each helix. In contrast, the repeat distance for a *parallel* double-chain structure is 180° turn for each strand.

When these two features had been taken into consideration and a model of the sugar–phosphate chain built, the final piece in the jigsaw was the concept of base pairing, and the rest, as they say, is history.

> While examining oriented films of DNA, I saw in the polarising microscope, extremely thin uniform fibres giving clear extinction between crossed Nicols. I found that the fibres had been produced unwittingly while I was manipulating each gel with a glass rod and on removing the rod, a thin almost invisible fibre of DNA was drawn out like a filament of a spider's web. The perfection and uniformity of the fibres suggested that the molecules in them were regularly arranged. I immediately thought the fibres might be excellent objects to study by X-ray diffraction analysis.
>
> Maurice Wilkins

In this story, as so often in biochemistry, the liquid crystalline state is never far below the surface. DNA [7] (and for that matter, RNA and unpolymerized nucleotides) form chromonic phases. The fibres mentioned by Maurice Wilkins were drawn out of a viscous, liquid crystalline gel. The ordering of the B-form is more or less that of a polymer chromonic N phase and that of the A-form, an chromonic M phase. If chromonic mesophase systems had been studied earlier (as they easily could have been) the A and B polymorphism would have been expected. There is a wonderfully eclectic article in the first volume of *Mol. Cryst. Liq. Cryst.* by Conmar Robinson, describing the cholesteric phase of synthetic polypeptides and the iridescence of beetles where the occurrence of liquid crystalline phases of nucleic acids is mentioned.

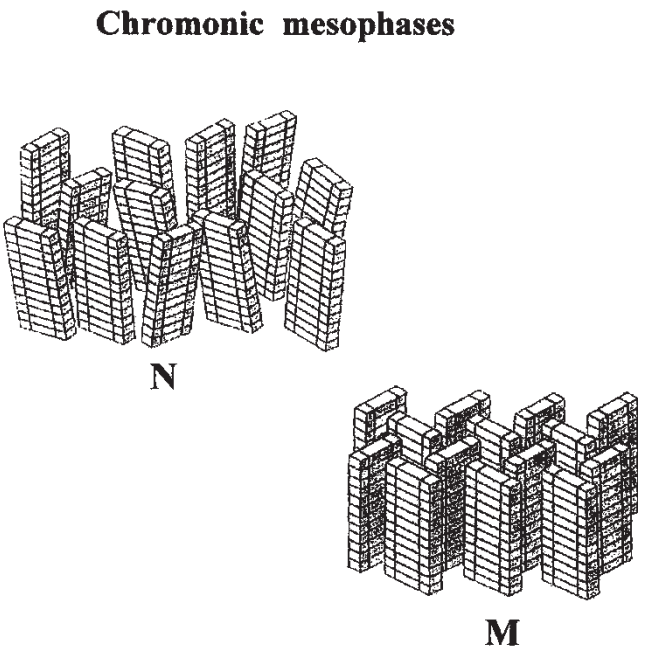

Figure 11. The chomonic N and M phases. Chromonic mesophases are the lyotropic analogues of the thermotopic discotic phases. They are formed by soluble aromatic molecules such as drugs, dyes and nucleic acids. The molecules stack into columns in solution. In the more dilute N phase the columns lie in a nematic array. In the more concentrated M phase the columns form a hexagonal lattice. The B-phase of DNA is essentially a polymerised chromonic N and the A-phase is a polymerised chromonic M.

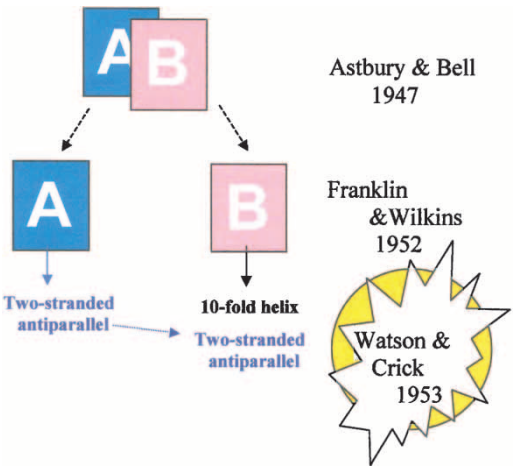

Figure 12. The overall scheme of this paper. It was the existence of the A and B forms of DNA which made Astbury's and Bell's diffraction photographs impossible to interpret (since their photographs were a superposition of both). But ironically, it was the existence of the two forms which made it possible for Watson and Crick to solve the double helix structure by combining information about the anti-parallelism from the A-form pictures, with details of 10-fold helix from the B-form pictures.

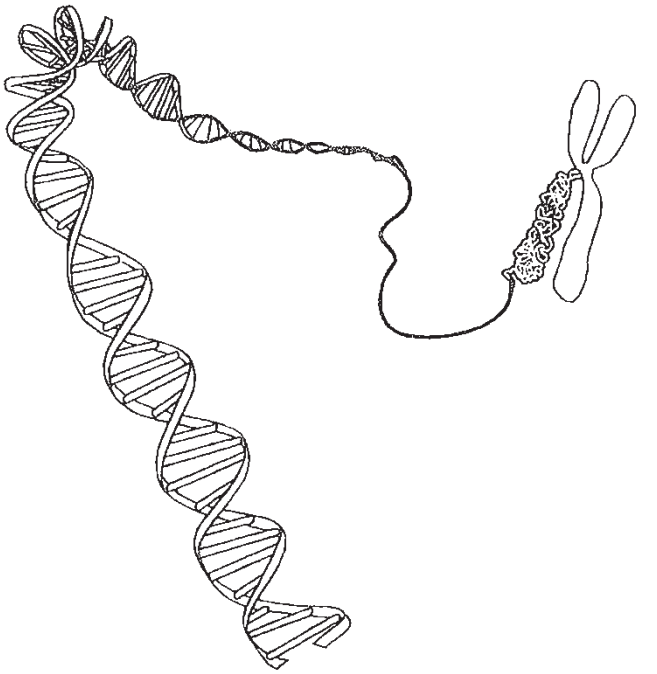

Figure 13. An old prediction and a current picture. A modern biochemistry textbook illustration of the structural hierarchy from the DNA double helix to the chromosome, and a prehistoric prediction.
"It has often been suggested that the hierarchy of coils seen under the microscope in chromosomes actually extends downwards to a molecular spiral."
Pollister 1947

At the beginning, I drew a parallel between the real conversation in the *Eagle* and the surreal conversation in the *Horse and Groom*, commenting on the similarity in response. But in other aspects they could not have been more different. In one the world was about to end; in the other, a brave new world was about to begin. Next time a noisy stranger tries to bend your ear in a pub, remember that there is just an outside chance that he might be worth listening to.

Postscript

I recall once hearing a poem in the style of Longfellow describing the Watson Crick story, but I could not track it down,† so to conclude: this is my attempt at its reconstruction.

†I have since tracked this down. (It's considerably better than my doggerel.) It is by J. Field and was originally published in the *Journal of Irreproducible Results*, 1968, **17**, 53. It is reprinted in *Principles of Nucleic Acid Structure* by Wolfram Saenger (Springer Verlag).

(With apologies to Hiawatha)

Sing the song of Crick and Watson
Wilkins, Franklin in Kings London
Tell of DNA chains spiral
And of ribose sugars (chiral).
Tell of Levene's theory, misbegotten
And three-chain models - best forgotten.
Tell of bases pairing
And our two heroes daring
To outthink the great L Pauling
Giving a structure calling
Loud and clear
The secret of all life, is here!

Notes and references

I have referenced only the more obscure sources. The sources of all other material mentioned can be found in the remarkable book, *The Eighth Day of Creation* by Horace Judson, published by Touchstone, 1979. This highly researched and beautifully written volume contains a wealth of detailed background of the DNA story. Its 600 plus pages read like a novel. Or in the earlier, more historical and less biographical account given in *The Path to the Double Helix* by Robert Olby published by Macmillan 1974.

[1] "Six pints of bitter" said Ford Prefect to the barman of the *Horse and Groom* "and quickly please, the world's about to end."

The barman of the Horse and Groom didn't deserve this sort of treatment, he was a dignified old man. He pushed his glasses up and blinked at Ford Prefect. Ford ignored him and stared out of the window, so the barman looked instead at Arthur who shrugged helplessly and said nothing. So the barman said "Oh yes, sir? Nice weather for it."

Hitchhikers Guide to the Galaxy by Douglas Adams, Chapter 2.

[2] Erwin Chargaff's less than complimentary comment about Francis Crick. His other famously acid quote about our two heroes was on the lines of "Never have two pigmies cast such a long shadow".

[3] This letter is quoted verbatim in *The Eighth Day of Creation* (p. 182).

[4] This was Francis Crick's prospective title. In his autobiography [5], he describes how he made a start at >this. In an echo of the beginning of the double helix, the opening sentence was to be "Jim was always clumsy with his hands. One had only to see him peel an orange." This was more or less as far as he got.

[5] Francis Crick's autobiography. An alternative title, suggested by Sidney Brenner, was *Brighter than a Thousand Jims*.

[6] See for example the beautiful collection of optical diffraction patterns given in Harburn, G., Taylor, C. A., and Welberry, T. R., 1975, *Atlas of Optical Transforms* (London: Bell & Sons).

[7] The elegant studies of Livolant, Levelut and others have extended these investigations.See for example, Livolant, F., Levelut, A. M., Doucet, J, and Benoit, J. P., 1989, *Nature*, **339**, 724.