The molecular configuration of nucleic acids

Nobel Lecture, December 11, 1962

Nucleic acids are basically simple. They are at the root of very fundamental biological processes, growth and inheritance. The simplicity of nucleic acid molecular structure and of its relation to function expresses the underlying simplicity of the biological phenomena, clarifies their nature, and has given rise to the first extensive interpretation of living processes in terms of macromolecular structure. These matters have only become clear by an unprecedented combination of biological, chemical and physical studies, ranging from genetics to hydrogen-bond stereochemistry. I shall not discuss all this here but concentrate on the field in which I have worked, and show how X-ray diffraction analysis has made its contribution. I shall describe some of the background of my own researches, for I suspect I am not alone in finding such accounts often more interesting than general reviews.

Early Background

I took a physics degree at Cambridge in 1938, with some training in X-ray crystallography. This X-ray background was influenced by J. D. Bernal, then at the Cavendish. I began research at Birmingham, under J. T. Randall, studying luminescence and how electrons move in crystals. My contemporaries at Cambridge had mainly been interested in elementary particles, but the organization of the solid state and the special properties which depended on this organization interested me more. This may have been a forerunner of my interest in biological macromolecules and how their structure related to their highly specific properties which so largely determine the processes of life.

During the war I took part in making the atomic bomb. When the war was ending, I, like many others, cast around for a new field of research. Partly on account of the bomb, I had lost some interest in physics. I was therefore very interested when I read Schrödinger’s book "What is Life?" and was struck by the concept of a highly complex molecular structure
which controlled living processes. Research on such matters seemed more ambitious than solid-state physics. At that time many leading physicists such as Massey, Oliphant, and Randall (and later I learned that Bohr shared their view) believed that physics would contribute significantly to biology; their advice encouraged me to move into biology.

I went to work in the Physics Department at St. Andrews, Scotland, where Randall had invited me to join a biophysics project he had begun. Stimulated by Muller's experimental modification, by means of X-radiation, of genetic substance, I thought it might be interesting to investigate the effects of ultrasonics; but the results were not very encouraging.

The biophysics work then moved to King's College, London, where Randall took the Wheatstone Chair of Physics and built up, with the help of the Medical Research Council, an unusual laboratory for a Physics Department, where biologists, biochemists and others worked with the physicists. He suggested I might take over some ultraviolet microscope studies of the quantities of nucleic acids in cells. This work followed that of Caspersson, but made use of the achromatism of reflecting microscopes. By this time, the work of Caspersson and Brachet had made the scientific world generally aware that nucleic acids had important biological roles which were connected with protein synthesis. The idea that DNA might itself be the genetic substance was, however, barely hinted at. Its function in chromosomes was supposed to be associated with replication of the protein chromosome thread. The work of Avery, MacLeod, and McCarty, showing that bacteria could be genetically transformed by DNA, was published in 1944, but even in 1946 seemed almost unknown, or if known, its significance was often belittled.

It was fascinating to look through microscopes at chromosomes in cells, but I began to feel that as a physicist I might contribute more to biology by studying macromolecules isolated from cells. I was encouraged in this by Gerald Oster who came from Stanley's virus laboratory and interested me in particles of tobacco mosaic virus. As Caspersson had shown, ultraviolet microscopes could be used to find the orientation of ultraviolet absorbing groups in molecules as well as to measure quantities of nucleic acids in cells. Bill Seeds and I studied DNA, proteins, tobacco mosaic virus, vitamin B₁₂, etc. While examining oriented films of DNA prepared for ultraviolet dichroism studies, I saw in the polarizing microscope extremely uniform fibres giving clear extinction between crossed nics. I found the fibres had been produced unwittingly while I was manipulating DNA gel. Each time
that I touched the gel with a glass rod and removed the rod, a thin and almost invisible fibre of DNA was drawn out like a filament of 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. I took them to Ray-
mond Gosling, who had our only X-ray equipment (made from war-surplus radiography parts) and who was using it to obtain diffraction photographs from heads of ram spermatozoa. This research was directed by Randall, who had been trained under W. L. Bragg and had worked with X-ray diffraction. Almost immediately, Gosling obtained very encouraging diffraction patterns (see Fig. 1). One reason for this success was that we kept the fibres moist. We remembered that, to obtain detailed X-ray patterns from proteins, Bernal had kept protein crystals in their mother liquor. It seemed likely that the configuration of all kinds of water-soluble biological macromolecules would depend on their aqueous environment. We obtained good diffraction patterns with DNA made by Signer and Schwander which Singer brought to London to a Faraday Society meeting on nucleic acids and which he generously distributed so that all workers, using their various techniques, could study it.

Realization that the Genetic Material was a Pure Chemical Substance and Signs that its Molecular Structure was Singularly Simple

Between 1946 and 1950 many lines of evidence were uncovered indicating that the genetic substance was DNA, not protein or nucleoprotein. For instance, it was found that the DNA content of a set of chromosomes was constant, and that DNA from a given species had a constant composition although the nucleotide sequence in DNA molecules was complex. It was suggested that genetic information was carried in the polynucleotide chain in a complicated sequence of the four nucleotides. The great significance of bacterial transformation now became generally recognized, and the demonstration by Hershey and Chase that bacteriophage DNA carried the viral genetic information from parent to progeny helped to complete what was a fairly considerable revolution in thought.

The prospects of elucidating genetic function in terms of molecular structure were greatly improved when it was known that the genetic substance was DNA, which had a well-defined chemical structure, rather than an ill-defined nucleoprotein. There were many indications of simplicity and regularity in DNA structure. The chemists had shown that DNA was a polymer in which the phosphate and deoxyribose parts of the molecule were regularly repeated in a polynucleotide chain with 3'-5' linkages. Chargaff discovered an important regularity: although the sequence of bases along the poly-
nucleotide chains was complex and the base composition of different DNA’s varied considerably, the numbers of adenine and thymine groups were always equal, and so were the numbers of guanine and cytosine. In the electron microscope, DNA was seen as a uniform unbranched thread of diameter about 20 Å. Signer, Caspersson, and Hammarsten showed by flow-birefringence measurements that the bases in DNA lay with their planes roughly perpendicular to the length of the thread-like molecule. Their ultraviolet dichroism measurements gave the same results and showed marked parallelism of the bases in the DNA in heads of spermatozoa. Earlier, Schmidt and Pattri had studied optically the remarkable ordering of the genetic material in sperm heads. Astbury made pioneer X-ray diffraction studies of DNA fibres and found evidence of considerable regularity in DNA; he correctly interpreted the strong 3.4 Å reflection as being due to planar bases stacked on each other. The electro-titrometric study by Gulland and Jordan showed that the bases were hydrogen-bonded together, and indeed Gulland suggested that the polynucleotide chains might be linked by these hydrogen bonds to form multi-chain micelles.

Thus the remarkable conclusion that a pure chemical substance was invested with a deeply significant biological activity coincided with a considerable growth of many-sided knowledge of the nature of the substance. Meanwhile we began to obtain detailed X-ray diffraction data from DNA. This was the only type of data that could provide an adequate description of the 3-dimensional configuration of the molecule.

*The Need for Combining X-ray Diffraction Studies of DNA with Molecular Model-Building*

As soon as good diffraction patterns were obtained from fibres of DNA, great interest was aroused. In our laboratory, Alex Stokes provided a theory of diffraction from helical DNA. Rosalind Franklin (who died some years later at the peak of her career) made very valuable contributions to the X-ray analysis. In Cambridge, at the Medical Research Council laboratory where structures of biological macromolecules were studied, my friends Francis Crick and Jim Watson were deeply interested in DNA structure. Watson was a biologist who had gone to Cambridge to study molecular structure. He had worked on bacteriophage reproduction and was keenly aware of the great possibilities that might be opened up by finding the molecular
structure of DNA. Crick was working on helical protein structure and was interested in what controlled protein synthesis. Pauling and Corey, by their discovery of the protein α-helix, had shown that precise molecular model-building was a powerful analytical tool in its own right. The X-ray data from DNA were not so complete that a detailed picture of DNA structure could be derived without considerable aid from stereochemistry. It was clear that the X-ray studies of DNA needed to be complemented by precise molecular model-building. In our laboratory we concentrated on amplifying the X-ray data. In Cambridge, Watson and Crick built molecular models.

The paradox of the regularity of the DNA molecule

The sharpness of the X-ray diffraction patterns of DNA showed that DNA molecules were highly regular - so regular that DNA could crystallize. The form of the patterns gave clear indications that the molecule was helical, the polynucleotide chains in the molecular thread being regularly twisted. It was known, however, that the purines and pyrimidines of various dimensions were arranged in irregular sequence along the polynucleotide chains. How could such an irregular arrangement give a highly regular structure? This paradox pointed to the solution of the DNA structure problem and was resolved by the structural hypothesis of Watson and Crick.

The Helical Structure of the DNA Molecule

The key to DNA molecular structure was the discovery by Watson and Crick that, if the bases in DNA were joined in pairs by hydrogen-bonding, the overall dimensions of the pairs of adenine and thymine and of guanine and cytosine were identical. This meant that a DNA molecule containing these pairs could be highly regular in spite of the sequence of bases being irregular. Watson and Crick proposed that the DNA molecule consisted of two polynucleotide chains joined together by base-pairs. These pairs are shown in Fig. 2. The distance between the bonds joining the bases to the deoxyribose groups is exactly (within the uncertainty of 0.1 Å or so) the same for both base-pairs, and all those bonds make exactly (within the uncertainty of 1° or so) the same angle with the line joining the C atoms of the deoxyribose (see Fig. 2). As a result, if two polynucleotide chains are
Watson and Crick built a two-chain molecular model of this kind, the chains being helical and the main dimensions being as indicated by the X-ray data. In the model, one polynucleotide chain is twisted round the other and joined by the base-pairs, the distance between the two chains is the same for both base-pans and, because the angle between the bonds and the C1-C1 line is the same for all bases, the geometry of the deoxyribose and phosphate parts of the molecule can be exactly regular.
Fig. 3. (Left): Molecular model of the B configuration of DNA. The sizes of the atoms correspond to Van der Waals diameters. (Right): Diagram corresponding to the model. The two polynucleotide chains, joined by hydrogen-bonded bases, may be seen clearly.
the sequence of atoms in one chain runs in opposite direction to that in the other. As a result, one chain is identical with the other if turned upside down, and every nucleotide in the molecule has identical structure and environment. The only irregularities are in the base sequences. The sequence along one chain can vary without restriction, but base-pairing requires that adenine in one chain be linked to thymine in the other, and similarly guanine to cytosine. The sequence in one chain is, therefore, determined by the sequence in the other, and is said to be complementary to it.

The structure of the DNA molecule in the B configuration is shown in Fig. 3. The bases are stacked on each other 3.4 Å apart and their planes are almost perpendicular to the helix axis. The flat sides of the bases cannot bind water molecules; as a result there is attraction between the bases when DNA is in an aqueous medium. This hydrophobic bonding, together with the base-pair hydrogen-bonding, stabilizes the structure.

It is essential for genetic material to be able to make exact copies of itself; otherwise growth would produce disorder, life could not originate, and favourable forms would not be perpetuated by natural selection. Base-pairing provides the means of self-replication (Watson and Crick). It also appears to be the basis of information transfer during various stages in protein synthesis.

Genetic information is written in a four-letter code in the sequence of the four bases along a polynucleotide chain. This information may be transferred from one polynucleotide chain to another. A polynucleotide chain acts as a template on which nucleotides are arranged to build a new chain. Provided that the two-chain molecule so formed is exactly regular, base-pairing ensures that the sequence in the new chain is exactly complementary to that in the parent chain. If the two chains then separate, the new chain can act as a template, and a further chain is formed; this is identical with the original chain. Most DNA molecules consist of two chains; clearly the copying process can be used to replicate such a molecule. It can also be used to transfer information from a DNA chain to an RNA chain (as is believed to be the case in the formation of messenger RNA).

Base-pairing also enables specific attachments to be made between part
of one polynucleotide chain and a complementary sequence in another. Such specific interaction may be the means by which amino acids are attached to the requisite portions of a polynucleotide chain that has encoded in it the sequence of amino acids that specifies a protein. In this case the amino acid is attached to a transfer RNA molecule and part of the polynucleotide chain in this RNA pairs with the coding chain.

Since the base-pairs were first described by Watson and Crick in 1953, many new data on purine and pyrimidine dimensions and hydrogen-bond lengths have become available. The most recent refinement of the pairs (due to S. Arnott) is shown in Fig. 2. We now take the distance between C atoms as 10.7 Å instead of the value used recently of 11.0 Å, mainly because new data on N-H...N bonds show that this distance is 0.2 Å shorter between ring nitrogen atoms than between atoms that are not in rings. The linearity of the hydrogen bonds in the base-pairs is excellent and the lengths of the bonds are the same as those found in crystals (these lengths vary by about 0.04 Å).

The remarkable precision of the base-pairs reflects the exactness of DNA replication. One wonders, however, why the precision is so great, for the energy required to distort the base-pairs so that their perfection is appreciably less, is probably no greater than one quantum of thermal energy. The explanation may be that replication is a co-operative phenomenon involving many base-pairs. In any case, it must be emphasized that the specificity of the base-pairing depends on the bonds joining the bases to the deoxyribose groups being correctly placed in relation to each other. This placing is probably determined by the DNA polymerizing enzyme. Whatever the mechanics of the process are, the exact equivalence of geometry and environment of every nucleotide in the double-helix should be conducive to precise replication. Mistakes in the copying process will be produced if there are tautomeric shifts of protons involved in the hydrogen-bonding or chemical alterations of the bases. These mistakes can correspond to mutations.

The Universal Nature and Constancy of the Helical Structure of DNA

After our preliminary X-ray studies had been made, my friend Leonard Hamilton sent me human DNA he and Ralph Barclay had isolated from human leucocytes of a patient with chronic myeloid leukaemia. He was studying nucleic acid metabolism in man in relation to cancer and had pre-
Fig. 4. X-ray diffraction pattern of cephalopod sperm. The DNA molecules in the sperm heads have their axes vertical. The 3.4 Å internucleotide spacing corresponds to the strong diffraction at the top and bottom of the pattern. The sharp reflections in the central part of the pattern show that the molecules are in crystalline array.

pared the DNA in order to compare the DNA of normal and leukaemic leucocytes. The DNA gave a very well-defined X-ray pattern. Thus began a collaboration that has lasted over many years and in which we have used Hamilton’s DNA, in the form of many salts, to establish the correctness of the double-helix structure. Hamilton prepared DNA from a very wide range of species and diverse tissues. Thus it has been shown that the DNA
double-helix is present in inert genetic material in sperm and bacteriophage, and in cells slowly or rapidly dividing or secreting protein (Hamilton et al.\textsuperscript{16}). No difference of structure has been found between DNA from normal and

Fig. 5. X-ray diffraction photograph of DNA fibres (\(\beta\) configuration) at high humidity. The fibres are vertical. The 3.4 Å reflection is at the top and bottom. The angle in the pronounced X shape, made by the reflections in the central region, corresponds to the constant angle of ascent of the polynucleotide chains in the helical molecule. (Photograph with H. R. Wilson; DNA by L. D. Hamilton.)
from cancerous tissues, or in calf thymus DNA separated into fractions of
different base composition by my colleague Geoffrey Brown.

We also made a study, in collaboration with Harriet Ephrussi-Taylor, of
active transforming principle from pneumococci, and observed the same
DNA structure. The only exception to double-helical DNA so far found
is in some very small bacteriophages where the DNA is single-stranded. We
have found, however, that DNA, with an unusually high content of adenine,
or with glucose attached to hydroxymethylcytosine, crystallized differently.

**DNA Structure is Not an Artefact**

It did not seem enough to study X-ray diffraction from DNA alone. Obvi-
ously one should try to look at genetic material in intact cells. It was pos-
sible that the structure of the isolated DNA might be different from that in
*vivo*, where DNA was in most cases combined with protein. The optical
studies indicated that there was marked molecular order in sperm heads and
that they might therefore be good objects for X-ray study, whereas chromo-
somes in most types of cells were complicated objects with little sign of
ordered structure. Randall had been interested in this matter for some years
and had started Gosling studying ram sperm. It seemed that the rod-shaped
cephalopod sperm, found by Schmidt to be highly anisotropic optically,
would be excellent for X-ray investigation. Rinne\(^1\), while making a study
of liquid crystals from many branches of Nature, had already taken diffra-
c tion photographs of such sperm; but presumably his technique was inade-
quate, for he came to the mistaken conclusion that the nucleoprotein was
liquid-crystalline. Our X-ray photographs (Wilkins and Randall\(^2\)) showed
clearly that the material in the sperm heads had 3-dimensional order, i.e. it
was crystalline and not liquid-crystalline. The diffraction pattern (Fig. 4)
bore a close resemblance to that of DNA (Fig. 5), thus showing that the
structure in fibres of purified DNA was basically not an artefact. Working
at the Stazione Zoologica in Naples, I found it possible to orient the sperm
heads in fibres. Intact wet spermatophore, being bundles of naturally ori-
ented sperm, gave good diffraction patterns. DNA-like patterns were also
obtained from T2 bacteriophage given me by Watson.
Fig. 6. X-ray pattern of microcrystalline fibres of DNA. The general intensity distribution is similar to that in Fig. 4 but the diffraction is split into sharp reflections owing to the regular arrangement of the molecules in the crystals. Sharp reflections extend to spacings as small as 1.7 Å. (Photograph with N. Chard; DNA by L. D. Hamilton.)
<table>
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<th>Configuration of molecule</th>
<th>Number of nucleotide pairs per turn of helix</th>
<th>Inclination of base pairs in molecule</th>
<th>Salt</th>
<th>Relative humidity and condition necessary</th>
<th>Crystal class</th>
<th>Crystallinity</th>
<th>Molecular positions</th>
<th>Uni-cell dimensions</th>
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X-ray diffraction analysis is the only technique that can give very detailed information about the configuration of the DNA molecule. Optical techniques, though valuable as being complementary to X-ray analysis, provide much more limited information - mainly about orientation of bonds and groups. X-ray data contributed to the deriving of the structure of DNA at two stages. First, in providing information that helped in building the Watson-Crick model; and second, in showing that the Watson-Crick proposal was correct in its essentials, which involved readjusting and refining the model.

The X-ray studies (e.g. Langridge et al., Wilkins) show that DNA molecules are remarkable in that they adopt a large number of different conformations, most of which can exist in several crystal forms. The main factors determining the molecular conformation and crystal form are the water and salt contents of the fibre and the cation used to neutralize the phosphate groups (see Table 1).

I shall describe briefly the three main configurations of DNA. In all cases the diffraction data are satisfactorily accounted for in terms of the same basic Watson-Crick structure. This is a much more convincing demonstration of the correctness of the structure than if one configuration alone were studied. The basic procedure is to adjust the molecular model until the calculated intensities of diffraction from the model correspond to those observed (Langridge et al.).

As with most X-ray data, only the intensities, and not the phases, of the diffracted beams from DNA are available. Therefore the structure cannot be derived directly. If the resolution of X-ray data is sufficient to separate most of the atoms in a structure, the structure may be derived with no stereochemical assumption except that the structure is assumed to consist of atoms of known average size. With DNA, however, most of the atoms cannot be separately located by the X-rays alone (see Fig. 7). Therefore, more extensive stereochemical assumptions are made: these take the form of molecular model-building. There are no alternatives to most of these assumptions but where there might be an alternative, e.g. in the arrangement of hydrogen bonds in a base-pair, the X-ray data should be used to establish the correctness of the assumption. In other words, it is necessary to establish that the structure proposed is unique. Most of our work in recent years has
Fig. 7. Fourier synthesis map (by S. Arnott) showing the distribution of electron density in the plane of a base-pair in the B configuration of DNA. The distribution corresponds to an average base-pair. The shape of the base-pair appears in the map, but individual atoms in a base-pair are not resolved. (The Fourier synthesis is being revised and the map is subject to improvement.)

been of this nature. To be reasonably certain that the DNA structure was correct, X-ray data, as extensive as possible, had to be collected.

The B configuration

Fig. 5 shows a diffraction pattern of a fibre of DNA at high humidity when the molecules are separated by water and, to a large extent, behave independently of each other. We have not made intensive study of DNA under these conditions. The patterns could be improved, but they are reasonably well-defined, and the sharpness of many of their features shows that the molecules have a regular structure. The configuration is known as B (see also Fig. 3); it is observed in vivo, and there is evidence that it exists when DNA is in solution in water. There are 10 nucleotide pairs per helix turn. There is no obvious structural reason why this number should be integral; if it is exactly so, the significance of this is not yet apparent.
When DNA crystallizes, the process of crystallization imposes restraints on the molecule and can give it extra regularity. Also, the periodic arrangement of the molecules in the microcrystals in the fibre causes the diffraction pattern to be split into sharp reflections corresponding to the various crystal planes (Fig. 6). Careful measurement of the positions of the reflections and deduction of the crystal lattice enables the directions of the reflections to be identified in three dimensions. Diffraction patterns from most fibrous substances resemble Fig. 5 in that the diffraction data are 2-dimensional. In contrast, the crystalline fibres of DNA give fairly complete 3-dimensional data. These data give information about the appearance of the molecule when viewed from all angles, and are comparable with those from single
crystals. Techniques such as 3-dimensional Fourier synthesis (see Fig. 7) can be used and the structure determination made reasonably reliable.

The A configuration

In this conformation, the molecule has nucleotide pairs per helix turn; the helix pitch is 28 Å. The relative positions and orientations of the base, and of the deoxyribose and phosphate parts of the nucleotides differ considerably from those in the B form; in particular the base-pairs are tilted 20° from perpendicular to the helix axis (Fig. 8).

The A form of DNA (Fig. 1) was the first crystalline form to be observed. Although it has not been observed in vivo, it is of special interest because helical RNA adopts a very similar configuration. A full account of A DNA will shortly be available. A good photograph of the A pattern is shown in Fig. 9.

The C configuration

This form may be regarded as an artefact formed by partial drying. The helix is non-integral, with about $9\frac{1}{2}$ nucleotide pairs per turn. The helices pack together to form a semi-crystalline structure; there is no special relation between the position of one nucleotide in a molecule and that in another. The conformation of an individual nucleotide is very similar to that in the B form. The differences between the B and C diffraction patterns are accounted for by the different position of the nucleotides in the helix. Comparison of the forms provides further confirmation of the correctness of the structures. In a way, the problem is like trying to deduce the structure of a folding chair by observing its shadow: if the conformation of the chair is altered slightly, its structure becomes more evident.

The Helical Structure of RNA Molecules

In contrast to DNA, RNA gave poor diffraction patterns, in spite of much effort by various workers including ourselves. There were many indications that RNA contained helical regions, e.g. optical properties of RNA solutions strongly suggested (e.g. Doty's) that parts of RNA molecules resembled DNA in that the bases were stacked on each other and the structure was helical; and X-ray studies of synthetic polyribonucleotides suggested that
RNA resembled DNA (Rich\textsuperscript{22}). The diffraction patterns of RNA (Rich and Watson\textsuperscript{23}) bore a general resemblance to those of DNA, but the nature of pattern could not be clearly distinguished because of disorientation and diffuseness. An important difficulty was that there appeared to be strong meridional reflections at 3.3 Å and 4 Å. It was not possible to interpret these in terms of one helical structure.
In early work, many RNA preparations were very heterogeneous. We thought that the much more homogeneous plant virus RNA might give better patterns, but this was not so. However, when preparations of ribosomal RNA and "soluble" RNA became available, we felt the prospects of structure analysis were improved. We decided to concentrate on "soluble" RNA largely because Geoffrey Brown in our laboratory was preparing large quantities of a highly purified transfer RNA component of soluble RNA for his physical and chemical studies, and because he was fractionating it into various transfer RNA’s specific for incorporation of particular amino acids into proteins. This RNA was attractive for other reasons: the molecule was
unusually small for a nucleic acid, there were indications that it might have a regular structure, its biochemical role was important, and in many ways its functioning was understood.

We found it very difficult to orient transfer RNA in fibres. However, by carefully stretching RNA gels in a dry atmosphere under a dissecting microscope, I found that fibres with birefringence as high as that of DNA could be made. But these fibres gave patterns no better than those obtained with other types of RNA, and the molecules disoriented when the water content of the fibres was raised. Watson, Fuller, Michael Spencer, and myself worked for many months trying to make better specimens for X-ray study. We made little progress until Spencer found a specimen that gave some faint but sharp diffraction rings in addition to the usual diffuse RNA pattern. This specimen consisted of RNA gel that had been sealed for X-ray study in a small cell, and he found that it had dried slowly owing to a leak. The diffraction rings were so sharp that we were almost certain that they were spurious diffraction due to crystalline impurity - this being common in X-ray studies of biochemical preparations. A specimen of RNA had given very similar rings due to DNA impurity. We were therefore not very hopeful about the rings. However, after several weeks Spencer eliminated all other possibilities: it seemed clear that the rings were due to RNA itself. By controlled slow drying, he produced stronger rings; and, with the refined devices we had developed for stretching RNA and with gels slowly concentrated by Brown, Fuller oriented the RNA without destroying its crystallinity. These fibres gave clearly defined diffraction patterns, and the orientation did not disappear when the fibres were hydrated. It appeared that the methods I had been using earlier, of stretching the fibres as much as possible, destroyed the crystallinity. If instead, the material was first allowed to crystallize slowly, stretching oriented the microcrystals and the RNA molecules in them. Single molecules were too small to be oriented well unless aggregated by crystallization. It was rather unexpected that, of all the different types of RNA we had tried, transfer RNA which had the lowest molecular weight, oriented best.

The diffraction patterns of transfer RNA were clearly defined and well-oriented (Spencer, Fuller, Wilkins, and Brown\textsuperscript{24}). These improvements revealed a striking resemblance between the patterns of RNA and A DNA (Fig. 10). The difficulty of the two reflections at 3.3 Å and 4 Å was resolved (Fig. 11): in the RNA pattern the positions of reflections on three layer-lines differed from those in DNA; as a result, when the patterns were poorly
Fig. II. Diffraction pattern of transfer RNA showing resolution of diffraction, in the regions of 3.3 Å and 4 Å, into three layer-lines indicated by the arrows and corresponding to the A DNA pattern. (Photograph with W. Fuller and M. Spencer; RNA by G. L. Brown.)

oriented, the three reflections overlapped and gave the impression of two. There was no doubt that the RNA had a regular helical structure almost identical with that of A DNA. The differences between the RNA and DNA patterns could be accounted for in terms of small differences between the two structures.

An important consequence of the close resemblance of the RNA structure to that of DNA is that the RNA must contain base sequences that are largely or entirely complementary. The number of nucleotides in the molecule is about 80. The simplest structure compatible with the X-ray results consists of a single polynucleotide chain folded back on itself, one half of the chain being joined to the other by base-pairing. This structure is shown in Fig. 12. While we are certain the helical structure is correct, it must be emphasized that we do not know whether the two ends of the chain are at the end of the molecule. The chain might be folded at both ends of the molecule with the ends of the chain somewhere along the helix. It is known that the amino acid attaches to the end of the chain terminated by the base sequence cytosine-cytosine-adenine.
Fig. 12. Molecular model and diagram of a transfer RNA molecule.
Molecular model-building shows that the number of nucleotides forming the fold at the end of a transfer RNA molecule must be three or more. In our model, the fold consists of three nucleotides, each with an unpaired base. It might be that this base-triplet is the part of the molecule that attaches to the requisite part of the coding RNA polynucleotide chain that determines the sequence of amino acids in the polypeptide chain of a protein. It is believed that a base-triplet in the coding RNA corresponds to each amino acid. The triplet in the transfer RNA could attach itself specifically to the coding triplet by hydrogen-bonding and formation of base-pairs. It must be emphasized, however, that these ideas are speculative.

We suppose that part of the transfer RNA molecule interacts specifically with the enzyme that is involved in attaching the amino acid to the RNA; but we do not know how this takes place. Similarly, we know little of the way in which the enzyme involved in DNA replication interacts with DNA, or of other aspects of the mechanics of DNA replication. The presence of complementary base sequences in the transfer RNA molecule, suggests that it might be self-replicating like DNA; but there is at present little evidence to support this idea. The diffraction patterns of virus and ribosome RNA show that these molecules also contain helical regions; the function of these are uncertain too.

In the case of DNA, the discovery of its molecular structure led immediately to the replication hypothesis. This was due to the simplicity of the structure of DNA. It seems that molecular structure and function are in most cases less directly related. Derivation of the helical configuration of RNA molecules is a step towards interpreting RNA function; but more complete structural information, e.g. determination of base sequences, and more knowledge about how the various kinds of RNA interact in the ribosome, will probably be required before an adequate picture of RNA function emerges.

**The Possibility of Determining the Base Sequence of Transfer RNA by X-ray Diffraction Analysis**

Since the biological specificity of nucleic acids appears to be entirely determined by their base sequences in them, determination of these sequences is probably the most fundamental problem in nucleic acid research today. The
number of bases in a DNA molecule is too large for determination of base sequence by X-ray diffraction to be feasible. However, in transfer RNA the number of bases is not too large. The possibility of complete structure analysis of transfer RNA by means of X-rays is indicated by two observations. First, we have observed (Fig. 13), in X-ray patterns of transfer RNA, separate spots each corresponding to a single crystal of RNA. We estimated their size to be about 10μ and have confirmed this estimate by observing, in the polarizing microscope, birefringent regions that probably are the crystals. It should not be too difficult to grow crystals several times larger, which is large enough for single-crystal X-ray analysis.

The second encouraging observation is that the X-ray data from DNA have restricted resolution almost entirely on account of disorientation of the microcrystals in DNA fibres. The DNA intensity data indicate that the temperature factor (B = 4Å) is the same for DNA as for simple compounds. It thus appears that DNA crystals have fairly perfect crystallinity and that, if
single crystals of DNA could be obtained, the intensity data would be ade-
quate for precise determination of all atomic positions in DNA (apart from
the non-periodic base sequence).

We are investigating the possibility of obtaining single crystals of DNA,
but the more exciting problem is to obtain single crystals of transfer RNA
with crystalline perfection equal to that of DNA, and thereby analyse base
sequence. At present, the RNA crystals are much less perfect than those of
DNA. However, most of our experiments have been made with RNA that
is a mixture of RNA’s specific for different amino acids. We have seldom
used RNA that is very largely specific for one amino acid only. We hope
that good preparations of such RNA may be obtained consisting of one type
of molecule only. We might expect such RNA to form crystals as perfect
as those of DNA. If so, there should be no obstacle to the direct analysis of
the whole structure of the molecule, including the sequence of the bases and
the fold at the end of the helix. We may be over-optimistic, but the recent
and somewhat unexpected successes of X-ray diffraction analysis in the nu-
cleic acid and protein fields, are cause for optimism.

Acknowledgements

During the past twelve years, while studying molecular structure of nucleic
acids, I have had so much help from so many people that all could not be
acknowledged properly here. I must, however, thank the following:

Sir John Randall, for his long-standing help and encouragement, and for
his vision and energy in creating and directing a unique laboratory;

all my co-workers at various times over the past twelve years; first,
Raymond Gosling, Alex Stokes, Bill Seeds, and Herbert Wilson, then Bob
Langridge, Clive Hooper, Max Feughelman, Don Marvin, and Geoffrey
Zubay; and at present, Michael Spencer, Watson Fuller, and Struther Arnott,
who with much ability, skill and persistence (often through the night) car-
rried out the X-ray, molecular model-building, and computing studies;

my late colleague Rosalind Franklin who, with great ability and expe-
rience of X-ray diffraction, so much helped the initial investigations on
DNA;

Leonard Hamilton for his constant encouragement and friendly coopera-
tion, and for supplying us with high-quality DNA isolated in many forms
and from many sources; Geoffrey Brown for giving me moral and intellec-
tual support throughout the work and for preparing RNA for X-ray study; Harriet Ephrussi-Taylor for the privilege of collaborating with her in studying crystallization of transforming principle; the laboratory technicians, mechanics and photographers, including P. J. Cooper, N. Chard, J. Hayward, Mrs. F. Collier, Z. Gabor, and R. Lerner, for having played a valuable part in the work at various stages.

I also wish to thank:

the Medical Research Council for their far-sighted and consistent support of our work; King’s College for being our base; I.B.M. United Kingdom Limited and I.B.M. World Trade Corporation and the London University Computer Unit for help with computing; The Rockefeller Foundation and The British Empire Cancer Campaign for financial support; the Sloan-Kettering Institute, New York, and the Stazione Zoologica, Naples, for use of facilities.

More generally, I thank:

Francis Crick and Jim Watson for stimulating discussion; Norman Simmons for having refined techniques of isolating DNA and thereby helping a great many workers including ourselves; many other workers for supplying us with DNA and RNA; and especially, Erwin Chargaff for laying foundations for nucleic acid structural studies by his analytical work and his discovery of the equality of base contents in DNA, and for generously helping us newcomers in the field of nucleic acids.

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