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# Molecular Structure of the Nucleic Acids

ALEXANDER RICH

*Department of Biology, Massachusetts Institute of Technology, Cambridge 39, Massachusetts*

ONE of the most useful, although simplified, views of the role of the nucleic acids in cells is that they function in a manner analogous to a punched tape in a computer. The punched tape and the nucleic acids are very long elements which direct the larger unit, either computer or cell, in which they are located. They both carry information through the linear arrangement of a few fundamental repeating units along their length. The nucleic acids are very long polymeric molecules which are built up by the repeated connection of a very few small molecules. It is generally assumed that the specificity of nucleic-acid function arises from the particular sequence of its constituent residues, as well as from their geometrical configuration.

There are two classes of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), and they differ in structure as well as in function. However, they are made up of similar though not identical chemical units. The fundamental building block of the nucleic acids is the nucleotide. It is a complex molecule consisting of a purine or pyrimidine base, a sugar residue, and a phosphate group. In DNA, the sugar is deoxyribose, while in RNA, it is ribose. These sugars differ by the presence of a hydroxyl group on $C_2'$. Both of the nucleic acids have four types of bases, two purines and two pyrimidines, and three of these are found both in the deoxyribose and in the ribose polymers. DNA and RNA contain the purines adenine (A) and guanine (G), as well as the pyrimidine, cytosine (C) (see Figs. 3 and 4). In addition, RNA has the pyrimidine uracil (U), whereas DNA has the closely related pyrimidine, 5 methyl-uracil (thymine, T). Thus, both of the nucleic acids have a similar chemical composition, and only differ by the presence of a systematic hydroxyl group on each nucleotide of RNA and by the absence of a methyl group on one of the bases. While this description of the chemical composition of the nucleic acids is roughly correct, it should be pointed out that some nucleic acids have modified purine or pyrimidine residues, such as a $-CH_2OH$ group or a glucose residue attached to cytosine in the bacteriophages.

Both ribose and deoxyribose are five-carbon sugars which are in the furanose form—i.e., in the form of a ring involving four of the carbons and one oxygen. The nucleotides of DNA and RNA are connected by the same linkage through the phosphate group which is attached to the $C_3'$ and the $C_5'$ atoms of successive sugar residues. In a schematic way, the polynucleotide chain for both RNA and DNA can be written as shown in Fig. 1. It should be pointed out that the chain in Fig. 1 is asymmetric in that it has a direction which is most easily seen by the sense of the $C_3'-C_5'$ linkage in the sugar residue.

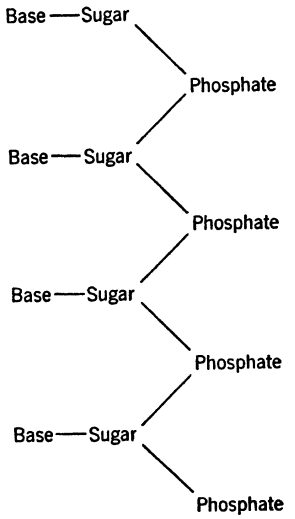


FIG. 1. Diagram of the nucleic-acid backbone. The bases are side chains on the repeating sugar-phosphate polymer chain.

## INFORMATION TRANSFER AND THE NUCLEIC ACIDS

It is generally believed that DNA alone functions as the carrier of genetic information. This understanding is based upon the classic experiments of Avery who discovered bacterial transformation—that is, the ability of purified DNA from one bacterial species to alter the metabolic characteristics of another bacterial species in an inheritable manner. This interpretation of DNA function was further strengthened by the demonstration by Hershey and Chase[1] that DNA alone is the infective component and hence the carrier of genetic information in the bacterial viruses.

All cells of a given organism have the same DNA content. The only exception to this statement is to be found in spermatozoa and ova, where the DNA content is one-half of the normal amount. Further, if one analyzes the chemical composition of the DNA in all tissues of a given animal, it is found to be the same. Thus, it is believed that all cells contain the same set of DNA molecules. The DNA is located in the chromosomal material of the nucleus, and during cell division the DNA is replicated in some manner so that an equal amount is found in the two daughter cells, and with the same chemical composition as that found in the parental cell. One of the goals of molecular structural work in the nucleic acids is to discover the fundamental interpretation for these phenomena.

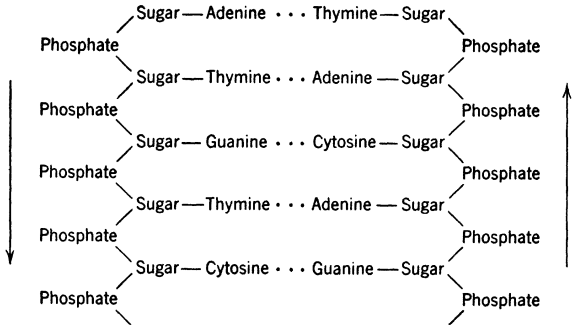


FIG. 2. Schematic diagram of DNA. The two chains are antiparallel, as shown by the arrows. The dotted lines between the bases represent hydrogen bonding. Although the chains are drawn as flat in the diagram, they are actually wound around each other in the molecule.

Although the amount of DNA is the same in each cell of a given organism, the amount varies from species to species. In general, the more complex species have more DNA. A bacterial cell has the order of $10^8$ nucleotides in its DNA, which would make a molecular strand about 2 cm in length. In mammalian species, there are the order of $10^{10}$ nucleotides which corresponds to a total molecular length of 1 to 2 m/cell. Thus, the actual length of the primary coding material in a living cell is in the range of macroscopic dimensions and is much longer than the metabolic machine (or cell) which it directs. The same is often true for the punched tapes which are fed into computers.

## MOLECULAR STRUCTURE OF DEOXYRIBONUCLEIC ACID

One of the most stimulating suggestions in molecular biology was a proposal made by Watson and Crick[2] that the molecular structure of DNA may consist of two polynucleotide chains helically wrapped around each other, with the sugar-phosphate chain on the outside and the purine and pyrimidine bases on the inside. They suggested that the purine and pyrimidine bases from the two chains are joined by hydrogen bonds to form specific pairs. Thus, the adenine residue hydrogen bonds with thymine, and guanine hydrogen bonds with cytosine. These hydrogen-bond pairs are specific in that only these combinations have the necessary stereochemistry to fit into the repeating lattice formed by the regular helical polynucleotide chains. In a schematic way, the DNA molecule is illustrated in Fig. 2 which shows the pairing relationship between the two polynucleotide chains. The arrows indicate the direction of the sugar-phosphate backbone. The two strands are organized in an antiparallel fashion so that the molecule looks the same even if it is turned about by 180°. If one ignores the varied base sequence, the backbone sugar-phosphate chains are organized about a diad axis perpendicular to the fiber axis and passing through the center of each base pair. The pairing of the bases is shown in Figs. 3 and 4. An important feature of the Watson-Crick hypothesis is the identity of the two types of base pairs. That is, the distance between the two sugar-phosphate chains must be the same both for the adenine-thymine pair and for the guanine-cytosine pair. In this way, both base pairs could fit into the helix interchangeably. Pauling and Corey[3] have made a critical survey of x-ray diffraction results obtained from crystals containing purines and pyrimidines. From this, they concluded that the cytosine and guanine residues are probably held together by three hydrogen bonds (Fig. 4), while the adenine-thymine residues are held together by two. The dimensions of the hydrogen-bond pairs suggested by Pauling and Corey are shown in Figs. 3 and 4. Within experimental error, the positions and angles of the two chains relative to the base pairs are identical.

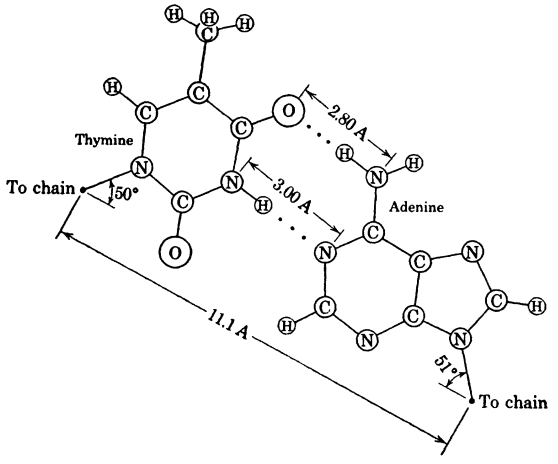


FIG. 3. Diagram showing the hydrogen bonding between adenine and thymine in DNA. The dimensions of the base pair are those discussed by Pauling and Corey.[3]

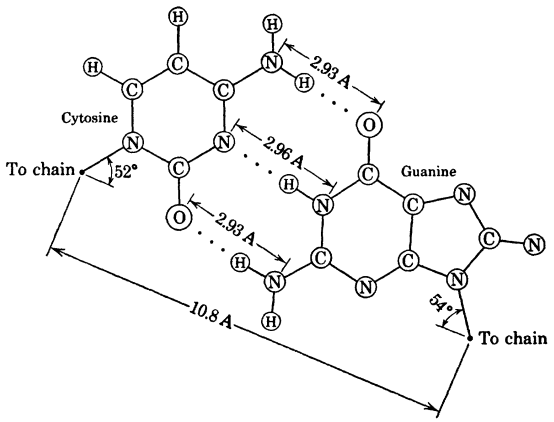


FIG. 4. Diagram showing the hydrogen bonding between guanine and cytosine in DNA. This pair is held together by three hydrogen bonds in contrast to the two found in the adenine-thymine pairing [from L. Pauling and R. B. Corey, Arch. Biochem. Biophys. **65**, 164 (1956)].

Early x-ray diffraction studies by Wilkins[4] and by Franklin,[5] and their collaborators showed that DNA is a helical molecule with 10 residues per turn. The gross features of the diffraction pattern were, at this early stage, shown to be compatible with the double-stranded form proposed by Watson and Crick. Wilkins and his collaborators have continued to carry out extensive studies on the diffraction patterns of DNA, and they are responsible for most of the knowledge concerning the detailed configuration of DNA.[6–8]

The DNA molecule exists in several forms. At lower relative humidities (about 70%), the molecule crystallizes in the *A*-form—that is, a face-centered monoclinic lattice with $a=22.2$ A, $b=40.0$ A, $c=28.1$ A, and $\beta=97.1$ A. This unit cell contains a repeat unit of two DNA molecules with the helical axis along $c$. The water content of this lattice is about 40%, and the bases in it are tilted about 25° from the fiber axis. In this form, the DNA is a true crystal and produces about 100 independent reflections. This implies that there is a high degree of regularity in the structure in all directions.

At higher relative humidities, the *B*-form which is paracrystalline appears with the molecules all parallel to each other but with random rotation about their molecular axes. The layer lines on these paracrystalline diffraction patterns show a continuous distribution of scattering intensity rather than the sharp spots characteristic of a crystalline lattice. In the *B*-form, the fiber-axis repeat is 34.6 A, and there is an extremely strong x-ray reflection on the meridian at 3.4 A. More recently, Wilkins and his collaborators[8] have been able to obtain truly crystalline diffraction patterns of the lithium salt of DNA in the *B*-configuration. In this form, the lithium DNA crystals are orthorhombic and have the dimensions $a=22.7$ A, $b=31.3$ A, and $c=33.6$ A. The axis of the helical molecules is along $c$, and two molecules pass through the unit cell.

The *B*-form of the DNA molecule is shown in Fig. 5 where atoms have been drawn with approximately their van der Waals radii. The base pairs are shown horizontally in the middle of the diagram, and the two sugar-phosphate chains are helically wrapped around the stacked bases. As can be seen in the diagram, there are two helical grooves which go round the DNA molecule. One of them is wider than the other because of the orientation of the sugar-base bonds shown in Figs. 3 and 4. The phosphates on the outside of the molecule are found at a radius of 9 A, and they are just over 7 A apart along a given chain. Wilkins and his co-workers have studied the organization of the polypeptide chain of protamine in a DNA-protamine combination.[7] The polypeptide chain is arranged as a third coaxial helix which fills the narrow groove in the DNA structure. In this position, the positively charged arginine side chains can interact with the negatively charged phosphate groups and stabilize the molecule.

Whenever a parent cell divides, the genetic information in that cell has to be replicated in order to insure continuity of inheritance to the daughter cell. The structural model of DNA suggested to Watson and Crick[9] a method for the replication of this molecule. They felt that the parent molecule could unwind as shown in Fig. 6 so that its two strands separated. These individual strands could then serve as a template for organizing the individual nucleotides which are necessary to make the second strand of the DNA daughter molecules. The template specificity is assured by the specificity of the hydrogen bonds between the purine-pyrimidine base pairs. In this way, a single molecule could twist about its axis, and two daughter helixes would form on the unraveled ends. Although this molecular model of genetic replication has not been established, recent experiments suggest that it is probably correct. This is a good example of the way in which a molecular structure can suggest a molecular mechanism.

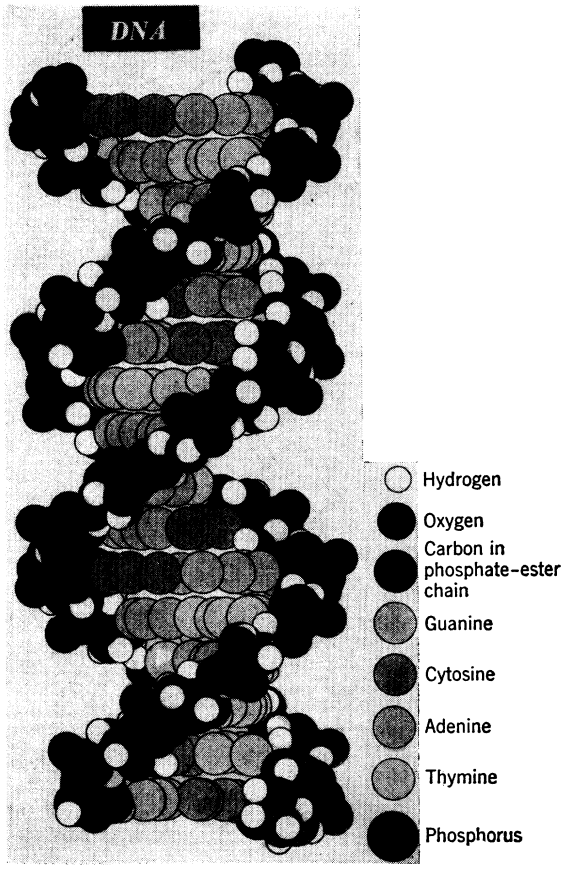


FIG. 5. A drawing of the DNA molecule using solid circles to illustrate atoms. It can be seen that there are two helical grooves of unequal size on the outside of the DNA molecule (after M. Feughelman *et al.*[7]).

## ROLE OF RIBONUCLEIC ACID

In addition to carrying out its replication activities, it is necessary for the DNA molecule, acting as a genetic material, to influence and guide the metabolism of the

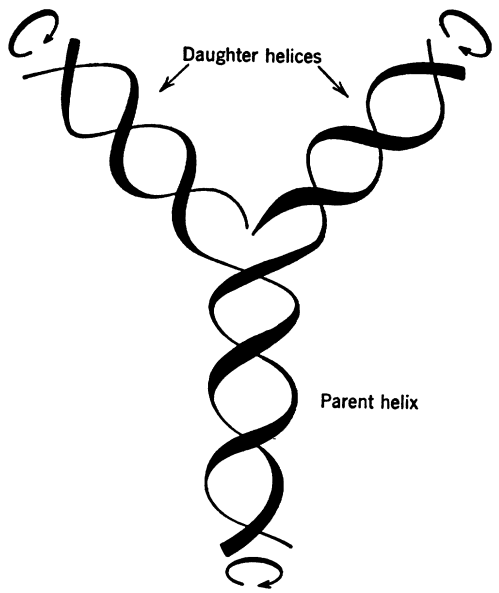


FIG. 6. Diagram showing a possible replication mechanism for DNA. The parent helix unwinds, and the two separated strands serve as sites for forming two daughter molecules. Both parent and daughter helices must wind simultaneously [from M. Delbrück and G. S. Stent in *The Chemical Basis of Heredity*, W. D. McElroy and B. Glass, editors (The Johns Hopkins Press, Baltimore, Maryland, 1957), p. 699].

cell. It is not known for certain how this is carried out. However, there is a large body of indirect information which suggests that this is carried out by using another nucleotide polymer which is somewhat similar to DNA—i.e., ribonucleic acid, which probably acts as an intermediary between DNA and the proteins which are synthesized and constitute the working chemical machinery of the cell.

The relationship between RNA and protein synthesis is not completely worked out at the present time. Proteins appear to be polymerized from their amino acids at the site of the small particulate bodies which are known as microsomal particles. These units are widely distributed in the cytoplasm, and appear as approximately spherical particles with a diameter of 180 A. They usually contain about half RNA and half protein, and it is likely that the RNA component plays a fundamental role in the protein-synthetic process, such as partly or wholly determining the sequence of amino acids. Thus, in order to control protein specificity, the information present in the DNA molecule in its nucleotide sequence may pass through the RNA molecule before ultimately emerging in a particular sequence of amino acids which define a particular protein. If these views are correct, one would like to know how it is that the DNA molecule "makes" RNA and how in turn this RNA molecule organizes amino acids. Unfortunately, at the present time, these questions cannot be answered.

The role of RNA in the metabolic cycle is not simple. In addition to being implicated in protein synthesis, it has also been demonstrated that RNA can function as a carrier of genetic information in a manner very similar to that of DNA. The pure RNA isolated from the tobacco mosaic virus is capable of infecting the tobacco leaf, and this infection ultimately produces a large number of new virus particles which carry the same genetic markers as those of the original virus.[10,11] Thus, RNA has some functions in common with DNA, but also appears to have unique activities as well.

Unfortunately, it is not possible to describe the molecular structure of RNA in a final manner, as is the case for DNA. RNA has been isolated in fibrous form and x-ray diffraction photographs taken of this material show a helical diffraction pattern with a fiber-axis repeat of approximately 26 to 28 A and strong meridional reflections at 3.3 and 4.0 A.[12] However, the diffraction photographs are not of sufficiently high resolution to allow one to make a unique structural interpretation. Experimental work on the structure of RNA offered little hope of achieving a solution to this molecular structural problem until the discovery of an enzyme capable of polymerizing synthetic polyribonucleotides.

## Synthetic Polyribonucleotides

Grunberg-Manago and Ochoa[13] discovered an enzyme which converts nucleotide diphosphates into polyribonucleotides. The enzyme removes the terminal phosphate groups from the diphosphates and assembles the resultant nucleotide residues to form polyribonucleotides. Polymers obtained in this fashion resemble naturally occurring ribonucleic acid in that they have the same covalent ribophosphate backbone, and they have been shown to undergo similar enzymatic hydrolysis.[14] Polymers have been made from all of the purine and pyrimidine bases which are found in RNA. In addition, the enzyme will also make polymers which contain other purine or pyrimidine bases; e.g., polyinosinic acid has been made which contains the purine hypoxanthene, and recently a polymer has been made which contains thymine—i.e., a normal constituent of DNA but not of RNA. These polynucleotide molecules can be made either as pure molecules involving only one residue, or as copolymers involving two or more of the purine-pyrimidine side chains. The similarity between RNA and the synthetic polyribonucleotides can be shown by an x-ray diffraction study of synthetic copolymers, since they produce an x-ray diffraction pattern identical with that of native RNA.[15] This suggests that it might be possible to study the molecular configuration of the synthetic polyribonucleotides and thereby learn something about the configuration of naturally occurring RNA.

The synthetic polyribonucleotides are very reactive molecules. Soon after they were polymerized, it was shown that a complex formed when polyadenylic acid was mixed with polyuridylic acid.[16] Using x-ray diffraction analysis, it was found that these two mole-

cules wrap around each other in solution to form a two-stranded helical molecule very similar to naturally occurring DNA.[17] The discovery of this remarkable interaction has been followed by a variety of similar discoveries among the other polynucleotides. At present, we know of the existence of several of these elongate macromolecules which form two-stranded and three-stranded helical complexes.

### Formation of Synthetic Two-Stranded Helical Molecules

If a dilute salt solution at neutral *p*H contains both polyuridylic acid (Poly U) and polyadenylic acid (Poly A), these two molecules complex together. The reaction is shown schematically in Fig. 7, where the bands represent the polynucleotide chains. On meeting each other, the molecules wrap about to form a two-stranded helix. Evidence for this reaction can be obtained from an x-ray diffraction study of a fiber drawn from a lyophilized mixture of the two polymeric species. The fiber has strong negative birefringence, and produces an x-ray diffraction pattern which has many similarities to a diffraction pattern of DNA. The distribution of scattering intensity is that which is characteristic of a helix: it has a large area on the meridian which is clear, and the scattering intensity is distributed in the form of a "cross" through the origin. The layer-line spacing varies slightly with humidity.[18] However, both DNA and (Poly A+Poly U) have a layer-line spacing of 34 A. This spacing represents the helical pitch of the molecule. From the strong meridional reflections in the region of 3 to 4 A, it can be shown that there are 10 residues per turn of the helix in both DNA and the (Poly-A+Poly-U) molecules. The birefringence of both materials is identical when the (Poly-A+Poly-U) molecules crystallize in a hexagonal lattice with a distance between the molecules of 28.8 A. This is approximately 6 A greater than that observed for the DNA molecule.

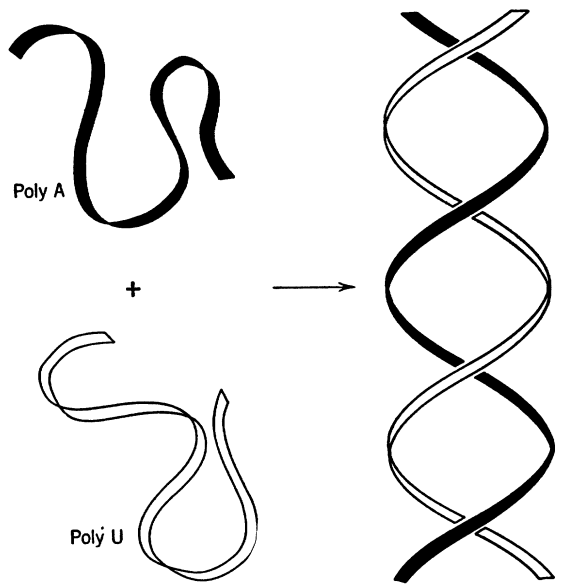


FIG. 7. Diagram illustrating the chemical reaction between the two polymers, polyadenylic acid (A) and polyuradylic acid (U). The irregular contours on the left represent the molecules in a random-coil configuration. After reacting on the right, the molecules are organized into a regular two-stranded helix. The bases connecting the two molecules are not shown in this diagram.

With the exception of the diameter of the molecule, the two diffraction patterns are similar enough to suggest that they arise from a similar helical structure. In the solution, the adenine residues of polyadenylic acid meet with and hydrogen bond onto the uracil residues of polyuridylic acid in a way which is identical to the kind of hydrogen bonding which occurs between adenine and thymine in DNA (Fig. 3). The only difference is that the uracil does not have the methyl group which is found on thymine. However, this does not affect the hydrogen bonding. Since the remainder of the molecule is similar to DNA, it forms the stablest structure possible—i.e., a DNA-like configuration. There is an additional hydroxyl group in the sugar residue of the polyribonucleotides relative to DNA, and this increases the diameter of the molecule slightly through its interaction with the other atoms of the sugar ring. This alters the hexagonal spacing mentioned above.

There are other methods which can be used to study the interaction between these two molecular species. When they react in solution, the optical density at 259 m$\mu$ decreases. This effect has been utilized in a quantitative study of the interaction.[19] A series of mixtures of polyadenylic acid and polyuridylic acid is made wherein the total concentration of phosphate groups remains constant, but the mole ratio of the two species varies continuously. The optical density is measured for this continuous series of solutions and the results are shown in Fig. 8. The dashed line shows the optical density of various mixtures of polyadenylic acid and polyuridylic acid at neutral *p*H in 0.1 molar sodium chloride, plotted as a function of mole ratio. It can be seen that the optical density falls quite sharply, and a minimum is reached at 50% mole ratio when the number of adenine residues in the solution just equals the number of uracil residues. This strongly suggests that a new species is being formed which is a 1:1 mixture of the two polymeric molecules. This interpretation is reinforced by studying this reaction in an ultracentrifuge, since there is an increase in molecular weight and sedimentation velocity when the two molecules combine.

Making careful measurements of the type shown in Fig. 8 for the 1:1 complex, it can be shown that over 95% of the residues have reacted, as judged by the sharpness of the drop in optical density. This is a measure of the high equilibrium constant for the reaction. One of the consequences of this figure is the inference that the reaction must be reversible in order to have all of these residues react.[20]

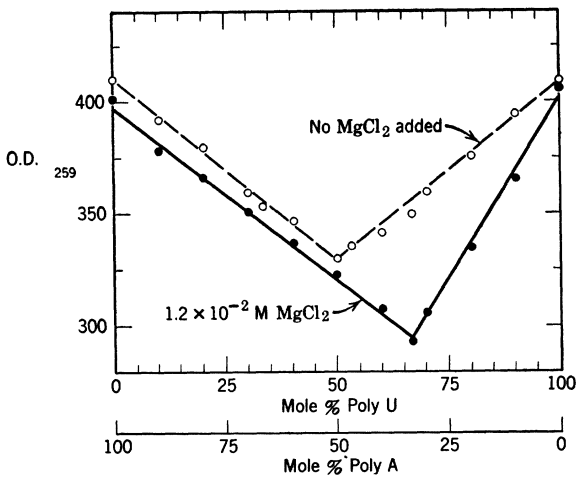


FIG. 8. The optical density of various mixtures of polyadenylic acid (A) and polyuradylic acid (U) at 259 mμ. The total number of moles of polymer is constant for all points, but the ratio of molecular species varies as indicated. All solutions are in 0.1 molar sodium chloride at neutral *p*H. The dashed line shows the formation of a 1:1 complex. The addition of a small number of divalent cations induces the formation of the three-stranded molecule [from G. Felsenfeld and A. Rich, Biochim. et Biophys. Acta **26**, 457 (1957)].

These experiments with mixtures of polyadenylic acid and polyuridylic acid clearly demonstrate the stability of the DNA configuration. In addition, they show that it is possible for the RNA covalent backbone to assume the form of a two-stranded complimentary duplex of the DNA type. This is significant because the work mentioned above on tobacco mosaic virus showed that the RNA molecule from the virus carries the genetic information residing in the virus. The molecule is also probably capable of carrying out the molecular replication necessary to virus multiplication in the leaf. In view of the fact that the RNA backbone can assume the DNA configuration, it seems quite reasonable to assume that the molecular replication of RNA may be carried out by a mechanism very similar to that involved in the molecular replication of DNA.

The reaction between polyadenylic acid and polyuridylic acid was perhaps not completely unexpected in view of the fact that DNA is composed of two strands held together by hydrogen-bonded purine-pyrimidine base pairs. Since uracil is so close to thymine, it is expected that the stability of (Poly A+Poly U) may be related to the stability of DNA itself. However, it is perhaps unexpected to find that it is possible to make a stable two-stranded helical molecule composed of polyadenylic acid and polyinosinic acid—i.e., a molecule very similar to the DNA molecule, except that it has purine-purine base pairs instead of purine-pyrimidine base pairs.

The evidence for this combination parallels that mentioned in the foregoing for polyadenylic acid and polyuridylic acid. When polyadenylic acid is mixed with polyinosinic acid under appropriate conditions, a lowering of the optical density occurs producing a minimum at the 1:1 mole ratio point, just as shown in Fig. 8.[21] Further evidence for the formation of this complex is also seen in ultracentrifuge experiments, since the complex has a larger molecular weight and sedimentation constant than either original molecule.

An x-ray diffraction pattern of a fiber of polyadenylic acid plus polyinosinic acid (Poly A+Poly I) is similar also to the *B*-form of deoxyribonucleic acid. The (Poly-A+Poly-I) molecules crystallize in a hexagonal array on the equator with $a = 24.4$ A. The fundamental screw operation for generating the (Poly-A+Poly-I) helix is a translation of 3.4 A and a rotation of 31.5°, just slightly less than the DNA and the (Poly-A+Poly-U) molecules.

The purine base in polyinosinic acid is hypoxanthine. It is closely related to guanine in that it has an oxygen on $C_6$ of the purine ring, even though it lacks the amino group present in guanine on $C_2$. It is likely that the hypoxanthine is in the keto tautomeric form and that it hydrogen bonds to the adenine residue as shown in Fig. 9. The keto oxygen of hypoxanthine is hydrogen bonded to the amino group of adenine, while the hydrogen on $N_3$ of hypoxanthine is bonded to the corresponding ring nitrogen in adenine. As can be seen when comparing this with Figs. 3 and 4, the hydrogen-bonding system has some similarities to what is observed in DNA. The major difference is the additional imidazole ring present in the hypoxanthine base. The hydrogen bonding shown in Fig. 9 could be used in the naturally occurring nucleic acids if the hypoxanthine base were replaced by guanine, since the additional amino group attached to $C_2$ of the purine ring would not introduce any steric interference.

## Three-Stranded Helical Molecules

It was found that small amounts of divalent salts had a profound effect on the optical density-composition diagram in the Poly-A and Poly-U system.[22] The solid line in Fig. 8 shows the change brought about by making the solution $10^{-2}$ molar in magnesium chloride. A new minimum appears at 67% polyuridylic acid and 33%

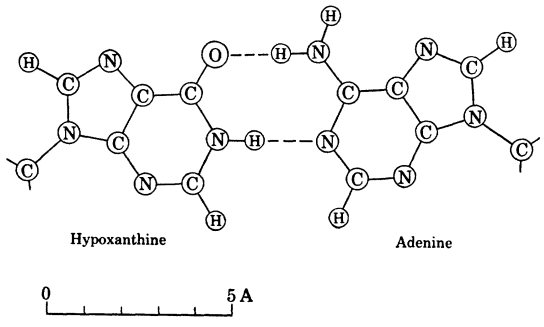


FIG. 9. Diagram showing the hydrogen bonding between the adenine base of polyadenylic acid and the hypoxanthine base of polyinosinic acid [from A. Rich, Nature **181**, 521 (1958)].

polyadenylic acid. The new minimum is quite sharp, indicating that the new complex is quite stable. This was the first indication that a three-stranded complex was forming from two strands of polyuridylic acid and one of polyadenylic acid.

Additional evidence for the formation of the three-stranded molecule is obtained by studying the sedimentation constant of the 1:1 complex as compared with the 2:1. The mean sedimentation constant for the three-stranded complex is about 50% greater than for the two-stranded complex. This increase would be expected if the two-stranded molecule were to take on a third strand. The third strand probably fills the deep helical groove in the two-stranded molecule which is similar to the deep groove seen in DNA. Since it would displace water molecules from that site and would not appreciably alter the frictional forces or shape factor of the molecule, the net density increment of the molecule over the solvent would result in approximately a 50% increment in sedimenting velocity.

It has been suggested that the second uracil residue is hydrogen bonding to the original adenine-uracil pair by forming two strong hydrogen bonds onto $N_7$ and $N_{10}$ of the adenine ring. Such an additional third strand would not involve an increase in radius or helical pitch of the molecule, but could account for the approximately 50% increase in sedimentation velocity. X-ray diffraction photographs have also been obtained from this three-stranded complex.

The kinetics of the formation of the three-stranded molecules from a mixture of two-stranded (Poly-A +Poly-U) molecules and single-stranded Poly-U molecules have been investigated by measuring the optical density at 259 mμ as a function of time. Study of these curves for various concentrations of magnesium chloride or manganese chloride has shown that the reaction is second order for divalent cations; i.e., two divalent cations are present for each triplet of bases.

It is important to note that divalent cations do not have a unique role in forming the three-stranded molecule, since this complex will fully form in sodium-chloride solutions which are 0.7 molar. Thus, it is likely that the cations are necessary to overcome the electrostatic repulsion between the negatively-charged phosphate groups in the three polynucleotide chains. Divalent cations are much more effective than monovalent cations, probably because they form stable complexes with phosphate groups. Nonetheless, they are not necessary, since monovalent cations alone are capable of carrying out this reaction.

In a completely analogous fashion, the two-stranded polyadenylic-acid plus polyinosinic-acid molecule will take on a third strand of polyinosinic acid to become three-stranded.[21] This has been shown both spectrophotometrically as well as in the ultracentrifuge.

Up to this point, the discussion has been concerned with two- and three-stranded molecules composed of

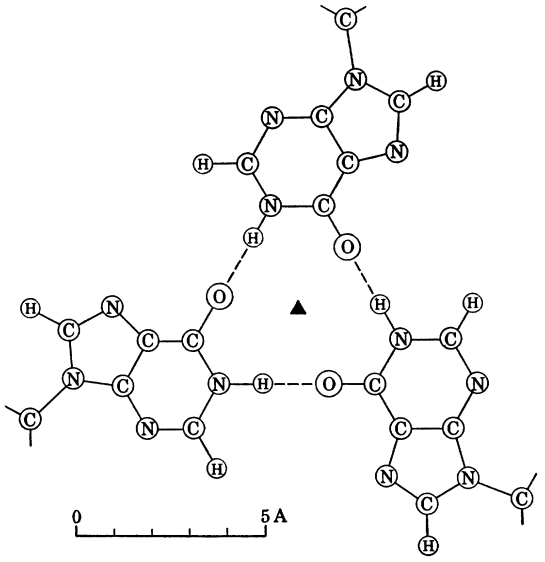


FIG. 10. Diagram showing the hydrogen bonding between three hypoxanthine bases in the three-stranded model of polyinosinic acid. The molecules are organized around a threefold rotation axis [from A. Rich, Biochim. et Biophys. Acta **29**, 502 (1958)].

different kinds of residues hydrogen bonded together. However, polyinosinic acid forms another kind of helical structure which involves only one type of molecule.[23]

If polyinosinic acid is prepared as a high molecular-weight polymer, it can be drawn into an oriented fiber which is negatively birefringent and which produces an unusual diffraction pattern when compared with the diffraction photograph of the mixtures described above. One unusual feature is that the first layer line is found at a spacing of 9.8 A, in contrast to the 30- to 40-A spacings discussed in the foregoing. Even more unusual is the appearance of the second layer line at 5.2 A and of the third intense meridional layer line at 3.4 A. These are nonintegral; i.e., they are not successive orders of one fundamental repeat distance. This feature and the fact that the first and second layer lines do not appear on the meridian of the diagram point uniquely to a helical configuration for the molecule. The meridional reflection at 3.4 A is undoubtedly attributable to the stacking of the purine residues at right angles to the fiber axis in agreement with the negative birefringence. The largest equatorial reflections occur at a spacing of 23.8 A.

This is an example of a helical diffraction pattern which points to a multiple-stranded structure, largely because it will not fit any single-stranded model. A three-stranded model will fit the diffraction data if all three strands are parallel to each other. The bases in this model are organized around a threefold rotation axis, utilizing the hydrogen-bonding system shown in Fig. 10. Here, the three hypoxanthine residues are hydrogen bonded in a cyclic manner, so that the keto oxygen attached to position 6 in the purine ring is hydrogen

bonded to the nitrogen at position 1 on an adjoining ring. The threefold rotation axis has the effect of decreasing the repeat distance along the fiber axis from 29.4 to 9.8 A.

Polyinosinic acid in solution can be converted from an organized helix into a random coil, and vice versa. This alteration is characteristic of all the polynucleotide molecular complexes. There are certain conditions under which they form stable, organized aggregates, and other conditions under which they separate in solution and are no longer organized. It is only when polyinosinic acid is in a single-chain random coil that it is able to react with polyadenylic acid to form the two- and three-stranded molecules already described; if polyadenylic acid and polyinosinic acid are mixed together in high salt concentration, no reaction occurs at all.

Several other combinations of polynucleotides have been studied and are mentioned only. Polyadenylic-acid chains combine with themselves to form two-stranded helixes in which both chains are parallel to each other. Polyinosinic acid, in addition to combining with itself and with polyadenylic acid, will also combine with polycytidylic acid.[24] Recently, it has been possible to synthesize polyribothymidylic acid.[25] This is a polymer with an RNA backbone even though it contains a DNA base. This molecule also combines with polyadenylic acid to form two- and three-stranded molecules, in a manner analogous to the combination with polyuridylic acid.[26]

## DISCUSSION

The synthetic polyribonucleotides are extraordinarily reactive, and they can readily form a variety of multi-stranded helical structures, in some cases with molecules which are all alike, and in other cases with molecules which are different. There are, however, some generalities which emerge from this study which undoubtedly reflect some of the fundamental stabilizing features found in helical nucleic-acid molecules. For example, all of the helical molecules discovered so far have been either two- or three-stranded, but in no case has there been a stable single-stranded molecule. In the structures described in the foregoing, including DNA, the over-all architecture has been similar in that the purine or pyrimidine bases appear to be largely on the inside of the molecule, whereas the charged sugar-phosphate chain is on the outside of the molecule. The bases are hydrogen bonded together, usually with two or occasionally three strong hydrogen bonds holding each base. The molecules are stabilized by the van der Waals packing of the stacked planar purines or pyrimidines, in addition to the hydrogen bonding. It may be that a single-chain helix cannot form in a polyelectrolyte molecule such as the nucleic acids, because of the electrostatic repulsion. When two or more chains are present, the electrostatic repulsion in the helical molecule usually tends to pull the two chains closer to each other since they are coiled. In a single-stranded molecule, the effect of electrostatic repulsion would be only to elongate the molecule and break up any organized helical structure.

All of the synthetic organized helices are formed reversibly, and they can be made to assume a random coil in solution when conditions are appropriately modified. In most cases, a simple reduction of ionic strength is enough to drive the two chains apart; in other cases, altering the *p*H is sufficient. Usually, there is only a given *p*H range over which a multistranded molecule is stable. For example, the polyinosinic-acid helix breaks up when the *p*H is raised above 10 owing to the fact that a proton necessary in the hydrogen-bonding system is removed. Similarly, the other helices are stable only over *p*H ranges in which the necessary tautomeric forms exist. Studies such as these are useful in developing an understanding of the stability of the naturally occurring nucleic acids and, since several of these molecules are similar to DNA, they can be used as model systems for studying nucleic-acid reactivity.

In this regard, an especially attractive hypothesis may be made concerning the three-stranded molecules. It is possible that these may be analogs for the formation of a single-stranded RNA by a two-stranded DNA. DNA has a deep helical groove in it between the two strands. It is this groove which is filled in the (polyadenylic plus polyuridylic) molecule by the oncoming strand of polyuridylic acid, and, as such, it may be an example of a physiologically important type of reaction. Since DNA itself has two kinds of base pairs, there are a total of four different sites on the DNA molecule. These four types of sites may serve as templates for the four kinds of ribonucleotides which must be polymerized together to make the RNA molecule. Schemes such as this have been worked on by several investigators for many years, since it is an attractive and simple method for transferring sequence information from DNA to RNA. However, there has been as yet no convincing demonstration of a detailed molecular mechanism which has the requisite specificity. Further work is necessary to fully evaluate mechanisms of this sort.

Despite the variety of polynucleotide structures which are now understood, the structure of naturally occurring RNA itself remains unknown. There is undoubtedly a difference in the configuration of the RNA which is found in the microsomal particle from that found in the small RNA molecules present in the soluble supernatant. In addition, it is possible that the nuclear RNA is in yet a different configuration. The most interesting configuration for RNA is perhaps that which is found in the microsomal particle. According to the current hypothesis, the nucleotide sequence in this molecule is translated by some means into the amino-acid sequence in the proteins which are synthesized at that site. The configuration of RNA in these particles is indeed an interesting problem, and there is no way of

knowing whether or not the studies on the synthetic polyribonucleotides will yield an answer which is at all applicable to the problem. Nonetheless, these studies are producing a variety of structures and configurations and it can only be hoped that future work will yield promising results in this most interesting aspect of the problem.

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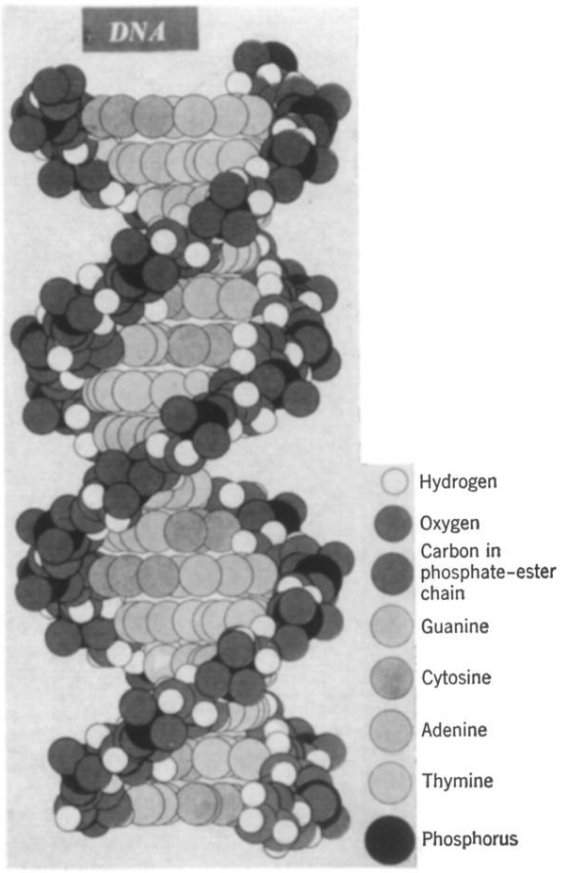


FIG. 5. A drawing of the DNA molecule using solid circles to illustrate atoms. It can be seen that there are two helical grooves of unequal size on the outside of the DNA molecule (after M. Feughelman *et al.*[7]).