The Three-dimensional Structure of Transfer RNA

This nucleic acid plays a key role in translating the genetic code into the sequence of amino acids in a protein. The determination of its structure has clarified the mechanism of protein synthesis

by Alexander Rich and Sung Hou Kim

It is now widely known that the instructions for the assembly and organization of a living system are embodied in the DNA molecules contained within the living cell. The sequence of nucleotide bases along the linear chain of the DNA molecule specifies the structure of the thousands of proteins that are the construction materials of the cell and the catalysts of its intricate biochemical reactions. By itself, however, a DNA molecule is rather like a strip of magnetic recording tape: the information embodied in its structure cannot be expressed without a decoding mechanism.

The development of such a decoding mechanism was one of the crucial events in the origin of life some four billion years ago. A basic biochemical system gradually evolved in which the nucleotide sequence of DNA is first transcribed into the complementary sequence of messenger RNA (abbreviated mRNA). The messenger RNA then directs the assembly of amino acids into the specific linear sequence characteristic of a given protein, a process called translation.

A central role in translation is played by another kind of RNA: transfer RNA (tRNA). The molecules of transfer RNA form a class of small globular polynucleotide chains (as distinct from fibrous polynucleotide chains such as DNA and mRNA) about 75 to 90 nucleotides long. They act as vehicles for transferring amino acids from the free state inside the cell into the assembled chain of the protein. This vital function as an intermediary between the nucleic acid language of the genetic code and the amino acid language of the working cell has made transfer RNA a major subject of research in molecular biology. Recently, in an important step toward the goal of understanding the process of translation in precise molecular terms, the three-dimensional structure of a tRNA molecule has been worked out at high resolution.

The translation of the nucleotide sequence of messenger RNA into protein proceeds in two major steps. First an amino acid molecule is attached to a particular transfer-RNA molecule, a reaction catalyzed by a large enzyme called an aminoacyl-tRNA synthetase. There are many different types of synthetase in living cells, each specific for one of the 20 different amino acids found in proteins. For example, leucyl-tRNA synthetase selectively binds to itself both the amino acid leucine and

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SPACE-FILLING MODEL of yeast phenylalanine tRNA approximates the actual shape of the molecule. It was constructed on the basis of X-ray-diffraction analyses conducted in the authors' laboratories at the Massachusetts Institute of Technology and the Duke University School of Medicine. The polynucleotide chain of tRNA is folded into a compact L-shaped structure. During protein synthesis the amino acid phenylalanine is joined to the end of the horizontal arm of the L. Three nucleotide bases at the end of the vertical arm then recognize the genetic code for phenylalanine on the strand of messenger RNA (mRNA). Finally the amino acid is transferred to the growing protein chain. In this molecular model carbon is black, oxygen red, nitrogen blue, phosphorus yellow and hydrogen white.

FOLDING PATTERN of the polynucleotide chain in yeast phenylalanine transfer RNA is diagrammed. The sugar-phosphate backbone of the molecule is represented as a coiled tube, with the cross rungs standing for the nucleotide base pairs in the stem regions. The short rungs indicate bases that are not involved in base-base hydrogen bonding. The shading refers to cloverleaf diagram on opposite page.
FUNCTION OF TRANSFER RNA in the synthesis of a protein molecule is to make a chain of amino acids that reflects the nucleotide sequence of the template represented by messenger RNA. First a large enzyme called an aminoacyl-tRNA synthetase joins a specific transfer-RNA molecule to its corresponding amino acid with a covalent bond (1-3). The transfer RNA with the amino acid attached to it binds at the A site to the ribosome: the organelle where the amino acids are linked into the polypeptide chain of a protein. This interaction requires specific hydrogen bonding between the three codon bases on the messenger-RNA strand that specify an amino acid and the three anticodon bases of the transfer RNA (4). A transfer-RNA molecule in the adjacent P site then transfers the growing polypeptide chain to the tRNA in the A site (5). The “empty” tRNA leaves the P site and the ribosome moves along the messenger RNA a distance of one codon, so that the transfer RNA carrying the polypeptide chain is shifted from the A site to the P site (6, 7). Then the cycle begins anew.
the tRNA for leucine; a complex of leucine and leucine tRNA is then formed and released. Once a tRNA has an amino acid attached to it, it is ready to participate in the second major step of protein synthesis.

This second step, the joining of the amino acids into a chain, is carried out inside the cellular organelle known as the ribosome, an aggregate of more than 50 different protein molecules and three RNA molecules. The ribosome is an intricate piece of molecular machinery designed to help translate the polynucleotide sequence of messenger RNA into the polypeptide sequence of protein. Although the exact details of the process have not been worked out, its general features are known.

Each amino acid in a protein is specified by a group of three adjacent nucleotide bases, designated a codon, on the messenger-RNA strand. There are four kinds of nucleotide base in messenger RNA, and so there is a total of 4^3 or 64, possible codons. The relation between the codons and the amino acids they specify is the genetic code. The fact that the code appears to be the same in all living organisms is a remarkable proof of the unity of life at the molecular level.

Inside the ribosome are two sites that are involved in translation. One of them is the A site, which stands for amino-acyl-tRNA binding site. It is at this position that the transfer-RNA molecule and its attached amino acid are bound to the ribosome. The tRNA is positioned there partly by a set of specific interactions with the messenger RNA, which has already become associated with the ribosome. Three special nucleotide bases in the transfer-RNA molecule, designated the anticodon, interact with three complementary codon bases in the messenger RNA. The interaction involves the weak directional bonds known as hydrogen bonds, in which a hydrogen atom with a slight positive charge is shared by two other atoms with a slight negative charge. Hydrogen bonding is also the force that holds together the complementary nucleotide bases in the double helix of DNA: the base guanine on one strand of the helix is always paired with the base cytosine on the other strand, and the base adenine is always paired with the base thymine.

Immediately adjacent to the A site in the ribosome is the peptidyl-tRNA binding site, or P site. The transfer-RNA molecule with the growing chain of amino acids attached to it is bound to this site and specifically interacts with the next codon triplet of bases on the messenger-RNA chain. In the course of protein synthesis the growing polypeptide chain is cleaved from the tRNA molecule in the P site and is transferred to the end of the single amino acid attached to

**NUCLEOSIDES**, consisting of a nucleotide base attached to the sugar ribose, are joined by negatively charged phosphate (PO₄) groups to form the polynucleotide chain of transfer RNA. The four major nucleosides in the molecule are adenosine, guanosine, cytidine and uridine. Transfer RNA also incorporates many modified nucleosides, more than 50 of which have been identified. The commonest modification is the replacement of a hydrogen atom by a methyl group (CH₃). This reaction is catalyzed by special enzymes and occurs at the sites indicated by an asterisk. Other structural modifications also occur. For example, the nucleoside pseudouridine (ψ) has its base attached to the ribose through a carbon atom instead of a nitrogen atom.
the tRNA molecule in the A site. Once the transfer has been accomplished (the cleavage and rejoining reactions are carried out by an enzyme in the ribosome) the growing polypeptide chain has been elongated by one amino acid. The "empty" tRNA molecule is then released from the P site, and the messenger RNA and the newly elongated peptidyl-tRNA are shifted from the A site to the P site. A new transfer RNA with an amino acid attached to it now finds its way into the ribosome and becomes lodged in the vacated A site through the specific interaction between its anticodon bases and those of the next codon on the messenger-RNA strand. The system is now back to its starting point, ready to begin another cycle of events in which one more amino acid will be added to the chain. This stepwise addition is repeated until the complete protein has been synthesized.

The process of polypeptide-chain elongation is fairly rapid: it occurs as many as 20 times a second in a bacterial cell and about once every second in a mammalian cell. For example, the hemoglobin molecule is a large protein consisting of four polypeptide chains with about 140 amino acids each. The synthesis of one such chain would take seven seconds in a bacterial cell and two or three minutes in a mammalian cell. Even though this rate of synthesis is fairly high there are surprisingly few errors in translation, because the machinery of the ribosome ensures a careful fit between each transfer-RNA molecule and the messenger RNA. The process is also very efficient, because there are usually several ribosomes at work translating a single strand of messenger RNA.

In order to understand how transfer RNA carries an amino acid into the ribosome and transfers it to the growing polypeptide chain it is essential to have a knowledge of the three-dimensional structure of the tRNA molecule. One of the first clues to that structure emerged from the nucleotide sequence of a yeast tRNA specific for the amino acid alanine, which was determined in 1965 by Robert W. Holley and his colleagues at Cornell University. These workers noted that there were certain regions of the sequence that would be complementary if the chain were folded back on itself. Specifically, these regions could form hydrogen bonds with each other, much like the base pairing in the double helix of DNA (except that in RNA's the base adenine is paired with uracil instead of thymine). The polynucleotide chain of transfer RNA could thus be arranged in such a way that it would contain hydrogen-bonded double-strand regions called stems and nonbonded regions called loops. The postulated combination of stems and loops resembled a four-leaf clover, and so it became known as the cloverleaf diagram.

One feature of the nucleotide sequence of transfer RNA is that it includes many unusual bases, most of them common RNA bases that have been modified by the addition of one or more methyl groups (CH₃). Because of this feature some parts of the cloverleaf diagram have been named for the modified bases that occur in them. For example, the T loop is so named because it includes thymine (T), which is found in DNA but is not found in any RNA species other than transfer RNA. Similarly, the D loop usually includes the modified base dihydrouracil (D). Other regions of the cloverleaf are the variable loop, which in different tRNA's has different numbers of nucleotides (ranging from four to 21), the anticodon loop, which includes the three bases of the anticodon, and the acceptor stem, which accepts the amino acid specific to that particular tRNA.

An interesting feature of the cloverleaf diagram is the presence of nucleotide sequences that are constant in all 100 of the tRNA sequences that have been determined so far. The number of base pairs in the stem regions is also constant: seven in the acceptor stem, five in the T stem, five in the anticodon stem and three or four in the D stem. These features are maintained in tRNA molecules from plants, animals, bacteria and viruses. Indeed, the pattern of stems, loops and constant nucleotides found in the tRNA cloverleaf appears to have the same universality as the genetic code. Much of the explanation for this constancy was later provided by the three-dimensional structure of tRNA.

Today the three-dimensional structure of large biological molecules is commonly determined by means of the X-ray-diffraction analysis of molecular crystals. A molecular crystal is an assembly of molecules packed together in a regular three-dimensional array. When X rays with a wavelength comparable to the distance between atoms are directed into the crystal, they are diffracted, or scattered, in a variety of directions by the electron clouds of the atoms in the crystal lattice. The diffraction pattern of the crystal can be detected as a series of spots on a piece of X-ray film, with the blackening of the emulsion being proportional to the intensity of each scattered beam.

This pattern contains a great deal of information about the addition of new parts to the crystal. For one thing, the amplitude of the wave scattered by an atom is proportional to the number of electrons in the atom, so that a carbon atom will scatter...
DETAILED SKELETAL MODEL of yeast phenylalanine tRNA shows the hydrogen-bonding interactions between the nucleotide bases. It was derived in 1974 from an X-ray-crystallographic study at a resolution of three angstroms. Projection shown here was generated on a computer by one of the authors (Kim). Ribose-phosphate backbone of the molecule is shaded in color; the bases are shaded in gray.
X rays six times more strongly than a hydrogen atom. Secondly, the scattered waves recombine inside the crystal lattice; depending on whether they are in phase or out of phase, they will either reinforce or cancel one another. The way the scattered waves recombine depends only on the arrangement of the atoms in the crystal, and so it is possible to reconstruct the image of a molecule from its diffraction pattern.

To analyze the three-dimensional structure of a large protein or nucleic acid molecule a crystal of the substance is first prepared. Then the crystal is mounted in a capillary tube and positioned in a precise orientation with respect to the X-ray beam and the film. The crystal is rotated along each of its axes to yield a series of X-ray photographs in which there is a regular array of spots of various intensities. Each of these photographs is actually a two-dimensional section through a three-dimensional array of spots.

Next the intensities of all the spots in the diffraction patterns are measured, either from the film or through the use of a Geiger counter. Additional information is needed, however, before one can establish the three-dimensional structure, namely the phases of the scattered X-ray beams with respect to an arbitrary fixed point in the crystal. This information is obtained by inserting heavy-metal atoms such as those of platinum or gold into the crystal lattice as markers. The addition of these atoms changes the diffraction pattern slightly and enables one to calculate the phases of the diffracted beams.

With this information in hand it is possible to calculate the density of the electrons at a large number of regularly spaced points in the crystal, making use of a Fourier series: a sum of sine and cosine terms. A high-speed computer is needed to handle the enormous number of terms (more than a billion) involved in determining the structure of a large protein or nucleic acid molecule. The first such molecule whose structure was determined in this way was the protein myoglobin; the feat was accomplished in 1958. Today the technique is almost routinely exploited for the structural analysis of large molecules.

The end product of the technique is a three-dimensional map showing the distribution of the electron density in the molecule. For example, a map at a resolution of six angstroms, derived from the innermost spots of the diffraction pattern, may reveal the general shape of the molecule but few additional structural details. (An angstrom is $10^{-10}$ meter, about the diameter of a hydrogen atom.) Maps of higher resolution are needed to delineate groups of atoms, which may be three to four angstroms apart, or individual atoms, which are from one to two angstroms apart. A large molecule is usually analyzed at different levels of resolution, making it possible to visualize different features of the structure. The ultimate resolution of an X-ray analysis, however, is determined by the degree of perfection of the crystal. For large biological molecules the best resolution one can usually obtain is about two angstroms.

With transfer RNA the first step of the process—crystallizing the molecule—turned out to be a major hurdle. In 1968 our group at the Massachusetts Institute of Technology and workers in five other laboratories discovered that it was possible to crystallize different species of tRNA by dissolving them in various mixtures of solvents and allowing the solvents to evaporate slowly. This advance caused great excitement among molecular biologists, since it seemed that the major hurdle had been overcome and that the three-dimensional structure of transfer RNA was within reach. Our elation was soon followed by some degree of despair when it was realized that although many different species of tRNA had been crystallized, most of the crystals were quite disordered. As a result the crystals provided diffraction patterns with very low resolution (usually between 10 and 20 angstroms) and hence could reveal little of the detailed structure of the molecule. Although it was exciting to discover that tRNA was crystallizable, it was frustrating to realize that further work had to be done before suitable material was available for X-ray-diffraction analysis.

Together with Gary J. Quigley and Fred L. Suddath we made a concerted
effort to find conditions where tRNA would form a well-ordered crystal that would produce an X-ray-diffraction pattern with sufficient resolution to reveal the three-dimensional structure of the molecule. For two years we surveyed a large number of different tRNA species and crystallizing conditions. Finally we made an important discovery: the addition of spermine, a small positively charged molecule, resulted in the formation of a highly ordered crystal of a tRNA extracted from yeast cells that was specific for the amino acid phenylalanine. The spermine-stabilized crystal had a diffraction pattern that extended out to a resolution of nearly two angstroms.

Late in 1972, working with Alexander McPherson, Daryll Sneden, Jung-Ja Park Kim and Jon Weinzierl, we obtained an electron-density map of the crystal in which we were able to trace the backbone of the polynucleotide chain of the tRNA at a resolution of four angstroms. At that resolution it was not possible to perceive the individual bases of the polynucleotide chain, but the electron-dense phosphate (PO₄) groups along the backbone of the molecule could be seen as a string of beads coiled in three-dimensional space. To our great surprise the polynucleotide chain was organized in such a way that the molecule was shaped like an L, with one arm of the L made up of the acceptor stem and the T stem and the other arm made up of the D stem and the anticodon stem. The complementary hydrogen-bonded sequences that had been identified in the cloverleaf diagram were clearly seen as RNA double helixes. The various loops occupied strategic positions either at one end of the molecule or at the corner of it, where the T and D loops were coiled together in a complex manner.

This folding of the molecule was entirely unexpected. Over the preceding few years a number of investigators had recognized the features common to the cloverleafs of all transfer RNA’s and had tried to predict how the tRNA molecule might be folded. As is so often the case, however, nature proved to be subtler than had been imagined. The L-shaped folding served to explain a number of chemical observations that had accumulated, and it also made people wonder what functional purpose was served by this unusual shape.

By mid-1974, together with Joel L. Sussman, Andrew H.-J. Wang and Nadrian C. Seeman, we had interpreted the electron-density map at a resolution of three angstroms. The overall form of the molecule was the same as the one apparent at four angstroms, but now many more details were visible, including the positions of most of the nucleotide bases. At about this time Jon Robertus, Brian F. C. Clark, Aaron Klug and their colleagues at the British Medical Research Council Laboratory of Molecular Biology in Cambridge described their X-ray-crystallographic analysis of a transfer RNA at a resolution of three angstroms. Their tRNA was the same spermine-stabilized yeast phenylalnine tRNA, but it was in a different crystal form. Even though the molecule was packed differently in the crystal lattice, comparison of the two three-dimensional structures resulting from the analyses showed that the structures were virtually identical. This agreement between the findings of the two groups provided important evidence that the structure of the tRNA molecule is independent of how it is packed in a crystal.

The map of the tRNA molecule at a resolution of three angstroms confirmed our earlier finding that it is organized into two columns of nucleotide bases stacked at right angles to each other. These columns have both helical and nonhelical regions corresponding to the stems and loops of the cloverleaf diagram. The high-resolution map further revealed that the two helical regions each consist of about 10 base pairs, corresponding to one turn of the double helix, and possess the same type of hydrogen bonding between complementary nucleotide bases as that found in the double helix of DNA.

In the nonhelical parts of the tRNA molecule many of the nucleotide bases are oriented with their hydrogen-bonding groups pointed toward the interior of the molecule, where they participate in a variety of unusual hydrogen-bonding interactions known as tertiary interactions. Such bonds may occur between two or three bases that are not usually considered complementary, between a base and the ribose-phosphate backbone of the transfer-RNA chain or even between different parts of the backbone itself. The fact that several tertiary interactions in tRNA involve the hydroxyl (OH) groups of the sugar ribose is of particular interest, because hydroxyl groups are absent from the sugar molecules of DNA. Such tertiary interactions...
UNUSUAL INTERACTIONS between bases stabilize the folding pattern of the transfer-RNA molecule. In the acceptor stem the normally noncomplementary bases guanine and uracil are held together by two hydrogen bonds as the result of a slight lateral "wobble," or displacement, in one of the bases (a). In the T loop 1-methyladenine is paired with thymine, a modified form of uracil that has an added methyl group (b). In the core region of the molecule, immediately below the corner, guanine and cytosine are paired, but with two hydrogen bonds instead of the usual three. This pairing is of the trans type because the ribose groups fall on opposite sides of the pair (c).

Also in the core region are two complex systems of hydrogen bonding involving three bases in the same plane (d, e). In the region joining the D stem and the anticodon stem a dimethylated guanine is paired with an adenine by two hydrogen bonds (f). Because of the bulky methyl groups on the guanine this base pair is not planar; the two bases are tilted about 25 degrees away from each other like the blades of a propeller. The dimethyl guanine is stacked at the bottom of the D stem and the adenine is stacked at the top of the anticodon stem, an arrangement that stabilizes the junction between the two stems. For a more schematic view of these interactions see the diagram on page 62.
are simply not needed in a regular linear nucleotide chain such as that of DNA, but they are essential for stabilizing the complex coiling of the polynucleotide chain in tRNA.

One unusual hydrogen-bonding arrangement was found in the anticodon of the tRNA, where the pair of nucleotide bases guanine-uracil occurs in place of the normal pair guanine-cytosine or adenosine-uracil. The possibility of such a pairing had been suggested several years earlier when Francis H. C. Crick made the observation that it was likely certain additional types of base pairing would be found at the position of the third base in the interaction between the messenger-RNA codon and the transfer-RNA anticodon. One of the "unconventional" arrangements Crick had postulated was a guanine-uracil pair that would be connected by two hydrogen bonds as a result of a "wobble," or slight lateral displacement, in one of the bases. Continued analysis and refinement of the electron-density map at a resolution of 2.5 angstroms confirmed the wobble type of pairing between guanine and uracil in the acceptor stem.

Several other novel arrangements of hydrogen bonds have been discovered among the tertiary base-base interactions in the transfer-RNA molecule [see illustration on opposite page]. The variety of these interactions was one of the most surprising findings to emerge from our structure-determination work.

Most of the flat nucleotide bases in transfer RNA are organized in two stacked columns that form the arms of the L-shaped molecule. This arrangement explains the unusual stability of tRNA. If one heats a solution containing tRNA molecules, they will denature, that is, the polynucleotide chain will unravel and assume random conformations in the solution. As soon as the solution cools, however, the molecule will immediately snap back to its native conformation. This behavior is quite different from that exhibited by most proteins, which denature irreversibly; egg albumin, for example, turns white and opaque when the egg is boiled and stays that way when the egg is cooled.

Why does the transfer-RNA molecule revert so readily to its native structure? It is known that the stacking interaction between the adjacent nucleotide bases in the interior of the DNA double helix is one of the major stabilizing features of that molecule. Similarly, the bases of tRNA are predominantly hydrophobic (water-repelling), so that they retreat from the surrounding solvent into the interior of the folded polynucleotide chain; this behavior helps to return the tRNA molecule to its native and stabilizes—conformation. In proteins there is usually no comparable interaction that will make the polypeptide chain refold spontaneously. Thus it appears that the structure of tRNA is organized to preserve the stabilizing feature of the stacking interactions between bases. At the same time some very complex molecular architecture holds the two stacked columns at right angles to each other.

An important aspect of the tertiary interactions found in yeast phenylalanine tRNA is the fact that many of them involve bases that are the same in the polynucleotide sequences of all tRNA's. Moreover, bases occurring in regions of the polynucleotide chain that have variable numbers of nucleotides are usually unstacked and located in loops that protrude from the surface of the tRNA molecule. These findings suggest that the structural framework of yeast phenylalanine tRNA may accommodate the nucleotide sequences found in other tRNA's. For example, in yeast phenylalanine tRNA one variable region of the D loop contains two nucleotides, and this segment of the polynucleotide chain arches away from the molecule and returns. If there were more nucleotides in this region, it is likely that the bulge would be larger; conversely, if there were fewer nucleotides, it would be smaller. The size of such variable loops, however, would not affect the overall folding pattern of the molecule.

A number of important problems concerning the three-dimensional structure of transfer RNA's in general remain unsolved. It is not clear, for example, what the detailed structure will be for tRNA's with very large variable loops. The structure of "initiator" tRNA's, which start the synthesis of proteins by laying down the first amino acid, is also of interest. Some initiator tRNA's have polynucleotide sequences that depart somewhat from the sequences common to other tRNA's, particularly in the T loop. It is quite likely that these differences are associated with a specific rather than a universal function of that region of the tRNA molecule.

Our crystals of yeast phenylalanine tRNA contain almost 75 percent water. It is important to ask whether the molecule has the same form in solution (where it is biologically active) that it has in the crystal. Fortunately there have been numerous investigations of yeast phenylalanine tRNA synthesized in solution. These studies make it possible to correlate the structure observed in the crystal with various chemical characteristics of the molecule. For example, one of the features of yeast phenylalanine tRNA in solution is that some nucleotides seem to be readily available for chemical modification when chemical reagents are added to the solution, whereas other nucleotides are not. This disparity was puzzling until the structure of the molecule in the crystal emerged. Then it became apparent that only certain nucleotides, such as those that protrude from the molecule in the crystalline state, are readily available for chemical modification.

In general there is an excellent correlation between the susceptibility of a region of the tRNA molecule to chemical modification and the accessibility of that region of the molecule in the crystalline state.

Several other types of experiments carried out in solution can be interpreted in the light of the three-dimensional structure, including experiments based on nuclear magnetic resonance, which is sensitive to the three-dimensional structure of a molecule. Several investigators have found a good correlation between the nuclear-magnetic-resonance signals obtained from transfer-RNA molecules in solution and the three-dimensionsal structure deduced from X-ray-diffraction analysis of yeast phenylalanine tRNA in the crystal. These and other findings provide convincing evidence that the structure of the tRNA molecule in the crystal is the structure of the biologically active form of the molecule.
the messenger-RNA chain. This second tRNA would then take the chain onto its own amino acid and in turn pass the chain along. The considerable distance between the end of the acceptor stem and the anticodon loop of the tRNA molecule may also be functionally important in that different ribosomal proteins can simultaneously interact with several regions of the tRNA in order to help maintain the precision of protein synthesis.

The view of the tRNA molecule that has been obtained from X-ray-diffraction analyses of molecules in a crystal is essentially a static one. In its natural environment within the cell the molecule may undergo conformational changes, particularly when it interacts with large molecular structures such as the ribosome. Recent experiments suggest that inside the ribosome the D loop and the T loop of the tRNA molecule may move away from each other when the molecule shifts from the A site to the P site. It is also possible that the shape of the anticodon loop is modified when it comes in contact with the messenger RNA inside the ribosome. A fuller evaluation of these proposals will have to await the results of further research.

At the beginning of this article we described the role of transfer RNA in protein synthesis in some detail because that is the molecule’s most essential role in biological systems. Without the tRNA molecule genetic information could not be expressed in the synthesis of proteins. In addition tRNA participates in a variety of other processes, some of which are of great importance. For example, tRNA molecules can donate amino acids to preformed protein molecules or to the molecular structure of the cell wall in bacteria independently of the ribosome.

Another process in which tRNA participates is the control of gene expression. Certain tRNA’s with an amino acid attached to them are known to determine whether or not genes, that is, segments of DNA, will be expressed by regulating their transcription into messenger RNA. The detailed mechanism is not known, but in some systems the control function is associated with a particular modified nucleotide in the tRNA molecule, for example a uracil that has been converted into a pseudouracil. It is thought that tRNA helps to control the expression of many different genes, although the exact number is not known. The instances that have been most intensively studied are those of the genes that regulate the synthesis of amino acids, where tRNA-mediated regulation plays a major role.

Other observations suggest that transfer RNA may be involved in still more types of biochemical regulation. For example, in the course of embryonic development one kind of modification of certain nucleotides in a tRNA gives way to another kind. Similarly, when a normal cell becomes cancerous, the kinds of modifications of nucleotides in its tRNA molecules change substantially. It is not yet known whether these transformations are associated with the regulatory functions of tRNA.

Another mysterious area concerns the relatively high number of modifications in the nucleotide sequences of the D loop as well as in those of the variable loop. Why has nature gone to such trouble to vary the nucleotides that project from the surface of the molecule? It is generally believed these sequences are not required for the specificity of protein synthesis; instead they may be involved in the regulatory functions of tRNA molecules, since the variable regions could provide sites for specific recognition by other molecules.

Finally, tRNA is associated not only with the synthesis of polypeptide chains but also with that of polynucleotide chains. This synthesis is carried out by special enzymes such as reverse transcriptase, which was discovered a few years ago as a constituent of several tumor viruses. Reverse transcriptase synthesizes a strand of DNA from a template of single-strand RNA, a direction of information flow that is the reverse of the normal one. Surprisingly, a specific type of tRNA first binds to the enzyme and to the viral RNA and signals the synthesis of the DNA copy to begin. Why a tRNA serves this purpose is completely unknown.

It is probable that tRNA-like molecules were an essential component of the earliest living systems. Once these molecules were formed their unusual stability may have resulted in their gradually being utilized to serve purposes other than their main function in protein synthesis. Although the elucidation of the three-dimensional structure of tRNA has been an important step forward, a great deal remains to be learned about this versatile molecule and its many roles in the living cell.
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