tertiary structure of the molecule. These seem to be in the region of the 'hinge' between dihydrouridine and the ribosylthymine-pseudouridine loop. Further, the n.m.r. studies allow one to propose a detailed structural model for the anticodon loop (Ts'o et al., 1975).

The problem of the specificity of aminoacylation has been approached by equilibrium and kinetic studies. A striking feature of this is the 2'- and 3'-specificity. Thus one can divide tRNA species into two classes, one accepting the amino acid at the 2'-position (about 60%) and the other at the 3'-position (about 40%) (Sprinzl & Cramer, 1973, 1975).

Introduction of chemically modified labels into tRNA either by chemical reactions or by incorporation of modified nucleotides allows studies of the functions of different parts of the molecule. In this way labels for tRNA-binding sites can be introduced (H. Sternbach, M. Sprinzl & F. von der Haar, unpublished work).

Ribosomes also show specificity with respect to the position of the amino acid on the tRNA, i.e. whether it is at the 2'- or the 3'-position. Thus the Tu-factor seems to require a 2'-aminoacyl-tRNA, whereas the ribosomal sites have a preference for 3'-aminoacyl-tRNA (Chinali et al., 1974).


The Three-Dimensional Structure of Yeast Phenylalanine Transfer Ribonucleic Acid and its Interaction with Aminoacyl Synthetases

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Knowledge of the three-dimensional structure of tRNA is a necessary prelude to understanding its function during protein synthesis. Although tRNA molecules crystallize readily, the crystals are generally imperfect for X-ray diffraction analysis in that they do not yield a diffraction pattern with sufficient order. An important exception to this was found in 1971 as yeast phenylalanine tRNA was stabilized through the addition of spermine to produce crystals that yielded X-ray diffraction patterns with a resolution between 0.2 and 0.3 nm (2 and 3 Å) (Kim et al., 1971). Over 2 years ago, the electron-density map of orthorhombic crystals of yeast phenylalanine tRNA at 0.4 nm (4 Å) resolution (Kim et al., 1973) showed that the molecule contains four double-helical regions which correspond to the stems of the familiar clover-leaf diagram for tRNA (Fig. 1). The molecule was seen to have a flattened L-shaped conformation in which the acceptor and TYC stems are aligned approximately parallel to each other along one arm of the L and the dihydrouracil (D) stem and anticodon stems are arranged along the other arm of the L. The 3'-terminal adenosine to which the amino acid is attached during aminoacylation was found at one end of the L, and the anticodon is 7.6 nm (76 Å) away at the other end of the L. More recent X-ray diffraction analysis of yeast phenylalanine tRNA at 0.3 nm (3 Å) resolution in both orthorhombic (Kim et al., 1974a) and monoclinic (Robertus et al., 1974) crystal forms revealed a series of additional tertiary hydrogen-binding interactions involving nucleotides in the loop regions. The folding of the molecule is very similar in the two different lattices.

The sequence of yeast phenylalanine tRNA is shown in Fig. 1 (RajBhandary & Chang, 1968), and the folding of the molecule is shown diagrammatically in Fig. 2 (Kim et al., 1974a,b). The ribose-phosphate backbone is represented as a coiled tube in Fig. 2, and the cross-rungs represent bases. The black segments indicate tertiary interactions.
The positions occupied by constant nucleotides (■), or which always contain purines or pyrimidines (□), are indicated. The solid lines indicate tertiary interactions with one, two or three hydrogen bonds (Kim et al., 1974a).

As shown in Fig. 2, the stacking of the base pairs in the TYC stem is extended into the TYC loop. Four different layers are involved in the stacking. These include: the hydrogen-bonded pair T54 and m'A58; the pair Y55 and G18 (of the D loop); G57 by itself; and finally at the outer edge C56 hydrogen-bonded to G19 (of the D loop). Two other residues of the TYC loop, U59 and C60, are oriented almost at right-angles to the other bases in the TYC stem and loop so that they are parallel to the bases in the D stem. U59 is stacked on the base pair G15–C48, which in turn is stacked on the pairing between U8 and A14. The adenine of A21 is stacked in the U8–A14 plane, where it probably hydrogen-bonds to ribose 8. The base pairs in the D stem and anticodon stem are stacked approximately along the same axis with residues A9 and m7G46 involved in hydrogen-
bonding interactions with base pairs in the major groove of the D stem. Many of the hydrogen bonds in the tertiary interactions are non-standard and have been seen previously only in model compounds. The stacking interactions are continued down one side of the anticodon loop and include the three bases of the anticodon. All but four nucleotides in the yeast phenylalanine tRNA molecule are involved in two hydrophobic stacking domains oriented more or less at right-angles to each other. The overall stability of the molecule appears to be related to the large number of stacking interactions as well as hydrogen bonds which stabilize the entire structure. Many of the tertiary interactions involve nucleotides which are common to all tRNA sequences (Barrell & Clark, 1974).

A study of the available tRNA sequences in relation to the three-dimensional structure of yeast phenylalanine tRNA has led to the conclusion that it is likely that all tRNA species have a similar structure with a similar three-dimensional folding of the
polynucleotide chain (Kim et al., 1974b). There are likely to be some changes in the hydrogen-bonding in the D stem in some tRNA structures, but the hydrophobic stacking is likely to be similar. However, some tRNA sequences contain different numbers of nucleotides in certain places (Barrell & Clark, 1974) as shown by the dotted regions in Fig. 2. These are in the variable loop, which may have four to 21 nucleotides and in the D loop. The structure is able to accommodate extra nucleotides by forming a bulge in the polynucleotide chain just as is seen in the region of D16–D17 in yeast phenylalanine tRNA. tRNA molecules can be described as having a region of variability in terms of the numbers of nucleotides on the right-hand side of the molecules as shown in Fig. 2, whereas the other parts of the molecule, especially the diagonal left-hand side of the molecule, will have constant numbers of nucleotides.

There is an extensive literature on the interactions between tRNA and the aminoacyl synthetases (Soll & Schimmel, 1974; Kisselev & Favorova, 1974). One can ask whether the interaction of the aminoacyl synthetases with their cognate molecules are unique for each pair or whether there is a common type of interaction which may be found in most systems. The most striking phenomenon in this respect is that, in addition to interacting with their cognate tRNA molecules, the synthetases generally are able to interact with and even aminoacylate a number of other tRNA species, the so-called 'non-cognate' interactions. This occurs even though these different tRNA species have different numbers of nucleotides in the variable loop or in the D loop. In a recent analysis of these interactions (A. Rich & P. R. Schimmel, unpublished work), we conclude that there may be a common structural basis for these interactions, namely the tRNA is recognized by most synthetases along the side of the tRNA which has a constant folding in all species. This is shown by the dashed line in Fig. 2, which represents in schematic fashion a cleft on the surface of the synthetase enzyme in which the tRNA molecule rests during aminoacylation. It should be pointed out that the schematic representation in Fig. 2 suggests that the synthetases may interact with the entire length of the tRNA molecule from the 3'-end all the way down to the anticodon. However, this is not necessarily the case for all enzymes. Some of the recognition sites may not extend as far as the anticodon, whereas in other cases they do.

Three principal sites have been suggested as recognition loci for tRNA–synthetase interactions. These include the acceptor stem, the D stem or the anticodon in different species of tRNA (Kisselev & Favorova, 1974). It is likely that these are all correct and the apparent conflicts can be resolved by recognizing that these are all on the same side of the tRNA molecule. There may thus be a common approach between most tRNA molecules and their synthetases, even though specificity is determined at different regions in different synthetases.

The interactions between the synthetase and its tRNA substrate may be of two types. Interactions between the protein and the ribose-phosphate backbone of the tRNA molecule are likely to have a large electrostatic component and are mostly non-specific in that they involve structural features that are likely to be common to all tRNA molecules. As such, they provide the physical basis for the interactions between synthetases and non-cognate tRNA species. Another class of interactions are those involving the amino acids of the protein and particular nucleotide bases or base pairs. These provide the specificity of aminoacylation. Thus the structure of the synthetase clefts in different enzymes is not likely to be the same even though most of them may interact with the same side of the tRNA molecule.


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The Relation of Structure and Function of Transfer Ribonucleic Acid, with Special Reference to Phenylalanine and Methionine Transfer Ribonucleic Acids

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Generalized primary structure for tRNA

tRNA plays a central role in protein biosynthesis. Each molecule carries an amino acid to the ribosome before peptide-bond formation and decodes the genetic information embodied in the mRNA. Although there is increasing evidence that tRNA has several other cellular functions, for example in cell-wall biosynthesis, at present we are mostly concerned with relating its structure with its function in protein biosynthesis.

The information from 62 different primary structures (Barrell & Clark, 1974; plus 14 new structures) has been incorporated into a standard generalized 'clover-leaf' form as shown in Fig. 1. This remarkable feature of all the primary structures was first proposed by Holley et al. (1965) and is based on Watson–Crick base-pairing. As shown, the Watson–Crick base pairs give rise to four constant stem regions a, b, c and e, three of which are closed by non-base-paired loop regions I, I1 and IV. Another point of nomenclature which is illustrated in Fig. 1 is that a stem plus a loop can also be called an arm. Most of the tRNA clover-leaf forms have remarkably constant regions. At one end there is a 5'-phosphate whereas at the 3'-end, where the amino acid is attached, there is a common sequence CpCpA. Stems a, c and e contain seven, five and five base pairs respectively and loops II and IV each contain seven non-base-paired nucleotides. The variable regions are confined to stems b and d and loops I and I1. The arms can also be referred to for convenience by trivial historical names as shown in Fig. 1: e.g. loop I + stem b as the D arm since it usually contains some D bases; loop II + stem c as the anticodon (ac) arm since the loop contains the anticodon; loop III + stem d as the variable finger or extra arm; loop IV + stem e as the TψC arm. Stem a is also called the amino acid (aa) stem, since this is where the amino acid is attached. There are also many invariant and semi-invariant nucleotide positions in the generalized structure. The invariant positions are shown by nucleoside letters whereas the semi-invariant positions are shown by R (signifying a purine nucleoside, A or G) or Y (signifying a pyrimidine, C or U). It is now becoming evident from the three-dimensional structure of tRNA at 0.3 nm (3 Å) resolution (Robertus et al., 1974a; Kim et al., 1974) that most of these invariant and semi-invariant nucleotides play roles in forming the tertiary structure. There are several exceptions to the standardized structure of Fig. 1. However, most of the exceptions are tRNA species with special functions such as initiator tRNA or tRNA involved in cell wall metabolism. Other tRNA species that may be considered as exceptions are those that contain a strikingly non-Watson–Crick base pair such as A·C or Y·Ψ in a stem region. In this respect a G·U base pair and a base pair where Ψ replaces U are considered to be allowable and not exceptions.

Classes of tRNA secondary structure

tRNA is usually classified according to significant changes of size in the variable regions. Class 1 (38 structures) contains four Watson–Crick base pairs in the D stem.