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# Structural organization of complexes of transfer RNAs with aminoacyl transfer RNA synthetases*

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ABSTRACT

A variety of experimental data on synthetase-tRNA interactions are examined. Although these data previously had no direct explanation when viewed only in terms of the tRNA cloverleaf diagram, they can be rationalized according to a simple proposal that takes account of the three dimensional structure of tRNA. It is proposed that a major part of the binding site for most or all synthetases is along and around the diagonal side of the tRNA structure, which contains the acceptor stem, dihydrouridine stem, and anticodon. This side of the tRNA molecule contains structural features likely to be common for all tRNAs. Depending on the system, an enzyme may span a small part or all of the region of this side of the molecule. Interactions with other parts of the structure may also occur in a manner that varies from complex to complex. These interactions may be determined, in part, by the angle at which the diagonal side of the flat tRNA molecule is inserted onto the surface of the synthetase.

## INTRODUCTION

One of the crucial steps in translating nucleotide sequences into specific amino acid sequences during protein synthesis is the aminoacylation of specific transfer RNA molecules by their cognate amino acids. This reaction is catalyzed by the aminoacyl tRNA synthetases (1, 2). For each amino acid there is at least one tRNA and an aminoacyl tRNA synthetase. The synthetase matches its corresponding amino acid to the appropriate tRNA species. This process must be carried out with a high degree of fidelity, since the attachment of a given amino acid to a noncognate tRNA can lead to errors in protein synthesis (3). Thus, the precision of synthetase-tRNA interactions is a critical factor in assuring the accuracy of translation.

The question of the molecular basis for the strength and specificity of synthetase-tRNA interactions has been pursued for many years. The full complexity of this problem has only been appreciated in recent years as a wide number of experimental approaches have failed to reveal the

structural organization of the complexes or the critical elements associated with specificity. On the other hand, many of these earlier studies were interpreted without the benefit of a detailed structural model for tRNA, such as the one now available for yeast $tRNA^{Phe}$ (4-9). As outlined below, when many of these earlier observations are re-examined in the light of the high resolution tRNA structure, apparently disparate findings can be rationalized by postulating a rather simple model for the structural organization of these enzyme-tRNA complexes.

The heart of our proposal is that a major binding site for most or all synthetases is along and around the side of the tRNA molecule which contains the acceptor stem, dihydrouridine stem, and anticodon. This side of the molecule has structural features likely to be common to virtually all tRNAs (10). The area on this side of the molecule that is actually covered by the synthetase probably varies from enzyme to enzyme, so that some may span the entire distance from the 3'-terminus to the anticodon, while others may confine their principal contacts to a smaller area, such as the acceptor stem. The essential feature is that in most or all cases a major part of the binding is to one particular side of the tRNA molecule. In addition to this essential feature, interactions with other parts of the structure may occur in a manner that varies from complex to complex. This proposal explains a diversity of indirect experimental observations; in addition, certain of its features are also directly supported by recent experiments in which synthetases have been photocrosslinked to specific regions of bound tRNA molecules (11-13) and by isotope labeling studies (14). This proposal has been briefly described previously (15-17).

## REVIEW AND INTERPRETATION OF EXPERIMENTAL RESULTS

### Common tRNA structures.

Over seventy-five tRNA molecules have been sequenced up to the present time (8, 18). The striking feature of all these sequences is that they can be organized in a cloverleaf arrangement as first pointed out by Holley and co-workers (19). The cloverleaf arrangement such as that shown for yeast phenylalanine tRNA (Fig. 1) contains stems and loops. The stems are generally composed of regions with complementary base pairing. About four years ago an x-ray diffraction analysis of orthorhombic crystals of yeast phenylalanine tRNA at 4Å resolution showed the molecule contains four double helical regions which correspond to the stems of the cloverleaf (4). The molecule was seen to have a flattened L-shaped conformation in which the

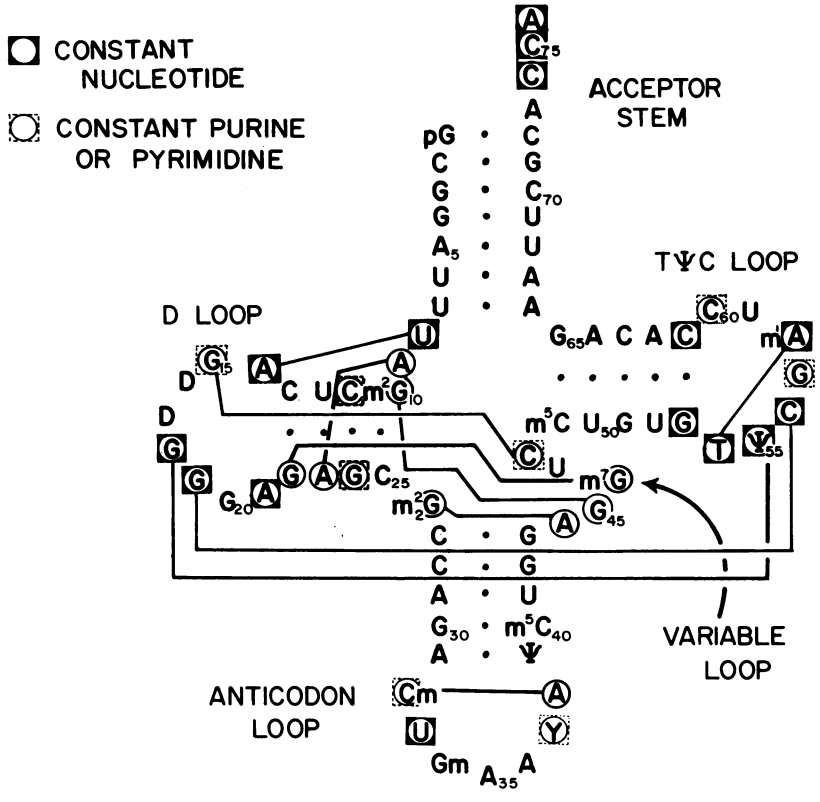


Figure 1.

The nucleotide sequence of yeast phenylalanine tRNA (18). The positions occupied by constant nucleotides and constant purines or pyrimidines are indicated for tRNAs active in polypeptide chain elongation. The solid lines indicate tertiary interactions between bases with one, two or three hydrogen bonds (5, 9).

CCA and TψC stems are aligned approximately parallel to each other along one arm of the L while the D stem and anticodon stem are arranged along the other arm of the L.† The 3'-terminal adenosine to which the amino acid is attached in the aminoacylation reaction and the anticodon are about 76Å apart at opposite ends of the L, while the molecule is only 20-25Å thick. A stereoscopic view showing the three dimensional form of yeast phenylalanine tRNA is shown in Fig. 2 (9).

†Abbreviations used: D, dihydrouridine; xRS, a particular aminoacyl tRNA synthetase, where x is the standard three letter abbreviation for an amino acid.

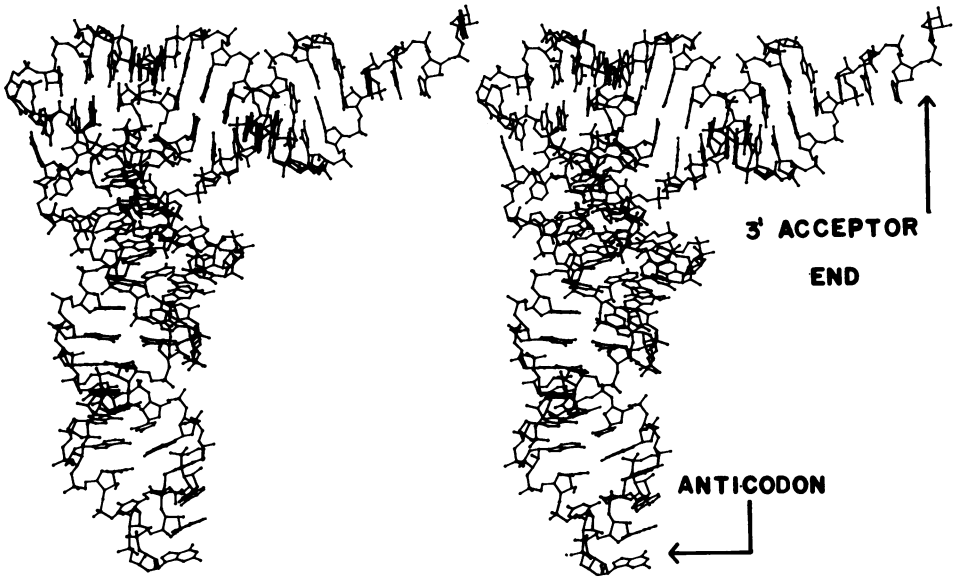


Figure 2.

A stereoscopic diagram of yeast phenylalanine $tRNA^{Phe}$ (9). This diagram can be seen in three dimensions by using stereoscopic glasses. However, the three dimensionality can also be seen without glasses by simply letting the eye muscles relax so that the eyes diverge slightly until the two images fuse.

Another feature of tRNA sequences is that certain positions contain nucleotides common to all species (8, 18). More recent x-ray diffraction analysis of yeast phenylalanine tRNA at 3Å and 2.5Å resolution in both orthorhombic and monoclinic crystal forms revealed a series of additional teritary hydrogen bonding interactions many of which involve the common nucleotides (5-7, 9, 20). The yeast phenylalanine molecule as a whole is also stabilized by the large system of stacking interactions involving most of the purines and pyrimidines (10). Because the double helical stems and most of the common nucleotides found in all tRNA sequences are used in stabilizing the three-dimensional structure of yeast phenylalanine tRNA, it is reasonable to believe that this structure is a useful model for understanding the three-dimensional structure of all tRNAs (10).

In surveying tRNA sequence data, important differences between species emerge due to the variable numbers of nucleotides in two different regions in the molecule. One of these is the variable loop which has four or five nucleotides in most cases, but in approximately 20% of the tRNAs there are

very large structures containing 13 to 21 nucleotides (8, 18). In addition, there are variations in the number of nucleotides in the dihydrouracil (D) loop. The variations in nucleotide number in the D loop occur in two different regions, each containing from one to three nucleotides (10). These regions are found flanking the two constant guanine residues (18 and 19 in Fig. 1) common to all tRNA sequences. The variable number of nucleotides in the D loop and in the variable loop is likely to be accommodated into tRNA structures by a bulging out of the polynucleotide chain in those regions, as seen in yeast phenylalanine tRNA (10).

Another feature of interest in the molecule is that those regions with variable numbers of nucleotides are located along the vertical right side of the molecule as shown by the dotted segments of the backbone in the schematic diagram in Fig. 3. However, the remainder of the molecule and especially the diagonal lefthand side of Fig. 3 is a region containing the same number of nucleotides in all transfer RNA molecules and their conformation is likely to be similar (10).

Proposal for structural organization of synthetase-tRNA complexes.

We propose that a major binding site for most or all synthetases is along the side of the tRNA that has a constant number of nucleotides in all species. Figure 3 schematically illustrates by dashed lines the regions that may be spanned on this part of the tRNA molecule by the various synthetases. The idea is that each enzyme attaches to the same side of the molecule, but depending on the particular system the extent of contact with tRNA may encompass the entire area from the 3'-terminus to the anticodon or may involve a much smaller region. Three examples are illustrated by the dashed lines A-C which show varying lengths of synthetase recognition regions. Since enzymes with macromolecular substrates often have an elongated depression corresponding to the substrate binding site (21), it is likely that parts of the protein extend out and around the nucleic acid, so that potential contact sites are not simply confined to the narrow segments indicated by the dashed lines in Figure 3. Figure 2 shows the three-dimensional features of the tRNA molecule. Although the extent to which the binding site of the enzymes wrap around the tRNA structures cannot be surmised, it is unlikely that they extend far enough to surround major portions of the variable loop, dihydrouridine loop, or TψC loop as discussed below.

There are two important variables which need to be pointed out. One of these cited above involves the length of the recognition site. This is

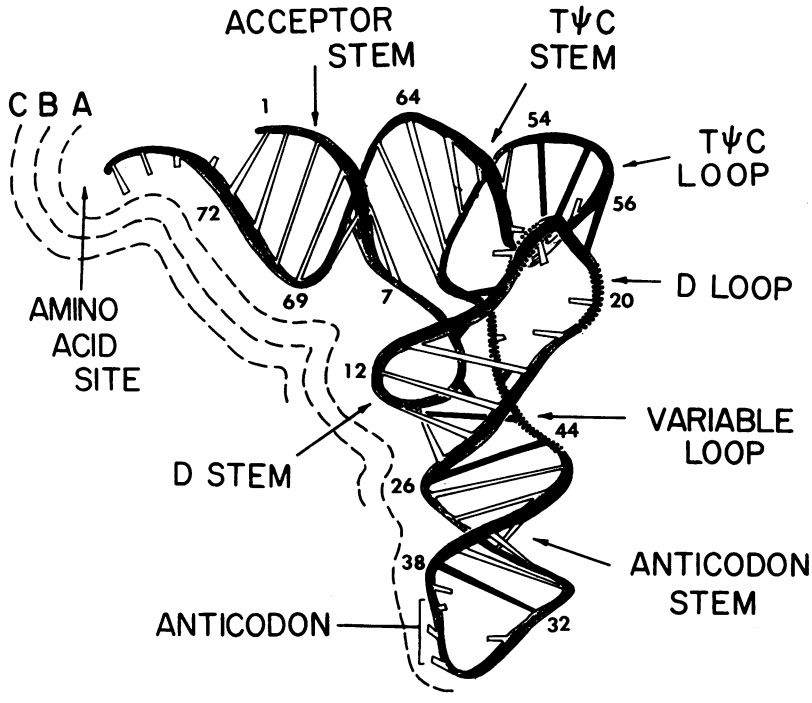


Figure 3.

A schematic diagram illustrating the folding of the polynucleotide chain in yeast phenylalanine tRNA (5, 9) and its proposed interaction with the synthetase surface. The ribose-phosphate backbone is drawn as a continuous tube and crossbars indicate the bases. Nucleotide numbers correspond to Figure 1. Secondary base-base hydrogen bonding interactions have unshaded crossbars, while tertiary ones are black. The regions that are variable in terms of the number of nucleotides in different tRNA molecules are shown in dotted outline. Three different synthetase surfaces are shown by the dashed lines (A-C). These represent different sizes of the recognition region, as discussed in the text. Interactions are not intended to be viewed as simply occurring on an edge of the flattened tRNA molecule, but also involve parts of the tRNA on either side of the indicated lines. As mentioned in the text, the plane of the tRNA is not necessarily perpendicular to the surface of the synthetase.

shown schematically in Fig. 3, where the three dashed lines, A-C represent the recognition region of three different types of synthetases. Type A reaches all the way to the anticodon; type C is much shortened and type B is intermediate. However, another variable concerns the angle at which

the flat tRNA molecule is inserted onto the surface of the larger synthetase. One can imagine a plane which passes through the tRNA molecule intersecting the 3' terminal adenosine, the anticodon, and the corner of the molecule near cytosine 56 (Fig. 3). The insertion angle of this plane could be normal to the surface of the synthetase, but it is more likely to be tilted somewhat, so that the dashed lines A-C in Fig. 2 would not represent the central part of the contact area. This may differ among different synthetases.

Observations that support the model.

With regard to our proposal, a critical question is whether the various synthetase-tRNA complexes have a common basic structural organization or whether the mode of attachment of enzyme to tRNA varies substantially from complex to complex. In this connection, the most pertinent observation is that in addition to interacting with cognate tRNAs, synthetases generally have the ability to interact with and occasionally aminoacylate a number of other tRNA species, the so-called "non-cognate" interactions (1, 2). This occurs even though these species have different numbers of nucleotides in the variable loop or in the D loop. We suspect that these non-cognate interactions occur because most or all synthetases bind along the side of the tRNA that has a uniform folding of the polynucleotide chain with a constant number of nucleotides in all species. By binding to this side of the molecule, steric barriers are minimized for non-cognate interactions.

There are several examples that illustrate the common occurrence of non-cognate associations. For example, Roe, Sirover and Dudock (22) have shown that yeast PheRS acylates not only homologous $tRNA^{Phe}$, but also ten specific tRNAs from other organisms. The misacylation of several E. coli tRNAs by this enzyme proceeds with roughly similar $K_m$ values, but with substantial variations in $V_{max}$ (22). Ebel et al. (23) have also studied non-cognate interactions and, like Roe et al. (22), point out the importance of $V_{max}$ in determining the specificity of aminoacylation. This suggests that a similar system of general binding interactions may be shared by many enzyme-tRNA pairs; variations in $V_{max}$ may arise from subtle differences in a few specific interactions, possibly with individual bases. This conclusion is reinforced by observations of Yarus and of Ebel and their colleagues that extensive mischarging with many different enzymes results from the addition of organic solvents to the reaction mixture (23-29). For example, in the presence of 20% dimethyl sulfoxide, yeast

PheRS aminoacylates virtually all E. coli tRNAs (26). This would occur if, for example, the organic solvent encourages a subtle relaxation or readjustment of a similar basic geometric pattern of the complexes so that the 3'-termini of the non-cognate tRNAs are brought into proper register with the catalytic groups of the enzyme. It is known that tRNA conformation is perturbed by minor proportions of organic solvent mixed with aqueous solutions (30).

It should be pointed out that the observations cited above are not likely to be due solely to the existence of a common tRNA structure, but are likely to arise from a somewhat similar orientation of tRNAs on various enzymes. If, for example, different enzymes bound to the native tRNA structure in radically different ways, it is not likely that addition of an organic solvent could induce subtle changes in tRNAs that would facilitate misacylations by different enzymes. The similar effects of special reaction conditions on a variety of enzymes implies that structural differences between enzyme-tRNA complexes are subtle but not large (23-29).

The variety of observations on misacylation reactions are thus consistent with the idea that many synthetase-tRNA complexes have a common structural organization. This in turn suggests that a major portion of the binding must occur along the part of the tRNA molecule that is similar among the various tRNA species. This conclusion is given strong support by recent photochemical crosslinking experiments in which enzymes and tRNAs are directly linked under the action of uv light, without the disadvantage of extraneous reagents or affinity labels that can force or bias crosslinking to occur at a particular site. In six complexes that have been studied, involving both cognate and non-cognate systems, the dihydrouridine stem or its immediate vicinity is one of the sections on the tRNA that crosslinks in every instance (11-13). This not only suggests some common features to the structural organization of these six complexes, but it also indicates that the enzymes make contact with the diagonal side of the tRNA molecule, where the dihydrouridine stem region is spatially located (See Figure 3).

The combined evidence thus leads to the conclusion that many or possibly all synthetase-tRNA complexes share some common structural features. This concept allows us to examine a variety of data on different systems to test further whether a common mode of binding occurs along and around the diagonal side of the tRNA molecule.

First, it should be noted that three principal regions of the tRNA

molecule have been suggested as crucial for synthetase recognition by different investigators (1). These are the acceptor stem, the D stem, and the anticodon. The three-dimensional structure of tRNA (Fig. 2) shows that these all lie along the side of the molecule proposed as the synthetase binding area (Fig. 3).

The significance of the acceptor stem and 3'-terminal nucleotides is suggested by several considerations and observations. Since the 3'-terminus is the site of amino acid attachment, contact by the enzyme must be made at this site. In addition, Crothers et al. (31) have given evidence supporting the concept that the fourth base from the 3'-terminus serves as a discriminator site which synthetases use to distinguish between broad categories of tRNAs. Finally, the role of the acceptor stem has been convincingly demonstrated in the studies of Shimura et al. (32), Hooper et al. (33), and Smith and Celis (34). These authors showed that mutations in the amino acid acceptor arm of E. coli $su^{+}_{III}$ $tRNA^{Tyr}$ lead to mischarging.

The role of the D stem in the interactions of yeast phenylalanyl tRNA has been suggested by Dudock and co-workers (22, 35). Strong evidence that this region is in close contact with the enzyme comes from the substantial enhancement of the aminoacylation $V_{max}$ which accompanies methylation of guanine 10 in a non-cognate tRNA lacking the methyl group found in the cognate tRNA (22). This position on the D stem is located just below the bend in the L, and it can be easily grasped by the enzyme when it is oriented as shown in Figure 3. Also as discussed above, photochemical crosslinking studies have directly demonstrated the significance of this general area in a variety of systems (11-13).

Several studies suggest that for many synthetases the anticodon is a prime point of contact. Bayev, Mirzabekov and co-workers (36) have shown that modification or removal of bases from the anticodon of yeast $tRNA^{Val}$ gives loss of acceptor activity. Squires and Carbon (37) discovered that an anticodon mutation in an E. coli $tRNA^{Gly}$ subspecies produces a $10^{4}$-fold depression in the rate of aminoacylation by the cognate synthetase. And Yaniv et al. (38) found an anticodon mutation of E. coli $tRNA^{Trp}$ which enables this tRNA to accept glutamine. Other data bearing on the significance of the anticodon are reviewed by Kisselev and Favorova (1) and Söll and Schimmel (2).

Further support for these ideas comes from the work of Clarke and Carbon (39) who determined the sequence of an E. coli $tRNA^{Thr}$. This tRNA

has over 67% homology with certain E. coli isoleucine and glycine tRNAs as well as two valine isoacceptors. The major differences between these tRNAs are in the TψC-amino acid acceptor helix and in the anticodon stem and loop. Many of these differences would easily be sensed by enzymes which bind predominantly along the diagonal part of the L as illustrated in Figure 3, without regard for the TψC, D and extra loops.

The above observations are consistent with the idea that synthetase binding involves the parts of the tRNA molecule indicated in Figure 3. Data are also needed, however, that test whether the enzymes predominantly bind to the opposite side of the tRNA molecule, in contrast to our proposal. This side includes the TψC, D, and variable loops.

Consider first the variable loop. The number of bases in this loop of four serine specific tRNAs varies as follows: rat liver $tRNA^{Ser}$ and yeast $tRNA^{Ser}$ have 14; E. coli $tRNA_1^{Ser}$ has 16; and E. coli $tRNA_3^{Ser}$ has 21 (8, 18). However, all of these are aminoacylated by the liver and by the yeast seryl-tRNA synthetase (40-42). This implies that the variation in length of the extra loop is of no consequence to the tRNA synthetase interaction. Likewise, Rigler et al. (43) showed that yeast seryl-tRNA synthetase not only interacts with yeast $tRNA^{Ser}$ (14 bases in the variable loop), but also with yeast $tRNA^{Phe}$ (5 bases in the variable loop). In addition, Wintermeyer and Zachau (44) and Thiebe et al. (45) have demonstrated that the extra loop does not even need to be intact in order for the yeast Phe synthetase-$tRNA^{Phe}$ interaction to occur. Similarly, yeast $tRNA^{Val}$ will interact with its synthetase when two bases are removed from the extra loop (46). Finally, Ebel et al. (23) have shown that yeast Phe, Val and Asp synthetases appear to misacylate tRNAs which contain extra loops considerably larger than the ones associated with their respective cognate species. All of these findings support the notion that some of the extra loop is oriented away from the main part of the enzyme-tRNA interaction.

In the three dimensional tRNA structure, the TψC and D loops interact closely (4). Various observations suggest a non-critical role in synthetase recognition for this area of the molecule. In comparing sequence homologies of tRNAs aminoacylated by the same enzyme, Dudock et al. (35) and Roe et al. (22) have indicated the unimportance of the TψC and D loops. There are also several examples where cleavages, removal of bases, or chemical modification in one of these loops does not prevent synthetase recognition (45, 47-49). For example, removal of large seg-

ments of the D loop or TψC loop does not prevent recognition of yeast $tRNA_1^{Val}$ (46). Moreover, the four serine tRNAs aminoacylated by yeast and liver seryl-tRNA synthetases cited above have different numbers of nucleotides (10 or 11) in their D loops (8, 18). Thus, the TψC and D loops are probably not a locus of important enzyme-tRNA interactions.

The nucleotides in the two variable regions flanking the two constant G residues in the D loop (dotted in Fig. 3) are close to each other on the surface of the tRNA molecule. Ladner et al. (7) have suggested that these variable regions of the D loop may constitute part of the synthetase recognition system. However, this seems unlikely since isoacceptor tRNA species have different numbers and types of nucleotides in these regions even though they are acylated by the same synthetase.

Other considerations.

It is important to note that although non-cognate tRNAs frequently associate strongly with a synthetase, this association can also be weak. For example, the $K_m$ for the misacylation of E. coli $tRNA^{Phe}$ by E. coli IleRS is less than $10^{-4}$ M under normal aminoacylation conditions (25). And direct determination of enzyme-tRNA association constants by fluorescence quenching has shown that several non-cognate E. coli tRNAs bind to E. coli IleRS several orders of magnitude weaker than the cognate species (50). This indicates that some aspect of tRNA or enzyme structure may obstruct or weaken complex formation. This hinderance to binding could be explained by repulsive or steric effects due to the presence of a particular base or amino acid in the region where critical enzyme-tRNA contacts occur. In the case studied by Yarus (25), addition of organic solvent greatly enhances the non-cognate binding. Here again, it appears that a conformational or steric restriction is alleviated by subtle changes in the tRNA or protein introduced by the solvent. The unique discrimination of base pairs in the RNA double helix by an enzyme has a number of inherent ambiguities which the protein may overcome by careful positioning of certain amino acid side chains (51). Thus, it is not surprising if slight changes in tRNA (or protein) conformation substantially modify the recognition interactions.

Possible involvement of other parts of the tRNA structure.

The discussion above outlines some of the experimental observations that support the notion that a major part of the synthetase attachment is along the diagonal side of the tRNA structure. This is likely to be a common feature of most synthetase tRNA systems. However, it must be recog-

nized that interactions with other parts of the structure may be superimposed on this basic mode of binding. These interactions may vary considerably from system to system.

For example, even though results from photocrosslinking of six enzyme-tRNA pairs (11-13) and isotope labeling studies (14) have provided support for some of the main features of our proposal, these data also indicate that some parts of the tRNA structure not on the diagonal face may also have contact with the synthetase. For example, the first base of the 3'-side of the D loop of E. coli $tRNA^{Ile}$ has been implicated as close to the bound Ile-tRNA synthetase (14). Although not on the diagonal surface of the tRNA structure, it is not far removed from it. In addition, in certain photocrosslinked complexes one of the coupling sites occurs within a T1 ribonuslease segment that is derived entirely or in part from the variable loop (11, 13). However, it should be appreciated that part of the variable loop is close to the D stem region (Figure 2). For example, $m^7G46$ is bonded to the major groove of the D stem in yeast $tRNA^{Phe}$ (5, 6). If a synthetase makes contact with the variable loop, it is possibly near the edge of the binding site of the enzyme. In this way, variations in size of the extra loop could occur without steric interference from the enzyme as discussed above.

These examples illustrate that parts of the molecule adjacent to the diagonal surface may interact with a bound synthetase. These interactions might be determined in part by the angle at which the diagonal side of the flat tRNA molecule is inserted onto the surface of the synthetase, as discussed above.

Aminoacylation of viral RNAs.

It is worth mentioning the observation that certain large viral RNAs can be aminoacylated by specific synthetases (52-57). It is possible that the 3'-end of the viral chain can adopt the native tRNA conformation. If that were to happen, aminoacylation can easily occur in our model since the remainder of the large viral RNA is attached to the 5'-end of the tRNA segment, where it is in a position to project away from the proposed region in which synthetase-tRNA interactions occur (Figure 3). Thus, the surprising observations with viral RNAs fit into the proposal made here.

## DISCUSSION

Review of some assumptions made.

The proposed organization for synthetase-tRNA interactions is able to rationalize a variety of experimental data, much of which was previously

interpreted without the aid of a three dimensional model for tRNA. Without such a model, some observations were confusing and even suggested that each enzyme binds in a quite different way to its respective tRNA. But as pointed out above, many of the different regions that have been implicated as important for recognition fall along the diagonal side of the tRNA molecule. Conversely, the opposite side of the molecule contains sections that often appear to be non-essential for recognition.

The model proposed here rests heavily on the assumption that all tRNAs are folded into the same basic structural pattern as that determined for yeast $tRNA^{Phe}$. Strong evidence supports this assumption (10). Because of this assumption, it was possible to interpret non-cognate interactions and photo-crosslinking data as showing that most or all synthetases bind to the same side of the tRNA structure. This in turn enabled us to examine collectively a variety of data on different synthetase tRNA complexes, and thus to interpret these data in terms of a general class of models for the complexes.

We have also assumed that the conformation of tRNA in the crystal is similar to the form of the molecule in solution, where it is biologically active. This is suggested by the good correlation between the structure of yeast $tRNA^{Phe}$ in the crystal with the structure in solution as revealed by chemical modification studies, NMR studies, oligonucleotide binding studies and enzymatic susceptability. These correlations have been extensively reviewed (8). In considering the nature of the tRNA synthetase interactions, we also imply that the tRNA does not undergo a large conformational change when it is bound to the enzyme. NMR studies of E. coli $tRNA^{Gln}$ bound to its homologous synthetase suggest that the conformation of the tRNA molecule does not change greatly from that which is found in solution (58). Furthermore, many photo-crosslinked species of E. coli tRNA can be aminoacylated by synthetases even though the bases at positions 8 and 13 are joined by an intramolecular covalent bond (59). This also implies the absence of large conformational changes on aminoacylation.

Synthetase structure in relation to tRNA binding.

Some synthetases appear to be single polypeptide chains while others are comprised of subunits (1, 2). The smallest subunits found for a synthetase are on the order of 30,000-40,000 daltons. If a subunit of this size interacts with the entire region from the 3'-terminus to the anticodon 76Å away, it must be elongated and not spherical. The largest single-chain enzymes are near 100-120,000 daltons (1, 2). These also must be

somewhat elongated in order to span the entire distance from the 3'-terminus to the anticodon. But as discussed above, some enzymes may not span this entire region.

It is possible that the recognition region is in the crevice between subunits of the synthetase or that it may span two subunits. In discussing these interactions, Kim (60) has suggested that the tRNA may interact with two synthetase subunits using the pseudo two-fold axis between the two arms of the L-shaped tRNA molecule. He suggests that the pseudo symmetry may be important in these interactions. However, our proposal is not restricted to this assumption.

The crystal structure of tyrosine tRNA synthetase from B. stearothermophilus at 2.7Å resolution has recently been reported by Irwin et al. (61). This is a dimeric enzyme with a molecular weight of 90,000. The monomer has dimensions of about 60 x 60 x 40 Å, and the longest dimension of the dimer is approximately 130 Å. Although no structural data are available on a synthetase tRNA complex, the information available on Tyr-tRNA synthetase combined with the proposal put forth here might make it possible to construct a molecular model of the complex.

Types of bonding in a complex.

Interactions between the synthetase and its tRNA substrate may be divided into two categories. First, interactions doubtless occur between the protein and the ribose-phosphate backbone of the tRNA. In general, these are likely to be largely electrostatic and non-specific, especially if they involve structural features that are common to all tRNA molecules. As such, they provide the physical basis for the interactions between synthetases and non-cognate tRNAs. In addition to these general bonding sites, a second type of interaction involving specific contacts between the polypeptide chain and free bases or base pairs in the helical grooves must also occur; these could provide incremental interactions that account for the specificity of aminoacylation.

Along the diagonal side of the tRNA, the orientation of backbone phosphate groups should be approximately the same for the various tRNA species. Therefore, a spatial arrangement of electrostatic bonds between positive charges on the enzyme with tRNA phosphate groups, as well as hydrogen bonds to the ribose-phosphate backbone, may provide a general system of interactions. Since electrostatic bonds in aqueous solution have positive entropies of formation owing the liberation of solvating water molecules (62), these ionic interactions would account for the

large favorable entropy changes accompanying synthetase tRNA complex formation (50, 63). In this regard, the ribose-phosphate residues of the D stem and of nucleotides 67-71 in yeast $tRNA^{Phe}$ are in a favorable position to interact with the synthetase. It is likely that the total number of backbone interactions will not be very large.

The second class of interactions mentioned above are between the protein and particular nucleotide bases; these are likely to provide a major portion of the specificity. Some of these interactions may involve single unpaired bases such as one or more of those in the anticodon, the 3'-terminal adenosine, and perhaps the fourth base from the 3'-end. However, other differentiating interactions could involve bases in the tRNA stems, which are usually hydrogen bonded to each other. For base pairs in double helical stems, this may occur by specific recognition by amino acid side chains that are placed in either the major or minor grooves of the helix (51). For example, a number of specific interactions between base pairs in double helical stems and side chains of glutamine, asparagine, and arginine have been postulated. Considering the geometry of the molecule, these may occur in the wide or narrow grooves at the top of the acceptor stem or in the narrow groove of the D stem. It is possible that these interactions may be more sensitive to changes in solvent composition and that this in turn leads to the misacylation discussed above.

Conclusions.

The main hypothesis here is that the major locus of synthetase-tRNA interaction is along one side of the tRNA molecule. The interactions are of two classes, both non-specific and specific. Since the active site on the synthetase is likely to be in the form of a cleft, the interactions with the tRNA are not solely along an edge, but also involve adjacent parts of the molecule. It should be emphasized that we do not suggest that the structures of synthetase clefts are all identical to each other. Furthermore, the angle at which the diagonal side of the flat tRNA molecule inserts into the synthetase is left undefined, and may vary.

The major value of the hypothesis given here is that it serves as a useful model for designing further experiments. If indeed further experiments show that most or all synthetases interact in this fashion, then this gives a useful basis for understanding the evolution of tRNA-synthetase interactions. With the passage of time, there may have occurred a diversification of the specific sites on the tRNA molecule that are used for recognition by different synthetases.

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