FIVE SPECIFIC PROTEIN-TRANSFER RNA INTERACTIONS*

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I. INTRODUCTION

Transfer RNAs have been subjected to intensive investigation during the past decade. These studies have ranged from investigations of primary and three-dimensional structure, to recognition of tRNA by diverse systems of proteins, and to tRNA biosynthesis and gene organization.[1,2] While early investigations of biological function concentrated on the role of tRNA in translation of messenger RNA, there has been an increasing awareness of the participation of tRNA in a variety of other cellular processes.[2]

For each amino acid, there is one or more specific tRNA species and because of the existence of isoaccepting species for a given amino acid, there are on the order of 60 distinct tRNA species in the typical cell. To date, over 100 tRNA species (from various organisms) have been sequenced and these range in length from 73 to 93 nucleotides.[3,4] Figure 1 gives a cloverleaf representation of tRNAs that participate in the elongation of polypeptide chains. Bases that are common to all or almost all of these species are indicated by a capital letter. In this diagram, R represents purine, Y designates pyrimidine, and H denotes hypermodified purine. In addition, landmarks found in all tRNAs are indicated; these are the amino acid acceptor terminus, dihydrouridine loop (so named because of the common occurrence of dihydrouridine [D] residues), anticodon loop, variable loop, and TψC loop. Open circles designate bases that commonly vary among the various tRNAs and a thin line connects those that are associated through cloverleaf hydrogen bonds.

The variation in length among the various tRNAs occurs in three specific regions. Two of these are in the dihydrouridine loop and are designated α and β; these regions immediately flank the two contiguous Gs in this loop (see Figure 1). The remaining region that varies in length is known as the variable loop. It is in these three regions, and only in these regions, that length variations occur. In general, the value of α and β may be between 1 and 3 and the variable loop is at least 4 but may be as large as 21 nucleotides.[3,4]

A schematic illustration of the three-dimensional structure of yeast $tRNA^{Phe}$ is shown in Figure 2.[5-7] The molecule is approximately L-shaped and is made up of two helical sections that join roughly at a right angle. These helical sections arise from the cloverleaf hydrogen-bonding pattern that is shown in Figure 1. In addition, there are a large number of tertiary interactions which bridge together the two branches of the L-shaped structure. In this structure, the largest dimension is from the 3′-terminus to the anticodon, a distance of 75A. From what is known at present, all tRNA molecules may

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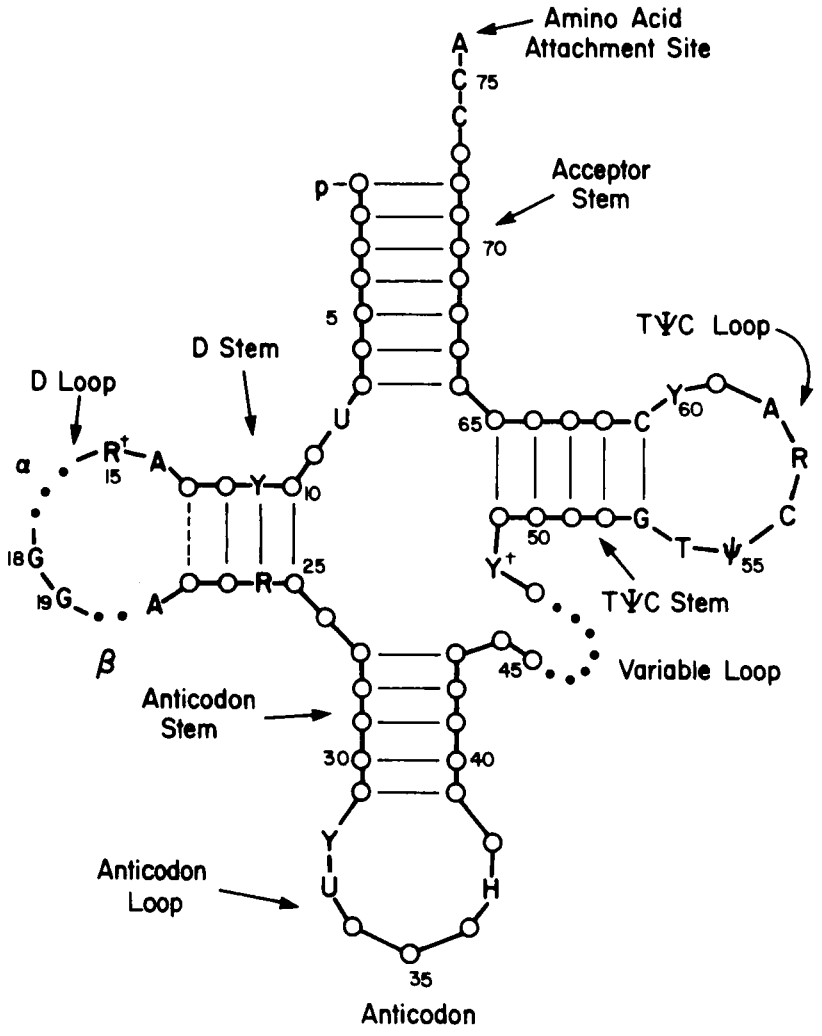

FIGURE 1. Diagrammatic representation of all noninitiator tRNAs. Invariant and semi-invariant residues are designed by capital letters. R is purine, Y is pyrimidine, and H is hypermodified purine. Length variations in different tRNA species occur in regions designated α, β, and variable loop. Major landmarks on the structure are indicated.

be folded in this general pattern, with length variations being accommodated by the dotted outlines shown in Figure 1. These regions (α, β, and the variable loop) bulge out and away from the structure, where they do not interfere with the basic pattern of folding. For this and other reasons, it is believed that the great variety of tRNA sequences and lengths can all be accommodated into the basic folding arrangement shown in Figure 2.[8]

Although relatively small, single-stranded RNAs, they are designed to present receptor sites to a variety of proteins with which they interact during protein synthesis. In addition, transfer RNAs participate in the regulation of gene expression,[9-12] in cell wall biosynthesis,[13,14] in the priming of reverse transcriptase,[15,16] and possibly in amino acid transport.[17] In some cases, these diverse phenomena involve interactions of tRNAs with proteins not normally associated with protein synthesis (e.g., reverse transcriptase), and such interactions further emphasize the unusual versatility of tRNA molecules.

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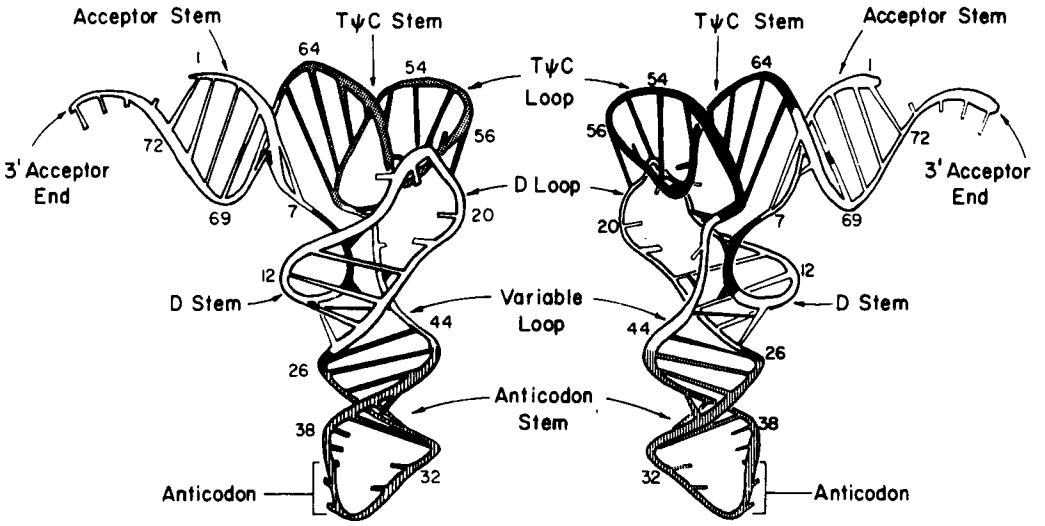

FIGURE 2. Schematic illustration of the yeast $tRNA^{Phe}$ three-dimensional structure. Two views are shown, together with major landmarks (compare with Figure 1). The tRNA backbone is represented as a continuous tube with base pairs designated by crossbars. (Courtesy of Quigley, G. J., Wang, A. H.-J., Seeman, N. C., Suddath, F. L., Rich, A., Sussman, J. L., and Kim, S. H., *Proc. Natl. Acad. Sci. U.S.A.*, 72, 4866, 1975.)

Here we consider the interaction of tRNAs with five different proteins. Four of these are proteins that tRNA molecules encounter during the process of protein synthesis. These are: aminoacyl tRNA synthetases, elongation factor Tu, $tRNA_f^{Met}$ transformylase (only in prokaryotes), and peptidyl hydrolase. In addition, we consider the interaction of a specific tRNA species with reverse transcriptase; it is an interaction between reverse transcriptase and a specific tRNA species that is responsible for the priming of DNA synthesis off of an RNA template.[15,16] This interaction is presumably unrelated to the role of tRNA in protein synthesis.

A problem we face is that because the various tRNA species must all interact with the same protein synthesis machinery (e.g., ribosome system) and therefore must conform to the constraints imposed on it by this machinery, the tRNAs have much in common from a structural standpoint. The similar structural features of all tRNAs are apparent in Figures 1 and 2. The problem is that for proteins that differentiate between tRNAs, this differentiation must be done against a backdrop of similarities, and it is against this backdrop that we must sort out the features that are critical for a specific recognition reaction.

Our knowledge about each of the five systems described below is quite uneven. For example, there is considerably more understanding of the interactions of tRNAs with aminoacyl tRNA synthetases and elongation factor Tu than there is of its interactions in other systems. In spite of this, enough has been done in all five cases to indicate clearly that each system has unique recognition characteristics. For example, what is of no consequence to aminoacyl tRNA synthetases may be of crucial importance for elongation factor Tu. This simply emphasizes the diversity of receptor sites that are perceived by the different proteins that interact with tRNAs.

Although five specific protein-tRNA interactions are considered here, there are additional ones that will not be considered. However, the five cases treated here represent those that are best characterized and give a good picture of the current state of methodological approaches and of mechanistic concepts that are a crucial part of this important field.

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II. AMINOACYL tRNA SYNTHETASES

The aminoacyl tRNA synthetases catalyze the first step in protein synthesis in which each amino acid is covalently attached to its cognate tRNA.[18] In the expression of the genetic code, this is the first place where information in a nucleic acid (tRNA) is interpreted in terms of an amino acid. The connection between nucleic acid information and an amino acid is done with great precision by the aminoacyl tRNA synthetases. A mistake in this reaction can result in the incorporation of the wrong amino acid into a growing polypeptide chain.[19]

The enzymes vary considerably in subunit structure and molecular weight. There appear to be four different types of structures: α, α_2, α_4, and $\alpha_2\beta_2$.[18] This size heterogeneity may not reflect as great a heterogeneity in the enzymes as seems superficially apparent. It is well known that the large, single-chain α enzymes and the large subunits of multichain enzymes contain repeated sequences so that, in some sense, the enzymes may not be as different as suggested by their gross structural parameters.[20-24] For example, a large single-chain α enzyme, with sequence repeats, may bear close similarity to an α_2 enzyme. But in any event, in spite of the gross structural differences, each of these enzymes is designed to carry out a highly specific tRNA recognition reaction.

The recognition of transfer RNAs by aminoacyl tRNA synthetases has recently been reviewed in several articles.[18,25-30] Because of this, no attempt is made here to repeat in detail this material. Instead, some of the major concepts are concisely summarized and attention is then directed to two areas currently of great research interest: the possibility of covalent bond formation in synthetase-tRNA complexes, and the use of oligonucleotides to probe the role of specific sequences in the synthetase-tRNA recognition reactions.

A. Summary of Some Major Conclusions

1. Thermodynamics of Synthetase-tRNA Interactions

Many investigators have studied synthetase-tRNA complex formation at equilibrium.[18] Dissociation constants for cognate enzyme-tRNA complexes (that is, cases where the enzyme and tRNA are specific for the same amino acid) indicate that the dissociation constants typically fall in the range of 10^{-6} to $10^{-8}M$, depending upon conditions.[18] In general, complex formation is stronger on the acid side of neutral pH.

Interactions also occur between noncognate enzyme-tRNA pairs. For example, it has been estimated that *Escherichia coli* Ile-tRNA synthetase interacts with *E. coli* $tRNA^{Phe}$ with a dissociation constant of about $10\mu M$ at pH 5.5, 17°C; with the cognate *E. coli* $tRNA^{Ile}$, under the same conditions, the dissociation constant is about 10 nM.[31] Therefore, in this case, the noncognate complex is about three orders of magnitude weaker than the cognate one. Even greater differences have been observed in some cases.[31] However, in heterologous systems (where the enzyme and tRNA come from different organisms, such as yeast and *E. coli*, respectively) noncognate complexes can be much stronger relative to the homologous cognate one.[31] Thus, at least at the level of binding, discrimination is less exact in the heterologous systems.

The temperature dependence of synthetase-tRNA complex stability has also been investigated. Homologous, cognate and heterologous, and noncognate complexes have been studied. In all cases, complex formation is accompanied by a large, positive change in ΔS^0, while ΔH^0 values tend to be small or even positive.[31,32] Thus, complex formation is entropically driven. This can be rationalized by assuming that electrostatic interactions play an important role in stabilizing the complexes, and that the ΔS^0 values reflect liberation of solvating water molecules (from charged sites) upon complex formation.[31] An analogous situation has been found for the interaction of the *lac* repressor-*lac* operator interaction, where complex formation is also entropically driven.[33]

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2. Kinetics of Synthetase-tRNA Association

A limited number of studies of synthetase-tRNA interactions have been done with stopped-flow and temperature-jump methods. The major conclusion from these studies is that for cognate complexes, association between enzyme and tRNA occurs in a two-step reaction.[32,34,35] The first is an approximate diffusion control bimolecular association between protein and nucleic acid. The second step is a unimolecular conformational change of the initially formed enzyme-tRNA complex. This process occurs on the order of milliseconds.

Some data indicate that the second step is a crucial part of the recognition process and represents a "reading" or discrimination step. Support for this idea comes from studies of the heterologous, noncognate complex that forms between yeast Phe-tRNA synthetase and *E. coli* $tRNA^{Tyr}$. Although a rapid association occurs between these two species, no subsequent unimolecular conformational change has been detected.[32] Apparently the second, "reading" step cannot occur because the enzyme and tRNA are mismatched.

3. Concept of Dual Discrimination

Steady state kinetic studies of the aminoacylation reaction have shown that the maximal velocity plays an important part in discriminating between tRNA species. For example, yeast Val-tRNA synthetase, under normal reaction conditions, can do some aminoacylation of yeast $tRNA^{Ala}$.[36] The Km for this reaction is about 60-fold higher than it is for the cognate aminoacylation of $tRNA^{Val}$. However, the V_{max} is about 5,000-fold lower for the noncognate aminoacylation. Therefore, even when the noncognate tRNA binds to the synthetase, there is a low statistical likelihood of it being aminoacylated with the wrong amino acid. This concept has been strengthened by other studies.[37]

As mentioned earlier, studies of complex formation at equilibrium have shown that, in a homologous system, noncognate complexes can have dissociation constants 100-fold or more higher than that of the cognate complex. Thus, at the level of binding alone, there can be a good bias in favor of cognate complexes. However, the steady-state kinetic data suggest that the maximal velocity plays at least as great, if not a greater, role in the discrimination process. These results have given rise to the concept of dual discrimination, or a two-part process for recognition specificity.

It is not hard to rationalize the phenomenon of dual discrimination. Transfer RNA molecules have many structural similarities, as mentioned earlier. This enables each of them to cycle through the same ribosomal machinery in the process of protein synthesis. But this structural similarity makes it difficult for the various aminoacyl-tRNA synthetases to distinguish, simply on the basis of binding, the different tRNA species. The discrimination at the level of maximal velocity enables the enzymes to achieve a substantial extra increment in their recognition specificities.

The recognition that occurs at the level of the rate-determining step in catalysis is remarkable, because some experiments indicate that there are close similarities in the structural organization of cognate and noncognate complexes (see below). That is, a noncognate tRNA may bind to an enzyme in approximately the same orientation as the cognate one. Therefore, the distinction between various tRNAs, once they are bound to an enzyme, must be extremely subtle. It is likely that the placement of the 3′-terminus of the tRNA in the catalytic site of the enzyme has severe stereochemical requirements that must be met in order for catalysis to occur.

4. Role of the Anticodon

Because each anticodon is unique, this is the most obvious area for synthetases to

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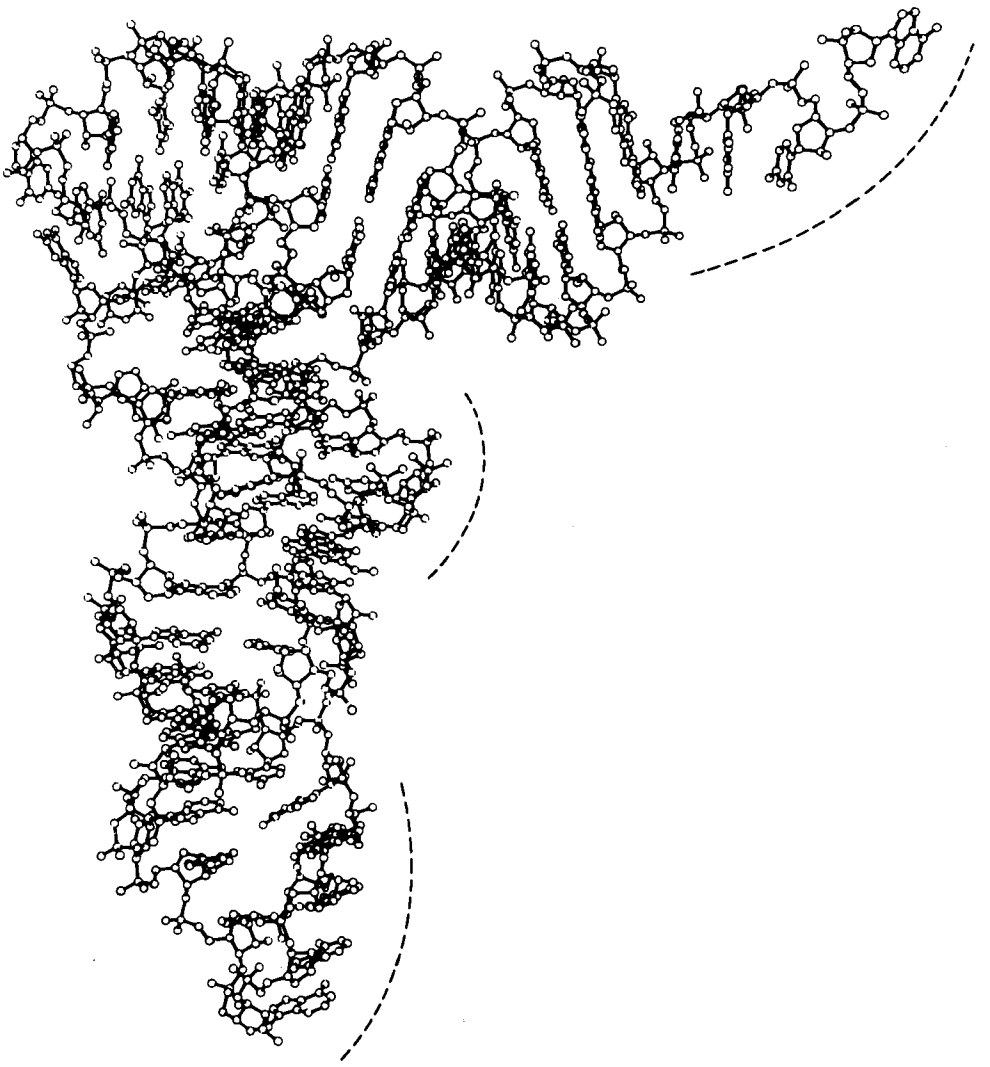

FIGURE 3. Skeletal model of yeast $tRNA^{Phe}$. The illustration may be compared with Figure 2. General areas found important for synthetase interactions in a number of studies are roughly indicated by dotted lines. (The diagram of the skeletal model was kindly provided by Dr. S. H. Kim.)

showed that in the same region of the $tRNA^{Tyr}$ structure, Tyr-tRNA synthetase catalyzes a similar exchange.[66] This raises the possibility that synthetase-catalyzed exchange at uridine 8 may be a common phenomenon.

Extensive model studies have been done on the H-5 exchange reaction of pyrimidines.[67] The mechanism that is generally visualized for this exchange is nucleophilic attack at C-6 to give a transient saturated adduct; reversal of this reaction can lead to tritium exchange. This is depicted in Figure 5, where it is envisioned that a nucleophilic group is attached to the enzyme. It should be noted that formation of the transient covalent adduct does not necessarily insure that exchange will occur. If adduct formation is stereospecific, so that, for example, enzyme and tritium enter from opposite faces of the pyrimidine ring, then reversal of the reaction will result in removal of the tritium that was incorporated (by microscopic reversibility). However, if tritium can

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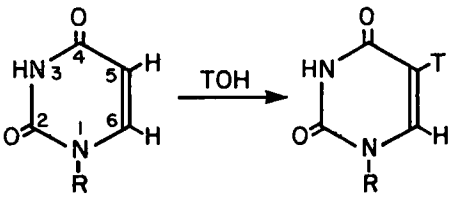

FIGURE 4. H-5 exchange reaction of uridine.

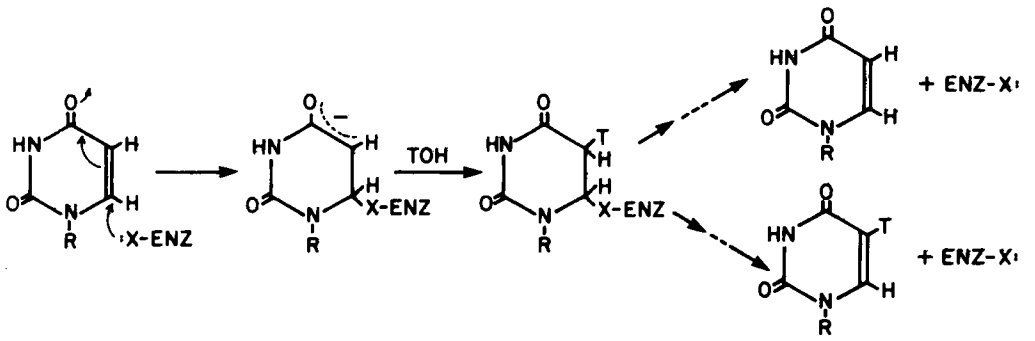

FIGURE 5. A mechanism for H-5 exchange.

occasionally add to either face of the ring, then on occasion the reverse reaction will result in some tritium incorporation. Therefore, the rate of tritium incorporation is not a quantitative measure of the extent of adduct formation, but rather is a suggestive indicator of the formation of a transient covalent bond.

This kind of mechanism has been demonstrated for thymidylate synthetase, an enzyme which catalyzes the formation of thymidylate from dUMP.[67] In this case, the existence of the transient adduct of enzyme with C-6 of the pyrimidine ring is well established. As a result of the formation of this adduct, the enzyme does stimulate tritium incorporation into C-5 of dUMP.

Figure 6 designates the sequence and cloverleaf structure of *E. coli* $tRNA^{Ile}$. The arrow designates the position of uridine 8. According to the published X-ray crystallographic data on yeast $tRNA^{Phe}$, the base in this position can participate in tertiary hydrogen bonding interactions with A14.[5,6] Poised in this conformation, the 5,6-side of U8 faces the solvent, and is easily accessible to attack by an approaching enzyme. Moreover, this part of the structure is precisely the region that other studies have indicated is important for synthetase-tRNA interactions; that is, it is believed that the synthetase spans this position in the tRNA structure (Figure 3).[26,59] Therefore, available evidence is consistent with the possibility that the synthetase comes close enough to this part of the structure to make a transient covalent bond with U8. With this in mind, further experiments have been done to determine more definitively whether this in fact occurs and, if so, its significance.

2. Further Observations

There is a major difficulty associated with working with the intact tRNA and proving that, with it, a transient covalent adduct forms with the bound synthetase. It is technically difficult to study an isolated interaction at one specific base among the background of the other nucleotides in the chain. For example, an enormous technical

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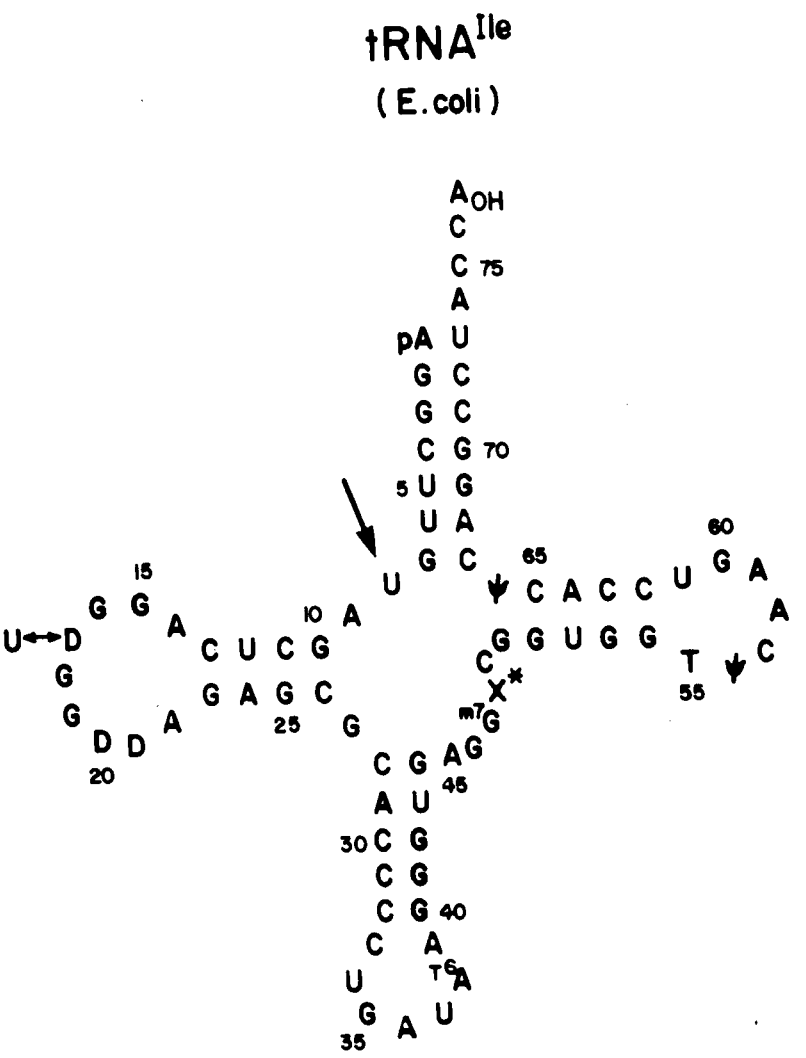

FIGURE 6. Sequence and cloverleaf structure of *E. coli* $tRNA^{Ile}$ with an arrow designating the 8-position.

effort was required in order to prove that synthetase catalyzed exchange occurs at uridine 8 and not at a variety of other pyrimidines in the structure.[66] Likewise, considerable technical difficulties are associated with trapping, or otherwise proving, a covalent adduct with enzyme occurs at a specific position. For this reason, attention has been turned towards smaller substrates.

Preliminary experiments have indicated that Ile-tRNA synthetase can catalyze H-5 exchange simply with uridine.[68] If the exchange with the mononucleoside involves the same catalytic groups which give rise to the exchange at U8 in the intact tRNA, then it is clearly advantageous to do further experiments with the monomeric substrate.

When working with uridine, a major concern is whether the exchange catalyzed by synthetase is a nonspecific phenomenon, which is unrelated to the reaction that occurs with tRNA. That is, one can imagine that any protein laden with nucleophilic groups (such as SH groups) could catalyze such an exchange. To check this point, experiments were also done with serum albumin and with phosphorylase *a*. In the absence of denaturing agents, the latter protein contains approximately 14 titratable SH groups,[69]

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and would be expected to catalyze exchange if nonspecific nucleophilic groups are capable of stimulating this reaction. However, when employed at concentrations comparable to Ile-tRNA synthetase, neither serum albumin nor phosphorylase *a* catalyzes any significant H-5 exchange with uridine.[68] These data suggest that regardless of its exact significance, Ile-tRNA synthetase has an apparently unique capability to carry out this reaction.

A second experiment compared the catalytic efficiency of Ile-tRNA synthetase with one of the best known chemical catalysis of the H-5 exchange reaction. It is well known that cysteine is an excellent catalyst, particularly around pH 9.[70] However, the reported rate of cysteine catalyzed exchange at pH 9, 37°C, is considerably lower than that of Ile-tRNA synthetase at pH 7.5, 37°C. In fact, on a per molecule basis, our preliminary data suggest that the synthetase at pH 7.5 appears to be approximately 1,000-fold more efficient than cysteine at pH 9; if the comparison is made with cysteine at pH 7.5, then the relative catalytic efficiency of the enzyme is even greater.[68] These data suggest the enzyme possesses unusual chemical properties with regard to catalysis of this exchange reaction.

3. Significance for Recognition

In order to establish that the interaction of synthetase with U8 is crucial for aminoacylation, it is necessary to show that when this interaction cannot occur, aminoacylation also does not take place. Conversely, if it can be shown that blocking the ability of the synthetase to make the putative adduct with U8 does not prevent aminoacylation, it is safe to conclude that the presumed adduct formation is not essential to the catalytic mechanism. With these objectives in mind, there are several different ways to approach this problem.

One approach is to show that when no H-5 exchange occurs there is also a lack of aminoacylation. It has been shown that when Ile-tRNA synthetase is mixed with $tRNA^{Tyr}$, there is no stimulation of tritium incorporation into the pyrimidines of $tRNA^{Tyr}$; on the other hand, when Tyr-tRNA synthetase is mixed with $tRNA^{Tyr}$, there is a stimulation and this largely occurs in the region of U8.[66] These experiments argue that there is some specificity to the H-5 exchange reaction and because Ile-tRNA synthetase does not aminoacylate $tRNA^{Tyr}$, it is consistent with the idea that H-5 exchange and aminoacylation are intimately linked.

A second approach is to modify U8 so the 5,6-double bond is destroyed or blocked from attack by enzyme, or to remove the base from the tRNA structure. The latter possibility has been attempted in two different systems. In cases where a thiouridine occurs at Position 8 (such as in many *E. coli* tRNAs[4]), it is possible to carry out a specific reaction with this base. (It appears that, for the H-5 exchange reaction, 4-thiouridine and uridine are roughly equivalent, and that exchange can go on with either base.[66]) It is well known that 4-thiouridine can be reduced with sodium borohydride and that subsequent treatment of the reduced base with acid results in fracture of the glycosidic bond.[71] Therefore, this is a way to reduce the 5,6-double bond and even remove the base from Position 8.

This experiment has been carried out with two *E. coli* tRNAs that contain thiouridine: $tRNA^{Tyr}$ and $tRNA^{Val}$.[66,72,73] The reduction and acid cleavage steps were carried out with the tRNAs containing either thiouridine or uridine at Position 8. (The latter can be produced from the former through a simple treatment.) The purpose of doing the experiment with the uridine-containing tRNA is to serve as a control; the reduction and the acid cleavage step should not have any effect on a uridine residue. In both cases, the reduction and acid cleavage result in a decrease in tRNA aminoacylation capacity; from an analysis of the experiment, it appears that all tRNA molecules con-

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taining a thiouridine at the 8-position are inactivated by this treatment.[72,73] However, experiments with tRNA containing uridine at Position 8, show that the reduction procedure per se leads to some inactivation; this is undoubtedly due to modification of other sites in the molecule such as dihyrouridine and m^7G. However, even after the acid treatment step, this tRNA material still retains significant, albeit reduced activity. These experiments are supportative of the idea that an intact uridine (or thiouridine) at Position 8 is essential for activity. On the other hand, because other sites are damaged in the modification procedure, the experiment is not clear-cut and unambigious in its interpretation.

It is well known that it is possible to cross-link a s^4U8 with C13 under the action of ultraviolet light.[74,75] The cross-linking occurs through the 4-position of the uridine ring and it can occur without a major disruption of the tRNA structure.[5,6] This modification does not prevent aminoacylation, although it can affect the rate of charging.[75] However, because the 5,6-double bond is not affected by the cross-linking reaction, it is difficult to assess the meaning of this finding.

In summary, therefore, there is still no definitive proof that a transient covalent bond must form at uridine 8 in order for aminoacylation to occur. What is clear is that the synthetases come into close proximity with this residue, and that, because a similar interaction at this site has been observed with two different aminoacyl tRNA synthetases, the interaction at U8 is common to most synthetase-tRNA complexes.

What purpose might a covalent interaction at U8 serve? Because all tRNAs contain this base, it is clearly not the recognition site per se. It is possible that residues flanking uridine 8 play a role in recognition, and this is discussed in the following section. Bases in other parts of the structure, and/or those flanking uridine 8, might be specificity sites which determine whether a synthetase is oriented in such a way as to make a transient bond with U8. The bond at U8 itself may serve the purpose of coordinating the recognition sites with the catalytic site on the enzyme which attaches amino acid to the 3′-terminus of the tRNA. This may be especially important in a situation where the "specificity sites" on the tRNA substrate are well separated spatially from the site of catalytic action. The many degrees of freedom which a large, bound macromolecule substrate can have may require the enzyme to "clamp" the substrate in a fixed orientation in order to align exactly the 3′-terminus with the catalytic groups on the enzyme.

The necessity for proper alignment could be an important part of the discrimination process. As mentioned earlier, the recognition process is in two steps and occurs both at the binding step and at the catalytic step associated with V_{max}. One can imagine that for a noncognate complex, it may be difficult to make the transient adduct at U8, and for that reason the 3′-terminus of the incorrectly bound tRNA cannot line up precisely enough with the catalytic groups of the enzyme so as to achieve aminoacylation. In this way the interaction at uridine 8 could play a crucial role in the discrimination process.

C. Oligonucleotide Binding

As mentioned earlier, work on aminoacyl tRNA synthetases has suggested that the recognition sites on tRNA for these enzymes may be scattered on various parts of the tRNA structure. Thus, it would seem that a single block of consecutive nucleotides does not comprise the recognition site of any aminoacyl tRNA synthetase. On the other hand, it is reasonable to suppose that short sequences in scattered parts of the structure may play a role in recognition. In this connection, because there is clear evidence that at least two synthetases interact with the region around uridine 8, it is reasonable to test the binding of oligonucleotides derived from the uridine 8 region of the tRNA.

This kind of experiment has been done with $tRNA^{Ile}$ and Ile-tRNA synthetase. The

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oligonucleotide U-A-G corresponds to the 8-9-10 position in the $tRNA^{Ile}$ structure (see Figure 6). Using equilibrium dialysis, binding of this oligonucleotide and the reverse sequence G-A-U has been tested. It was found that at pH 7.5, 4°C, U-A-G binds to Ile-tRNA synthetase with a dissociation constant of about 90 μM; under the same conditions, no binding of G-A-U could be detected.[68] This argues for a specific interaction of U-A-G with Ile-tRNA synthetase. Because the interaction is specific, it is plausible that the oligonucleotide sits in the same site on the enzyme that is occupied by the corresponding sequence in the bound tRNA.

A different approach to the interaction of oligonucleotides with synthetases has been taken by Vlassov and Khodyreva.[76] These authors simply scanned the entire tRNA sequence for oligonucleotides which might interact with synthetase. This was done with *E. coli* $tRNA^{Phe}$ which was treated with ribonuclease A. This produced oligonucleotides which were then dialyzed against enzyme. After dialysis, the oligonucleotides on both sides of the dialysis membrane were separated and analyzed. The main objective was to determine whether certain oligonucleotides were more concentrated on the enzyme side of the membrane than on the opposite side.

In this experiment, two oligonucleotides were found to be substantially concentrated on the enzyme-containing side of the membrane. These are designated in Figure 7, which gives the sequence and cloverleaf structure of *E. coli* $tRNA^{Phe}$ together with oligonucleotides found to bind strongly to the enzyme enclosed by a solid line. One of these oligonucleotides includes the uridine 8 region; the other is in the anticodon stem. Although the authors do not calculate dissociation constants for these enzyme-oligonucleotide complexes, it would appear that they would need to be less than 100 μM in order to account for the binding they observe. Finally, a third oligonucleotide, derived from the 3′-side of the dihydrouridine stem, was also found concentrated on the enzyme side of the dialysis membrane; this oligomer appeared to interact significantly less strongly than the other two and it is indicated by a dotted outline in Figure 7.

There are three questions that can be raised in connection with these experiments. First, it is unknown whether the oligonucleotides interact separately with the enzyme or whether combinations of two of them, or all three of them, must be simultaneously bound in order to get strong binding. That is, the experiment does not tell whether the interactions are synergistic or whether they occur independently.

The second question is related to the first. As mentioned, it would appear that in order to account for the binding that is observed, the dissociation constants of the strongly binding oligonucleotides would need to be less than 100 μM. Assuming that the weaker binding oligonucleotide has a dissociation constant of 1 mM or less, then a paradox arises when one tries to relate these data to the dissociation constants normally encountered when synthetases bind to tRNAs. In particular, synthetase-tRNA dissociation constants are typically in the range of 1 μM to 1 nM.[18] If the free oligonucleotides independently interact with the synthetase with the same interaction free energy as the corresponding oligonucleotide sequences in the tRNA, then we would predict that the dissociation constant for the synthetase-tRNA interaction would be at least 0.01 nM (which is the product of the three maximal dissociation constants estimated for each of the oligonucleotide-synthetase interactions). Unless there are other oligonucleotide sequences in the tRNA which tend to decrease synthetase-tRNA binding, it is clear that the dialysis results do not fit well with the overall synthetase-tRNA dissociation constant. Of course, as previously mentioned, it is possible that the interaction of these oligonucleotides is synergistic, so that it is not legitimate to calculate independent dissociation constants for each oligonucleotide. In any event, this question needs to be cleared up.

A third question has to do with the specificity of the interaction of these oligonu-

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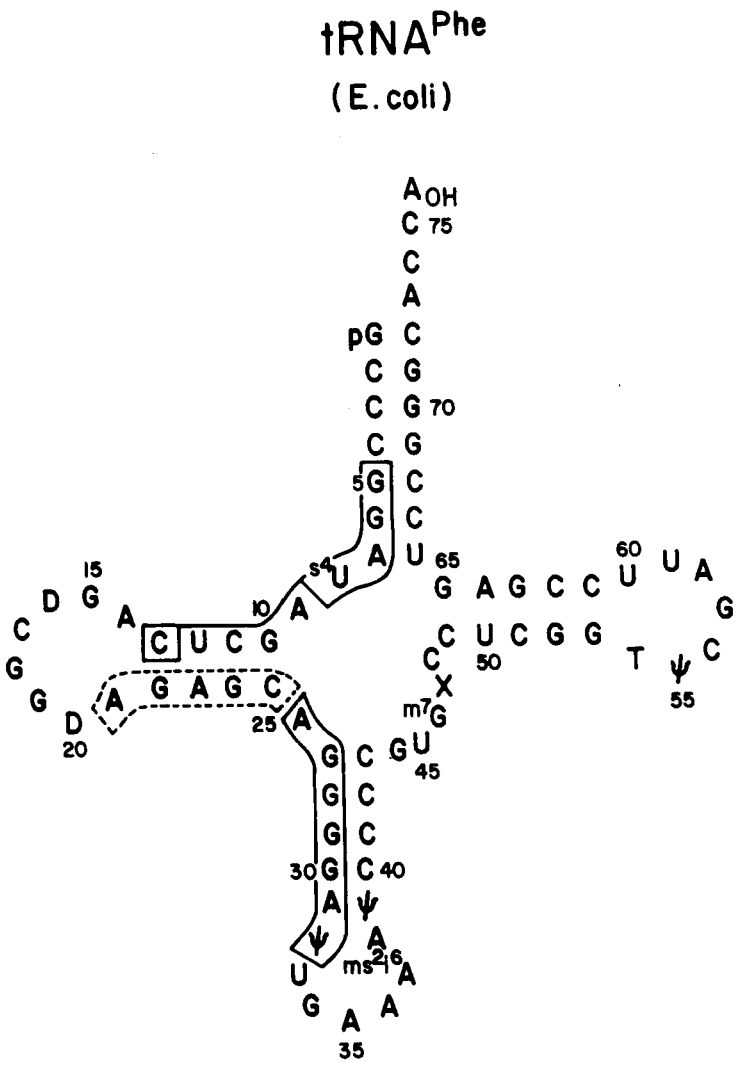

FIGURE 7. Sequence and cloverleaf structure of *E. coli* $tRNA^{Phe}$. Oligonucleotides found to bind strongly to Phe-tRNA synthetase are enclosed by a solid line, while a less tightly binding fragment is enclosed by a dashed line. Every fifth base in the sequence is designated by a lower case letter. In the case of the fragment that runs from G5 to s^4U8, it is apparently joined to C13 as indicated, presumably through a photo-cross-link between s^4U8 and C13. (From Vlassov, V. V. and Khodyreva, S. N., *FEBS Lett.*, 96, 95, 1978. With permission.)

cleotides with the synthetase. In particular, one of these oligomers contains four consecutive Gs (see Figure 7). It is possible that the interactions of this oligonucleotide with the synthetase are nonspecific, and arise from the predominance of consecutive G residues which might interact with a variety of proteins.

Nevertheless, it is interesting that these investigators find that an oligonucleotide from the U8 region associates significantly with the synthetase. This fits well with the data on Ile-tRNA synthetase and, clearly, their approach represents a rapid screening method for determining sequences in the tRNA that may bind as isolated oligonucleotides to the synthetase. This approach hopefully will be used in studying a variety of other systems.

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These results on oligonucleotide binding to Ile-tRNA synthetase and to Phe-tRNA synthetase have been sufficiently encouraging so as to warrant expansion of this approach to other systems. For example, as mentioned earlier, mutations in the anticodon of *E. coli* $tRNA^{Gly}$ and of *E. coli* $tRNA^{Trp}$ significantly affect recognition.[40,41] Equilibrium dialysis experiments with anticodon oligonucleotides would be of considerable interest in these cases. Also, it is conceivable that oligonucleotide binding might influence enzyme conformation in a way that is crucial for catalysis. For example, if the interaction of synthetase with a particular oligonucleotide sequence in the intact tRNA somehow influences the conformation of the enzyme so that it can more easily aminoacylate the 3′-terminus, then the same conformational change might occur upon oligonucleotide binding. In this event, it would be interesting to see whether the 3′-terminal oligonucleotide C-C-A, which is present in all tRNAs, could be aminoacylated by a synthetase which has been "triggered" by the specific binding of an oligonucleotide from another part of the structure.

III. ELONGATION FACTOR Tu

A. Basic Aspects

The bacterial elongation factor Tu has been under intense study for several years. It is involved in the delivery of aminoacyl tRNA (AA-tRNA) to the ribosomes. It participates in the following series of reactions:[77,78]

1. Tu·GTP + AA-tRNA → Tu·GTP·AA-tRNA
2. Tu·GTP·AA-tRNA·ribosomes Tu·GDP + P_i + ribosome bound AA-tRNA
3. Tu·GDP + Ts→ Tu·T_s + GDP
4. Tu·T_s + GTP → Tu·GTP + Ts

In the first step, aminoacyl tRNA (AA-tRNA) is bound to a complex of Tu with GTP. This complex binds to the ribosomes, resulting in the release of AA-tRNA and the hydrolysis of GTP to GDP. The Tu·GDP complex then interacts with another protein, T_s. This results in the release of GDP and the formation of the Tu·TS complex. Finally, the cycle is completed by the binding of GTP to Tu and the release of Ts from Tu.

The designation Tu means "temperature unstable", while Ts denotes "temperature stable".[77] The two proteins have molecular weights of 45,000 and 30,000, respectively.[79-81] It appears that the main function of Ts is to catalyze the exchange of GDP for GTP.[82] This exchange is apparently accelerated by 1500-fold or more by Ts.[77]

Both GTP and GDP bind strongly to Tu, with the diphosphate having over a 100-fold stronger affinity (dissociation constant of about 3 n*M* at 20°C, in the presence of Mg^{2+}).[77,79] Interestingly enough, only the GTP form of Tu associates strongly with aminoacyl tRNA.[83,84] This implies that a conformational difference exists between Tu·GTP and Tu·GDP. There is direct experimental evidence for such a difference.[85,86]

The binding of aminoacyl tRNA to Tu·GTP is sometimes detected by a nitrocellulose filter assay.[83] In the absence of AA-tRNA, Tu·GTP adheres to a nitrocellulose filter, while AA-tRNA does not stick. When mixed together to form a complex, the filtration properties of the AA-tRNA apparently predominate because the entire complex passes through the filter. Thus, the extent of complex formation can be monitored by measuring the amount of Tu·GTP that passes through the filter upon addition of AA-tRNA. This is generally done by using radioactively labeled GTP to detect the Tu·GTP. Using a variation of this assay, it is estimated that the dissociation constant of AA-tRNA with Tu·GTP is about 10 to 100 n*M*.[87,88]

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Tu·GTP associates strongly with charged noninitiator tRNAs.[83,89] That is, neither the nature of the amino acid nor the specific noninitiator tRNA species is discriminated by the protein-nucleotide complex. On the other hand, Tu·GTP does discriminate against the charged initiator tRNA, Met-tRNA$_f$Met. This tRNA species is not bound while the analogous noninitiator, Met-tRNA$_m$Met, is bound by Tu·GTP.[90] Thus, the protein is able to distinguish between a charged initiator vs. a charged noninitiator tRNA.

Another important aspect of the interaction of AA-tRNA with Tu·GTP is the stringent requirement for an aminoacyl group; the protein does not interact with uncharged tRNA.[83] Moreover, blocking of the amino group of the aminoacyl moiety of AA-tRNA prevents Tu binding.[89] Thus, there is a strong requirement for a free amino group on the amino acid. But it is interesting to note that the discrimination against Met-tRNA$_f$Met occurs even when the charged initiator species lacks the formyl group on its methionine residue.[90] This means that information in the tRNA structure itself is recognized by the protein.

Several studies have attempted to understand in more detail the nature of the interaction between Tu·GTP and AA-tRNA. One objective has been to define the architectural features of the complex and to define what parts of the tRNA are sensed by the protein. In addition, efforts have been directed at the nature of the protein-tRNA interaction at the 3′-end of the tRNA molecule, where the amino acid is attached. Because aminoacylation is the signal that triggers binding of Tu·GTP, it is reasonable to explore features of the protein-nucleic acid complex in this part of the tRNA. Some of these studies are considered below.

B. Nuclease Digestion Studies

Jekowsky and Jekowsky et al. have done several experiments to map the architectural design of the complex.[91,92] Evidence was obtained showing that the 3′-end is shielded by bound Tu. For example, the solvent mediated spontaneous hydrolysis of the amino acid from aminoacyl tRNA is strongly retarded if Tu is bound to the charged tRNA. This suggests that the protein shields the aminoacyl moiety from the solvent. Moreover, in free AA-tRNA the 3′-end can be rapidly cleaved by pancreatic ribonuclease A; this occurs because of the single-stranded cytidine residues at the 3′-end. However, in the presence of saturating amounts of Tu·GTP, the cleavage can be inhibited by over a factor of 15. Moreover, no protection is afforded by Tu·GTP. Thus, the protection by Tu·GTP of the 3′-terminus can be used to assay for the extent of complex formation.[92]

Further nuclease digestion studies were done with T1 ribonuclease. This enzyme specifically cleaves after G's. The idea was to digest the complex with T1 ribonuclease with the hope of obtaining a nuclease-digested core that remains bound to Tu. Digestion was carried out for 30 min at 37°C; after this time, a nuclease-digested section of tRNA remained bound to Tu. This was subjected to structural analysis to determine which parts of the intact tRNA had been cleaved.

The results of this analysis are shown in Figure 8. The figure gives the cloverleaf structure of yeast tRNA^{Phe} and indicates by solid arrows the sites at which T1 ribonuclease cleaves the aminoacyl tRNA that is bound to Tu·GTP. These cleavages occur after G15, G18, and G57. The dashed arrows represent additional cleavages that occur at either G19 or G20 or both. It is clear from this diagram that a chunk of the dihydrouridine loop is removed and that a cleavage is introduced into the TψCG loop; but even with these cleavages, the charged tRNA remains firmly bound to Tu·GTP.

It should be noted that it is not possible for T1 ribonuclease to introduce a cleavage in the anticodon loop, where there is only one G residue and this is modified with a

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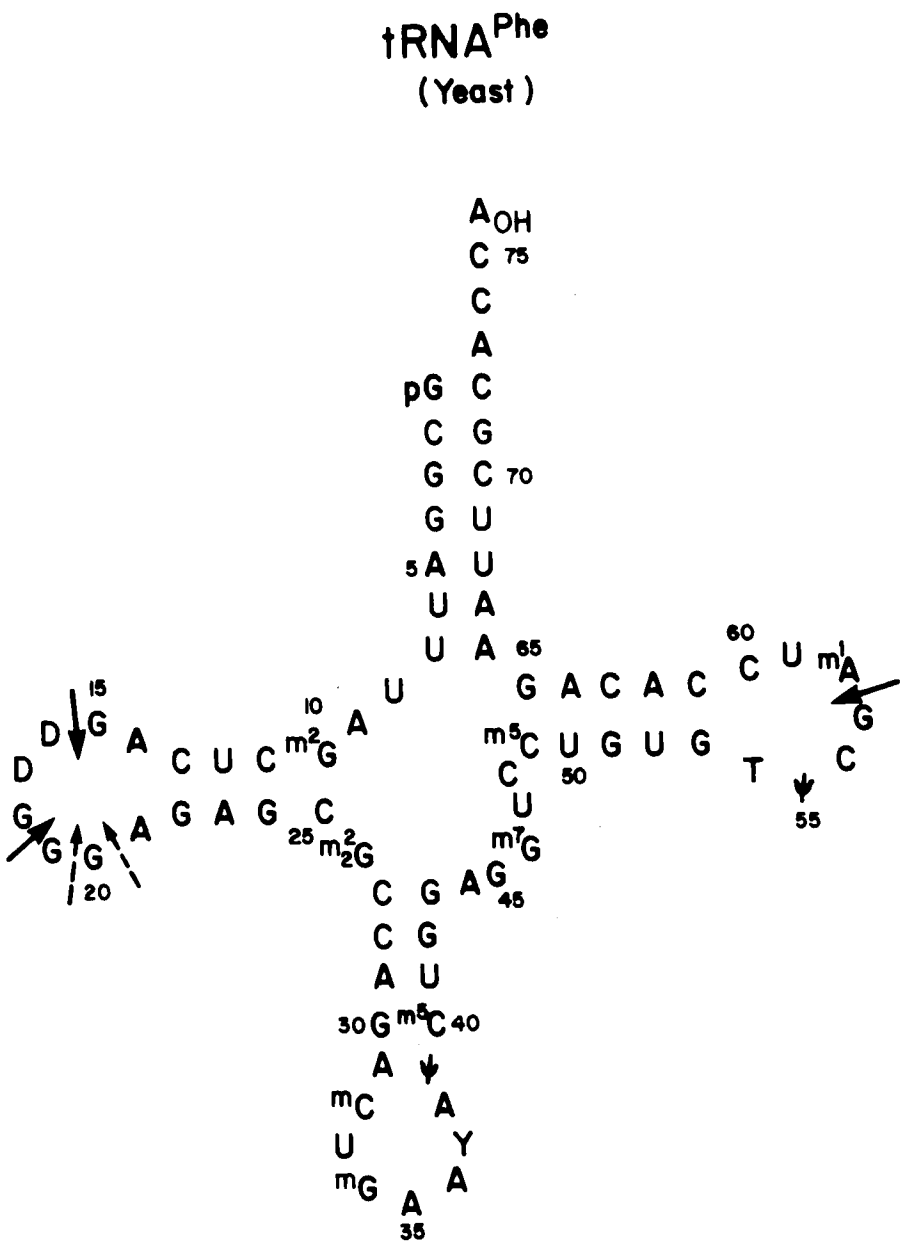

FIGURE 8. Sequence and cloverleaf structure of yeast tRNA^{Phe}. Cleavages in a T1 ribonuclease digested complex of this tRNA with elongation factor Tu are indicated by solid arrows. Dashed arrows denote an additional cleavage that occurs at G19 or G20 or both. (With permission from Jekowsky, E., Miller, D. L., and Schimmel, P. R., *J. Mol. Biol.*, 114, 451, 1977. Copyright by Academic Press, Inc.)

2′-O-methyl group; the modification blocks nuclease action. There is only one G in the TψC loop and cleavage occurs at that point; cleavage occurs at least at three, if not at all four, Gs in the dihydrouridine loop. Most of the remaining Gs are in double helical sections where they are less susceptible to T1 ribonuclease attack.

The significance of these cleavages is seen better in Figure 9, which gives a schematic illustration of the 3′-dimensional structure of yeast tRNA^{Phe}. In this figure, the tRNA

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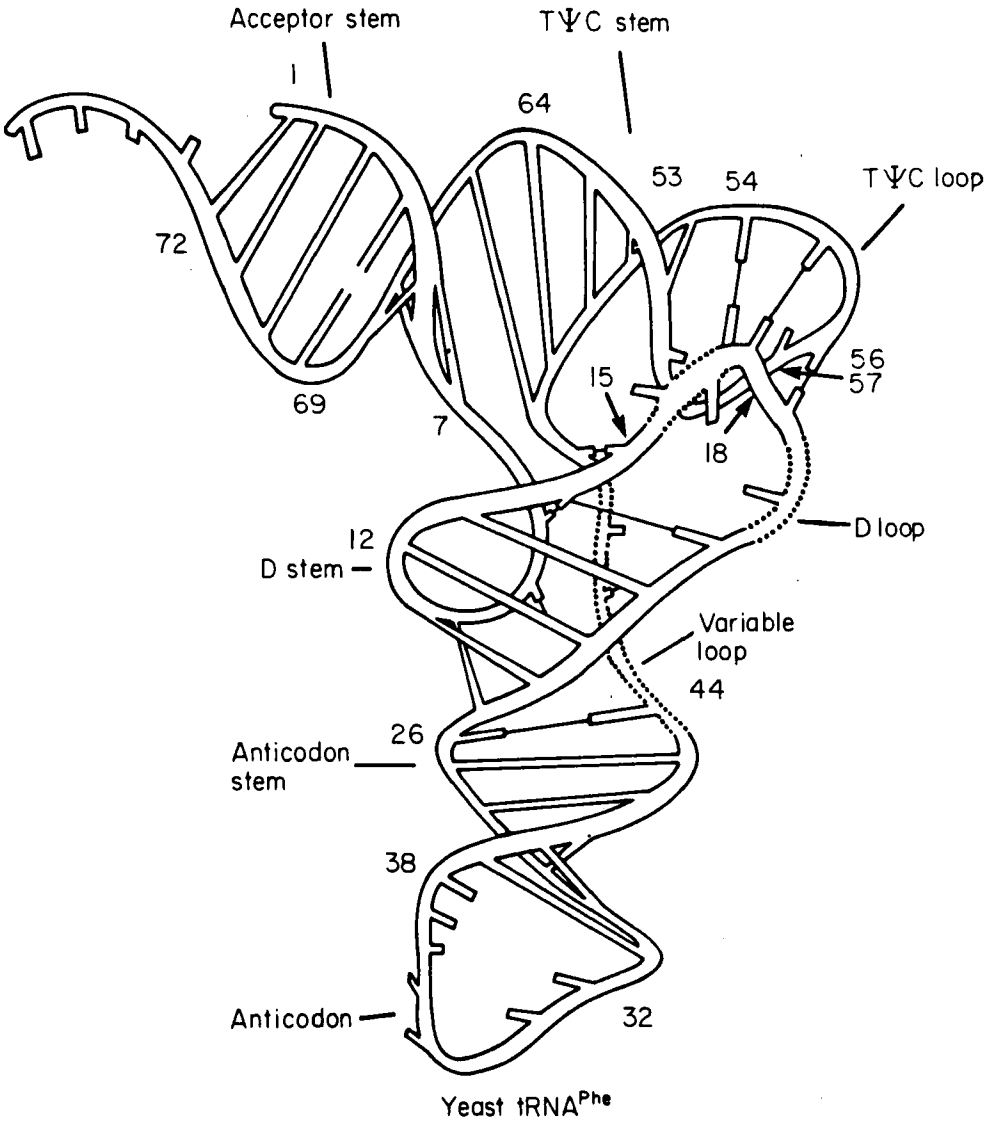

FIGURE 9. Schematic illustration of yeast $tRNA^{Phe}$ structure with arrows indicating positions of three of the T1 ribonuclease cleavages of the nuclease digested complex of the tRNA with elongation factor Tu. The arrows correspond to the solid ones in Figure 8. The tRNA structure is represented as a continuous tube with cloverleaf hydrogen bonds designated with open crossbars and thin lines joining groups that bond in tertiary interactions. (With permission from Jekowsky, E., Miller, D. L., and Schimmel, P. R., *J. Mol. Biol.*, 114, 451, 1977. Copyright by Academic Press, Inc.)

backbone is represented as a continuous tube, while cloverleaf hydrogen bonds are designated by open crossbars. Thin lines join bases that are bonded together through tertiary interactions. The solid arrows in this figure designate the same cleavage points that are indicated by solid arrows in Figure 8. From this figure, it is clear that removal of Residues 16, 17, and 18 from the dihydrouridine loop seriously disrupts the tertiary interactions of this loop with the TψC loop. In addition, the cleavage at G57 is spatially close to the dihydrouridine loop cleavages, and should also disrupt the tertiary structure. Because Tu is able to bind to a tRNA species which is cleaved in these areas, the results indicate that serious damage to the tertiary structure does not destroy the recep-

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tor sites for the elongation factor. This result is compatible with the idea that the protein bonds primarily to the aminoacyl acceptor-TψC helical stem of the tRNA. In this way, it would avoid contact with both the D and the TψC loop, so that cleavages in these loops would not seriously affect its interaction with the tRNA. Also, because cleavages that disturb the tertiary structure might affect the orientation of the helical D stem and anticodon stem with respect to the acceptor-TψC helix that runs approximately at a right angle, it seems likely that the interaction does not extend to a region significantly beyond the acceptor-TψC helical axis. This would appear to be the simplest explanation of the data.

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C. Chemical Modification Studies

As mentioned earlier, Tu is able to distinguish between Met-tRNA^{Met} and Met-tRNA$_f$Met. Using a chemical modification approach, Schulman et al. have explored the physical basis for this discrimination.[90] This in turn has led to considerable insight into the structural features important for recognition of aminoacyl tRNAs by Tu.

The basic approach takes advantage of a relatively gentle chemical modification that can be introduced by bisulfite treatment. By use of bisulfite, cytidine residues are deaminated and converted to uridines, according to the scheme of Figure 10.[93-95] This is a relatively gentle modification, especially because no bulky groups are left attached to the nucleotide units.

In the case of *E. coli* tRNA$_f$Met, there are 26 cytidine residues. When treated with 3 *M* sodium bisulfite at pH 6 and 10 m*M* $MgCl_2$ at 25°C, only six of the cytidine residues are converted to uridines.[90] The six modified residues are located at Positions 1, 16, 17, 35, 75, and 76. The modifications at Positions 35 and 76 lead to loss of amino acid acceptor activity. The remaining modifications have no effect on the acceptor activity. The sequence and cloverleaf structure of tRNA$_f$Met and the location in the structure of the four modifications that do not affect aminoacylation are illustrated in Figure 11.

A unique feature of the initiator tRNA is the lack of a base pair for the 5′-terminal residue. In noninitiator tRNAs, this residue is paired with a complementary base on the 3′-side of the tRNA; but in tRNA$_f$Met, Cl is matched with A73, so that no Watson-Crick base pair can form. However, the bisulfite- induced conversion of C to U enables this terminal residue to base pair through a U · A match. This is the most striking result of the bisulfite modification of tRNA$_f$Met.

1. Role of Terminal Base Pair

Many experiments involving chemical modifications seek to alter specific residues and then test the effect of the modification on the binding of a particular protein; in general, one looks for modifications that either diminish or leave unaltered the binding characteristics. If an active tRNA molecule can be modified at a specific site and thereby be made inactive, one can tentatively assume that the modified site represents a point important for binding with the protein under investigation. This is not a hard and fast conclusion because it is possible that modifications introduce structural changes which in turn cause inactivation. As a starting point in the analysis, however, it is useful to consider the possibility that the modification destroys the ability of a base to act as a receptor site.

In contrast to the usual situation, in working with tRNA$_f$Met one *starts* with a species that is inactive (with respect to Tu binding). Therefore, modifications are introduced to see whether some of them might induce a change which would permit Tu to bind strongly to the charged, modified tRNA.

The basic experimental approach used was as follows. *E. coli* tRNA$_f$Met was treated

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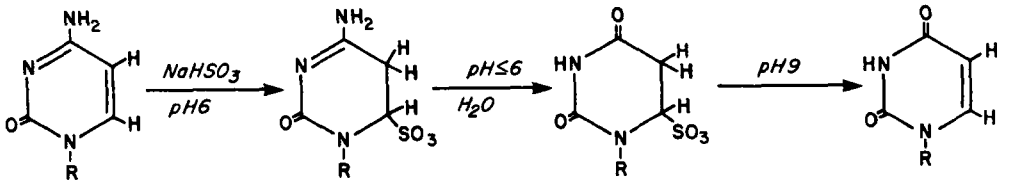

FIGURE 10. Schematic illustration of the chemical conversion of cytidine to uridine.

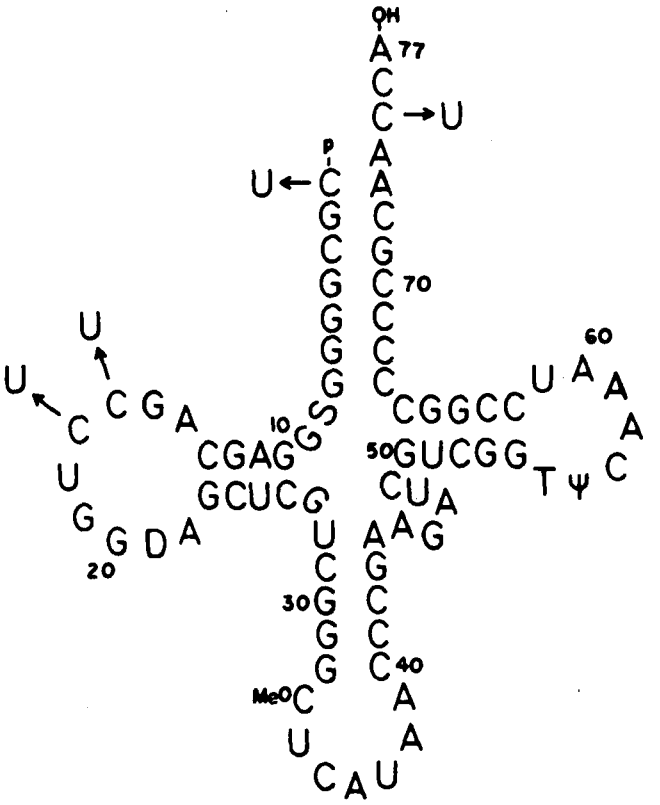

FIGURE 11. Cloverleaf representation of *E. coli* $tRNA_f^{Met}$. Every tenth base in the sequence is numbered. Four C → U conversions that can be achieved by bisulfite treatment (see Figure 10) are indicated, and these four do not affect amino acid acceptance activity. (From Schulman, L. H., Pelka, H., and Sundari, R. M., *J. Biol. Chem.*, 249, 7102, 1974. With permission.)

with bisulfite to give partial modification of each of the reactive sites and loss of over half of the original methionine acceptor activity. The active molecules were selected by aminoacylating the modified species and testing the ability of these molecules to form a complex with Tu·GTP. The molecules that were not aminoacylated represent ones that have modifications at C35 and, additionally or alternatively, C76. As mentioned earlier, these cannot be aminoacylated.

The results of this experiment are shown in Figure 12. The figure shows the amount

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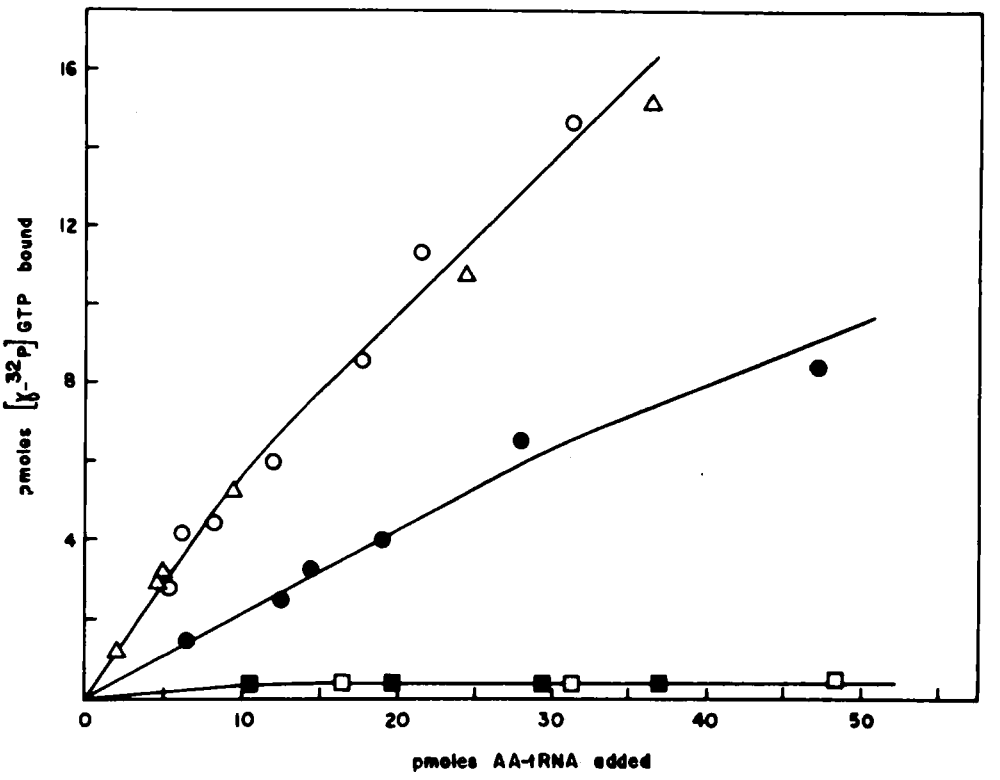

FIGURE 12. Association of modified and unmodified tRNAs with elongation factor Tu. The vertical axis gives the amount of protein-nucleic acid complex formed. Bottom line is for unmodified Met-tRNA$_f$Met and modified fMet-tRNA$_f$Met. The top line is for Phe-tRNA^{Phe} and Met-tRNA$^{Met}_m$. The middle curve is for partially modified (see text) Met-tRNA$_f$Met. (From Schulman, L. H., Pelka, H., and Sundari, R. M., *J. Biol. Chem.*, 249, 7102, 1974. With permission.)

of complex formed vs. the amount of aminoacyl tRNA added. The bottom line shows results with unmodified Met-tRNA$_f$Met and modified fMet-tRNA$_f$Met. In both cases, there is no association of the tRNA species with the elongation factor. The top curve shows the good complex formation obtained with Phe-tRNA^{Phe} and Met-tRNA$_m$Met. Between the two curves is a curve which represents the interaction of a partially modified Met-tRNA$_f$Met with elongation factor Tu. It is clear that the bisulfite modification of tRNA$_f$Met has introduced a substantial alteration in the ability of the charged initiator tRNA to interact with elongation factor. Thus, here is a clear case where a chemical modification gives activation of a biological function of tRNA rather than deactivation.

Subsequent experiments showed that the binding curve for modified Met-tRNA$_f$Met could be brought into coincidence with that for Phe-tRNA^{Phe} and Met-tRNA$_m$Met by a simple treatment of the modified initiator tRNA. One of the C to U transitions that occurs is at Position 75. By an enzymatic procedure, it is possible to remove modified C75 (which is now a U) and replace it with its normal counterpart, C. When this is done, the modified Met-tRNA$_f$Met binds to elongation factor with an affinity that is indistinguishable from that of unmodified Met-tRNA$_m$Met.[90] Thus, normal binding of the initiator tRNA to the elongation factor is achieved by the introduction of one or more of the changes that are possible at Positions 1, 16, and 17.

An attempt was made to pinpoint precisely which base was responsible for the acti-

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vation of tRNA$_f$Met with respect to Tu binding. In particular, attention was directed at the nature of the base at Position 1 in the modified tRNA that binds to Tu. Bound tRNA was separated from free tRNA on a gel filtration column, and the 5′-terminus was analyzed. It was found that in the material bound to Tu, there was a strong enrichment for the modified species that had a terminal 5′-U relative to the starting modified material. In the fractions from the filtration column which represented tRNA molecules that did not bind to Tu, there was no enrichment in the amount of Terminal U. Although C to U modification also occurs in the dihydrouridine loop, these are less frequent than the extent of conversion of Cl to Ul, in the material that binds to Tu. Collectively, these results suggest that the C to U conversion of the 5′-terminus is a necessary condition for activation of the initiator tRNA with respect to Tu binding.

In the gel filtration separation of bound, modified tRNA from free, modified tRNA, some 5′-terminal C residues were present in the material that presumably represents complex. This raises the possibility that the charged initiator can be induced to bind to Tu·GTP, even without a terminal base pair. However, the separation between the free and the bound tRNA was not perfect, and the amounts of 5′-terminal C detected in the complex fraction could be due to contamination from the unbound tRNA.

2. Role of 5′-Phosphate

Schulman et al. have also done experiments to test out the significance of another structural feature of the 5′-terminus.[90] The question was raised as to the significance for Tu binding of the 5′-terminal phosphate group. Tu binding was tested with tRNAs which either contained or lacked this terminal group. The results are shown in Figure 13. In Panel A, it is shown that no complex formation occurs with Met-tRNA$_f$Met, regardless of the presence or absence of phosphate. However, in the case of Phe-tRNA^{Phe} and Met-tRNA$_m$Met, two noninitiator tRNAs, there is a clear absolute requirement for the presence of the 5′-terminal phosphate in order for Tu binding to occur (Panels B and C). This result adds further weight to the conclusion, established through chemical modification studies, that the 5′-terminus is a key point for Tu recognition.

The importance of the terminal base pair in the acceptor stem, the observation that conversion of C75 to a uridine results in a serious decrease in the ability of Tu to bind, the obvious significance of the 5′-phosphate, and the nuclease protection results clearly indicate that the acceptor end of the molecule is crucial for the recognition process. In support of this are results obtained several years ago with a modified yeast Phe-tRNA^{Phe}. An extra cytidylic acid residue was introduced at the 3′-terminus to give C-C-C-A instead of the usual C-C-A. The charged form of this species binds poorly to Tu·GTP.[96]

D. 2′ Versus 3′ Aminoacyl Specificity

Much effort has been directed at identifying the initial position of aminoacylation (2′ or 3′) by aminoacyl tRNA synthetases. It is now clear that some enzymes preferentially aminoacylate the 2′ position, some aminoacylate the 3′ OH, and a few aminoacylate at either position.[97-100] Because elongation factor Tu discriminates between charged vs uncharged tRNA, it seems clear that some aspect of the aminoacyl moiety must be sensed by bound Tu. If the recognition involves a precise stereochemistry, one can imagine that Tu binds to an aminoacyl moiety specifically attached to the 2′ or 3′ hydroxyl. For example, if the amino group of an amino acid itself is sensed by Tu as an important recognition site on aminoacyl tRNA, its orientation with respect to the rest of the terminal adenosine residue would clearly be different in the 2′ vs. the 3′ position. Therefore, it is plausible to think that Tu might preferentially bind one of

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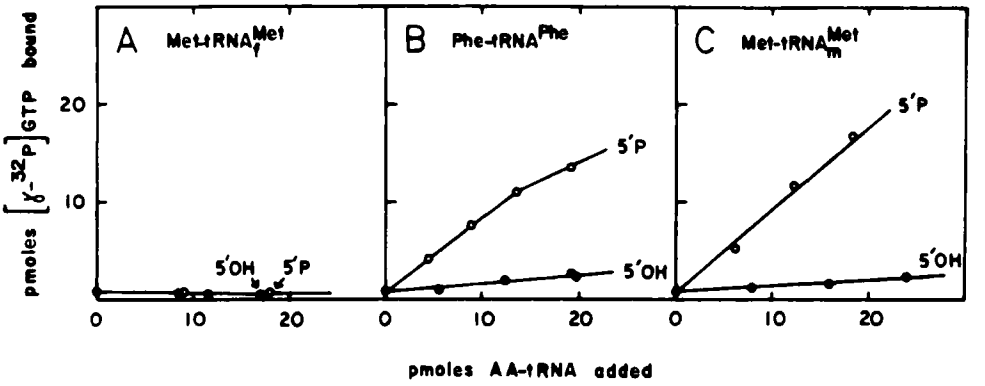

FIGURE 13. Effect of 5'-phosphate on complex formation of charged tRNAs with elongation factor Tu. Vertical axis gives amount of complex formed. From Schulman, L. H., Pelka, H., and Sundari, R. M., *J. Biol. Chem.*, 249, 7102, 1974. With permission.)

the aminoacyl-tRNA isomers and should the initial position of amino acid attachment be incorrect for Tu recognition, rapid acyl migration to the adjacent hydroxyl would yield the species that is active for T-factor binding.

This question has been examined in some detail by taking advantage of special procedures that enable the preparation of isomeric tRNA species with the aminoacyl moiety stably attached specifically to the 2′ or 3′ position. For example, use has been made of tRNAs that terminate in 2′ or 3′ deoxyadenosine. Such tRNAs can be prepared in their aminoacylated form and tested for binding to Tu·GTP.

The results of extensive investigations indicate that the T-factor shows little preference for the 2′ or 3′ aminoacylated form.[100-103] Experiments have been designed to give good sensitivity for detection of any preference by using molecular sieve gel filtration of two isomeric tRNAs mixed with Tu·GTP. One positional isomer is labeled with tritium and the other with ^{14}C. Using this approach with isomeric tRNA^{Tyr}, little preference for either isomer was found.[103] Hecht has pointed out that the small preferences which are sometimes seen in an experiment of this sort can easily be reversed by simply altering the experimental conditions — for example, by introducing a change as simple as the nature of the gel filtration matrix.[103] On the other hand, although there is little preference for either of the isomeric species, it appears that the *unmodified* aminoacyl tRNA, which has both cis hydroxyl groups available for the aminoacyl moiety, is the best ligand for Tu.[100-103]

E. Conclusions and Unresolved Issues

The experiments cited above have given considerable insight into the areas on aminoacyl tRNA that are important for Tu recognition. It is clear that the acceptor terminus is the key element. The evidence is also fairly good that the protein is able to discriminate against initiator tRNAs by virtue of their lack of a base pair at the first position in the acceptor helix. What still remains unclear, however, is how the factor is able to distinguish between aminoacylated vs. unacylated tRNA.

One obvious possibility is that the tRNA undergoes a subtle conformational change upon aminoacylation. The question of the effect of aminoacylation on the conformation of tRNA has been investigated for a number of years. Some of the evidence has produced conflicting conclusions and, as a result, no clear picture has emerged. Cohn and co-workers investigated proton magnetic relaxation rates of water protons surrounding tRNA; they found changes upon aminoacylation of bulk tRNA and of

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$tRNA^{Phe}$ which they interpreted as due to a change in structure at some of the manganese binding sites.[104] Ninio used small angle X-ray scattering and found no intensity changes upon aminoacylation of *E. coli* $tRNA^{Val}$, but at large angles found that aminoacylation did cause some intensity differences which they attributed to altered Mg^{+2} binding and to changes in the cation distribution.[105] More recently, Potts et al. have used laser light scattering techniques to measure accurately the diffusion constant of charged and uncharged yeast $tRNA^{Phe}$, under different solution conditions.[106,107] Some data are plotted in Figure 14, which gives the translational diffusion constant of yeast $tRNA^{Phe}$ at different extents of aminoacylation. The diffusion constants were monitored along with the extent of deacylation, as the charged tRNA deacylated spontaneously. Solutions contained 0.1 *M* Tris·Cl, 0.1 *M* NaCl, at pH 7.2 and 20°C. In one case, the magnesium concentration was 10 m*M*, while in the other case it was 1 m*M*. At the higher magnesium concentration, there is a clear decrease in the translation diffusion constant accompanying aminoacylation; at the lower magnesium concentration, there is no effect of aminoacylation. These results show that effects of aminoacylation are extremely sensitive to the ionic environment. At the higher Mg^{2+} concentrations, the results have been interpreted in terms of an intrinsic molecular change in addition to altered hydration and ion atmosphere.[106,107] Relating these results to the intracellular environment of an organism at 37°C is not simple, but the results at least indicate that under some conditions a conformational perturbation is possible. It is conceivable that such a change would be the signal that enables Tu to discriminate between charged and uncharged tRNA.

On the other hand, the experiments of Potts et al. have not been repeated with other charged tRNA species. A possible problem with Phe-$tRNA^{Phe}$ is the aromatic side chain of the amino acid. It is conceivable that upon aminoacylation and under some conditions, this aromatic side chain may be able to intercalate with a portion of the amino acid acceptor helix. That this is possible is suggested by fluorescence polarization experiments with Ile-$tRNA^{Ile}$ which was modified on the amino group of the amino acid through the attachment of a naphthyl ring.[108] The environment of the naphthyl group can be studied by fluorescence. Conditions were found under which the fluorescence is strongly polarized, suggesting that the naphthyl group was immobilized somehow through an interaction with the tRNA structure. A likely mechanism for immobilization of a planar aromatic group is formation of an intercalation complex. This should also be possible with the phenyl ring of phenylalanine. Thus, it will be of great interest to see whether the results of Potts et al. will be reproduced with other aminoacyl tRNA species.

A difficulty with the idea that a conformational change in aminoacyl tRNA is responsible for the ability of Tu to bind has to do with the data cited above on the binding of Tu to 2′ and 3′ aminoacyl tRNA isomers. If conformational changes occur, and if they are due to interactions involving the aminoacyl moiety with a portion of the tRNA structure, then one might expect that this interaction would be quite sensitive to the position of the aminoacyl moiety on the terminal ribose. For example, if the 5′-terminal phosphate interacts with the amino moiety (which should be at least partially protonated at pH 7) of the amino acid, the geometry of this interaction will clearly depend upon whether the amino acid is attached to a 2′ or 3′ OH. As already mentioned, however, the discrimination by Tu between 2′ and 3′ aminoacyl isomers is not great.

It is also possible that the free amino group itself is a key feature in aminoacyl tRNA that is recognized by Tu. This is suggested by experiments that show a lack of binding of Tu to *N*-blocked aminoacyl to tRNA.[89] However, there is also a problem with this idea. Several years ago it was shown by Fahenstock et al. that replacement of the NH_2

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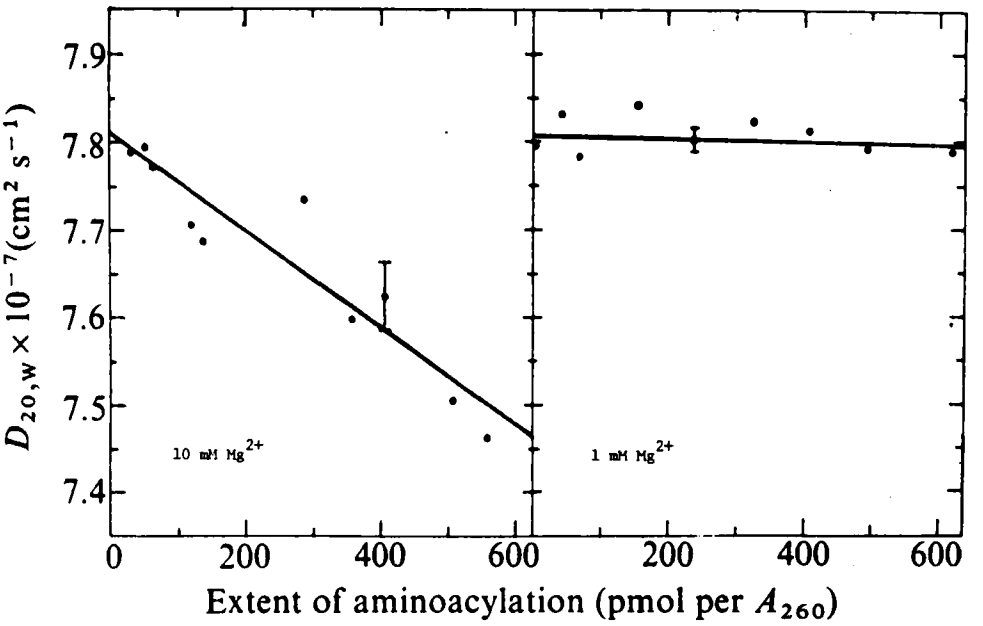

FIGURE 14. Translation diffusion constant ($D_{20,w}$) of yeast $tRNA^{Phe}$ as a function of the extent of aminoacylation, at pH 7.2. Experiments are for two different Mg^{2+} concentrations. (From Potts, R., Fournier, M. J., and Ford, N. C., Jr., *Nature (London)* 268, 563, 1977. With permission.)

(of the aminoacyl moiety of AA-tRNA) with a hydroxyl gives a species that still reacts with Tu·GTP.[109] This would seem to rule out the possibility that, for example, a protonated amino group on the aminoacyl moiety participates in a crucial electrostatic interaction with Tu; obviously, a similar positive charge cannot be put on an OH group.

As just mentioned, blocking by acetylation of the amino group of the aminoacyl moiety prevents Tu binding.[89] One interpretation of this result is that the amino group projects directly into a pocket in the structure of Tu; any substituent added to the amino group would disrupt the complex because it could not be sterically accommodated into the pocket designed for it. However, an OH group could be acceptable because it is reasonably close in diameter to an amino group. According to this viewpoint, the amino group plays a passive role, but strict steric requirements for its accommodation into a pocket on Tu prevent groups significantly larger than $-NH_2$ from being bound.

In summary, much progress has been made on identifying the structural features of tRNA that are important for interaction with Tu. But the critical question of how aminoacylation facilitates binding remains unanswered. At present, we can only suggest that a conformational change occurs which facilitates binding, or that the free amino group itself is a critical part of the interaction; in the latter case, any interaction of the protein with the amino group would also have to be able to accommodate an OH group.

IV. PEPTIDYL tRNA HYDROLASE

Peptidyl tRNA hydrolase catalyzes the hydrolysis of *N*-acetyl aminoacyl tRNAs and oligopeptidyl tRNAs.[110-114] The enzyme appears to be a single-polypeptide chain with

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a molecular weight of about 13,000.[115] Unlike the elongation factor Tu, the biological function of peptidyl-tRNA hydrolase is not well understood. A possibility is that when peptidyl-tRNAs are prematurely released from the ribosomes, they are rapidly cleaved by this enzyme so as to regenerate free tRNA which may be recycled in protein synthesis. Additionally, or alternatively, because the enzyme does not attack fMet-tRNA$_f$Met, it may also be used to assure that no *N*-blocked charged tRNAs are used for initiation, other than the methionine specific initiator tRNA.

A. Characteristics of and Studies of Recognition

With one exception, the hydrolysis is completely nonspecific with regard to the attached peptidyl moiety of tRNA. The exception is fMet-tRNA$_f$Met which is the only known *N*-substituted aminoacyl tRNA which is not attacked by the hydrolase.[111-114,116,117] This has nothing to do with the formyl methionine group per se because fMet-tRNA$_m$Met and other peptidyl-tRNA$_m$Met species are readily cleaved by the enzyme.[115-117] In short, the enzyme displays a kind of negative recognition; it discriminates against a particular tRNA species. This contrasts with the recognition of tRNAs by aminoacyl tRNA synthetases which specifically select one tRNA species for aminoacylation. However, the peptidyl tRNA hydrolase must have some awareness of the tRNA structure, because *N*-substituted aminoacyl-oligonucleotides are not as readily cleaved by the enzyme.[110,111,113,114]

The protein has not been investigated nearly as extensively as aminoacyl tRNA synthetases or the elongation factor Tu. The most extensive study of the mechanism by which the protein discriminates against fMet-tRNA$_f$Met appears to be that of Schulman and Pelka.[118] These authors specifically searched for the structural feature in the initiator tRNA that prevents catalysis by the peptidyl tRNA hydrolase.

Using the bisulfite modification approach, by which cytidine residues are converted to uridines, it was possible to alter the tRNA$_f$Met structure. Modifications that were introduced are shown in Figure 11. These modifications are the same as those introduced in connection with the study of the recognition mechanism of elongation factor Tu.

It was found that partial bisulfite modification of the residues shown in Figure 11 gave rise to a fMet-tRNA$_f$Met species that is hydrolyzed readily by the peptidyl tRNA hydrolase. This is exactly reminiscent of the results obtained with elongation factor Tu, where it was found that bisulfite modification of Met-tRNA$_f$Met activated the species with regard to recognition by Tu. Further characterizations were done to determine which of the modifications were most important for producing activation of the peptidyl tRNA hydrolase. Analysis showed that after 17% of the partially modified fMet-tRNA$_f$Met had been hydrolyzed by the enzyme, over 40% of those containing a 5′-terminal uridine were cleaved, whereas only a few percent of those with 5%-terminal cytidine had been hydrolyzed. Furthermore, after about 40% of the total partially modified fMet-tRNA$_f$Met was cleaved, virtually all of the molecules containing 5′-terminal uridine were hydrolyzed, and less than 20% of those with a 5′-terminal cytidine were cleaved. Plots of log of the rate vs the mole fraction of Ul gave good linearity, suggesting that a single hit was responsible for the activation of the tRNA. Analogous plots involving residues C16, C17, or C75 gave nonlinear results. These results appear to indicate that the lack of a base pair at the 5′-terminus is largely responsible for the discrimination against fMet-tRNA$_f$Met by the peptidyl hydrolase.[118]

Other experiments indicated that the 5′-terminal phosphate is also important for catalysis by peptidyl-tRNA hydrolase. With the 5′-phosphate removed from *N*-acetyl Phe-tRNA^{Phe}, the rate of hydrolysis by the enzyme is sharply reduced; this also is true when the 5′-phosphate is removed from partially bisulfite-modified fMet-tRNA$_f$Met.[118]

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B. Comparison of Recognition of tRNA by Hydrolase and Elongation Factor Tu

From these results, and the analogous ones on the recognition properties of elongation factor Tu, it appears that the lack of a terminal base pair in the initiator tRNA enables this species to be distinguished by both the elongation factor and peptidyl tRNA hydrolase. Moreover, for both proteins, the 5′-terminal phosphate plays a crucial role in the interaction between the protein and tRNA. In these respects, the two proteins have closely analogous recognition properties; beyond that, the analogy breaks down. In the case of elongation factor Tu, the amino group of the aminoacyl-tRNA must not be blocked, in order to have efficient elongation factor binding. Conversely, for the peptidyl-tRNA hydrolase, the amino group must be blocked, either by the attachment of an acetyl group or of additional amino acid residues. It is precisely this aspect of the recognition characteristics of both proteins that we still do not understand. In the case of the peptidyl-tRNA hydrolase, it must somehow recognize the presence of a peptide bond at the 3′-end of the tRNA. Because peptidyl moieties of different sizes are cleaved from a peptidyl-tRNA, it is apparent that however recognition is achieved, the main axis of the peptide chain must not project into a binding site on the protein; otherwise, some large peptidyl groups would be sterically excluded. Instead, the enzyme must be able to sense the presence of a peptide group, perhaps by hydrogen bonding with the planar, *trans* amide portion of the peptidyl backbone, and it may accomplish this by binding along the "side" of the peptidyl chain rather than "end-on".

V. *E. COLI* METHIONYL-tRNA FORMYLASE

In prokaryotes, protein synthesis is initiated with the formyl methionine residue donated from fMet-tRNA$_f$Met.[119-123] The only known exception occurs in *Halo-bacterium cutirubrum* where initiation occurs with an unformylated Met-tRNA species.[124] The formyl group is derived from formyl tetrahydrofolic acid and the transfer to Met-tRNA$_f$Met is catalyzed by the transformylase enzyme.[125] This is a small protein with a molecular weight of about 25,000.[125] This enzyme specifically recognizes Met-tRNA$_f$Met; it does not catalyze the formylation of Met-tRNA$_m$Met or of any other charged tRNA species.[123] Moreover, while it can form a stable complex with fMet-tRNA$_f$Met or Met-tRNA$_f$Met, it does not form stable complexes with uncharged initiator tRNA (tRNA$_f$Met) nor with various aminoacyl-tRNA species.[126] This means it recognizes a specific tRNA and it requires that this tRNA be aminoacylated.

In the case of elongation factor Tu and peptidyl tRNA hydrolase, it appears that these enzymes do not interact with initiator tRNA because of the lack of a terminal base pair in the acceptor stem. We can surmise that this unique structural feature could also be the means by which the Met-tRNA formylase distinguishes between Met-tRNA$_f$Met and Met-tRNA$_m$Met. However, initiator tRNAs from eucaryotic species such as yeast and vertebrates have a terminal base pair in the acceptor stem, and the *E. coli* Met-tRNA formylase is able to formylate these charged initiator tRNAs.[127] This indicates that the presence or absence of a terminal base pair in the initiator tRNA is not essential for recognition by the formylase.

Attempts to determine sites on initiator tRNA important for recognition by the formylase have been confined to two types of investigations. First, it has been shown that a variety of charged initiator tRNAs can be formylated by the enzyme. Sequence comparisons of these tRNAs have revealed those sites that are common, and those that are different, to the various initiators. Figure 15 gives a composite structure of the various tRNAs that are recognized by the Met-tRNA formylase.[127] This composite is

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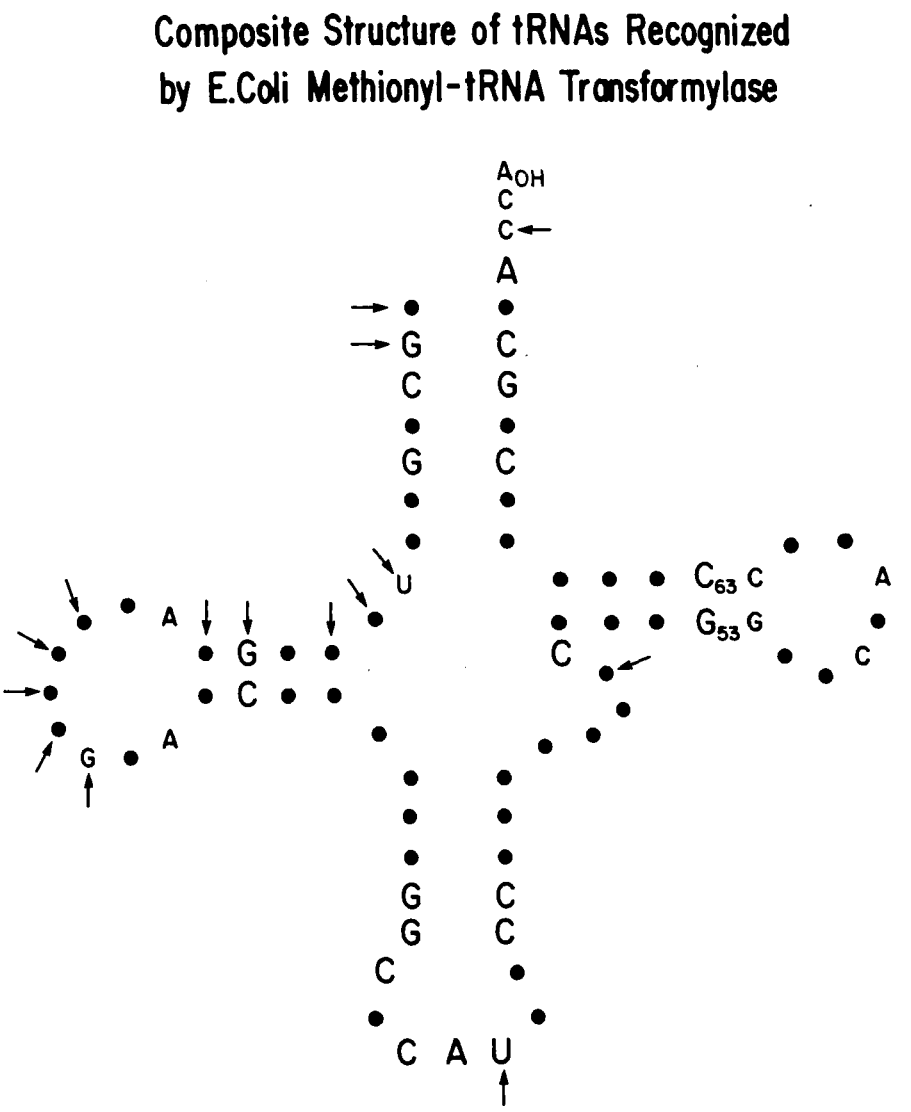

FIGURE 15. Composite of tRNAs recognized by *E. coli* methionyl-tRNA transformylase. The composite is based on sequences of a variety of bacterial and eukaryote initiator tRNAs. Nucleotides common to these tRNAs are shown by large letters, while small letters designate nucleotides found in all tRNAs. Bases that are not common to these initiators are designated by dots. Arrows designate sites that can be chemically modified without loss of transformylase recognition." (In part from Gillum, A. M., Hecker, L. I., Silberklang, M., Schwartzbach, S. D., RajBhandary, U. L. and Barnett, W. E., *Nucleic Acids Res.*, 4, 4109, 1977. With permission. Updated in part with data from Heckman, J. E., Hecker, L. I., Schwartzbach, S. D., Barnett, W. E., Baumstark, B., and RajBhandary, U. L., *Cell*, 13, 83, 1978.)

based on sequences from *E. coli*, yeast, *Bacillus subtilis*, *Neurospora crassa*, and vertebrates. Lower-case letters designate nucleotides that are found in the same position in all tRNAs, while large letters show those common to only the initiator tRNAs. It is seen that bases uniquely common to the initiator tRNA are scattered throughout the structure. No obvious pattern emerges from this sequence comparison.

A second approach to the question of recognition has been chemical modification studies. Schulman and co-workers have carried out many studies with *E. coli*

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tRNA$_f$Met using the chemical modification approach.[27] Of the sites which are common to the initiator tRNAs, six can be chemically modified without destroying the substrate properties of this tRNA. These six sites are designated by arrows in Figure 15. A number of other modifications have also been carried out and, in spite of the identification of 15 different sites of modification, so far no site has been found which inactivates *E. coli* tRNA$_f$Met with respect to its ability to be acted upon by the formylase.[27]

It is clear that from the available data there is no obvious pattern to the recognition by the formylase. Although it comes from a prokaryote source, it can formylate at least some eukaryote initiators. From Figure 15 it is apparent that a minor fraction of the bases are identical in the various tRNAs that can be formylated by the *E. coli* enzyme. A common feature to all of these tRNAs are G:C base pairs in specific positions in each of the four stems of the cloverleaf; interestingly, there are no conserved A:U base pairs. However, there is no way, with the present data, to come to any plausible scheme for recognition by the transformylase.

In some ways, the recognition properties of the transformylase incorporate features of the recognition reactions of both the aminoacyl tRNA synthetases and elongation factor Tu. It is like the synthetases (and unlike Tu) in that it recognizes a particular tRNA species, and it is like elongation factor Tu (and unlike synthetases) in that it recognizes charged vs. uncharged tRNA. It is yet another example of the diversity of the critical characteristics of the recognition reactions of the various protein-tRNA system, and presumably of the different kinds of receptor sites on tRNAs that are differentiated by these systems.

A basic problem with the current state of the formylase problem is that we have no concrete data on which parts of the tRNA make contact with the enzyme. Thus, nuclease digestion, oligonucleotide hybridization, photochemical cross-linking, and tritium labeling studies — analogous to those done with aminoacyl tRNA synthetases and elongation factor Tu — would aid greatly in narrowing down the parts of the tRNA that are important for recognition. As it stands now, virtually all areas are possible, because bases uniquely common to all initiator tRNAs are scattered throughout the structure (Figure 15), and because chemical modification studies have not been able to locate any sites at which inactivation can be induced.

VI. REVERSE TRANSCRIPTASE

Reverse transcriptase is a RNA-dependent DNA polymerase found in the virions of RNA tumor viruses.[128] The function of the polymerase is to copy the viral RNA into complementary DNA. The 70S tumor virus RNA has several 4S RNA species associated with it; these can be dissociated by raising the temperature.[129,130] Interestingly enough, the transcription process requires a low-molecular-weight 4S RNA species as a primer.[130-133]

Harada et al. showed that tRNA^{Trp} is the RNA primer used for initiation of Rous sarcoma virus DNA synthesis.[15] This tRNA is found in the virions and also in the cells of chickens, which are the viral host. This tRNA also associates tightly with the 35S RNA subunits of the 70S viral RNA;[134,135] in fact, it appears to associate more strongly with the viral RNA than do any of the other RNA species encapsulated with the virion.[129,130]

A. Interaction with Viral RNA and with tRNA

The question can be raised as to whether the priming by tRNA^{Trp} is due to a strong and specific association of the tRNA with the enzyme. This question was investigated

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by Panet et al., who employed gel chromatography on Sephadex® G-100 and sedimentation in glycerol gradients to investigate the possible association between reverse transcriptase and $tRNA^{Trp}$.[136] ^{32}P-tRNA from chicken embryo cells was mixed with reverse transcriptase and run on a Sephadex® G-100 column. A small amount of the tRNA ran in the excluded volume with the enzyme. This tRNA was then analyzed by a two-demensional gel electrophoresis procedure that separated individual tRNAs. The analysis showed that the fraction excluded by Sephadex® in the presence of reverse transcriptase mainly contains $tRNA^{Trp}$. The other significant species was $tRNA_4$Met.[136]

It was also shown that binding of $tRNA^{Trp}$ to reverse transcriptase shifts the enzyme's sedimentation coefficient in a glycerol gradient. Demonstrating that the enzyme activity actually shifts in the gradient leaves little doubt that the tRNA binds directly to the protein and not to some contaminating material.[136]

Reverse transcriptase is comprised of two different types of subunits.[137,138] There is an α chain with a molecular weight of 63,000 and a β chain with a molecular weight of 81,000.[138] It is likely that the α chain is derived from a proteolytic cleavage of the β chain.[139,140] The predominant form of the enzyme is an $\alpha\beta$ dimer, although it can also exist as a free α chain and as a β_2 dimer.[138] All three forms are enzymatically active, although they differ in their catalytic behavior.

The question of the association of these forms with RNA was investigated by Hizi et al.[141] These investigators used a nitrocellulose filter binding assay. Some of the results obtained with various RNA species for the $\alpha\beta$ enzyme are tabulated in Table 1. Strong association is found with the 70S RNA (which contains bound $tRNA^{Trp}$) for which the dissociation constant is about .01 n*M*. The dissociation constant is about a factor of 10 larger for 35S RNA (which lacks $tRNA^{Trp}$) and is only slightly increased by the hybridization of $tRNA^{Trp}$ to the 35S RNA (data not shown). For $tRNA^{Trp}$ alone, the dissociation constant is about 30 n*M*, which is only about a factor of 5 smaller than the dissociation constant of $\alpha\beta$ enzyme withwhole tRNA that lacks $tRNA^{Trp}$.

Results with each of the two other molecular forms of the enzyme were also obtained.[141] From these results it is evident that except for $tRNA^{Trp}$, there is little difference in the affinity of the three forms for the various RNA species. In the case of $tRNA^{Trp}$, there is a roughly six-fold smaller dissociation constant for the complex with the $\alpha\beta$ enzyme than with the α enzyme. Except for this difference, in the other instances all enzyme forms show little difference among themselves for association with the various RNA species.

The results of Hizi et al.[141] contrast with those of Panet et al.[136] In the latter case, gel filtration of a mixture of cellular tRNAs with reverse transcriptase showed that only two species, $tRNA^{Trp}$ and $tRNA_4^{Met}$, were prominent in the binding. However, the nitrocellulose filter results of Hizi et al. suggest that the bulk cellular tRNA has an association with the $\alpha\beta$ enzyme which is only about a factor of 5 lower than that for the association with $tRNA^{Trp}$. Because about 90 to 95% of the bulk 4S RNA should contain species other than $tRNA^{Trp}$ and $tRNA_4$Met, this would seem an ample amount to make up for the approximately five-fold lower association of these tRNAs. Thus, it would be expected that in a gel filtration, considerable amounts of tRNAs other than $tRNA^{Trp}$ should be bound to the enzyme. The reason for this discrepancy is not apparent.

B. Regions on tRNA Important for Interaction

Some attempt has been made to identify on $tRNA^{Trp}$ the sites important for its binding to reverse transcriptase. Using avian myeloblastosis virus (AMV) reverse transcriptase, experiments were done with modified forms of $tRNA^{Trp}$.[142] For example, the tRNA was split into 5′ and 3′ halves by using S1 nuclease partial cleavage. The halves

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Table 1
DISSOCIATION CONSTANTS FOR $\alpha\beta$ FORM OF AVIAN SARCOMA VIRUS REVERSE TRANSCRIPTASE WITH VARIOUS RNAS [141]

Viral RNAs			Nonviral RNAs				
70S RNA	35S RNA	$tRNA^{Trp}$	4S RNA	5S RNA	f2 phage RNA	18S HeLa cell ribosomal RNA	28S HeLa cell ribosomal RNA
0.01	0.16	29	140	15	5.6	1.4	0.67

Note: Dissociation constants are given in units of n*M* and were measured (by a nitrocellular filtration method) at, approximately, pH 8, 0°C.

of the molecule were separated and tested for their association with reverse transcriptase using gel filtration and glycerol gradient sedimentation. No complex formation could be detected with the half molecules. A similar result was obtained by using fragments produced by cleavage, at m^7G. Neither the 5′ nor the 3′ fragments resulting from this cleavage, which produces one third and two third molecules, bound to the enzyme. On the other hand, neither periodate oxidation of the 3′-terminus, nor elimination of the 3′-terminal adenosine, prevented binding of the reverse transcriptase. Furthermore, elongation of the $tRNA^{Trp}$ primer by the addition of seven deoxynucleotides to the 3′-terminus gives a tRNA species that still binds firmly to reverse transcriptase. These results indicate that even though the enzyme uses the 3′-terminus as a site of elongation, this region does not seem to be crucial for binding to the enzyme. On the other hand, the experiments with the fragments argue that both halves of the tRNA are required for recognition by the enzyme. Of course, this result must be interpreted with caution because a half molecule has a very different conformation as an isolated species than does the corresponding half in the intact tRNA. This conformational difference in itself could strongly affect recognition by the protein.

In the case of murine viral 70S RNA, the primer is $tRNA^{Pro}$.[143] The avian enzyme can be used to synthesize DNA from the murine 70S RNA. In this case, the avian enzyme, which normally uses $tRNA^{Trp}$ to prime DNA synthesis from avian 70S RNA, uses $tRNA^{Pro}$ as its primer for DNA synthesis from murine 70S RNA.[143] In addition, it is possible to show an association between the avian enzyme and $tRNA^{Pro}$ derived from Moloney murine leukemia virus virions or NRK cells.[142] Curiously enough, no binding of the Moloney murine leukemia virus reverse transcriptase to $tRNA^{Pro}$ or to $tRNA^{Trp}$ could be detected.

Further work on the nature of the complex formed between AMV reverse transcriptase and $tRNA^{Trp}$ has been carried out by Araya and Litvak.[144] Using UV irradiation, it has been possible to photo-cross-link beef $tRNA^{Trp}$ to the enzyme. The beef $tRNA^{Trp}$ has a sequence similar to the chicken $tRNA^{Trp}$ and it has also been shown that the beef tRNA can serve as a primer.[145]

As shown in Figure 16, cross-linking of $tRNA^{Trp}$ from beef to AMV reverse transcriptase is efficient and results in the joining of about 1.7 to 1.8 mol of tRNA per mole of enzyme. Substantially lower values are obtained with yeast $tRNA^{Trp}$ and beef $tRNA^{Val}$. These tRNAs differ markedly in sequence from beef $tRNA^{Trp}$.[4,145] In addition, bovine serum albumin does not cross-link to $tRNA^{Trp}$ from beef.

The joining of almost 2 mol of $tRNA^{Trp}$ to AMV reverse transcriptase checks well with nitrocellulose filter assays which show that close to 2 mol of tRNA can be titrated onto the enzyme. Moreover, it appears from Figure 16 that there is some specificity

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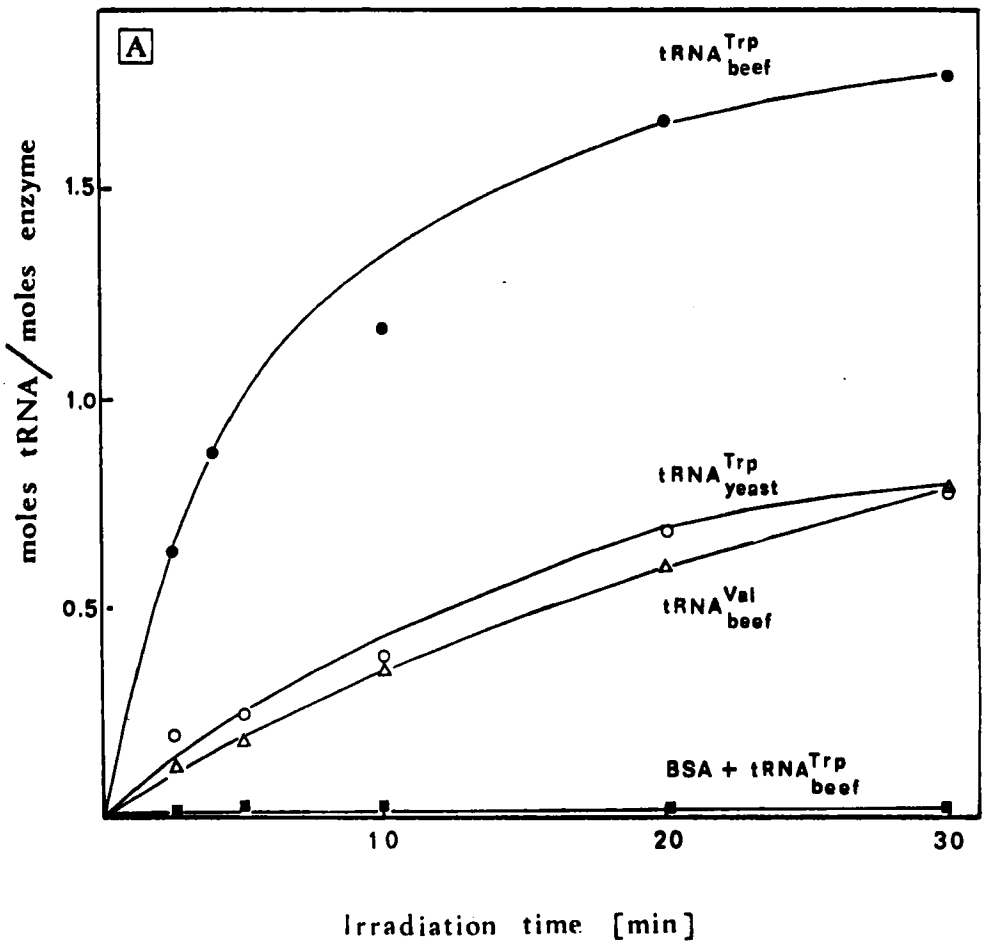

FIGURE 16. Photo cross-linking of various tRNAs to avian myeloblastosis reverse transcriptase. The vertical axis indicates the moles of tRNA joined to enzyme per mole of enzyme. (From Araya, A. and Litvak, S., *Cold Spring Harbor Symp. Quant. Biol.*, 43, in press. With permission.)

to the cross-linking process. It will be of considerable interest to learn which regions on the tRNA have been cross-linked to the enzyme. These presumably are the contact sites in the protein-nucleic acid complex.

C. Significance of Interaction With tRNA

When the results discussed above are considered collectively, the significance of the association of AMV reverse transcriptase with $tRNA^{Trp}$ is still not clear. While the gel filtration results of Panet et al.[136] and the preliminary photo-cross-linking results of Araya and Litvak[144] indicate a definite specificity in the binding of $tRNA^{Trp}$ to reverse transcriptase, the findings of Hizi et al. suggest that the specificity over other cellular tRNAs is not that great.[141] Moreover, it is clear that the association of the enzyme with 70S RNA or 35S RNA subunits is orders of magnitude stronger than it is with $tRNA^{Trp}$. Thus, in vivo, the enzyme should show a strong preference for the high-molecular-weight viral RNA, and the amount of binding to free $tRNA^{Trp}$ alone may be insignificant. On the other hand, the results of Panet et al. do suggest that, in a mixture of cellular tRNAs, the enzyme will specifically select $tRNA^{Trp}$.[136] Because this is also the species used to prime DNA synthesis, it seems too much to ask for the selective binding of $tRNA^{Trp}$ to be mere coincidence.

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More recent experiments by Cordell et al. shed further light on these issues.[146] It had been shown previously that, beginning with the penultimate nucleotide at the 3′-terminus of $tRNA^{Trp}$, there is a sequence of at least 16 nucleotides which binds the tRNA to the avian sarcoma virus genome.[147,148] A duplex of this small portion of the tRNA and a sequence in the virus genome does not detectably bind to the enzyme.[146] Neither do a large variety of tRNA fragments, of varying sizes, bind to reverse transcriptase.[146] However, a fragment of 27 residues, starting at the 3′-terminus, primes DNA synthesis from the viral genome. Interestingly enough, this fragment includes a span of ten consecutive residues which are homologous to the analogous positions in the $tRNA^{Pro}$ species that is used as a primer for the genome of the Moloney murine leukemia virus.[146] As mentioned above, the avian enzyme can bind to and initiate synthesis from the murine virus genome with this tRNA.

Because the 3′-fragment of 27 residues primes DNA synthesis and yet shows no detectable association with the enzyme, Cordell et al.[146] argue that the binding of free $tRNA^{Trp}$ to reverse transcriptase may not be important for the initiation of DNA synthesis from the viral genome.[146] Rather, it is the sequence of nucleotides on the 3′-side of the tRNA that is probably of primary importance, because this sequence makes possible the association of the tRNA with the viral RNA and the subsequent initiation of DNA synthesis. This is not to say that the enzyme-tRNA association has no significance whatsoever. The Rous sarcoma avian virus contains roughly comparable amounts of the polymerase and the $tRNA^{Trp}$ species that selectively binds to it,[133,149] and the enhanced abundance of this tRNA species may reflect, at least in part, its preferential incorporation into the virion as directed by reverse transcriptase.

The question may be raised as to whether one of the subunits of reverse transcriptase comes from Trp-tRNA synthetase. The Trp-tRNA synthetase of human placenta, bovine pancreas, and yeast appears to be an α_2 enzyme with an α-subunit molecular weight of 50,000 to 60,000.[18] This is close to the molecular weight of the α subunit of AMV reverse transcriptase. However, Panet et al. found no Trp-tRNA synthetase activity present in their AMV reverse transcriptase preparation.[136] Also, antireverse transcriptase IgG prepared from rat serum inhibited both the DNA polymerase and the $tRNA^{Trp}$ binding activity of the enzyme, while nonspecific IgG did not. These results do not rigorously exclude the possibility that a subunit of Trp-tRNA synthetase is also a subunit of reverse transcriptase, but neither do they offer any encouragement for such a viewpoint. It would be worthwhile to do a few more experiments to rule out the possibility that one of the subunits of reverse transcriptase is derived from Trp-tRNA synthetase.

VII. CONCLUSIONS

It is evident from the foregoing discussion that transfer RNA has been designed so as to present receptor sites to a variety of different proteins. Five different protein-tRNA interactions have been considered here, and this is not a complete survey of all possible protein-tRNA specific associations. In the process of protein synthesis, there are many special interactions with ribosomal proteins that occur as tRNA moves through the acceptor and peptidyl sites during polypeptide chain elongation. These protein-tRNA interactions presumably take advantage of features that are common to all tRNAs that function in elongation of polypeptide chains. In addition to these systems associated with protein synthesis, there are probably additional protein-tRNA interactions that are yet uncharacterized. For example, the involvement of transfer RNAs in regulation of gene expression,[9-12] cell wall biosynthesis,[13,14] and possibly membrane transport phenomena[17] suggest that a variety of other interactions with proteins will be discovered at some point in the future.

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It is striking that the characteristics of the five recognition reactions discussed here are remarkably different. For example, aminoacyl tRNA synthetases discriminate between the various amino acid specific tRNA species, and have evolved a "dual" discrimination scheme which enables them to differentiate between tRNAs at both the level of binding and of catalysis. On the other hand, the elongation factor Tu apparently makes no distinction between the various noninitiator tRNA species and simply requires that these species be aminoacylated. The difference between synthetase and the elongation factor recognition is further underscored by evidence that synthetases do not show much discrimination between charged vs. uncharged tRNA.[150] Likewise, unusual and striking features are associated with the recognition reactions of the other proteins described (transformylase, peptidyl hydrolase, and reverse transcriptase).

Clearly, the greatest amount of information has been gained on specific synthetase-tRNA interactions. Here it is clear that, for many of the enzymes, the inner part of the L-shaped tRNA structure is an important part of the protein binding region (Figure 3). In this part of the structure, the uridine at the 8-Position comes into close contact with bound synthetase and may make a transient covalent adduct with the protein. If this proves to be the case, then it will be of considerable interest to test whether transient covalent interactions occur in some of the other specific protein-tRNA systems.

In studying protein-RNA recognition, the ultimate objective is to provide a molecular picture and explanation of the recognition specificity. This objective has not been reached so far in any of the systems that have been studied. There are inherent limitations associated with techniques for studying molecules in solutions that make it extremely difficult to come up with a precise molecular picture. Therefore, in the long run, we expect that the greatest surge in new information in this area may come from X-ray crystallographic studies of protein-tRNA complexes. However, at present this objective does not appear to be near fruition. Numerous attempts have been and continue to be made to crystallize especially synthetase-tRNA complexes. Although hopes have been raised on a number of occasions, at the time of this writing no crystals of a complex suitable for X-ray analysis have been obtained.

In spite of the lack of information, it is helpful to speculate on the kinds of interactions that might occur between atomic groups on proteins and complementary sites on nucleic acids. With single-stranded sections of nucleic acids, it is not hard to construct stereospecific complexes involving hydrogen bonding groups on bases with complementary groups on a polypeptide backbone or with certain of the amino acid side chains that have hydrogen bonding potential. For example, in the complex between actinomycin D and deoxyguanosine, two hydrogen bonds form between the peptide backbone of the antibiotic to the ring N3 and the exocyclic amino acid group of guanine.[151] Many other such interactions can also be visualized with unpaired bases.

But the real challenge lies in deducing how specific interactions might occur between double-helical nucleic acids and protein groups. This is because many important protein-nucleic acid interactions appear to involve sections of double-helical nucleic acids. For example, the interaction between *lac* repressor and *lac* operator presumably involves double-helical DNA.[152] Moreover, if synthetases bind, at least in part, along the inner part of the L-shaped tRNA structure, then the protein is confronted with, largely, double-helical RNA segments.

To get at this issue, some interesting model building studies have already been carried out. One of the earliest efforts was by Carter and Kraut.[153] These authors pointed out that in polypeptides, anti-parallel β chains can form a gradual right-handed double helix, having a long pitch. Interestingly enough, these structures have a pitch and radius approximately in the same range of nucleic acid double helices. Furthermore, such polypeptide "double helices" can be found in the structures of a number of proteins.[153]

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With this in mind, they constructed a model in which a hydrogen-bonded complex forms between a polypeptide double chain β-sheet and a RNA double helix. In the double-stranded polypeptide sheet, amide NH and carbonyl oxygens alternately turn inward, where they hydrogen bond with opposite members of the sheet, and turn outward, where they can interact with solvent or other groups. Carter and Kraut showed it was possible to build a model in which hydrogen bonds can be made between ribose 2′-hydroxyls and the outwardly turned carbonyl oxygens on the polypeptide chains. Moreover, from their models, it appeared possible for water molecules to connect (via hydrogen bonds) alternate peptide NH groups with furanose ring oxygen atoms and with 2′-hydroxyl groups. This structure, which would appear devoid of specificity because it involves interactions between the polypeptide and nucleic acid backbones, can be conferred with specificity because not all amino acid side chains can be sterically accommodated into the arrangement dictated by the backbone interactions.[153] It should also be noted that a structure involving the polypeptide double stranded β-sheet interacting with double stranded DNA has been built by Church et al.[154]

The question of sequence-specific recognition has been approached by Seeman et al.[155] These authors looked for specific interactions that might occur between amino acid side chains and hydrogen bonded nucleotide bases in a double helix. Their studies suggest that to uniquely identify a particular base pair (for example, A ·U vs. U·A) requires at least two hydrogen bonds between an amino acid side chain and a base. A proposed scheme of interactions for the major groove is given in Figure 17A. Here it is shown that asparagine or glutamine may specifically bond to the adenine portion of an A·U pair, while arginine may bond to the guanine portion of a G·C pair. In Figure 17B, a proposed interaction for the minor group is shown. In this case the interaction involves the side chain of asparagine or glutamine with the guanine part of the G·C pair.

We can expand upon these ideas by looking at the recognition surface of tRNA, regarding all exposed potential hydrogen bond acceptors and donors on the bases as possible receptor sites for interactions with proteins. The distribution of these sites will vary from tRNA to tRNA, owing to their sequence differences. By interacting specifically with these hydrogen bonding sites in single- and double-stranded regions, a protein should be able to discriminate between the various tRNA species.

These ideas are illustrated in Figure 18, which shows two views of a space-filling model of tRNA (compare with Figure 2) and where we have used shading to indicate the locations of free hydrogen bond acceptors and donors (on the bases only). The light stippled areas are the acceptors and the darker ones are the donors. These sites scatter all over the surface and provide a rich diversity of regions for hydrogen bonding associations with proteins. The potential bonding sites occur in single-stranded sections such as the anticodon and the 3′-terminus, which clearly are accessible to an approaching protein side chain, and also in some of the double-stranded regions where interactions like those shown in Figure 17 may occur.

These model building studies of protein-nucleic acid complexes give concreteness to our ideas about protein-nucleic acid recognition. Also, more interactions than what has been discussed above can certainly be visualized. For example, it is obvious that aromatic side chains of proteins can intercalate into helical sections. What is not clear, however, is whether such an interaction can provide any specificity. The same question holds for the interaction of positively charged side chains, such as lysine and arginine, with the charged phosphate backbone of nucleic acids. In any event, however, it appears enough diverse interactions are possible so that, in principle, recognition is not difficult to visualize or rationalize. Further, if it turns out that one or more transient covalent bonds can form in these protein-nucleic systems, this will, of course, open up a whole new dimension to our way of viewing these interactions.

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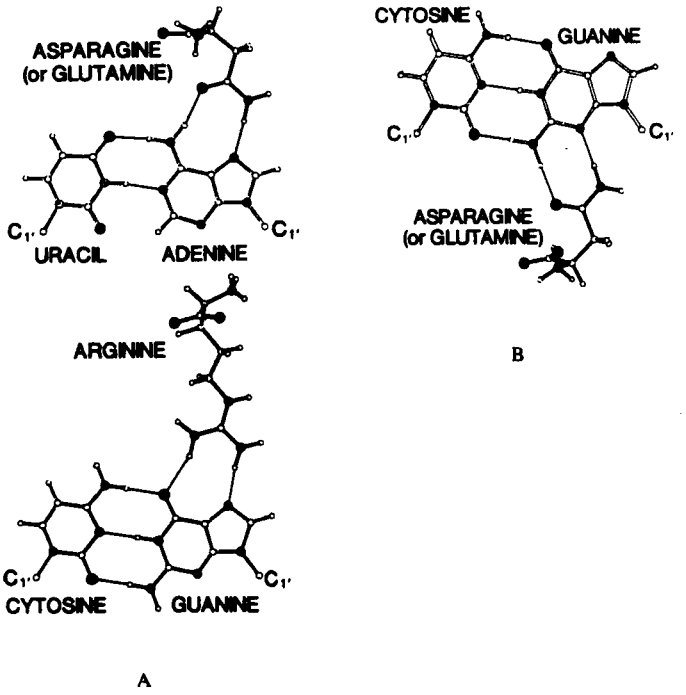

FIGURE 17. Schemes for specific base-amino acid hydrogen bonded complexes in the major groove (A) and minor groove (B). (From Seeman, N. C., Rosenberg, J. M., and Rich, A., *Proc. Natl. Acad. Sci. U.S.A.*, 73, 804, 1976. (With permission.)

It should also be appreciated that the X-ray crystallographic determination of one of these complexes may not completely clarify the mechanism of recognition. The reason for this is that it may be difficult to sort out which interactions are crucial for recognition and which are not. In other words, it may be that clearly defined rules for recognition in these systems may only emerge after a number of complexes have been analyzed at the molecular detail and compared against each other.

Some of the most interesting questions that remain have to do with the molecular architecture and organization of the proteins which carry out the recognition reactions. For example, consider the tRNA recognition sites on aminoacyl tRNA synthetases. If these recognition sites largely use the same part of the RNA structure, it is reasonable to speculate that these sites share a great deal in common. That is, the architectural features of these sites may be closely similar, with specificity achieved by the variation of a few amino acids in critical positions. This might be like the situation with immunoglobulins, where specificity is controlled by residues in the variable region.[156] We can imagine that synthetases have a portion of their structure which is devoted to RNA recognition and that alterations in this portion (through amino acid changes) could lead to changes in recognition specificity. This idea is currently being pursued, and it appears that mutant synthetases with altered tRNA recognition have been obtained.[30] The structural analysis of these mutants, and of the synthetase genes themselves, will obviously provide a great deal of new insight and information on this important issue.

In conclusion, the recognition of transfer RNA by proteins has been studied by a variety of imaginative approaches which have served both to delineate structural features on the nucleic acid important for recognition, and to bring to light the remarkable differences (see above) in the recognition characteristics of the various proteins. The

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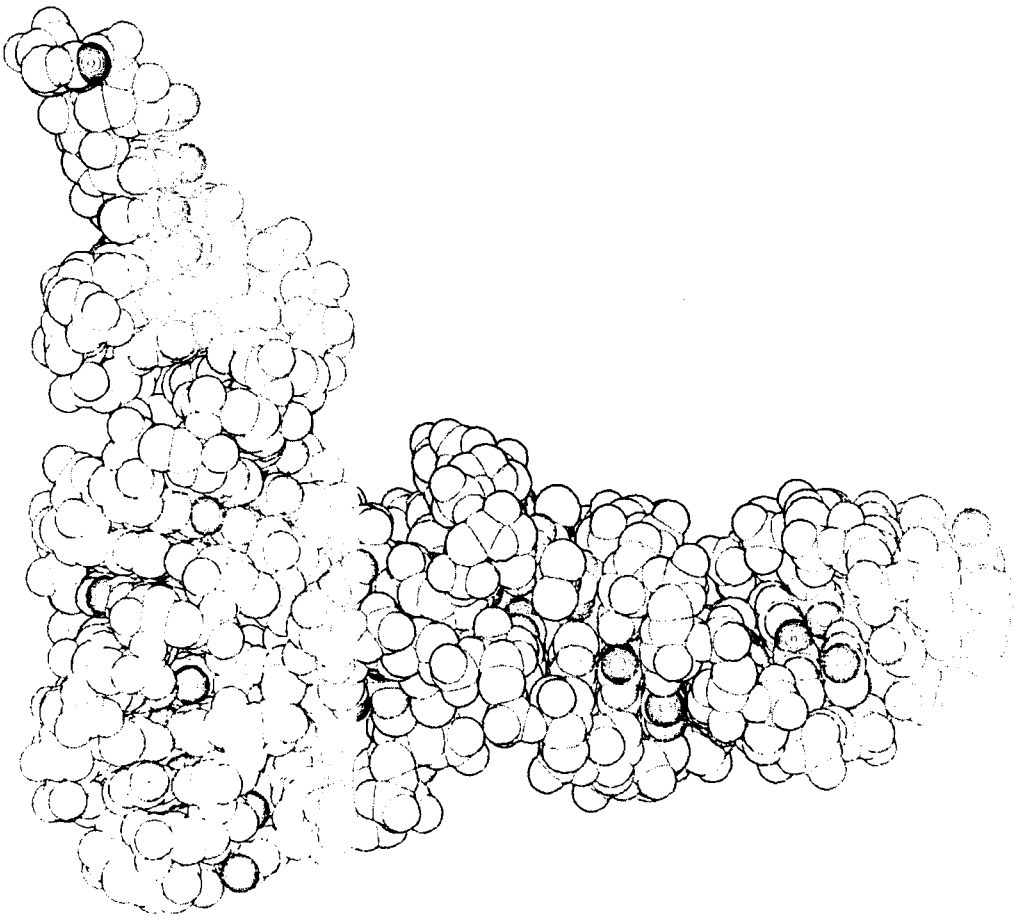

View 1

FIGURE 18. Space filling diagram of tRNA structure, shown in two views. Location on the bases of potential hydrogen bond acceptors (dotted radial lines) and donors (solid concentric lines) is indicated. (Kindly provided by Quigley, G. J., Teeter, M., and Rich, A.)

continued efforts in this area should bring a considerable amount of new insight, particularly with regard to the molecular basis for the interactions and to the structural organization of the RNA binding sites on the protein themselves.

VIII. SUMMARY

Transfer RNAs have specific interactions with a variety of proteins. Some of these have to do with the role of tRNA in protein synthesis, while others are due to the participation of tRNAs in processes other than protein synthesis. Five specific protein-tRNA interactions are reviewed here. The protein systems are: aminoacyl tRNA synthetases, elongation factor Tu, peptidyl tRNA hydrolase, methionyl-tRNA formylase, and reverse transcriptase. The first four are proteins which interact with tRNAs during the course of protein synthesis while, in the last case, reverse transcriptase uses a specific tRNA to prime the synthesis of DNA off of an RNA template. Each of these five systems has been studied with the aim of identifying on tRNAs the receptor sites (e.g., nucleotide units) responsible for a particular specific association. The most informa-

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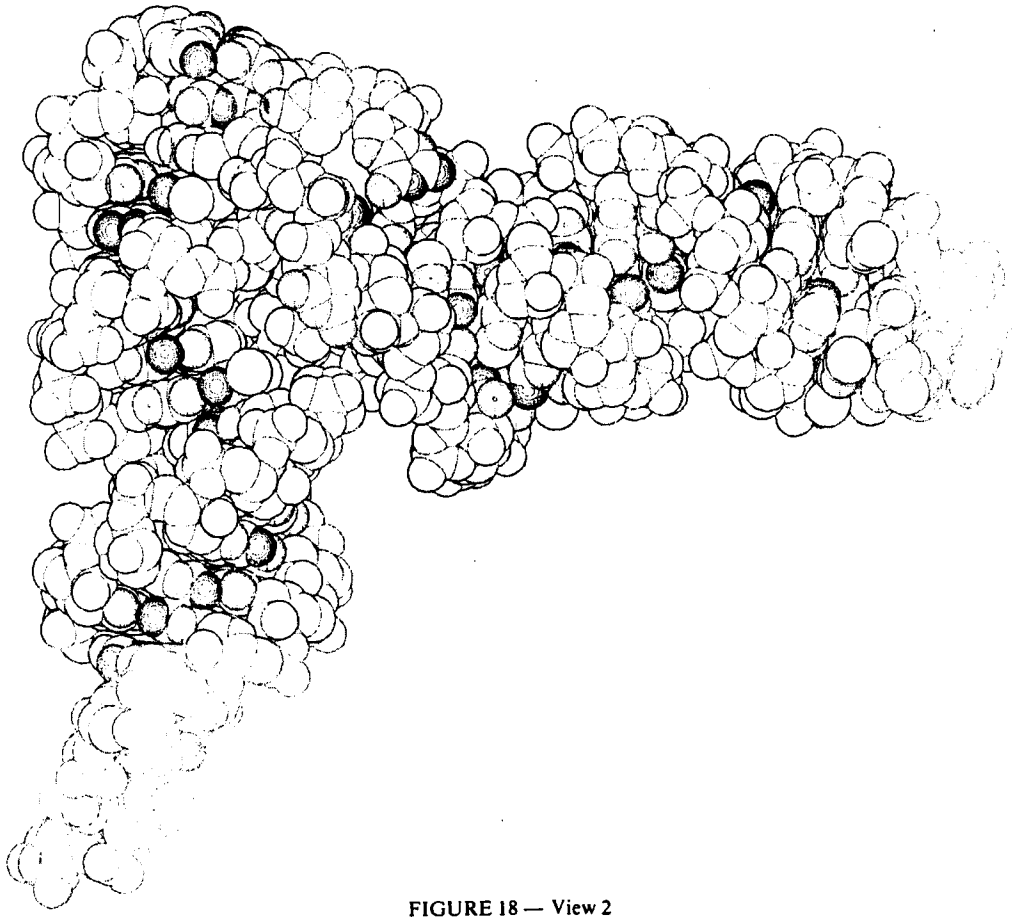

FIGURE 18 — View 2

tion has been obtained with aminoacyl tRNA synthetases, where some parts of the tRNA surface that make contact with a bound synthetase have been identified. Considerable progress has also been made on the other systems and, in some cases, regions crucial for the interaction with a specific protein have been pinpointed. It appears that, at least in part, each system has its own pecularities and a given protein may recognize features of the tRNA structure — or the aminoacyl- or peptidyl-tRNA structure — which are of no significance or of totally opposite significance in some of the other systems. For example, elongation factor Tu binds selectively to aminoacyl tRNAs, and shows little affinity for unacylated tRNAs, while a given aminoacyl tRNA synthetase may show little preference for its aminoacyl vs. non-acylated cognate tRNA. Also, elongation factor Tu does not significantly associate with N-blocked (e.g., N-acetyl) aminoacyl tRNAs while, in contrast, peptidyl tRNA hydrolase will not act on aminoacyl-tRNAs but will hydrolyze N-blocked (e.g., N-acetyl, peptidyl, etc.) tRNAs. Therefore, each protein system must be viewed separately and it appears that the tRNA molecule is designed to have a variety of receptor sites which, in conjunction with moieties attached to its 3′-terminus, are used — perhaps in various combinations — so as to make tRNA a truly multifaceted and multifunctional molecule. From a space-filling model of the tRNA structure it can be seen that, even in double-stranded regions, there is considerable potential for the formation of hydrogen bonds between bases on tRNAs and protein side chains, and that the spatial distribution of such po-

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tential hydrogen bonding sites may vary considerably from tRNA species to species. And while hydrogen bonds are not the only kind of interaction that can stabilize a protein-nucleic acid complex, they serve to demonstrate one way that specificity might be achieved in the various protein-tRNA recognition reactions. In addition, with at least the aminoacyl tRNA synthetases, there may be a transient covalent bond between a base on the tRNA and the bound synthetase.

ADDENDUM

There have been at least two important developments since the submission of this article for publication. In the area of aminoacyl-tRNA synthetases, strong further evidence has been obtained indicating that these enzymes can make a transient covalent bond with uridine residues. In particular, it was discovered that the mononucleoside 5-bromo-uridine is cleaved at the glycosyl linkage by various synthetases to yield 5-bromouracil plus free ribose.[157] This unusual reaction, which is believed to proceed through a Michael adduct intermediate in which a synthetase has been added to C-6 of the pyrimidine ring, was shown to occur with all four *E. coli* aminoacyl-tRNA synthetases that were tested, and also with a mammalian synthetase that was studied. Thus, there appears to be a common catalytic center on all synthetases, both in bacteria and in higher organisms, which can undergo Michael addition with a uridine ring.

In the case of reverse transcriptase, A. Araya, L. Sarih, and S. Litvak have presented evidence showing that the avian myeloblastoris virus transcriptase plays an active role in hybridizing $tRNA^{Trp}$ to the 35S viral RNA.[158] Moreover, as evidenced by nuclease digestion with RNAase T1 and micrococcal nuclease, the transcriptase was shown to unwind part of the acceptor stem of the tRNA. Because the 3′-side of the tRNA, involving the 3′-side of acceptor stem, is the part which hybridizes to 35S RNA, it would appear that the transcriptase catalyzes this unwinding as a step in its mechanism of mediating hybridization between the tRNA and 35S RNA.

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REFERENCES

1. **Schimmel, P., Söll, D., and Abelson, J.**, Eds. *Transfer RNA: Structure, Properties, and Recognition*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1979.
2. **Söll, D., Abelson, J., and Schimmel, P.**, Eds. *Transfer RNA: Biological Aspects*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1980.
3. **Rich, A. and RajBhandary, U. L.**, Transfer RNA: Molecular structure, sequence, and properties, *Annu. Rev. Biochem.*, 45, 805, 1976.
4. **Sprinzl, M., Gruter, F., and Gauss, D. H.**, Collection of published tRNA sequences, *Nucleic Acids Res.*, 5, r15, 1978.

Critical Reviews in Biochemistry and Molecular Biology Downloaded from informahealthcare.com by 89.163.34.136 on 01/07/12
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5. Kim, S. H., Suddath, F. L., Quigley, G. J., McPherson, A., Sussman, J. L., Wang, A. H.-J., Seeman, N. C., and Rich, A., Three-dimensional tertiary structure of yeast phenylalanine transfer RNA, *Science*, 185, 435, 1974.
6. Robertus, J. D., Ladner, J. E., Finch, J. T., Rhodes, D., Brown, R. S., Clark, B. F. C., and Klug, A., Structure of yeast phenylalanine tRNA at 3Å resolution, *Nature (London)*, 250, 546, 1974.
7. Quigley, G. J., Wang, A. H.-J., Seeman, N. C., Suddath, F. L., Rich, A., Sussman, J. L., and Kim, S. H., Hydrogen bonding in yeast phenylalanine transfer RNA, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 4866, 1975.
8. Kim, S. H., Sussman, J. L., Suddath, F. L., Quigley, G. J., McPherson, A., Wang, A. J.-J., Seeman, N. C., and Rich, A., The general structure of transfer RNA molecules, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 4970, 1974.
9. Calvo, J. M. and Fink, G. R., Regulation of biosynthetic pathways in bacteria and fungi, *Annu. Rev. Biochem.*, 40, 943, 1971.
10. Littauer, U. Z. and Inouye, H., Regulation of tRNA, *Annu. Rev. Biochem.*, 47, 439, 1973.
11. Brenchley, J. E. and Williams, L. S., Transfer RNA involvement in the regulation of enzyme synthesis, *Annu. Rev. Microbiol.*, 29, 251, 1975.
12. Umbarger, H. E., Amino acid biosynthesis and its regulation, *Annu. Rev. Biochem.*, 47, 533, 1978.
13. Gould, R. M. and Lennarz, W. J., Metabolism of phosphatidylglycerol and lysyl phosphatidylglycerol in *Staphylococcus aureus*, *J. Bacteriol.*, 104, 1135, 1970.
14. Strominger, J. L., Penicillin-sensitive enzymatic reactions in bacterial cell wall synthesis, *Harvey Lect.*, 64, 179, 1970.
15. Harada, F., Sawyer, R. C., and Dahlberg, J. E., A primer ribonucleic acid for initiation of *in vitro* rous sarcoma virus deoxyribonucleic acid synthesis, *J. Biol. Chem.*, 250, 3487, 1975.
16. Water, L. C., Mullin, B. C., Ho, T., and Yang, W. K., Ability of tryptophan tRNA to hybridize with 35S RNA of avian myeloblastoris virus and to prime reverse transcriptase *in vitro*, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 2155, 1975.
17. Yoo, S. H., Pratt, M. L., and Shive, W., Evidence for a direct role of tRNA in an amino acid transport system, *J. Biol. Chem.*, 254, 1013, 1979.
18. Schimmel, P. R. and Söll, D., Aminoacyl-tRNA synthetases. General features and recognition of transfer RNAs, *Annu. Rev. Biochem.*, 48, 601, 1979.
19. Chapeville, F., Lipmann, F., von Ehrenstein, G., Weisblum, B., Ray, W. J., Jr., and Benzer, S., On the role of soluble ribonucleic acid in coding for amino acids, *Proc. Natl. Acad. Sci. U.S.A.*, 48, 1086, 1962.
20. Kula, M. R., Structural studies on isoleucyl-tRNA synthetase from *E. coli*, *FEBS Lett.*, 35, 299, 1973.
21. Koch, G. L. E., Boulanger, Y., and Hartley, B. S., Repeating sequences in aminoacyl-tRNA synthetases, *Nature (London)*, 249, 316, 1974.
22. Waterson, R. M. and Konigsberg, W. H., Peptide mapping of aminoacyl-tRNA synthetases: evidence for internal sequence homology in *Escherichia coli* leucyl-tRNA synthetase, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 376, 1974.
23. Bruton, C. J., Jakes, R., and Koch, G. L. E., Repeated sequence in methionyl-tRNA synthetase from *E. coli*, *FEBS Lett.*, 45, 26, 1974.
24. Robbe-Saul, S., Fasiolo, F., and Boulanger, Y., Phenylalanyl-tRNA synthetases from Baker's yeast. Repeated sequences in the two subunits, *FEBS Lett.*, 84, 57, 1977.
25. Ofengand, J., tRNA and aminoacyl-tRNA synthetases, in *Molecular Mechanisms of Protein Biosynthesis*, Weissbach, S. and Pestka, S., Eds., Academic Press, New York, 1977, 7.
26. Schimmel, P. R., Approaches to understanding the mechanism of specific protein-transfer RNA interactions, *Acc. Chem. Res.*, 10, 411, 1977.
27. Schulman, L. H., Chemical approaches to the study of protein-tRNA recognition, in *Transfer RNA: Structure, Properties, and Recognition*, Schimmel, P., Söll, D., and Abelson, J., Eds., Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1979, 311.
28. Ebel, J.-P., Renaud, M., Dietrich, A., Fasiolo, F., Keith, G., Favorova, D., Vassilenko, S., Baltzinger, M., Ehrlich, R., Remy, P., Bonnet, J., and Giegé, R., Interaction between tRNA and aminoacyl-tRNA synthetase in the valine and phenylalanine systems from yeast, in *Transfer RNA: Structure, Properties, and Recognition*, Schimmel, P., Söll, D., and Abelson, J., Eds., Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1979, 325.
29. Schimmel, P. R., Understanding the recognition of transfer RNAs by aminoacyl transfer RNA synthetases, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 49, 187, 1979.
30. Schimmel, P. R., Recent results on how aminoacyl transfer RNA synthetases recognize specific transfer RNAs, *Mol. Cell. Biochem.*, 25, 3, 1979.
31. Lam, S. S. M. and Schimmel, P. R., Equilibrium measurements of cognate and noncognate interactions between aminoacyl transfer RNA synthetases and transfer RNA, *Biochemistry*, 14, 2775, 1975.

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32. **Krauss, G., Riesner, D., and Maass, G.,** Mechanism of discrimination between cognate and non-cognate tRNAs by phenylalanyl-tRNA synthetase from yeast, *Eur. J. Biochem.*, 68, 81, 1976.
33. **Riggs, A. D., Bourgeois, S., and Cohn, M.,** The *lac* repressor-operator interaction. III. Kinetic studies, *J. Mol. Biol.*, 53, 401, 1970.
34. **Rigler, R., Pachmann, U., Hirsch, R., and Zachau, H. G.,** On the interaction of seryl-tRNA synthetase with $tRNA^{Ser}$. A contribution to the problem of synthetase-tRNA recognition, *Eur. J. Biochem.*, 65, 307, 1976.
35. **Riesner, D., Pingoud, A., Boehme, D., Peters, F., and Maass, G.,** Distinct steps in the specific binding of tRNA to aminoacyl-tRNA synthetase, *Eur. J. Biochem.*, 68, 71, 1976.
36. **Ebel, J.-P., Giegé, R., Bonnet, J., Kern, D., Befort, N., Bollack, C., Fasiolo, F., Gangloff, J., and Dirheimer, G.,** Factors determining the specificity of the tRNA aminoacylation reaction. Non-absolute specificity of tRNA-aminoacyl-tRNA synthetase recognition and particular importance of the maximal velocity, *Biochimie*, 55, 547, 1973.
37. **Roe, B., Sirover, M., and Dudock, B.,** Kinetics of homologous and heterologous aminoacylation with yeast phenylalanyl transfer ribonucleic acid synthetase, *Biochemistry*, 12, 4146, 1973.
38. **Thiebe, R., Harbers, K., and Zachau, H. G.,** Aminoacylation of fragment combinations from yeast $tRNA^{Phe}$, *Eur. J. Biochem.*, 26, 144, 1972.
39. **Abelson, J. N., Gefter, M. L., Barnett, L., Landy, A., Russell, R. L., and Smith, J. D.,** Mutant tyrosine transfer ribonucleic acids, *J. Mol. Biol.*, 47, 15, 1970.
40. **Carbon, J. and Squires, C.,** Studies on genetically altered transfer RNA species in *Escherichia coli*, *Cancer Res.*, 31, 663, 1971.
41. **Yaniv, M., Folk, W. R., Berg, P., and Soll, L.,** A single mutational modification of a tryptophan-specific transfer RNA permits aminoacylation by glutamine and translation of the codon UAG, *J. Mol. Biol.*, 86, 245, 1974.
42. **Cory, S., Marcker, K. A., Dube, S. K., and Clark, B. F. C.,** Primary structure of a methionine transfer RNA from *Escherichia coli*, *Nature (London)*, 220, 1039, 1974.
43. **Blank, H. U. and Söll, D.,** The nucleotide sequence of two leucine tRNA species from *Escherichia coli* K12, *Biochem. Biophys. Res. Commun.*, 43, 1192, 1971.
44. **Yaniv, M. and Barrell, B. G.,** Sequence relationship of three valine acceptor tRNAs from *Escherichia coli*, *Nature (London) New Biol.*, 233, 113, 1971.
45. **Myers, G., Blank, H. U., and Söll, D.,** A comparative study of the interaction of *Escherichia coli* leucyl-, seryl-, and valyl-transfer ribonucleic acid synthetases with their cognate transfer ribonucleic acids, *J. Biol. Chem.*, 246, 4955, 1971; *J. Biol. Chem.*, 247, 6011, 1972.
46. **McCutchan, T., Silverman, S., Kohli, J., and Söll, D.,** Nucleotide sequence of phenylalanine transfer RNA from *Schizosaccharomyces pombe*: implications for transfer RNA recognition by yeast phenylalanyl-tRNA synthetase, *Biochemistry*, 17, 1622, 1978.
47. **Schulman, L. H. and Pelka, H.,** Structural requirements for aminoacylation of *Escherichia coli* formylmethionine transfer RNA, *Biochemistry*, 16, 4256, 1977.
48. **Mirzabekow, A. D., Lastity, D., Levina, E. S., and Bayev, A. A.,** Localization of two recognition sites in yeast valine tRNA I, *Nature (London) New Biol.*, 229, 21, 1971.
49. **Wübbeler, W., Lossow, C., Fittler, F., and Zachau, H. G.,** Amino-acid incorporation into tRNA fragments and into heterologous combinations of fragments, *Eur. J. Biochem.*, 59, 405, 1975.
50. **Horz, W. and Zachau, H. G.,** Complexes of aminoacyl-tRNA synthetases with tRNAs as studied by partial nuclease digestion, *Eur. J. Biochem.*, 32, 1, 1973.
51. **Dube, S. K.,** Evidence for "three-point" attachment of tRNA to methionyl tRNA synthetase, *Nature (London) New Biol*, 243, 103, 1973.
52. **Dickson, L. A. and Schimmel, P. R.,** Structure of transfer RNA — aminoacyl transfer RNA synthetase complexes investigated by nuclease digestion, *Arch. Biochem. Biophys.*, 167, 638, 1975.
53. **Schoemaker, H. J. P. and Schimmel, P. R.,** Photo-induced joining of a transfer RNA with its cognate aminoacyl-transfer RNA synthetase, *J. Mol. Biol.*, 84, 503, 1974.
54. **Budzik, G. P., Lam, S. S. M., Schoemaker, H. J. P., and Schimmel, P. R.,** Two photo-cross-linked complexes of isoleucine specific transfer ribonucleic acid with aminoacyl transfer ribonucleic acid synthetases, *J. Biol. Chem.*, 250, 4433, 1975.
55. **Schoemaker, J. J. P., Budzik, G. P., Giegé, R., and Schimmel, P. R.,** Three photo-cross-linked complexes of yeast phenylalanine specific transfer ribonucleic acid with aminoacyl transfer ribonucleic acid synthetases, *J. Biol. Chem.*, 250, 4440, 1975.
56. **Rosa, J., Rosa, D., and Sigler, P. B.,** Photocross-linking analysis of the contact surface of $tRNA^{Met}$ in complexes with *Escherichia coli* methionine: tRNA-ligase, *Biochemistry*, 18, 637, 1979.
57. **Schimmel, P. R., Uhlenbeck, O. C., Lewis, B., Dickson, L. A., Eldred, E. W., and Schreier, A. A.,** Binding of complementary oligonculeotides to free and aminoacyl transfer ribonucleic acid synthetase bound transfer ribonucleic acid, *Biochemistry*, 11, 642, 1972.

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58. Schoemaker, H. J. P. and Schimmel, P. R., Isotope labeling of free and aminoacyl transfer RNA synthetase-bound transfer RNA, *J. Biol. Chem.*, 251, 6823, 1976.
59. Rich, A. and Schimmel, P. R., Structural organization of complexes of transfer RNAs with aminoacyl transfer RNA synthetases, *Nucleic Acids Res.*, 4, 1649, 1977.
60. Giegé, R., Kern, D., Ebel, J.-P., and Taglang, R., Incorrect heterologous aminoacylation of various yeast tRNAs catalysed by *E. coli* valyl-tRNA synthetase, *FEBS Lett.*, 15, 281, 1971.
61. Yarus, M., Solvent and specificity binding and isoleucylation of phenylalanine transfer ribonucleic acid *(Escherichia coli)* by isoleucyl transfer ribonucleic acid synthetase from *Escherichia coli*, *Biochemistry*, 11, 2352, 1972.
62. Kern, D., Giegé, R., and Ebel, J.-P., Incorrect aminoacylations catalysed by the phenylalanyl- and valyl-tRNA synthetases from yeast, *Eur. J. Biochem.*, 31, 148, 1972.
63. Giegé, R., Kern, D., and Ebel, J.-P., Incorrect aminoacylations catalysed by *E. coli* valyl-tRNA synthetase, *Biochimie*, 54, 1245, 1972.
64. Yarus, M. and Mertes, M., The variety of intraspecific misacylations carried out by isoleucyl transfer ribonucleic acid synthetase of *Escherichia coli*, *J. Biol. Chem.*, 248, 6744, 1973.
65. Giegé, R., Kern, D., Ebel, J.-P., Grosjean, H., DeHenau, S., and Chantrenne, H., Incorrect aminoacylations involvoing tRNAs or valyl-tRNA synthetase from *Bacillus stearothermophilus*, *Eur. J. Biochem.*, 45, 351, 1974.
66. Schoemaker, H. J. P. and Schimmel, P. R., Effect of aminoacyl transfer RNA synthetases on H-5 exchange of specific pyrimidines in transfer RNAs, *Biochemistry*, 16, 5454, 1977.
67. Pogolotti, A. L., Jr. and Santi, D. V., The catalytic mechanism of thymidylate synthetase, in *Bioorganic Chemistry*, van Tamelen, E. E., Ed., Academic Press, New York, 1977, 277.
68. Koontz, S. W. and Schimmel, P. R., unpublished data, 1979.
69. Battell, M. L., Smillie, L. B., and Madsen, N. B., The sulfhydryl groups of muscle phosphorylase. I. Number and reactivity, *Can. J. Biochem.*, 46, 609, 1968.
70. Wataya, Y., Hayatsu, H., ad Kawazoe, Y., Cysteine-catalyzed hydrogen isotope exchange at the 5 position of uridylic acid, *J. Am. Chem. Soc.*, 94, 8927, 1972.
71. Cerutti, P., Holt, J. W., and Miller, N. J., Detection and determination of 5, 6-dihydrouridine and 4-thiouridine in transfer ribonucleic acid from different sources, *J. Mol. Biol.*, 34, 505, 1968.
72. Schoemaker, H. J. P., Investigations of structure-function relationships of transfer RNA's and their complexes with aminoacyl transfer RNA synthetase, Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, 1975.
73. Bedrosian, C. R. and Schimmel, P. R., unpublished data, 1978.
74. Favre, A., Yaniv, M., and Michelson, A. M., The photochemistry of 4-thiouridine in *Escherichia coli* tRNA^{Val}, *Biochem. Biophys. Res. Commun.*, 37, 266, 1969.
75. Carre, D. S., Thomas, G., and Favre, A., Conformation and functioning of tRNAs: cross-linked tRNAs as substrate for tRNA nucleotidyl-transferase and aminoacyl synthetases, *Biochimie*, 56, 1089, 1974.
76. Vlassov, V. V. and Khodyreva, S. N., Equilibrium screening-dialysis investigation of the nucleotide sequences in the tRNA^{Phe} recognized by phenylalanyl-tRNA synthetase *(Escherichia coli)*, *FEBS Lett.*, 96, 95, 1978.
77. Miller, D. L. and Weissbach, H., Factors involved in the transfer aminoacyl-tRNA to the ribosome, in *Molecular Mechanisms of Protein Biosynthesis*, Pestka, S. and Weissbach, H., Eds., Academic Press. New York, 1977, 323.
78. Lucas-Lenard, J. and Lipmann, F., Protein biosynthesis, *Annu. Rev. Biochem.*, 40, 409, 1971.
79. Miller, D. L. and Weissbach, H., Studies on the purification and properties of factor Tu from *E. coli*, *Arch. Biochem. Biophys.*, 141, 26, 1970.
80. Blumenthal, T., Landers, T. A., and Weber, K., Bacteriophage Qβ replicase contains the protein biosynthesis elongation factors EF Tu and EF Ts, *Proc. Natl. Acad. Sci. U.S.A.*, 69, 1313, 1972.
81. Hachmann, J., Miller, D. L., and Weissbach, H., Purification of factor Ts: studies on the formation and stability of nucleotide complexes containing transfer factor Tu, *Arch. Biochem. Biophys.*, 147, 457, 1971.
82. Weissbach, H., Miller, D. L., and Hachmann, J., Studies on the role of factor Ts in aminoacyl-tRNA binding to ribosomes, *Arch. Biochem. Biophys.*, 141, 384, 1970b.
83. Gordon, J., A stepwise reaction yielding a complex between a supernatant fraction from *E. coli*, guanosine 5′-triphosphate, and aminoacyl-sRNA, *Proc. Natl. Acad. Sci. U.S.A.*, 59, 179, 1968.
84. Shulman, R. G., Hilbers, C. W., and Miller, D. L., Nuclear magnetic resonance studies of protein-RNA interactions. I. The elongation factor Tu·GTP aminoacyl-tRNA complex, *J. Mol. Biol.*, 90, 601, 1974.
85. Pritz, M. P. and Miller, D. L., Evidence for conformational changes in elongation factor Tu induced by GTP and GDP, *Biochem. Biophys. Res. Commun.*, 53, 149, 1973.

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86. **Crane, L. J. and Miller, D. L.**, Guanosine triphosphate and guanosine diphosphate as conformation-determining molecules. Differential interaction of a fluorescent probe with the guanosine nucleotide complexes of bacterial elongation factor Tu, *Biochemistry*, 13, 933, 1974.
87. **Miller, D. L., Cashel, M., and Weissbach, H.**, The interaction of guanosine 5′-diphosphate, 2′(3′)-diphosphate with the bacterial elongation factor Tu, *Arch. Biochem. Biophys.*, 154, 675, 1973.
88. **Arai, K., Kawakita, M., and Karizo, Y.**, Studies on the polypeptide elongation factors from *E. coli*, *J. Biochem. (Tokyo)*, 76, 293, 1974a.
89. **Ravel, J. M., Shorey, R. L., and Shive, W.**, Evidence for a guanine nucleotide-aminoacyl-tRNA complex as an intermediate in the enzymatic transfer of aminoacyl-RNA to ribosomes, *Biochem. Biophys. Res. Commun.*, 29, 68, 1967.
90. **Schulman, L. H., Pelka, H., and Sundari, R. M.**, Structural requirements for recognition of *Escherichia coli* initiator and non-initiator transfer ribonucleic acids by bacterial T factor, *J. Biol. Chem.*, 249, 7102, 1974.
91. **Jekowsky, E.**, The interaction of aminoacyl-tRNA with elongation factor Tu-GTP, Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, 1976.
92. **Jekowsky, E., Miller, D. L., and Schimmel, P. R.**, Isolation, characterization and structural implications of a nuclease-digested complex of aminoacyl transfer RNA and *Escherichia coli* elongation factor Tu, *J. Mol. Biol.*, 114, 451, 1977.
93. **Shapiro, R., Servis, R. E., and Welcher, M.**, Reactions of uracil and cytosine derivatives with sodium bisulfite. A specific deamination method, *J. Am. Chem. Soc.*, 92, 422, 1970.
94. **Hayatsu, H., Wataya, Y., and Kai, J.**, The addition of sodium bisulfite to uracil and to cystosine, *J. Am. Chem. Soc.*, 92, 724, 1970.
95. **Hayatsu, H., Wataya, Y., Kai, K., and Iida, S.**, Reaction of sodium bisulfite with uracil, cytosine, and their derivatives, *Biochemistry*, 9, 2858, 1970.
96. **Thang, M. N., Dondon, L., Thang, D. C., and Rether, B.**, Effect of the presence of a pCpCpCpA 3′-terminus in Phe-tRNA$_{yeast}^{Phe}$ on the interaction with elongation factors and with the poly U-ribosome system, *FEBS Lett.*, 26, 145, 1972.
97. **Fraser, T. H. and Rich, A.**, Amino acids are not all initially attached to the same position on transfer RNA molecules, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 3044, 1975.
98. **Sprinzl, M. and Cramer, F.**, Site of aminoacylation of tRNAs from *Escherichia coli* with respect to the 2′- or 3′-hydroxyl group of the terminal adenosine, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 3049, 1975.
99. **Hecht, S. M. and Chinault, A. C.**, Position of aminoacylation of individual *Escherichia coli* and yeast tRNAs, *Proc. Natl. Acad. Sci. U.S.A.*, 73, 405, 1976.
100. **Julius, D. J., Fraser, T. H., and Rich, A.**, Isomeric specificity of aminoacylation of wheat germ transfer ribonucleic acid and the specificity of interaction of elongation factor Tu with aminoacyl transfer ribonucleic acid, *Biochemistry*, 18, 604, 1979.
101. **Hecht, S. M., Tan, K. H., Chinault, A. C., and Arcari, P.**, Isomeric aminoacyl-tRNAs are both bound by elongation factor Tu, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 437, 1977.
102. **Sprinzl, M., Kucharzewski, M., Hobbs, J. B., and Cramer, F.**, Specificity of elongation factor Tu from *Escherichia coli* with respect to attachment of the amino acid to the 2′ or 3′-hydroxyl group of the terminal adenosine of tRNA, *Eur. J. Biochem.*, 78, 55, 1977.
103. **Hecht, S. M.**, 2′-versus 3′-OH specificity in tRNA aminoacylation, in *Transfer RNA: Structure, Properties, and Recognition*, Schimmel, P., Söll, D., and Abelson, J., Eds., Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1979, 345.
104. **Cohn, M., Danchin, A., and Grunberg-Manago, M.**, Proton magnetic relaxation studies of manganous complexes of transfer RNA and related compounds, *J. Mol. Biol.*, 39, 199, 1969.
105. **Ninio, J., Luzzatti, V., and Yaniv, M.**, Comparative small-angle X-ray scattering studies on unacylated, acylated and cross-linked *Escherichia coli* transfer RNA,Val, *J. Mol. Biol.*, 71, 217, 1972.
106. **Potts, R., Fournier, M. J., and Ford, N. C. Jr.**, Effect of aminoacylation on the conformation of yeast phenylalanine tRNA, *Nature (London)*, 268, 563, 1977.
107. **Potts, R. O., Wang, C-C., Fritzinger, D. C., Ford, N. C., Jr., and Fournier, M. J.**, Effects of aminoacylation and solution conditions on the structure of ribonucleic acid, in *Transfer RNA: Structure, Properties, and Recognition*, Schimmel, P., Söll, D. and Abelson, J., Eds., Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1979, 207.
108. **Lynch, D. C. and Schimmel, P. R.**, Effects of abnormal base ionizations on Mg^{2+} binding to transfer ribonucleic acid as studied by a fluorescent probe, *Biochemistry*, 13, 1852, 1974.
109. **Fahnestock, S., Weissbach, H., and Rich, A.**, Formation of a ternary complex of phenyllactyl-tRNA with transfer factor Tu and GTP, *Biochim. Biophys. Acta*, 269, 62, 1972.
110. **Cuzin, F., Kretchmer, N., Greenberg, R. E., Hurwitz, R., and Chapeville, F.**, Enzymatic hydrolysis of *N*-substituted aminoacyl-tRNA, *Proc. Natl. Acad. Sci. U.S.A.*, 58, 2079, 1967.

Critical Reviews in Biochemistry and Molecular Biology Downloaded from informahealthcare.com by 89.163.34.136 on 01/07/12
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111. **Kössel, H. and RajBhandary, U. L.**, Studies on polynucleotides. LXXXVI. Enzymatic hydrolysis of *N*-acylaminoacyl-transfer RNA, *J. Mol. Biol.*, 35, 539, 1968.
112. **Vogel, Z., Zamir, A., and Elson, D.**, On the specificity and stability of an enzyme that hydrolyzes *N*-substituted aminoacyl-transfer RNA's, *Proc. Natl. Acad. Sci. U.S.A.*, 61, 701, 1968.
113. **Chapeville, F., Yot, P., and Paulin, D.**, Enzymatic hydrolysis of *N*-acylaminoacyl transfer RNAs, *Cold Spring Harbor Symp. Quaint. Biol.*, 34, 493, 1969.
114. **Menninger, J. R., Mulholland, M. C., and Stirewalt, W. S.**, Peptidyl-tRNA hydrolase and protein chain termination, *Biochim. Biophys. Acta*, 217, 496, 1970.
115. **Kössel, H.**, Purification and properties of peptidyl-tRNA hydrolase from *Escherichia coli*, *Biochim. Biophys. Acta*, 204, 191, 1970.
116. **Vogel, Z., Zamir, Z., and Elson D.**, The specific resistance of *N*-substituted initiator methionyl-tRNA to enzymatic hydrolysis, *Biochim. Biophys. Res. Commun.*, 33, 94, 1968.
117. **Lapidot, Y., Inbar, D., DeGroot, N., and Kössel, H.**, Enzymatic hydrolysis of *N*-substituted Met-tRNA$_M$ and Met-tRNA$_F$, *FEBS Lett.*, 3, 253, 1969.
118. **Schulman, L. H. and Pelka, H.**, The structural basis for the resistance of *Escherichia coli* formyl-methionyl transfer ribonucleic acid to cleavage by *Escherichia coli* peptidyl transfer ribonucleic acid hydrolase, *J. Biol. Chem.*, 250, 542, 1975.
119. **Lengyel, P. and Söll, D.**, Mechanism of protein biosynthesis, *Bacteriol. Rev.*, 33, 264, 1969.
120. **Adams, G. M. and Capecchi, M. R.**, *N*-formylmethionyl-sRNA as the initiator of protein synthesis, *Proc. Natl. Acad. Sci. U.S.A.*, 55, 147, 1966.
121. **Thach, R. E., Dewey, K. F., Brown, J. C., and Doty, P.**, Formylmethionine codon AUG as an initiator of polypeptide synthesis, *Science*, 153, 416, 1966.
122. **Webster, R. E., Engelhardt, D. L., and Zinder, N. D.**, *In vitro* protein synthesis: chain initiation, *Proc. Natl. Acad. Sci. U.S.A.*, 55, 155, 1966.
123. **Clark, B. F. C. and Marcker, K. A.**, The role of *N*-formyl-methionyl-sRNA in protein biosynthesis, *J. Mol. Biol.*, 17, 394, 1966.
124. **White, B. N. and Bayley, S. T.**, Methionine transfer RNAs from the extreme halophile, *Halobacterium cutirubrum*, *Biochim. Biophys. Acta*, 272, 583, 1972.
125. **Dickerman, H. W., Steve, E., Jr., Redfield, B. G., and Weissbach, H.**, Methionyl soluble ribonucleic acid transformylase. I. Purification and partial characterization, *J. Biol. Chem.*, 242, 1522, 1967.
126. **Dickerman, H. W. and Smith, B. C.**, Formylmethionyl transfer ribonucleic acid transformylase. The specific interaction of one enzyme with its transfer ribonucleic acid substrate, *J. Mol. Biol.*, 59, 425, 1971.
127. **Gillum, A. M., Hecker, L. I., Silberklang, M., Schwartzbach, S. D., RajBhandary, U. L., and Barnett, W. E.**, Nucleotide sequence of *neurospora crassa* cytoplasmic initiator tRNA, *Nucleic Acids Res.* 4, 4109, 1977.
128. **Temin, H. and Baltimore, D.**, RNA-directed DNA synthesis and RNA tumor viruses, in *Advances in Virus Research*, Vol. 17, Smith, K. M., Lauffer, M. A., and Bang, F. B., Eds., Academic Press, New York, 1974, 129.
129. **Canaani, E. and Duesberg, P.**, Role of subunits of 60 to 70S avian tumor virus ribonucleic acid in its template activity for the viral deoxyribonucleic acid polymerase, *J. Virol.*, 10, 23, 1972.
130. **Dahlberg, J. E., Sawyer, R. C., Taylor, J. M., Faras, A. J., Levinson, W. E., Goodman, H. M., and Bishop, J. M.**, Transcription of DNA from the 70S RNA of rous sarcoma virus. I. Identification of a specific 4S RNA which serves as primer, *J. Virol.*, 13, 1126, 1974.
131. **Bishop, J. M., Tsan-Deng, C., Faras, A. J., Goodman, H. M., Levinson, W. E., Taylor, J. M., and Varmus, H. E.**, Transcription of the rous sarcoma virus genome by RNA-directed DNA polymerase, in *Virus Research*, Robinson, W. S. and Fox, C. F., Eds., Academic Press, New York, 1973, 13.
132. **Faras, A. J., Taylor, J. M., Levinson, W. E., Goodman, H. M., and Bishop, J. M.**, RNA-directed DNA polymerase of rous sarcoma virus: initiation of synthesis with 70S viral RNA as template, *J. Mol. Biol.*, 79, 163, 1973.
133. **Sawyer, R. C. and Dahlberg, J. E.**, Small RNAs of rous sarcoma virus: characterization by two-dimensional polyacrylamide gel electrophoresis and fingerprint analysis, *J. Virol.*, 12, 1226, 1973.
134. **Faras, A. J. and Dibble, N. A.**, RNA-directed synthesis by the DNA polymerase of rous sarcoma virus: structural and functional identification of 4S primer RNA in uninfected cells, *Proc. Nat. Acad. Sci. U.S.A.*, 72, 859, 1975.
135. **Taylor, J. M., Cordell-Stewart, B., Rhode, W., Goodman, H. M., and Bishop, J. M.**, Reassociation of 4S and 5S RNA's with the genome of avian sarcoma virus, *Virology*, 65, 248, 1975.
136. **Panet, A., Haseltine, W. A., Baltimore, D., Peters, G., Harada, F., and Dahlberg, J. E.**, Specific binding of tryptophan transfer RNA to avian myeloblastosis virus RNA-dependent DNA polymerase (reverse transcriptase), *Proc. Natl. Acad. Sci. U.S.A.*, 72, 2535, 1975.

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137. **Kacian, D. L., Watson, K. F., Burny, A., and Spiegelman, S.,** Purification of the DNA polymerase of avian myeloblastosis virus, *Biochim. Biophys. Acta*, 246, 365, 1971.
138. **Hizi, A. and Joklik, W. K.,** RNA-dependent DNA polymerase of avian sarcoma virus B77, *J. Biol. Chem.*, 252, 2281, 1977.
139. **Gibson, W. and Verma, I.,** Studies on the reverse transcriptase of RNA tumor viruses, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 4991, 1974.
140. **Rho, H. M., Grandgenett, D. P., and Green, M.,** Sequence relatedness between the subunits of avian myeloblastosis virus reverse transcriptase, *J. Biol. Chem.*, 250, 5278, 1975.
141. **Hizi, A., Leis, J. P., and Joklik, W. K.,** The RNA-dependent DNA polymerase of avian sarcoma virus B77, *J. Biol. Chem.*, 252, 6878, 1977.
142. **Haseltine, W. W., Panet, A., Smoler, D., Baltimore, D., Peters, G., Harada, F., and Dahlberg, J. E.,** Interaction of tryptophan tRNA and avian myeloblastosis virus reverse transcriptase: further characterization of the binding reaction, *Biochemistry*, 16, 3625, 1977.
143. **Peters, G., Harada, F., Dahlberg, J. E., Panet, A., Haseltine, W. A., and Baltimore, D.,** Low-molecular-weight RNAs of moloney murine leukemia virus: identification of the primer for RNA-directed DNA synthesis, *J. Virol.*, 21, 1031, 1977.
144. **Araya, A. and Litvak, S.,** Studies on the interaction of tRNA and avian myeloblastosis DNA polymerase, *Cold Spring Harbor Symp. Quant. Biol.*, 43, in press.
145. **Baroudy, B. M., Fournier, M., Labouesse, J., Papas, T. S., and Chirikjian, J. G.,** $tRNA^{Trp}$ (bovine) binding to the reverse transcriptase of avian myeloblastosis virus and function as a heterologous primer, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 1889, 1977.
146. **Cordell, B., Swanstrom, R., Goodman, H. M., and Bishop, J. M.,** $tRNA^{Trp}$ as primer for RNA-directed DNA polymerase: structural determinants of function, *J. Biol. Chem.*, 254, 1866, 1979.
147. **Cordell, B., Stavnezer, E., Friedrich, R., Bishop, J. M., and Goodman, H. M.,** Nucleotide sequence that binds primer for DNA synthesis to the avian sarcoma virus genome, *J. Virol.*, 19, 548, 1976.
148. **Eiden, J. J., Quade, K., and Nichols, J. L.,** Interaction of tryptophan transfer RNA with rous sarcoma virus 35S RNA, *Nature (London)*, 259, 245, 1976.
149. **Panet, A., Baltimore, D., and Hanafusa, T.,** Quantitation of avian RNA tumor virus reverse transcriptase by radioimmunoassay, *J. Virol.*, 16, 146, 1975.
150. **Yarus, M. and Berg, P.,** Recognition of tRNA by aminoacyl tRNA synthetases, *J. Mol. Biol.*, 28, 479, 1967.
151. **Jain, S. C. and Sobell, H. M.,** Stereochemistry of actinomycin binding to DNA. I. Refinement and further structural details of the actinomycindeoxyguanosine crystalline complex, *J. Mol. Biol.*, 68, 1, 1972.
152. **Gilbert, W., Maizels, N., and Maxam, A.,** Sequences of controlling regions of the lactose operon, *Cold Spring Harbor Symp. Quant. Biol.*, 38, 845, 1973.
153. **Carter, C. W. and Kraut, J.,** A proposed model for interaction of polypeptides with RNA, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 283, 1974.
154. **Church, G. M., Sussman, J. L., and Kim, S-H.,** Secondary structural complementarity between DNA and proteins, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 1458, 1977.
155. **Seeman, N. C., Rosenberg, J. M., and Rich, A.,** Sequence-specific recognition of double helical nucleic acids by proteins, *Proc. Natl. Acad. Sci. U.S.A.*, 73, 804, 1976.
156. **Williamson, A. R.,** The biological origin of antibody diversity, *Annu. Rev. Biochem.*, 45, 467, 1976.
157. **Koontz, S. W. and Schimmel, P. R.,** Aminoacyl-tRNA synthetase-catalyzed cleavage of the glycosidic bond of 5-halogenated uridines, *J. Biol. Chem.*, 254, 12277, 1979.
158. **Araya, A., Sarih, L., and Litvak, S.,** Reverse transcriptase mediated binding of primer tRNA to the viral genome, *Nucl. Acids Res.*, 6, 3831, 1979.

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