The Transfer RNA Identity Problem: A Search for Rules

Margaret E. Saks, Jeffrey R. Sampson, John N. Abelson

Correct recognition of transfer RNAs (tRNAs) by aminoacyl-tRNA synthetases is central to the maintenance of translational fidelity. The hypothesis that synthetases recognize anticodon nucleotides was proposed in 1964 and had considerable experimental support by the mid-1970s. Nevertheless, the idea was not widely accepted until relatively recently in part because the methodologies initially available for examining tRNA recognition proved hampering for adequately testing alternative hypotheses. Implementation of new technologies has led to a reasonably complete picture of how tRNAs are recognized. The anticodon is indeed important for 17 of the 20 *Escherichia coli* isoaccepting groups. For many of the isoaccepting groups, the acceptor stem or position 73 (or both) is important as well.

The study of recognition of transfer RNAs (tRNA) by aminoacyl-tRNA synthetases is an old problem in molecular biology. Its beginning was foretold when Crick hypothesized the existence of adaptor molecules that would carry amino acids and interact with messenger RNA (mRNA) codons by way of complementary base pairing and thereby translate the genetic code (*1*). By 1965, the genetic code was solved (*2*), the existence of adaptor molecules was confirmed (*3*), the nucleic acid sequence of one adaptor molecule, or transfer RNA as it became known, was reported by Holley (*4*), and the decoding specificity of the tRNA on the ribosome was attributed to the nature of the tRNA rather than to its associated amino acid (*5*). Concurrently, it was shown that synthetases attach amino acids to tRNAs (*6*). These observations established that interactions between tRNAs and synthetases are central to maintaining the fidelity of translation and set the stage for investigating how synthetases recognize and aminoacylate their cognate tRNAs.

Detailed characterizations of synthetases and tRNAs began immediately. We now know that synthetases are a family of enzymes that catalyze the same type of reaction by the same mechanism (*7*) but that they share only a few common structural features. Although they can be divided into two distinct classes on the basis of the architecture of the catalytic domain (*8*), synthetases within each class vary in size and quaternary structure (*9*). In contrast, tRNAs are a family of molecules of similar sizes and tertiary structures (*10*), presumably because they interact interchangeably with components of the protein synthesis machinery. The elements that permit synthetases to recognize and aminoacylate their cognate tRNAs and to avoid misacylating those from the 19 noncognate groups must be embedded within the nucleotide sequence of the tRNA and within any subtle structural variations that might exist. However, these recognition elements are not obvious because tRNAs that accept the same amino acid (isoacceptors) often have different nucleotide sequences (*11*).

Among the pioneers who initially joined the quest to identify the tRNA elements that direct correct aminoacylation was L. H. Schulman. Her early studies of *Escherichia coli* methionine tRNA ($tRNA^{fMet}$), which is the tRNA responsible for translation initiation (Fig. 1), provided crucial support for the idea that anticodon nucleotides are important to recognition by synthetases. Her subsequent detailed in vitro and in vivo studies of anticodon recognition in other *E. coli* isoaccepting groups revealed that anticodon recognition is a general characteristic of tRNA recognition systems. This article focuses on the development of ideas about the elements in tRNAs that dictate recognition by synthetases and highlights Schulman's contributions (*12*). Additional information relevant to this field can be found in reviews (*13, 14*).

The Early Years

The early development of the tRNA recognition field was driven by a belief in the existence of a common set of rules that dictate recognition in all tRNA-synthetase cognate systems. Although the idea of rules was conceptually simple, the hypothesis was difficult to test because data for most, if not all, of the 20 cognate tRNA-synthetase systems were required. An attractive initial hypothesis held that recognition elements were located at the same site in all tRNAs and that synthetases discriminated among tRNAs on the basis of a particular and distinctive nucleotide sequence at such a site. In this way, the tRNA recognition system would mirror the simplicity and elegance of the genetic code.

Speculation about the location of the recognition site primarily focused on two regions of the tRNA. The acceptor stem and the "discriminator" base at position 73 were attractive candidates because their proximity to the tRNA 3′ terminus, where amino acids are attached, places them in the vicinity of the synthetase active site (*15, 16*). On the other hand, Kisselev pointed out that the anticodon was an obvious choice for an "encoded" recognition site because it is a defining feature of each isoaccepting group (*17*). Moreover, anticodon recognition would ensure translational fidelity because an anticodon mutation would simultaneously change the identity of the tRNA and its mRNA coupling capacity.

In large measure, the initial hypotheses concerning tRNA recognition defined the questions that needed to be answered. However, as in any emerging field, the latitude and precision of experimental exploration were both driven and constrained by the available methods (Table 1) (*7, 15,*

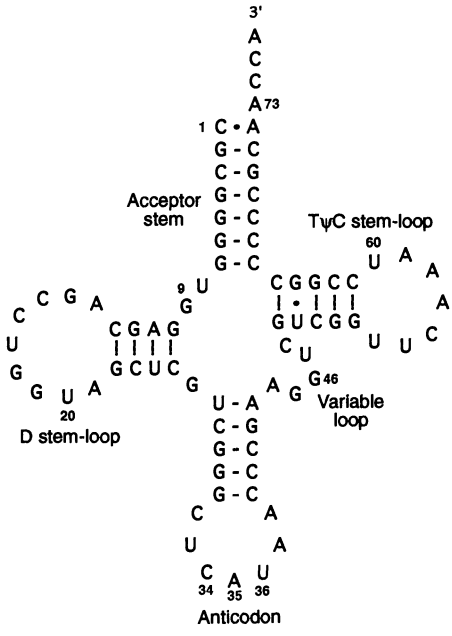

Fig. 1. The nucleotide sequence of *E. coli* $tRNA^{fMet}$. The standard cloverleaf structure and conventional numbering system are used. Modified nucleotides normally present in the tRNA have been omitted.

M. E. Saks and J. R. Sampson are Senior Research Fellows and J. N. Abelson is George Beadle Professor of Biology in the Division of Biology at the California Institute of Technology, Pasadena, CA 91125. This article was written after the untimely death of LaDonne H. Schulman on 12 August 1992 to commemorate her work demonstrating the importance of anticodon nucleotides to recognition by aminoacyl-tRNA synthetases. The substance of the article was presented by J. N. Abelson at the First Annual LaDonne Schulman Memorial Lecture at the Albert Einstein College of Medicine on 17 June 1993.

18, 19). In spite of concerns about the limitations of the methods that were initially available, most early results have been confirmed and extended with the use of the more direct methodologies that were later developed.

Schulman initially used chemical and photochemical modification of tRNAs to approach the problem of tRNA recognition. The results she obtained for yeast alanine transfer RNA ($tRNA^{Ala}$) and *Escherichia coli* $tRNA^{fMet}$ provide an interesting example of the difficulties attendant on understanding tRNA recognition. Her initial results for both isoaccepting groups showed that modification of acceptor stem nucleotides decreased aminoacylation by the cognate synthetase (*20, 21*) and thus supported the acceptor stem recognition hypothesis. Indeed, much later, other investigators used additional methods to confirm the participation of acceptor stem nucleotides in the recognition of tRNAs from both isoaccepting groups (*22–25*).

Although Schulman had demonstrated acceptor stem recognition for $tRNA^{fMet}$, she soon learned that this was only part of the story. By 1977 she had systematically modified nearly half of the $tRNA^{fMet}$ nucleotides (*26, 27*). Whereas modification of acceptor stem and variable loop nucleotides reduced aminoacylation by MetRS (*28*), modification of the anticodon nucleotides C34 and A35 abolished it. The strongest evidence for the central role of anticodon nucleotides in dictating $tRNA^{fMet}$ recognition was obtained when Schulman created a $tRNA^{fMet}$ having a modification of only C34 and showed that the tRNA was neither bound nor aminoacylated by MetRS (*29*).

The data obtained in numerous laboratories by the late 1970s for yeast and *E. coli* tRNAs indicated that recognition elements could be located in the acceptor stem, position 73 and the anticodon, as well as in the variable loop and D stem (*30*). Because the data did not completely support a single hypothesis about tRNA recognition, it was possible that the universal recognition site had not yet been detected or that tRNA recognition systems were idiosyncratic (*31*). By this time, there was sufficient evidence to reject the hypothesis that the anticodon was a universal recognition site because in vitro studies of $tRNA^{Ala}$ (*32*) and $tRNA^{Ser}$ (*33*) failed to implicate any anticodon nucleotides in aminoacylation. Moreover, in vivo genetic studies showed that two naturally occurring amber-suppressing tRNAs (anticodon CUA) retained their correct amino acid identity even though they arose from wild-type tyrosine and glutamine tRNA genes by a single anticodon nucleotide mutation (*34*). Nevertheless, the available results supported the idea that anticodon recognition was important. Because each naturally occurring amber suppressor arose by a single mutation, each still retained two wild-type anticodon nucleotides. In fact, there was evidence that GlnRS recognized U35 of the anticodon because it aminoacylated a $tRNA^{Trp}$ and a $tRNA^{Gln}$ amber suppressor, as well as a wild-type $tRNA^{Gln}$, which all contain this nucleotide (*35, 36*). Moreover, Schulman had established anticodon recognition for $tRNA^{fMet}$ and anticodon nucleotides were implicated in recognition in most isoaccepting groups that had been studied in vitro (*30*).

In spite of the considerable evidence supporting anticodon recognition, it appears that many investigators in the field were hesitant to formulate generalizations about tRNA recognition systems. This is evident from the cursory treatments of the tRNA recognition problem in textbooks published in the 1980s and from statements therein that the anticodon does not contain recognition elements. The omissions persisted into the 1990s even after publication of an article that reviewed the extensive evidence supporting anticodon recognition (*37*). It is worth considering why this occurred. Perhaps the answer lies in concerns about the nature of tRNA recognition systems as well as concerns about the interpretation of results obtained by the methodologies that were initially available.

The crystal structure of yeast $tRNA^{Phe}$ that was solved in 1974 (*38*) had a major impact. It revealed that tRNA nucleotides, except those in the 3′ terminus, variable pocket (*39*), and anticodon loop, take part in base pairs or tertiary interactions (Fig. 2). Thus, it was possible that the "universal" recognition site had not yet been elucidated because it existed in one of the structured regions that were generally unreactive to chemical modification, sequence-specific ribonucleases, and oligonucleotide binding. It was also possible that the results of chemical modification and ribonuclease digestion studies were misleading about the

Table 1. Methods for studying questions about tRNA in vitro recognition and in vivo identity.

Questions	Methods available in the 1960s and 1970s	Methods currently available
Do distinctive nucleotides define tRNAs from each isoaccepting group?	Comparisons of tRNA sequences Heterologous misacylation studies	Computer-assisted searches of tRNA sequences for nucleotides that are distinctive either individually or in sets
What is the minimal RNA sequence that promotes aminoacylation?	Aminoacylation of tRNA fragments obtained by limited nuclease digestion	Aminoacylation and binding of RNAs that represent discrete tRNA domains obtained by enzymatic or chemical synthesis
Which nucleotides contribute to tRNA in vitro aminoacylation?	Aminoacylation of tRNAs altered by nucleotide-specific chemical reagents Oligonucleotide inhibition of aminoacylation	Aminoacylation of engineered tRNA mutants obtained by T7 transcription or in vivo expression
Which nucleotides contribute to tRNA in vivo identity?	Genetic selection and RNA sequencing of mutant nonsense suppressing tRNAs that insert a particular amino acid	Sequencing of a reporter protein to determine the amino acid inserted by an engineered tRNA mutant
What is the contribution of tRNA structure to aminoacylation?	Aminoacylation of cognate and noncognate tRNAs in the presence of organic solvents to relax tRNA structure	Aminoacylation of tRNA structural variants obtained by T7 transcription
Which nucleotides are in close proximity or in direct contact with the cognate synthetase?	Chemical and photochemical crosslinking of tRNA-synthetase complexes	X-ray crystallographic and NMR analysis of tRNA-synthetase complexes
What is the range of possible tRNA variants that can be bound or aminoacylated by a particular synthetase?	No method available	Isolation of RNAs, from a pool of randomized sequences, that bind or are aminoacylated by a particular synthetase (SELEX)

locations of tRNA recognition sites because these methods could have inactivated a tRNA by altering its structure rather than by changing or eliminating a nucleotide required for contact by a synthetase.

The available methods also had technical drawbacks. The difficult and time-consuming process of purifying and sequencing individual isoaccepting tRNAs from bulk cellular RNA limited the types of tRNAs that were available for heterologous misacylation and chemical modification studies. Regions other than the anticodon had been studied in vivo where genetic selection was used for the insertion of particular amino acids by nonsense suppressing tRNAs (*40*), but it was nearly impossible to obtain tRNAs with multiple mutations. Moreover, only a subset of tRNA variants could be studied because many mutants were either not processed or were unstable (*41*).

As an alternative to mutational studies of tRNAs, those who wished to pursue tRNA recognition examined tRNA-synthetase binding topologies by crosslinking the two molecules (*19, 42*). The available methods allowed nucleotides in the crosslinks to be identified but it was difficult to determine the corresponding crosslinked amino acids. Schulman developed more versatile methods that overcame some of the problems (*43*) and used them to define the orientation of $tRNA^{fMet}$ with respect to the NH_2- and COOH-terminal domains of MetRS (*44*). By the late 1980s she had created MetRS mutants by site-directed mutagenesis and identified amino acids involved in anticodon recognition (*45, 46*). Studies of the $tRNA^{Met}$-MetRS topology by Schulman and others, in conjunction with the MetRS crystal structure, provide the basis for modeling the binding topology of a system which, to date, lacks a cocrystal structure (*45–47*).

By the late 1970s, methods were developed for manipulating RNA sequences not involved in highly structured regions (*48*). Uhlenbeck and Schulman independently adapted these methods for studying anticodon recognition (*49*). They used sequence-specific ribonucleases to cleave and remove a portion of the anticodon loop and then used T4 RNA ligase to insert RNA oligomers with the desired sequence. Uhlenbeck studied the in vitro aminoacylation of yeast $tRNA^{Phe}$ and $tRNA^{Tyr}$ variants having altered anticodons and showed that their anticodon nucleotides were important recognition elements for the cognate synthetases (*50*). Schulman generated a series of *E. coli* $tRNA^{fMet}$ variants representing all but two single-base changes at each anticodon position and showed that U36, as well as C34 and A35, contributed to aminoacylation by MetRS (*51*).

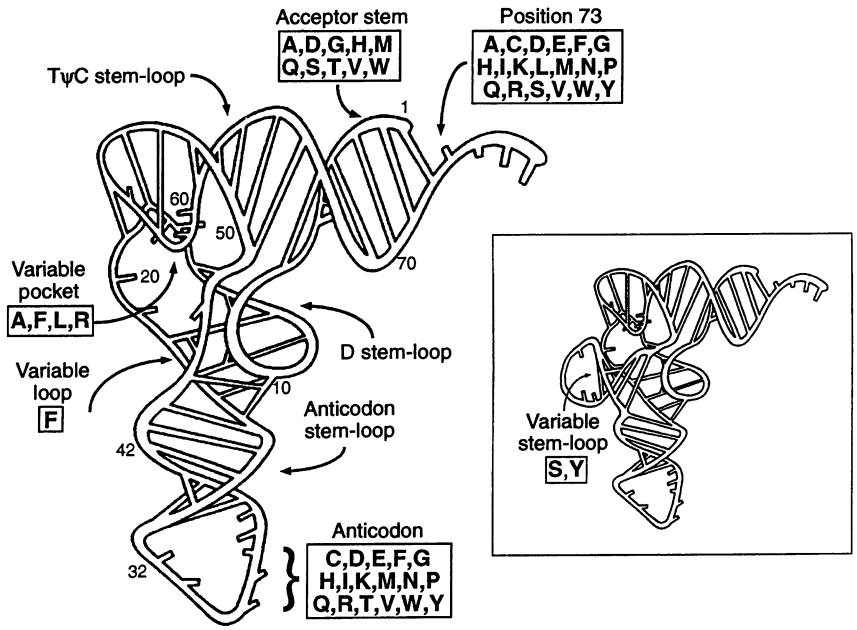

Fig. 2. Ribbon diagram of a type I tRNA showing the common locations of nucleotides that contribute to recognition and identity for each of the 20 isoaccepting groups in *E. coli*. Isoaccepting groups are indicated by the single-letter amino acid designations. The inset represents type II tRNAs that have a variable loop consisting of a stem-loop structure. Table 1 gives the references for anticodon recognition in each isoaccepting group. Selected references for elements in other regions that contribute to recognition are as follows: Ala (*23, 60, 73, 124*); Arg (*65, 110, 125*); Asn (*87, 111*); Asp (*112, 127*); Cys (*78, 111, 113*); Glu (*111*); Gln (*80, 90, 126*); Gly (*60, 72, 116*); His (*60, 128*); Ile (*111*); Leu (*74, 117*); Lys (*71, 110*); Met (*21, 24, 25*); Phe (*70, 79*); Pro (*111*); Ser (*63, 68, 74, 118*); Thr (*66, 119*); Trp (*120, 121*); Tyr (*74, 118*); and Val (*123*). Whether the indicated elements provide a direct contact for a synthetase or dictate the spatial orientation of nucleotide(s) that are directly contacted is a necessary distinction (*13, 14*), but it remains unclear in many cases. Moreover, the magnitude of the contribution to aminoacylation of elements at each location is not known for all isoaccepting groups and can depend on the RNA sequence background in which it is tested (*79, 109*). Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Revitalization of tRNA Recognition Studies

Up until the mid-1980s, it was still difficult to make mutations at will throughout the entire tRNA. Thus comprehensive studies of the locations of tRNA recognition elements were still constrained. Although the de novo synthesis of $tRNA^{fMet}$ had been achieved by a combination of chemical and enzymatic methods (*52*), this labor-intensive process was not suitable for generating the quantity and number of tRNA variants that was necessary. Most such obstacles were overcome in the mid-1980s when DNA synthesis and recombinant DNA technologies made it possible to synthesize tRNA genes with any desired sequence. This technology led to the development of methods for the in vitro transcription and in vivo expression of tRNAs.

Among the several methods developed for the in vitro transcription of RNAs active for aminoacylation (*53, 54*), was that of Uhlenbeck whereby tRNA genes having an upstream T7 RNA polymerase promoter and a suitable restriction endonuclease site at the 3′ terminus were cloned into a plasmid. This allowed the mature tRNA, including its 3′-terminal adenosine, to be synthesized by run-off transcription from linearized plasmid DNA. The method was validated by showing that a T7 transcript of yeast $tRNA^{Phe}$, which lacked the normal modified bases, had aminoacylation kinetics (*54*) and structure (*55*) similar to that of native $tRNA^{Phe}$. Subsequently, T7 transcription was used in many laboratories to directly establish that (i) subtle variations in tRNA structure can contribute to recognition (*56, 57*); (ii) some synthetases can specifically aminoacylate RNAs that represent only the acceptor-TΨC stem domain (*24, 58–63*); and (iii) nucleotides in many different regions of the tRNA can contribute to recognition by synthetases (Fig. 2).

The T7 transcription technique contributed directly to the study of anticodon recognition. Schulman showed that ValRS as well as MetRS recognized anticodon nucleotides by "transplanting" a valine an-

ticodon (UAC) into a $tRNA^{Met}$ and showing that the tRNA became an excellent substrate for ValRS and was no longer aminoacylated by MetRS (64). In subsequent studies, she used the transplant approach to show that $tRNA^{Arg}$ (65) and $tRNA^{Thr}$ (66) anticodon nucleotides make large contributions to recognition by their cognate synthetases. Later studies by others confirmed Schulman's results and brought the number of *E. coli* isoaccepting groups for which anticodon recognition is important to 17 (Table 2).

The in vivo method developed by Abelson (67, 68) utilized tRNA genes having amber anticodons that were constructed from synthetic DNA oligonucleotides and cloned into and expressed from high copy number plasmids. A reporter protein gene having an engineered amber codon at residue 10 of dihydrofolate reductase was expressed in *E. coli* along with the tRNA gene and the in vivo amino acid identity of the amber suppressing tRNA was determined by sequencing the NH_2-terminus of the purified translated protein. This system made it possible to express amber suppressing tRNAs having virtually any sequence so long as the tRNA-like structure that is required for processing and translation was maintained, and to quantify the amount of any amino acid that was inserted into the reporter protein by the suppressor tRNA. Abelson used this method to demonstrate that the in vivo identity of an amber suppressing $tRNA^{Leu}$ could be changed from leucine to serine by transplanting nucleotides common to *E. coli* serine tRNAs into a $tRNA^{Leu}$ sequence background and therefore showed that in vivo amino acid "identity swaps" were possible (68).

The in vivo method was not quite as versatile as the in vitro method because nucleotides involved in maintaining tRNA structure and in the anticodon could not be changed at will. Nevertheless, because all other nucleotides could be changed, the method provided a means for elucidating the requirements for specific tRNA amino acid identities within the cellular environment where all 20 synthetases are competing for substrates. In vivo identity sets comprise positive elements that are recognized by a particular synthetase as well as negative elements that disrupt potential productive interactions with the 19 other synthetases in the cell (36, 69). Studies of the identity sets (70–74) and identity elements of *E. coli* isoaccepting groups indicate that positive or negative elements in regions other than the anticodon often contribute to tRNA identity (Fig. 2).

In spite of the requirement for either amber or opal (71) anticodons, the in vivo method has contributed to our understanding of the relation of anticodon nucleotides to tRNA identity. The most comprehensive study in this regard involved determining the amino acid identities of amber suppressing tRNAs from each of the 20 *E. coli* isoaccepting groups (67, 75, 76) (Table 2). When the anticodons of tRNAs from nine isoaccepting groups were changed to amber, their amino acid identities were also changed, indicating that a recognition element for the cognate synthetase was lost. Because these amber suppressors were misacylated with either glutamine or lysine, the results additionally indicated that the U35 held in common between the amber and the wild-type anticodons of $tRNA^{Gln}$ and $tRNA^{Lys}$ was a recognition element for GlnRS, as shown earlier (35, 36, 77), and also for LysRS (76). That some of the amber suppressors retained their identity presented an interesting problem. One explanation was that these tRNAs did not have an anticodon recognition element. Alternatively, because in many cases an anticodon nucleotide was held in common between the wild-type and amber anticodons, it was possible that this nucleotide was recognized by the cognate synthetase and accounted for the maintenance of tRNA identify. However, it soon became clear that neither explanation completely accounted for the results. In vitro aminoacylation studies of $tRNA^{Cys}$ (57, 78), $tRNA^{Phe}$ (79), $tRNA^{Gln}$ (80), and $tRNA^{Tyr}$ (81) showed that aminoacylation by the

Table 2. Recognition of anticodon nucleotides by *E. coli* aminoacyl-tRNA synthetases; +, anticodon nucleotides contribute to in vitro aminoacylation or in vivo identity; −, changing the wild-type anticodon did not affect in vitro aminoacylation or in vivo identity. ND, not determined; Y, pyrimidine; N, any nucleotide.

Isoaccepting group	Anticodon	In vitro aminoacylation	In vivo	
			Nonsense suppression	Protein initiation
Alanine	NGC	− (*32, 58*)	− (*76*)	ND
Arginine	NCG YCU	+ (*65, 110*)	+ (*71, 76*)	ND
Asparagine	GUU	+ (*87, 111*)	?*	−†
Aspartic acid	GUC	+ (*112*)	+ (*76*)	ND
Cysteine	GCA	+ (*57, 78*)	− (*67, 78*)	+ (113)
Glutamic acid	YUC	+ (*99*)	+ (*76*)	ND
Glutamine	YUG	+ (*80*)	+‡ (*76*)	+ (*114, 121*)
Glycine	NCC	+ (*115*)	+ (*72, 76*)	+ (*113, 116*)
Histidine	GUG	+ (*111*)	− (76)	ND
Isoleucine	GAU CAU§	+ (*98*)	+ (*76*)	+ (86)
Leucine	YAA NAG	− (*117*)	− (*68*)	ND
Lysine	YUU	+ (*110*)	+‡ (*71, 76*)	ND†
Methionine	CAU	+ (*51*)	+ (*76*)	+ (*85*)
Phenylalanine	GAA	+ (*79*)	− (*67*)	+ (*85, 86*)
Proline	NGG	+ (*111*)	− (*76*)	ND
Serine	NGA GCU	− (*63, 118*)	− (*68*)	ND
Threonine	NGU	+ (*66, 119*)	+ (*76*)	ND
Tryptophan	CCA	+ (*120*)	+ (*35*)	+ (*121*)
Tyrosine	GUA	+ (*81, 118*)	− (*34*)	+ (*122*)
Valine	NAC	+ (*64, 123*)	+ (*76*)	+ (*85, 86*)

*The tRNA was an inactive amber suppressor (*75*). †Anticodon recognition was shown by a gel shift assay that detected in vivo aminoacylation (*87*). ‡Anticodon recognition inferred because GlnRS and LysRS misacylated noncognate amber suppressor tRNAs (*76*) §The cytidine residue is modified to lysidine (*98*).

cognate synthetase was decreased when the wild-type anticodons were changed to amber even though each amber suppressor had retained its in vivo amino acid identity (Table 2).

The expression of tRNAs from high copy number plasmids may, in part, explain why a recognition element that makes a significant contribution to in vitro aminoacylation can be dispensable with respect to in vivo identity. More than 20 years ago, Yarus proposed that a proper balance among tRNA and synthetase concentrations is critical to maintaining aminoacylation specificity (*82*). He later pointed out that, because of potential cross-reactions between synthetases and noncognate tRNAs, tRNA elements that increase interactions with cognate synthetases (positive elements) and decrease interactions with noncognate synthetases (negative elements) are critical to the tRNA's ultimate amino acid identity in the cell (*36*). Subsequent studies by other investigators involving the manipulation of tRNA and synthetase concentrations further demonstrated that tRNA in vivo identity depends on properly balanced concentrations of the two macromolecules (*81, 83*).

The results of competition studies as well as the discrepancies between the in vitro recognition and the in vivo identity sets (Table 2) illustrated the need for parallel in vivo and in vitro studies of each isoaccepting group. However, it was not possible to directly examine the contributions of anticodon nucleotides to in vivo amino acid identity because the existing methods required that tRNAs have nonsense anticodons. This prompted Schulman to develop a new in vivo system. Central to its design were Schulman's in vitro studies of anticodon recognition in $tRNA^{fMet}$ (*51*) and other isoaccepting groups (*64–66, 77*) and RajBhandary's studies of the $tRNA^{fMet}$ characteristics required for translation initiation (*84*). Under normal circumstances, only $tRNA^{fMet}$ initiates protein synthesis in *E. coli* due to structural features in its anticodon and acceptor stems (*84*). In addition, changing the $tRNA^{fMet}$ anticodon disrupts productive interactions with MetRS and allows interactions with other synthetases to be examined; yet it does not affect the ability of $tRNA^{fMet}$ to initiate protein synthesis so long as a complementary initiation codon exists in a reporter mRNA (*85*). Thus, in Schulman's new in vivo system (*85, 86*) a gene for $tRNA^{fMet}$ is constructed with an anticodon of interest and a reporter gene containing the complementary initiation codon is constructed as well. Both genes are expressed in *E. coli* and the amino acid identity of the tRNA is revealed by determining the NH_2-terminal amino acid of the purified reporter protein.

To date, Schulman has shown that the anticodons for nine isoaccepting groups (including methionine) direct the insertion of some of the corresponding amino acid into the NH_2-terminus of the reporter protein (Table 2). For the initiator tRNAs having either a glycine, cysteine, or tryptophan anticodon, the initiation efficiency and proportion of the correct amino acid inserted was increased when position 73 was changed to that found in the respective wild-type tRNA. In contrast, a $tRNA^{fMet}$ having the asparagine anticodon (GUU) did not initiate protein synthesis (*87*), possibly because the asparagine residue attached to the $tRNA^{fMet}$ was not formylated and thus the tRNA was not competent for protein initiation (*88*). However, by expressing mutants of wild-type $tRNA^{Asn}$ in vivo and using a gel shift assay that distinguishes between acylated and deacylated tRNAs (*89*), Schulman showed that all three anticodon nucleotides are important for $tRNA^{Asn}$ identity (*87*). Moreover, the aminoacylation by LysRS of a $tRNA^{Asn}$ having a lysine anticodon (CUU) confirmed the importance of U35 to $tRNA^{Lys}$ identity and showed that a pyrimidine at position 34 is also important for $tRNA^{Lys}$ identity. Schulman's in vivo protein initiation system is invaluable for determining the contributions of anticodon nucleotides to in vivo amino acid identity, and it is also attractive because contributions to identity from nucleotides in other regions of the tRNA can be evaluated within the context of the wild-type anticodon.

The co-crystal structure of *E. coli* $tRNA^{Gln}$ and GlnRS (*90, 91*) and of yeast $tRNA^{Asp}$-AspRS (*92*) confirmed the involvement of anticodon nucleotides in tRNA recognition. In particular, the $tRNA^{Gln}$-GlnRS structure revealed an individual binding pocket for each anticodon nucleotide where specific hydrogen bonding and van der Waals interactions account for base specificity. Interactions with the acceptor stem are also evident in both structures; yet GlnRS contacts the acceptor stem minor groove whereas AspRS approaches it from the major groove. Although the tRNA-synthetase binding topologies of the two complexes conformed to the general model that Rich and Schimmel had proposed (*93*), differences in the details of synthetase structures and biochemical data for other tRNA-synthetase interactions indicate that other tRNA-synthetase binding topologies probably exist (*92a*).

Summary of Available Results: Implications and Future Directions

All 20 isoaccepting groups from *E. coli* have now been studied to some extent by in vivo or in vitro methods (or both). The results of both recent and older studies confirm the essence of Schulman's conviction that anticodon nucleotides make important contributions to recognition. Although the anticodon does not "encode" the amino acid identity for all isoaccepting groups, at least one anticodon nucleotide contributes to recognition in 17 of the 20 isoaccepting groups in *E. coli* (Table 2).

Recognition elements are not limited to the anticodon. They also can be located in the acceptor stem, position 73, the variable loop, and the variable pocket (Fig. 2). It is intriguing that tRNAs from most isoaccepting groups have recognition elements in at least two locations, with the most common being the anticodon and the acceptor stem or position 73 (or both). The existence of overlapping recognition elements for several synthetases near the tRNA 3′ terminus (*60*) and the observation that the active site domains of some synthetases can specifically aminoacylate RNAs comprised of only the acceptor-TΨC stem (*94*) has led to the proposal that the ancestral tRNA-synthetase recognition system primarily involved the acceptor stem and position 73 and a portion of present-day synthetases (*95*). However, a recognition system entirely based on nucleotides in this region seems to have been lost as the translation machinery evolved and the genetic code was set; anticodon recognition has been added and, in some cases, seems to make a larger contribution to aminoacylation specificity than does the acceptor stem or position 73.

Experimental results and a computer-assisted analysis of *E. coli* tRNA sequences (*96*) reveal that tRNA recognition and identity for most isoaccepting groups is governed by a distinctive set of elements rather than by a single distinctive nucleotide or base pair. Although the sets are distinctive, members of a set frequently occur in noncognate tRNAs. For example, Ozeki's compilation of all *E. coli* tRNA gene sequences (*97*) shows that G2, an acceptor stem nucleotide that is recognized by *E. coli* GlnRS (*90*), occurs in 22 noncognate *E. coli* tRNAs from 11 additional isoaccepting groups. Moreover, no single anticodon nucleotide is distinctive to each isoaccepting group, yet in most instances anticodon nucleotides are required for tRNA in vitro recognition and in vivo amino acid identity.

A variety of factors can dictate the extent to which recognition elements for one isoaccepting group can be tolerated in noncognate tRNAs. Competition among synthetases and the magnitude of an element's contribution to the aminoacylation reaction can be important. Posttranscriptional base modifications, which essentially elaborate the nucleotide alphabet, contribute to the recognition of $tRNA^{Ile}$ (*98*) and $tRNA^{Glu}$ (*99*) by their cognate synthetase. Subtle

structural variations among tRNAs can affect the "presentation" of nucleotides that are directly contacted by the cognate synthetase (*14*). Finally, negative elements in tRNAs can potentially block misacylation. These complexities of tRNA recognition systems underscore the necessity to elucidate more than just the locations and nucleotide identities of recognition elements.

It is not yet clear whether there is a correlation between a recognition element's location and the way in which it contributes to the aminoacylation reaction. Although it is thought that in vivo aminoacylation specificity is primarily determined at the catalytic step (*100*), in vitro aminoacylation studies indicate that a recognition element can contribute to either binding or catalysis (k_{cat}). Based on structure-function considerations, recognition elements located in the acceptor stem or position 73 could affect k_{cat} due to their close proximity to the active site. However, because the anticodon is distant from the active site the mechanism by which it contributes to k_{cat} is problematic. The anticodon could contribute to k_{cat} if conformational changes in the tRNA, the protein, or both are induced by anticodon binding and are transmitted to the synthetase active site (*46, 61, 91, 101*). Studies of the effects of recognition elements on the kinetic pathway and structural studies of tRNA-synthetase complexes should help clarify this issue.

Elucidating the nucleotide functional groups that are responsible for recognition by the cognate synthetase is a logical and necessary step toward understanding how specificity is achieved. Schulman began addressing this problem in the 1970s using the data from her chemical modification (*27*) and nucleotide substitution studies of $tRNA^{fMet}$ (*51*). Recent developments in the chemical synthesis of RNA and in the availability of nucleotide analogs have made it possible to directly delimit important nucleotide functional groups in $tRNA^{Ala}$ (*102*) and $tRNA^{Gln}$ (*103*). Moreover, the co-crystal structures of *E. coli* $tRNA^{Gln}$-GlnRS and yeast $tRNA^{Asp}$-AspRS not only reveal the nucleotide and amino acid functional groups that are directly involved in recognition but also illustrate the complexity of these interactions (*90–92*). For example, bifurcated hydrogen bonds, hydrogen bonding of a single amino acid side chain with the functional groups of adjacent base pairs, and water-mediated hydrogen bonding are apparent in these tRNA-synthetase complexes. It is likely that these types of interactions dictate recognition in other cognate systems.

An in vitro selection technique (SELEX) (*104*) that allows RNAs that interact with proteins to be isolated from a pool of randomized RNA sequences provides an efficient means for sampling a large number of RNA variants. Uhlenbeck modified the SELEX procedure to isolate tRNAs that were bound and aminoacylated by *E. coli* PheRS (*105*). Transfer RNAs having some, but not all, of the nucleotides that conventional methods had previously shown to be important for recognition by PheRS (*79*) were selected from an RNA library representing a randomization of $tRNA^{Phe}$ recognition elements. Functional tRNAs isolated from another randomized library included some having unexpected combinations of nucleotides involved in tertiary interactions, indicating that PheRS can accommodate a much broader range of nucleotide sequence variation than is normally exhibited in its cognate tRNAs. SELEX paves the way for examining whether the nucleotides of recognition sets are functionally interdependent and for investigating constraints that might be imposed on tRNA sequence evolution.

Systematic studies of recognition sets in several organisms make it possible to speculate about mechanisms of tRNA evolution. The $tRNA^{Ala}$ G3-U70 base pair is conserved in all organisms (*11*) and contributes significantly to aminoacylation by AlaRS in three distantly related organisms (*106*). However, for both $tRNA^{Phe}$ (*79, 107*) and $tRNA^{Trp}$ (*108*), recognition element locations but not their nucleotide identities have been conserved. The data for $tRNA^{Phe}$ and $tRNA^{Trp}$ indicate that mutations in recognition elements can be tolerated. Perhaps in vivo competition suppresses the effects of some mutations on the identity of the expressed tRNAs under normal cellular conditions. This would permit evolutionary divergence in the nucleotide identity of a recognition element within an isoaccepting group lineage that could be subsequently compensated by coevolutionary changes in the amino acid residues of the cognate synthetase.

Analyses of the available tRNA gene sequences provide another perspective on tRNA gene evolution. They reveal that tRNAs from different isoaccepting groups, within a single organism, are often more similar to one another than they are to their isoaccepting counterparts (*109*). This may simply reflect the accumulation of neutral mutations at sites that do not contribute to tRNA recognition. Alternatively, it may reflect the consequences of anticodon mutations. Because anticodon mutations have a high probability of changing both the amino acid identity and the mRNA coupling capacity of the expressed tRNA, they have a high probability of being tolerated. Anticodon mutations could therefore recruit tRNA genes from one isoaccepting group to another and thereby intermix tRNA gene sequences. Thus, the properties of the tRNA recognition system may permit tRNA gene sequences to be evolutionarily labile without sacrificing the high degree of translational fidelity that is necessary in the cell.

Our current appreciation for the importance of anticodon nucleotides to tRNA recognition is, in large part, due to Schulman's persistence in studying this problem. The results obtained by Schulman and other researchers have provided insights and prompted questions about the mechanism of tRNA recognition and the evolution of tRNAs. Thus, in the tradition of all good science, Schulman's research helped to answer long-standing questions, and in so doing, raised new questions that are certain to stimulate further investigations.

REFERENCES AND NOTES

1. F. H. C. Crick, "A Note for the tRNA Tie Club," 1955, as quoted by M. B. Hoagland, in *The Nucleic Acids*, E. Chargaff and J. N. Davidson, Eds. (Academic Press, New York, 1960), vol. 3, p. 400.
2. M. W. Nirenberg and J. H. Matthaei, *Proc. Natl. Acad. Sci. U.S.A.* **47**, 1588 (1961); H. G. Khorana *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 39 (1966).
3. M. B. Hoagland *et al.*, *J. Biol. Chem.* **231**, 241 (1958).
4. R. W. Holley *et al.*, *Science* **147**, 1462 (1965).
5. F. Chapeville *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **48**, 1086 (1962).
6. M. B. Hoagland, P. C. Zamecnik, M. L. Stephenson, *Biochim. Biophys. Acta* **24**, 215 (1957); J. Preiss *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **45**, 319 (1959).
7. D. Söll and P. R. Schimmel, in *The Enzymes*, P. D. Boyer, Ed. (Academic Press, New York, ed. 3, 1974), vol. 10, pp. 489–538.
8. G. Eriani *et al.*, *Nature* **347**, 203 (1990).
9. C. W. Carter, *Annu. Rev. Biochem.* **62**, 715 (1993).
10. S.-H. Kim, in *Transfer RNA: Structure, Properties, and Recognition*, P. Schimmel, D. Söll, J. Abelson, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1979), p. 83.
11. M. Sprinzl, N. Dank, S. Nock, A. Schön, *Nucleic Acids Res.* **19** (suppl.), 2127 (1991).
12. For simplicity, we refer to work accomplished in Schulman's laboratory as if it were hers alone. This is not meant to detract from the contributions of her co-workers. A similar convention is used for other research groups.
13. L. H. Schulman, *Prog. Nucleic Acid Res. Mol. Biol.* **41**, 23 (1991); L. Pallanck and L. H. Schulman, in *Transfer RNA in Protein Synthesis*, D. L. Hatfield, B. J. Lee, R. Pirtle, Eds. (CRC Press, Boca Raton, FL, 1992), pp. 279–318.
14. R. Giegé, J. D. Puglisi, C. Florentz, *Prog. Nucleic Acid Res. Mol. Biol.* **45**, 129 (1993).
15. R. W. Chambers, *ibid.* **11**, 489 (1971).
16. D. M. Crothers, T. Seno, D. G. Söll, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3063 (1972).
17. V. A. Engelhardt and L. L. Kisselev, in *Current Aspects of Biochemical Energetics*, N. O. Kaplan and E. P. Kennedy, Eds. (Academic Press, New York, 1966), pp. 213–225.
18. Y. Shimura and H. Ozeki, *Adv. Biophys.* **4**, 191 (1973); L. L. Kisselev and O. O. Favorova, *Adv. Enzymol.* **40**, 141 (1974).
19. P. R. Schimmel, *Acc. Chem. Res.* **10**, 411 (1977).
20. L. H. Schulman and R. W. Chambers, *Proc. Natl. Acad. Sci. U.S.A.* **61**, 308 (1968).
21. L. H. Schulman, *ibid.* **69**, 3594 (1972).
22. N. Imura, G. B. Weiss, R. W. Chambers, *Nature* **222**, 1147 (1969); N. E. Prather, E. J. Murgola, B. H. Mims, *J. Mol. Biol.* **172**, 177 (1984).
23. W. H. McClain and K. Foss, *Science* **240**, 793 (1988); Y.-M. Hou and P. Schimmel, *Nature* **333**, 140 (1988).

24. S. A. Martinis and P. Schimmel, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 65 (1992).
25. T. Meinnel *et al.*, *J. Mol. Biol.* **229**, 26 (1993).
26. L. H. Schulman and H. Pelka, *Biochemistry* **15**, 5769 (1976).
27. ———, *ibid.* **16**, 4256 (1977).
28. Aminoacyl-tRNA synthetases for each amino acid are abbreviated with the standard three-letter amino acid abbreviation (for example, MetRS, methionyl-tRNA synthetase; AlaRS, alanyl-tRNA synthetase). The anticodon of tRNA consists of the three single-stranded nucleotides at positions 34, 35, and 36 that pair with complementary mRNA codons during translation. Isoacceptors are tRNAs that are aminoacylated with the same amino acid.
29. L. Stern and L. H. Schulman, *J. Biol. Chem.* **252**, 6403 (1977).
30. J. P. Goddard, *Prog. Biophys. Mol. Biol.* **32**, 233 (1977).
31. R. B. Loftfield, *Prog. Nucleic Acid Res. Mol. Biol.* **12**, 87 (1972).
32. N. Imura, H. Schwam, R. W. Chambers, *Proc. Natl. Acad. Sci. U.S.A.* **62**, 1203 (1969).
33. G. Sundharadas *et al.*, *ibid.* **61**, 693 (1968).
34. H. Ozeki *et al.*, in *Transfer RNA: Biological Aspects*, D. Söll, J. N. Abelson, P. R. Schimmel, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1980), pp. 341–362.
35. M. Yaniv, W. R. Folk, P. Berg, L. Soll, *J. Mol. Biol.* **86**, 245 (1974).
36. M. Yarus, R. Knowlton, L. Soll, in *Nucleic Acid–Protein Recognition*, H. J. Vogel, Ed. (Academic Press, New York, 1977), pp. 391–408.
37. L. L. Kisselev, *Prog. Nucleic Acid Res. Mol. Biol.* **32**, 237 (1985).
38. S. H. Kim *et al.*, *Science* **185**, 435 (1974); J. D. Robertus *et al.*, *Nature* **250**, 546 (1974).
39. J. E. Ladner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4414 (1975).
40. Y. Shimura *et al.*, *FEBS Lett.* **22**, 144 (1972); M. L. Hooper, R. L. Russell, J. D. Smith, *ibid.*, p. 149.
41. J. N. Abelson *et al.*, *J. Mol. Biol.* **47**, 15 (1970).
42. J.-P. Ebel *et al.*, in *Transfer RNA: Structure, Properties, and Recognition*, P. R. Schimmel, D. Söll, J. N. Abelson, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1979), pp. 325–343.
43. A. K. Sarkar and L. H. Schulman, *Methods Enzymol.* **59**, 156 (1979).
44. O. Leon and L. H. Schulman, *Biochemistry* **26**, 5416 (1987).
45. G. Ghosh, H. Pelka, L. H. Schulman, *ibid.* **29**, 2220 (1990); H. Y. Kim, H. Pelka, S. Brunie, L. H. Schulman, *ibid.* **32**, 10506 (1993).
46. G. Ghosh *et al.*, *ibid.* **30**, 11767 (1991).
47. P. Mellot *et al.*, *J. Mol. Biol.* **208**, 429 (1989); T. Meinnel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 291 (1991); C. Zelwer, J. L. Risler, S. Brunie, *J. Mol. Biol.* **155**, 63 (1982); J. J. Perona *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 2903 (1991).
48. R. I. Gumport and O. C. Uhlenbeck, in *Gene Amplification and Analysis*, J. G. Chirikjian and T. S. Papas, Eds. (Elsevier, New York, 1981), vol. 2, pp. 314–358.
49. A. G. Bruce and O. C. Uhlenbeck, *Biochemistry* **21**, 855 (1982); L. H. Schulman, H. Pelka, M. Susani, *Nucleic Acids Res.* **11**, 1439 (1983).
50. A. G. Bruce and O. C. Uhlenbeck, *Biochemistry* **21**, 3921 (1982); L. A. Bare and O. C. Uhlenbeck, *ibid.* **25**, 5825 (1986).
51. L. H. Schulman and H. Pelka, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6755 (1983).
52. E. Ohtsuka *et al.*, *ibid.* **78**, 5493 (1981).
53. T. W. Dreher, J. J. Bujarski, T. C. Hall, *Nature* **311**, 171 (1984); T. Samuelsson, T. Borén, T.-I. Johansen, F. Lustig, *J. Biol. Chem.* **263**, 13692 (1988).
54. J. R. Sampson and O. C. Uhlenbeck, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1033 (1988).
55. K. B. Hall, J. R. Sampson, O. C. Uhlenbeck, A. G. Redfield, *Biochemistry* **28**, 5794 (1989); L. S. Behlen, J. R. Sampson, A. B. DiRenzo, O. C. Uhlenbeck, *ibid.* **29**, 2515 (1990).
56. J. R. Sampson, A. B. DiRenzo, L. S. Behlen, O. C. Uhlenbeck, *ibid.*, p. 2523, R. Giegé *et al.*, *Biochimie* **72**, 453 (1990); V. Perret, C. Florentz, J. D. Puglisi, R. Giegé, *J. Mol. Biol.* **226**, 323 (1992).
57. Y.-M. Hou, E. Westhof, R. Giegé, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6776 (1993).
58. C. Francklyn and P. Schimmel, *Nature* **337**, 478 (1989).
59. ———, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8655 (1990).
60. C. Francklyn, J.-P. Shi, P. Schimmel, *Science* **255**, 1121 (1992).
61. M. Frugier, C. Florentz, R. Giegé, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 3990 (1992).
62. J. Rudinger, C. Florentz, T. Dreher, R. Giegé, *Nucleic Acids Res.* **20**, 1865 (1992).
63. J. R. Sampson and M. E. Saks, *ibid.* **21**, 4467 (1993).
64. L. H. Schulman and H. Pelka, *Science* **242**, 765 (1988).
65. ———, *ibid.* **246**, 1595 (1989).
66. ———, *Nucleic Acids Res.* **18**, 285 (1990).
67. J. Normanly *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6548 (1986).
68. J. Normanly, R. C. Ogden, S. J. Horvath, J. Abelson, *Nature* **321**, 213 (1986).
69. L. H. Schulman and J. Abelson, *Science* **240**, 1591 (1988).
70. W. H. McClain and K. Foss, *J. Mol. Biol.* **202**, 697 (1988).
71. ———, R. A. Jenkins, J. Schneider, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 9260 (1990).
72. ———, *ibid.* **88**, 6147 (1991).
73. ———, *ibid.*, p. 9272.
74. J. Normanly, T. Ollick, J. Abelson, *ibid.* **89**, 5680 (1992).
75. L. G. Kleina *et al.*, *J. Mol. Biol.* **213**, 705 (1990).
76. J. Normanly *et al.*, *ibid.*, p. 719.
77. L. H. Schulman and H. Pelka, *Biochemistry* **24**, 7309 (1985).
78. G. Komatsoulis and J. Abelson, *ibid.* **32**, 7435 (1993).
79. E. T. Peterson and O. C. Uhlenbeck, *ibid.* **31**, 10380 (1992).
80. M. Jahn, M. J. Rogers, D. Söll, *Nature* **352**, 258 (1991).
81. Y.-M. Hou and P. Schimmel, *Biochemistry* **28**, 4942 (1989).
82. M. Yarus, *Nature New Biol.* **239**, 106 (1972).
83. R. Swanson *et al.*, *Science* **242**, 1548 (1988); M. J. Rogers and D. Söll, *Prog. Nucleic Acid Res. Mol. Biol.* **39**, 185 (1990).
84. B. L. Seong and U. L. RajBhandary, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 334 (1987); *ibid.*, p. 8859.
85. R. Chattapadhyay, H. Pelka, L. H. Schulman, *Biochemistry* **29**, 4263 (1990).
86. L. Pallanck and L. H. Schulman, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 3872 (1991).
87. S. Li, H. Pelka, L. H. Schulman, *J. Biol. Chem.* **268**, 18335 (1993).
88. U. Varshney, C.-P. Lee, B. Seong, U. L. RajBhandary, *ibid.* **266**, 18018 (1991).
89. Y.-S. Ho and Y. W. Kan, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2185 (1987); U. Varshney, C.-P. Lee, U. L. RajBhandary, *J. Biol. Chem.* **266**, 24712 (1991).
90. M. A. Rould, J. J. Perona, D. Söll, T. A. Steitz, *Science* **246**, 1135 (1989).
91. M. A. Rould, J. J. Perona, T. A. Steitz, *Nature* **352**, 213 (1991).
92. M. Ruff *et al.*, *Science* **252**, 1682 (1991); J. Cavarelli *et al.*, *Nature* **362**, 181 (1993).
92a. V. Biou, A. Yaremchuk, M. Tukalo, S. Cusack, *Science*, in press.
93. A. Rich and P. R. Schimmel, *Nucleic Acids Res.* **4**, 1649 (1977).
94. D. D. Buechter and P. Schimmel, *Biochemistry* **32**, 5267 (1993).
95. P. Schimmel, R. Giegé, D. Moras, S. Yokoyama, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8763 (1993).
96. W. H. McClain and H. B. Nicholas, *J. Mol. Biol.* **194**, 635 (1987).
97. Y. Komine, T. Adachi, H. Inokuchi, H. Ozeki, *ibid.* **212**, 579 (1990).
98. T. Muramatsu *et al.*, *Nature* **336**, 179 (1988).
99. L. A. Sylvers *et al.*, *Biochemistry* **32**, 3836 (1993).
100. J. P. Ebel *et al.*, *Biochimie* **55**, 547 (1973); P. R. Schimmel and D. Söll, *Annu. Rev. Biochem.* **48**, 601 (1979).
101. J. Pütz, J. D. Puglisi, C. Florentz, R. Giegé, *Science* **252**, 1696 (1991).
102. K. Musier-Forsyth *et al.*, *ibid.* **253**, 784 (1991).
103. Y. Hayase *et al.*, *EMBO J.* **11**, 4159 (1992).
104. C. Tuerk and L. Gold, *Science* **249**, 505 (1990).
105. E. T. Peterson, J. Blank, M. Sprinzl, O. C. Uhlenbeck, *EMBO J.* **12**, 2959 (1993).
106. Y.-M. Hou and P. Schimmel, *Biochemistry* **28**, 6800 (1989).
107. J. R. Sampson, A. B. DiRenzo, L. S. Behlen, O. C. Uhlenbeck, *Science* **243**, 1363 (1989); I. A. Nazarenko *et al.*, *Nucleic Acids Res.* **20**, 475 (1992).
108. H. Xue, W. Shen, R. Giegé, J. T.-F. Wong, *J. Biol. Chem.* **268**, 9316 (1993).
109. M. E. Saks, J. R. Sampson, J. N. Abelson, unpublished data.
110. K. Tamura *et al.*, *Nucleic Acids Res.* **20**, 2335 (1992).
111. M. Shimizu *et al.*, *J. Mol. Evol.* **35**, 436 (1992).
112. N. Nameki *et al.*, *Biochem. Biophys. Res. Commun.* **189**, 856 (1992).
113. L. Pallanck, S. Li, L. H. Schulman, *J. Biol. Chem.* **267**, 7221 (1992).
114. L. Pallanck and L. H. Schulman, unpublished data.
115. C. Squires and J. Carbon, *Nature New Biol.* **233**, 274 (1971).
116. L. Pallanck, S. Li, L. H. Schulman, *FASEB J.* **6**, A360 (1992).
117. H. Asahara *et al.*, *J. Mol. Biol.* **231**, 219 (1993).
118. H. Himeno *et al.*, *Nucleic Acids Res.* **18**, 6815 (1990).
119. T. Hasegawa *et al.*, *Biochem. Biophys. Res. Commun.* **184**, 478 (1992).
120. H. Himeno *et al.*, *Nucleic Acids Res.* **19**, 6379 (1991); M. J. Rogers, T. Adachi, H. Inokuchi, D. Söll, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 3463 (1992).
121. M. Pak, L. Pallanck, L. H. Schulman, *Biochemistry* **31**, 3303 (1992).
122. L. Pallanck, M. Pak, L. H. Schulman, unpublished data.
123. K. Tamura *et al.*, *Biochem. Biophys. Res. Commun.* **177**, 619 (1991).
124. J.-P. Shi, C. Francklyn, K. Hill, P. Schimmel, *Biochemistry* **29**, 3621 (1990); K. Tamura *et al.*, *J. Mol. Recogn.* **4**, 129 (1991).
125. W. H. McClain and K. Foss, *Science* **241**, 1804 (1988).
126. M. J. Rogers and D. Söll, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6627 (1988).
127. T. Hasegawa, H. Himeno, H. Ishikura, M. Shimizu, *Biochem. Biophys. Res. Commun.* **163**, 1534 (1989).
128. H. Himeno *et al.*, *Nucleic Acids Res.* **17**, 7855 (1989).

129. We thank H. Pelka, L. Pallanck, S. Li, and M. Pak of LaDonne H. Schulman's laboratory for their careful reading of earlier drafts of this article and for providing unpublished results; along with S. Brunie, R. Giegé, L. L. Kisselev, W. H. McClain, U. L. RajBhandary, P. Schimmel, D. Söll, O. C. Uhlenbeck, and M. Yarus, who were contemporaries of L. H. Schulman, for their invaluable insights about the development of the tRNA recognition field; and S. Cusack, F. Gould, J. Hey, H. Himeno, E. T. Peterson, M. Shimizu, and T. A. Steitz for their comments. Supported by NIH grant GM 48560.

17 March 1993; accepted 15 October 1993