Perspective

Recent Excitement in Understanding Transfer RNA Identity

LaDonne H. Schulman and John Abelson

There was a recent flurry of articles in the press about the "second genetic code." Many scientists wondered what this could be. It is an unfortunate term recently coined (*1*) to describe the sites on each transfer RNA (tRNA) that determine which amino acid will be coupled to it by an aminoacyl tRNA synthetase (AAS). The correct attachment of amino acids to specific tRNA's is crucial to the accurate translation of genetic information from nucleic acid to protein. The "first" genetic code deciphers the rules which govern insertion of specific amino acids in response to the sequence of the messenger RNA. This occurs by alignment of aminoacyl-tRNA's along the mRNA template by base pairing between the anticodon of the tRNA and codons in the template. Because tRNA molecules must interact interchangeably with the protein synthesis apparatus, they all have very similar secondary and tertiary structures, but within this framework variation must exist so that each tRNA is recognizable to its cognate AAS. The term "second genetic code" implies that a common set of rules governs tRNA recognition by AAS's; however, this does not appear to be the case. A better term, "tRNA identity" is in common use, which describes the features of a tRNA molecule which make that tRNA recognizable to one AAS and prevent its recognition by all other AAS's.

Although the search for AAS recognition sites has been going on for over 20 years, until recently the basis of tRNA identity largely remained a mystery (*2*). In the past 3 years, new technology has resulted in rapid progress on this problem. Recent advances by six laboratories (*3*) were presented at the American Society of Microbiology meeting in Miami on 9 May. We briefly summarize here the exciting results reported at that meeting.

The approach that has proved most fruitful in the establishment of tRNA identity is one in which an attempt is made to alter, with the fewest possible changes, the specificity of the tRNA. Those changes are by definition elements of the identity of the tRNA. This experiment was attempted some 16 years ago by two groups, J. D. Smith and S. Brenner at Cambridge and H. Ozeki and Y. Shimura at Kyoto. Genetic selections were devised which forced identity changes in an amber suppressor allele of an *Escherichia coli* $tRNA^{Tyr}$ gene (*4, 5*). Transformations to glutamine specificity were observed. Further changes could not be achieved, however, probably because multiple base changes were required. With the advent of automated DNA synthesizers, it became possible to construct any tRNA gene, and it is this technology that has made possible the current progress.

LaDonne H. Schulman is in the Department of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York 10461. John Abelson is in the Division of Biology, California Institute of Technology, Pasadena, CA 91125.

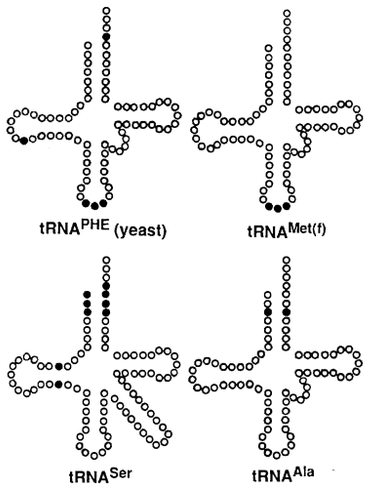
Two different ways to assay changes in specificity have been devised. The first, an in vitro method, assays the substrate activity of the altered tRNA. Sampson and Uhlenbeck (*6*) have synthesized tRNA genes that are linked directly to a synthetic bacteriophage T7 late promoter. Transcription of this DNA in vitro by T7 RNA polymerase yields a transcript that is identical in sequence to tRNA but that lacks the nucleoside modifications of the mature tRNA. These unmodified tRNA's are, fortunately, near normal substrates for the cognate AAS. Specificity is monitored by determination of the kinetic parameters of acylation of the tRNA by cognate and noncognate AAS. An advantage of this approach is that milligram quantities of the tRNA can be obtained for physical studies. However, in this method the effects of competition between AAS's for a given substrate are not determined.

In the second method, Normanly *et al.* (*7*) have returned to alteration of amber suppressor specificity, but instead of genetic selection for alterations in the tRNA, the suppressor genes are synthesized de novo as in the pioneering work of Khorana (*8*). Specificity is assayed by suppressing an amber mutation near the NH_2-terminus of dihydrofolate reductase. The protein is purified and sequenced, thus establishing directly the specificity of the suppressor. This approach is rapid and the in vivo activity of the tRNA is assayed; however, it also has disadvantages. The anticodon must be CUA recognizing the amber codon UAG. Thus the contribution of the anticodon in AAS recognition cannot be assessed by this method. In addition, base changes in a tRNA may affect suppression efficiency (an important parameter in these experiments) by altering steps other than AAS recognition. In the end it will be desirable to use both methods.

The most logical sequence to define the identity of a tRNA is the anticodon (*9*). The fact that many tRNA's could be altered to amber suppressors without loss of identity led to the proposal (stated as fact in some textbooks) that the anticodon is not a recognition element for AAS. Schulman's laboratory has known for 15 years that the anticodon of $tRNA^{Met}$ is a crucial element in recognition by the Met AAS (*10*). Using in vitro methods, Schulman and Pelka have now shown that alteration of the anticodons of either $tRNA^{Trp}$ or $tRNA^{Val}$ to the methionine anticodon CAU (recognizing AUG) transforms each of those tRNA's into a $tRNA^{Met}$. Furthermore, changing the $tRNA^{Met}$ anticodon to that of $tRNA^{Val}$ causes a complete loss of methionine acceptor activity and results in a near normal $tRNA^{Val}$. Clearly, the identity elements of $tRNA^{Met}$ and $tRNA^{Val}$ are principally, if not entirely, in the anticodon.

The anticodon as an identity element may actually be quite common. Miller and Abelson have been constructing a complete set of amber suppressor tRNA genes in *E. coli* (*11*). While many of these suppressors (11 of 20) function well and insert the correct amino acid, others either mischarge completely or insert a mixture of amino acids. The mischarging is due entirely to either Gln AAS or Lys AAS. The first example of this phenomenon was discovered 15 years ago by Berg and co-workers (*12*) who found that a $tRNA^{Trp}$ amber suppressor inserts mostly glutamine. Yarus (*13*) has proved that the anticodon is a major recognition element for both Gln AAS and Trp AAS. The new suppressor tRNA's constructed for Asp, Ile, and Val insert only lysine. This result implies that the anticodon contains elements of identity of those tRNA's and also that the amber anticodon is recognized by Lys AAS.

In some cases the anticodon comprises a portion, but not all of the identity of a tRNA. Sampson and Uhlenbeck have made an extensive in vitro study of the substrate recognition properties of yeast $tRNA^{Phe}$. This is an ideal target for study because a high resolution x-ray crystal structure of $tRNA^{Phe}$ is available. Five bases have been shown to be identity elements of yeast $tRNA^{Phe}$. They are the three bases of the anticodon, G20 in the D loop and A73, the



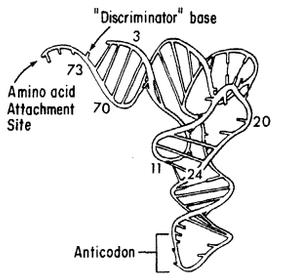


Fig. 1 (**left**). Major identity elements in four tRNA's. Each base in the tRNA is represented by a circle. Filled circles indicate positions in the cloverleaf that have been shown to be identity elements for the cognate AAS. In each case additional identity elements may yet be discovered. **Fig. 2** (**right**). Location of elements in the tertiary structure of tRNA. A representation of the x-ray crystal structure of yeast $tRNA^{Phe}$ is shown (*21*). The positions of the major recognition elements shown in Fig. 1 are indicated.

fourth base from the 3′ end of the molecule. The latter base had been termed the "discriminator" by Crothers *et al.* (*14*), and it does appear that this base may often play a role in tRNA identity. These assignments were proved by identity changes from *E. coli* $tRNA^{Phe}$ to yeast $tRNA^{Phe}$ and from yeast $tRNA^{Tyr}$, $tRNA^{Met}$, and $tRNA^{Arg}$ to yeast $tRNA^{Phe}$. Alteration of any single base of the five leads to a drop in K_{cat}/K_m for Phe AAS (about tenfold), and the effects at each site are independent. Early mischarging studies of a variety of noncognate tRNA's by yeast Phe AAS were nicely rationalized by Sampson and Uhlenbeck on the basis of these data.

Abelson's laboratory has used the suppression assay to study $tRNA^{Ser}$ whose identity elements do not apparently include the anticodon. The six serine codons—UCU, UCC, UCA, UCG, AGU, and AGC—dictate the use of at least four different tRNA's with different anticodons. Normanly and Abelson have now reported the complete conversion of $tRNA^{Leu}$ to $tRNA^{Ser}$. The minimum set of recognition elements are G73, the three base pairs at the end of the acceptor stem and C11-G24, a base pair in the D stem. Further conversions should lead to a complete identity profile for $tRNA^{Ser}$.

A particularly simple identity has been discovered in the case of $tRNA^{Ala}$. Several years ago Murgola and co-workers had discovered, through genetic experiments, that changing the 3–70 base pair in the acceptor stem of $tRNA^{Lys}$ from G-C to G-U allowed this tRNA to be misacylated with either alanine or glycine (*15*). Hou and Schimmel reported that this G-U pair is a principal element of alanine identity (*16*). A $tRNA^{Cys}$ amber suppressor was converted entirely to alanine specificity by insertion of the G3-U70 base pair. Inserting the G3-U70 pair into a $tRNA^{Phe}$ amber suppressor resulted in recognition by Ala AAS, but did not block recognition by Phe AAS (*16*). A similar identity change from $tRNA^{Phe}$ to $tRNA^{Ala}$ had been described by McClain and Foss (*17*).

McClain's laboratory has been determining the basis of identity of several *E. coli* tRNA's by the suppression assay, making site specific nucleotide changes based on computer analysis of tRNA sequences (*18*). Important identity elements have been assigned for $tRNA^{Phe}$, $tRNA^{Gly}$, and $tRNA^{Arg}$. It is also apparent from McClain's work that total tRNA identity may involve "fine tuning" by a number of other nucleotides in addition to major identity elements.

Excellent progress on determination of the identity elements for $tRNA^{Gln}$ was also reported by Rogers and Söll. This is an especially attractive system for study since cocrystals of $tRNA^{Gln}$ and Gln AAS have recently been obtained by Söll and Steitz (*19*). In addition, interesting mischarging mutants of Gln AAS have been isolated (*20*). One of these shows normal kinetic parameters for charging $tRNA^{Gln}$, but mischarges one or more noncognate tRNA's, illustrating that the interactions that lead to mischarging are not necessarily identical to those involved in cognate tRNA selection.

These examples serve to illustrate the progress that has been made in this field. The goal is a complete catalog of the identity elements for all 20 tRNA's. Clearly the methods are now at hand to complete the catalog and we expect that to happen in the next several years.

Figure 1 summarizes major recognition elements in four tRNA's and Fig. 2 indicates the locations of these elements in the three-dimensional structure of tRNA (*21*). Several generalizations concerning tRNA identity have already emerged:

1) A small number of nucleotides comprise the major identity elements in a tRNA molecule.

2) The anticodon often contains one or more identity elements. Therefore AAS which must necessarily interact with the 3′ end of the tRNA must also interact with positions that are at the opposite end of the molecule. Available data suggest that recognition elements are present in the anticodons of more than half of the tRNA's.

3) In addition to the positive elements in a tRNA that dictate interaction with the cognate synthetase, negative elements contribute to tRNA identity by blocking the recognition by other AAS.

4) The identity elements for a tRNA are not necessarily conserved in evolution. Thus, U20 in *E. coli* $tRNA^{Phe}$ must be altered to G in order to allow good recognition by the yeast Phe AAS. On the other hand the G3-U70 base pair is present in all cytoplasmic $tRNA^{Ala}$ that have been sequenced, from archaebacteria to eukaryotes.

The recognition elements in a tRNA appear to reflect events that occurred early in evolution, perhaps at the time the genetic code evolved. Whether an understanding of tRNA identity will provide a rationale for the relation between an amino acid and its codons remains to be seen.

REFERENCES AND NOTES

1. C. de Duve, *Nature* **333**, 117 (1988).
2. P. Schimmel, *Annu. Rev. Biochem.* **56**, 125 (1987).
3. John Abelson, California Institute of Technology; William H. McClain, University of Wisconsin; Paul Schimmel, Massachusetts Institute of Technology; LaDonne H. Schulman, Albert Einstein College of Medicine; Dieter Söll, Yale University; Olke Uhlenbeck, University of Colorado.
4. Y. Shimura, H. Aono, H. Ozeki, A. Sarabhai, H. Lamfrom, J. Abelson, *FEBS Lett.* **22**, 144 (1972).
5. M. L. Hooper, R. L. Russell, J. D. Smith, *ibid.*, p. 149.
6. J. R. Sampson and O. C. Uhlenbeck, *Proc. Natl. Acad. U.S.A.* **85**, 1033 (1988).
7. J. Normanly, R. C. Ogden, S. J. Horvath, J. Abelson, *Nature* **321**, 213 (1986).
8. H. G. Khorana, *Science* **203**, 614 (1979).
9. L. Kisselev, *Prog. Nucleic Acid Res. Mol. Biol.* **32**, 237 (1985).
10. L. H. Schulman and H. Pelka, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6755 (1983), and references cited therein.
11. J. Normanly, J.-M. Masson, L. G. Kleina, J. Abelson, J. H. Miller, *ibid.* **83**, 6548 (1986); J.-M. Masson and J. F. Miller, *Gene* **47**, 179 (1986).
12. M. Yaniv, W. R. Folk, P. Berg, L. Soll, *J. Mol. Biol.* **86**, 245 (1974).
13. M. Yarus, R. G. Knowlton, L. Soll, in *Nucleic Acid Protein Recognition*, H. Vogel, Ed. (Academic Press, New York, 1977), p. 391.
14. D. M. Crothers, T. Seno, D. G. Söll, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3063 (1972).
15. N. E. Prather, E. J. Murgola, B. H. Mims, *J. Mol. Biol.* **172**, 177 (1984).
16. Y.-M. Hou and P. Schimmel, *Nature* **333**, 140 (1988).
17. W. H. McClain and K. Foss, *Science* **240**, 793 (1988).
18. W. H. McClain and H. B. Nicholas, Jr., *J. Mol. Biol.* **194**, 635 (1987).
19. J. Perona, R. Swanson, T. Steitz, D. Söll, *ibid.*, in press.
20. H. Inokuchi, P. Hoben, F. Yamao, H. Ozeki, D. Söll, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5076 (1984).
21. S. H. Kim *et al.*, *ibid.* **71**, 4970 (1974); A. Klug, J. Robertus, J. Ladner, R. Brown, J. Finch, *ibid.*, p. 3711.