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Eight base changes are sufficient to convert a leucine-inserting tRNA into a serine-inserting tRNA

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ABSTRACT **Each aminoacyl-tRNA synthetase must functionally distinguish its cognate tRNAs from all others. We have determined the minimum number of changes required to transform a leucine amber suppressor tRNA to serine identity. Eight changes are required. These are located in the acceptor stem and in the D stem.**

Correct recognition of tRNAs by aminoacyl-tRNA synthetases (AASs) is a prerequisite for accurate protein synthesis in the cell. Each of the 20 AASs must recognize and aminoacylate its own set of isoaccepting tRNAs and reject all others. Elements that facilitate the aminoacylation of a tRNA by its cognate AAS are termed recognition elements and must play a major role in maintaining the amino acid specificity (identity) of all tRNAs *in vivo* (1, 2). Recognition elements include nucleotides that are specifically contacted by an AAS (2, 3). They can also be nucleotides with indirect effects. For example, some nucleotides dictate a particular tRNA conformation that is required for proper orientation of nucleotides with which the AAS specifically interacts (3–5). Finally, recognition elements can be distinctive structural features that are directly recognized by an AAS (6, 7). Most nonmitochondrial tRNAs have similar structural features, including an extra loop that contains 4 or 5 nucleotides (type I). In *Escherichia coli*, however, serine, tyrosine, and leucine tRNAs are distinguished from all other isoaccepting groups by the presence of a large extra stem–loop (type II). This structural feature could be an important determinant of their recognition with respect to both cognate and noncognate AASs.

One way to determine the requirements for tRNA identity is to change the tRNA so that it is aminoacylated with a different amino acid. This approach was taken 20 years ago when attempts were made to genetically select variants of the *E. coli* tyrosine amber suppressor gene, which would insert a different amino acid in response to the amber anticodon (8–10). Several such mutants were isolated. They occurred in the first and second base pairs of the acceptor stem and in the fourth nucleotide from the 3′ end of the tRNA. In every case, the amino acid inserted was glutamine (8, 9, 11–14). Attempts to find other changes in identity failed, probably because of the requirement for multiple changes in order to effect other identity changes. When it became possible to synthesize tRNA genes, we returned to this approach and showed that we could change the identity of a $tRNA^{Leu}$ amber suppressor to $tRNA^{Ser}$ by making 12 nucleotide changes (15). Subsequently, this approach was taken to show that the G3·U70 base pair in the acceptor stem of $tRNA^{Ala}$ is required for its *in vivo* identity (16, 17). Similar studies have provided important information about the requirements for the *in vivo* identity of $tRNA^{Phe}$ (18), $tRNA^{Arg}$ (19), $tRNA^{Lys}$ (20), and $tRNA^{Gln}$ (21).

An intrinsic limitation of the *in vivo* approach is the requirement for the CUA anticodon. Introduction of this anticodon into a tRNA can itself lead to a change in identity. In retrospect, this should have been clear early on when it was discovered that the amber suppressor allele of a $tRNA^{Trp}$ gene inserts glutamine (22). Recently, we have synthesized amber suppressor tRNA genes for all 20 isoaccepting groups in *E. coli* (23, 24). Five of these tRNAs (Ile-1, Gly-2, Met_f, Glu, and Trp) insert glutamine and six others (Ile-2, Arg, Met_m, Asp, Thr, and Val) insert lysine (25). This result confirms Kisselev and Frolova's (26) and Schulman and Goddard's (27) long-held conviction that AASs recognize anticodon nucleotides, and there is now much additional evidence to support that notion (2). Recently, Schulman and coworkers (28, 29) have inserted different anticodons into the *E. coli* $tRNA^{Met}$ initiator and have shown that it will initiate polypeptide chains with the amino acid corresponding to the identity of the new anticodon. Thus, the *in vivo* approach has been a powerful tool for detecting recognition of elements in the anticodon.

A successful identity swap necessitates supplying correct positive elements for the desired AAS. In addition, it is necessary to discourage aminoacylation by the original AAS by removing its positive recognition elements from the tRNA. We found that 12 nucleotide changes were required to change the $tRNA^{Leu}$ amber suppressor to $tRNA^{Ser}$ identity (15). However, it was not established that this was the minimum set of changes required to change the $tRNA^{Leu}$ amber suppressor to $tRNA^{Ser}$ identity. Moreover, because $tRNA^{Leu}$ contains a long extra stem–loop, as does $tRNA^{Ser}$, it was not possible to directly test the effect of this structural feature on recognition.

In this paper, we describe our efforts to determine the minimum number of base changes required to effect the complete change of identity of $tRNA^{Leu}$ to $tRNA^{Ser}$ and to determine the effect of structural features on the identity of $tRNA^{Ser}$, $tRNA^{Leu}$, and $tRNA^{Tyr}$. As in our original work, synthetic tRNA amber suppressor genes, expressed in *E. coli*, were assayed for their *in vivo* specificity by determining the amino acid sequence of dihydrofolate reductase (DHFR) synthesized from an engineered gene containing an amber mutation at codon 10. A second, more diagnostic assay for serine insertion utilized an amber mutation at the active site serine in the β-lactamase gene. Since this serine is essential for β-lactamase activity, growth of *E. coli* containing this gene on ampicillin indicates the presence of an active serine amber suppressor.

MATERIALS AND METHODS

Strains and Plasmids. *E. coli* XAC-1 is $F'lacI^{q}_{373}lacZ_{u118am}$-$proB^{+}/\Delta(lacproB)_{x111}$, *nalA, rif, ara,* $argE_{am}$. Plasmid pJN10 is derived from plasmids pBR322 and a derivative of pEMBL8^{+} (30), pEMBL8$^{+}\beta^{-}$T. pEMBL8$^{+}\beta^{-}$T has an amber mutation at the active-site serine (residue 68) of the *amp*

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Abbreviations: AAS, aminoacyl-tRNA synthetase; DHFR, dihydrofolate reductase; LSM6, leucine to serine mutant 6.

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gene and a transcription termination sequence (15) inserted into the *Pst* I/*Hin*dIII sites of the polylinker. The 1769-base-pair (bp) *Pvu* I/*Pvu* II fragment from pEMBL8$^+\beta^-$T containing a portion of the *amp* gene, the replication origin, and the polylinker was ligated to the 2820-bp *Pvu* I/*Pvu* II fragment of pBR322 that carries the *tet* gene. Before pJN10 was constructed, the *Eco*RI site in pBR322 was removed by digesting the plasmid with *Eco*RI, filling in the 5′ termini with Klenow, and ligating. Plasmid pDAYQ encodes the gene conferring resistance to chloramphenicol, the gene for the *lac* repressor, *lacIQ*, as well as the *fol* gene encoding DHFR, with an amber mutation at residue 10 (25). The *fol* gene is under control of the *tac* promoter (31).

Gene Synthesis. The tRNA genes were constructed from synthetic oligonucleotides (made by the California Institute of Technology Microchemical Facility) and were assembled as described (15) and ligated into the unique *Eco*RI/*Pst* I sites located in the polylinker region of pJN10, which are flanked by the *lac* promoter and *rrnC* terminator, 5′ and 3′, respectively.

Determination of Suppressor Efficiency. Suppression efficiencies of the mutant tRNAs were determined by assaying β-galactosidase activity (32) in strain XAC-1, which had been transformed with pJN10 carrying a suppressor tRNA gene.

Purification of DHFR. DHFR was isolated from *E. coli* strain XAC-1 carrying both the mutant tRNA gene borne on plasmid pJN10 and plasmid pDAYQ. Cells were cultured in L broth (10 g of Bactotryptone per liter, 10 g of NaCl per liter, 5 g of yeast extract per liter) supplemented with 5 μg of tetracycline and 30 μg of chloramphenicol per ml to an A_{600} of 0.4–0.8, at which time they were diluted 1:50 into minimal M9 glycerol medium (32), which had been supplemented with 5 μg of tetracycline per ml, 30 μg of chloramphenicol per ml, and 1 mM isopropyl β-D-thiogalactopyranoside. The culture was incubated, shaking at 37°C for 12–18 hr, and the cells were harvested. DHFR was purified from the cells as described (15). N-terminal sequence analysis of DHFR was carried out at either the University of Southern California Microchemical Core Laboratory or the California Institute of Technology Microchemical Facility.

RESULTS

Fig. 1*A* shows the nucleotides that are highly conserved in tRNA^{Ser} (or, as in the case of base pair 3·70, highly correlated with tRNA^{Ser}) and that are not found in tRNA^{Leu5}—our original starting tRNA. Fig. 1*B* shows the original leucine to serine identity conversion, tRNA$^{Leu\rightarrow Ser}_{CUA}$, with the 12 changes indicated. In tRNA$^{Leu\rightarrow Ser}_{CUA}$, we had shifted the position of the conserved nucleotides G18 and G19. The position of these nucleotides relative to the D stem is conserved in serine tRNAs. In tRNA^{Leu}, the position of G18 and G19 is different. We speculated that this structural feature could play a role in recognition by serine AAS and, therefore, altered the leucine D loop to introduce a serine-like configuration (Fig. 1*B*). We examined the contribution of these mutations to serine identity in LSM6 (leucine to serine mutant 6; Fig. 2). The D loop has the original wild-type tRNA^{Leu} sequence configuration, while the mutations in the D stem and acceptor stem are retained. This suppressor (LSM6) inserted serine exclusively at 40% efficiency (Table 1). Therefore, the changes originally made to the D loop of tRNA$^{Leu5}_{CUA}$ were not only unnecessary for serine identity but were also likely responsible for its poor suppression efficiency. The eight changes in LSM6 successfully converted the identity of tRNA^{Leu} to tRNA^{Ser}. The task then was to determine which of these changes were necessary.

In all tRNA^{Ser}, base pair 11·24 is a C·G and in tRNA^{Leu5} it is a U·A. We examined the contribution of this D-stem base pair to serine identity by altering LSM6 to create a new mutant, LSM4 (Fig. 2) with a U11·A24 base pair. This variant, while an efficient suppressor (Table 1), inserted a mixture of amino acids: serine (16%), glutamine (39%), and leucine (38%). Clearly, base pair C11·G24 plays a role in serine identity. We wondered whether a U·G base pair would be acceptable at this position. This variant (LSM11; Fig. 2) suppressed the β-lactamase amber sufficiently to enable growth in the presence of ampicillin; however, the specificity assay revealed that LSM11 inserts leucine (62%), glutamine (33%), and <1% serine (Table 1). This result suggests that C11 plays an important structural or chemical role in serine identity.

We then set out to determine which of the original changes in the acceptor stem were actually necessary, by changing LSM6 one base pair or nucleotide at a time. In LSM10 (Fig. 2), position 73 has been changed back to an A. This tRNA variant inserts exclusively leucine (Table 1). In LSM12, base pair 2·71 was changed to the original leucine base pair (C·G) and in LSM13, position 72 was changed to the original U. Both of these mutants insert little or no serine and predominantly leucine and glutamine, indicating that these positions are important for serine identity. In LSM9, the base pair at

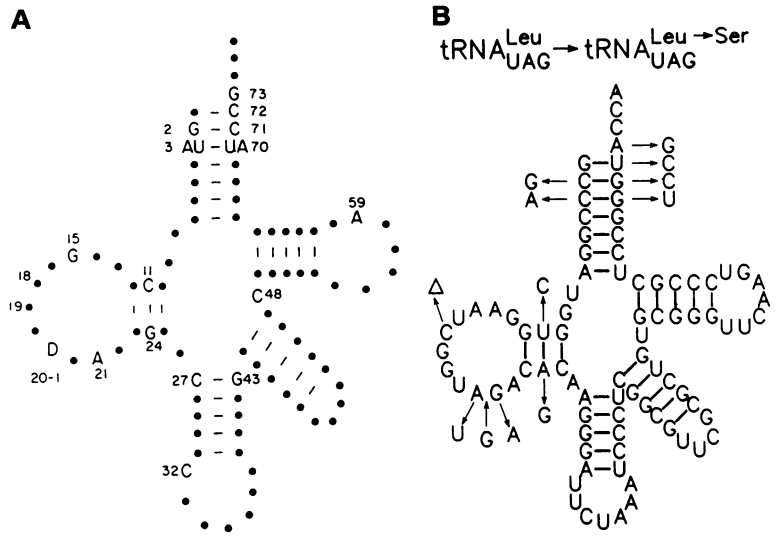

FIG. 1. (*A*) Composite of *E. coli* tRNA^{Ser}. Nucleotides highly conserved in or correlated with tRNA^{Ser} and not found in tRNA$^{Leu5}_{UAG}$ are indicated. Adapted from ref. 15. (*B*) tRNA$^{Leu5}_{UAG}$ with 12 base changes predicted to convert the tRNA to serine identity. Adapted from ref. 15.

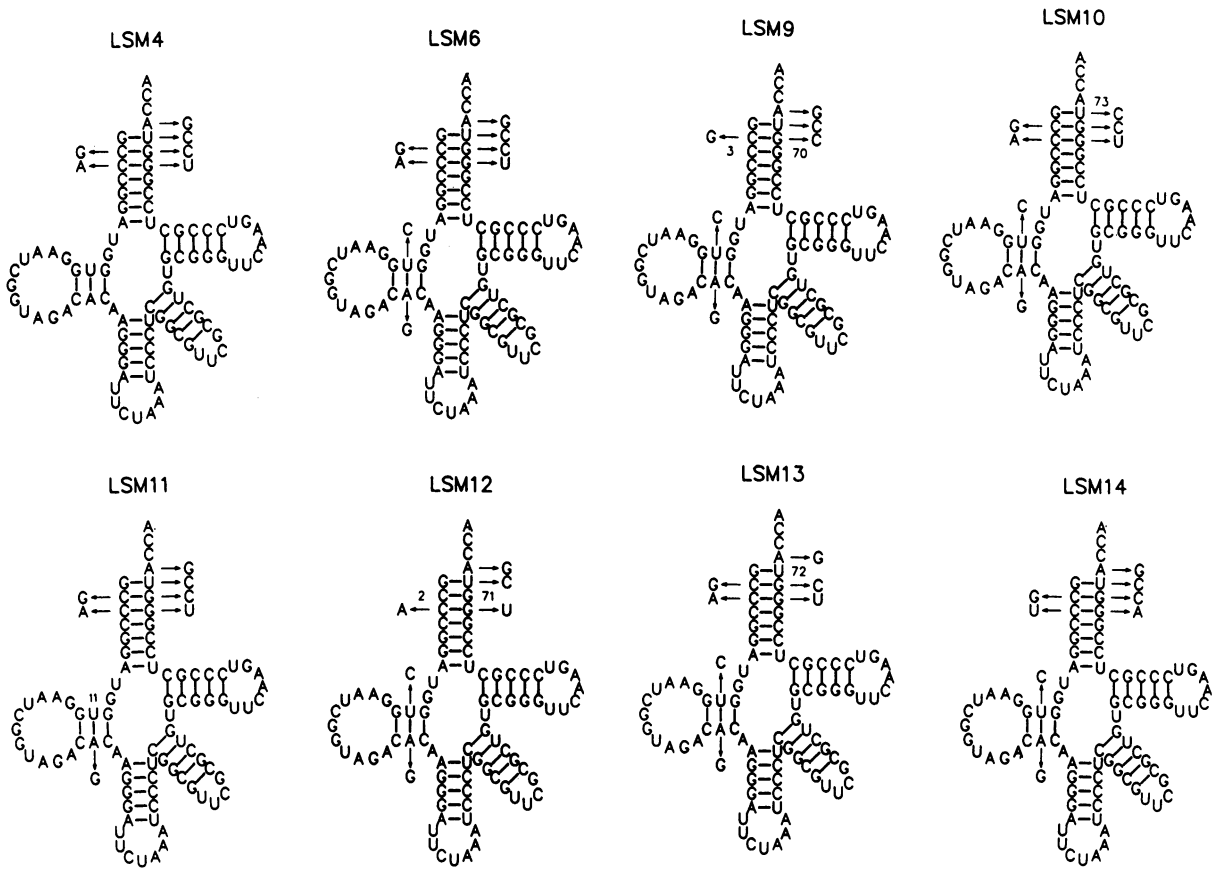

FIG. 2. Sequence of LSM variants.

3·70 has been changed to the C·G found in $tRNA^{Leu5}_{CUA}$. This tRNA inserts predominantly leucine (72%) and some serine (20%) and glutamine (6%), suggesting that this position is also important for serine identity. Because serine isoacceptors have not only A·U at position 3·70 (LSM6) but also U·A, we created LSM14 (Fig. 2). Surprisingly, this tRNA inserts predominantly leucine (65%) along with glutamine (19%) and serine (11%) (Table 1). This unexpected result will be addressed in the *Discussion*. From these reversion studies, it is apparent that all of the original changes made in the acceptor stem of $tRNA^{Leu5}_{CUA}$ were necessary to effect its conversion from leucine to serine identity. The eight changes in LSM6 are therefore sufficient and necessary to alter $tRNA^{Leu5}$ to serine identity *in vivo*.

The eight changes in LSM6 do not necessarily constitute all of the elements recognized by serine AAS. Some recognition elements could already have been present in $tRNA^{Leu5}$. One obvious feature is the large type II extra stem–loop common to both isoaccepting groups. To investigate the role of this structural feature in tRNA identity, we have constructed $tRNA^{Ser}$, $tRNA^{Leu}$, and $tRNA^{Tyr}$ amber suppressor genes in which the large type II extra stem–loop is replaced by a consensus type I extra loop.

A survey of the *E. coli* type I extra loop sequences (33) revealed a consensus sequence of RRGUC (where R is purine). Using methods previously described (15), we synthesized altered amber suppressor genes in which the extra loop sequences of $tRNA^{Tyr}_{GUA}$, $tRNA^{Ser}_{CGA}$, and $tRNA^{Leu}_{CAA}$ were

Table 1. Suppression efficiency of various LSM constructs

$tRNA_{CUA}$	Suppression efficiency, % wild-type β-galactosidase	Growth on ampicillin	Specificity (% amino acid inserted at $DHFR_{am10}$)
Leu	52–59	−	Leu (99)
Ser	34–52	+	Ser (92)
Leu→Ser	0.5–1	+	Ser (90), Leu (10)
LSM6	33–49	+	Ser (92)
LSM4	35–48	+	Leu (38), Gln (39), Ser (16)
LSM11	11–19	+	Leu (62), Gln (33), Ser (<1)
LSM10	20–35	−	Leu (99)
LSM12	5–9	−	Leu (91), Gln (9)
LSM13	12	+	Leu (15), Gln (78), Ser (<1)
LSM9	11–12	+	Leu (72), Gln (6), Ser (20)
LSM14	14–16	+	Leu (65), Gln (19), Ser (11)

Values for percentage amino acid inserted may not total 100%, because background levels of <5% for any amino acids are not included, with the exception of serine. While the values given for serine inserted are <5% in some cases, the ability to confer ampicillin resistance indicates that the mutant must be inserting some low level of serine. Estimates for percentage glutamine inserted include glutamic acid.

Table 2. Suppression efficiency of extra loop deletion mutants

Suppressor	Efficiency,* %	Specificity
Tyr	64–73	Tyr
TyrΔ	4	80% Gln, 20% Tyr
Ser	75	Ser
SerΔ	28–34	95% Gln, 5% Ser
Leu	64–78	Leu
LeuΔ	26–35	Leu

*This experiment was performed independently of the one described in Table 1 and gave somewhat higher efficiencies for the serine and leucine suppressors.

altered to have the 5-base consensus sequence: A^{44}-G^{45}-G^{46}-U^{47}-C^{48}. These tRNAs were designated $tRNA^{Ser}\Delta$, $tRNA^{Tyr}\Delta$, and $tRNA^{Leu}\Delta$. Each tRNA suppressor was assayed for suppression efficiency and for specificity (Table 2). Both $tRNA^{Ser}\Delta$ and $tRNA^{Leu}\Delta$ are reasonably efficient suppressors, although somewhat less efficient than their wild-type controls. $tRNA^{Tyr}\Delta$ is only 4% efficient. Both $tRNA^{Tyr}\Delta$ and $tRNA^{Ser}\Delta$ insert predominantly glutamine, whereas $tRNA^{Leu}\Delta$ inserts exclusively leucine. This suggests that the extra stem–loop may be a more important recognition element for serine and tyrosine AAS than for leucine AAS.

DISCUSSION

The results of this study confirm and extend our earlier conclusion that elements necessary for serine identity *in vivo* reside in the acceptor stem and D stem of the tRNA. It is shown here that the extra stem–loop is an additional element that contributes to serine identity. A wide distribution of recognition elements is known for yeast $tRNA^{Phe}$ (34) and *E. coli* $tRNA^{Gln}$ (3). This situation stands in contrast to the distribution of recognition elements in other tRNAs such as *E. coli* alanine (16, 17), methionine, and valine (35), for which recognition elements seem to be primarily restricted to a single region. Because the *in vivo* identity of $tRNA^{Ser}$ depends on elements in three distinct regions, and within the acceptor stem involves at least six nucleotides, changing the identity of any tRNA to that of serine presents a particularly challenging task. On the other hand, a swap to serine identity does not encounter a difficulty seen in other tRNAs. The anticodon is not expected to be a recognition element in $tRNA^{Ser}$ so that the necessity to have the amber anticodon is not a problem.

The relationship between the *in vivo* identity of a tRNA and its suppression efficiency is not necessarily straightforward. The four changes that were originally made in the D loop of $tRNA^{Leu5}$ were shown here to have no measurable effect on serine identity when they were eliminated in LSM6. Thus, the special context of the conserved nucleotides in the D loop of serine tRNAs is not a feature of $tRNA^{Ser}$ identity *per se*. However, the configuration of these D-loop nucleotides in LSM6 did have a large positive effect on suppression efficiency. Because the original $tRNA^{Leu\rightarrow Ser}$ and LSM6 differed in suppression efficiency but not in identity, it seems that poor suppression efficiency did not reflect a deficiency in the requisite recognition elements. Rather, the low suppression efficiency of the original mutant may reflect problems associated with processing (19), stability, translation efficiency (24, 36), or presentation of recognition elements. Under some circumstances, however, poor suppression efficiency could directly reflect a deficiency in the recognition elements of a variant. A variant containing only a subset of positive elements for any one of the 20 AASs could have a low suppression efficiency simply because of a low efficiency of aminoacylation. LSM12, a low efficiency leucine suppressor, may be an example of such a tRNA.

Of the original 12 changes made to convert $tRNA^{Leu}$ to $tRNA^{Ser}$, only those at base pairs 11·24, 2·71, and 3·70, and at positions 72 and 73 were absolutely required. The majority of these changes involved nucleotides within the acceptor stem. With the exception of base pair 3·70, all nucleotide changes necessary to change $tRNA^{Leu}$ to $tRNA^{Ser}$ were to bases that are absolutely conserved in *E. coli* serine tRNAs. In two variants, changing a nucleotide that conferred serine identity in LSM6 to the wild-type nucleotide for $tRNA^{Leu5}$ reduced serine identity and increased leucine identity. Changing G73 to A (LSM10) gave a tRNA with complete leucine identity. Similarly, changing G2·C71 to C2·G71 (LSM12) gave a tRNA with an identity that was 91% leucine and 9% glutamine. There are two possible, but not mutually exclusive explanations for these types of results. G73 and G2·C71 may be positive elements for serine AAS and the alternatives A73 and C2·G71 may be positive elements for leucine AAS. Thus, the presence of A73 and C2·G71 in LSM10 and LSM12 would effectively discourage aminoacylation by serine AAS and would facilitate aminoacylation by leucine AAS. Although G73 and G2·C71 are clearly required for serine identity *in vivo*, it is difficult from these experiments alone to assign their effects on identity to recognition by leucine and serine AAS. Changing the identity of a $tRNA^{Ser}$ amber suppressor to that of leucine (the reverse identity swap) and measuring the aminoacylation kinetics of various mutants may help define the leucine and serine recognition elements.

We were surprised that two variants, LSM6 (A3·U70) and LSM14 (U3·A70), differed in serine identity. These mutants differed only at base pair 3·70 and each contained a base pair at this position that is found in *E. coli* $tRNA^{Ser}$. It is believed that proteins recognize the minor but not the major groove of internal stretches of helical RNA (37). In the minor groove, the constellation of hydrogen bond acceptors and donors does not differ between A·U and U·A base pairs (38). Consequently, based on current ideas about RNA–protein interactions, it does not seem that the observed *in vivo* identity of LSM6 and LSM14 can be strictly explained based on differential recognition of A·U and U·A at position 3·70. It is possible, however, that both LSM6 and LSM14 lack some element that has not yet been defined and, therefore, are not optimal substrates for serine AAS. In this case, the large difference in their *in vivo* identity could be due to subtle differences in their interaction not only with serine AAS but also with the competing leucine and glutamine AASs. Clearly, *in vitro* kinetic analyses of these mutants will help to resolve this problem.

Several amber suppressor tRNAs are misacylated by glutamine AAS (8, 9, 11–14, 25) as are several of the LSMs. The structure of the $tRNA^{Gln}$–glutamine AAS complex now provides some explanation for that fact (3). The crystal structure reveals specific contacts between glutamine AAS and all 3 bases of the anticodon (39). The amber anticodon CUA has 2 bases in common with the CUG glutamine anticodon. Therefore, for an amber suppressor to retain its original identity, it must be a better substrate for its cognate AAS than it is for glutamine AAS. The $tRNA^{Gln}$–glutamine AAS complex also reveals that fraying of the first base pair in the acceptor stem is an important element for recognition by glutamine AAS. This could explain, in part, the high level of glutamine inserted (78%) by LSM13, which has a G1·U72 pair.

$tRNA^{Ser}\Delta$ and $tRNA^{Tyr}\Delta$ amber suppressors were both misacylated by glutamine AAS, whereas $tRNA^{Leu\Delta}_{CUA}$ retained its identity. This suggests that the extra stem–loop is a more important recognition element for serine and tyrosine AAS than it is for leucine AAS. Because the structure of the $tRNA^{Gln}$–glutamine AAS complex shows that glutamine AAS binds along the inside of the L structure of the tRNA and makes no obvious contacts with the extra loop, it is not likely

that glutamine AAS recognizes either the size or configuration of this structure. Thus, the misacylation of $tRNA^{Tyr}\Delta$ and $tRNA^{Ser}\Delta$ by glutamine AAS is best explained as the consequence of a loss of a positive element for serine and tyrosine AAS.

The extra stem–loop is clearly indicated as a serine recognition element by footprinting studies of the *E. coli* (40) and yeast (41) $tRNA^{Ser}$–serine AAS complex. Moreover, our observed *in vivo* effect of the extra stem–loop on serine identity is consistent with recent *in vitro* studies of $tRNA^{Tyr}$ and $tRNA^{Ser}$, which show that certain alterations of the extra stem–loop can affect the k_{cat}/K_m for aminoacylation by serine AAS (7, 42). It is likely that the positive effect of the extra stem–loop on serine identity is due to its structure rather than its sequence since the extra stem–loop nucleotides are not strictly conserved in *E. coli* serine isoacceptors.

The *in vivo* approach that we have used in this work measures the outcome of competition between all AASs for a particular tRNA. Thus, tRNAs with single identities as well as those with multiple identities are revealed by this method. By examining the amino acid identity of a bank of mutants, it is possible to delineate the sites in a tRNA that determine its identity. Moreover, because the results of these types of experiments reveal which AASs are competing for a given substrate, they provide information about the elements that are recognized by competing AASs. With a detailed knowledge of the suppression efficiency, identity, and, additionally, the aminoacylation kinetics of a series of systematically related mutants, it should be possible to delimit the elements necessary for maintaining specificity. Measurement of the *in vitro* aminoacylation kinetics of T7 transcripts corresponding to the LSM series remains to be done. Such studies, along with the three-dimensional structure of serine AAS (43), should further our understanding of $tRNA^{Ser}$ recognition.

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1. Normanly, J. & Abelson, J. (1989) *Annu. Rev. Biochem.* **58**, 1029–1049.
2. Schulman, L. H. (1991) *Progr. Nucleic Acid Res. Mol. Biol.* **41**, 23–87.
3. Rould, M. A., Perona, J. J., Söll, D. & Steitz, T. A. (1989) *Science* **246**, 1135–1142.
4. Sampson, J. R., DiRenzo, A. B., Behlen, L. S. & Uhlenbeck, O. C. (1990) *Biochemistry* **29**, 2523–2532.
5. Giegé, R., Florentz, C., Garcia, A., Grosjean, H., Perret, V., Puglisi, J., Théobald-Dietrich, A. & Ebel, J. P. (1990) *Biochimie* **72**, 453–461.
6. Edwards, H., Trézéguet, V. & Schimmel, P. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1153–1156.
7. Himeno, H., Hasegawa, T., Ueda, T., Watanabe, K. & Shimizu, M. (1990) *Nucleic Acids Res.* **18**, 6815–6819.
8. Hooper, M. L., Russell, R. L. & Smith, J. D. (1972) *FEBS Lett.* **22**, 149–155.
9. Shimura, Y., Aono, A., Ozeki, H., Sarabhai, A., Lamfrom, H. & Abelson, J. (1972) *FEBS Lett.* **22**, 144–148.
10. Ghysen, A., Reyes, O., Allende, C. C. & Allende, J. E. (1973) in *Gene Expression and Its Regulation*, eds. Kenney, F. T., Hamkalo, B. A., Favelukes, G. & August, J. T. (Plenum, New York), pp. 209–216.
11. Smith, J. D. & Celis, J. E. (1973) *Nature New Biol. (London)* **243**, 66–71.
12. Celis, J. E., Hooper, M. L. & Smith, J. D. (1973) *Nature New Biol. (London)* **244**, 261–264.
13. Inokuchi, H., Celis, J. E. & Smith, J. D. (1974) *J. Mol. Biol.* **85**, 187–192.
14. Ghysen, A. & Celis, J. E. (1974) *J. Mol. Biol.* **83**, 333–351.
15. Normanly, J., Ogden, R. C., Horvath, S. J. & Abelson, J. (1986) *Nature (London)* **321**, 213–219.
16. McClain, W. H. & Foss, K. (1988) *Science* **240**, 793–796.
17. Hou, Y.-M. & Schimmel, P. R. (1988) *Nature (London)* **333**, 140–145.
18. McClain, W. H. & Foss, K. (1988) *J. Mol. Biol.* **202**, 697–709.
19. McClain, W. H. & Foss, K. (1988) *Science* **241**, 1804–1807.
20. McClain, W. H., Foss, K., Jenkins, R. A. & Schneider, J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9260–9264.
21. Rogers, J. M. & Söll, D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6627–6631.
22. Yaniv, M., Folk, W. R., Berg, P. & Soll, L. (1974) *J. Mol. Biol.* **86**, 245–260.
23. Normanly, J., Masson, J.-M., Kleina, L. G., Abelson, J. & Miller, J. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6548–6552.
24. Kleina, L. G., Masson, J.-M., Normanly, J., Abelson, J. & Miller, J. H. (1990) *J. Mol. Biol.* **213**, 705–717.
25. Normanly, J., Kleina, L. G., Masson, J.-M., Abelson, J. & Miller, J. H. (1990) *J. Mol. Biol.* **213**, 719–726.
26. Kisselev, L. L. & Frolova, L. Yu. (1964) *Biokhimiya (Moscow)* **29**, 1177–1189.
27. Schulman, L. H. & Goddard, J. P. (1973) *J. Biol. Chem.* **248**, 1341–1345.
28. Chattapadhyay, R., Pelka, H. & Schulman, L. H. (1990) *Biochemistry* **29**, 4263–4268.
29. Pallanck, L. & Schulman, L. H. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3872–3876.
30. Dente, L., Cesareni, G. & Cortese, R. (1983) *Nucleic Acids Res.* **11**, 1645–1655.
31. Amann, E., Brosius, J. & Ptashne, M. (1983) *Gene* **25**, 167–178.
32. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
33. Spinzl, M., Hartmann, T., Weber, J., Blank, J. & Zeidler, R. (1989) *Nucleic Acids Res.* **17**, r1–r172.
34. Sampson, J. R., DiRenzo, A. B., Behlen, L. S. & Uhlenbeck, O. C. (1989) *Science* **243**, 1363–1366.
35. Schulman, L. H. & Pelka, H. (1988) *Science* **242**, 765–768.
36. Raftery, L. A. & Yarus, M. (1987) *EMBO J.* **6**, 1499–1506.
37. Delarue, M. & Moras, D. (1989) in *Nucleic Acids and Molecular Biology*, eds. Eckstein, F. & Lilley, D. M. J. (Springer, Berlin), Vol. 3, pp. 182–196.
38. Seeman, N. C., Rosenberg, J. M. & Rich, A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 804–808.
39. Rould, M. A., Perona, J. J. & Steitz, T. A. (1991) *Nature (London)* **352**, 213–218.
40. Schatz, D., Leberman, R. & Eckstein, F. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6132–6136.
41. Dock-Bregeon, A. C., Garcia, A., Giegé, R. & Moras, D. (1990) *Eur. J. Biochem.* **188**, 283–290.
42. Asahara, H., Himeno, H. & Shimizu, M. (1991) *Chem. Lett.* **3**, 363–366.
43. Cusack, S., Berthet-Colominas, C., Härtlein, M., Nassar, N. & Leberman, R. (1990) *Nature (London)* **347**, 249–255.