Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 9272–9276, October 1991
Biochemistry

Four sites in the acceptor helix and one site in the variable pocket of $tRNA^{Ala}$ determine the molecule's acceptor identity

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Communicated by Aaron Klug, August 8, 1991 (received for review June 14, 1991)

ABSTRACT **The structural features that determine $tRNA^{Ala}$ acceptor identity have been studied with amber-suppressor tRNAs in *Escherichia coli* cells. Previous work established that a wobble pair composed of guanosine at position 3 and uridine at position 70 (G3·U70) in the acceptor helix of $tRNA^{Ala}$ is a determinant of the molecule's acceptor identity. We show that additional determinants are located at three other sites in the acceptor helix and at one site in the variable pocket of $tRNA^{Ala}$. These latter determinants are less important than G3·U70 since their individual alterations in mutants of $tRNA^{Ala}$ have smaller degrading effects on the functions of the molecules, and subsets of the determinants, when combined with G3·U70, are sufficient to switch the identities of several other tRNAs to that of $tRNA^{Ala}$. Other workers are using fragments of the $tRNA^{Ala}$ acceptor helix to study the molecule's acceptor identity. Our demonstration that the variable pocket contributes to $tRNA^{Ala}$ acceptor identity means that such fragments do not faithfully replicate the structure–function relationship of the cellular process.**

Molecules of tRNA mediate the translation of genetic information into amino acid sequences of proteins. In this process, the tRNAs are first aminoacylated with amino acids, their anticodons then interact with complementary codons in the mRNA, and the amino acids are inserted into the growing protein chain. The matching of amino acid to the cognate tRNA is essential for faithful translation. The specificity of tRNA aminoacylation, termed tRNA acceptor identity, is determined by part of the tRNA structure as it is recognized by and interacts with an aminoacyl-tRNA synthetase enzyme. Each of 20 enzymes catalyzes the aminoacylation reaction and recognizes the correct amino acid as well as the cognate tRNA species. The tRNA interacts productively with its cognate aminoacyl-tRNA synthetase and nonproductively with the other 19 noncognate enzymes (1). Here we are interested in the structural features that determine the acceptor identity of *Escherichia coli* $tRNA^{Ala}$.

Background and Rationale

A wobble pair composed of guanosine at position 3 and uridine at position 70 (G3·U70) in the acceptor helix of $tRNA^{Ala}$ is a determinant of the molecule's acceptor identity. This was shown *in vivo* by replacing the wild-type nucleotide pairs in amber suppressors of $tRNA^{Phe}$, $tRNA^{Cys}$, and $tRNA^{Lys}$ with G3·U70 of $tRNA^{Ala}$ and observing the insertion of some alanine into proteins (2–4). Also, the *in vitro* aminoacylation of a fragment of $tRNA^{Ala}$ containing only the acceptor and T helices and T loop was dependent on G3·U70 (7). Fig. 1*A* shows the location of G3·U70 in the cloverleaf representation of $tRNA^{Ala}$ (6).

However, we demonstrated that G3·U70 is not sufficient for $tRNA^{Ala}$ acceptor identity since a G3·U70 mutant of amber suppressor $tRNA^{Gly}$ (see Fig. 2*A*) was unable to insert alanine into proteins (2). This observation led us to predict additional determinants of $tRNA^{Ala}$ identity. Here we identify several of those determinants, not only to confirm our prediction and obtain a more complete description of the system, but also to learn if any determinants lie outside the acceptor helix since ongoing investigations of $tRNA^{Ala}$ identity by other researchers focus on fragments containing only this part of the molecule (7–10).

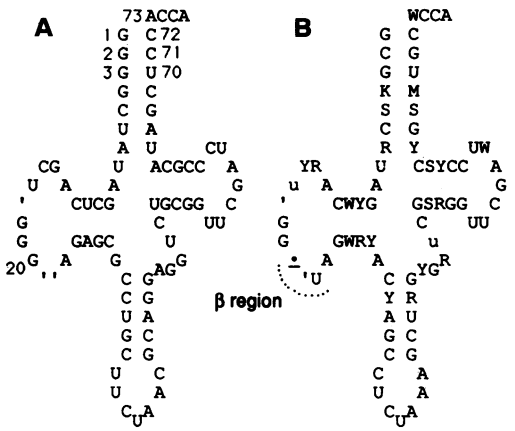

FIG. 1. Cloverleaf arrangement of nucleotide sequences in amber suppressor $tRNA^{Ala}$ (*A*) and a composite tRNA sequence (5) showing the nucleotides common to tRNAs that insert alanine ($tRNA^{Ala}$, GLY_3G3·U70, and G3·U70 mutants of $tRNA^{Phe}$, $tRNA^{Cys}$, and $tRNA^{Lys}$) and other tRNAs that do not (ARGG3·U70 and GLY_1G3·U70) (*B*). The modified nucleotides (6) are not indicated and an alignment gap is indicated by '. The wild-type anticodon sequence UGC in *A* was changed to CUA so the tRNA could translate UAG in the mRNA. The nucleotide positions relevant to this study are numbered and the β region is indicated. The position in *B* where the two composites do not contain a common nucleotide is underlined and marked ·. Incompletely specified nucleotides in *B* are designated as follows: M, A or C; R, A or G; W, A or U; S, C or G; Y, C or U; K, G or U. A lowercase letter indicates that the position contains either an alignment gap or the indicated nucleotide.

Our approach for locating additional determinants was, first, to find other tRNAs where the substitution of G3·U70 did not confer the ability to insert alanine into proteins. We inspected the sequences of those tRNAs for a distinguishing structural characteristic and then probed that structure with secondary tRNA mutants. This process located one additional determinant. Three others were subsequently uncovered by further correlation of tRNA structure and identity and by proximity to G3·U70.

Additional G3·U70 Variants

The additional determinants of $tRNA^{Ala}$ acceptor identity may be rather subtle since the molecules of $tRNA^{Gly}$, $tRNA^{Phe}$, $tRNA^{Cys}$, $tRNA^{Lys}$, and $tRNA^{Ala}$ are members of class I tRNAs (6) and share a common secondary structure.

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A G3·U70 mutant of $tRNA^{Tyr}$ described previously did not insert alanine in proteins (11) and could lack a determinant, or the large extra helix in this class II tRNA could act negatively in interactions with Ala-tRNA synthetase.

We constructed two genes for class I-type tRNAs containing G3·U70. Mutant ARGG3·U70 (Fig. 2*B*) was derived from $tRNA^{Arg}$ (wild-type anticodon ICG) and mutant GLY_3-G3·U70 (Fig. 2*C*) was derived from $tRNA^{Gly}$ (wild-type anticodon CCC) (6). The previous mutant, GLY_1G3·U70 (Fig. 2*A*), was derived from a different isoacceptor of $tRNA^{Gly}$ (wild-type anticodon UCC) (2). For an experimental analysis of amber suppressor tRNAs in *E. coli* cells, each gene was further altered to produce tRNA with a CUA anticodon capable of pairing with and translating the amber triplet UAG in mRNA. The mutant genes and a reference *ALA* gene (encoding amber suppressor $tRNA^{Ala}$) were separately inserted in expression plasmid pGFIB (12) and transformed into cells containing compatible plasmid pD3am (13) expressing dihydrofolate reductase protein with an amber mutation in the third codon from the protein's amino terminus. The acceptor identity of the tRNA was ascertained from the third amino acid in the protein's sequence. Using a *lacI–Z* fusion system (12), the suppression efficiency for transmitting the growing protein chain beyond the amber triplet was also determined for each suppressor tRNA. The suppression efficiency reflects the cellular level of the aminoacylated tRNA and its ribosomal performance. Amber suppressors of different wild-type tRNAs exhibit various suppression efficiencies for unknown reasons. The importance of this property is minimized here since we compare wild-type and mutant forms of the same tRNA.

The reference amber suppressor, $tRNA^{Ala}$, inserted only alanine into the suppressed protein (Table 1, experiment 1). In contrast, the tRNA transcribed from the ARGG3·U70 gene inserted 86% arginine and 7% lysine; alanine was not detected and thus was <5% of the inserted amino acids (Table 1, experiment 2). We then attempted to block $tRNA^{Arg}$ acceptor identity by changing A20U20a to U20U20a (24), but were still unable to elicit alanine insertion (Table 1, experiment 3). The lysine inserted by both tRNA mutants reflects the presence of U35, a $tRNA^{Lys}$ determinant (15, 16), in the middle anticodon position of the amber suppressor tRNA. The tRNA transcribed from the GLY_3G3·U70 gene inserted 22% alanine and 78% glycine (Table 1, experiment 4), indicating that this molecule had a partial $tRNA^{Ala}$ identity. This contrasts with GLY_1G3·U70 encoding the other $tRNA^{Gly}$ isoacceptor, which does not insert alanine into protein (ref. 2; Table 1, experiment 5). We note that the insertion of more than one amino acid by several of these tRNA species reflects a competition by the corresponding synthetases (1, 11, 17, 18).

Covariation of tRNA Structure and Function

We looked for a systematic difference between the G3·U70-containing tRNAs that did not insert alanine into proteins and others that did by assembling composite sequences so that the nucleotides at corresponding cloverleaf positions could be compared (5, 13). This revealed that the $tRNA^{Ala}$-like molecules contained 1 nucleotide residue in the β region of the D loop, whereas the non-$tRNA^{Ala}$-like molecules contained 2 residues in this region (Fig. 1*B*, underlined residue). The β region, which lies to the 3′ side of the constant G18 and G19 residues, is a site of natural variation of length (1–3 residues) and nucleotide type in tRNA molecules (6, 19). No other systematic difference exists between the two composite tRNA sequences. The results suggest that two residues in the β region of $tRNA^{Arg}$ and $tRNA^{Gly}$ preclude their conversion to a molecule able to insert alanine into proteins.

To test this idea, we mutated the genes of ARGG3·U70 and GLY_1G3·U70 so the β region of the transcribed tRNAs would contain 1 residue rather than 2. Analysis of the suppressed protein revealed that the tRNA produced by ARGG3·U70Δ20 inserted 23% alanine and 65% lysine (Table 1, experiment 6), whereas that produced by mutant GLY_1G3·U70Δ20 inserted 7% alanine, 71% glutamine, and 19% glycine (Table 1, experiment 7). The inserted glutamine reflects the presence of U35, a $tRNA^{Gln}$ determinant (20, 21), in the anticodon of the amber suppressor tRNA. Most importantly, both tRNAs acquired an ability to insert some alanine into protein after the lengths of their β regions were reduced from 2 nucleotide residues to 1.

Variable Pocket Contributes to $tRNA^{Ala}$ Identity

The results described above demonstrate that the size of the β region of $tRNA^{Arg}$ and $tRNA^{Gly}$ limits the ability of the molecules to insert alanine into protein. The redesigned β regions of both tRNAs contain U20, whereas $tRNA^{Ala}$ contains G20. To test the contribution of G20 to the productive interaction of tRNA with the Ala-tRNA synthetase, we made a $tRNA^{Arg}$ gene containing G20 and G3·U70 and then deter-

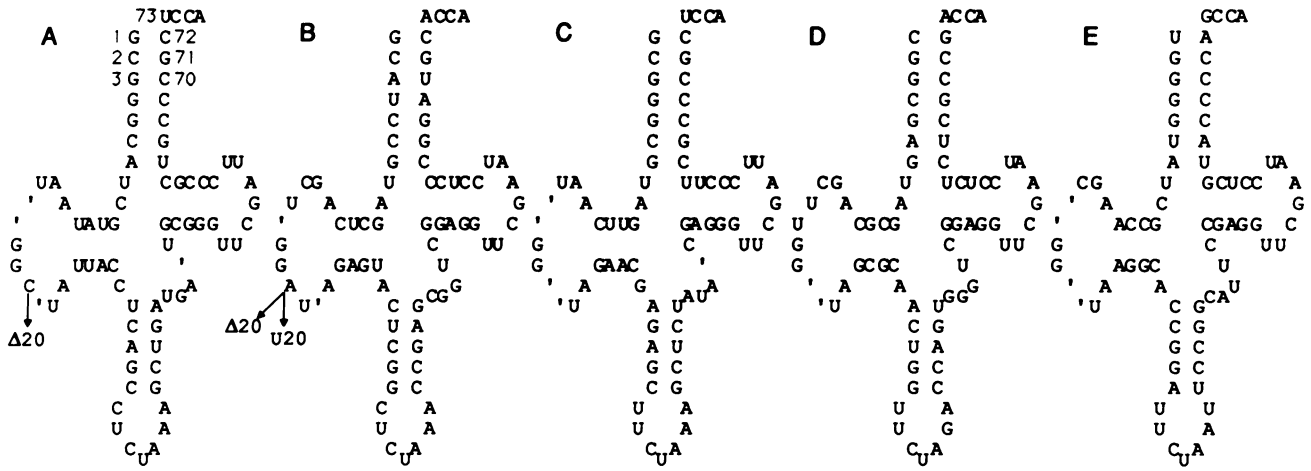

FIG. 2. Cloverleaf arrangement of nucleotide sequences of amber suppressor tRNAs transcribed from genes encoding GLY_1 (*A*), ARG (*B*), GLY_3 (*C*), PRO (*D*), and GLN (*E*). The wild-type anticodon sequences (UCC in *A*, ICG in *B*, CCC in *C*, UGG in *D*, and CUG in *E*) were changed to CUA so the tRNAs could translate UAG in the mRNA. Other nucleotide changes in specific mutants are indicated in the text. The nucleotide positions relevant to this study are numbered. The modified nucleotides (6) are not indicated. The symbol ′ indicates an alignment gap. Arrows in *A* and *B* point to deleted (Δ) or substituted nucleotides.

Table 1. Structure and function of amber suppressor tRNAs

Exp.	Mutant	Identity with nucleotide or nucleotide pair of $tRNA^{Ala}$					Amino acid(s) in suppressed protein		Suppression efficiency, %
		G20	G3·U70	G2·C71	G1·C72	A73	Ala, %	Other (%)	
1	ALA	√	√	√	√	√	94	—	13
2	ARGG3·U70	A20U20a	√	C2·G71	√	√	—	Arg (86), Lys (7)	41
3	ARGG3·U70U20U20a	U20U20a	√	C2·G71	√	√	—	Lys (90)	14
4	GLY_3G3·U70	U20	√	C2·G71	√	U73	22	Gly (78)	10
5	GLY_1G3·U70	C20U20a	√	C2·G71	√	U73	—	Gln (95), Gly (5)	37
6	ARGG3·U70Δ20	U20	√	C2·G71	√	√	23	Lys (65)	26
7	GLY_1G3·U70Δ20	U20	√	C2·G71	√	U73	7	Gln (71), Gly (19)	23
8	ARGG3·U70G20	√	√	C2·G71	√	√	57	Lys (35)	22
9	ARGG3·U70Δ20G2·C71	U20	√	√	√	√	95	—	31
10	ARGG3·U70G2·C71	A20U20a	√	√	√	√	94	—	26
11	PROG3·U70	U20	√	√	C1·G72	√	ND		0.3
12	PROG3·U70G1·C72	U20	√	√	√	√	95	—	2
13	GLNG3·U70	U20	√	√	U1·A72	G73	4	Gln (93)	10
14	GLNG3·U70G1·C72	U20	√	√	√	G73	8	Gln (91)	9
15	ALAC1·G72	√	√	√	C1·G72	√	ND		1
16	GLNG3·U70A73	U20	√	√	U1·A72	√	54	Gln (38)	5
17	GLY_3G3·U70A73	U20	√	C2·G71	√	√	100	—	25
18	GLNG3·U70G1·C72A73	U20	√	√	√	√	97	—	18
19	ALAG73	√	√	√	√	G73	91	Gln (7)	11

Sequences of amber suppressor tRNAs transcribed from the indicated genes are given in Figs. 1*A* and 2. The structures of ALA (experiment 1), ALAC1·G72 (experiment 15), and ALAG73 (experiment 19) contain C38 of wild-type $tRNA^{Ala}$ rather than A38 in the previous ALA gene (2). Current and former versions of ALA insert only alanine, but the C38 version gives a higher suppression efficiency. The structure of GLY_1G3·U70 differs from that of a similar mutant GLY13 described previously (2) in containing G51·C63 of wild-type $tRNA^{Gly}$ (anticodon UCC) rather than A51·C63. Neither mutant inserts alanine, but GLY_1G3·U70 gives a higher suppression efficiency. Methods for construction of mutant genes, purifying and sequencing of dihydrofolate reductase protein, measurement of suppression efficiency, and analysis of the data have been described (14). The tRNAs were expressed from plasmid pGFIB (12) and the proteins were expressed from plasmid pD3am (13). Mutant ARGG3·U70 (experiment 2) was characterized at residue 10 of dihydrofolate reductase protein (12); all other mutants were characterized at residue 3. An amino acid yield of <5% was retained but not reported and the combined yield of reported residues was usually <100%. The glutamine values include ≤13% glutamate. Cysteine was not analyzed. Suppression efficiency measurements used UAG allele *A16* in a *lacI–Z* fusion; the reported value is the percentage of enzyme activity relative to a wild-type *I–Z* fusion (which averaged 217 units) and has not been corrected for the value of cells without a suppressor tRNA (≤0.009%). Nucleotide sequence of $tRNA^{Ala}$ at several positions is shown. In experiments 1–19, the corresponding nucleotide is either identical with that of $tRNA^{Ala}$ and indicated by a check mark or the alternative nucleotide is shown. —, None detected; yield therefore is <5%. ND, not determined.

mined its function. Analysis showed that the resulting mutant, ARGG3·U70G20, inserted 57% alanine and 35% lysine into protein, with a suppression efficiency of 22% (Table 1, experiment 8). This represents a 2.5-fold increase in alanine specificity while retaining suppression efficiency relative to $tRNA^{Arg}$ with U20 and G3·U70.

We conclude that G20 contributes to the productive interaction of $tRNA^{Ala}$ with the Ala-tRNA synthetase. Residue G20 can be replaced by U20, but not by two residues, without severe consequences on $tRNA^{Ala}$ acceptor identity. Neither single residue C20 nor A20 has been tested for its effect on $tRNA^{Ala}$ identity (see Table 1 and refs. 2–4 and 11). Two residues in the β region may perturb the productive interaction by causing steric or ionic clashes with the Ala-tRNA synthetase or by causing different local or distant conformations in the tRNA. Consistent with the importance of residue G20 described here, we previously reported a small degrading effect on $tRNA^{Ala}$ identity and suppression efficiency in a multiple mutant of amber suppressor $tRNA^{Ala}$ in which wild-type residues C16, U17, and G20 were changed to U16, C17, and U20 (2).

In the three-dimensional structure of yeast $tRNA^{Phe}$, the nucleotide at position 20 and others in the D and T loops arch out from a constant part of the tRNA structure, forming a hypothetical variable pocket that might be part of a protein recognition system (19). Different workers explicitly rejected this hypothesis (22). However, it has emerged that residue 20 in the variable pocket is one of several determinants of the acceptor identities of yeast $tRNA^{Phe}$ (23) and the molecules of *E. coli* $tRNA^{Phe}$ (14) and $tRNA^{Arg}$ (24, 25), as well as $tRNA^{Ala}$ (ref. 2; this work). The involvement of the variable pocket in $tRNA^{Ala}$ identity means that an incomplete picture of the relationship between structure and acceptor identity is obtained through the *in vitro* aminoacylation of fragments of $tRNA^{Ala}$ containing only the acceptor helix (7–10).

Four Sites in the Acceptor Helix Contribute to $tRNA^{Ala}$ Identity

The importance of G3·U70 to $tRNA^{Ala}$ acceptor identity was established previously (2, 3). We now show that other nucleotides in the acceptor helix also contribute. This was demonstrated by their requirement for normal $tRNA^{Ala}$ function and by their ability to switch the acceptor identities of other tRNAs to that of $tRNA^{Ala}$. Since different nucleotides in the corresponding positions are determinants in other tRNAs (13, 15), substitutions of $tRNA^{Ala}$-derived nucleotides in their sequences potentiate the desired changes. Thus, a switch in tRNA identity reflects two mutually reinforcing processes, an enhancement of the new tRNA identity and damage to the old tRNA identity. If modifications to a tRNA are not accompanied by an identity switch, then the redesigned tRNA structure is not a good enough substrate with the new aminoacyl-tRNA synthetase, or a sufficiently poor enough substrate with the old aminoacyl-tRNA synthetase, to promote the change. Notice that a modified tRNA can lack one of several determinants and still exhibit an identity switch if the tRNA structure is sufficient to both generate the new and block the old identity functions. Thus, the nucleotides that determine tRNA identity must be characterized not only for their sufficiency for switching other tRNA identities but also for their necessity for maintaining the functions of the tRNA under study.

We surmise that the tRNA transcribed from the gene of ARGG3·U70G20 still lacks a determinant of $tRNA^{Ala}$ acceptor identity since both alanine and lysine are inserted into protein (Table 1, experiment 8). In contrast, a G3·U70 variant of amber suppressor $tRNA^{Lys}$ inserts only alanine (4). A comparison of the transcribed tRNA sequences of G3·U70-containing molecules shows that while ALA and LYS contain G2·C71, ARG contains C2·G71 (compare Figs. 1*A* and 2*B*). The G2·C71 and/or G4·C69 pairs in $tRNA^{Ala}$ were previously implicated in $tRNA^{Ala}$ identity, since mutating both nucleotide pairs simultaneously had a small degrading effect on alanine specificity and suppression efficiency (4). Since both ARG and LYS contain U4·A69 (6), we disregarded this pair here and made a new derivative of ARG containing G2·C71 in place of C2·G71. This mutant, derived from ARGG3·U70Δ20 and named ARGG3·U70Δ20G2·C71, inserted only alanine into protein, with a suppression efficiency of 31% (Table 1, experiment 9). Thus, $tRNA^{Arg}$ has been converted into an alanine-specific amber suppressor tRNA on reducing the length of the β region to one residue and replacing two nucleotide pairs in the acceptor helix (G3·U70 and G2·C71) with the corresponding $tRNA^{Ala}$-derived residues.

We constructed a new mutant of amber suppressor $tRNA^{Arg}$ to determine whether two β residues (A20U20a) in $tRNA^{Arg}$ are sufficient to block $tRNA^{Ala}$ acceptor identity when the molecule contains G2·C71 and G3·U70. We found that the resulting mutant (named ARGG3·U70G2·C71) inserted only alanine into protein, with a suppression efficiency of 26% (Table 1, experiment 10). Thus, two β residues in $tRNA^{Arg}$ do not preclude a $tRNA^{Ala}$ identity. Rather, the β region is limiting when $tRNA^{Ala}$ determinant G2·C71 is absent.

The above results and earlier studies show that the 2nd and 3rd nucleotide pairs on the acceptor helix contribute to $tRNA^{Ala}$ acceptor identity. The flanking nucleotide pair in $tRNA^{Ala}$, G1·C72, had not been tested, since every other tRNA mutated to G3·U70 contained G1·C72. We therefore examined G3·U70 variants of tRNAs that lack G1·C72. Genes for amber suppressors of $tRNA^{Pro}$ (wild-type C1·G72; Fig. 2*D*) and $tRNA^{Gln}$ (wild-type U1·A72; Fig. 2*E*) were modified so that the transcribed tRNA molecules would contain G3·U70; another version of each tRNA gene combined G3·U70 with G1·C72. We observed that the G3·U70 mutant of $tRNA^{Pro}$ (named PROG3·U70) had a low suppression efficiency; the suppressed protein was not analyzed (Table 1, experiment 11). On introducing G1·C72 (mutant PROG3·U70G1·C72), the suppression efficiency increased and only alanine was found in the suppressed protein (Table 1, experiment 12). The G3·U70 mutant of $tRNA^{Gln}$ (named GLNG3·U70) inserted 4% alanine and 93% glutamine into protein (Table 1, experiment 13). On introducing G1·C72 (mutant GLNG3·U70G1·C72), 8% alanine and 91% glutamine were present in protein (Table 1, experiment 14). Finally, the construction of a $tRNA^{Ala}$ mutant with G1·C72 replaced by C1·G72 (named ALAC1·G72) confirmed the importance of these residues since the suppression efficiency was reduced 13-fold (Table 1, experiment 15).

The proximity of single-stranded residue A73 to the first 3 nucleotide pairs in the acceptor end of $tRNA^{Ala}$, and the identity role of this single-stranded residue in many other tRNAs (15), led us to determine the importance of A73 in changing the identity of $tRNA^{Gln}$ (wild-type G73; Fig. 2*E*) and $tRNA^{Gly}$ (wild-type U73; Fig. 2*C*) to that of $tRNA^{Ala}$. Mutants of each gene were constructed containing G3·U70 and A73 and then tested for function. The mutant of $tRNA^{Gln}$ (named GLNG3·U70A73) inserted 54% alanine and 38% glutamine into protein (Table 1, experiment 16), while that of $tRNA^{Gly}$ (named GLY_3G3·U70A73) inserted only alanine into protein (Table 1, experiment 17). As described above, the corresponding single mutant of $tRNA^{Gln}$ (GLNG3·U70) inserted 4% alanine (Table 1, experiment 13) while the single mutant of $tRNA^{Gly}$ inserted 22% alanine (Table 1, experiment 4). A new $tRNA^{Gln}$ mutant (named GLNG3·U70G1·C72A73) with all known determinants of $tRNA^{Ala}$ except residue G20 inserted only alanine into protein, with a suppression efficiency of 18% (Table 1, experiment 18). This tRNA is functionally superior to the other three versions of the molecule containing G3·U70 alone or in combination with either G1·C72 or A73. Finally, the construction of a $tRNA^{Ala}$ mutant with G73 (named ALAG73) revealed that 91% alanine and 7% glutamine were inserted into protein (Table 1, experiment 19), thus confirming the importance of A73 in $tRNA^{Ala}$.

Different workers concluded that A73 does not contribute to $tRNA^{Ala}$ acceptor identity since a U73 mutant of amber suppressor $tRNA^{Ala}$ inserted only alanine into protein while the suppression efficiency was reduced 1.7-fold (3). In our view, these workers have not clearly realized that a mutated tRNA will manifest a new acceptor identity only if the modified tRNA substrate (e.g., U73 in $tRNA^{Ala}$) is at least minimally acceptable with some other enzyme. They also constructed G73 and C73 mutants of $tRNA^{Ala}$ and observed suppression of *E. coli* allele *trpA234*, indicative of alanine or glycine insertion; however, these results do not exclude the simultaneous insertion of glutamine or other amino acids. Finally, a reinvestigation revealed that wild-type residue A73 is important for *in vitro* enzymatic attachment of alanine to fragments of acceptor helices of $tRNA^{Ala}$ (10) and a G3·U70 mutant of $tRNA^{Cys}$ (8). Our *in vivo* work reported here conclusively demonstrates that A73 contributes to $tRNA^{Ala}$ acceptor identity.

Concluding Remarks

In the cellular context of dynamic competition among aminoacyl-tRNA synthetases, $tRNA^{Ala}$ acceptor identity is determined by the nucleotides at four sites in the acceptor helix and at one site in the variable pocket. Of these, G3·U70 in the acceptor helix is the most important. The other determinants are less important since (*i*) their individual alterations in mutants of $tRNA^{Ala}$ have smaller degrading effects on the functions of the molecules and (*ii*) subsets of the minor determinants, when combined with G3·U70, are sufficient to switch the identities of several other tRNAs to that of $tRNA^{Ala}$. The complexity of the system, and the importance of G3·U70 in particular, makes it possible that additional determinants exist.

The recognition of tRNAs by the aminoacylating enzymes offers an interesting contrast with recognition of tRNA precursors by processing enzymes. Whereas enzymes of the latter type sense a common structure in different molecules, the former enzymes sense the differences so they can discriminate between tRNA molecules. *E. coli* RNase P, for example, recognizes a specific part of the tertiary structure in precursor RNAs, the tRNA acceptor helix, and the enzyme cleaves fragments containing only tRNA acceptor helices *in vitro* (26). However, other domains of the tRNA help orient and present the acceptor helix to the enzyme *in vivo*. In RNAs with other metabolic fates (e.g., rRNAs and mRNAs), stem–loop structures similar to tRNA acceptor helices may be quite common, but the flanking sequences in these RNAs may shield them from RNase P cleavage (26).

The aminoacylating enzymes, in contrast, recognize specific nucleotides in helical and single-stranded segments in two or more well-separated sites in the acceptor helix, the variable pocket, or the anticodon. Other parts of the tRNA determine the overall shape and orient these sites for recognition. Three examples suggest how recognition sites mediate tRNA acceptor identity. (*i*) The three-dimensional structure

of a complex of *E. coli* $tRNA^{Gln}$ with its cognate enzyme and ATP reveals base-specific interactions with the 3′ acceptor helix and anticodon loop of $tRNA^{Gln}$. While the tRNA retains much of its overall shape, the distal base pair in the acceptor helix is broken and the 3′ end loops back rather than continuing its helical path as in free tRNAs (21). The anticodon loop also adopts a new conformation. (*ii*) The structure of a complex between yeast $tRNA^{Asp}$ and Asp-tRNA synthetase shows interactions with the acceptor helix and anticodon stem–loop (27). The acceptor helix is maintained, but the anticodon region adopts a new conformation, which begins after a G·U wobble pair in the anticodon stem and extends into the loop. While $tRNA^{Asp}$ and $tRNA^{Gln}$ contain similar sequences in distal parts of their acceptor helices, the conformation of $tRNA^{Gln}$ is distinctive. This suggests that the nucleotides contained within the distal portions of the acceptor helices are themselves not sufficient to determine conformation (27). (*iii*) Indirect experiments suggest that a geometrically distinctive functional group on G3·U70 of *E. coli* $tRNA^{Ala}$ interacts with Ala-tRNA synthetase (2–4, 8). Also, the G3·U70 wobble pair may allow the acceptor helix to adopt a new conformation (4). In summary, the emerging picture suggests that tRNA recognition results from direct interactions between an aminoacyl-tRNA synthetase and the bases of the cognate tRNA. Conformational changes in the tRNA arising from distinctive and subtle aspects of its sequence contribute to this interaction.

These considerations and our demonstration here that the variable pocket of $tRNA^{Ala}$ contributes to the molecule's acceptor identity mean that *in vitro* aminoacylation experiments with fragments of $tRNA^{Ala}$ containing only the acceptor helix (7–10) provide an incomplete picture of the structure–function relationship of the cellular process.

We thank S. Altman, W. Ross, D. Söll, M. Yarus, and others for comments on the manuscript; C. Mattison and J.-C. Wang for technical assistance; R. Niece and M. Strickland at the University of Wisconsin Biotechnology Center for oligodeoxynucleotide synthesis and HPLC analysis; and L. Williams at the University of Southern California Microchemical Core Laboratory for protein sequencing. This work was supported by National Institutes of Health Grant GM42123.

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