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Genetics

Glycine tRNA mutants with normal anticodon loop size cause −1 frameshifting

(protein synthesis/translocation/frameshift suppressor/acceptor stem/TFC loop)

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ABSTRACT **Mutations in the acceptor stem, the 5-methyluridine-pseudouridine-cytidine (TFC) arm, and the anticodon of *Salmonella* $tRNA_2^{Gly}$ can cause −1 frameshifting. The potential for standard base pairing between acceptor stem positions 1 and 72 is disrupted in the mutant *sufS627*. This disruption may interfere with the interaction of the tRNA with elongation factor-Tu·GTP or an as-yet-unspecified domain of the ribosome. The potential for standard base pairing in part of the TFC stem is disrupted in mutant *sufS625*. The nearly universal C-61 base of the TFC stem is altered in mutant *sufS617*, and the TFC loop is extended in mutant *sufS605*. These changes are expected to interfere with the stability of the TFC loop and its interaction with the D arm. The mutation in mutant *sufS605*, and possibly other mutants, alters nucleoside modification in the D arm. Three mutants, *sufS601*, *sufS607*, and *sufS609*, have a cytidine substituted for the modified uridine at position 34, the first anticodon position. None of the alterations grossly disrupts in-frame triplet decoding by the mutant tRNAs. The results show that −1 frameshifting *in vivo* can be caused by tRNAs with normal anticodon loop size and suggest that alternative conformational states of the mutant tRNAs may allow them to read a codon in frame or to shift reading frame.**

The normal triplet progression of translation can be perturbed by ribosomes shifting reading frame. The phenomenon was initially detected as frameshift mutant leakiness, but there are examples in which the phenomenon is seen in the decoding of normal cellular or viral genes. Several examples have shifts into the +1 frame (1–5). Shifts to the −1 frame have been found in decoding of the DNA phages T7 (6) and φX174 (7), the *gag–pol* regions of several retroviruses (ref. 8 and the references therein), in a coronavirus (9), and in the RNA phage MS2, at least *in vitro* (2). In the latter example certain normal tRNAs (with seven-membered anticodon loops) promoted frameshifting, but the tRNA–mRNA interaction was noncognate; although the noncognate interaction was suggested as an explanation for one *in vivo* case (7), that interaction is distinct from the cognate type of frameshifting implicated in the other examples cited. In investigated examples in which high-level frameshifts naturally occur, specific mRNA signals have been shown to promote the frameshifting (8, 10, 11). Whether a subset of normal tRNAs plays a special role as well is an open question.

Mutants of tRNA and other translational components that promote frameshifting have been isolated. These mutants provide insight into the roles of translational macromolecules in normal reading frame maintenance and in frameshifting. The mutants have been detected as external suppressors of frameshift mutations. Many suppressors of +1 frameshift mutations in both bacteria and yeast have been characterized as tRNAs with increased anticodon loop size (refs. 12–14 and the references therein). In contrast, few −1 frameshift mutant suppressors have been described (15–20). The weaker −1 suppressors previously characterized are mutants of protein-coding genes (see ref. 20). Some of them, the *tuf* classes (17), carry alleles of either gene for elongation factor Tu. Two classes of tRNA suppressors have also been described. The *hopR/hopE* class are mutants of one or other of the four genes for $tRNA_1^{Val}$ (18), and the other class is *sufS* (16, 20), which is dealt with in this paper. *sufS* mutants were isolated as suppressors for a *Salmonella* frameshift mutant (*trpE91*) but have since been shown to suppress a series of constructed −1 frameshift mutants in *Escherichia coli lacZ*. The suppressor site is GGA (20). The sequences flanking this site in the *trpE91* and *lacZ* frameshift mutants are very different and do not appear important for suppression, with the exception of the immediately 5′ base, which, when guanosine, maximizes suppression (20).

MATERIALS AND METHODS

Suppressor Mutants. *sufS601*, *sufS605*, *sufS607*, *sufS609*, and *sufS617* were *sup-601*, *sup-605*, *sup-607*, *sup-609*, and *sup-617*, respectively, in the original publication (16), having been subsequently renamed (20). *sufS625* and *sufS627* were recently isolated (20).

Cloning and DNA Sequencing. Restriction endonucleases, T4 DNA ligase, T4 polynucleotide kinase, and DNA polymerase 1 (Klenow fragment) were from Boehringer Mannheim and used according to the supplier's instructions. Hybond-N and radio nucleotides were obtained from Amersham. Chromosomal DNA was prepared as described (21) from *Salmonella* strains. *sufS601*, *sufS625*, and wild-type *glyT* were cloned as follows: Chromosomal DNA was digested with *Bss*HII and *Cla* I and size-fractionated on low-melting-temperature agarose gel. The DNA was then cloned into pMBS2, a derivative of pBR322, which contained suitable cloning sites in a promoterless *metB* gene (gift of Y. McKeown, Trinity College, Dublin). The DNA was then transfected into *E. coli* JM103 by using standard procedures (22). Hybridization screening of the resulting cloned DNA with 5′-end-labeled oligonucleotides complementary to a noncoding region of the *Salmonella tufB* operon located between *thrT* and *tufB* followed published protocols (22). *sufS605*, *sufS607*, *sufS617*, and *sufS627* mutants were cloned by a different procedure. A 145-base-pair (bp) DNA fragment was amplified by standard polymerase chain reaction (23) with oligonucleotides complementary to the flanking sides of *glyT* (24). This DNA was treated with T4 polynucleotide

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Abbreviation: TFC, 5-methyluridine–pseudouridine–cytidine.

kinase and cloned into pUC19, which had been cut with *Sma* I and dephosphorylated. Irrespective of how the DNA was cloned, double-stranded DNA of the inserts present in each class of recombinants was sequenced by the same described procedure (25). The primers used for sequencing were complementary to different parts of the *Salmonella tufB* operon tRNA genes (refs. 12 and 24; L. Bossi and D. M. Dunn, personal communication).

RNA Isolation and Analysis. RNase T_2 was obtained from Sankyo through Calbiochem. Bacteriophage T4 polynucleotide kinase was from BRL, PEI-cellulose thin-layer plates were from J. T. Baker, and [γ-^{32}P]ATP was from ICN. Procedures for tRNA isolation, reversed phase (RPC-5) column chromatography, two-dimensional gel electrophoresis, and RNA sequence analysis have been described (26–30).

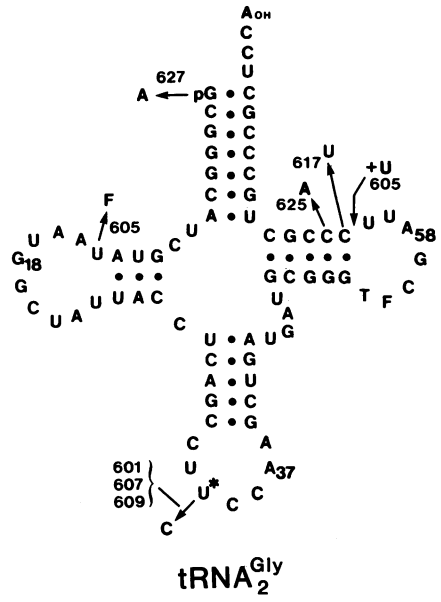
RESULTS

Cloning and DNA Sequence Analysis. Mutant class *sufS* maps to *glyT*, the gene for $tRNA_2^{Gly}$ (20), which reads GGA and GGG. A 575-bp *Salmonella* restriction fragment (12, 31, 32) that contains *glyT* and three flanking tRNA genes was cloned into *E. coli* from *sufS601*- and *sufS625*-containing strains, and their isogenic parent. The promoter for the tRNA genes was not included because of possible deleterious effects of high levels of suppressor. Sequencing of the 575-bp DNA fragments, presented in detail elsewhere (24), revealed that the only change in the genes cloned from the mutant *sufS601* and the isogenic parent strain was in *glyT*. In *sufS601* cytidine was substituted for thymidine at the site corresponding to position 34 of that tRNA. With confirmation that *sufS* mutants were alleles of *glyT*, polymerase-chain-reaction amplification was used to simplify cloning and sequencing of the other alleles. Fig. 1 shows the results, together with tRNA sequence determinations (see below). Mutant *sufS607* has the same DNA sequence as *sufS601*. The DNA sequence of mutant *sufS605* shows the insertion of thymidine at the positions corresponding to the two uridines at positions 59 and 60 at the 3′ end of the 5-methyluridine–pseudouridine–cytidine (TFC) loop. *sufS617* and *sufS625* each have a single substitution, one at position 61 and the other at 62 of the TFC stem. In contrast, the *sufS627* DNA sequence has a single substitution, guanosine to adenosine at position 1 in the tRNA.

Isolation of Glycine tRNAs. The *Salmonella* glycine tRNA isoacceptors ($tRNA_1^{Gly}$, $tRNA_2^{Gly}$, and $tRNA_3^{Gly}$) were separated by RPC-5 column chromatography and visualized as peaks of radioactively labeled glycine acceptor activity. Fig. 2 displays the profiles of each of two mutants, *sufS601* and *sufS605*, co-chromatographed with tRNA from the suppressor negative parental strain. Two other suppressors, *sufS609* and *sufS617*, were analyzed in the same way, but the results are not shown. The profile of *sufS609* was indistinguishable from that of *sufS601*, and the profile of *sufS617* was indistinguishable from that of *sufS605*. The wild-type profile we observed was essentially the same as that reported for *Salmonella* by Hill *et al.* (ref. 33; and see legend to Fig. 2).

As expected, it was the $tRNA_2^{Gly}$ species that was altered in each of the four mutants examined. *sufS601* $tRNA_2^{Gly}$ appeared not to be affected in its aminoacylation with glycine, but its mobility was changed, causing it to migrate more slowly, behind $tRNA_1^{Gly}$ (Fig. 2*A*). The same was true for *sufS609*. Fig. 2*B* indicates that, in *sufS605*, the $tRNA_2^{Gly}$ species is no longer apparent as glycine-accepting activity. The same result was obtained with *sufS617*. This phenomenon of "lost" peaks has been seen with most glycine tRNA suppressor mutants and seems to be due to a large decrease in ability of the mutant tRNAs to be aminoacylated with glycine (see ref. 30 for review).

To obtain glycine tRNAs for sequence analysis, the tRNAs from wild-type and mutant *Salmonella* were separated by two-dimensional gel electrophoresis. The wild-type two-dimensional pattern was sufficiently similar to that of *E. coli* to allow us to locate all three glycine tRNAs at the positions characteristic of the *E. coli* tRNAs. The four mutants differed with respect to the migration of $tRNA_2^{Gly}$. The $tRNA_2^{Gly}$ species from *sufS601* and *sufS609* were found at essentially the wild-type location, whereas those from *sufS605* and *sufS617* could not be found at, or near, that position. Considering the possibility that the latter mutant tRNA species are produced in lesser amounts (due either to decreased maturation of the $tRNA_2^{Gly}$ precursor or, less likely, to



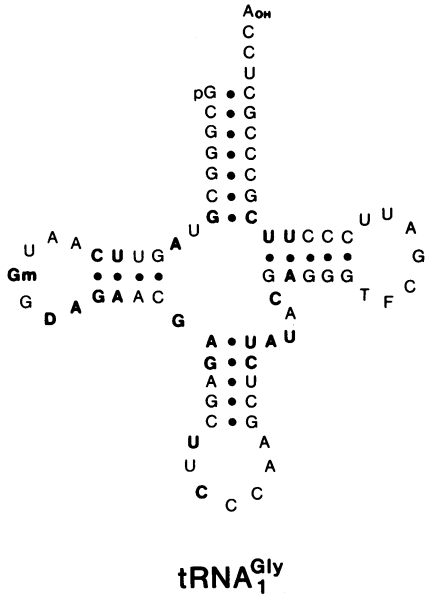


FIG. 1. Wild-type *Salmonella* $tRNA_2^{Gly}$, with the *sufS* changes indicated, and $tRNA_1^{Gly}$. The sequences of *sufS607*, *sufS617*, *sufS625*, and *sufS627* were determined at the DNA level only; for *sufS617*, *sufS625*, and *sufS627* changes in the modified nucleoside pattern may also occur (*sufS607* has the same primary sequence as *sufS601* and *sufS609*, which were analyzed at the tRNA level). F, formerly Ψ (pseudouridine); Gm, 2′-*O*-methylguanosine; U*, an unidentified modification of uridine. Positions in $tRNA_1^{Gly}$ at which differences from $tRNA_2^{Gly}$ occur are in bold face.

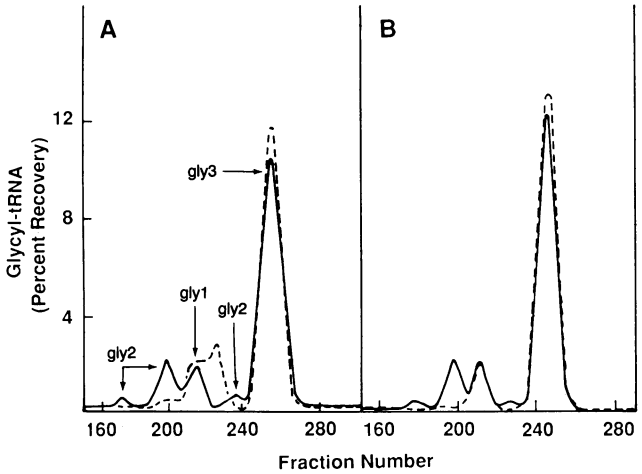


FIG. 2. *Salmonella* glycyl-tRNA profiles obtained after RPC-5 column chromatography. Percent recovery = (cpm from [^{3}H]glycine or [^{14}C]glycine of each fraction divided by the total cpm eluted from the column) × 100. (*A*) Wild-type tRNA (—) co-chromatographed with *sufS601* tRNA (––). Numbers over each peak indicate the isoacceptor species, $tRNA_1^{Gly}$, (gly1), $tRNA_2^{Gly}$ (gly2), or $tRNA_3^{Gly}$ (gly3). RPC-5 chromatography separates three differently modified forms of $tRNA_2^{Gly}$, which is encoded by a single-copy gene *glyT*. (*B*) Wild-type tRNA (—) co-chromatographed with *sufS605* tRNA (––).

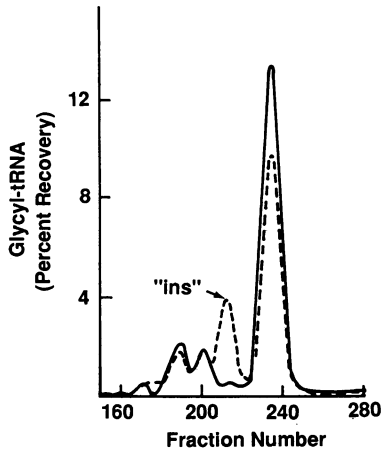


FIG. 3. Glycyl-tRNA profiles obtained after RPC-5 column chromatography. —, *Salmonella trpE91* (otherwise wild-type) parental strain; ––, an *ins*-containing *E. coli* strain.

decreased transcription of the operon including $tRNA_2^{Gly}$), we sought to enrich for these mutant species by running an RPC-5 column, pooling fractions in two groups (the first containing the $tRNA_1^{Gly}$ and $tRNA_2^{Gly}$ regions of the Gly-tRNA profile and the second, the $tRNA_3^{Gly}$ region) and then loading each group onto a gel. All the spots from each gel were then eluted, and the anticodon region was sequenced to identify the $tRNA_2^{Gly}$. In this way, we determined that the $tRNA_2^{Gly}$ from *sufS605* was retarded and recoverable in the $tRNA_3^{Gly}$ group of pooled fractions. Visualization of the appropriate spot on the gel, however, required longer than usual staining time. The $tRNA_2^{Gly}$ from *sufS617* was not recoverable from either of the two groups. The mutant *sufS617* $tRNA_2^{Gly}$ either is highly hydrophobic and runs very slowly on the column (in the "high-salt wash") or is produced in even less quantity than the $tRNA_2^{Gly}$ of *sufS605*. Mutants *sufS625* and *sufS627* have not yet been analyzed at the tRNA level.

RNA Sequence Analysis of Wild-Type and Mutant Glycine tRNAs. The three wild-type *Salmonella* glycine tRNA species were eluted from gel spots, and their nucleotide sequences were determined. The $tRNA_1^{Gly}$ sequence was as previously determined for *Salmonella* (33). The $tRNA_2^{Gly}$ sequence was identical to that of *E. coli*, and the same was true for the portion of *Salmonella* $tRNA_3^{Gly}$ that we analyzed (the 5′ portion up to the extra arm).

The $tRNA_2^{Gly}$ nucleotide sequences from three *sufS* mutants, *sufS601*, *sufS605*, and *sufS609*, differed from the wild type. The $tRNA_2^{Gly}$ species from *sufS601* and *sufS609* were identical, displaying the change of modified uridine (U*) to C-34 (Fig. 1). We detected no other alteration in those molecules, not even in the nature and extent of nucleoside modification. The *sufS605* $tRNA_2^{Gly}$, which was located at an uncharacteristic position in the 2-dimensional gel, differed from wild-type $tRNA_2^{Gly}$ in two respects. The primary sequence change was the insertion of a uridine into the TFC loop of $tRNA_2^{Gly}$ to make three adjacent uridines (Fig. 1). In addition, a new modified nucleoside was seen: pseudouridine for U-13. We consider it likely that the primary sequence change leads to a decrease in $tRNA_2^{Gly}$ maturation. For example, the insertion of a nucleotide into the TFC loop may decrease RNase P activity or interfere with 3′-end processing. The mutational alteration in mutant *sufS617* may result in a similar, but more severe, defect in tRNA processing. The reduced level of aminoacylated mutant tRNA suggests a possible explanation for the frameshifting by *sufS605*. There may be starvation-induced frameshifting at the decoding site, as has been documented for a very different example (34).

Does Wild-Type *Salmonella* Have a GGA-Reading Mutant Form of $tRNA_3^{Gly}$? *In vivo* the *E. coli* $tRNA_2^{Gly}$, which reads GGA and GGG and is encoded by the single-copy gene *glyT*, has been shown to be the only GGA-reading glycine-inserting tRNA (35). In *E. coli*, one of the three *glyV* genes (*glyV* codes for $tRNA_3^{Gly}$, which reads GGU and GGC) can mutate to a GGA-reading form by a guanosine to uridine change at nucleotide 34 of the tRNA. The mutant *glyV*-encoded tRNA, originally designated "*ins*" (36, 37) but later *glyV55* tRNA (38), is visible in the RPC-5 profile as a peak of glycine-accepting tRNA between the peaks for $tRNA_1^{Gly}$ and $tRNA_3^{Gly}$. Fig. 3 displays the profiles of glycylated wild-type *Salmonella* tRNA co-chromatographed with *glyV55*-containing *E. coli* tRNA. From this figure and reexamination of Figs. 2*A* and *B* our profiles of wild-type and *sufS Salmonella* tRNA clearly exhibit no *ins* (*glyV55*) peak. The small peak that follows $tRNA_1^{Gly}$ is a form of $tRNA_2^{Gly}$; profiles for mutants *sufS605* and *sufS617* "lose" not only the main $tRNA_2^{Gly}$ peak but also the small leading peak and the small peak that follows $tRNA_1^{Gly}$ and is "under" *ins* tRNA in Fig. 3. Finally, in our standard two-dimensional gel system, the *E. coli ins* tRNA does not migrate differently from $tRNA_3^{Gly}$. Consequently, sequence analysis of the tRNA extracted from the $tRNA_3^{Gly}$ spot from an *ins*-containing strain reveals heterogeneity at position 34: guanosine for wild-type $tRNA_3^{Gly}$ and uridine for the *ins* tRNA (data not shown). No such heterogeneity was seen, however, in the $tRNA_3^{Gly}$ spots of three *Salmonella* strains, the *trpE91*-containing (otherwise wild-type) parental strain and the mutant strains *sufS601* and *sufS605*. We conclude that these *Salmonella* strains do not have a mutant form of $tRNA_3^{Gly}$ that reads GGA.

DISCUSSION

The alterations in $tRNA_2^{Gly}$ that promote −1 frameshifting and are responsible for the investigated *sufS* suppressors are in the acceptor stem, the TFC arm and the anticodon.

In the acceptor stem the potential for positions 1 and 72 pairing is present in all elongator tRNAs but absent in initiator tRNAs (39). Normally $tRNA^{fMet}$ interacts with initiator factor IF2 and binds directly to the ribosomal P site. However, mutants of $tRNA^{fMet}$ that allow position 1 to

position 72 base pairing can bind directly to elongation factor-Tu·GTP, and their functioning as elongators is facilitated. The acceptor stem mutant, *sufS627*, has a G → A substitution at the first base of the stem, which may disrupt the position 1 to position 72 base pairing. The mutant $tRNA_2^{Gly}$, nevertheless, still functions as an elongator (see below), albeit with defective codon-framing properties. Whether the mutant tRNA has reduced efficiency of interacting with elongation factor-Tu·GTP and this directly causes the defective framing property has not been investigated. Absence of position 1 to position 72 pairing may also make such a mutant peptidyl tRNA a poor substrate for peptidyl tRNA hydrolase (40), which is associated with the proposed P-site editing (41). Position 1 is not the only site in the acceptor stem where alterations can cause frameshifting, however, because a substitution at position 70, found in a missense suppressor lysine tRNA, has also recently been shown to have this property (42). The behavior of either, or both, of these acceptor stem mutants may reflect interaction of the tRNA with an as-yet-unidentified domain of the ribosome, perhaps a ribosomal RNA.

Three alleles have their primary change in the TFC arm. Position 61 in the TFC arm is cytidine in 99.5% of tRNAs except those in mitochondria (43). In isolated $tRNA^{Phe}$ and $tRNA^{Asp}$ this base, through its N-4 amino group, is hydrogen bonded with the phosphate at position 60 (44). A variant of $tRNA^{Asp}$ with C-61 → U substitution has been constructed. This alteration prevents interaction with the phosphate at position 60 and probably weakens hydrogen bonding of that phosphate to the ribose at position 58, with a resultant weakening of the TFC loop structure (44). In mutant *sufS617* the cytidine normally at position 61 is also replaced by uridine, and this substitution provides the first indication of a role of TFC arm sequences or structure affecting reading frame maintenance. In another mutant, *sufS605*, the two adjacent uridines at positions 59 and 60 are extended to three uridines. The adenosine at position 58, which is found in most tRNAs, forms a reverse Hoogsteen pair with T-54 (uridine at 58, or 58a, is only found in certain mitochondrial tRNAs and is incapable of such pairing) (see ref. 44). Pairing of A-58 to T-54 is important for maintaining the distinctive TFC loop structure (44). In $tRNA_2^{Gly}$, by analogy with $tRNA^{Asp}$, the bases at position 59 and 60 are presumed to bulge out. Increasing the number of bases to be bulged out is likely to affect both stability of the reverse Hoogsteen pair and hydrogen bonding of the phosphate at position 60. In a third mutant, *sufS625*, substitution of adenosine for C-62 breaks a conserved base pair in the stem and is expected to influence the structure of the loop. In both *sufS605* and *sufS625* the resultant TFC loop is likely to have increased flexibility and to more resemble an anticodon loop in structure (see ref. 44). Such a structure could prevent intercalation of the D loop G-18 between residues 57 and 58. Because of normal interaction between the D and TFC loops, it is not surprising that alterations in the primary structure of the TFC arm affect the nucleoside modification pattern of the D arm. The modification pattern of the mutant $tRNA_2^{Gly}$ from *sufS617* and *sufS625* has not yet been examined, but such analysis of *sufS605* shows a nucleoside modification that is not detectable in wild-type $tRNA_2^{Gly}$—namely, pseudouridine for U-13 (see Fig. 1 and legend) in the D arm.

We do not know how the mutations in the TFC arm influence frame maintenance, but among the possibilities, apart from a direct conformational influence on the anticodon, are alterations in the proposed interactions of this arm with either elongation factor-Tu·GTP (45), the D arm, or directly with ribosomal RNA (46). Interestingly, wild-type yeast mitochondrial $tRNA^{Phe}$ has an extra nucleotide in the TFC stem that cannot be base paired (47), and runs of uridine, which it decodes, are known to be especially prone to ribosomal frameshifting (48). It is even possible that the modification change in the D arm is directly responsible for the frameshifting. Previously a change in the D stem was shown to enhance UGA reading by an otherwise wild-type UGG-reading tryptophan tRNA (see ref. 49). The D-stem mutant appears to be the only example of a change in the coding specificity of a tRNA that does not alter the anticodon arm (see ref. 49) prior to this work and our concurrent studies (ref. 42; M. O'Connor, R. F. Gesteland, and J.F.A., personal communication). Evidence that the D-stem mutant acts to reduce the rate at which the ribosome can reject tRNA, which is partially noncognate for UGA reading, has been presented (49).

Three independent representatives of the most efficient *sufS* suppressors, *sufS601*, *sufS607*, and *sufS609*, show a single alteration, a U*-34 → C base change in the anticodon. In wild-type $tRNA_2^{Gly}$ a modified uridine at position 34 pairs with the third base, adenosine, of the codon read by the tRNA, namely GGA. With cytidine at position 34 rather than the modified uridine there is sometimes doublet reading of the first two bases, GG, of GGA, for the insertion of glycine, with consequent frameshifting (20). Thus cytidine at position 34 rather than the modified uridine sometimes results in the mRNA 3′ codon base adenosine being the first base of the following codon rather than the third base of the glycine codon (19). Mutant *sufS601* does not cause frameshifting when GGA is altered to GGG (20); presumably because its $tRNA_2^{Gly}$ anticodon is CCC, there is stable pairing of the anticodon base at position 34, cytidine, with the third codon base, guanosine, preventing frameshifting. These site-of-action studies have not been extended to the other suppressor mutants.

Besides causing frameshifting, the *sufS*-encoded mutant tRNAs must also be able to read GGA in a triplet mode sufficiently well for the cell to grow. *In vivo E. coli* $tRNA_2^{Gly}$ has been shown to be the only GGA-reading glycine-inserting tRNA (35), and we conclude that this is also the case in *Salmonella* (see *Results*). The dual capabilities of frameshifting and in-frame triplet decoding could be achieved by two conformations of each tRNA. A characteristic even of wild-type *Salmonella* $tRNA_2^{Gly}$ that may contribute to alternate conformations is that the D-arm sequence may be able to form an alternative secondary structure, as has been pointed out for the glycine tRNA of phage T4 (50). The D arm is known to interact with the TFC arm in those tRNAs where the structure has been determined.

Mutants *sufS601*, *sufS607*, and *sufS609*, which have the CCC anticodon, invite another consideration for GGA reading as a triplet. Several other cases are known of the decoding of a codon ending in adenosine by a tRNA the anticodon of which begins (5′ end) with cytidine at position 34 (51–54). The mechanism for decoding is not known. One suggestion (55) is that some conformational feature of the tRNA allows the tautomerization of C-34 to the rarer imino form. Such a change could, in turn, allow a Watson–Crick type hydrogen bonding between C-34 and the 3′ adenosine residue of the codon, resulting in an A·C pair that is sterically equivalent to an A·U pair. It is noteworthy that wild-type *E. coli* $tRNA_1^{Gly}$, which has the same anticodon as *sufS601*, *sufS607*, and *sufS609*, does not read GGA *in vivo* well enough for the cells to grow (35), and this situation is likely to be true for *Salmonella* also (see above). Consequently, the different context of the $tRNA_1^{Gly}$ anticodon (5′ -CCC- 3′)—i.e., in the $tRNA_2^{Gly}$ "body," somehow allows the tRNA to substantially "mis"read GGA as a triplet (as well as a doublet). [The nucleotide sequences of $tRNA_1^{Gly}$ and $tRNA_2^{Gly}$ are ≈30% nonhomologous (ref. 43; see Fig. 1).]

Whatever the mechanism of action of these various suppressors, their study shows that tRNAs with normal anticodon loop sizes can cause frameshifting at cognate codons.

This conclusion is reinforced by the very recent finding that several missense and nonsense suppressors with seven-membered anticodon loops can act to correct +1 frameshift mutants (42), in clear contrast to the classical +1 suppressors, which contain enlarged anticodon loops. The inference is that the special characteristic of a tRNA involved in promoting frameshifting, be it in a wild-type or in a mutant situation, is more subtle than solely an anticodon loop enlarged by one nucleotide for +1 frameshifting, or decreased by one nucleotide for −1 frameshifting. Involvement of parts of a tRNA molecule outside the anticodon in action-at-a-distance effects on reading-frame maintenance is indicated and provides new criteria to consider when characterizing or searching for tRNAs responsible for natural frameshifting.

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