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Research Articles

Reading Frame Selection and Transfer RNA Anticodon Loop Stacking

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Messenger RNA's are translated in successive three-nucleotide steps (a reading frame), therefore decoding must proceed in only one of three possible frames. A molecular model for correct propagation of the frame is presented based on (i) the measured translational properties of transfer RNA's (tRNA's) that contain an extra nucleotide in the anticodon loop and (ii) a straightforward concept about anticodon loop structure. The model explains the high accuracy of reading frame maintenance by normal tRNA's, as well as activities of all characterized frameshift suppressor tRNA's that have altered anticodon loops.

MAINTENANCE OF THE TRANSLATIONAL READING FRAME is essential for useful gene expression. However, the detailed mechanism by which ribosomes, transfer RNA's (tRNA's), and the message interact to minimize frameshifts has not been clearly defined.

Studies of the activities of tRNA's with eight, rather than the normal seven, nucleotides (nt's) in the anticodon loop have suggested that the length of the translational step is metered by the tRNA. Several such tRNA's have been isolated by selective suppression of single nucleotide insertion (frameshift) mutations in *Salmonella* (*1–3*) and yeast (*4, 5*).

However, previous data do not suggest a unified set of translocational properties. For example, some frameshift suppressor tRNA's act only when four anticodon nucleotide pairs can be formed, while others do not require a fourth nucleotide pair (*1–5*). None of these tRNA's has been tested for translation of 3-nt codons. Missense suppressors exist (*6, 7*) that contain an extra nucleotide in the anticodon loop, but decode 3-nt codons. Finally, there is a wild-type yeast mitochondrial tRNA with an 8-nt anticodon loop that presumably favors 3-nt codons (*8*). The rule that unites these observations was not evident.

We surmised that measurement of both 3-nt and 4-nt translation by individual 8-nt anticodon loop tRNA's would clarify reading frame maintenance. To systematically study the translational activities of such tRNA's, we constructed tRNA genes that contain each nucleotide inserted 5′ to the anticodon of an amber suppressor tRNA. Each of these tRNA's was tested for both 3- and 4-nt decoding efficiencies of an amber codon (UAG) created in *Escherichia coli lacZ*. We varied the nucleotide 3′ to the amber codon in the message in order to detect possible fourth nucleotide pair interactions between each tRNA's anticodon loop and the message.

Our mutant tRNA's translate the same 4-nt message sequence in these messages as a 3- or a 4-nt codon. We suggest that two readily

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interconvertible tRNA conformations corresponding to the structural isomers of Bossi and Smith (*3*), which differ in the number of stacked nucleotides on the 3′ side of the anticodon loop, determine whether three or four nucleotides are translated. By reference to the stereochemistry of stacks in RNA hairpin loops, the model explains the apparent decoding activities of a variety of frameshift suppressor tRNA's, and suggests how tRNA's with normal-sized anticodon loops translate the message in 3-nt steps with the observed high degree of accuracy.

The assay for translational step size. We constructed tRNA's with each of the four nucleotides inserted 5′ to the normal anticodon of Su7 (Fig. 1). We chose Su7, an amber suppressor, because we can quantitate decoding efficiencies at amber codons in vivo. Such measurements are more difficult at sense codons. Our suppressors insert glutamine. In order to promote efficient aminoacylation, we used a cloning vector that encodes the *E. coli* glutaminyl tRNA synthetase gene (*GlnRS*). Because of the high copy number of the vector, *GlnRS* activity is 40 times its normal level in extracts of these strains (*9*).

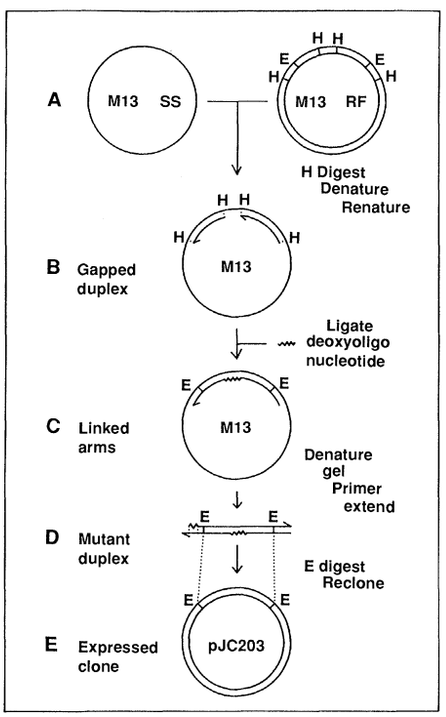

Fig. 1. Insertions of each nucleotide into the anticodon loop of Su7. This procedure is an extension of the gap-filling procedure (*34*). Details of the protocols used are available on request. The objective is to replace an Hpa II fragment that contains the anticodon loop with mutant sequences encoded by synthetic oligonucleotides. (**A**) An M13 mp8 (*35*) clone encoding the Su7 gene on an Eco RI fragment was used to obtain both a single-stranded (SS) genome preparation (from the virions) and a double-stranded (RF) preparation. The RF was digested with Hpa II (H), and the fragments that flank the anticodon region of Su7 (Hpa II arms) were purified from a polyacrylamide gel. (**B**) To generate the partially duplex molecule containing a gap in the anticodon region shown, the Hpa II arms were denatured in the presence of the single-stranded genome, then annealed. This mixture was precipitated with ethanol and the DNA was dissolved in a small volume of buffer. (**C**) For placement of the mutagenic oligonucleotide in the gap, the oligonucleotide was added to the gapped molecule preparation. The mixture was incubated at 75°C for 5 minutes, then allowed to cool to room temperature over 20 minutes, diluted into an appropriate buffer, and then treated with T4 DNA ligase (E, Eco RI). (**D**) The ligation step above covalently links the single-stranded Hpa II arms to the mutagenic oligonucleotide. The linked single-stranded arms fragment was separated from the single-stranded genome and all unlinked arms by fractionation on a 5 percent denaturing polyacrylamide gel. This fragment was then made double-stranded by primer extension. DNA synthesis with this fragment as template causes the mutations encoded by the oligonucleotide to be copied into the newly made strand. As a result, 100 percent mutants may theoretically be obtained among the tRNA's that are generated. (**E**) The Eco RI fragment containing the Su7 gene was then cloned with either pOP203 (*36*) or pJC203. Plasmid JC203 differs from pOP203 only in that pJC203 encodes the *GlnRS* gene. All mutations were verified by DNA sequencing. The mutant tRNA's are named by the inserted nucleotide, followed by 33.5 to indicate the location of insertion between nucleotides 33 and 34; A33.5 is the mutant with an A inserted 5′ to the anticodon.

The tRNA's were assayed at amber codons created by site-directed mutagenesis in a plasmid-encoded *lacZ* gene. The altered codon (codon number 366, coding for glutamine), is deep within the *lacZ* gene to avoid activity due to reinitiation of translation. The plasmids carrying the *lacZ* amber alleles are compatible with those carrying the cloned tRNA genes. Suppression of the *lac* amber mutations by the altered tRNA's restores enzyme activity, providing an assay for decoding efficiency.

We made three sets of four *lacZ* alleles (Table 1). We expected that suppression (and thus β-galactosidase activities) would be low in at least some cases. To facilitate comparison of low activities, we compared message sequence changes that conserve, as much as possible, the amino acid sequences of the active suppressor-dependent products.

One message set was used to examine 4-nt decoding at message sequences UAG:N. That set (termed UAG:N4) was made by converting codon number 366 (CAG) to an amber (UAG) codon and inserting each nucleotide 3′ to the amber codon. Translation of the 4-nt codons, UAG:N4, by our Su7 derivatives should not result in any alteration of amino acid sequence in β-galactosidase.

A second set of messages (UAG:N3) was used to measure 3-nt translocation at sequence UAG:N. Rather than inserting a nucleotide 3′ of the amber codon, we made this set by altering the first nucleotide of the codon 3′ to the UAG (codon number 367, coding for valine in the wild type). Since our suppressors insert glutamine, translation of the amber codon by our mutant suppressors does not alter the amino acid normally inserted at that position. However, because changing the first nucleotide of codon number 367 alters the amino acid inserted at that sense codon, possibly affecting enzyme activity, we made a third set of alleles (termed CAG:N3) to control for such effects. That set has the altered 3′ codons, but retains the normal glutamine codon at position 366 (sequence CAG:N). These messages were used to determine the effects on enzyme function of amino acid substitution at codon 367. Those substitutions have small, but measurable effects on β-galactosidase activity (legend to Table 2).

With these message sets, we can determine 3- and 4-nt decoding efficiencies at each 4-nt sequence, UAG:N. For example, the UAG:A3 message contains the sequence UAG:A and requires 3-nt reading at that sequence to yield active β-galactosidase. In contrast, UAG:N4 requires 4-nt reading at UAG:A for active *lacZ* product. By measuring *lacZ* activities produced from these two messages in two strains containing a particular cloned tRNA, we determined the activity of that tRNA for 3- and 4-nt reading at UAG:A.

Table 1. Sequences of the message mutants used to determine 3- and 4-nt decoding efficiencies shown in Table 2.

Allele	Sequence	Used for testing
UAG:A4	GGU UAGA GUC	4-nt translation at UAG:N
UAG:G4	GGU UAGG GUC	
UAG:U4	GGU UAGU GUC	
UAG:C4	GGU UAGC GUC	
UAG:A3	GGU UAG AUC	3-nt translation at UAG:N
UAG:G3	GGU UAG GUC	
UAG:U3	GGU UAG UUC	
UAG:C3	GGU UAG CUC	
CAG:A3	GGU CAG AUC	Effects of amino acid changes at codon 367
CAG:G3	GGU CAG GUC	
CAG:U3	GGU CAG UUC	
CAG:C3	GGU CAG CUC	

The *lacZ* mutations were made by site-directed mutagenesis (*20*). Mutants for testing 3-nt decoding were made by converting the CAG (Gln) codon at position 366 of *lacZ* to UAG and changing the first nucleotide of the next 3′ codon appropriately. To make mutants for measuring 4-nt decoding, codon 366 was converted to UAG and a nucleotide was inserted 3′ to the amber codon.

Our UAG:N messages also control partially for message context effects on suppression. Since all mutations were made at the same site, and with a minimal number of nucleotide substitutions and insertions, all messages were identical with the exception of the nucleotide inserted (UAG:N4) or altered (UAG:N3) 3′ of the amber codon. Furthermore, the nucleotide 3′ of UAG:N in the 4-nt messages is always G. However, for the 3-nt messages, the nucleotide following UAG is varied. Thus, for the UAG:G pair of messages, the contexts are identical. The other pairs of 3- and 4-nt messages have only a single nucleotide difference in their contexts. We show below that these small context variations have relatively small effects on suppression.

Decoding properties of our tRNA mutants. All our mutant tRNA's translate both 3- and 4-nt codons with a consistent set of tendencies although magnitudes of suppression vary among tRNA's. However, the absolute translational efficiency of tRNA's with 8-nt loops is generally much lower than those with 7-nt anticodon loops (Table 2). One tRNA insertion derivative, G33.5, shows no dependable increase in suppression above the control (no tRNA column of Table 2). Low efficiencies for U33.5, C33.5, and A33.5 are attributable in part to poor aminoacylation, which is improved by including the GlnRS gene on the vehicle. However, our conclusions depend on the ratio of activities of the same tRNA in translation of two different messages, UAG:N3 and UAG:N4. The ratio of 3- to 4-nt translation is therefore unaffected by difficulties in maturation or aminoacylation encountered by these mutant tRNA's.

Su7 U33.5 translates both 3- and 4-nt codons at all sequences UAG:N when compared to the "no tRNA" control (compare columns 1 and 3 of Table 2), thus both 3- and 4-nt decoding can occur at the same 4-nt sequence. When the fourth message nucleotide is G, C, or U, 3-nt decoding is more common than 4-nt reading. However, when a fourth nucleotide pair is possible, 4-nt reading is predominant (compare UAG:A4 to UAG:A3 in column 3, Table 2). Even in this case triplet translation remains about half as frequent as 4-nt translation despite the possibility of a fourth nucleotide pair.

Su7 C33.5 shows a similar pattern (Table 2). This tRNA carries out significant 3- and 4-nt reading on all messages. However, at UAG:G where four nucleotide pairs are possible, 4-nt translation is more than 40 times as frequent as 3-nt translation (after background is subtracted). Thus, again, a fourth Watson-Crick nucleotide pair increases the relative frequency of 4-nt translation. There is a peculiarity most evident in this column. Suppression by 3 and 4 nt is greater for the UAG:A messages than for the UAG:G messages, despite the possibility of a standard nucleotide pair in the latter messages. Thus, the possibility of a fourth nucleotide pair does not necessarily increase the probability that a tRNA will act in translation, although the ratio of 3- to 4-nt translation is as usual for both UAG:A and UAG:G.

Particularly inefficient are tRNA's with a purine inserted between 33 and 34. As mentioned above, we cannot measure the activity of G33.5 tRNA. However, A33.5 shows a fragment of the same pattern as the pyrimidine insertion mutants. That is, 3-nt translation predominates at UAG:A, but 4-nt translation is more frequent at UAG:U where a complementary nucleotide pair is possible.

Message context effects do not account for the predominance of 4-nt translation when four nucleotide pairs are possible. An effect of message context, uncontrolled in these comparisons of 3- and 4-nt messages, could alter relative frequencies of 3- and 4-nt suppression.

However, a predominance of 4-nt translation was not associated with any particular message comparison, nor with any particular tRNA; instead, it was associated with the possibility of four nucleotide pairs between tRNA and message. Further, the preference for 4-nt decoding with four nucleotide pairs was marked in the comparison of UAG:G3 and UAG:G4, a comparison in which the codons have the same 3′ message nucleotide neighbor. Those results suggest that context differences in our messages cannot account for the observed strongly ordered decoding pattern.

Two summary rules for translational step size by 8-nt anticodon loops. (i) When only three nucleotide pairs are possible between tRNA and message, 3-nt translation is most probable, although 4-nt translation may also occur. (ii) Where four Watson-Crick nucleotide pairs are possible between tRNA and message, 4-nt translation is most probable, although 3-nt translation may also occur.

Molecular model for translational step-size determination. We now combine these rules with a model for anticodon arm structure which obeys the constraints intrinsic to an A-form helix and loop and thus provides a simple molecular explanation for reading frame selection. For this model, we make two principal assumptions regarding the active structure of tRNA.

1) We assume that an anticodon arm helix of the A type, continued by an extended stack of nucleotides on the 3′ side of the loop, is the effective structure of normal tRNA in the ribosomal P site (shown schematically in Fig. 2A). That structure is observed for tRNA's in crystals (*10–13*), and a 3′ stack is likely to be the active form in both the A and P sites (*9, 14*).

2) In all normal tRNA's, the anticodon consists of the three distal nucleotides of the 5-nt 3′ stack (Fig. 2A). Our model is based on the assumption that all anticodons will be at this same position with respect to the anticodon arm.

Under the first assumption, upper limits to the size of a continuous 3′ stack in anticodon loops can be deduced from the calculations of Pleij *et al.* (*15*), who determined the phosphate-to-phosphate distances required to bridge the strands of an A-form helix across the large groove; that is, to close a stacked loop like the one that

Table 2. *lacZ* activities due to suppression by mutant tRNA's. All values are percentage activity relative to a CAG:N control. Activities were determined for averages of 4 to 16 assays for each strain. Standard errors of the mean were always less than 10 percent. All 4-nt messages are referred to CAG:G (17,000 beta-galactosidase units). The 3-nt messages are referred to the appropriate CAG:N message (CAG:A gives 13,500 units; CAG:U gives 18,600 units; CAG:C gives 9,800 units). Boldfaced values on the diagonal are from strains where the inserted tRNA nucleotide is complementary to the nucleotide 3′ to the amber codon. "No tRNA" control strains contained pJC203. These controls have not been subtracted from the measurements in the table. Beta-galactosidase assays were performed as described (*20*), except that a correction was made to account for loss of enzyme activity during the course of the assay. Beta-galactosidase activity decays with first-order kinetics with a half-life of 988 minutes (average of eight determinations, J.F.C., unpublished results). The unit calculation is as follows:

$$\text{Units} = 1000\, k\, A_{420}/[V(1 - e^{-kt})A_{550}]$$

where A_{420} is absorbance of the assay mixture at time T; A_{550} is turbidity of the culture at time of assay; V is the culture volume assayed; and k (0.693/988 minutes) is the first-order decay constant for β-galactosidase activity.

Message	No tRNA	tRNA				
		Su7	Su7 U33.5	Su7 C33.5	Su7 A33.5	Su7 G33.5
UAG:A3	0.026	49	**0.38**	4.4	0.11	0.024
UAG:A4	0.008	0.01	**0.66**	1.1	0.024	0.025
UAG:G3	0.019	55	0.48	**0.038**	0.019	0.025
UAG:G4	0.006	0.005	0.27	**0.82**	0.016	0.019
UAG:U3	0.012	29	0.13	0.28	**0.01**	0.007
UAG:U4	0.009	0.009	0.065	0.12	**0.024**	0.007
UAG:C3	0.082	54	0.71	2.6	0.012	**0.023**
UAG:C4	0.013	0.005	0.09	0.1	0.013	**0.014**

contains the anticodon. Closure of a 7-nt anticodon loop requires a minimum of two 5′ nucleotides (in the 3′-endo conformation, bridging 7.5 to 8 angstroms). Thus the 3′ stack can contain no more than 5 nt. The 5′ nucleotides that close the loop are unavailable for nucleotide pairing with the message (*10*).

The first assumption is also supported by our previous observation that when the nucleotide 5′ of the anticodon is complementary to the nucleotide 3′ of the in-phase codon, amber suppression efficiency for 7-nt loops is unaffected for all combinations of complementary nucleotides (*16*). Thus a fourth nucleotide pair, which would require breaking the 3′ stack, seems to be forbidden when a 7-nt tRNA loop is in the ribosomal coding sites.

Our model allows for normal tRNA's to have anticodon loop 3′ stacks that contain fewer than 5 nt. However, it is likely that the 5-nt anticodon stack is usually preserved by nucleotide pairing with the P-site codon.

The second assumption is supported by the properties of missense suppressors isolated by Murgola (*6, 7*) that contain an extra nucleotide in the anticodon loop. The mutations that create these tRNA's differ from our insertion mutants in that the extra nucleotide is inserted 3′, rather than 5′, of the normal anticodon. When such tRNA's assume a 5-nt 3′ stack conformation, the nucleotides at the normal position of the anticodon within the stack are offset from the normal anticodon sequence by a single nucleotide (Fig. 2D). These tRNA's have a missense suppressor phenotype because they translate codons complementary to the new set of shifted anticodon nucleotides, and thereby insert a novel amino acid at missense codons.

Eight-nucleotide anticodon loops may assume two active conformations. In contrast to the single active structure likely for 7-nt anticodon loops on ribosomes, we suggest that 8-nt anticodon loops may assume either of two 3′ stack conformations that correspond to either 3- or 4-nt translation (below and shown schematically in Fig. 2, B and C). We have adopted structural isomers similar to those suggested by Bossi and Smith (*3*).

1) An 8-nt anticodon loop tRNA can contain a 5-nt 3′ stack and use three 5′ nucleotides to close the loop (the 5-3 conformation). We suggest that this stack which, like normal tRNA, has only 3 nt available for interaction with the message, always translates in 3-nt steps (compare Fig. 2, A and C). The predominance of 3-nt translation that occurs when a fourth nucleotide pair is not possible therefore reflects a preference for the 5-nt 3′ stack under those conditions.

2) An 8-nt anticodon loop may contain a 6-nt 3′ stack and close the loop with the remaining 2 nt (the 6-2 conformation, Fig. 2B). We suggest that this structure causes 4-nt frameshift errors because the extra nucleotide stacked against the 5′ side of the normal anticodon can occupy a fourth message nucleotide when the tRNA resides in the P site (Fig. 3B). Thus the next triplet available for translation is shifted 1 nt 3′ relative to the initial reading frame.

Concerning the 5-3 conformation: Bossi and Smith (*3*) supposed that this stack could cause 4-nt frameshifts. However, because the relative frequency of 4-nt translation is always increased by the possibility of a fourth nucleotide pair, we suggest that only a 6-nt 3′ stack (described below) decodes in 4-nt steps. Furthermore, if a 5-nt 3′ stack could cause 4-nt translation, it is difficult to explain the occurrence of a yeast mitochondrial tRNA that has an 8-nt anticodon loop and presumably selectively decodes 3-nt codons (*8*). That tRNA may exist predominantly in the 5-nt 3′ stack anticodon loop arrangement, even when presented with the possibility of nucleotide pairing at the fourth position.

With regard to the 6-2 conformation, we argue that the predominance of 4-nt translation when four nucleotide pairs are possible between tRNA and message is a consequence of stabilization of the extended stack by a fourth nucleotide pair.

However, four nucleotide pairs are not required for 4-nt translation. We observe that even in the absence of a stable interaction at the fourth tRNA:codon position, the enlarged 3′ stack is always sufficient to force the frameshift (Table 2 and Fig. 3B). Therefore, it is the size of the anticodon stack, and not the number of codon-anticodon nucleotide pairs, that is the determinant of translational step-size.

An explicit model for step-size determination. We suppose that during translocation, the body of the tRNA is moved from the A to the P site (from the aminoacyl tRNA selection to the peptidyl site) making contacts at fixed positions within the ribosome. That movement results in an approximate 3-nt displacement of the anticodon-codon complex relative to the ribosome (Fig. 3A). An extended stack of an 8-nt anticodon loop tRNA formally causes a 3-nt translocation because the codon-anticodon nucleotide pairs are at the normal position with respect to the body of the tRNA (the second assumption, above) (compare Fig. 3, A and B). The next codon is, operationally, the first triplet not occluded by the anticodon stack of the P-site tRNA (Fig. 3, A and B). A frameshift occurs if the next available triplet is out of phase because the next translocation results in a 4-nt displacement of the message (Fig. 3, C and D), as a consequence of the unvaried movement of the body of the tRNA.

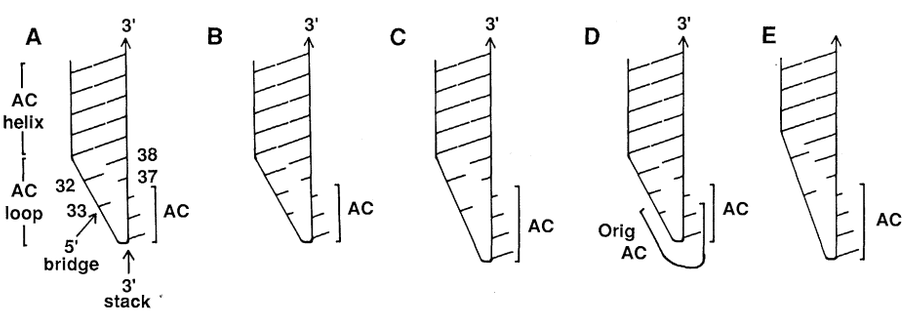

Fig. 2. Schematic anticodon arm structures for 7-, 8-, and 9-nt tRNA loops. The brackets marked AC show the anticodon nucleotides. In every case the 3′ nucleotide of the anticodon is at the same position with respect to the top of the helix or the body of the tRNA. (**A**) A normal-sized anticodon loop in a 3′ stack. The 5 nt on the 3′ side of the loop are in a stack that is continuous with the anticodon stem helix. The backbone is kinked 5′ of the anticodon, with nucleotides 32 and 33 bridging the major groove of the A-form helix to close the loop. (**B**) The 5-nt 3′ stack. One likely conformation (called 5-3 in the text) of an 8-nt anticodon loop. In this conformation, the extra nucleotide exists 5′ of the backbone kink that separates the anticodon stack from the nucleotides on the 5′ side of the loop. This tRNA, which has a nearly normal anticodon stack, is likely to cause 3-nt translations. (**C**) The 6-nt 3′ stack. In this conformation (called 6-2), the nucleotide inserted between 33 and 34 joins the anticodon stack. Pairing between the inserted nucleotide and the fourth message nucleotide stabilizes this conformation, which predisposes the tRNA to 4-nt translation. (**D**) A missense suppressor (*6*) that contains a nucleotide inserted 3′ of the anticodon. When this tRNA assumes a 5-nt 3′ stack, the nucleotides which occupy the anticodon position are offset from the wild-type anticodon by 1 nt. (**E**) A possible conformation for a frameshift suppressor with a disrupted nucleotide pair at the distal position of the anticodon stem (*18*). In this mutant, which contains a 9-nt loop, a 7-nt 3′ stack is possible. A minimum of 2 nt is required to close an anticodon loop that contains a 7-nt 3′ stack (see text). The 7-nt stack provides a 4-nt anticodon at the same position as the 6-nt 3′ stack in (C), which reads 4-nt codons.

This view is appealing because of its simplicity. In particular, it is easy to imagine how this mechanism for frame determination could originate on primitive ribosomes and subsequently evolve. As another corollary, this model explains why a 7-nt anticodon loop is an almost universally conserved feature of tRNA.

In the model we favor, the extended stack of an 8-nt loop has its effect in the P site by positioning the P/A boundary at a novel ribosomal locus. Translocation itself is viewed as a repetitive, unvarying displacement of the body of the tRNA. However, there is another point of view, also roughly consistent with our results, in which the P/A boundary is a fixed ribosomal locus. The 5′-most nucleotide of the anticodon stack is moved past that fixed point by translocation (Fig. 3).

We prefer the first model presented because it is simpler, and requires postulation of no unknown ribosomal apparatus. In addition, this second frameshift mechanism requires displacing or straining the strong bonds between the P site and the rest of the tRNA that has an 8-nt loop. It seems unlikely that a stacked nucleotide would be stable enough to support this strain, as required by our observations. In contrast, it is easy to imagine an extended stack trapped sterically by the entry of the next tRNA, as in the model we prefer.

The model unifies the diverse decoding activities of other tRNA's that have abnormal anticodon loops. Several tRNA's that contain an extra nucleotide in the anticodon loop suppress frameshift mutations apparently only when four Watson-Crick nucleotide pairs are possible between the tRNA and the message (*1*). Those tRNA's may exist in a 6-nt 3′ stack conformation only when a fourth message nucleotide pair is possible. Certain other frameshift suppressors do not require a fourth nucleotide pair to translate four message nucleotides (*2, 3, 5*). We suggest that those tRNA's may assume the 6-nt 3′ stack conformation with a significant frequency at most message contexts. It would be of interest to determine whether and with what frequency these tRNA's can also translate in 3-nt steps.

Our model also accommodates the translational activities of two tRNA's that have 9-nt anticodon loops. In those tRNA's, the anticodon arm contains the usual number of nucleotides. However, the usually paired nucleotides at the distal position of the anticodon helix are mismatched and thus cannot nucleotide pair. Our model suggests that those 9-nt anticodon loops could take alternative active conformations that correspond to either 3- or 4-nt translation. One conformation uses the same number of stacked nucleotides on the 3′ side of the anticodon arm as typical tRNA and should therefore decode 3-nt steps. In fact, the predicted 3- and 4-nt translation has been observed, although in different tRNA's. The $tRNA^{Leu}_{UUA}$ from the mitochondria of *Schizosaccharomyces pombe*, which is the only wild-type tRNA with a 9-nt anticodon loop, presumably decodes 3-nt codons (*17*) via that structure.

The other 9-nt loop tRNA (*suf*8) contains a mutation that disrupts the distal nucleotide pair of the anticodon stem of *Saccharomyces cerevisiae* $tRNA^{Pro}_{CCA}$ (*18*). That tRNA suppresses a frameshift mutation by a previously unknown mechanism. We expect that, like other frameshift suppressors, 4-nt translation by that tRNA is a result of a longer-than-normal anticodon loop stack. A 9-nt loop in an A-form hairpin may form a stack of 7 nt on the 3′ side, and bridge across the major groove with the other two nucleotides (*15*). This allows the formation of a stacked 4-nt anticodon at the normal position with respect to the rest of the tRNA (compare Fig. 2, E and C). The proposed anticodon can translate a completely complementary 4-nt codon within the suppression window of the frameshift used to characterize the tRNA (*19*), and thereby give the suppressor phenotype.

The potential accuracy of frame determination is often subverted by other ambiguities. We previously measured very low in vivo translocation error rates for normal tRNA's reading their cognate codon in phase [error frequencies $<3 \times 10^{-5}$ (*20*)]. That high accuracy was observed to be insensitive to nucleotide substitutions that saturate the anticodon loop (except the anticodon itself) and proximal helix, while preserving the 2° structure. Thus it appeared that a correctly paired anticodon-codon is sufficient to ensure accurate frame maintenance even when strongly perturbed by all possible changes in the ten other nucleotides proximal to the anticodon (*20*). Our current model accounts for this high and imperturbable accuracy as a consequence of the existence of the normal anticodon loop stack despite the nucleotide substitutions in all the tRNA variants tested.

Frameshifts generally are rare, but they do occur readily in a few exceptional cases. However, frameshifts in cells that lack suppressors can be explained without reference to aberrant anticodon stacks. For example, certain genes require high frequency frameshifts for normal expression (*21–23*). Those frameshifts occur when the message slips and then rephases against an anticodon stack when alternative nucleotides pairing arrangements are possible (*24–26*).

Frameshifts may also occur when the ribosome accepts a normal tRNA incorrectly paired in the A site (*27*). Kurland (*28*) observed that rates of reading frame errors are related to those of missense errors. For example, such errors can be increased by streptomycin (*29*). In addition, frameshifts due to tRNA selection errors can be induced by reduced concentrations of the charged cognate tRNA in vivo (*30–32*), or by increased concentrations of misreading species in vitro (*33*). Such errors may not require that P-site tRNA's suffer abberant translocations; instead, they are a result of missense errors because incorrect tRNA's or tRNA's paired to the wrong frame have been accepted.

Thus, we conclude that reading frame maintenance is potentially highly accurate because of an intrinsic stereochemical property of anticodon loop structure. However, the potential for high accuracy

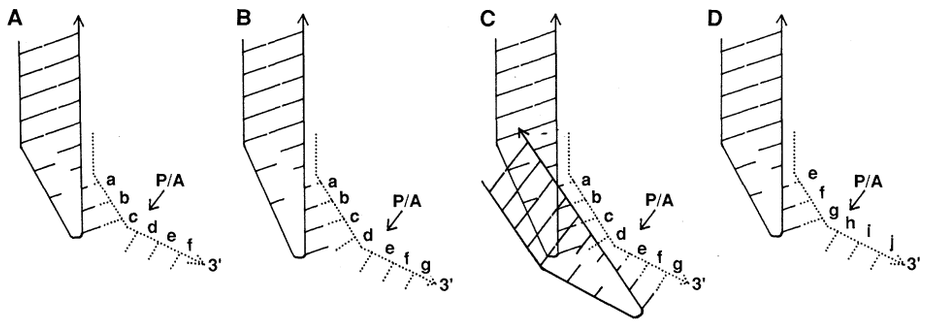

Fig. 3. The anticodon loop stack as the determinant of translocation step size. (**A**) The state of the anticodon arm–message complex of normal-sized tRNA after translocation. The 5-nt 3′ stack of a normal tRNA occupies three message nucleotides. Other stacking arrangements are not likely to be accessible to normal-sized anticodon loops on the ribosome. Thus normal tRNA is constrained to maintain the reading frame. (**B**) The anticodon loop of an 8-nt loop P-site tRNA in a 6-nt 3′ stack obscures the message nucleotide 3′ to the normal 3-nt codon. Thus, the next available message triplet is shifted 1 nt 3′-ward. (**C**) The next available triplet is occupied by a tRNA during the next translational cycle. (**D**) Translocation of the A-site tRNA paired to the shifted message triplet causes a 4-nt displacement of the message, which consummates the frameshift.

cannot always be realized because the ribosome does not select tRNA's paired to the correct reading frame with an equivalently high degree of accuracy, and also because messages may rephase against the anticodon stack when alternative nucleotide pairing arrangements are possible.

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Star Formation in W49A: Gravitational Collapse of the Molecular Cloud Core Toward a Ring of Massive Stars

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High-resolution molecular line and continuum radio images from the Hat Creek Radio Observatory and the Very Large Array suggest that the core of the W49A star-forming region is undergoing gravitational collapse. The radio continuum shows a 2-parsec ring of at least ten distinct ultracompact H-II regions, each associated with at least one O star. The ring is a region of large-scale, organized massive star formation. Recombination line velocities and HCO^+ excitation requirements indicate that the ring is rotating around 50,000 solar masses of material. Because the HCO^+ (1-0) line shows red-shifted absorption but blue-shifted emission, the molecular cloud core is believed to be collapsing toward the center of the ring. The HCO^+ radial velocities, as well as H-I, H_2CO, and magnetic-field measurements, fit a simple model of inside-out gravitational collapse of a once magnetically supported cloud.

ALTHOUGH IT IS A COMMON EXPECTATION THAT STARS form as a result of the collapse of molecular clouds, direct evidence of this process (*1*) is hard to find. The difficulties in finding a cloud in the stage of collapse are fourfold. First, molecular clouds are opaque to visible light, so they must be studied at infrared and radio wavelengths. Second, the time required for a cloud core to collapse (about 10^5 to 10^6 years) is small compared with the lifetimes of molecular clouds (about 10^7 to 10^8 years). Therefore, the core must be observed just as it undergoes a relatively short-lived phase in its history. Third, astronomers are limited to obtaining two-dimensional images of three-dimensional objects. Consequently, it is sometimes difficult to separate the components of a cloud that lie along the same line of sight. Finally, the small systematic

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