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Recognition and Positioning of mRNA in the Ribosome by tRNAs with Expanded Anticodons

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Mutant tRNAs containing an extra nucleotide in the anticodon loop are known to suppress +1 frameshift mutations, but in no case has the molecular mechanism been clarified. It has been proposed that the expanded anticodon pairs with a complementary mRNA sequence (the frameshift sequence) in the A site, and this quadruplet ''codon-anticodon'' helix is translocated to the P site to restore the correct reading frame. Here, we analyze the ability of tRNA analogs containing expanded anticodons to recognize and position mRNA in ribosomal complexes *in vitro*. In all cases tested, 8 nt anticodon loops position the 3′ three-quarters of the frameshift sequence in the P site, indicating that the 5′ bases of the expanded anticodon (nucleotides 33.5, 34, and 35) pair with mRNA in the P site. We also provide evidence that four base-pairs can form between the P-site tRNA and mRNA, and the fourth base-pair involves nucleotide 36 of the tRNA and lies toward (or in) the 30 S E site. In the A site, tRNA analogs with the expanded anticodon ACCG are able to recognize either CGG or GGU. These data imply a flexibility of the expanded anticodon in the A site. Recognition of the 5′ three-quarters of the frameshift sequence in the A site and subsequent translocation of the expanded anticodon to the P site results in movement of mRNA by four nucleotides, explaining how these tRNAs can change the mRNA register in the ribosome to restore the correct reading frame.

Keywords: ribosome; tRNA; mRNA; frameshift; suppressor

Introduction

Many mutations were isolated in *Salmonella typhimurium* on the basis of their ability to suppress single base-pair insertion (+1 frameshift) mutations.[1] These suppressor of frameshift (*suf*) mutations were classified by distinct suppression activities and mapped to separate loci.[2–5] Interestingly, most of the characterized *suf* alleles were found to encode tRNAs with anticodon loops expanded by one nucleotide. Alleles of *sufA*, *sufB*, *sufD*, *sufG*, and *sufJ* encoded 8 nt anticodon loop variants of $tRNA^{Pro1}$, $tRNA^{Pro2}$, $tRNA^{Gly1}$, $tRNA^{Gln1}$, and $tRNA^{Thr3}$ (8 nt loop tRNAs).[6–9] Analogous tRNA variants have since been isolated in *Escherichia coli* and *Saccharomyces cerevisiae*.[10–13] Among the first characterized was a *sufD* allele of *S. typhimurium* encoding a mutant $tRNA^{Gly1}$ with anticodon CCCC that induced ribosomal frameshifts at runs of guanosine bases.[7] This led to an elegant quadruplet-pairing model to explain +1 frameshift suppression in which the expanded anticodon pairs with four bases of the mRNA and facilitates translocation of the mRNA by four nucleotides, thus allowing the ribosome to re-enter the correct reading frame. Consistent with this model, optimal suppression activity typically requires complementarity between the 4 nt expanded anticodon and the 4 nt frameshift sequence in the mRNA.[11,14–16] A notable exception is the *sufJ* suppressor, which functions efficiently without the potential to pair with the fourth nucleotide of the frameshift sequence.[8,17]

This classical quadruplet-pairing model has been challenged.[18] Molecular analysis of tRNAs encoded by the *sufA6* and *sufB2* alleles revealed that, in each case, the 3′-most base of the putative quadruplet anticodon was methylated on its Watson–Crick face. Base methylation was therefore predicted to preclude quadruplet pairing. An alternative model was proposed in which the 8 nt loop tRNA alters the balance of isoacceptors in the cell and allows a near-cognate

Abbreviation used: ASL, anticodon stem–loop.

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wild-type tRNA to enter the A site and cause a +1 frameshift event to restore the correct reading frame.

Although *sufA6* and *sufB2* may promote frameshifting without quadruplet pairing, the suggestion that 8 nt loop tRNAs act indirectly to promote frameshifting is inconsistent with the dominant nature of most *suf* alleles.[3] Furthermore, it has been shown *in vitro* and *in vivo* that 8 nt loop tRNAs can direct incorporation of unnatural amino acids in response to a complementary quadruplet mRNA sequence.[19–21] These studies demonstrate that 8 nt loop tRNAs can act directly to promote +1 frameshifting and lend support to the classical quadruplet-pairing model.

According to the quadruplet-pairing model, the expanded anticodon forms four base-pairs with the mRNA in the A site, and this 4 bp codon-anticodon helix is then translocated to the P site, resulting in restoration of the correct reading frame.[1] However, no biochemical evidence for four base-pairs between tRNA and mRNA has been reported, and whether an additional base-pair can be accommodated in either ribosomal site is unclear. An alternative possibility consistent with the genetic data is that the first and fourth bases of the mRNA frameshift sequence are recognized sequentially in the A and P sites, respectively.[16] Here, we use purified components to test the ability of tRNA analogs with expanded anticodons to recognize and position mRNA in the ribosome. This study provides new insights concerning the 30 S subunit A and P sites and the molecular mechanism of frameshift suppression.

Results

Positioning of mRNA by $tRNA^{Ala2}_{ACCG}$ and $tRNA^{Ala2}_{GCCG}$ bound to the P site

Two $tRNA^{Ala2}$ variants were generated containing expanded anticodons ACCG and GCCG (Figure 1). (Both codon and anticodon sequences are written 5′ to 3′ throughout). Analogous variants of yeast $tRNA^{Phe}$ have been shown to promote efficient (>20%) +1 frameshifting during translation *in vitro*.[19] We chose $tRNA^{Ala2}$ because its anticodon stem–loop (ASL) lacks modifications and is not used as a specificity determinant for AlaRS.[22,23] Control 7 nt anticodon loop variants with anticodons ACC, GCC, and CCG were also made. A 9 nt anticodon loop variant with the sequence GCCCG between U33 and A37 was fortuitously obtained during the mutagenesis. Earlier work demonstrated that certain tRNAs with 9 nt anticodon loops can also promote frameshifting.[20,24]

Using a set of model mRNAs derived from phage T4 gene *32* mRNA, we bound each $tRNA^{Ala2}$ derivative to the P site and mapped the position of mRNA in the resulting ribosomal complexes by toeprinting (Figures 2 and 3). Because toeprints map precisely 16 nucleotides from the first nucleotide of the P codon, this method allows the mRNA nucleotides that physically occupy the P site to be assigned.[25–27] Three pairs of mRNAs were used for this analysis (Figure 2). Messages m408 and m410 contain two nearby GGU codons, but only m408 has C at position

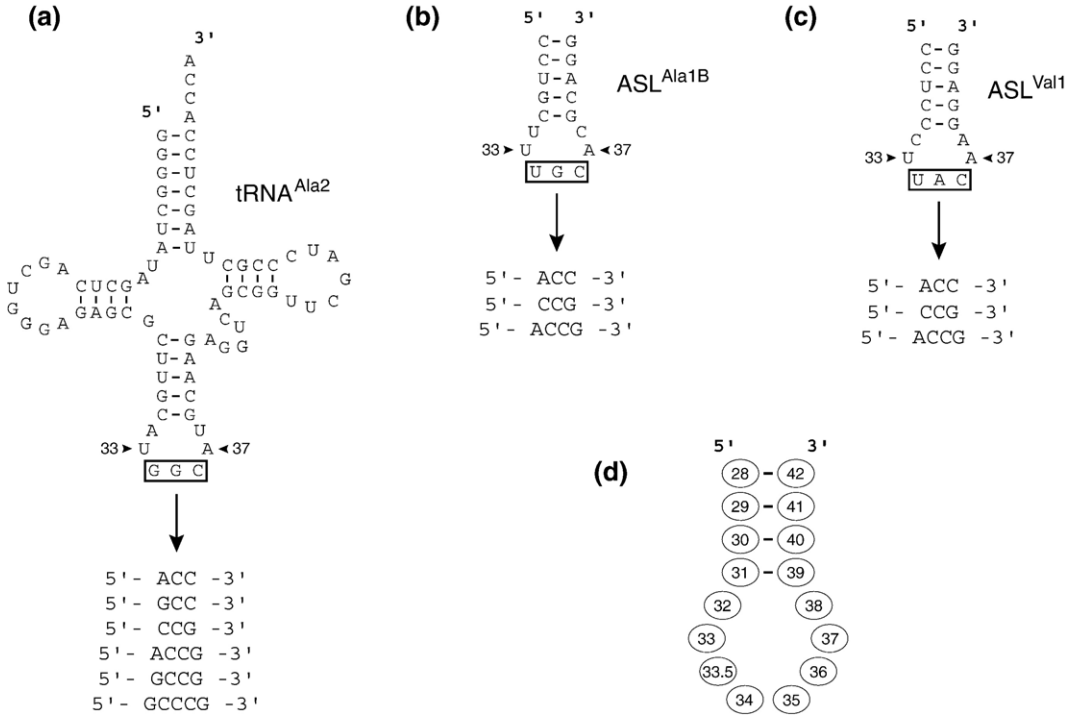

Figure 1. Derivatives of (a) $tRNA^{Ala2}$, (b) ASL^{Ala1B}, and (c) ASL^{Val1} used in this study. (d) Nucleotides of 8nt anticodon loops are numbered as shown.