Insights into reading of isoleucine codon AUA by an archaeal isoleucine tRNA

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The genetic code table consists of sixteen four codon boxes. In fourteen of the boxes, the four codons either code for the same amino acid or are split into two sets of two codons, with each set coding for a different amino acid. The AUN box is an exception in that it is split into 3 and 1 with AUU, AUC and AUA coding for isoleucine and AUG coding for methionine. Bacteria and eukaryotes use different strategies for the isoleucine tRNA ($tRNA^{Ile}$) to read the isoleucine codon AUA without also misreading the methionine codon AUG. Bacteria use a $tRNA^{Ile}$ with the anticodon LAU (L = lysidine), L is a modified cytidine in which the C2-oxo group of cytidine is replaced by lysine. Eukaryotes use either one of two tRNAs with the anticodon IAU or ΨAΨ to read AUA. Whether the archaeal $tRNA^{Ile}$ follows the bacterial strategy or the eukaryotic strategy has, therefore, been a question of much interest.

We have purified $tRNA^{Ile}$ from the haloarchaeon *Haloarcula marismortui* and have confirmed that it binds specifically to the AUA isoleucine codon but not to the methionine codon AUG or to the other isoleucine codons, AUU and AUC. We have shown that the tRNA contains a modified C in the first anticodon position, in which the 2-oxo group of C is replaced by agmatine (decarboxy arginine) and have named the modified C, agmatidine.

Agmatidine is quite similar to lysidine found in the corresponding $tRNA^{Ile}$ of bacteria, suggesting that bacteria and archaea use similar mechanisms for the modified C to base pair specifically with A, of the AUA codon, but not with G of the AUG codon. To investigate the molecular mechanism behind the specific base pairing of agmatidine to A, we determined the crystal structure of the archaeal $tRNA^{Ile}$ bound to 70S ribosomes in the presence of an AUA containing mRNA. Surprisingly, agmatidine forms only a single H-bond with the A of the AUA codon. This single H-bond is likely augmented by another H-bond between the long side chain of agmatidine and the mRNA backbone.

Work in collaboration with the laboratories of Drs. Patrick Limbach, Venki Ramakrishnan and Dieter Söll.

Nonsense suppression in archaea

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Bacterial strains carrying nonsense suppressor tRNA genes played a crucial role in early work on bacterial and bacterial viral genetics. In eukaryotes too, suppressor tRNAs have played important roles in the genetic analysis of yeasts and worms. Surprisingly, little is known about genetic suppression in archaea, and there has been no characterization of suppressor tRNAs or identification of nonsense mutations in any of the archaeal genes.

There could be several reasons why suppressor tRNAs have not been identified in archaea: (1) suppression could be weak and difficult to detect. (2) Suppressor tRNAs, particularly those expressed constitutively, may be inherently toxic in archaea. (3) In contrast to bacteria and eukaryotes, archaea contain very few tRNA genes that are redundant. Therefore, mutation of any archaeal tRNA gene to produce a suppressor tRNA could abrogate the normal function of the tRNA and therefore, be lethal.

To distinguish between the possibilities above and as a first step towards the goal of generating archaeal strains carrying suppressor tRNA genes on the chromosome, we mutated the serine and tyrosine tRNA genes of the archaeon *Haloferax volcanii* to amber, ochre, and opal suppressors and studied their activities in suppression of amber, ochre, and opal stop codons using β-galactosidase genes carrying the corresponding mutations as reporters. The suppressor tRNAs are active in suppression of the corresponding codons in *H. volcanii*. Using a promoter for tRNA expression regulated by tryptophan, we also established inducible and regulated suppression of all three stop codons.

Finally, using *hdrB* as a selectable marker (thymidine prototrophy) along with *hdrB* deficient *H. volcanii* strains, we isolated strains carrying amber, ochre, and opal suppressor tRNA genes, respectively, stably integrated into the chromosome. The availability of these strains should facilitate genetic analysis of *H. volcanii* and viruses that infect *H. volcanii*. Our results also suggest possibility 2 as an unlikely explanation for why suppressor tRNAs have not been identified in archaea.