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Shape Selective RNA Recognition by Cysteinyl-tRNA Synthetase

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Cysteinyl-tRNA synthetase (CysRS) is the enzyme responsible for attaching cysteine (Cys) to the 3'-end of cysteine-specific transfer RNA (tRNA^{Cys}). The synthesis of Cys-tRNA^{Cys} is a crucial preliminary step required to incorporate cysteine into new proteins. We determined the three-dimensional structure of the complex formed between CysRS and tRNA^{Cys} at high resolution, revealing that the complex has an extensive enzyme-RNA recognition interface. An intricate network of hydrogen-bonding, steric, and electrostatic interactions was found at the inner-corner of the tRNA L-shape, near an unusual tertiary base-pair previously implicated in tRNA aminoacylation. Our combined mutational analysis of enzyme and tRNA groups showed that tRNA recognition by CysRS is shape-selective.

Aminoacyl-tRNA synthetases are the class of enzymes responsible for the covalent attachment of amino acids to the 3'-ends of cognate transfer RNA molecules (tRNAs) — the process known as aminoacylation. Cysteinyl-tRNA synthetase (CysRS) is the smallest monomeric tRNA synthetase in *E. coli*, and is an excellent model for exploring how the enzymes discriminate among a large number of structurally similar L-shaped tRNAs. Biochemical data has previously suggested that CysRS may rely on the distinctive globular shape of the tRNA^{Cys} core to recognize critical molecular elements, via a general mechanism known as "indirect readout." A striking feature of the tRNA^{Cys} core is the presence of a rare G15:G48 noncanonical tertiary base-pair. To provide the essential framework for interpreting this type of functional data, we crystallized *E. coli* CysRS bound to tRNA^{Cys} and determined the crystal structure to 2.3 Å resolution.

Our previously determined structure of the unbound CysRS revealed that it has an elongated shape. It features a Rossman fold catalytic domain, an inserted domain adjacent to the tRNA-acceptor end, a bridging stem-contact fold, and an α -helical bundle domain conserved among a subclass of other tRNA synthetases. While the relative orientations of these four domains are identical in CysRS bound to tRNA, a fifth domain — consisting of approximately 60 amino acids forming a mixed α/β fold — appears at the C-terminus of the helical domain, adjacent to the anticodon loop (**Figure 1**). This is a unique feature not previously observed in tRNA synthetases.

A significant fraction of the binding surface between CysRS and tRNA^{Cys} consists of interactions between the CysRS α -helical bundle domain and the tRNA's D and anticodon domains (which together form the vertical arm of the L-shape). The structure of the arm shows that there are no base-specific contacts at the key G15-G48 base-pair (where G is the nucleotide guanine). Instead, hydrogen bonds are made at a non-bridging phosphate oxygen atom located on the G15 and at the 2'OH on A14 (the nucleotide adenine) by the side-chains of Asn351 and Glu354 (the amino acids asparagine and glutamic acid, respectively) (**Figure 2**). In addition, a water-mediated hydrogen bond links G15 with the side chains of Asp348 (aspartic acid) and Lys12 (lysine), which form an adjacent salt-bridge.



Authors (from left) Scott Hauenstein and John Perona

To test how cysteinylolation relies on these sugar-phosphate backbone contacts at G15, we carried out a combined mutational analysis of the CysRS enzyme and tRNA in this region. The data revealed that variant enzymes mutated at Asn351 were no longer able to distinguish between tRNAs possessing the unusual G15-G48 pair and a canonical G15-C48 pair found in most tRNAs. Asn351 mutants are just six times more likely to prefer G15:G48 tRNA^{Cys}, compared with a 125-fold preference that native CysRS has for the wild-type tRNA. Together with the highly complementary sugar-phosphate backbone interactions observed in the structure, these data indicate that the Asn351-G15 phosphate contact mediates tRNA recognition by indirect readout.

Because the 3'-terminus of the tRNA is disordered in the crystals, we do not yet know how interactions with the sugar-phosphate backbone are transmitted to the enzyme active site. The extent to which tRNA selectivity may be coupled to a unique zinc ion-mediated mechanism for amino acid selection is also unresolved. We are currently performing crystallographic and enzymological experiments to address these questions.

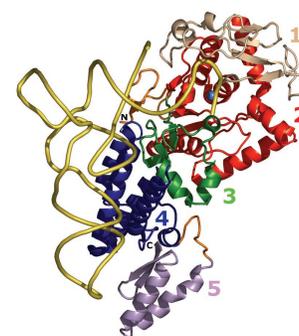


Figure 1. Ribbon representation of the *E. coli* CysRS-tRNA^{Cys} structure. The CP domain (1), Rossmann fold (2), SC fold (3), helical bundle domain (4), and AB domain (5) are colored tan, red, green, blue, and light purple, respectively. The tRNA molecule is shown as a gold tube and the active-site zinc ion as a blue sphere.

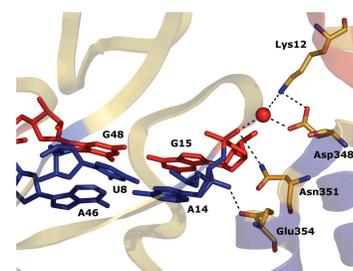


Figure 2. Indirect readout of the tertiary core. Asn351 makes a hydrogen bond with the phosphate of G15 in the G15-G48 Levitt pair (red). Glu354 makes secondary contact with the 2'OH group of A14 from the U8-A14-A46 pair (blue) stacked directly beneath the G15:G48 pair. A red sphere represents the water molecule involved in hydrogen bonding between the phosphate of G15 and Asp348/Lys12.