TWO DIFFERENT DOUBLE-SIEVING PRINCIPLES IN MOLECULAR RECOGNITION BY AMINOACYL-tRNA SYNTHETASES AS REVEALED BY PROTEIN CRYSTALLOGRAPHY

Accurate translation of genetic code is dependent upon accurate charging of a tRNA molecule with its cognate amino acid. This accuracy is maintained by editing mechanisms carried out by aminoacyl-tRNA synthetases (aaRSs). AaRSs catalyze a two-step aminoacylation reaction: (i) activation of the amino acid with adenosine triphosphate (ATP), forming an aminoacyl-adenylate intermediate, and (ii) the transfer of the aminoacyl moiety to the 3' terminal adenosine (A76) of tRNA. In the aminoacylation reaction, amino acid selection is the least accurate step because of the enzyme's difficulties in fine discrimination between structurally similar amino acids solely by the intrinsic binding interaction. As a result, errors of aminoacylation occur (error rates of 4 to 10^2). These errors are corrected by a resident editing activity of the aaRS, which catalyzes hydrolysis of the mischarged products in a tRNA-dependent manner. Two prominent aaRSs which possess this editing activity are isoleucyl- and valyl-tRNA synthetases (IleRS and ValRS, respectively). IleRS activates not only the cognate L-isoleucine but also L-valine, which is smaller than L-isoleucine by only one methylene group. IleRS edits the misactivated valine as follows:

$$\text{IleRS} + \text{Val} + \text{ATP} \rightarrow \text{IleRS}\bullet\text{Val-AMP} + \text{PPi} \quad (1)$$

$$\text{IleRS}\circ\text{Val-AMP} + \text{tRNA}^{\text{Ile}} \rightarrow \text{IleRS} + \text{Val} + \text{AMP} + \text{tRNA}^{\text{Ile}} \quad (2)$$

where PPi is inorganic pyrophosphate. In Eq. 1, the L-valine is activated. In Eq. 2, tRNA-dependent editing occurs. Two editing mechanisms are seen: Val-AMP can be directly hydrolyzed to Val + AMP ("pre-transfer editing"), while the Val-tRNAIle that forms can be deacylated to Val and tRNAIle ("post-transfer editing"). In an analogous set of reactions, ValRS misactivates L-threonine, which is isosteric to L-valine but has a hydrophilic hydroxyl group in its side chain:

$$\text{ValRS} + \text{Thr} + \text{ATP} \rightarrow \text{ValRS}\circ\text{Thr-AMP} + \text{PPi} \quad (3)$$

$$\text{ValRS}\bullet\text{Thr-AMP} + \text{tRNA}^{\text{Val}} \rightarrow \text{ValRS} + \text{Thr} + \text{AMP} + \text{tRNA}^{\text{Val}} \quad (4)$$

These editing reactions reduce the overall error rates to $1/10^4$. Fersht first proposed a "double-sieve" (two-step substrate selection) model for the molecular mechanism of the editing reaction seen in IleRS and ValRS [1]. In IleRS, amino acids larger than the cognate L-isoleucine are strictly excluded by the amino acid activation site which serves as the "first, coarse sieve", and smaller ones, such as L-valine, are strictly eliminated at the hydrolytic editing site by the "second, fine sieve." (Fig. 1). In ValRS, amino acids larger than the cognate L-valine are excluded in the first sieve, and smaller (or isosteric) and hydrophilic ones, such as L-threonine, are eliminated in the second sieve.

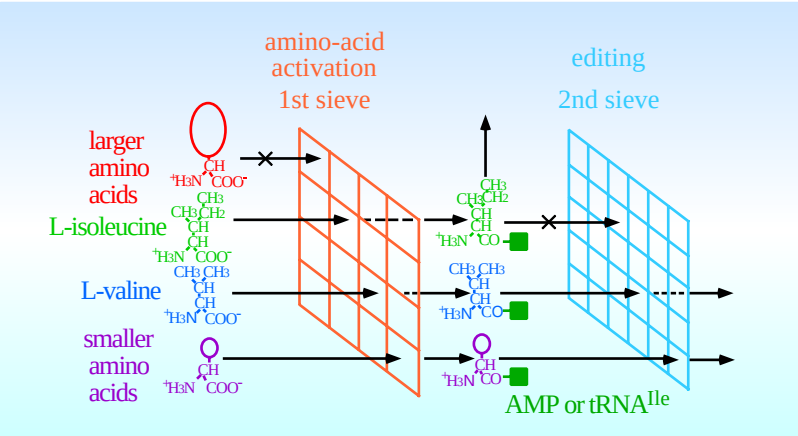

Fig. 1. "Double-sieve" (two-step subtrate selection) model of IleRS.

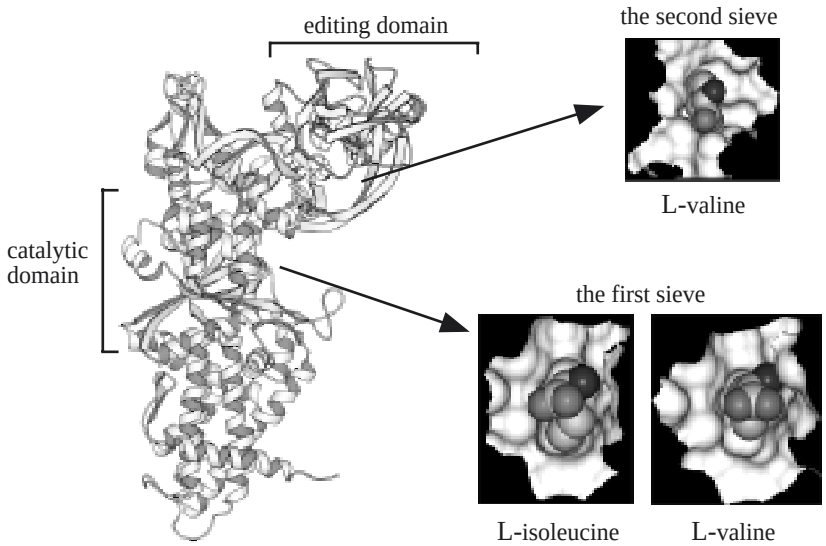

Fig. 2. The double sieve in the crystal structure of IleRS. The first sieve accommodates both L-isoleucine and L-valine, and the second sieve specifically selects L-valine.

Therefore, IleRS selects the cognate L-isoleucine only on the basis of the size of its hydrophobic side chain, whereas ValRS selects L-valine by size exclusion at the first step followed by hydrophobicity exclusion at the second step.

We previously reported the crystal structure of *Thermus thermophilus* IleRS in a complex with L-isoleucine or L-valine, which elucidated the mechanism of "double-sieve" amino-acid selection [2]. The first sieve which accommodates both L-isoleucine and L-valine is on the aminoacylation domain containing the Rossmann fold, whereas the second sieve, which is specific to L-valine, is found in a globular β-barrel CP domain that protrudes from the aminoacylation domain (Fig. 2). In addition, we crystallized *T. thermophilus* ValRS in a complex with $tRNA^{Val}$ and a Val-AMP analogue. The crystals belong to the $P4_22_12$ space group with unit cell parameters of $a=b=410$ Å and $c=82.8$ Å. Recently, the strong X-ray beam at the SPring-8 synchrotron radiation facility enabled us to solve the crystal structure at 2.8 Å resolution (**BL41XU**) [2]. The striking feature of ValRS complex structure is the atomic resolution of the Val/Thr "double-sieve" selection apparatus. The initial size exclusion sieve was observed in the aminoacylation site and the second hydrophobicity filter was seen in the editing site. The Val-AMP analogue is bound in the aminoacylation site, with the valyl moiety bound in a hydrophobic pocket. This pocket packs tightly against L-valine and thus can exclude larger amino acids such as L-isoleucine (Fig. 3). The CCA terminus of $tRNA^{Val}$ is not bound in the aminoacylation domain but in the editing domain, where the 3'-A is specifically recognized by the editing site (Fig. 4), which may represents a snapshot of Thr-$tRNA^{Val}$ editing. The editing domain possesses a hydrophilic pocket for L-threonine next to the 2'-OH group of A76 (Fig. 5). We propose a novel plausible model of the transition between the aminoacylation and editing states of an aaRS, in which the position of the ATP-binding signature loop and the orientation of the editing domain cooperate to determine whether the tRNA CCA terminus enters the aminoacylation site or the editing site (Fig. 6).

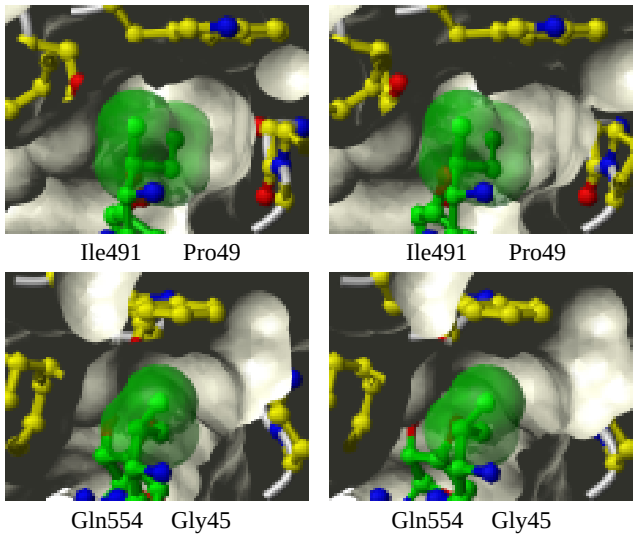

Fig. 3. Comparison of the first sierve of ValRS (upper) and IleRS (lower). Replacements in IleRS of Gln554 and Gly45 with Ile491 and Pro41 (valine-specific residues ValRS), respectively, narrow the space of the amino-acid binding pocket to exclude larger amino acids such as L-isoleucine.

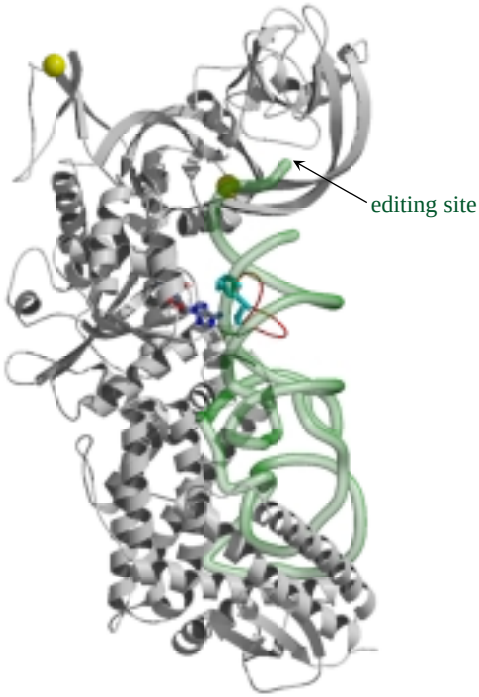

Fig. 4. Crystal structure of ValRS•tRNA Val•Val-AMP ternary complex, representing the "editing complex".

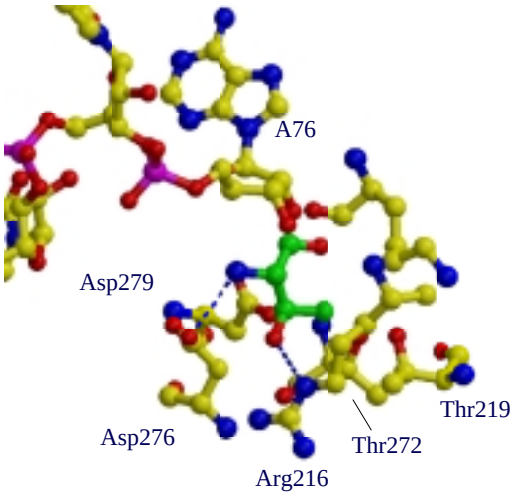

Fig. 5. A hydrophilic pocket specific for L-threonine next to the 2'-OH group of A76 at the ValRS editing site.

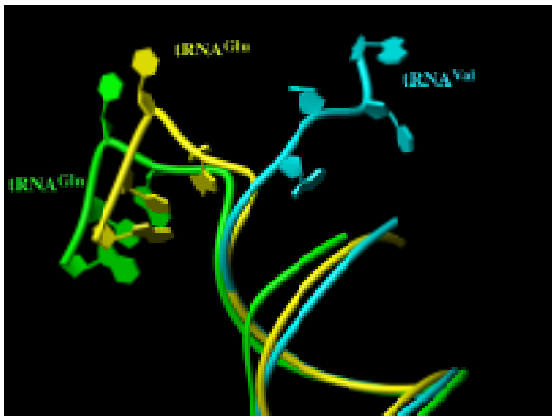

Fig. 6. Transition between the aminoacylation and editing states as revealed by the comparison of CCA terminus conformations of $tRNA^{GLU}/tRNA^{Gln}$ in the "aminoacylation complex" and of $tRNA^{VAL}$ in the "editing complex".

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