Crystal structure of bacterial selenocysteine synthase SelA in complex with tRNA^{Sec} reveals the selenocysteine formation mechanism in bacteria

Selenium is an essential human micronutrient. It is present in proteins in the form of the special amino acid selenocysteine (Sec). Sec is structurally similar to cysteine and serine, and the selenium replaces the sulfur and oxygen atoms of cysteine and serine, respectively. Among the 25 human Sec-containing proteins (selenoproteins), most are redox proteins. Since the selenol group of Sec is easily deprotonated as compared to the cysteine thiol group (pKa 5.2 *vs* 8.3), selenoproteins have stronger redox activities than their cysteine-containing counterparts.

Sec is the 21st amino acid that is incorporated into proteins translationally. It is encoded by the UGA codon, and the Sec-specific transfer RNA (tRNA^{Sec}) has the UCA anticodon (Fig. 1), which is complementary to UGA in mRNA. The UGA codon is a stop codon, and it usually encodes a translation termination signal. Depending on the special mRNA sequence downstream of UGA, the UGA is recoded as a Sec codon.

In the usual translation system, the canonical 20 amino acids are ligated to their tRNAs by their cognate aminoacyl-tRNA synthetases (aaRSs); e.g., seryl-tRNA synthetase (SerRS) ligates serine to serine tRNA (tRNA^{Ser}) (Fig. 1). In contrast, Sec lacks its own aaRS, and is synthesized by the tRNA-dependent conversion of serine (Fig. 1). The first step in Sec synthesis is the ligation of serine to tRNA^{Sec} to form seryl-tRNA^{Sec} (Ser-tRNA^{Sec}) by SerRS, which is also responsible for ligating serine to tRNA^{Ser} (Fig. 1). In Eukaryotes (including human) and Archaea, *O*-phosphoseryl-tRNA^{Sec} kinase (PSTK) phosphorylates the Ser moiety of Ser-tRNA^{Sec}, and then the phosphoserine is converted to Sec by Sep-tRNA^{Sec}:Sec-tRNA^{Sec} synthase (SepSecS) (Fig. 1). Archaea are prokaryotic organisms, but they are evolutionally closer to Eukaryotes than Bacteria. In contrast, in Bacteria, the Sec synthase SelA directly converts Ser to Sec without phosphorylation (Fig. 1). The crystal structures of PSTK [1] and SepSecS [2] in complex with tRNA^{Sec} have clarified the mechanisms of substrate recognition and catalysis. In contrast, the crystal structure of SelA has not been determined due to its huge molecular mass, as described below.

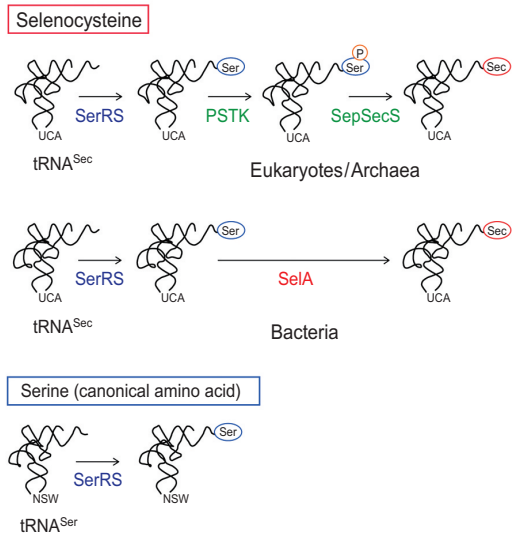

Fig. 1. Sec synthesis system. The schemes of the tRNA^{Sec}-dependent Ser-to-Sec conversions in Eukaryotes/Archaea and Bacteria. Phosphorylation is required in the eukaryotic/archaeal system. SerRS is responsible for serine ligation to both tRNA^{Sec} and tRNA^{Ser}.

We crystallized SelA from the bacterium *Aquifex aeolicus*, and determined the crystal structures of the full-length and an N-terminally truncated mutant of SelA at 3.2 and 3.9 Å resolutions, respectively [3]. The diffraction data were collected at beamline **BL41XU**. SelA is a homodecameric enzyme composed of a pentamer of intimate dimers, and the 10 subunits form a star-shaped ring structure (Fig. 2(a)). The

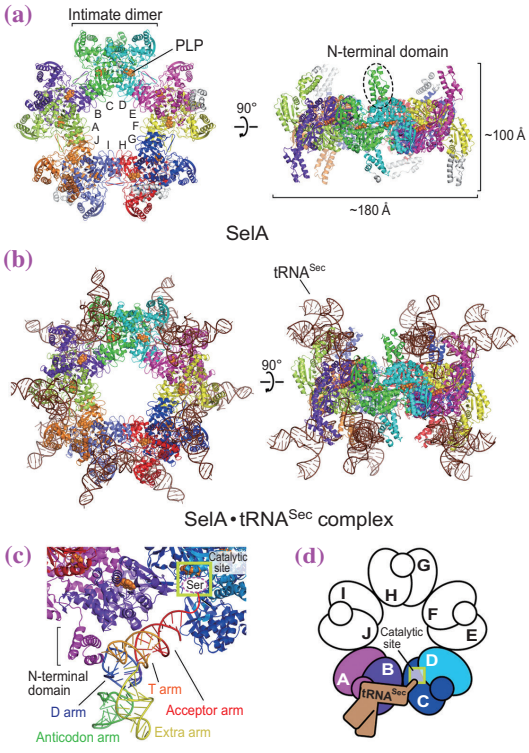

Fig. 2. Crystal structures of SelA. **(a,b)** Overall structures of SelA (without tRNA^{Sec}) and the Sel•tRNA^{Sec} complex. **(c,d)** Close-up view and scheme of the interaction between the SelA subunits and tRNA^{Sec}.

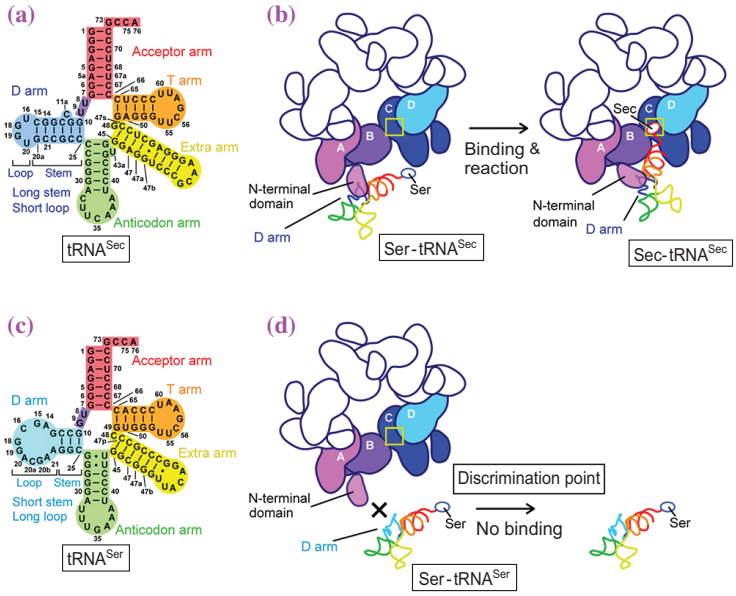

Fig. 3. tRNA discrimination mechanism. **(a,c)** Secondary structures of $tRNA^{Sec}$ and $tRNA^{Ser}$. **(b,d)** SelA discriminates $tRNA^{Sec}$ from $tRNA^{Ser}$ by interacting with the D arm of $tRNA^{Sec}$.

total molecular mass exceeds 500 kDa. The SelA decamer has regular pentagonal symmetry, since the 10 subunits are identical to each other. Each subunit consists of the N-terminal, core, and C-terminal domains, and the N-terminal domains are flexible and protrude from the pentagon (Fig. 2(a)). There are 10 catalytic sites located in the subunit interfaces within the intimate dimers. The cofactor pyridoxal phosphate (PLP) is bound to each catalytic site. PLP is the active form of vitamin B_6, and is a cofactor for many enzymes that function in amino acid metabolism.

We also crystallized SelA in the complex with $tRNA^{Sec}$, and determined its structure at 7.5 Å resolution (Fig. 2(b)). The decameric SelA binds 10 $tRNA^{Sec}$s, and the total molecular mass is about 800 kDa, which is comparable to the smaller particle of the bacterial ribosome (30S, 900 kDa). Each $tRNA^{Sec}$ interacts with four SelA subunits; i.e., subunits A–D (Fig. 2(a,d)). The N-terminal domain of subunit A interacts with the D arm of $tRNA^{Sec}$, subunit B interacts with the acceptor stem, and subunit C interacts with the acceptor arm end. The 3′-terminus of $tRNA^{Sec}$, which carries the ligated Ser, is likely to be able to reach the active site located on the subunit C-D interface, although the $tRNA^{Sec}$ used for crystallization lacked the ligated Ser.

The mutant SelA that lacked the N-terminal domain did not bind $tRNA^{Sec}$, while the dimeric mutant retained the $tRNA^{Sec}$ binding ability [3], indicating that the N-terminal domain is responsible for $tRNA^{Sec}$ binding. However, both of the mutants lacked activity, and thus not only $tRNA^{Sec}$ binding but also the decameric quaternary structure is essential for SelA catalysis.

The Ser-to-Sec conversion requires strict specificity. If SelA interacted with serine tRNA ($tRNA^{Ser}$), then it would convert the Ser-ligated $tRNA^{Ser}$ (Ser-$tRNA^{Ser}$) to Sec-$tRNA^{Ser}$, and Sec would be mis-incorporated into Ser codons. Since serine is a canonical amino acid, the amount of $tRNA^{Ser}$ in cells is much larger than that of $tRNA^{Sec}$, suggesting that it is essential for SelA to discriminate $tRNA^{Sec}$ from $tRNA^{Ser}$. $tRNA^{Sec}$ is the largest tRNA, and it has unique secondary and tertiary structures [4,5]. The D arm of $tRNA^{Sec}$ has longer stem and shorter loop regions, as compared to the canonical tRNAs (Fig. 3(a,c)). The SelA·$tRNA^{Sec}$ complex structure shows that the N-terminal domain of SelA recognizes the unique structure of the $tRNA^{Sec}$ D arm, and thus discriminates $tRNA^{Sec}$ from $tRNA^{Ser}$ (Fig. 3(b,d)).

Most PLP-dependent enzymes function as dimers or tetramers. In contrast, SelA is a decamer, and its four subunits work together with one Ser-$tRNA^{Sec}$. In the conversion reaction, subunit A recognizes Ser-$tRNA^{Sec}$ (i), subunits A and B bind Ser-$tRNA^{Sec}$ (ii), subunit C interacts with the acceptor-arm end of $tRNA^{Sec}$ (iii), and subunits C and D convert the ligated Ser to Sec (iv) (Fig. 3(b)). Since SelA is a ring structure, subunits C–F work on the adjacent Ser-$tRNA^{Sec}$, where subunit C plays the roles of (i) and (ii). Each subunit can perform the four roles. The proper arrangement of the five intimate dimers is important for catalysis and discrimination, and the decameric ring structure stabilizes this arrangement.

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