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## PROTEIN STRUCTURE REPORT

# The crystal structure of leucyl/phenylalanyl-tRNA-protein transferase from *Escherichia coli*

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### Abstract

Leucyl/phenylalanyl-tRNA-protein transferase (L/F-transferase) is an N-end rule pathway enzyme, which catalyzes the transfer of Leu and Phe from aminoacyl-tRNAs to exposed N-terminal Arg or Lys residues of acceptor proteins. Here, we report the 1.6 Å resolution crystal structure of L/F-transferase (JW0868) from *Escherichia coli*, the first three-dimensional structure of an L/F-transferase. The L/F-transferase adopts a monomeric structure consisting of two domains that form a bilobate molecule. The N-terminal domain forms a small lobe with a novel fold. The large C-terminal domain has a highly conserved fold, which is observed in the GCN5-related N-acetyltransferase (GNAT) family. Most of the conserved residues of L/F-transferase reside in the central cavity, which exists at the interface between the N-terminal and C-terminal domains. A comparison of the structures of L/F-transferase and the bacterial peptidoglycan synthase FemX, indicated a structural homology in the C-terminal domain, and a similar domain interface region. Although the peptidyltransferase function is shared between the two proteins, the enzymatic mechanism would differ. The conserved residues in the central cavity of L/F-transferase suggest that this region is important for the enzyme catalysis.

**Keywords:** crystal structure; N-end rule pathway; proteolysis; GNAT superfamily fold

Leucyl/phenylalanyl-tRNA-protein transferase (EC: 2.3.2.6., L/F-transferase) (Kaji et al. 1965) is one of the essential enzymes controlling the half-lives of proteins in vivo in the N-end rule pathway (Tobias et al. 1991; Varshavsky 1992; Shrader et al. 1993). The two proteolytic systems, the Clp-dependent and the ubiquitin-dependent, are both referred to as the N-end rule pathways in prokaryotes and eukaryotes, respectively (Hwang et al. 1988; Katayama et al. 1988).

In bacteria with the N-end rule pathway, the primary destabilizing N-terminal residues, Leu and Phe, are recognized by the ATP-dependent protease ClpAp (Tobias et al. 1991; Goldberg 1992). The analogous eukaryotic protein, arginyl-tRNA-protein transferase (*ATE1*, R-transferase), mediates arginyl transfer to Asp and Glu (Kaji et al. 1963). One of the primary destabilizing N-terminal residues, Arg, is recognized by the ubiquitin proteolytic system (Hochstrasser 1996; Haas and Siepmann 1997; Varshavsky 1997). Despite the enzymological similarities between the eukaryotic R-transferase and the prokaryotic L/F-transferase, there is no significant sequence similarity between them.

The L/F-transferase is encoded by the *aat* gene, which was first isolated from *Escherichia coli* (JW0868) (Leibowitz and Soffer 1970). It also exists in actinobacteria,

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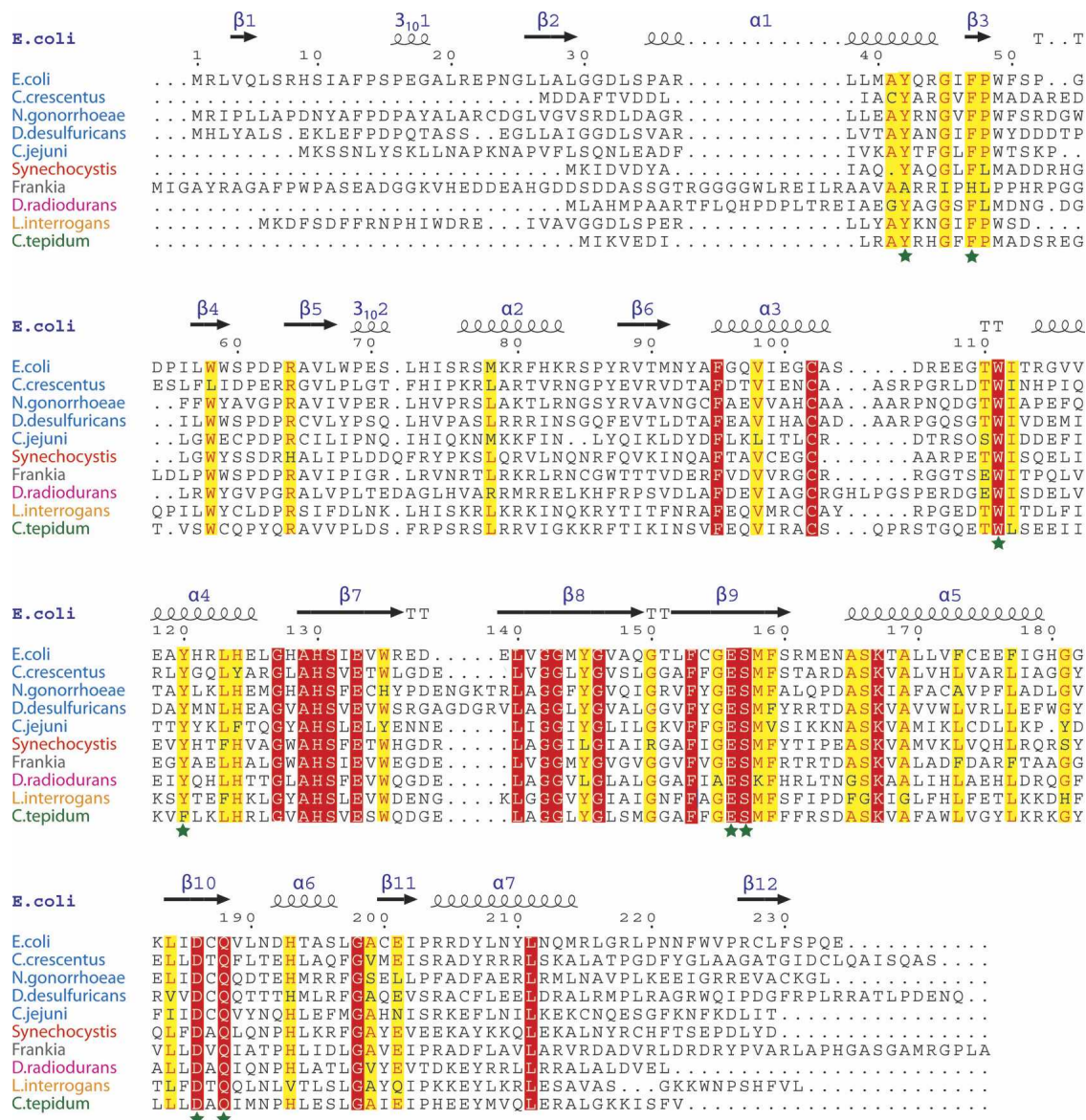
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cyanobacteria, proteobacteria, chlorobi, spirochaetes, and thermus-deinococcus (Fig. 1) and is widely distributed in eubacteria (Ichetovkin et al. 1997). L/F-transferase catalyzes the transfer of Leu and Phe from aminoacyl-tRNAs to exposed N-terminal Arg or Lys residues of acceptor proteins (Leibowitz and Soffer 1971).

Several previous studies have explored the biochemical and kinetic properties of L/F-transferase (Abramochkin and Shrader 1995, 1996). The L/F-transferase preferred Leu-, Phe-, and Met-tRNA as substrates, thus suggesting that an unbranched  $\beta$ -carbon and the side-chain hydro-

phobicity of the aminoacyl group of the aminoacyl-tRNA were recognized by the enzyme (Kaji et al. 1965; Scarpulla et al. 1976; Abramochkin and Shrader 1995). The research suggested that the recognition of the aminoacyl-tRNA by L/F-transferase started from the 5' end to the single-stranded 3'-terminal CCA, where no base pairs were formed at all (Abramochkin and Shrader 1996).

The L/F-transferase enzymatic activity core domain encompasses  $\sim 120$  amino acids (Ichetovkin et al. 1997), but the critical residues that catalyze the peptidyltransferase reaction are still unclear. Here, we determined the



crystal structure of *E. coli* L/F-transferase, the first three-dimensional structure of an L/F-transferase. The structure of L/F-transferase contains two domains and revealed the location of the enzyme catalytic region at the domain interface.

## Results and Discussion

The L/F-transferase was crystallized by the hanging-drop vapor diffusion method. The L/F-transferase crystal belonged to the space group C2, with unit cell dimensions of  $a = 68.3 \text{ \AA}$ ,  $b = 44.8 \text{ \AA}$ , and  $c = 76.4 \text{ \AA}$ . The structure of L/F-transferase was solved by the MAD method using the Se-Met crystal, and was refined to an R factor of 18.7% and an R-free factor of 21.7% to 1.6 Å resolution (Table 1). There was no observable electron density for the region encompassing residues 105–109, and the two C-terminal residues, and thus these residues were not included in the final structure. The L/F-transferase is a monomeric protein consisting of two domains that form a bilobate molecule, with dimensions of  $54 \times 32 \times 34 \text{ \AA}$  (Fig. 2A). The smaller lobe of the molecule is formed by four β strands, with two parallel (β1, β2) and two anti-parallel (β3, β4), surrounded by an α helix (α1) and a

$3_{10}$ -helix ( $3_{10}1$ ) of the N-terminal domain (residues 1–63). The larger lobe of L/F-transferase comprises eight β strands with two parts of an anti-parallel β-sheet (β5, β10, β11; β6–β9, β12), and with six α helices (α2–α7) and a  $3_{10}$ -helix ( $3_{10}2$ ) (Fig. 2). An analysis of the molecular surface electrostatic potential indicated a long shallow cleft at the surface, running across the N-terminal and C-terminal domains. A large central cavity exists at the interface of the N-terminal and C-terminal domains, with the characteristic positive charge contributed by the corner of the cleft on the C-terminal domain surface (Fig. 3A), suggesting that they probably complement the negatively charged phosphate backbone of the substrate aminoacyl-tRNA. A structure database search was performed using the Dali server (Holm and Sander 1993). Three similar structures were found, including GCN5 histone acetyltransferase (Protein Data Bank [PDB] code 1PU9, Z-score = 10.3, sequence identity = 6%) (Clements et al. 2003), FemX transferase (PDB code 1XE4, Z-score = 9.8, sequence identity = 12%) (Maillard et al. 2005), and aminoglycoside 6'-N-acetyltransferase (PDB code 1S3Z, Z-score = 9.0, sequence identity = 12%) (Vetting et al. 2004). Although they lack significant sequence identity, the C-terminal domains of L/F-transferase and the others consist of the structurally conserved core region formed by four α helices and six β strands (α3–α6, β6–β11 in L/F-transferase), which is observed in the GCN5-related N-acetyltransferase (GNAT) family (Fig. 2B). In addition, the GNAT superfamily fold usually indicates the binding of acetyl-CoA, which donates the acetyl group that is transferred to a primary amine (Vetting et al. 2005).

L/F-transferase and FemX catalyze similar peptidyl-transferase reactions, involving the transfer of an uncharged amino acid from a donor aminoacyl-tRNA to an acceptor peptide. The FemX UDP-MurNAc-pentapeptide (UDP-MPP):L-alanine transferase from *Weissella viridescens* is a cell-wall peptidoglycan biosynthesis-related protein, which consists of two domains, and it has a long substrate binding cleft (Hegde and Shrader 2001; Biarrotte-Sorin et al. 2004). Based on their structural and peptidyltransferase reaction similarities, we superimposed the L/F-transferase and FemX structures. The N-terminal domains of the two structures indicated a very different structure, in which the N-terminal domain of L/F-transferase appeared to have a novel βαβαββ fold and to be smaller than that of FemX (Fig. 4). In contrast, the C-terminal domains of the two structures can be superimposed with a root-mean-square deviation (RMSD) of 2.7 Å for the structurally conserved GNAT superfamily fold (Fig. 4C; Dyda et al. 2000; Vetting et al. 2005). In addition, the two domains of the FemX structure share a similar fold structure, but the L/F-transferase did not exhibit the structural homology between the two domains. The FemX enzyme adds L-Ala to the

**Table 1.** X-ray crystallography statistics

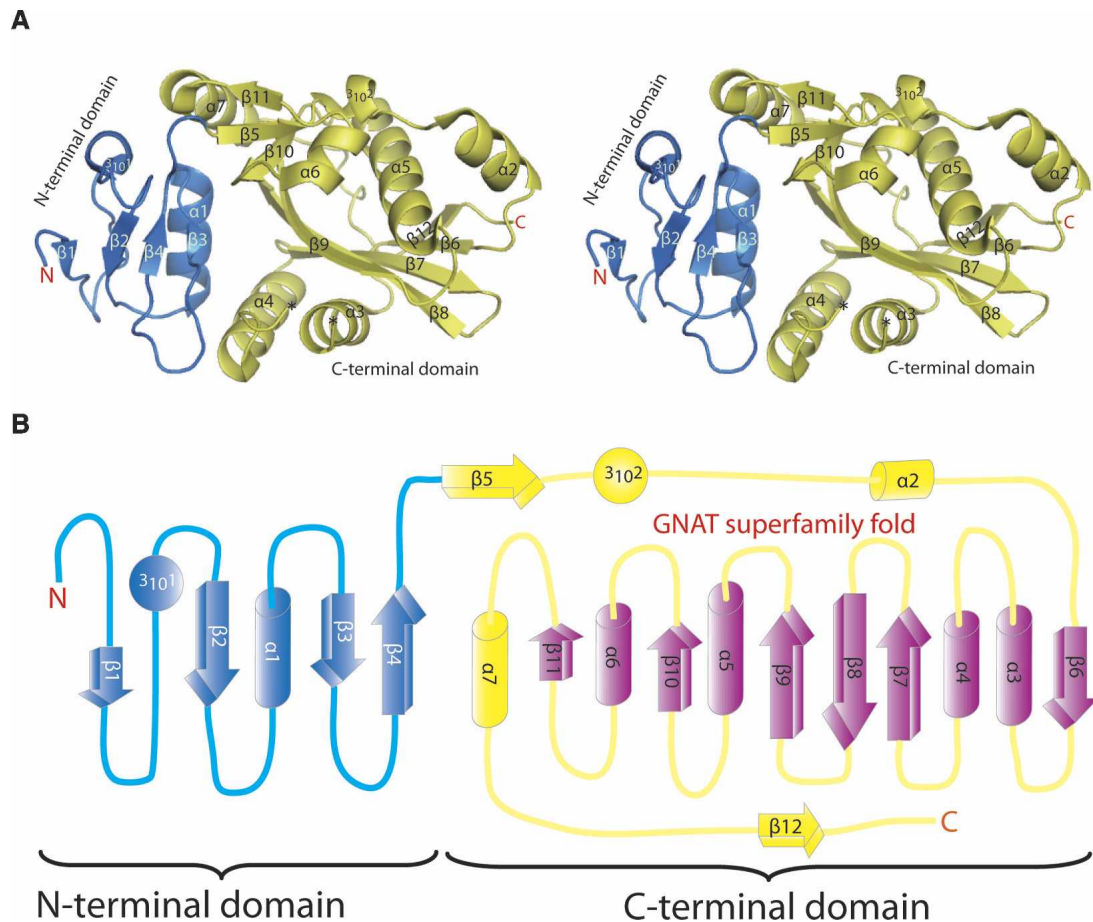
Data collection and processing statistics			
Space group	C2		
Cell constant (Å)	$a = 68.3 \text{ \AA}$ $b = 44.8 \text{ \AA}$ $c = 76.4 \text{ \AA}$		
	Remote	Peak	Edge
Wavelength (Å)	0.9680	0.9795	0.9797
Resolution limit (Å)	50.0–1.6	50.0–1.6	50.0–1.6
Redundancy <sup>a</sup>	3.9	7.5	3.9
No. of unique reflections <sup>a</sup>	28,862	28,737	28,791
Completeness <sup>a</sup> (%)	98.7 (93.9)	99.1 (98.3)	99.1 (97.9)
$\langle I/\sigma(I) \rangle^a$	20.2 (4.5)	23.3 (8.6)	18.2 (5.3)
$R_{\text{merge}}^{\text{a,b}}$ (%)	0.051 (0.242)	0.065 (0.225)	0.054 (0.229)
Figure of merit (FOM)	0.58 (after resolve modification, 0.74)		
Refinement statistics			
Resolution limits (Å)	36.8–1.6		
No. of unique reflections	28,814		
R-factor <sup>c</sup> (%)	18.7		
$R_{\text{free}}^{\text{d}}$ (%)	21.7		
No. of protein atoms	1850		
Root-mean-square deviation from ideal values			
Bond lengths (Å)	0.01		
Bond angles (degree)	1.6		
Dihedral angles (degree)	23.9		
Improper angles (degree)	1.05		

<sup>a</sup> Values in parentheses are for the highest-resolution shell.

<sup>b</sup>  $R_{\text{merge}} = 100(\sum_i |I_{\text{av}} - I_i| / \sum_i I_i)$ , where  $I_{\text{av}}$  is the average of all individual observations  $I_i$ .

<sup>c</sup>  $R = 100(\sum |F_o - F_c| / \sum |F_o|)$ , where  $F_o$  and  $F_c$  are the observed and calculated structure factor amplitudes.

<sup>d</sup>  $R_{\text{free}} = 100(\sum |F_o - F_c| / \sum |F_o|)$ , calculated using a test data set consisting of 10% of the total data randomly selected from the observed reflections.

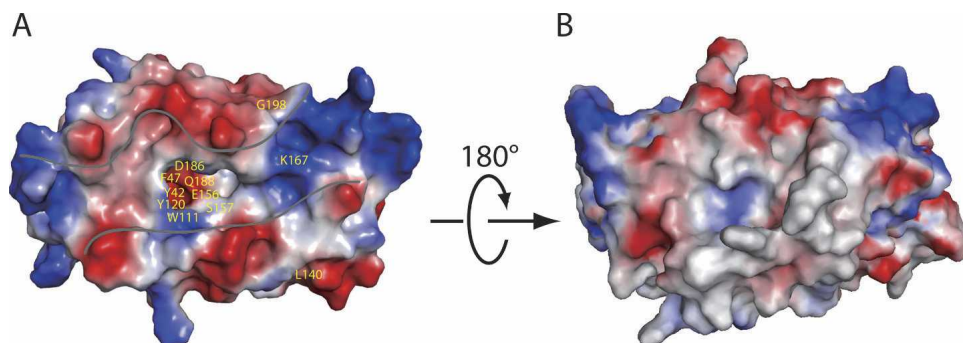


**Figure 2.** The structure of L/F transferase. (A) Stereo-view ribbon diagram of the overall structure of L/F-transferase. The N-terminal domain (1–63) is colored blue, and the C-terminal domain (64–232) is colored yellow. The part of the loop that is missing is marked by asterisks. (B) A cartoon demonstrating the folded topology of L/F-transferase. The orientation and the color scheme of the domains are the same as in the ribbon diagram. The conserved GNAT superfamily fold is colored pink.

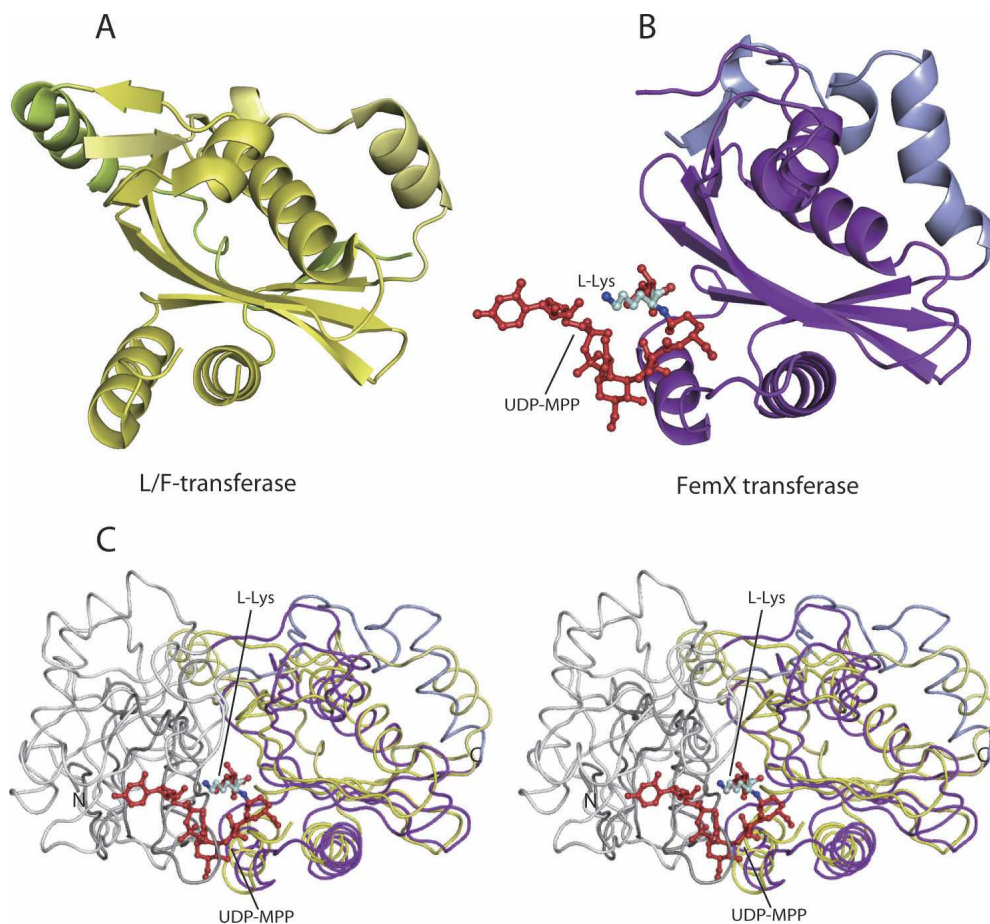
$\epsilon$ -amino group of L-Lys (Fig. 4B, C) in the UDP-MPP. It is bound in the interface of the two domains, thus implying the existence of a catalytic site in this region. Although the L/F-transferase and FemX appear to utilize the different sub-

strates, the similar structural features revealed that the two proteins may share the substrate binding and active regions.

Thus, in the L/F-transferase structure, the domain interface region involves a broad space surrounded by



**Figure 3.** Molecular surface representation of L/F-transferase. The front view (A) and the back view (B) are colored by electrostatic potential (blue, +70KT; red, -70KT). The proposed substrate binding cleft is indicated by a gray line.



**Figure 4.** Structural comparison of the L/F-transferase and the FemX transferase. The conserved GNAT superfamily fold regions are shown in yellow and purple-blue color, respectively, in the structure of the C-terminal domain of L/F-transferase (A) and FemX transferase (B), belonging to the peptidyltransferase enzymes. The UDP-MPP bound in the FemX transferase structure is colored red and depicted as a ball-and-stick model. The L-Lys of UDP-MPP is shown in pale cyan. (C) Superposition of the overall backbone structures of L/F-transferase and FemX transferase (stereo view). The structures are indicated by a main-chain, with the N-terminal domains of L/F-transferase and FemX colored dark gray and gray, respectively. The C-terminal domains of L/F-transferase and FemX are colored as in A and B, respectively.

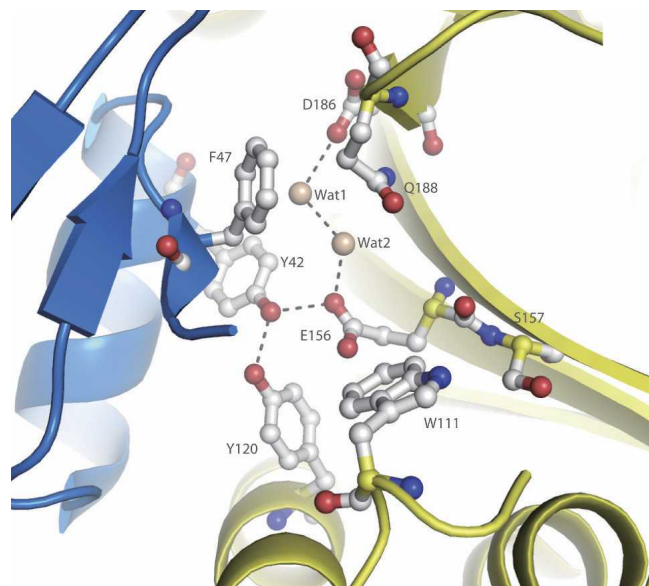
$\alpha 1$  and  $\beta 3$  of the N-terminal domain, and  $\alpha 4$ ,  $\beta 5$ ,  $\beta 9$ , and  $\beta 10$  of the C-terminal domain (Fig. 2A). Most of the conserved residues are extensively distributed within the cleft of L/F-transferase. Especially, the conserved residues Tyr42, Phe47, Trp111, Tyr120, Glu156, Ser157, Asp186, and Gln188 are assembled in the central cavity of the long cleft (Figs. 1, 3A). Notably, the completely conserved residue Glu156 is located at the central position of the cavity, and its side-chain is directed toward Tyr42 and Tyr120, respectively belonging to  $\alpha 1$  and  $\alpha 4$ , which are highly conserved in the L/F-transferase family (Fig. 1). Thus, Tyr42 is located within hydrogen-bonding distance to Tyr120 and Glu156. It seems that Tyr120 and Glu156 are structurally similar to the critical residues for UDP-MPP binding in the FemX complex structure (Hegde and Shrader 2001; Biarrotte-Sorin et al. 2004). The other completely conserved residues, Asp186

and Gln188, are located at the end of  $\beta 5$ , and Asp186 is positioned within hydrogen-bonding distance to Glu156 via two water molecules (Fig. 5). Interestingly, two aromatic residues, Phe47 and Trp111, are located at the entrance of the cavity of the L/F-transferase, and both of their side-chains point toward the inside of the cavity, implying that the aromatic rings of the residues should be favorable for tRNA stacking. The domain interface region of L/F-transferase contains many key conserved residues that form a hydrogen-bond network, suggesting that the cavity region is important for enzyme catalysis.

## Materials and methods

### *Protein expression, purification, and crystallization*

The selenomethionine-labeled L/F-transferase was expressed from the cloning vector pCA24N in *E. coli* strain B834(DE3)



**Figure 5.** Detailed view of the domain interface region of L/F-transferase. The L/F-transferase structure is shown as a cartoon model, and the side-chains of the residues are shown as ball-and-stick models. The conserved residues of L/F-transferase are colored gray, and the two water molecules are shown by wheat-colored spheres. The hydrogen bonds are indicated by dashed black lines.

(Novagen). The cells were harvested and disrupted by sonication in 20 mM of Tris-HCl buffer (pH 8.0), containing 500 mM NaCl, 5 mM of 2-mercaptoethanol, and 1 mM of PMSF. The lysate was cleared by centrifugation for 30 min at 15,000 rpm. The supernatant was purified by two chromatography steps, on HiTrap Chelating HP5 and Hiload 16/60 Superdex 200 columns. The crystals of L/F-transferase were grown using the hanging-drop vapor diffusion method at 293 K. The protein solution was dialyzed against 20 mM of MES buffer (pH 6.0), containing 200 mM NaCl and 20 mM of DTT, and was concentrated to 1.1 mg/mL with an Amicon Ultra-15 Centrifugal Filter (Millipore). The best crystallization conditions employed a reservoir solution containing 100 mM of Tris-HCl buffer (pH 8.5), 200 mM NaCl, and 25% PEG 3350. The crystals were grown within 5 d to maximum dimensions of  $0.1 \times 0.1 \times 0.2$  mm.

#### Data collection, structure determination, and refinement

For data collection, all crystals were transferred to a cryoprotectant solution including 12% (v/v) of PEG 400, picked up in a 0.2-mm nylon loop, and then flash frozen in a cold nitrogen stream at 100 K. The MAD data sets were collected at three wavelengths, 0.9797 Å (edge), 0.9795 Å (peak), and 0.9680 Å (remote), to 1.6 Å resolution on the NW12A beamline at PF-AR (Tsukuba). Diffraction data were processed and scaled using the HKL2000 program (Otwinowski and Minor 1997). Data collection statistics are presented in Table 1.

For phase determination, eight selenium sites were located by using SOLVE (Terwilliger and Berendzen 1999). The resulting electron density map (figure of merit 0.58) was considerably improved by density modification with the program RESOLVE (Terwilliger 2002) (figure of merit 0.74). The model building

was completed using the program O (Jones et al. 1991). Rigid-body, simulated annealing, energy minimization, and individual B-factor refinements were carried out using CNS (Brunger et al. 1998). The stereochemical quality of the final structural models was checked with PROCHECK (Laskowski et al. 1993). All figures were made with PyMOL (DeLano 2002), and superpositions of structures were prepared with LSQMAN (Kleywegt 1996).

The coordinates and structure factors have been deposited in the RCSB Protein Data Bank, with the accession code 2CXA.

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