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Crystal structure of the leucyl/phenylalanyl-tRNA-protein transferase, an N-end rule pathway enzyme from *Escherichia coli*

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The N-end rule pathway, which relates the *in vivo* half-life of a protein to identity of its N-terminal residues are present in many organisms. 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 in Bacteria. N-terminal residue Leu, Phe, Trp or Tyr is directly recognized and cleaved by ATP-dependent protease Clp in protein degradation processes. L/F-transferase encodes by aat gene adjacent to Clp, that protein expressions are regulated each other were found. Arginyl-tRNA protein transferase (R-transferase) in eukaryotes has been shown to transfer Arg to N-terminal of Asp or Glu proteins as same N-end rule pathway that regulated upstream most of ubiquitin pathway. Although the bacteria lacks ubiquitin system, L/F-transferase and R-transferase catalyze reaction of same type and utilize the same source of activated amino acid.

In this study, the 1.6 Å resolution crystal structure of L/F-transferase (JW0868) has been determined from *Escherichia coli*. This is the first structure of aminoacyl-tRNA protein transferase, that indicated monomer structure consisting N-terminal and C-terminal domain, separated by a big cleft. 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. We suggest that the catalytic site located at bottom of cleft that substrate of protein and aminoacyl-tRNA was bound. The analysis of L/F-transferase structure would lead to the understanding of the mechanism that couples recognition of the N-terminal amino acid and the protein's half-lives.