Substrate Recognition by the Leucyl/Phenylalanyl-tRNA-protein Transferase

CONSERVATION WITHIN THE ENZYME FAMILY AND LOCALIZATION TO THE TRYSIN-RESISTANT DOMAIN

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The leucyl/phenylalanyl-tRNA-protein transferase (L/F-transferase) from Escherichia coli catalyzes a peptidyltransferase reaction that results in the N-terminal aminoacylation of acceptor proteins using Leu-, Phe-, and Met-tRNAs as amino acid donors. We demonstrated that L/F-transferase homologs are widely distributed throughout the eubacteria, supporting our proposal that the enzyme family is ancient and catalyzes early peptide bond synthesis. However, here we present data suggesting that the L/F-transferase is not a homolog of the peptidyltransferase enzymes involved in cell wall peptidoglycan biosynthesis in Gram-positive species, such as Staphylococcus aureus. A sequence comparison of the known L/F-transferase homologs began to identify the essential residues required to catalyze a peptidyltransferase reaction and revealed that <20% of the residues were invariant within the L/F-transferase family. Despite this sequence variation, substrate specificity was broadly conserved, and L/F-transferase homologs from Providencia stuartii, Vibrio cholerae, Neisseria gonorrhoeae, and the cyanobacterium Synechocystis sp. all complemented an E. coli aat mutant (lacking L/F-transferase activity) for the degradation of N-end rule substrates. In vitro comparison of the most divergent L/F-transferase homologs, from E. coli and the cyanobacterium Synechocystis sp., revealed near-complete conservation of both substrate specificity and secondary structure. Finally, we demonstrated that variants of the E. coli L/F-transferase, lacking either 33 or 78 N-terminal residues, retained measurable peptidyl-transferase activity and wild type substrate specificity. Overall, our results identified an essential core of an L/F-transferase and revealed that a peptidyltransferase catalyst may be constructed from ~120 amino acids.

The L/F-transferase catalyzes the transfer of Leu, Phe, and Met from aminoacyl-tRNAs to the N termini of acceptor proteins (1, 2). Recognition of aminoacyl-tRNA substrates is via the aminoacyl moiety and ~five nucleotides at the 5’ terminus of the tRNA (3). Recognition of acceptor proteins requires an exposed N-terminal Arg or Lys residue (2). In Escherichia coli cells, a β-galactosidase variant, engineered to expose an N-terminal Arg (Arg-βgal), is modified to form Leu-Arg-βgal by the L/F-transferase (4). Subsequently, the Leu-Arg-βgal is degraded by the Clp A/P (Ti) protease (5, 6). This fate of degradation for L/F-transferase substrates identified the enzyme as a component of the N-end rule pathway of protein degradation (7). The N-end rule pathway degrades cytoplasmic proteins on the basis of their N-terminal residues. Distinct versions of the N-end rule have been identified in E. coli, S. cerevisiae, and mammalian cells, suggesting that the cellular turnover of cytoplasmic proteins bearing certain N-terminal residues is broadly conserved (7). However, prior to this study, the aat gene, which encodes the L/F-transferase, had been identified only in E. coli (4).

Several lines of evidence suggest that the L/F-transferase is the molecular fossil of an ancient enzyme responsible for peptide bond synthesis (3, 8). For example, the enzyme is unusually small (234 residues), and we demonstrate below that ~50% of the enzyme is dispensable without abolition of peptidyltransferase activity and without a detectable loss in substrate specificity. Nonetheless, the L/F-transferase recognizes activated amino acids (aminoacyl-tRNAs) and acceptor polypeptides, each with degenerate specificity (9, 10), allowing the enzyme to synthesize multiple different peptide bonds. In addition, although the E. coli L/F-transferase utilizes two large macromolecular substrates, the determinants recognized in each substrate are relatively simple. For example, aminoacyl-tRNAs are recognized via their aminoacyl side chain and ~5 single-stranded nucleotides at the 5’ terminus of the tRNA acceptor stem (3, 9). The lack of importance of the remainder of the tRNA for L/F-transferase recognition suggests that ancient versions of the enzyme recognized amino acids activated via conjugation to much simpler molecules.

Our proposal that an L/F-transferase homolog was an ancient enzyme and its inclusion within the broadly conserved N-end rule pathway (7) suggest that the L/F-transferase should be widely distributed throughout the eubacteria. To test experimentally the prediction that the L/F-transferase enzyme is distributed widely among the eubacteria, we searched, using degenerate PCR, for genes encoding additional L/F-transferase homologs. We identified two homologs using this approach, and during the course of our work, additional homologs were identified by genome sequencing projects. We present below the first sequence comparison of the L/F-transferase family of peptidyltransferase enzymes. In addition, we demonstrated that although there is significant sequence divergence within the L/F-transferase enzyme family, the complex process of substrate recognition is broadly conserved.
EXPERIMENTAL PROCEDURES

Bacterial Strains and Media—E. coli strain MC1061 (hisD merB araD139 Δ[araAB-loci]/7697 galU galK rpsL thi) was used for plasmid preparation and protein overexpression (11). Determinations of in vitro L/F-transferase activity employed strain TS351 (MC1061 aat:mininet) containing plasmid pUBF-R-fgag (4). This strain expresses, but cannot degrade, a β-galactosidase variant bearing an N-terminal Arg residue (Arg8). Therefore, colonies of TS351 pUBF-R-fgag cells are blue on solid medium containing the chromogenic β-galactosidase substrate 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (Boehringer Mannheim), and lysates from the cells contain increased β-galactosidase activities (8). Where appropriate, E. coli cells were grown in Luria broth or solid medium containing antibiotics at the following concentrations: Amp, 100 μg/ml; Kan, 20 μg/ml; Tet, 10 μg/ml; and Cm, 54 μg/ml.

PCR Amplifications—The degenerate oligonucleotide primers used for L/F-transferase peptides were as follows: L/F-1, EPNGL, 5'-CCGCTGGATCCGATATGAGTATGGGCAT-3'; L/F-2, IFPWF, 5'-CCGCTGGATCCARTCYTCYTTNGCCAT-3'; L/F-3, FSFES, 5'-CCGCTGGATCCGCCTGCTGGTCATGAGCCCTGCGCGCCTG-3'; L/F-4, AATGTGCCG-3'; L/F-5, 5'-CCGCTGGATCCYTCNATNGARTGNGC-3'; L/F-6a, (antisense of ESMFS), 5'-CCGCTGGATCCNCAYTCNCCRCARAA; L/F-6s (EGTWI), 5'-GCGTCAGGATCCYTCNATNGARTGNGC-3'; L/F-7a (antisense of SRSMK), 5'-GCGTCAGGATCCCNCTGATCNGCTC-3'; and L/F-8 (WWSPDP), 5'-GCGTCAGGATCCGCGTCAGGATCCYTCNATNGARTGNGC-3'.

The deoxynucleotides used for completion of the Provisiona stuartii aat gene were as follows: psD-int, 5'-CCGCTGGATCCCTTGAATAGTATGGGCAT-3'; psD-invA, 5'-CCGCTGGATCCATGAAAATTGAATCAG. The deoxynucleotides used for the E. coli aat gene were as follows: vcint-1, 5'-CCGCTGGATCCATGAAAATTGAATCAG. The deoxynucleotides used for the P. stuartii aat gene (ps-int) and a second degenerate primer (ps-invA), were synthesized as described previously (15). Large scale rRNA transcription reactions were carried out without induction of protein expression systems (no IPTG). Transcription products were synthesized as described previously (8). Complementation experiments were carried out with the T7 RNA polymerase expression plasmid pET-28a (Novagen; called pEI-7) and pEI-8, respectively. pG-2a is a derivative of pQE-8 (Qiagen) containing a lacZβ gene.

Expression of the L/F-transferase Family—In vivo expression of L/F-transferase genes coding region was expressed from the tightly regulated expression plasmid pET-28a (Novagen; called pEI-7) and pEI-8, respectively. pG-2a is a derivative of pQE-8 (Qiagen) containing a lacZβ gene.

In Vivo and In Vitro Assays for L/F-Transferase Activity—Plasmid clones containing aat homologs were tested for in vivo L/F-transferase activity by determining their ability to complement the E. coli aat mutant TS351 (MC1061 aat:mininet) for its defect in the degradation of Arg-β-gal as described previously (8). Complementation experiments were carried out with the T7 RNA polymerase expression plasmid pET-28a (Novagen; called pEI-9).
Expression of N-terminal Deletion Fragments of the L/F-transferase Family—Fragments encoding the N-terminally deleted L/F-transferase variants were generated using PCR with primers ecN-33 or ecN-78, and ecC. These coding regions were expressed either as fusions to glutathione S-transferase (GST) (18) using plasmid pGEX-2T (Pharmacia Biotech Inc.), or as fusions to maltose binding protein (MBP) using plasmid pMAL-c2 (New England Biolabs). Due to the cellular insolubility of the N-33 variant and the extreme insolubility of the N-78 variant, their GST fusions were purified from MC1061 cells also overexpressing the L/F-transferase (GST) (18) using plasmid pGEX-2T (Pharmacia Biotech Inc.; fusions were purified according to the manufacturer’s protocols). GST fusions were purified from MC1061 cells also overexpressing the L/F-transferase (GST) (18) using plasmid pGEX-2T (Pharmacia Biotech Inc.; fusions were purified according to the manufacturer’s protocols).

RESULTS AND DISCUSSION

The L/F-transferase Enzyme Family—During their characterization of genes involved in the regulation of 2'-N-acetyltransferase expression in the enterobacterium P. stuartii, MacInaga and Rather (12) reported a nucleotide sequence encoding an N-terminal fragment of an L/F-transferase homolog. To confirm or disprove the notion that the L/F-transferase is confined to the Enterobacteriaceae, we used degenerate PCR to search for aat homologs in other eubacteria. Using PCR primers that encoded peptides conserved between the E. coli and P. stuartii L/F-transferases, aat homologs were identified in the Gram-negative eubacteria S. typhimurium and V. cholerae (overall, seven species were tested; see “Experimental Procedures”). Identification of an L/F-transferase homolog in V. cholerae (a Gram-negative organism more divergent from E. coli than H. influenzae) demonstrated that the aat gene is not restricted to the family Enterobacteriaceae. The deduced amino acid sequence of the V. cholerae L/F-transferase is shown in Fig. 1. Partial sequence analysis of the S. typhimurium aat homolog revealed that this gene is nearly identical to the E. coli aat gene, rendering sequence comparisons uninformative (data not shown). During the course of this work, the genome sequencing projects for both N. gonorrhoeae (a Gram-negative organism more divergent from E. coli than H. influenzae) and Synechocystis sp. (a cyanobacterium, maximally divergent than H. influenzae) demonstrated that the aat gene is not restricted to the family Enterobacteriaceae. The deduced amino acid sequence of the V. cholerae L/F-transferase is shown in Fig. 1. Partial sequence analysis of the S. typhimurium aat homolog revealed that this gene is nearly identical to the E. coli aat gene, rendering sequence comparisons uninformative (data not shown). During the course of this work, the genome sequencing projects for both N. gonorrhoeae (a Gram-negative organism more divergent from E. coli than H. influenzae) and Synechocystis sp. (a cyanobacterium, maximally divergent from E. coli within the eubacterial kingdom) (21) identified aat homologs. The deduced amino acid sequences of the V. cholerae (a Gram-negative organism more divergent from E. coli than H. influenzae) demonstrated that the aat gene is not restricted to the family Enterobacteriaceae.

Enterobacteriaceae due to more complex growth requirements. Nonetheless H. influenzae is closely related to E. coli. Overall, these results suggested that the L/F-transferase is either a very recent enzyme, confined to the narrow family of the Enterobacteriaceae, or a more widely distributed, potentially much older enzyme that is missing from the genomes of H. influenzae and probably other eubacteria.

To confirm or disprove the notion that the L/F-transferase is confined to the Enterobacteriaceae, we used degenerate PCR to search for aat homologs in other eubacteria. Using PCR primers that encoded peptides conserved between the E. coli and P. stuartii L/F-transferases, aat homologs were identified in the Gram-negative eubacteria S. typhimurium and V. cholerae (overall, seven species were tested; see “Experimental Procedures”). Identification of an L/F-transferase homolog in V. cholerae (a Gram-negative organism more divergent from E. coli than H. influenzae) demonstrated that the aat gene is not restricted to the family Enterobacteriaceae. The deduced amino acid sequence of the V. cholerae L/F-transferase is shown in Fig. 1. Partial sequence analysis of the S. typhimurium aat homolog revealed that this gene is nearly identical to the E. coli aat gene, rendering sequence comparisons uninformative (data not shown). During the course of this work, the genome sequencing projects for both N. gonorrhoeae (a Gram-negative organism more divergent from E. coli than H. influenzae) and Synechocystis sp. (a cyanobacterium, maximally divergent from E. coli within the eubacterial kingdom) (21) identified aat homologs. The deduced amino acid sequences of the V. cholerae (a Gram-negative organism more divergent from E. coli than H. influenzae) demonstrated that the aat gene is not restricted to the family Enterobacteriaceae. The deduced amino acid sequence of the V. cholerae L/F-transferase is shown in Fig. 1. Partial sequence analysis of the S. typhimurium aat homolog revealed that this gene is nearly identical to the E. coli aat gene, rendering sequence comparisons uninformative (data not shown). During the course of this work, the genome sequencing projects for both N. gonorrhoeae (a Gram-negative organism more divergent from E. coli than H. influenzae) and Synechocystis sp. (a cyanobacterium, maximally divergent from E. coli within the eubacterial kingdom) (21) identified aat homologs. The deduced amino acid sequences of the V. cholerae (a Gram-negative organism more divergent from E. coli than H. influenzae) demonstrated that the aat gene is not restricted to the family Enterobacteriaceae. The deduced amino acid sequence of the V. cholerae L/F-transferase is shown in Fig. 1. Partial sequence analysis of the S. typhimurium aat homolog revealed that this gene is nearly identical to the E. coli aat gene, rendering sequence comparisons uninformative (data not shown). During the course of this work, the genome sequencing projects for both N. gonorrhoeae (a Gram-negative organism more divergent from E. coli than H. influenzae) and Synechocystis sp. (a cyanobacterium, maximally divergent from E. coli within the eubacterial kingdom) (21) identified aat homologs. The deduced amino acid sequences of the V. cholerae (a Gram-negative organism more divergent from E. coli than H. influenzae) demonstrated that the aat gene is not restricted to the family Enterobacteriaceae.
(both are Gram-positive species, more divergent from E. coli than are any Gram-negative species but less divergent from E. coli than are the cyanobacteria). Overall, these results demonstrate that aat homologs are found throughout the eubacteria but are missing from the chromosome of many species.

A sequence comparison of the known members of the L/F-transferase family reveals that only about 15% of the residues are invariant (Fig. 1). This modest sequence conservation is striking in light of the fact that each member of this family is able to use the aminoacyl-tRNA and acceptor protein substrates of the E. coli L/F-transferase (see below). Fig. 1 also reveals a natural N-terminal deletion of the Synechocystis sp. L/F-transferase and a natural C-terminal deletion of the V. cholerae L/F-transferase. These deletions suggest that the minimal size of a functional peptidyltransferase enzyme is significantly less than the 234 residues of the E. coli L/F-transferase (see below). Fig. 1 also reveals for the first time the amino acid residues likely to be essential for a peptidyltransferase reaction. For example, both peptide G-E-S-M-F and peptide A-S-K, which contain potential catalytic residues, are invariant. In addition, two His residues and a Cys residue, which might act as metal ion binding ligands, are also invariant. However, both the E. coli and the Synechocystis L/F-transferases are active in assays containing 10 mM EDTA, suggesting that a metal ion is not required for L/F-transferase activity.

In addition, the chromosomal neighbors of the E. coli aat gene are conserved only in P. stuartii. The aat genes of both E. coli and P. stuartii lie at the end of three gene operons, the other members of which are homologous to the P-glycoproteins responsible for multidrug resistance in mammalian cells (4, 12). In E. coli cells, these multidrug-resistant homologs are essential for exit from the stationary phase (22) and for the proper expression of the cytochrome d terminal oxidase (23). However, neither the Synechocystis sp. (21) nor the N. gonorrhoeae aat genes lie downstream of P-glycoprotein homologs.

New aat homologs encode L/F-transferases—Our identification of new aat homologs raised the question of whether or not these genes encode L/F-transferases that retain the substrate specificity of the E. coli enzyme. We determined the ability of each of the new aat homologs to complement TS351 cells for their defect in the degradation of Argβ-gal (a β-galactosidase variant engineered to expose an N-terminal Arg residue), and restore a functional N-end rule pathway (4). To complement the E. coli aat mutant, each new L/F-transferase homolog must recognize either E. coli Leu-tRNAs or Phe-tRNAs, as well as the N-terminal Arg residue of the Argβ-gal. Fig. 2 reveals that introduction of each of the new aat homologs into TS351 cells (bars Ps, Vc, Ng, and Sy) reduces the steady-state β-galactosidase level of the cell to levels at or below those of cells expressing the E. coli L/F-transferase (bar Ec). We conclude that each of the new aat genes encodes a functional L/F-transferase and restores the N-end rule pathway in TS351 cells by catalyzing the N-terminal aminooacylation of Argβ-gal with either Leu or Phe. Overall these results suggest that despite the significant sequence divergence within the L/F-transferase family (see Fig. 1), substrate recognition is broadly conserved. We conclude that within the L/F-transferase family, a small number of conserved residues are able mediate the specific recognition of aminooacyl-tRNA and acceptor protein substrates.

Characterization of the Divergent L/F-transferase Homolog from Synechocystis sp.—To characterize more fully the substrate specificity of the L/F-transferase from the cyanobacterium Synechocystis sp., we overexpressed and purified the enzyme and determined its ability to transfer specific amino acids from E. coli aminoacyl-tRNAs to the N terminus of α-casein (a standard L/F-transferase substrate bearing an N-terminal Arg residue) (8). The results of these experiments are indicated in Fig. 3. The L/F-transferase from Synechocystis sp. transfers Leu from Leu-tRNAs to the N terminus of α-casein, which accounts for its ability to complement the E. coli aat mutant (see Fig. 2). In addition, like the E. coli L/F-transferase, the Synechocystis sp. L/F-transferase discriminates against Val-, Ala-, Arg-, and Thr-tRNAs as substrates. However, the Synechocystis sp. enzyme also discriminates against Phe-tRNA as a substrate, revealing a slight increase in substrate specificity over the E. coli enzyme (Fig. 3, second cluster of bars). The physiological rationale for the increased specificity of the Synechocystis sp. L/F-transferase remains to be determined. However, under the assumption that the sole function of the L/F-transferase is to tag proteins for subsequent degradation by the ClpAP protease (24), this result suggests that unlike the situation in E. coli, the Synechocystis sp. ClpAP protease does not recognize N-terminal Phe as a signal for degradation (the genome of Synechocystis sp. contains four homologs of the ClpAP protease) (21).

The L/F-transferase of E. coli is a monomeric enzyme of 234 residues that lacks a detectable RNA component or other organic cofactors (8, 25). Circular dichroism analysis demonstrates that the E. coli L/F-transferase is ~50% α-helical and lacks detectable β-sheet structure (8). Circular dichroism spectra of the Synechocystis sp. L/F-transferase again reveal an enzyme with predominantly α-helical secondary structure (~60% after deconvolution by the method of Yang (26)) and no detectable β-sheet content. The absence of detectable β-sheet structure in both L/F-transferase homologs strongly suggests that the members of the L/F-transferase family recognize the nucleotide portion of aminooacyl-tRNAs using a domain other than the ribonucleotide RNA binding domain, found throughout the prokaryotic and eukaryotic kingdoms (the ribonucleoprotein domain is a β-sheet structure) (27).
contain essential catalytic residues (see Figs. 2 and 3). To define further the minimum size of a functional L/F-transferase, we expressed variants of the E. coli L/F-transferase containing N-terminal deletions of either 33 or 78 residues (called N-33 and N-78, respectively; see “Experimental Procedures”). The N-78 variant corresponds to the previously defined tryspin-resistant core of the E. coli L/F-transferase (8).

The N-33 and N-78 variants were tested in vivo for their ability to complement TS351 cells (MC1061 aat::minitet) for their defect in the degradation of Arg-β-gal as described above. Expression of the N-33 variant in strain TS351, which also expresses Arg-β-gal, resulted in white colonies of cells on LB plates containing the chromogenic β-galactosidase substrate 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside, indicating that the cell degrades Arg-β-gal efficiently (24). We conclude that removal of 33 residues from the N terminus of the L/F-transferase leaves the enzyme active. By contrast, expression of the N-78 variant in the same strain resulted in blue colonies of cells. This result might have suggested that residues essential for a peptidyltransferase reaction were contained within the first 78 residues of the E. coli L/F-transferase. However, the N-78 L/F-transferase variant was extremely insoluble in vivo, making it possible that the observed lack of L/F-transferase activity reflected enzyme-fragment insolubility rather than deletion of essential residues.

To increase the solubility of the N-33 and N-78 variants, their coding regions were recloned as fusions to either MBP (N-33M and N-78M, respectively) or GST (N-33G and N-78G, respectively) (18). Both MBP fusions partially complemented the degradation defect of TS351 cells in vivo (see Fig. 4). In addition, we partially purified the N-33G and N-78G GST fusions and assayed their L/F-transferase activity. The data in Fig. 5 reveal that both the N-33G and N-78G fusions were measurably active in the transfer of Leu from Leu-tRNAs to the N terminus of α-casein. In addition, both N-33G and N-78G discriminate against Thr-tRNAs or Val-tRNAs as substrates. This discrimination reveals that although the overall catalytic activities of N-33G and N-78G are reduced, these enzyme fragments do not exhibit relaxed substrate recognition.

The L/F-transferase activity of the N-78 variant of the L/F-transferase, corresponding to the tryspin-resistant domain (8), as part of both GST and MBP fusions, reveals that the first 78 residues of the enzyme are not essential to catalyze a peptidyltransferase reaction. In addition, the natural C-terminal deletion of ~30 residues of the V. cholerae L/F-transferase enzyme suggests that this region is also dispensable. Together, these results suggest that at least 50% of the E. coli L/F-transferase is dispensable and that a peptidyltransferase enzyme may be encoded by as few as ~120 amino acids.

Although the GST domain probably increased the solubility of the N-33G and N-78G fusions, we were unable to recover L/F-transferase activity from TS351 cells expressing the N-78G fusion without concomitant overexpression of the GroEL/GroES molecular chaperonin. This approach has been used previously to increase the solubility of recombinant enzymes prone to in vivo aggregation (28). Our implementation of chaperonin overexpression, employing plasmid pGroESL-911 (see “Experimental Procedures”), is likely to be of general utility and differs from previous constructs in that GroESL expression occurs via its own promoter and does not rely on induction of the expression system for GroESL overexpression (29).

Relationship to Other Nonribosomal Peptidyl Transferase Enzymes—The L/F-transferase encodes the only known example of a nonribosomal peptidyltransferase in E. coli. However, several species of Gram-positive bacteria contain peptidyltransferase enzymes involved in cell wall peptidoglycan biosynthesis. For example, the peptidoglycan of S. aureus contains a
Gly₅ interchain peptide that cross-links adjacent L-Ala-d-Glu-L-Lys-d-Ala muramyl-pentapeptides (30). This interchain peptide is synthesized using Gly-tRNA as the amino acid donor (30). This reaction further resembles the L/F-transferase reaction in that an uncharged amino acid (Gly) is transferred from an aminoacyl-tRNA to a basic residue of an acceptor peptide (Lys) (9). Based on this substrate similarity, the rarity of peptidyltransferase enzymes within bacterial cells, and our demonstration that the L/F-transferase is widely distributed throughout the eubacteria, it seemed reasonable to assume that this interchain peptide was synthesized by an L/F-transferase homolog. We employed degenerate PCR to search for L/F-transferase homologs in bacteria that synthesize an interchain peptide. Numerous PCR products were generated but none showed significant similarity to the L/F-transferase family. In addition, the E. coli L/F-transferase showed no detectable ability to incorporate radiolabeled amino acids into purified S. aureus muramyl-pentapeptide. Finally, the sequence of the S. pyogenes genome is now >90% complete, and no aat homolog has been found (S. pyogenes contains an Ala₂ interchain peptide) (32). These results allow us to conclude, with a reasonable degree of certainty, that if that the L/F-transferase and the interchain peptide peptidyltransferase are homologs, they are highly diverged in both primary sequence and substrate specificity.

During the course of this work, two S. aureus mutations were characterized (called femA and femB) that resulted in an incomplete formation of the Gly₅ interchain peptide (33). The femA femB double mutant forms only a Gly₁ interchain residue, strongly implicating the FemA and FemB proteins in the synthesis of the complete cell wall cross-link (33). As expected, based on our results, the sequences of FemA and FemB proteins are not related to the sequences of the L/F-transferase family members (data not shown). A lack of homology between enzymes with peptidyl transferase activity but differing substrate specificities was also observed during sequence comparisons of the L/F-transferase with the R-transferase of eu­karyotes (4, 34). The R-transferase catalyzes the transfer of Arg from Arg-tRNA to Asp or Glu at the N terminus of acceptor proteins (34) and is also a component of the N-end rule pathway (7).

Overall, these data suggest that it is very difficult to change the specificity of a peptidyltransferase reaction. Large variations in the sequences of L/F-transferase homologs result in, at most, very moderate changes in substrate specificity. For example, the 80% sequence deviation between the E. coli and cyanobacterial L/F-transferase results only in an increased discrimination against Phe-tRNA substrates, whereas recogni-

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