Aminoacyl-tRNA recognition by the FemX<sub>Wv</sub> transferase for bacterial cell wall synthesis

Matthieu Fonvielle<sup>1,2,3</sup>, Maryline Chemama<sup>4,5</sup>, Régis Villet<sup>1,2,3</sup>, Maxime Lecerf<sup>1,2,3</sup>, Ahmed Bouhss<sup>6,7</sup>, Jean-Marc Valéry<sup>4,5</sup>, Mélanie Ethève-Quelquejeu<sup>4,5</sup> and Michel Arthur<sup>1,2,3,*</sup>

<sup>1</sup>Centre de Recherche des Cordeliers, LRMA, Equipe 12, INSERM, <sup>2</sup>Université Pierre et Marie Curie – Paris 6, <sup>3</sup>Université Paris Descartes, UMR S 872, <sup>4</sup>Synthèse, Structure et Fonction de Molécules Bioactives, CNRS UMR 7613, <sup>5</sup>Université Pierre et Marie Curie – Paris 6, UMR 7613, F-75006, Paris, <sup>6</sup>Laboratoire des Enveloppes Bactériennes et Antibiotiques, Institut de Biochimie et Biophysique Moléculaire et Cellulaire, UMR 8619 CNRS and <sup>7</sup>Université Paris-Sud, F-91405, Orsay, France

Received October 8, 2008; Revised December 11, 2008; Accepted December 12, 2008

ABSTRACT

Transferases of the Fem family catalyse peptide-bond formation by using aminoacyl-tRNAs and peptidoglycan precursors as donor and acceptor substrates, respectively. The specificity of Fem transferases is essential since mis-incorporated amino acids could act as chain terminators thereby preventing formation of a functional stress-bearing peptidoglycan network. Here we have developed chemical acylation of RNA helices with natural and non-proteinogenic amino acids to gain insight into the specificity of the model transferase FemX<sub>Wv</sub>. Combining modifications in the RNA and aminoacyl moieties of the donor substrate revealed that unfavourable interactions of FemX<sub>Wv</sub> with the acceptor arm of tRNA<sub>Gly</sub> and with L-Ser or larger residues quantitatively accounts for the preferential transfer of L-Ala observed with complete aminoacyl-tRNAs. The main FemX<sub>Wv</sub> identity determinant was identified as the penultimate base pair (G<sup>2</sup>-C<sup>71</sup>) of the acceptor arm instead of G<sup>3</sup>/C<sup>15</sup>U<sup>70</sup> for the alanyl-tRNA synthetase. FemX<sub>Wv</sub> tolerated a configuration inversion of the C<sub>a</sub> of L-Ala but not the introduction of a second methyl on this atom. These results indicate that aminoacyl-tRNA recognition by FemX<sub>Wv</sub> is distinct from other components of the translation machinery and relies on the exclusion of bulky amino acids and of the sequence of tRNA<sub>Gly</sub> from the active site.

INTRODUCTION

Peptidoglycan is a giant macromolecule, in the order of 3 × 10<sup>9</sup> to 30 × 10<sup>9</sup> Da, that completely surrounds the cytoplasmic membrane and thereby provides a mechanical protection against the turgor pressure of the cytoplasm. Since the osmoprotective function is required in continuity throughout the cell cycle, peptidoglycan metabolism is intimately involved in cell division (1). Peptidoglycan also provides a scaffold to anchor various surface polymers that interact with host cells and the immune system (2,3). These multiple functions are fulfilled by polymerization of a relatively simple subunit, a disaccharide peptide, that was recently shown to display little conformational heterogeneity by solid-state nuclear magnetic resonance of the intact polymer (4). Formation of the peptidoglycan network involves two main enzyme activities, glycosyltransferase and D,D-transpeptidase, that are often combined in multifunctional proteins belonging to the penicillin-binding protein family (PBP). The glycosyltransferases polymerize glycan strands made of alternating β,1→4-linked N-acetyl-glucosaminyl (GlcNAc) and N-acetyl-muramyl (MurNAc) residues. The 3D nature of the peptidoglycan network is provided by the cross-linking of short stem peptides born by MurNAc residues from adjacent glycan strands (5).

Formation of the peptidoglycan network (Figure 1) involves different types of peptide and amide bond synthesizing enzymes. The Mur synthetases form cytoplasmic UDP-MurNAc-pentapeptide by sequential addition of amino acids with alternating L and D configurations except for the terminal d-Ala-d-Ala dipeptide (6).
These enzymes use ATP as a cofactor and activate the carboxyl of the acyl donor by formation of an acyl phosphate. The precursors of many Gram-positive bacteria contain an additional side chain linked to the third amino acid of the stem pentapeptide. The side chain comprises from one to seven amino acids belonging both to the l. and d series (7). Glycine and L-amino acids are activated as aminoacyl-tRNAs by the aminoacyl-tRNA synthetases involved in protein synthesis and are transferred to the peptidoglycan precursors by Fem transferases that belong to the GCN5-related N-acetyltransferase (GNAT) protein superfamily (8,9). The side chain carboxyl of D-Asp and D-Glu is activated as an acyl-phosphate and subsequently ligated to the precursors by members of the ATP-Grasp protein superfamily (10).

The final cross-linking step is performed by active-site serine peptidases that cleave the d-Ala4-d-Ala2 peptide bond of an acyl donor (transpeptidase of the D,D specificity) and link the carbonyl of d-Ala2 to the amine located at the extremity of the side chain of an acyl acceptor (11). In enterococci resistant to β-lactams and in Mycobacterium tuberculosis, these members of the PBP family can be replaced by active-site cysteine peptidases that cleave the L-Lys4-L-Ala3 peptide bond of an acyl donor (transpeptidase of the L,D specificity) and link the carbonyl of L-Lys3 to the amine of the acyl acceptor (12,13).

The specificity of peptide and amide forming enzymes is essential for bacteria since mis-incorporated amino acids can act as chain terminators (6,14) and block the final cross-linking step of peptidoglycan polymerization (15,16). The sequence of the peptide network has a critical impact on the activity of amidases and peptidases that fulfill a wide variety of physiological functions including peptidoglycan recycling, separation of daughter cells after division and partial hydrolysis of the peptidoglycan network thought to be required both for insertion of novel subunits into the pre-existing material and for crossing of macromolecular structures such as pili and flagella through the peptidoglycan layer (17). Finally, the sequence of the peptide network determines the susceptibility of the peptidoglycan to hydrolases produced by competing bacteria (18).

Fem transferases are considered as attractive targets for the development of novel antibiotics active against multi-resistant bacteria (14). These enzymes have a unique catalytic mechanism (19,20) and are essential either for viability (21) or for expression of β-lactam resistance mediated by low-affinity PBPs (22–24). Characterized members of this family include FemABX from Staphylococcus aureus that sequentially add one (FemX) or two (FemA and FemB) glycines (25) and homologues from Streptococcus pneumoniae (MurMN) (22,26) and Enterococcus faecalis (BppA1A2) (27,28) for incorporation of single residues into L-Ala (or L-Ser)-L-Ala side chains. In addition, FemXWv from Weissella viridescens has been widely used as a model transferase since the UDP-MurNAc-pentapeptide substrate of this enzyme...
(Figure 1) is more easily obtained than the lipid intermediates used by other members of the family (20,27). FemXWv catalysis proceeds by an ordered bi-bi mechanism with sequential fixation of the UDP-MurNAc-pentapeptide and Ala-\(\text{tRNA}^{\text{Ala}}\) substrates and sequential release of the \(\text{tRNA}^{\text{Ala}}\) and UDP-MurNAc-hexapeptide products (19). Structure-based site-directed mutagenesis of the UDP-MurNAc-pentapeptide-binding cavity of FemXWv (19) revealed that a complex hydrogen bond network connects two residues of the enzyme (Lys\(^{36}\) and Arg\(^{211}\)) with two regions of UDP-MurNAc-pentapeptide (both phosphate groups and both \(\text{d-Ala}\) residues) and constrains the substrate in a bent conformation essential for the aminoaeryl transferase activity (8,29). Analysis of the interaction of FemXWv with the second substrate (Ala-\(\text{tRNA}^{\text{Ala}}\)) showed that the acceptor stem of \(\text{tRNA}^{\text{Ala}}\) is sufficient for aminoaetyl transfer (30). Saturation mutagenesis of this region of the substrate and modelling of the acceptor stem in the FemXWv catalytic cavity suggested that the enzyme only interacts with the two distal base pairs (\(G^2-C^7\) and \(G^1-C^2\)) and the single-stranded 3'-end \(7^{\text{ACC}}A^6\) (30). We have analysed the specificity of FemXWv in the aminoaeryl transfer reaction by systematically exploring the impact of modifications in the aminoaetyl residue and RNA sequence on the catalytic efficiency of FemXWv.

**MATERIALS AND METHODS**

**Enzyme purification**

FemXWv (29), alanyl-\(\text{tRNA}^{\text{Ala}}\) synthetase (\(\text{AlaRS}\)) (27), T4 RNA ligase (30) and T7 RNA polymerase (30) were purified according to previously published procedures.

**Substrates**

Full-length \(\text{tRNA}^{\text{Ala}}\) (5'-GGGGCCUUAGGCUACCGUGGGAGCUGCGCUUUUGCATGAGAGGAUGGCUGGGCUUCGAUCCCGCUAGGCUCCACCA-3') corresponds to the three identical sequences annotated as \(\text{tRNA}^{\text{Ala}}\) in the genome sequence of *E. faecalis* strain V583 (http://www.tigr.org/). This 76-nucleotide RNA was obtained by *in vitro* transcription using T7 RNA polymerase (30). The choice of the *E. faecalis* rather than a *W. viridescens* \(\text{tRNA}^{\text{Ala}}\) sequence was dictated by the fact that the sequence of the genome of the latter bacteria is unknown.

The peptidoglycan precursor UDP-MurNAc-\(\text{l-Ala}_{1-}\text{d-iGlu}_{2-}\text{l-Lys}_{3-}\text{d-Ala}_{4-}\text{d-Ala}_{5}\) (UDP-MurNAc-pentapeptide) was synthesized as previously described (31). Labelled UDP-MurNAc-\(\text{l-[C^{14}]Ala}_{1-}\text{d-iGlu}_{2-}\text{l-Lys}_{3-}\text{d-Ala}_{4-}\text{d-Ala}_{5}\) was prepared by sequential addition of \(\text{l-[C^{14}]Ala}\) (6.3 GBq:mmol\(^{-1}\); Perkin Elmer), \text{d-Glu}, \text{l-Lys} and \text{d-Ala}-\text{d-Ala} by the purified MurC, D, E, F synthetases (32).

**Reagents and materials for organic synthesis**

Solvents were dried using standard methods and distilled before use. Unless otherwise specified, materials were purchased from commercial suppliers and used without further purification. TLC: precoated silica gel thin layer sheets 60 F\(_{254}\) (Merck). Flash chromatography: silica gel 60 A, 180–240 mesh from Merck. \(^1\text{H}\) (250.13 MHz), \(^13\text{C}\) (62.90 MHz) spectra were recorded on Brüker ARX 250 spectrometer in CDCl\(_3\). Chemicals shifts (\(\delta\)) are expressed in ppm relative to residual CDCl\(_3\) (\(\delta 7.26\)) for \(^1\text{H}\), CDCl\(_3\) (\(\delta 77.16\)) for \(^13\text{C}\) as internal references. Signals were attributed based on COSY and DEPT 135 (\(^1\text{C}\)). High-resolution mass spectrometry (HRMS) spectra were carried out on a LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific Inc.) in the positive or negative electrospray ionization modes (ESI) at the Mass Spectrometry Centre of the University Pierre & Marie Curie (Paris). High-performance liquid chromatography (HPLC) was performed with reverse phase C-18 columns (analytical column: 250 × 4.6 mm, HYPERSIL-100 C18; semipreparative column: 250 × 21.2 mm, HYPERSIL HS C18; Thermoelectron Corporation). Compounds were eluted at flow rates of 1 and 17 ml min\(^{-1}\) (for the analytical and semipreparative columns, respectively) with a linear gradient of CH\(_3\)CN (0–33% in 45 min) in 50 mM aqueous NH\(_4\)OAc (pH 4.5).

**Synthesis of [\(\text{N-(4-pentenoyl)]-aminoaeryl\text{-pdCpAs\ (compounds 2a-d)}\)**

The dinucleotides acylated by protected Gly, \(\text{t-Ala}\), \(\text{d-Ala}\) and 2-aminobutyrate (Abu) (compounds 2a–d) in Figure 2A) were synthesized according to a previously described method (33,34). A solution of freshly distilled DMF (100 \(\mu\)l) containing 10 mg of pdCpA tetrabutyrammonium salt (7.35 \(\mu\)mol) was added to 39.7 \(\mu\)mol of \(\text{N-(4-pentenoyl)-}\)amino-acid cyanomethyl esters (1a–d). The reaction mixtures were stirred at room temperature and monitored by rp-HPLC. Purification on the semipreparative C18 reversed phase column led to compounds 2a–d (retention times: 2a, 13.8 min; 2b, 14.7 min; 2c, 14.9 min; 2d, 16.3 min) that were recovered as colourless solids after lyophilization and analysed by ESI-HRMS. 2a: 44% yield (2.5 mg); observed and calculated \(m/z\) of 774.1655 and 774.1650 for the [\(\text{M} + \text{H}\)]\(^+\) ion, respectively; 2b: 48% yield (2.8 mg); \(m/z\) 790.1959 [\(\text{M} + \text{H}\)]\(^+\) (calculated 790.1963); 2c: 52% yield (3.0 mg); \(m/z\) 788.1785 [\(\text{M} + \text{H}\)]\(^+\) (calculated 788.1806); 2d: 60% yield (3.6 mg); \(m/z\) 802.1959 [\(\text{M} + \text{H}\)]\(^+\) (calculated 802.1963).

**Synthesis of \(\text{N-(4-pentenoyl)-l-serine methyl ester\ (compound 4)}\)**

NaHCO\(_3\) (135 mg; 1.61 mmol) was added to an aqueous solution (4 ml) of l-serine methylester hydrochloride 3 (250 mg; 1.61 mmol) (Figure 2B). A solution of 4-pentenoic acid succinimide ester (35) (177 mg in 4 ml of dioxane) was added under stirring at room temperature. After overnight stirring at room temperature, the reaction mixture was diluted with 8 ml of EtOAc and 8 ml of 1 M NaHSO\(_4\), and then extracted with EtOAc. The combined organic extracts were dried over Na\(_2\)SO\(_4\) and concentrated under diminished pressure. The crude product was purified by flash chromatography on a silica gel column. Elution with 9:1 CH\(_2\)Cl\(_2\)/MeOH gave 4 as a colourless oil: 95% yield (307 mg); \(^1\text{H}\) NMR...
Compound 4 (286 mg; 1.42 mmol) was dissolved in 5 ml of anhydrous DMF. After sequential addition of TBSCl (321 mg; 2.13 mmol) and imidazole (160 mg; 2.34 mmol), the reaction mixture was stirred under argon overnight at room temperature. The solution was diluted with 50 ml of brine and extracted with EtOAc. The combined organic phase was washed with water, dried over Na₂SO₄ and concentrated under diminished pressure. Purification by flash chromatography on a silica gel column (95:5 CH₂Cl₂/MeOH) gave 5 as a colourless oil: 88% yield (395 mg); 1H NMR (250 MHz, CDCl₃) δ 6.35 (br d, J = 7.9, 1H), 5.92–5.67 (m, 1H), 5.12–4.90 (m, 2H), 4.70–4.57 (m, 1H), 3.88 (ddd, J = 2.9, 10.1, 60.3, 2H), 3.69 (s, 3H), 2.45–2.24 (m, 4H), 0.81 (s, 9H), –0.02 (s, 3H), –0.03 (s, 3H). 13C NMR (63 MHz, CDCl₃) 173.6, 171.0, 136.6, 115.5, 62.5, 54.4, 52.5, 35.1, 29.3 ppm.

**Figure 2.** Semi-synthesis of Ala-tRNAₐ₁ₐₐₐ₁ₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐ}_{100}^{100} \text{deoxycytidine-}(5^{100})^{100}\text{phosphoadenine. (B) Organic synthesis of pdCpA acylated by L-Ser protected with a pentenoyl group. (C) Ligation of acylated dinucleotides to RNA helix.}
helices, which did not contain the terminal pCpA, were synthesized by the phosphoramidite method and purified by polyacrylamide gel electrophoresis (Eurogentec). The inhibitors containing the oxadiazole ring were purified by anion exchange chromatography (DEAE column, DNAPac-100, Dionex) with a linear gradient of ammonium acetate pH 8.0 (25–2500 mM) containing 0.5% acetonitrile. Fractions containing the ligand product were identified by denaturing polyacrylamide gel electrophoresis (37), lyophilized, resuspended in RNAse free water (Sigma) and stored at −20°C. The concentration of the inhibitors was determined spectrophotometrically (ε = 2.26 × 10^3 M^−1 cm^−1 at 260 nm). The substrates containing the aminoacyl-pentenoyl groups were purified by ethanol precipitation (34). The pentenoyl group was removed with iodine prior to enzyme assay (35).

### Determination of the relative activity of FemX_Wv with aminoacylated RNA helices

FemX_Wv activity was determined in 50 mM ammonium acetate (pH 6.5) containing 12.5 mM mgCl_2, 0.1 mg/ml bovine serum albumin, enzyme (0.75 nM to 10 μM), UDP-MurNAc-[14C]pentapeptide (78 μM) and acylated helices (3–10 μM). The reaction was allowed to proceed for 30 min at 37°C and an incubation of 10 min at 96°C was used to stop the reaction. UDP-MurNAc-[14C]hexapeptides produced by FemX_Wv were determined by rp-HPLC coupled to a radioflow detector using a C18 column (Nucleosil; 25 cm, 3 μM; Machery–Nagel). Elution was performed at a flow rate of 0.5 ml/min with a linear gradient of acetonitrile (0–4%) in 50 mM ammonium acetate pH 5.0, which was applied between 15 and 55 min. Reproducibility was assessed with two to three independent preparations of aminoacylated RNA helices and standard deviations were calculated by linear regression using a set of data generated with the same batch of substrate.

### Inhibition of FemX_Wv by analogues of Ala-tRNA^{Ala}

The assay contained Tris–HCl (50 mM, pH 7.5), alanyl-tRNA synthetase of *E. faecalis* (800 nM), ATP (7.5 mM), MgCl_2 (12.5 mM), t-[14C]Ala (50 μM, 3700 Bq/nmol; ICN, Orsay, France), FemX_Wv (2 nM), UDP-MurNAc-pentapeptide (5 μM), tRNA^{Ala} (0.4 μM) and inhibitors (0–200 μM). The reaction was performed at 37°C for 10 min with a preincubation of 2 min in the absence of FemX_Wv for synthesis of Ala-tRNA^{Ala} by the auxiliary system. The reaction was stopped at 96°C for 10 min and analysed by descending paper chromatography (Whatman 4 mm, Elancourt) with isobutyric acid-ammonia, 1 M (5:3 per vol). Radioactive spots were identified by autoradiography, cut out and counted by liquid scintillation.

### RESULTS

#### Synthesis of aminoacyl-tRNA analogues and design of a FemX_Wv assay for the resulting substrates

Acylated RNA helices were obtained by semi-synthesis (Figure 2). The main steps involved organic synthesis of...
dinucleotides acylated by protected amino acids (Figure 2A and B). The acylated dinucleotides were ligated with T4 RNA ligase to 22-nt RNA helices that mimic the acceptor arm of tRNAs (Figure 2C). The extent of the transfer of amino-acid residues from acylated helices to UDP-MurNAc-[14C]pentapeptide (reaction depicted in Figure 3A) was determined by rp-HPLC coupled to a radioflow detector (Figure 3B). Since the ester link connecting the amino-acid residues to the RNA helices was unstable, the deprotection step (Figure 2C) was performed immediately prior to the assay and the initial concentration of the aminoacylated helices was determined in each experiment. For this purpose, high concentrations of FemXWv and an excess of UDP-MurNAc-pentapeptide were used to obtain full transfer of the residue (Figure 3C). The concentration of UDP-MurNAc-[14C]hexapeptide obtained after full transfer was used to evaluate the initial concentrations of the acylated RNA helices that varied between experiments according to the yield (30–80%) of the ligation and deprotection steps. For each substrate, a range of FemXWv concentrations was identified in which formation of the product was proportional to the time of reaction (data not shown) and to the enzyme concentration (Figure 3D). The slopes of the
linear portion of the curves were used to determine the relative efficiency of the transfer reaction with the different acylated donor substrates (Figure 3E).

**Relative activity of FemXWv for the transfer of L-Ala, L-Ser and Gly from helixAla to UDP-MurNAc-pentapeptide**

Comparison of the efficiency of transfer of L-Ala and L-Ser from helixAla to UDP-MurNAc-[14C]pentapeptide indicated that FemXWv efficiently discriminated between the methyl and hydroxymethyl side chains of the residues (7.0% relative efficiency). A similar relative efficiency (6.0%) was previously reported for the complete aminoacyl-tRNAs (Ala-tRNAAla versus Ser-tRNASer) (30). Thus, exclusion of Ser from the active site of FemXWv was sufficient to account for enzyme specificity since the Ala-helixAla and Ser-helixAla only differed by the aminoacyl residue. In contrast, Gly-helixAla was efficiently used by FemXWv (33% relative efficiency) whereas Gly-tRNAGly was a poor substrate (2.6%). Thus, discrimination between Ala-tRNAAla and Gly-tRNAGly involved different interactions between the FemXWv and the RNA moiety of the substrate.

**Transfer of L-Ala from helices mimicking tRNAAla, tRNASer and tRNAGly**

The sequence of tRNAAla, tRNASer and tRNAGly differs at four positions in the distal portion of the acceptor arm (boxed in Figure 4A). Nucleotide substitutions were introduced in these positions of helixAla to generate helices that mimic the acceptor arm of tRNAAla and tRNAGly (designated helixAla, helixSer and helixGly, respectively). Thus, exclusion of Ser from the active site of FemXWv was sufficient to account for enzyme specificity since the Ala-helixAla and Ser-helixAla only differed by the aminoacyl residue. In contrast, Gly-helixAla was efficiently used by FemXWv (33% relative efficiency) whereas Gly-tRNAGly was a poor substrate (2.6%). Thus, discrimination between Ala-tRNAAla and Gly-tRNAGly involved different interactions between the FemXWv and the RNA moiety of the substrate.

To gain insight into the interaction of FemXWv with helixAla, helixSer and helixGly, stable analogues of the aminoacylated helices were synthesized by introducing an oxadiazole-containing substituent at the 3'-end of the helices (Figure 5A). The relative efficiency of transfer of L-Ala from the RNA helices to UDP-MurNAc-[14C]pentapeptide was estimated as described in Figure 3E.
the amino-acid residue to the tRNA (37). The oxadiazole ring was substituted by a 2-amino-ethyl group mimicking L-Ala (oxa). The IC₅₀s for the oxa-helix Ser and oxa-helix Gly were 11- and 48-fold higher than the IC₅₀ of the oxa-helix Ala, respectively (Figure 5B). These results show that differences in the sequence of the acceptor arm of tRNAAla, tRNA Ser and tRNA Gly affect binding of FemX₇v to the RNA substrate. The largest effect was observed between helix Ala and helix Gly underscoring again the key role of differences in the mode of recognition of the acceptor arm of the tRNA Ala and tRNA Gly for the specificity of FemX₇v.

Figure 5. Inhibition of FemX₇v by substrate analogues. (A) Structure of the Ala-helixAla and analogues containing an oxadiazole moiety linked to helixAla, helixSer and helixGly. (B) IC₅₀ values were determined in the AlaRS-FemX₇v coupled assay.

FemX₇v identity determinants in the acceptor arm of tRNAAla
Substitutions were introduced in the distal portion of the acceptor arm of Ala-helix Ala to evaluate the role of specific bases on FemX₇v activity (Figure 6). The discriminator base (position 73) was not a key element for FemX₇v specificity since a residual activity of 62% was observed for the modified Ala-helix Alaa containing the A₇₃→G substitution. The C₇₂→G substitution in the first base pair of the acceptor stem led to a 100-fold decrease in the catalytic activity of FemX₇v. The impact of the substitution involved at least in part the destabilization of the RNA helix since introduction of a second substitution G₁→C, which restored a Watson–Crick base pair, increased the residual activity 6.5-fold. In contrast, a similar analysis of the 2-71 base pair led to the opposite conclusion since the replacement of G₂→C₇₁ by G₂→G₇₁ and C₂→G₇₁ led to residual activity of 38 and 1.8%, respectively. This result indicates that the C₂→G₇₁ base pair of tRNA Gly acts as a major anti-determinant to prevent incorporation of Gly into peptidoglycan precursors by FemX₇v. Conversely, the moderate impact of the G₃→A (45% residual activity) and A₇₃→G (62%) substitutions are in agreement with the comparison of misacylated tRNAs (Figure 4) that
led to the conclusion that Ser-tRNA^Ser^ is mainly excluded from the FemX_Wv active site due to unfavourable interaction with the aminoacyl residue.

**Stereospecificity of FemX_Wv**

The helix_Ala substituted by d-Ala instead of l-Ala was used as a substrate by FemX_Wv albeit with a 110-fold reduced catalytic efficiency (Figure 7). Thus, the configuration of the C2 of the l-alanyl residue was preferred but not essential for FemX_Wv activity. 2-Amino-isobutyrate, which combines two methyl groups at this position, was not used as a substrate.

**DISCUSSION**

The main function of aminoacyl-tRNAs is the interpretation of the genetic code by providing the interface between nucleic acid triplets in mRNA and the corresponding amino acids in proteins. Aminoacyl-tRNAs also participate in the synthesis of proteinogenic amino acids such as glutamine and asparagine by amidation of Glu-tRNA^{Gln} and Asp-tRNA^{Asn} or selenocysteine by conversion of Ser-tRNA^{Sec} into Sec-tRNA^{Sec} (38,39). The misacylated tRNA intermediates of the tRNA-dependent amino-acid biosynthesis pathways are not recognized by EF-Tu or by the corresponding specific elongation factors (38,39). The fidelity of translation is preserved by this mechanism and by the assembly of the participating biosynthetic enzymes in stable ribonucleoprotein complexes that channel the misacylated tRNAs through the different catalytic steps and prevent their interaction with the translation machinery (40). Independently from their role as translation substrates, certain aminoacyl-tRNAs act as aminoacyl donors in several biosynthesis pathways (41) including the assembly of the side chain of peptidoglycan precursors (42) (Figure 1).

In addition, Glu-tRNA^{Gln} participates in the synthesis of porphyrins (43), Lys-tRNA^{Lys} and Ala-tRNA^{Ala} are used for aminoacylation of phosphatidylglycerol (44) and PhetRNA^{Phe} and Leu-tRNA^{Leu} act as aminoacyl donors for labelling the N-terminus of proteins targeted for degradation by the proteasome-like protease ClpAP (45). The aminoacyl-tRNA substrates of all these reactions are correctly acylated and have therefore the dual capacity to participate in two pathways. Partial uncoupling mediated by

Figure 6. Specificity of FemX_Wv for the discriminator base and the first three base pairs of the acceptor arm. (A) Nucleotide substitutions were introduced into the distal portion of the RNA helix_Ala (boxed). (B and C) The relative efficiency of FemX_Wv was estimated for the transfer of L-Ala from the acylated helices to UDP-MurNAc-l[14C]pentapeptide. Data are presented in two graphs according to the range of FemX_Wv concentrations required to estimate enzyme activity.
dedicated tRNAs have been reported in rare instances. In Acidithiobacillus ferrooxidans for example, one of the three Glu-tRNA<sup>Glu</sup> that act as translation substrates does not participate in porphyrin synthesis probably ensuring an adequate supply of Glu-tRNA<sup>Glu</sup> upon high heme demand (46). In Staphylococcus epidermidis, an adequate supply of Gly-tRNA<sup>Gly</sup> to the synthesis of the side chain of peptidoglycan precursors could be provided by two closely related tRNA<sup>Gly</sup> that do not participate in protein synthesis (47). The remaining tRNA<sup>Gly</sup> are functional in both pathways as are a wide variety of tRNAs that have been investigated in other organisms (19, 20, 25, 26, 30). This implies that the tRNA-dependent transferases have developed their own interpretation of the genetic code to achieve specificity, a question typically investigated with substrates obtained by enzymatic acylation of tRNAs (19, 30, 48). Since the aminoacyl-tRNA synthetases are highly specific, the latter experimental approach is severely limited by the substrates that can be analysed. Here we have developed chemical acylation of RNA helices that allowed for the first time combining modifications in the aminoacyl residue with any base substitution in the RNA moiety of the substrate (Figure 2). Since the complex organic syntheses were not feasible with radiolabelled material, determination of FemX<sub>Wv</sub> activity with these substrates required to develop a novel assay in which the radioisotope is introduced in the peptidoglycan precursor rather than in the aminoacyl residue (Figure 3).

In a first set of experiments, the contribution of the aminoacyl residue to the FemX<sub>Wv</sub> specificity was investigated by comparing substrates obtained by misacylation of an RNA helix that mimics the acceptor arm of tRNA<sup>Ala</sup> (helix<sup>Ala</sup>). L-Ser and L-Ala were found to be transferred from acyl donors containing an identical helix with a relative efficiency of 7.0% (Figure 3). Since a relative efficiency of 6.0% was previously observed for comparison of the entire substrate (Ala-tRNA<sup>Ala</sup> versus Ser-tRNA<sup>Ser</sup>) (30), this observation indicates that exclusion of L-Ser from the FemX<sub>Wv</sub> active site is in itself sufficient to quantitatively account for discrimination between these two aminoacyl-tRNAs (Figure 8). In agreement, transfer of L-Ala from helices mimicking the acceptor arm of tRNA<sup>Ala</sup> and tRNA<sup>Ser</sup> occurred at relative efficiencies of 31% reflecting the moderate impact of the A<sup>73G</sup> (62%) and G<sup>3A</sup> (45%) substitutions that were also tested individually (Figure 6 and 8). Cys is likely to be similarly excluded from the FemX<sub>Wv</sub> active site although this was not directly tested.

Several lines of evidence indicate that discrimination against l-Ser, and presumably l-Cys, is due to sterice hindrance. Helix<sup>Ala</sup> charged with L-Ala and Gly were used with relative efficiencies of 33% indicating that recognition of the methyl group of L-Ala has a marginal role in the catalytic efficiency of FemX<sub>Wv</sub> (Figure 3). In addition, 2-aminobutyrate was not transferred by FemX<sub>Wv</sub> (<0.01%) although Δ-Ala-helix<sup>Ala</sup> was a substrate (0.9%) (Figure 7). N-acetylation of L-Ala and extension of the side chain from a methyl (L-Ala) to a CH<sub>2</sub>-CH<sub>2</sub>-S-CH<sub>3</sub> group (L-Met) both abolished FemX<sub>Wv</sub> activity (M. Lecerf, unpublished results). Together these results indicate that the specificity of FemX<sub>Wv</sub> involves steric hindrance rather than the recognition of the methyl group of L-Ala. This mode of substrate recognition implies that FemX<sub>Wv</sub> can discriminate between L-Ala and all other proteinogenic amino acids except Gly.

Since recognition of the L-Ala and Gly residues cannot account for the specificity of FemX<sub>Wv</sub>, we searched for anti-determinants in the tRNA<sup>Gly</sup> sequence (Figure 6). Similar residual activities were observed for the transplanation of the C<sup>2-G</sup><sup>71</sup> base pair of tRNA<sup>Gly</sup> into the Ala-Helix<sup>Ala</sup> (1.8%) and the comparison of Ala-tRNA<sup>Ala</sup> and Gly-tRNA<sup>Gly</sup> (2.6%) (Figure 8). Thus, the C<sup>2-G</sup><sup>71</sup> base pair of tRNA<sup>Gly</sup> acts as a major anti-determinant that quantitatively accounts for the specificity of FemX<sub>Wv</sub> independently from the discrimination between the Ala and Gly residues. The role of the recognition of the RNA moiety of the substrate is also supported by the inhibition of FemX<sub>Wv</sub> by stable Ala-tRNA<sup>Ala</sup> analogues containing a mimetic of the acceptor stem of tRNA<sup>Gly</sup> since substitution of helix<sup>Ala</sup> by helix<sup>Gly</sup> in the RNA moiety of the inhibitors led to a 48-fold increase in the IC<sub>50</sub> (Figure 5). Thus, the specificity of FemX<sub>Wv</sub> for the aminoacyl-tRNA donor substrate is based on excluding aminoacyl residues larger than L-Ala from its active site.
and exploiting the first divergent base pair in the acceptor stems of tRNA\textsubscript{Ala} and tRNA\textsubscript{Gly} as an antiderminant to prevent mis-incorporation of Gly (G\textsubscript{2}-C\textsubscript{71} in tRNA\textsubscript{Ala} versus C\textsuperscript{2}-G\textsubscript{71} in tRNA\textsubscript{Gly}). In agreement, modelling of the donor substrate in the catalytic cavity of FemX\textsubscript{Wv} indicated that the enzyme can interact with the first two base pairs (1-72 and 2-71) of the acceptor stem of Ala-tRNA\textsubscript{Ala}, the single stranded ACCA extremity and the aminoacyl residue (30). Efficient binding of elongation factor EF-Tu to aminoacyl-tRNAs also requires the correct combination of amino acid and tRNA body that have independent and compensatory thermodynamic contributions to the overall affinity thereby ensuring uniform binding to the 20 aminoacyl-tRNA types (49).

Aminoacyl-tRNA recognition by FemX\textsubscript{Wv} and the leucyl/phenyl-tRNA protein transferase (L/F-transferase) involved in the control of protein degradation are very different although the two enzymes share a similar fold in the absence of primary amino-acid sequence conservation (50,51). The catalytic cavity of the L/F-transferase contains a hydrophobic pocket that accommodates the side chain of Leu and Phe and efficiently excludes the β-branched side chains of Ile and Val (48,50). The size of the Ala and Pro side chains is not large enough to fit within the hydrophobic pocket whereas low activity was detected with Met-tRNA\textsubscript{Met} (50). Steric hindrance accounts for the low activity observed with Trp-tRNA\textsubscript{Trp} (50). In contrast to FemX\textsubscript{Wv}, biochemical studies led to the conclusion that the L/F-transferase recognizes the aminoacyl-tRNA substrates in a sequence-independent manner (48,51). A double-stranded acceptor stem is fully dispensable for the L/F-transferase and replacement of G-C by weaker base pairs in the acceptor stem reduces the Km of the aminoacyl-tRNA substrate (48). The opposite result was obtained for FemX\textsubscript{Wv} (Figure 6) since replacement of G\textsubscript{1}-C\textsubscript{72} by unpaired G\textsuperscript{1}•G\textsubscript{72} had a greater impact on FemX\textsubscript{Wv} activity than a double substitution that restored a Watson–Crick base pairing (C\textsuperscript{1}-G\textsubscript{72}) at this position (1.0% versus 6.5% residual activity, respectively).

Comparison of the L/F-transferase and FemX\textsubscript{Wv} indicates that the mode of recognition of the aminoacyl-tRNA substrate is not conserved among enzymes that use this type of activated substrate in non-translational processes. Aminoacyl-tRNA recognition by component of the translation machinery is also different, as exemplified by the analysis of the G\textsubscript{15}•U\textsubscript{70} base pair, which is essential for the activity of the alanyl-tRNA synthetase, but not of FemX\textsubscript{Wv} (45% relative efficiency; Figure 6). These observations suggest that Fem transferases are parasitic on the translation machinery that provides a ubiquitous and permanent supply of amino acids activated in the form of aminoacyl-tRNAs. Fem transferases may have therefore evolved from a translation-independent pathway involving aminoacyl activation by adenylation, although the enzymes may have lost the capacity to use a mono-nucleotide as the substrate. The L/F-transferase binds phenylalanyl-adesosine (rA-Phe) (45) and its analogue puromycine (47). Transfer of Phe from rA-Phe to the aminoacyl-tRNA is catalysed by the leucyl/phenyl-tRNA protein transferase (45,50). In contrast to FemX\textsubscript{Wv}, biochemical analyses of FemX\textsubscript{Wv} and L/F-transferase suggest that the aminoacyl-tRNA substrate is not conserved among enzymes that use this type of activated substrate in non-translational processes.

**Figure 8.** Relative contribution of the aminoacyl residue and of the nucleotide sequence to the specificity of FemX\textsubscript{Wv}. Previous comparisons of full tRNAs obtained by in vitro transcription indicated that Ser-tRNA\textsubscript{Ser} and Gly-tRNA\textsubscript{Gly} are used 17- and 38-fold less efficiently than Ala-tRNA\textsubscript{Ala} by FemX\textsubscript{Wv} (6.0% and 2.6%, respectively) (30). In this study, the main identity determinants of FemX\textsubscript{Wv} were identified based on independently testing the impact of substitutions of the aminoacyl residue and of nucleotides on FemX\textsubscript{Wv} activity. Substitutions were chosen according to the differences found in the distal portion of the acceptor arm of tRNA Ala, tRNA Ser and tRNA Gly. The discrimination between L-Ala and L-Ser is the main factor that prevents incorporation of Gly (G\textsubscript{2}-C\textsubscript{71} in tRNA Ala) whereas the C\textsuperscript{2}-G\textsubscript{71} base pair in the acceptor arm of tRNA Gly is the major anti-determinant that prevents incorporation of Gly.
spectrometry (51), although a larger portion of the aminoacyl-tRNA substrate is required for efficient aminoacylation (48). Recognition of the acceptor stem can be selectively advantageous, not only to provide specificity determinants (as shown for the 2–71 base pair in this work), but also to ensure sufficient affinity for the aminoacyl-tRNA substrates in order to compete with EF-Tu. The predominant recognition of the aminoacyl moiety of the substrates by the L/F-transferase and FemX, as well as the different modes of recognition of the acceptor stems suggest multiple origins for these aminoacyl transferases that are unrelated to any tRNA-interacting protein of the translation machinery. FemX, and the L/F-transferase should therefore be considered as enzymes that have developed their own interpretation of the genetic code but did not participate in its elaboration.

ACKNOWLEDGEMENTS

We thank Antoine P. Maillard for helpful discussion.

FUNDING

This work was supported by the European Community (EUR-INTAFAR, Project N° LSHM-CT-2004-512138, 6th PCRD) and by the Fondation Recherche Médicale (fin de thèse to Régis Villet). Funding for open access charge: INSERM.

Conflict of interest statement. None declared.

REFERENCES

1. den Blaauwen,T., de Pedro,M.A., Nguyen-Distèche,M. and Ayala,J.A. (2008) Morphogenesis of rod-shaped sacculi. FEMS Microbiol. Rev., 32, 321–344.
2. Dramsi,S., Magnét,S., Davison,S. and Arthur,M. (2008) Covalent attachment of proteins to peptidoglycan. FEMS Microbiol. Rev., 32, 307–320.
3. Vollmer,W. (2008) Structural variation in the glycan strands of bacterial peptidoglycan. FEMS Microbiol. Rev., 32, 287–306.
4. Kern,T., Hediger,S., Muller,P., Giustini,C., Joris,B., Bougault,C., Vollmer,W. and Simorre,J.P. (2008) Toward the characterization of peptidoglycan structure and protein-peptidoglycan interactions by solid-state NMR spectroscopy. J. Am. Chem. Soc., 130, 5618–5619.
5. Vollmer,W., Blanot,D. and de Pedro,M.A. (2008) Peptidoglycan structure and architecture. FEMS Microbiol. Rev., 32, 149–167.
6. Barreteau,H., Kovac,A., Boniface,A., Sova,M., Gobec,S. and Blanot,D. (2008) Cytoplastical steps of peptidoglycan biosynthesis. FEMS Microbiol. Rev., 32, 168–207.
7. Schleifer,K.H. and Kandler,O. (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriologica, 136, 407–477.
8. Biarrotte-Sorin,S., Maillard,A.P., Delettre,J., Sougakoff,W., Arthur,M. and Mayer,C. (2004) Crystal structures of Weisella viridescens FemX and its complex with UDP-MurNAc-pentapeptide: insights into FemABX family substrate recognition. Structure, 12, 257–267.
9. Benson,T.E., Prince,D.B., Mutchler,V.T., Curry,K.A., Ho,A.M., Sarver,R.W., Hagadorn,J.C., Choi,G.H. and Garlick,R.L. (2002) X-ray crystal structure of Staphylococcus aureus FemA. Structure, 10, 1107–1115.
10. Bellais,S., Arthur,M., Dubost,L., Hugonnet,J.E., Gutmann,L., Mainardi,J.L., Heijenoort,I., Legrand,R., Brouard,J.P., Rice,L. and Mainardi,J.L. (2006) Asfim, the D-aspartyl ligase responsible for the addition of D-aspartic acid onto the peptidoglycan precursor of Enterococcus faecium. J. Biol. Chem., 281, 11586–11594.
11. Sauvage,E., Kerfl,F., Terrak,M., Ayala,J.A. and Charlier,P. (2008) The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. FEMS Microbiol. Rev., 32, 234–258.
12. Mainardi,J.L., Fourgeaud,M., Hugonnet,J.E., Dubost,L., Brouard,J.P., Ouazzani,J., Rice,L.B., Gutmann,L. and Arthur,M. (2005) A novel peptidoglycan cross-linking enzyme for a beta-lactam-resistant transpeptidation pathway. J. Biol. Chem., 280, 38146–38152.
13. Lavollay,M., Arthur,M., Fourgeaud,M., Dubost,L., Marie,A., Veziris,N., Blanot,D., Gutmann,L. and Mainardi,J.L. (2008) The peptidoglycan of stationary-phase Mycobacterium tuberculosis predominantly contains cross-links generated by L,D-transpeptidation. J. Bacteriol., 190, 4360–4366.
14. Kopp,U., Roos,M., Wecke,J. and Labischinski,H. (1996) Staphylococcal peptidoglycan interpeptide bridge biosynthesis: a novel antistaphylococcal target? Microb. Drug. Resist., 2, 29–41.
15. Arbeloa,A., Hugonnet,J.E., Sentille,M.C., Josseaume,N., Dubost,L., Monsempes,C., Blanot,D., Brouard,J.P. and Arthur,M. (2004) Synthesis of mosaic peptidoglycan cross-bridges by hybrid peptidoglycan assembly pathways in gram-positive bacteria. J. Biol. Chem., 279, 41546–41556.
16. Magnét,S., Arbeloa,A., Mainardi,J.L., Hugonnet,J.E., Fourgeaud,M., Dubost,L., Marie,A., Delfosse,V., Mayer,C., Rice,L.B. et al. (2007) Specificity of L,D-transpeptidases from gram-positive bacteria producing different peptidoglycan chemotypes. J. Biol. Chem., 282, 13151–13159.
17. Vollmer,W., Joris,B., Charlier,P. and Foster,S. (2008) Bacterial peptidoglycan (murain) hydrolases. FEMS Microbiol. Rev., 32, 259–286.
18. Ehler,K., Tschierske,M., Mork,C., Schroder,W. and Berger-Bachi,B. (2000) Site-specific serine incorporation by Lif and Epr into positions 3 and 5 of the Staphylococcal peptidoglycan interpeptide bridge. J. Bacteriol., 182, 2635–2638.
19. Hegde,S.S. and Blanchard,J.S. (2003) Kinetic and mechanistic recombination of Lactobacillus viridescens FemX (UDP-N-acetylmuramol pentapeptide-lysine N6-alanyltransferase). J. Biol. Chem., 278, 22861–22867.
20. Hegde,S.S. and Shrader,T.E. (2001) FemABX family members are novel nonribosomal peptidyltransferases and important pathogen-specific drug targets. J. Biol. Chem., 276, 6998–7003.
21. Rohner,S., Ehler,K., Tschierske,M., Labischinski,H. and Berger-Bachi,B. (1999) The essential Staphylococcus aureus gene fnnB is involved in the first step of peptidoglycan pentaglycine interpeptide formation. Proc. Natl Acad. Sci. USA, 96, 9351–9356.
22. Filipe,S.R., Severina,E. and Tomasz,A. (2002) The mural gene operon: a functional link between antibiotic resistance and antibiotic tolerance in Streptococcus pneumoniae. Proc. Natl Acad. Sci. USA, 99, 1505–1550.
23. Filipe,S.R., Severina,E. and Tomasz,A. (2001) The role of mural gene operon in penicillin resistance and antibiotic tolerance of Streptococcus pneumoniae. Microb. Drug Resist., 7, 303–316.
24. Berger-Bachi,B., Barberis-Maino,L., Strassel,A. and Kayser,F.H. (1989) FemA, a host-mediated factor essential for meticillin resistance in Staphylococcus aureus: molecular cloning and characterization. Mol. Gen. Genet., 219, 263–269.
25. Schneider,T., Senn,M.M., Berger-Bachi,B., Tossi,A., Sahh,H.G. and Wiedemann,I. (2004) In vitro assembly of a complete, pentaglycine interpeptide bridge containing cell wall precursor (lipid II-Gly5) of Staphylococcus aureus. Mol. Microbiol., 53, 675–685.
26. Lloyd,A.J., Gilbey,A.M., Blewett,A.M., De Pascale,G., El Zobeiby,A., Levesque,R.C., Catherwood,A.C., Tomasz,A., Bugg,T.D., Roper,D.I. et al. (2008) Characterization of tRNA-dependent peptide bond formation by MurM in the synthesis of Streptococcus pneumoniae peptidoglycan. J. Biol. Chem., 283, 6402–6417.
27. Bouhs,A., Josseaume,N., Allanic,D., Crouvoisier,M., Gutmann,L., Mainardi,J.L., Menger-Lecreux,L., Van Heijenoort,J. and Arthur,M. (2001) Identification of the UDP-MurNAc-pentapeptide-L-alanine ligase for synthesis of branched...
peptidoglycan precursors in Enterococcus faecalis. J. Bacteriol., 183, 5122–5127.
28. Bouhss, A., Josseaume, N., Severin, A., Tabel, K., Hugonnet, J.E., Shlaes, D., Mengin-Lecreulx, D., Van Heijenoort, J. and Arthur, M. (2002) Synthesis of the L-alanyl-L-alanine cross-bridge of Enterococcus faecalis peptidoglycan. J. Biol. Chem., 277, 45935–45941.
29. Maillard, A.P., Biarrotte-Sorin, S., Villet, R., Mesnage, S., Bouhss, A., Sougakoff, W., Mayer, C. and Arthur, M. (2005) Structure-based site-directed mutagenesis of the UDP-MurNAc-pentapeptidate-binding cavity of the FmEx alanyl transferase from Weissella viridescens. J. Bacteriol., 187, 3833–3838.
30. Villet, R., Fontvielle, M., Busca, P., Chemama, M., Maillard, A.P., Hugonnet, J.E., Dubost, L., Marie, A., Josseaume, N., Mesnage, S. et al. (2007) Idiosyncratic features in tRNAs participating in bacterial cell wall synthesis. Nucleic Acids Res., 35, 6870–6883.
31. Babic, A., Patin, D., Boniface, A., Hervé, M., Mengin-Lecreulx, D., Pecar, S., Gobec, S. and Blanot, D. (2007) Chemoenzymatic synthesis of the nucleotide substrates of the Mur ligases. In Proceedings of the 5th Joint Meeting on Medicinal Chemistry, June 17-21, Portoroz, Slovenia, Medimond Srl, Bologna, Italy, Medimond Srl, Bologna, Italy, pp. 1–4.
32. Bouhss, A., Crouvoisier, M., Blanot, D. and Mengin-Lecreulx, D. (2004) Purification and characterization of the bacterial MraY translocase catalyzing the first membrane step of peptidoglycan biosynthesis. J. Biol. Chem., 279, 29974–29980.
33. Robertson, S.A., Noren, C.J., Anthony-Cahill, S.J., Griffith, M.C. and Schultz, P.G. (1989) The use of 5′-phospho-2′ deoxyribocytidylylribonuclease as a facile route to chemical aminoacylation of tRNA. Methods Enzymol., 179, 245–251.
34. Chemama, M., Fonvielle, M., Villet, R., Arthur, M., Valery, J.M. and Etheve-Quelquejeu, M. (2007) Stable analogues of aminoacyl-tRNA for inhibition of an essential step of bacterial cell-wall synthesis. J. Am. Chem. Soc., 129, 12642–12643.
35. Ibbasi, M. and Soll, D. (2004) Aminoacyl-tRNAs: setting the limits of the genetic code. Genes Dev., 18, 731–738.
36. Sheppard, K., Yuan, J., Hohn, M.J., Jester, B., Devine, K.M. and Soll, D. (2008) From one amino acid to another: tRNA-dependent amino acid biosynthesis. Nucleic Acids Res., 36, 1813–1825.
37. Baille, M., Blaise, M., Roy, H., Deniziat, M., Lorber, B., Birck, C., Becker, H.D. and Kern, D. (2008) tRNA-dependent asparagine formation in prokaryotes: characterization, isolation and structural and functional analysis of a ribonucleoprotein particle generating Asn-tRNA(Asn). Methods, 44, 146–165.
38. RajBhandary, U.L. and Soll, D. (2008) Aminoacyl-tRNAs, the bacterial cell envelope, and antibiotics. Proc. Natl Acad. Sci. USA, 105, 5285–5286.
39. Mainardi, J.L., Villet, R., Bugg, T.D., Mayer, C. and Arthur, M. (2008) Evolution of peptidoglycan biosynthesis under the selective pressure of antibiotics in Gram-positive bacteria. FEMS Microbiol. Rev., 32, 386–408.
40. Randau, L., Schauer, S., Ambrogelly, A., Salazar, J.C., Moser, J., Sekine, S., Yokoyama, S., Soll, D. and Jahn, D. (2004) tRNA recognition by glutamyl-tRNA reductase. J. Biol. Chem., 279, 34931–34937.
41. Roy, H. and Ibba, M. (2008) RNA-dependent lipid remodeling by bacterial multiple peptide resistance factors. Proc. Natl Acad. Sci. USA, 105, 4667–4672.
42. Watanebe, K., Toh, Y., Suto, K., Shimizu, Y., Oka, N., Wada, T. and Tomita, K. (2007) Protein-based peptide-bond formation by aminoacyl-tRNA protein transferase. Nature, 449, 867–871.
43. Levican, G., Katz, A., Valenzuela, P., Soll, D. and Orellana, O. (2005) A tRNA(Glu) that uncouples protein and tetrapyrrole biosynthesis. FEBS Lett., 579, 6383–6387.
44. Roberts, R.J. (1974) Staphylococcal transfer ribonucleic acids. II. Sequence analysis of isoaccepting glycine transfer ribonucleic acids IA and IB from Staphylococcus epidermidis Texas 26. J. Biol. Chem., 249, 4787–4796.
45. Abramochkin, G. and Shrader, T.E. (1996) Aminoacyl-tRNA recognition by the leucyl/phenylalanyl-tRNA-protein transferase. J. Biol. Chem., 271, 22901–22907.
46. LaRiviere, F.J., Wolfson, A.D. and Uhlenbeck, O.C. (2001) Uniform binding of aminoacyl-tRNAs to elongation factor Tu by thermodynamic compensation. Science, 294, 165–168.
47. Dong, X., Kato-Murayama, M., Muramatsu, T., Mori, H., Shirouzu, M., Besho, Y. and Yokoyama, S. (2007) The crystal structure of leucyl/phenylalanyl-tRNA-protein transferase from Escherichia coli. Protein Sci., 16, 528–534.
48. Suto, K., Shimizu, Y., Watanebe, K., Ueda, T., Fukai, S., Nureki, O. and Tomita, K. (2006) Crystal structures of leucyl/phenylalanyl-tRNA-protein transferase and its complex with an aminoacyl-tRNA analog. EMBO J., 25, 5942–5950.