Aminoacyl-tRNAs, the bacterial cell envelope, and antibiotics

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As adaptor molecules linking the codons in a mRNA to the amino acids that they specify, aminoacyl-tRNAs (AA-tRNAs) play a central role in protein biosynthesis. In addition to this critical role, AA-tRNAs are also involved in several other less well known but still important biochemical reactions (Fig. 1). For example, AA-tRNAs are used as substrates for transfer of a single amino acid to the N termini of proteins in a reaction catalyzed by the AA-tRNA-protein transferases (1, 2). The newly attached N-terminal amino acid then acts as a signal for degradation of the protein (3). In another example, the amino acid attached to the tRNA is reduced; glutamyl-tRNA reductase (4) converts the glutamyl residue of glutamyl-tRNA to glutamate 1-semialdehyde, the first precursor in the C_5-pathway of porphyrin biosynthesis (5). Two other important uses of AA-tRNA that affect the properties of the cell envelope are (*i*) the aminoacylation of phospholipids in the cell membrane and (*ii*) the crosslinking of the peptidoglycan in the cell walls of Gram-positive pathogens. Two recent papers (6, 7), including the one by Roy and Ibba in a recent issue of PNAS (6), focus on these last two reactions by highlighting the role of AA-tRNA in the biosynthesis of the bacterial cell envelope that affects how the cell interacts with antibiotics and antimicrobial peptides.

The presence of a variety of aminoacyl-phosphatidylglycerol (AA-PG) compounds in bacteria was first described over four decades ago (8). The amino acids identified included lysine, alanine, arginine, and ornithine. Cell-free studies on the enzymes involved in the synthesis of these various AA-PG compounds led to the discovery that lysyl-tRNA is the donor of the amino acid (9) that is esterified to one of the 3′-hydroxyl groups of the glycerol moiety in lysyl-phosphatidylglycerol (lysyl-PG) (10). A biochemical survey showed that lysyl-PG was formed by cell extracts of *Staphylococcus aureus*, *Bacillus megaterium*, *Bacillus cereus*, and *Clostridium welchii*, an organism that also synthesizes alanyl-PG from alanyl-tRNA. Furthermore, arginyl-PG

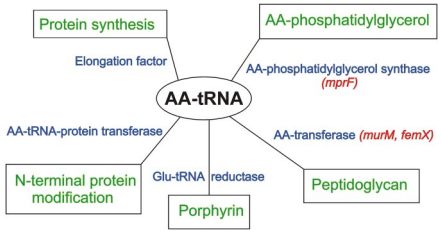

Fig. 1. Cellular processes that use AA-tRNA. The individual processes are shown in green, the enzymes/carrier proteins are shown in blue, and some relevant genes are shown in red.

was shown to be synthesized by extracts of *Enterococcus faecalis* (previously called *Streptococcus faecalis*) (11, 12), and ornithyl-PG was found in *B. cereus* (13). These studies suggested the existence of different enzymes for synthesis of alanyl-PG, lysyl-PG, and possibly other AA-PGs. The enzymes displayed some specificity for tRNA recognition, because Ala-tRNA^{Cys} (alanine attached to cysteine tRNA) was reported not to be a substrate for alanyl-PG formation (12). In addition, aminoethylcysteinyl-tRNA^{Lys}, an analogue of lysyl-tRNA^{Lys}, supported aminoethylcysteinyl-PG synthesis, whereas aminoethylcysteinyl-tRNA^{Cys} did not (14). The enzymes were not further characterized.

The next advance came 30 years later during studies of bacterial immune escape mechanisms, which are directed against antimicrobial peptides of the innate immune system such as defensins and which are conserved in several pathogens. Many compounds that affect bacteria (e.g., bacteriolytic enzymes or antimicrobial peptides) are cationic and bind to the bacterial cell membrane, which is mostly anionic. Bacteria can, however, modulate the net charge of their anionic cell membrane polymers (e.g., phospholipids) by introducing positively charged groups, which would lead to reduced binding and permeability of the cationic peptides. Examination of *S. aureus* resistance to defensins uncovered a new gene, *mprF*, of unknown function conserved in many pathogens (15). A staphylococcal *mprF* mutant strain was much more sensitive to defensins than was the wild-type strain. The gene product was named "multiple peptide resistance factor" (MprF) and was suggested to be a new virulence factor. Also, membrane lipid analysis revealed that the *mprF* mutant strain did not synthesize lysyl-PG. These findings led to the notion that lysyl-PG is important for pathogenicity of *S. aureus*, because its presence leads to reduced binding and cellular permeability of cationic antimicrobial peptides, leading to increased resistance to defensins. Another *S. aureus mprF* mutation sensitized the cells to vancomycin and other antibiotics, suggesting a role for lysyl-PG in the multidrug resistance of methicillin-resistant *S. aureus* (16), a growing problem in staphylococcal infections, and highlighting the important role of MprF.

The work of Roy and Ibba (6) presents a thorough analysis of two different *Clostridium perfringens* proteins, MprF1 and MprF2, as AA-PG synthases. *C. perfringens* MprF2 is an 851-aa protein with a membrane-inserted hydrophobic N-terminal domain and a hydrophilic C-terminal domain. MprF homologues are present in a large number of bacteria and even in some archaea. Using a special *Escherichia coli* strain that allows high expression of membrane proteins, Roy and Ibba characterized the *C. perfringens mprF1* and *mprF2* gene products *in vivo* and *in vitro*. Each enzyme was shown to have distinct amino acid specificity; MprF1 catalyzes alanyl-PG formation, whereas MprF2 catalyzes lysyl-PG formation. A careful analysis showed that, under physiological conditions, the affinity of MprF2 protein for Lys-tRNA^{Lys} was comparable to that of the elongation factor EF-Tu, the carrier of AA-tRNAs to the ribosome during protein synthesis. Studies with different tRNAs and a tRNA minihelix indicated that the primary determinant for AA-tRNA recognition by MprF1 and MprF2 was the amino acid moiety attached to the tRNA. In view of early studies suggesting some tRNA specificity

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in recognition of AA-tRNA by the AA-PG synthases (12, 14) and by enzymes involved in peptidoglycan synthesis (17), further work—particularly on the role of the tRNA acceptor stem nucleotides and the discriminator base—may, however, be desirable.

The paper by Lloyd *et al*. (7) focuses on the biosynthesis and properties of peptidoglycan. This essential cell wall component, located outside the cytoplasmic membrane, gives the bacterial cell wall strength and shape. The peptidoglycan layer is a linear carbohydrate polymer of alternating *N*-acetylmuramic acid and *N*-acetylglucosamine residues with an appended stem peptide of 4–5 aa linked to each of the *N*-acetylmuramic acid residues. The stem peptides are cross-linked either directly or through interpeptide bridges between the lysine of one chain and alanine of the other chain. The structure of the interpeptide bridges is to some degree genus-specific, and amino acids in the interpeptide bridges are inserted by AA-transferases using AA-tRNA as substrates (e.g., see refs. 18 and 19).

In *S. aureus*, including the methicillin-resistant strains, a pentaglycine bridge cross-links the peptidoglycan stem peptides (18, 20). Inhibition of pentaglycine bridge formation reduces methicillin resistance, leading to β-lactam hypersusceptibility (21). The enzyme catalyzing the first step in the synthesis of the pentaglycine bridge in *S. aureus* peptidoglycan was shown to be encoded by *fmhB* (also called *femX*), an essential gene (20). Similar work carried out in *Streptococcus pneumoniae* (7) has also shown that high-level penicillin resistance is associated with modifications in the structure of the peptidoglycan (22). Penicillin-resistant pneumococcal strains contained mostly abnormal branched stem peptides with Ala-Ala or Ser-Ala dipeptides linking the ε-amino group of the lysine residue in one stem peptide to alanine in the other stem peptide (22). In contrast, the penicillin-sensitive *Pneumococcus* strains had primarily linear stem peptides. Based on work done in *S. aureus*, the pneumococcal *murM* gene was identified from its sequence similarity with *S. aureus* FemX (23). A *murM* gene disruption in penicillin-resistant *S. pneumoniae* generated a penicillin-sensitive strain that contained mainly linear stem peptides. Thus, the presence of branched stem peptides in *S. pneumoniae* is critical for penicillin resistance. The recent work by Lloyd *et al.* (7) characterizes the *S. pneumoniae* MurM protein (406 aa) from penicillin-resistant and -sensitive clinical isolates. This enzyme catalyzes the first step in the synthesis of the branched stem peptide by attaching either alanine or serine to the ε-amino group of the stem peptide's lysine residue. The MurM enzyme from a penicillin-resistant strain was shown to have a much higher alanylation activity compared with one from the sensitive strain.

It is worth noting that peptidoglycan is covalently linked to wall teichoic acid, another class of polyanionic molecules in the cell walls of Gram-positive bacteria (24). Interestingly, D-alanine, covalently attached through ester linkages to teichoic acids (25), is also thought to modulate the net anionic charge of the teichoic acids. Furthermore, there is a good correlation between the D-alanyl ester content of teichoic acids and resistance of the bacteria to peptides of the innate immune system such as defensins and antibiotics such as vancomycin (26). Because it is D-alanine and not L-alanine that is linked to the teichoic acids, transfer of D-alanine does not involve AA-tRNA but involves a D-alanine carrier protein in which D-alanine is covalently linked to the 4′-phosphopantetheine prosthetic group of the carrier protein through a thioester bond (24).

In summary, recent studies on AA-PG synthases and the peptidoglycan related AA-transferases and the genes encoding them have highlighted the versatility of AA-tRNA in donating activated amino acids to very different acceptors in the cell. In addition, knowledge of the properties and important role of these enzymes and the genes encoding them has led to suggestions that inhibitors of these enzymes would increase the sensitivity of many bacterial pathogens to proteins of the innate immunity system and extend the action range of currently used antibiotics (27).

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