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Assembly of the monomer unit of bacterial peptidoglycan

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Abstract. The biosynthesis of peptidoglycan is a two-stage process. The first stage concerns the endocellular assembly of its monomer unit, whereas the second one concerns the exocellular polymerization steps. The continued interest for this system is due to (i) the emergence of new resistance mechanisms; (ii) the need of specific targets in the search for new antibacterials; and (iii) the steady progress in the study of the correlation of peptidoglycan metabolism with cell growth and division. The various steps of the assembly of the monomer unit will be discussed as well as the correlations between the two stages. Finally, the flexibility of the pathway will be exemplified in *Escherichia coli* and *Staphylococcus aureus*.

Key words. Bacterial peptidoglycan; precursor synthetases; catalytic mechanisms; regulations.

The biosynthesis of bacterial peptidoglycan is now a nearly half-century-old story. Its nucleotide precursors were first isolated and characterized by Park and Johnson [1, 2] at a time when its existence as an essential cell wall macromolecule was not yet established. The various steps of this biosynthesis have been studied in different species, and an overall view of the pathway valid for all eubacteria has emerged [3]. The continued interest in this system in recent years is primarily due to (i) the emergence of new resistance mechanisms against β-lactam and glycopeptide antibiotics involving subtle modifications in peptidoglycan synthesis; (ii) the need to overcome resistance mechanisms and to use specific targets in the search of new antibacterials; and (iii) the steady progress in the difficult problem of the correlation of peptidoglycan metabolism with cell growth and division.

The biosynthesis of peptidoglycan is a complex two-stage process [4]. The first stage concerns the assembly of its monomer unit by enzymes located in the cytoplasm or at the inner side of the cytoplasmic membrane. The final product is the lipid intermediate: disaccharide(pentapeptide)pyrophosphate undecaprenol. The second stage involves polymerization reactions that take place at the outer side of the cytoplasmic membrane and use the lipid intermediate as initial substrate. Concomitantly, nascent peptidoglycan is bound to the preexisting cell wall and undergoes maturation reactions that depend on the organism considered.

Three essential features characterize the assembly of the monomer unit. First, the high specificity of each step reflects the unusual structural characteristics of peptidoglycan, many of which are already encountered in its monomer unit (presence of *N*-acetylmuramic acid, D-glutamic acid involved in a γ linkage, a diamino acid and alternating D and L residues in peptide linkages). Second, the monomer unit is transferred from the cytoplasm to the externally located sites of polymerization. This implies a passage of the lipid intermediate through the hydrophobic environment of the membrane. Finally, the assembly of the monomer unit can be divided into four groups of reactions: formation of uridine 5′-pyrophosphate-*N*-acetylglucosamine (UDP-GlcNac), uridine 5′-pyrophosphate-*N*-acetylmuramic acid (UDP-MurNac), UDP-MurNac-peptides and lipid intermediates (fig. 1).

Formation of UDP-*N*-acetylglucosamine

Four successive steps are required for the synthesis of UDP-GlcNac from fructose-6-phosphate (fig. 2). The

first reaction is catalysed by glucosamine-6-phosphate synthase [5]. The next step concerns the conversion of glucosamine-6-phosphate into glucosamine-1-phosphate by the GlmM mutase coded by the recently identified *glmM* gene [6]. The last two steps, acetylation and uridylation, are catalysed by the *glmU* gene product, which is a bifunctional enzyme [7–9]. Its N-terminal domain catalyses uridylation, whereas its C-terminal domain catalyses acetylation [8, 9].

There is apparently no cooperativity between the two domains [9]. The pool of UDP-GlcNac is a limiting factor for the subsequent steps in the peptidoglycan pathway and is greatly increased on inhibition of protein synthesis [10]. The conversion of glucosamine-6-phosphate into glucosamine-1-phosphate may be the step regulating this process; only 5% of the abundant GlmM protein (1% of cell protein) is in an active state in growing cells, owing presumably to the phosphorylation of its active-site serine [6].

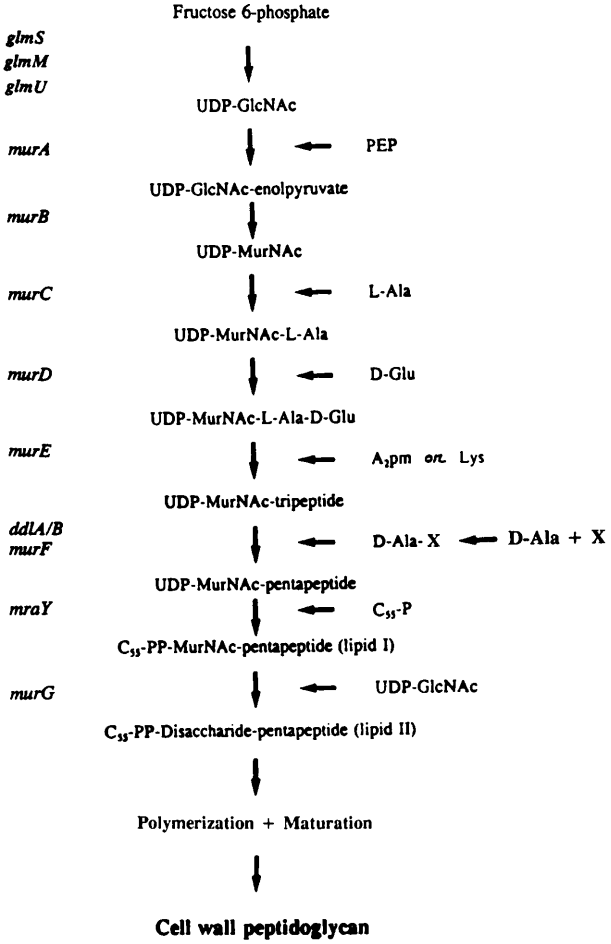

Figure 1. Assembly of the peptidoglycan monomer unit.

Formation of UDP-*N*-MurNac

The formation of UDP-MurNac from UDP-GlcNac is a two-step process (fig. 1). In the first step, the transfer of enolpyruvate from phosphoenolpyruvate to position 3 of the GlcNac residue is catalysed by transferase MurA to yield UDP-GlcNac-enolpyruvate. In the second step, the reduction of the enolpyruvyl moiety to D-lactoyl is catalysed by reductase MurB to yield UDP-MurNac. Both enzymes have been crystallized, and their three-dimensional (3D) structures determined. Their catalytic mechanisms are being actively investigated [11–15].

Formation of the UDP-MurNac-peptides

The assembly of the peptide subunit is carried out by the stepwise addition in most cases of L-alanine, D-glutamic acid, a diamino acid (usually diaminopimelic acid or lysine) and a dipeptide D-alanine-X (X = D-alanine, D-lactate, or D-serine) on the D-lactoyl group of UDP-MurNac (fig. 1). Each step is catalysed by a highly specific synthetase using adenosine 5′-triphosphate (ATP). These synthetases (MurC, MurD, MurE and MurF) have been extensively investigated in *Escherichia coli*

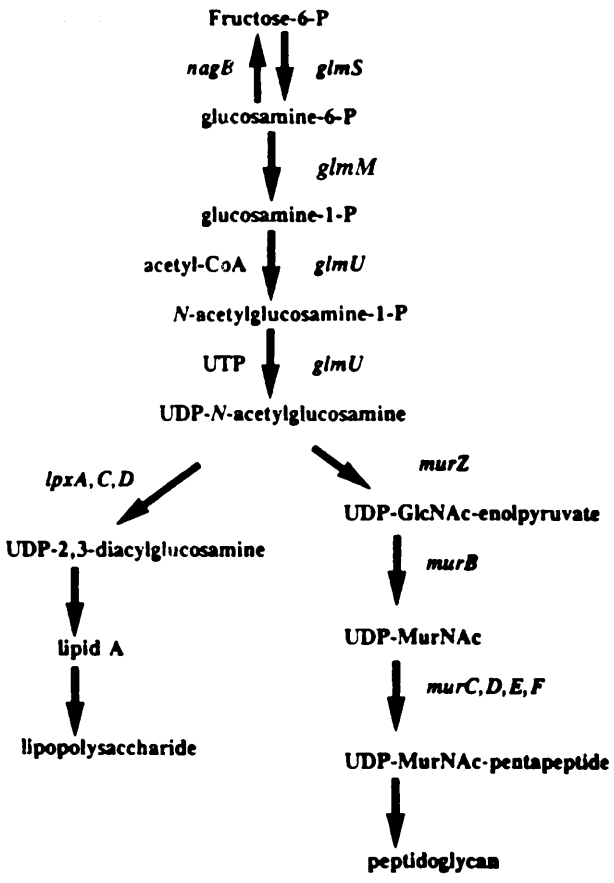

Figure 2. Metabolism of *N*-acetylglucosamine.

[16]. Their genes have been identified, cloned and sequenced, and their products overproduced and purified. They all catalyse the same type of reaction and operate via an essentially similar mechanism. This entails carboxyl activation of a C-terminal amino acid residue of the nucleotide substrate to an acyl-phosphate intermediate, followed by nucleophilic attack by the amino group of the condensing amino acid or dipeptide, with the elimination of phosphate and subsequent peptide bond formation. The existence of acyl-phosphate and tetrahedral intermediates has now been substantiated for these reactions [17–21].

The sequence comparison of 20 Mur synthetases from various bacterial organisms revealed common invariants: seven amino acids and the ATP-binding consensus sequence [22, 23]. The Mur synthetases thus appear as a well-defined class of functionally closely related proteins originating presumably from a common ancestor. The determination of the crystal structure of the MurD synthetase at 1.9-Å resolution [24] is the first step to the study of a possible common architecture for these enzymes. Moreover, it will be a great help in understanding not only their precise catalytic mechanisms but also the structural basis of their high specificity for their substrates.

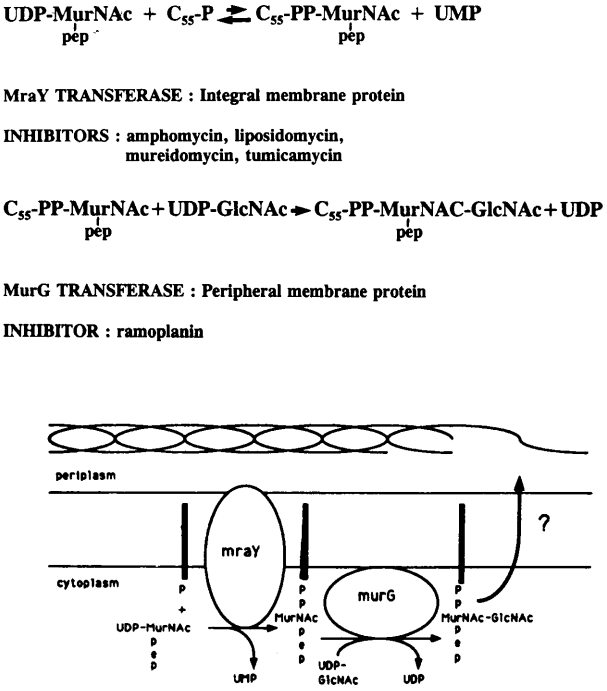

Figure 3. Formation of the peptidoglycan lipid intermediates.

Formation of the lipid intermediates

The membrane steps (fig. 1) involve, first, transferase MraY, which catalyses the transfer of the phospho-MurNac-pentapeptide moiety of UDP-MurNac-pentapeptide to the membrane acceptor undecaprenyl-phosphate to yield MurNac(pentapeptide)-pyrophosphoryl undecaprenol (lipid I). Thereafter, transferase MurG catalyses the addition of *N*-acetylglucosamine yielding GlcNac-MurNac(pentapeptide)-pyrophosphoryl-undecaprenol or lipid II [3, 4]. Comparison of amino acid sequences from six organisms clearly indicates a structure for MraY which has regularly repeated hydrophobic and hydrophilic domains. Computational modelling suggests that the protein has eight membrane segments and three main cytoplasm-oriented hydrophilic domains. The MraY transferases from *Bacillus megaterium* and *E. coli* have been solubilized and partially purified [25, 26]. Transferase MurG appears as a peripheral membrane protein located on the inner side of the cytoplasmic membrane [27] and has been partially purified [28].

In the formation of lipid I (fig. 3) the equilibrium is greatly in favour of the nucleotide precursor [4]. In growing *E. coli* cells, the ratio of UDP-MurNac-pentapeptide to lipid I is greater than 100:1 [29, 30]. This indicates that the pool level of undecaprenyl-phosphate is a main limiting factor in these membrane steps. The very low pool levels [31] of lipids I and II (700 and 2000 at most, respectively) imply high turnover rates of less than 1 s. This suggests that the lipid intermediates do not freely diffuse over the whole membrane and that they are in some way associated with transferases MraY and MurG. There could be a coupling between these two activities and also with the subsequent transport of lipid II across the membrane to the sites of polymerization. Evidence for lipid intermediate-protein interactions, which could indicate an association with the transferases in a peptidoglycan-synthesizing complex, has been obtained with *Staphylococcus aureus* [32].

It should be stressed that the described two-step formation of the lipid intermediates is valid only for peptidoglycan with a direct cross-linkage between its peptide subunits. However, in many organisms the final peptidoglycan material has more or less complex interpeptide cross-bridges between the subunits [3]. It has been shown in *S. aureus* that the extra cross-bridge amino acids were added to lipids I and II, thereby leading to a much more complex population of lipid intermediates [33].

Recently [34], analysis by high performance liquid chromatography (HPLC)-mass spectrometry of the UDP-MurNac-peptide pools of enterococci and staphylococci revealed that inhibition of the formation of lipid I by tunicamycin led to a sharp increase of the UDP-MurNac-pentapeptide pool and to the accumulation of UDP-MurNac-hexapeptide and -heptapeptides

carrying the extra cross-bridge amino acids. This indicates, first, that the pools of lipid intermediates are very limited and, second, that the transferases catalysing the addition of these extra amino acids are presumably located on the inner side of the cytoplasmic membrane.

Correlations between the two stages of peptidoglycan synthesis

In the formation of the lipid intermediates transferases MraY and MurG have a very low specificity for the peptide moiety of the monomer unit. In fact, UDP-MurNac-di-,tri-,tetrapeptides as well as UDP-MurNac-acylpeptides can be processed. This lack of specificity is true both in vivo and in cell-free systems, and extends to the steps of polymerization by transglycosylation [3, 4, 31]. This great flexibility of the pathway has recently been examplified in *E. coli* and *S. aureus*. In *E. coli* it was shown that the high accumulation of UDP-MurNac-tripeptide brought about by D-cycloserine leads to the in vivo formation of lipid II tripeptide and to the presence of stem tripeptides in the final peptidoglycan material [31]. It has been suggested that this lipid intermediate could normally be involved in the formation of septum peptidoglycan [35]. In *S. aureus* it has been shown that the gene coding for the lysine-adding enzyme is a *fem* gene essential for methicillin resistance [36]. Mutation RUSA235 in this gene had a considerably increased UDP-MurNac-L-Ala-D-Glu pool leading to a peptidoglycan with many stem dipeptides, presumably formed via a lipid II dipeptide intermediate. In both cases, a lower but still detectable pool of UDP-MurNac-pentpeptide provided the pentapeptide stems nessary for transpeptidation.

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