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Fortifying the wall: synthesis, regulation and degradation of bacterial peptidoglycan

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Growth and maintenance of the protective peptidoglycan cell wall are vital to bacterial growth and morphogenesis. Thus, the relative rate and spatiotemporal control of the synthesis and degradation of this net-like polymer defines bacterial cell shape. In recent years, our understanding of the processes that govern this delicate metabolic balance has improved and should lend insight into how to therapeutically target the system in the future.

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Introduction

Bacteria often thrive in hostile environments that demand defense against varying environmental stresses including temperature, osmolites and antibacterial agents. It is therefore critical that they are able to build, maintain, and remodel their defining and protective cell wall under these diverse conditions. In both Gram-negative and Gram-positive bacteria, the cell wall consists of peptidoglycan (PG), a mesh-like polymer composed of extended glycan chains of repeating disaccharide subunits harboring oligopeptides through which they are cross-linked. The upkeep of the PG superstructure is a metabolically expensive task that employs numerous enzymes forming a complex and coordinated assembly line. Many of these specialized enzymes have no mammalian orthologs and as such are attractive antibiotic drug targets, especially in the face of increased resistance to antibacterials. The object of this review is to provide an overview of recent studies on central players involved in PG synthesis, regulation, degradation and recycling, and to touch on the interactions that coordinate this complex series of events.

Peptidoglycan biosynthesis

PG biosynthesis initiates with the well-characterized Mur enzyme pathway (reviewed in [1]), responsible for the synthesis of the UDP-*N*-acetylmuramic acid (MurNAc) pentapeptide precursor. The pentapeptide moiety (attached at the C3 position of MurNAc and often termed the stem peptide) is typically comprised of L-alanine-γ-D-glutamate-diaminopimelate(meso-DAP)-D-alanine-D-alanine in Gram-negative bacteria and L-alanine-γ-D-glutamate-L-lysine-D-alanine-D-alanine in Gram-positive bacteria with a pentaglycine branch protruding from the L-lysine residue in the latter. This precursor is attached via a pyrophosphate linkage onto the membrane anchored C_{55} lipid carrier by the integral membrane phosphotransferase MraY. Subsequently, the glycosyltransferase MurG adds an *N*-acetylglucosamine (GlcNAc) to form C_{55}-PP activated GlcNAc-β-1,4-MurNAc pentapeptide, completing the formation of the PG precursor lipid II [1]. The polymerization of PG occurs on the outer leaflet of the cytoplasmic membrane, necessitating the translocation or 'flipping' of lipid II across the membrane barrier. The bioinformatic search for the 'flippase' responsible for facilitating the transfer of lipid II has been narrowed down to several promising candidates including MurJ, FtsW, and RodA [2,3]. Recently, it has been shown that FtsW is directly involved in the transport of lipid II across the membrane, although the possibility of other candidates performing complementary or redundant roles is not excluded [4•].

Lipid II precursors are stitched into the net-like structure of the cell-wall sacculus by high molecular weight penicillin binding proteins (HMW PBPs) in two successive steps followed by recycling of C_{55}-PP. Firstly, the non-reducing end of the lipid II disaccharide (acceptor) is attached via a β1,4-glycosidic linkage to the reducing end of a growing (donor) PG chain through the membrane anchored glycosyl transferase (GT) action of PBPs. Secondly, the newly incorporated pentapeptide of the growing chain is cross-linked to a preexisting glycopeptide chain by the transpeptidase (TP) action of PBPs (Figure 1a) [1]. Class A PBPs are bi-functional enzymes harboring both GT and TP activities in distinct catalytic sites, whereas class B PBPs have only TP activity (reviewed in [1]) and are thought to play more prominent roles during specialized cellular events such

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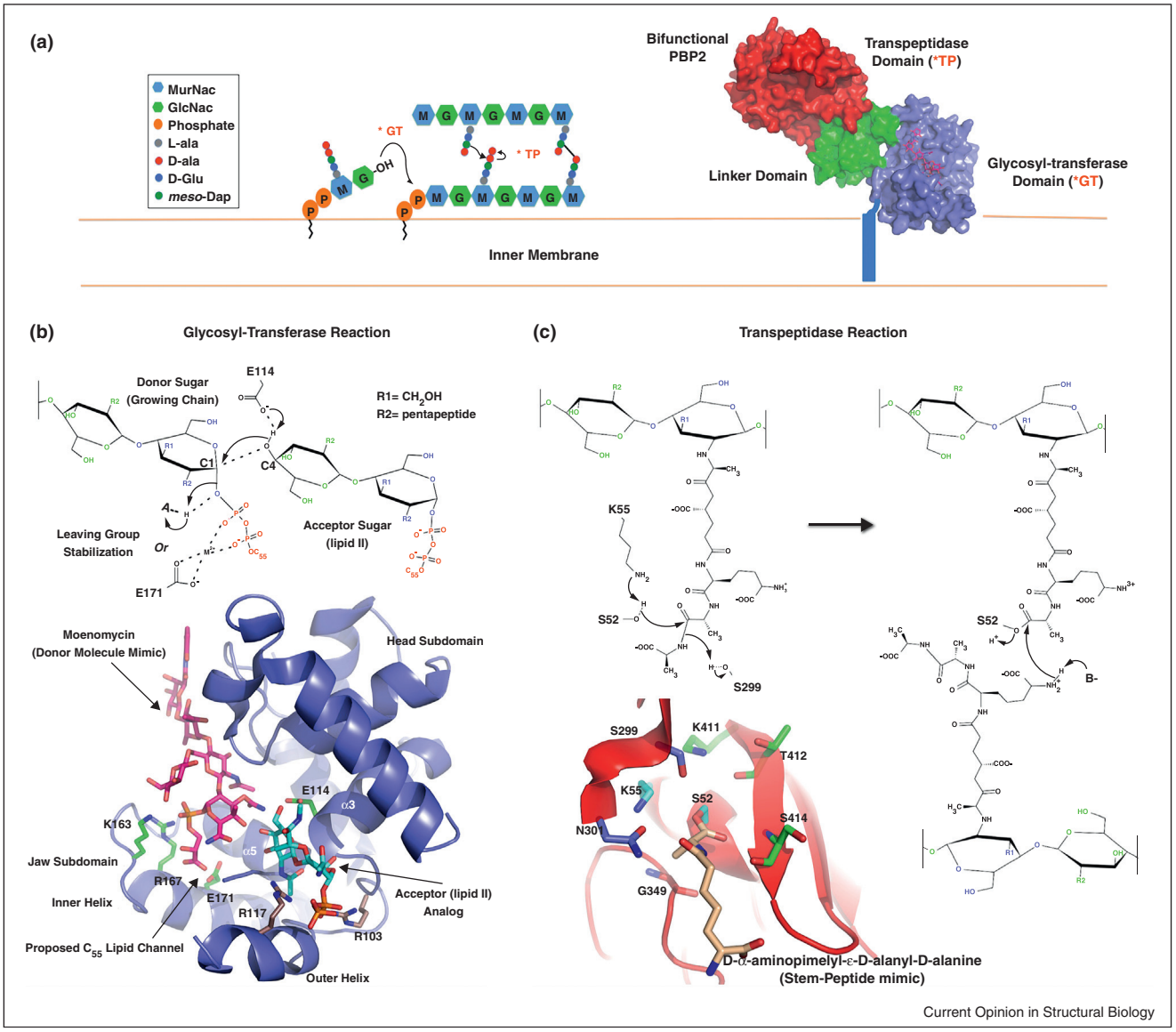
Figure 1



PBP catalyzed transglycosylation and transpeptidation. **(a)** Schematic of the GT and TP mediated incorporation of lipid II monomers into peptidoglycan. *Staphylococcus aureus* PBP2 (PDB ID: 2OLV [5]) is shown in a surface representation with GT, linker and TP domains colored blue, green and red respectively. **(b)** Active site close-up and mechanistic details of the GT reaction. The moenomycin bound PBP2 GT domain (PDB ID: 2OLV [5]) is depicted as a blue cartoon with selected active site residues shown as green sticks with atoms colored by type (N, blue; O, red; S, yellow). The donor molecule mimic (moenomycin) bound to PBP2 is depicted as pink sticks with atoms colored by type. The lipid II analog bound *S. aureus* monofunctional GT (PDB ID: 3VMT [7•]) is overlaid onto the PBP2 bound structure and the acceptor analog molecule, R103 and R117 are displayed as cyan and brown sticks with atoms colored by type. **(c)** Active site and mechanistic details of PG TP. The D-α-meso-DAP-ε-D-alanine-D-alanine bound PBP4a acyl enzyme complex protein backbone (PDB ID: 2J9P [60]) is shown as a red cartoon with the pentapeptide mimic depicted as beige sticks with atoms colored by type. Residues of the SXXK, SXN and KTG(S/T) motifs are represented in teal, blue and orange sticks respectively with atoms colored by type.

as division or in response to environmental cues including antibiotic stress.

Peptidoglycan transglycosylation

The peptidoglycan GT reaction occurs at the outer-leaflet of the cytoplasmic membrane. The seminal crystal structures of the full-length bi-functional *Staphylococcus aureus* PBP2 and *Aquifex aeolicus* PBP1a GT domain reveal that the PBP GT domain displays significant structural similarity to λ lysozyme [5,6]. An extended crater-like active site cleft is formed at the interface of a solvent exposed, predominantly α-helical 'head' subdomain, the

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