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New chemical tools to probe cell wall biosynthesis in bacteria

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Some of the most successful drugs in the antibiotic pharmacopeia are those that inhibit bacterial cell wall biosynthesis. However, the worldwide spread of bacterial antibiotic resistance has eroded the clinical efficacy of these drugs and the antibiotic pipeline continues to be lean as drug discovery programs struggle to bring new agents to the clinic. Nevertheless, cell wall biogenesis remains a high interest and celebrated target. Recent advances in the preparation of chemical probes and biosynthetic intermediates provide the tools necessary to better understand cell wall assembly. Likewise, these tools offer new opportunities to identify and evaluate novel biosynthetic inhibitors. This review aims to highlight these advancements and to provide context for their utility as innovative new tools to study cell wall biogenesis and for antibacterial drug discovery.

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Introduction

Antimicrobial agents that target bacterial cell wall biosynthesis are among the most successful and effective of those in the current antibiotic armamentarium. These agents, notably the β-lactam and glycopeptide classes of antibiotics that disrupt peptidoglycan assembly, have provided the mainstay of treatment regimes for bacterial infections in the clinic. However, rampant antibiotic resistance now threatens the clinical efficacy of these drugs worldwide.

Cell wall biosynthesis continues to be an attractive antibacterial target [1–3]; however, modern drug discovery programs have unearthed no new cell-wall active antibacterial drugs. It is clear that the effective utilization of this target requires novel tools and a thorough biochemical understanding of biosynthetic events. Recent development of chemical probes and simplified synthetic routes to important biosynthetic intermediates promises to aid these drug discovery efforts. Such resources have enabled both *in vitro* and *in vivo* study of cell wall assembly. In addition, they have been used to develop unique high-throughput screens and to evaluate the mode of action for several exciting non-protein targeting antibiotics. Herein we discuss these advancements and highlight their usefulness to modern drug discovery efforts.

Peptidoglycan assembly

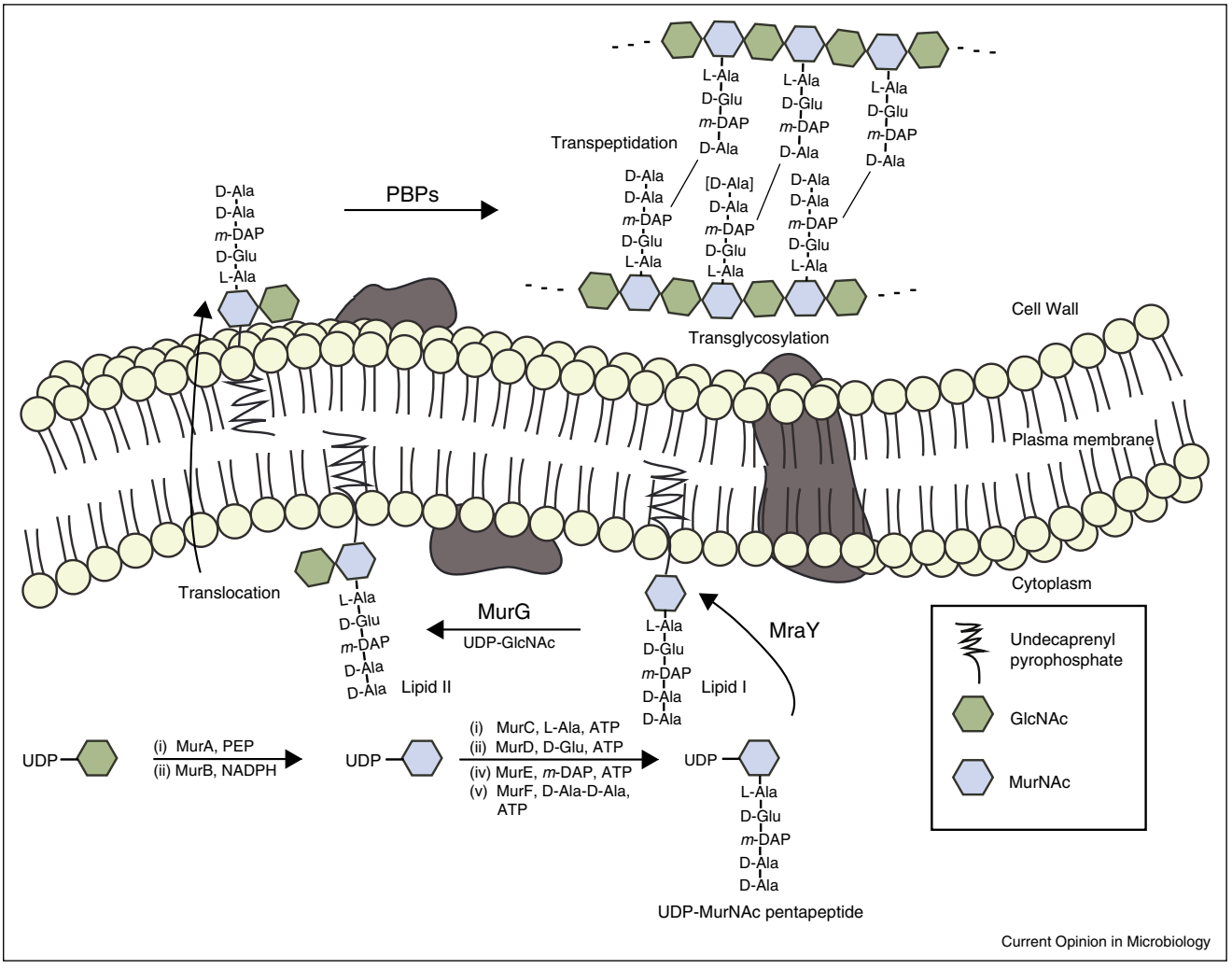
The biosynthesis of bacterial peptidoglycan is a validated and celebrated target of the clinical antibiotic pharmacopeia. The assembly of this macromolecule — a meshwork consisting of glycan heteropolymers connected via short peptides — begins with the cytoplasmic generation of UDP-*N*-acetylmuramic acid pentapeptide. This precursor is then coupled to a membrane-embedded undecaprenyl phosphate (C_{55}-P) lipid carrier on the cytoplasmic face of the bacterial membrane and elaborated to Lipid II. Lipid II is flipped across the bacterial membrane and subsequently polymerized and cross-linked through the transglycosylase and transpeptidase activities of penicillin-binding proteins (PBPs). This biosynthetic process is shown in Figure 1 and has recently been reviewed [4].

While many cytoplasmic stages of peptidoglycan assembly are considered viable targets for novel chemotherapeutic agents [1], the majority of successful antibiotics used in the clinic inhibit the later lipid-linked steps of the pathway. The emergence of resistance to these agents has sparked interest to better understand the molecular details of lipid-linked peptidoglycan assembly, and to develop new tools and assays that identify and progress novel biosynthetic inhibitors. Thorough biochemical studies of this nature require access to milligram quantities of peptidoglycan biosynthetic intermediates. Isolation of these compounds, namely Lipid I and Lipid II, from natural bacterial sources is futile due to their low abundance in cells [5]. Thus, research groups have focused efforts on synthetic strategies as a means to obtain these valuable intermediates.

Synthesis of lipid-linked peptidoglycan biosynthetic intermediates

Walker and co-workers along with VanNieuwenhze *et al.* have detailed chemical preparations for Lipid I [6,7]. VanNieuwenhze *et al.* and Schwartz *et al.* have outlined

Figure 1



Gram-negative bacterial peptidoglycan biosynthesis. The soluble cytoplasmic events of peptidoglycan assembly culminate with the preparation of UDP-MurNAc pentapeptide from precursor UDP-GlcNAc. The first step of this pathway involves linkage of an enolpyruvyl residue onto the C(3) hydroxyl moiety of UDP-GlcNAc in a reaction mediated by MurA. The resulting molecule is then reduced via the NADPH-dependent enolpyruvyl reductase MurB to yield UDP-MurNAc. ATP-dependent amino acid ligases (MurC-MurF) subsequently incorporate the stem peptide side chain on the lactate handle of UDP-MurNAc to produce UDP-MurNAc pentapeptide. UDP-MurNAc pentapeptide is anchored to membrane-embedded lipid carrier undecaprenyl phosphate in a pyrophosphate exchange reaction mediated by MraY to yield Lipid I. Lipid I is subsequently transformed to Lipid II via addition of GlcNAc to its C(4) hydroxyl group in a reaction facilitated by MurG. Lipid II is translocated to the extracellular leaflet of the cytoplasmic membrane (in a reaction currently thought to be mediated by either FtsW [23,24] or MurJ [25]), where it is polymerized, cross-linked and processed (shown in brackets) by the transglycosylase, transpeptidase and carboxypeptidase activities of penicillin-binding proteins (PBPs) [4]. The outer membrane is not shown. Abbreviations: GlcNAc, *N*-acetylglucosamine; MurNAc, *N*-acetylmuramic acid.

total syntheses for Lipid II [8,9]. Lipid II can also be prepared chemoenzymatically through MurG-mediated elaboration of Lipid I [6]. While these chemical and chemoenzymatic strategies are dependable and versatile, they involve lengthy chemical transformations that are impractical to conduct in most basic research laboratories. Therefore, a popular route to Lipid I and Lipid II employs a one-pot enzymatic reaction containing MraY and MurG-rich membrane extracts from *Micrococcus flavus* [10,11]. Braddick *et al.* used Lipid II prepared in this fashion to develop a quantitative assay for Lipid II polymerization by the *Staphylococcus aureus* transglycosylase MGT [12]. Schneider and co-workers utilized enzymatically prepared Lipid II to recapitulate, *in vitro*, events of *S. aureus* pentaglycine interpeptide assembly [11] — a process that maintains methicillin resistance *in vivo* [13]. Recently, the ligand preferences for the semisynthetic glycopeptide oritavancin (currently approved by the FDA to treat skin infections caused by several pathogenic Gram-positive bacteria) and the novel depsipeptide