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Undecaprenyl Pyrophosphate Phosphatase-Encoding Gene in Gram-Positive Bacteria as a New Antimicrobial Target

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Abstract

Antimicrobial agents that target bacterial cell wall biosynthesis are among the most successful and effective armamentaria against bacterial infections. However, the worldwide spread of antibiotic resistance in bacteria has eroded the clinical efficiency of these drugs and the antimicrobial pipeline continues to be lean. Nevertheless, cell wall biosynthesis of bacteria remains a high interest and celebrated targets for discovery of novel antimicrobial agents. Recent advances in genomics, including genome-wide mutagenesis analysis, genome sequencing, genotyping and gene expression profiling, have made considerable progress for our understanding of bacterial cell wall synthesis, promoting the discovery of novel antimicrobial targets.

Keywords: Antimicrobial agents; Biosynthesis; Mutagenesis

Introduction

Mankind fights microbial infections worldwide by using large quantities of antimicrobial agents. Indeed, many bacterial infections can be treated effectively with antibiotics since Alexander Fleming discovered the first antibiotic penicillin in 1928 [1]. The success of these therapies in infectious diseases is largely based on the availability of antimicrobial agents that aim to inhibit or kill target pathogens. In the battles of fighting against microbial infections today, however, we have found ourselves facing a severe threat: many antibiotics have lost their effectiveness in treating infectious diseases due to the growing crisis of development of antibiotic-resistant microbes [2]. The emergency of antibiotic resistance indicates the urgent need for new antimicrobial agents that selectively target specific pathogens but less likely cause antibiotic resistance. Therefore, application of high-throughput platforms to screen and discover new antimicrobial targets and antibiotics is highly needed to compete with rapid evolution of microbial resistance. In the post-genome era, the availability of various genomics-based platforms may speed up our pursuit and discovery of novel antimicrobial targets in bacteria.

A recent study published in Microbiology by Jalal et al. [3] is an excellent example of using a high throughput genomic approach to screen and identify potential antimicrobial targets involved in bacterial cell wall biosynthesis. These researchers constructed a genome-wide transposon mutant library in a Gram-positive bacterium, *Streptococcus mutans* that is considered as a leading pathogen of human tooth decay [4]. By screening the transposon mutant library combined with the tools of cell wall synthesis inhibitors and sequence analysis, the researchers identified nearly a dozen of transposon insertion mutants that are highly or moderately sensitive to bacitracin and several other cell wall synthesis antibiotics. Their work has clearly validated the strategy and methods used for screening and identification of transposon inserted genes under investigation. These researchers further characterized two identified mutants, which are highly sensitive to bacitracin and have two independent insertions in the same locus, SMU.244 encoding a putative undecaprenyl pyrophosphate phosphatase (UppP) required for the recycling and de novo synthesis of undercaprenyl phosphate (Up or C55-P) [3]. Topological analysis reveals that SMU.244-encoded protein is a highly hydrophobic membrane protein with seven or eight predicted transmembrane segments (TMSs), suggesting its association with the cytoplasmic membrane. In particular, two highly conserved regions specific to UppP and the enzymatic activity are identified in SMU.244-encoded protein (Figure 1).

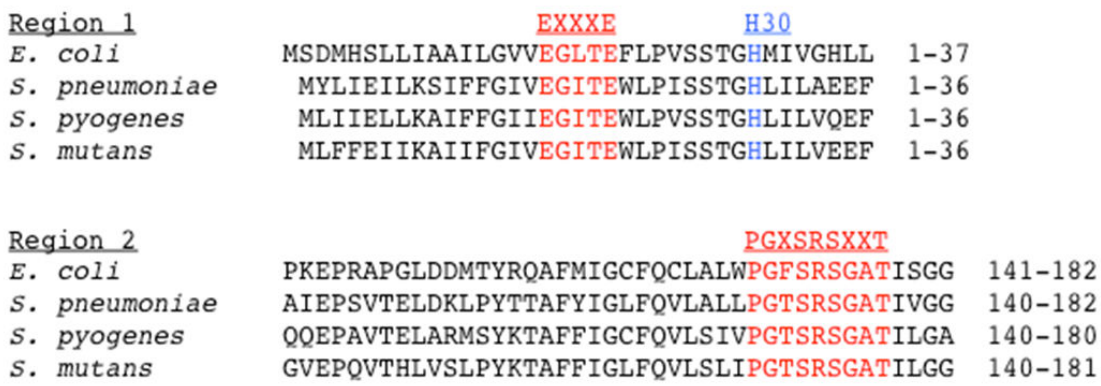

Figure 1: Bacterial undecaprenyl pyrophosphate phosphatase (UppP) is an integrated membrane protein consisting of seven or eight predicted transmembrane segments (TMSs) and two highly conserved regions. Sequence alignments indicate that these regions contain two well-conserved functional motifs across different species. Region one contains a highly conserved, glutamate-rich E/QXXXE motif (residue 17-21, red) along with a histidine (His-30, blue) in the first transmembrane segment. Region two contains a conserved PGXSRSXXT motif (residues 170-178, red) that forms a structural P-loop commonly found in many phosphate-binding enzymes.

One region contains a highly conserved glutamate-rich E/QXXXE (17-21) motif along with a histidine (His-30) in the first transmembrane segment. This region is suggested to be essential for catalytic activity and substrate binding. Another region contains a conserved PGXSRSXXT (170-178) motif that forms a structural phosphate-binding loop or P-loop commonly found in many phosphate-binding enzymes [5]. Sequence alignments also indicate that UppP proteins from the completed genome-sequence of five *S. mutans* strains share 99% of identity in their primary sequences, indicating that UppP proteins are highly conserved during evolution of *S. mutans*. Further work of these researchers confirms that purified recombinant UppP protein from *S. mutans* displays a strong activity of catalyzing dephosphorylation of a substrate farnesyl pyrophosphate.

In Gram-negative bacteria, such as *Escherichia coli*, UppP is not the only enzyme involved in the biosynthesis of Up or C55-P, but rather, four genes of pgpB, ybgG, lpxT and uppP have been found to encode integral membrane proteins with the UppP activity [5-7]. In contrast, PgpB, YbgG and LpxT homologues are not identified in many Gram-positive bacteria, including *S. mutans*. Although a protein, named BcrC, is reported to display an UppP activity in *Bacillus subtilis* [8], a gene encoding BcrC ortholog has not yet been identified in *S. mutans* and other Gram-positive bacteria. Thus, it is reasonable to assume that UppP may be the only enzyme that catalyzes dephosphorylation of its precursor undecaprenyl pyrophosphate (Upp) to up in Gram-positive bacteria, thereby, playing a central role in the recycling and de novo biosynthesis of the essential lipid carrier in these bacteria. It is not surprising that deletion of uppP gene in Gram-positive bacteria such as in *S. mutans* and *Streptococcus pneumoniae* results in mutants that are highly or super-sensitive to bacitracin [7]. Presumably, antibiotics that could selectively target UppP should have effective inhibition on the recycling and de novo biosynthesis of Up in Gram-positive bacteria. Unfortunately, except bacitracin, there are very few of such antibiotics that specifically target UppP protein in bacteria. Nevertheless, a recent study reveals that a ribosomally synthesized, unmodified two-peptide (class IIb) bacteriocin, lactococcin G, produced by some *Lactococcus lactis* strains can selectively bind to UppP protein (as a receptor), resulting in killing of sensitive *L. lactis* strains at a nano-mole concentrations [9]. Interestingly, lactococcin G is also found to bind to the UppP protein of *S. mutans*, resulting in a concentration-dependent killing [3]. A deletion of uppP (SMU.244) in *S. mutans* confers more resistance of the mutant to lactococcin G than the parent. Thus, it is possible that lactococcin G can be developed or modified as a new antibiotic that selectively targets Gram-positive bacteria.

Targeting the de novo biosynthesis and recycling of Up is a potentially important route for antibiotic discovery, because undercaprenol phosphate is an essential lipid carrier of glycan biosynthetic intermediates for various cell wall components, such as the peptidoglycan, teichoic acids, the O-antigen moiety of lipopolysaccharides (LPS) and other carbohydrate polymers [5-7]. Up is made available both by the de novo synthesis from the dephosphorylation of its precursor Upp or C55-PP in the cytoplasm, and by the recycling of released Upp after the transfer of the glycan component to other acceptor molecules outside the cytoplasmic membrane (Figure 2). During the de novo synthesis, Upp is synthesized by by undecaprenyl pyrophosphate synthase (UppS) through consecutive condensation reactions of eight molecules of isopentenyl pyrophosphate (Ipp) with farnesyl pyrophosphate (Fpp) in the cytoplasm. The product Upp is then dephosphorylated to Up by UppP. Up can be also available through the recycling process through dephosphorylation and translocation of released Upp cross the membrane by UppP. Clearly, both de novo synthesized and recyclable Upp rnust be dephosphorylated before it can be used or reused as.a carrier lipid for subsequent polymer biosynthesis. Given the high level of conservation of UppP proteins cross bacterial species; these insights open the door to new avenues of research targeting cell wall biosynthesis as a strategy for the development of new antibacterial agents.

In addition to the generation of Up through dephosphorylation of Upp by UppP, many Gram-positive bacteria, such as *Staphylococcus aureus, Streptococcus pyogenes* and *S. mutans*, are found to have an alternative pathway to generate Up from phosphorylation of undecaprenol (C55-OH) catalyzed by an undecaprenol kinase (UdpK), an ortholog of diacylglycerol kinase (DagK) [10]. However, the precise

role of C55-OH in Gram-positive bacteria remains unclear. Neither is known how this alternative pathway is regulated, especially in the presence of an intact UppP protein in bacteria. Limited evidence suggests that this pathway appears to allow the biosynthesis of limited amount of Up from phosphorylation of C55-OH catalyzed by undecaprenol kinase or DagK when bacteria are grown under stress conditions such as at low pH, indicating that C55-OH phosphorylation constitute a bypass pathway of the Up biosynthesis in stress conditions [10]. Further study of these pathways and regulatory mechanisms may promote the discovery of antimicrobial agents that target these enzymes required for the cell wall biosynthesis. It can be predicted that blocking one or more of these pathways in Gram-positive bacteria might show much more effective inhibition on the cell wall biosynthesis in these bacteria. This group of antimicrobial agents is highly desirable.

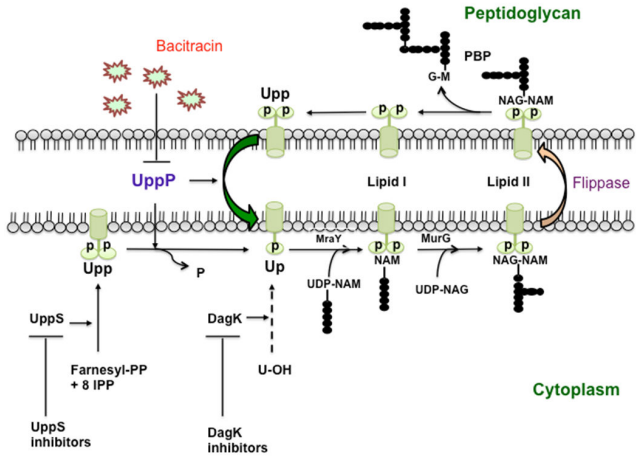

Figure 2: A schematic diagram describes the events and pathway of peptidoglycan synthesis emphasizing the essential role of undecaprenyl phosphate (Up or C55-P) as the critical lipid carrier molecule. The undecaprenyl pyrophosphate (Upp) is synthesized by undecaprenyl pyrophosphate synthase (UppS) through consecutive condensation reactions of farnesyl pyrophosphate (Fpp) with eight molecules of isopentenyl pyrophosphate (Ipp). The Upp is then dephosphorylated by undecaprenyl pyrophosphate phosphatase (UppP) to generate up (de novo synthesis). The sugar-pentapeptides are transferred to the Up by the enzymes MraY and MurG, producing lipid I and lipid II, respectively. Lipid II is then translocated across the cell membrane by flippase (FtsW) for the peptidoglycan assembly. In addition, the Upp released after the transfer of the glycan component to other molecules outside the membrane can be dephosphorylated as Up, which is flipped back to the cytoplasm to be reused via the recycling process.

Acknowledgments

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