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Renew or die: The molecular mechanisms of peptidoglycan recycling and antibiotic resistance in Gram-negative pathogens

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ABSTRACT

Antimicrobial resistance is one of the most serious health threats. Cell-wall remodeling processes are tightly regulated to warrant bacterial survival and in some cases are directly linked to antibiotic resistance. Remodeling produces cell-wall fragments that are recycled but can also act as messengers for bacterial communication, as effector molecules in immune response and as signaling molecules triggering antibiotic resistance. This review is intended to provide state-of-the-art information about the molecular mechanisms governing this process and gather structural information of the different macromolecular machineries involved in peptidoglycan recycling in Gram-negative bacteria. The growing body of literature on the 3D structures of the corresponding macromolecules reveals an extraordinary complexity. Considering the increasing incidence and widespread emergence of Gram-negative multidrug-resistant pathogens in clinics, structural information on the main actors of the recycling process paves the way for designing novel antibiotics disrupting cellular communication in the recycling-resistance pathway.

1. Introduction

"*Antimicrobial resistance in a wide range of infectious agents is a growing public health threat of broad concern to countries and multiple sectors. Increasingly, governments around the world are beginning to pay attention to a problem so serious that it threatens the achievements of modern medicine. A post-antibiotic era – in which common infections and minor injuries can kill – far from being an apocalyptic fantasy, is instead a very real possibility for the 21st century*" (WHO, 2014). This paragraph, elaborated by the World Health Organization, very precisely summarizes the present situation: infections from resistant bacteria are now too common, and some pathogens have even become resistant to multiple types or classes of antibiotics. Infectious diseases are now a leading cause of death worldwide. The increasing incidence and widespread emergence of pathogens that are resistant to antibiotics have reversed advances in the treatment of many infections (Fears and ter Meulen, 2014). Gram-negative bacteria are among the most problematic microorganisms due to their increasing resistance to antibiotics. These include a multitude of organisms, some of which cause respiratory problems such as *Hemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, urinary-tract infections caused by *Escherichia coli*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens* whereas *Helicobacter pylori*, *Salmonella enteritidis* and *Salmonella typhi* cause gastrointestinal problems. In addition, other bacteria such as *P. aeruginosa* are associated with hospital-acquired infections like meningitis and pneumonia in hospital intensive-care units.

It is estimated that in the European Union, antibacterial resistance causes more than 25,000 deaths per year, with estimated costs of €1500 million per year (WHO, 2014) and in the United States, at least 23,000 deaths occur every year due to antibiotic-resistant infections (Prevention, 2013). Understanding of the mechanisms that bacteria have evolved to survive in the presence of antibiotics is a primary requisite to tackle the problem of antibiotic resistance. This review focuses on the structural biology of the cell-wall recycling process in G(−) pathogens, a process that involves different lytic and transport machineries critical for bacterial fitness and antibiotic resistance.

Abbreviations: AnhNAM, anhydro N-acetylmuramic; CDD, Conserved Domain Database; DPBB, double-psi β barrel; EBD, effector-binding domain; EGV, endoglucanase V; G(−), Gram-negative; GH, glycosydehydrolase; LC, liquid chromatography; LT, lytic transglycosylases; LTTR, LysR-type transcriptional regulators; MS, mass spectrometry; Mlt, membrane-bound lytic transglycosylase; NAG, N-acetylglucosamine; NAG3, N,N′,N″-tri-acetyl-chitotriose; NAM, N-acetylmuramic; NMR, nuclear magnetic resonance; PBP, penicillin-binding protein; PDB, Protein Data Bank; PG, peptidoglycan; PGA, peptidoglycan amidase; PGRPs, peptidoglycan-recognizing proteins; RMSD, root-mean-square deviation; Slt, soluble lytic transglycosylase; SPR, surface plasmon resonance; VcNagZ, *Vibrio cholerae* NagZ.

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2. The peptidoglycan recycling processes and antibiotic resistance

The bacterial cell-wall is a huge biopolymer that ensures structural integrity to the bacterium to counter its inability to regulate its osmotic pressure. The major constituent of the cell-wall is the crosslinked peptidoglycan, which is assembled by polymerization of Lipid II to give a linear backbone of repeating N-acetylglucosamine (NAG)-N-acetylmuramic acid (NAM), with a pentapeptide stem attached to the NAM unit. The typical peptide stem in G(−) bacteria is L-Ala-γ-D-Glu-m-DAP-D-Ala-D-Ala (where DAP is the abbreviation for diaminopimelate), which experiences crosslinking to another peptide stem in a neighboring strand in construction of cell-wall by penicillin-binding proteins (PBPs).

The cell-wall is a dynamic matrix that it is constructed and recycled constantly in processes that involve a large number of enzymes (Fig. 1). Many G(−) bacteria remodel around half of their cell-wall per generation. These cell-wall fragments are recycled for cell-wall biosynthesis but also are used as messengers for bacterial communication and are detected by eukaryotes to initiate an immune response (Boudreau et al., 2012; Cho et al., 2007; Woodhams et al., 2013). Up to 20 muropeptides have been experimentally found in *P. aeruginosa*, the least abundant of these muropeptides being 100 molecules per bacterium and the most abundant at 55,000 molecules per bacterium (Lee et al., 2016a). While β-lactamase induction in G(−) bacteria has been known for many years, the link between cell-wall recycling and antibiotic resistance has been discovered only recently (for reviews see Johnson et al., 2013; Fisher and Mobashery, 2014). In the presence of antibiotics, muropeptide fragments accumulate in the cytoplasm and, in some G(−) bacteria, this results in derepression of the *ampC* gene encoding the AmpC β-lactamase. Recent studies on PBP4, a Penicillin-binding Protein presenting DD-carboxypeptidase and 4,3-endopeptidase activities, revealed its central role in detecting and responding to a β-lactam antibiotic challenge (Lee et al., 2015). The nature of the muropeptide as a signaling molecule capable of inducing AmpC, which plays roles in multiresistance, is also very relevant. Under induction of resistance to a β-lactam antibiotics in *P. aeruginosa* only 2 muropeptides (NAG-1,6-anhydroNAM

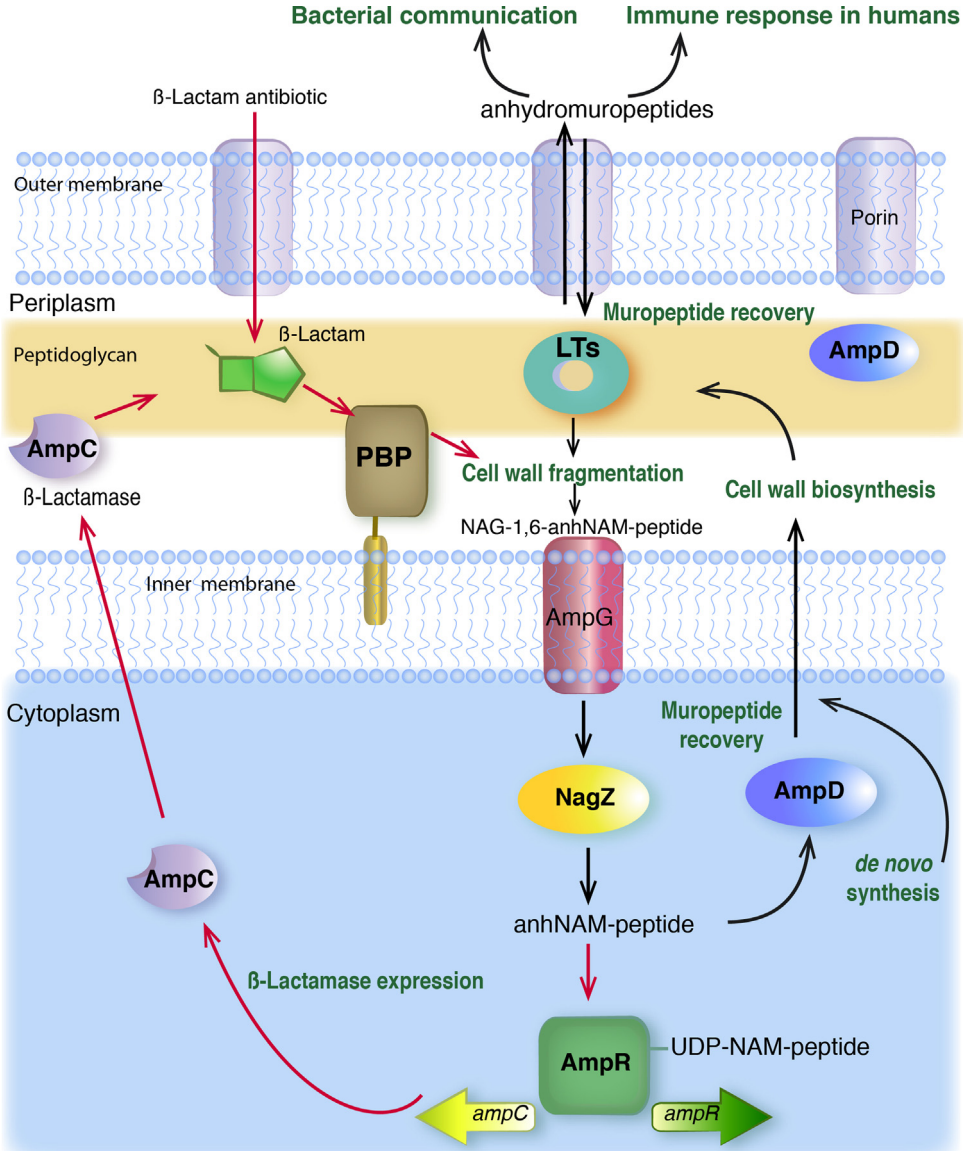

Fig. 1. Cell-wall recycling mechanism and antibiotic resistance in G(−) bacteria. Black arrows indicate cell-wall recycling pathways. In the presence of β-lactam antibiotics (red arrows) an antibiotic resistance pathway is activated to produce β-lactamase.