HHS Public Access

Author manuscript

Chem Rev. Author manuscript; available in PMC 2019 November 14.

Published in final edited form as:

Chem Rev. 2018 June 27; 118(12): 5952–5984. doi:10.1021/acs.chemrev.8b00277.

Cell-Wall Recycling of the Gram-Negative Bacteria and the Nexus to Antibiotic Resistance

David A. Dik, **Jed F. Fisher**, **Shahriar Mobashery***

Department of Chemistry and Biochemistry, McCourtney Hall, University of Notre Dame, Notre Dame, Indiana 46556, United States

Abstract

The importance of the cell wall to the viability of the bacterium is underscored by the breadth of antibiotic structures that act by blocking key enzymes that are tasked with cell-wall creation, preservation, and regulation. The interplay between cell-wall integrity, and the summoning forth of resistance mechanisms to deactivate cell-wall-targeting antibiotics, involves exquisite orchestration among cell-wall synthesis and remodeling and the detection of and response to the antibiotics through modulation of gene regulation by specific effectors. Given the profound importance of antibiotics to the practice of medicine, the assertion that understanding this interplay is among the most fundamentally important questions in bacterial physiology is credible. The enigmatic regulation of the expression of the AmpC β-lactamase, a clinically significant and highly regulated resistance response of certain Gram-negative bacteria to the β-lactama antibiotics, is the exemplar of this challenge. This review gives a current perspective to this compelling, and still not fully solved, 35-year enigma.

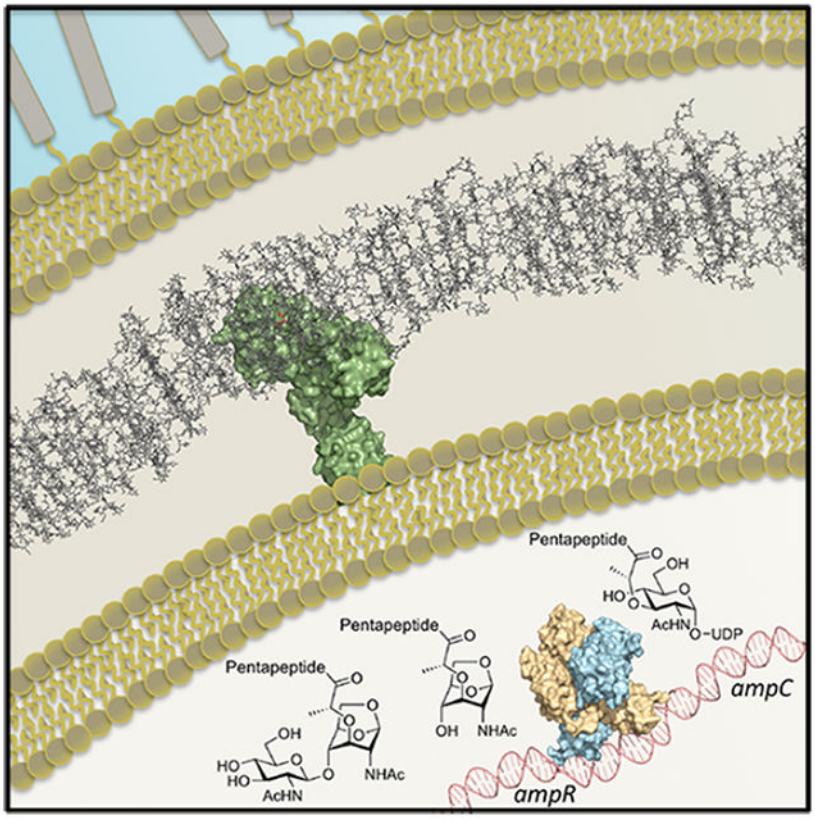
Graphical Abstract

* **Corresponding Author**: mobashery@nd.edu.

Author Contributions

This review was written by contributions from each author.

The authors declare no competing financial interest.



1. INTRODUCTION

Bacteria are unicellular microorganisms that reproduce by cell division without compromise of their internal osmotic cell pressure, as a consequence of their exquisite capability to maintain the structural integrity of their cell envelope throughout the processes of cell growth and cell division. An important contributing entity to their structural integrity is their “cell wall”. The bacterial cell wall is a polymeric single molecule that encases the bacterium. Its polymeric structure comprises glycan strands covalently linked to each other (in a perpendicular fashion with respect to the length of the glycan strands) by an interconnecting peptide stem attached to an alternating saccharide of the glycan strands. This alternating saccharide takes the form of an *N*-acetylglucosamine (GlcNAc, NAG) and *N*-acetylmuramic acid (MurNAc, NAM) connected by a β-1,4-glycosidic bond. A peptide stem projects from the lactyl ether moiety of the MurNAc saccharide. In Gram-negative bacteria the full-length stem structure is the pentapeptide L-Ala-γ-D-Glu-*meso*-diaminopimelate-D-Ala-D-Ala (lysine replaces the diaminopimelate in many Gram-positive cell walls). For these structural reasons the chemical character of the bacterial cell wall is described as that of a peptidoglycan (PG). A curious feature of the biosynthetic processes that lead to the mature cell wall (and especially for the most pathogenic Gram-negative bacteria) is that substantial portions of the PG of the old cell wall are excised, degraded, and recycled concurrently with the incorporation of new PG during bacterial duplication (Figure 1).[1] The reason for this requirement is not known. The customary thought is that the existing peptidoglycan polymer

is a template for the insertion of new peptidoglycan into the polymer.[2] The cell envelope of Gram-negative bacteria is demarcated by their two cell membranes, with the polymeric cell wall located between them. The PG precursors of the cell wall are biosynthesized in the bacterial cytoplasm (whether de novo or from recycled old PG), while the PG itself is assembled, remodeled, and degraded in the space between the two membranes (termed the periplasm). A requirement for pathways (and proteins) that coordinate precursor ingress to the cytoplasm through the inner membrane, for the coordinated assembly in the periplasm of the PG, and egress of degraded PG from the periplasm to the cytoplasm for precursor (re-) assembly, is evident. Only in the past decade has an initial understanding been achieved as to the identities of the proteins and enzymes that contribute to the ingress, assembly, and egress aspects of cell-wall assembly. A yet additional layer of complexity is presented by the rod-shaped Gram-negative bacteria. In these bacteria, the PG of the cell wall that comprises the length of the bacterium is distinct from the PG of the septal cell wall. The septal wall PG is made and then is split so as to create the new poles of the parent bacterium and the daughter bacterium. Accordingly, two different multiprotein assemblies for these events are required. Neither the breadth of the macromolecular composition, assembly, and coordinated motion with the cytoskeletal components of cell-wall synthesis, nor the exact circumstance of the transition from sidewall cell-wall synthesis to septal cell-wall synthesis, is fully known.[3]

With respect to virtually all measures, the bacteria defy generalization. Exceptions are made in that they are unicellular, and by recognition that most phyla of the bacteria divide between bacteria that are encased by a cell envelope having either a single membrane (the monoderm bacteria) or two membranes (the diderm bacteria).[6] The envelope structure surrounding the cytoplasm of the former is thus a single membrane in contact with the cytoplasm, followed (moving outward) by a thin interstitial space to accommodate the enzymes of cell-wall biosynthesis and culminating with the cell wall itself as a multilayer peptidoglycan (that is, composed of glycan strands interconnected through peptide stems) polymeric exoskeleton. The envelope structure of the latter bacteria is that of a cytoplasmic membrane, followed by a wider interstitial space (the periplasm), and culminating with a second exterior membrane. The cell wall of these bacteria is a thinner peptidoglycan polymer, located within the periplasm. Because of the very different character of the two cell surfaces (in the former, the peptidoglycan; in the latter, an outer leaflet composed of a specific glycolipid, called the lipopolysaccharide), the observation was made by Gram in the late 19th century that cationic dyes adhered more tightly to the surface of the monoderm bacteria (hence, Gram-positive) than to the diderm bacteria (Gram-negative).[7–9] Numerous species of both the Gram-positive and the Gram-negative bacteria are human pathogens, and numerous antibiotics act to control bacterial infection through mechanisms that compromise the cell-envelope structure.

The β-lactam antibiotics interfere with cell-wall biosynthesis. Their properties of selectively disrupting the bacterial cell wall without concurrent interference with any pathway of the eukaryotic cell is the basis for their extraordinary clinical efficacy and safety in the control of bacterial infection. The β-lactam antibiotic family encompasses the penicillin, cephalosporin, cephamycin, carbapenem, and monobactam subfamilies. Each structure within these subfamilies possesses an azetidin-2-one (β-lactam) cyclic amide core structure, which is engaged so as to inactivate critical members of an exclusively bacterial enzyme family, the penicillin-binding proteins (PBPs). Bacteria have several PBP enzymes.[10–13]

Although not all of these PBPs are essential, a subset is critical to the survival of the bacterium. In cell-wall biosynthesis, the PBPs ordinarily recognize the acyl-D-alanine-D-alanine and acyclic dipeptide motif, unusual in that the stereochemistry of the α-carbon of both alanines is *R*, and not the customary *S* stereochemistry of an L-amino acid, to give a D-alanyl acyl-enzyme (with departure of the distal D-alanine as the leaving group). These acylenzymes (depending on the PBP) are used efficiently for cell-wall synthesis in either a transpeptidase cross-linking reaction that is critical to the creation of the cell-wall polymer or in a carboxypeptidase reaction that is critical to the control of the extent of the cross-linking reaction. As the cyclic amide of the β-lactam antibiotic mimics exceptionally well the acyclic amide of the acyl-D-alanine-D-alanine substructure (Figure 2), the β-lactam efficiently acylates an active-site serine of the PBP enzymes. In contrast to the transient nature of D-alanyl-derived acyl-enzyme, the β-lactam-derived acyl-enzyme is stable. Neither the PBP that is a transpeptidase, nor the PBP that is a carboxypeptidase, is able to complete turnover once modified by the antibiotic. These PBPs persist, in a covalently inactivated state, as acyl-enzymes in association with the β-lactam antibiotic (Scheme 1). If for a particular bacterial pathogen the correct β-lactam structure is used therapeutically, so as to match well to the active site of an essential PBP, the loss of the catalytic activity of that PBP will culminate in the collapse of the integrity of the bacterial cell wall, as manifested by a bactericidal cell lysis. Notwithstanding the decades during which the β-lactams have been used to control bacterial infection, the precise sequence of events between the loss of the catalytic ability of an essential PBP, and the lytic event, remain largely unknown.

In recent decades, these bacteria have not idly succumbed to β-lactam chemotherapy. Each therapeutic intervention with a β-lactam is a learning experience for bacteria, and as a consequence, these same decades have witnessed an inexorable increase in bacterial resistance not only to the β-lactam antibiotics but also to all other antibiotics available clinically as well. Given the predominance of the β-lactams in modern chemotherapy, the loss of the β-lactams due to resistance would be devastating to human and animal health. The principal mechanisms used by bacteria to resist the antibiotic action are known. First, bacteria discriminate against β-lactam entry into their periplasm, where the PBPs are found, by deleting the specific porin protein used by the β-lactam for entry to the periplasm. If by fate the antibiotic reaches the periplasm, the site of its biological target, it must evade deportation from the cell by extremely effective multidrug efflux pumps.[14] Even more, and especially in Gram-positive bacteria, the PBP is mutated to evade inhibition by the β-lactam, or an intrinsically resistant PBP is acquired to replace the activity of the one lost to the β-lactam. This resistance mechanism was until now rare among the Gram-negative bacteria. Fourthly, the Gram-negative bacteria express a hydrolytic enzyme that recognizes and catalytically deactivates the β-lactam by hydrolysis of its β-lactams ring. These resistance enzymes, called β-lactamases, remain the primary mechanism used by the Gram-negative bacteria to withstand the antibiotic action of the β-lactams. Previously these bacteria might possess one β-lactamase, such that selection of a β-lactam that was still efficacious against the essential PBP, but not recognized by the β-lactamase, was a simple therapeutic alternative. The progressive loss of this alternative is now evident in the clinic.[15] Increasingly, bacteria are acquiring multiple β-lactamases, creating an ensemble of resistance enzymes collectively encompassing a breadth of β-lactam structures as substrates.

While the extensively β-lactam-resistant Gram-negative pathogen is by no means a rarity today, it is not yet common. Nonetheless, the emergence of these bacteria is ominous.

One particular β-lactamase remains a cornerstone in Gram-negative β-lactam resistance: the AmpC β-lactamase.[16–19] The AmpC β-lactamase is notably efficacious toward the deactivation of the extensively clinically used cephalosporin β-lactams. The mechanism of the hydrolysis of a penicillin by the AmpC β-lactamase is shown concisely in Scheme 2. This mechanism has been the subject of extensive experimental and computational study.[20–24] Although it is much less catalytically efficacious against the carbapenem β-lactams, it can be expressed in sufficient quantities so as to render, by a sequestration process (also involving a serine acyl-enzyme), the carbapenem impotent. The central feature of the action of the AmpC β-lactamase is that the enzyme is not expressed until the bacterium encounters the β-lactam antibiotic, as if the presence of the activity of this β-lactamase in the absence of a β-lactam is intrinsically deleterious to the bacterium or at least costly.[25] Indeed, Pérez-Gallego et al. have shown in *Pseudomonas aeruginosa* that the combination of hyper-expression of AmpC and the loss of the ability for the bacterium to recycle the peptidoglycan components of its cell wall together give a growth-motility-cytotoxicity phenotype (in contrast, overexpression of AmpC alone, did not give this phenotype).[25] For reasons not yet fully understood, AmpC overexpression in this bacterium is necessary but is not by itself sufficient for this growth- and motility-impaired phenotype. These observations lead to two conclusions. The first is the possible virtue of pairing a β-lactam with a chemotherapeutic agent that separately but simultaneously impairs cell-wall recycling.[25,26] The second is the importance of understanding the molecular relationship between cell-wall recycling and the induction of expression of the *ampC* β-lactamase as the key resistance determinant of the pathogenic *Enterobacteriaceae* (and *P. aeruginosa*) against the β-lactam antibiotics.

The direct connection of the recycling of the peptidoglycan of the cell wall to the induction of *ampC* expression in pathogenic Gram-negative bacteria, as a key β-lactam resistance event, has been known for more than 30 years.[27] Since its discovery, the identities of the key components of the pathway have been identified progressively.[5,28–31] These components include the periplasmic lytic transglycosylase enzymes (involved in the pathway in many of the *Enterobacteriaceae* species and in *P. aeruginosa);* the periplasmic penicillin-binding protein 4 (PBP4) in *P. aeruginosa* (a different PBP may be used by other bacteria); the proton-motive force-driven AmpG transporter of the inner membrane, used to bring the peptidoglycan segments formed in the periplasm into the cytoplasm for recycling; in the cytoplasm, the AmpD amidase(s) and the NagZ glucosaminidase, and the AmpR transcriptional regulator. This review is a perspective not only on recent studies with these protein components but also on the still inchoate effort to assemble this seemingly spare (in terms of the number of its components) pathway into the sequential transformation of structure for interpretation by the AmpR transcriptional regulator. AmpR is the regulatory nexus not only for β-lactam resistance but also for a host of other pathways.[31–33] AmpR controls directly AmpC expression and as such is an important example of the inscrutable LysR-type transcriptional regulators. Lastly, we provide a perspective on the place of this pathway in the broader pathway of peptidoglycan recycling and the possible mechanistic fit

of the peptidoglycan recycling pathways of the Gram-negative bacteria into the bactericidal mechanism of the β-lactam antibiotics.

In the eons of internecine bacterial encounter, both antibiotic structure and antibiotic-resistance mechanisms have evolved. The few decades of human use of antibiotics have resulted in the rapid coalescence of multiple, and complementary, resistance mechanisms into particular bacterial species, such that the future ability of the antibiotics to control their growth is uncertain. Of all of the cell-envelope-acting antibiotics, the β-lactams are arguably unparalleled with respect to their clinical safety and efficacy. Yet for precisely this reason, their antibiotic future is threatened, and the importance of understanding and unraveling their resistance network is paramount. In this section, the biosynthetic events that occur in the cytoplasm of the Gram-negative bacteria, as prefatory to the assembly in the periplasm of the target of the β-lactams, the cell wall, are introduced.

2. CYTOPLASMIC EVENTS: ASSEMBLY OF LIPID II

The bacterial cell wall is not an ordinary polymer. Although its peptidoglycan-derived structure uses a single structural building unit, lipid II (Figure 3), the resulting polymer exists in a ceaseless state of enzyme-catalyzed remodeling. This remodeling is a deliberate effort to impart to this polymer distinctive structural nuances for the purposes (among others) of defining sites for protein recognition, of imparting resistance to degradation (such as by the antibacterial enzyme and lysozyme), and of demarcating sites for mature peptidoglycan excision and new peptidoglycan insertion. With rare exception, neither the precise identity of the structural nuance, nor the correlation of that nuance to function, is known. One example of a structure—function correlation is the *N*-deacetylation and *O*-acetylation of the lipid II-derived saccharides of the peptidoglycan polymer, for the purposes of antibiotic resistance and for immune evasion by the bacterium.[34,35] An example where the structural change is known, but where the functional consequence is now just emerging, is the correlation between complete peptide stem removal from the peptidoglycan (as a recognition motif for peptidoglycan-modifying enzymes that have the sporulation-related repeat, or SPOR, domain) and protein recruitment to the division site for the purpose of daughter-cell separation.[36] The efficient, methodical, rapid, and seemingly flawless ability of the bacteria to replicate precisely both their size and shape,[37–40] with the implicit requirement for ordered peptidoglycan polymer modification, compels the belief that the peptidoglycan structure encodes structural nuances (such as the one recognized by the SPOR domain-containing enzymes) to direct these events.

Our knowledge of peptidoglycan structural transformation is considerably more complete with respect to the cytoplasmic pathways that mesh in Gram-negative bacteria to give lipid II as the final cytoplasmic intermediate (and first periplasmic intermediate) for the synthesis of the peptidoglycan of the cell wall. In addition to the de novo lipid II biosynthetic pathway, a second pathways merges the recycling of peptidoglycan-derived segments, copious amounts of which are liberated during new peptidoglycan insertion into existing peptidoglycan,[41] with this de novo synthesis. The pathway for de novo lipid II biosynthesis is very similar in all bacteria that have cell walls. In contrast, peptidoglycan recycling is predominantly a characteristic of the Gram-negative (diderm) bacteria. Moreover, depending on the particular

Gram-negative bacterium, either a catabolic (such as is used by *Escherichia coli,* a rod-shaped Gram-negative bacterium of the *Enterobacteriaceae* family) or an anabolic (such as is used by *P. aeruginosa,* a rod-shaped bacterium of the *Pseudomonadaceae* family) recycling pathway is used,[42,43] as further elaborated below. Both of these bacteria are human pathogens. A second pathogenic genus of the *Pseudomonadaceae* family is the *Acinetobacter.* Other pathogenic bacteria of the *Enterobacteriaceae* family include the *Salmonella, Klebsiella, Enterobacter, Yersinia,* and *Shigella* genera. The prevalence of *Klebsiella, Acinetobacter, Pseudomonas,* and *Enterobacter* ("KAPE") genera as multiantibiotic-resistant bacteria pathogens is captured by their designation by the Infectious Diseases Society of America as the "ESKAPE" (and where the initial ES signify the no less problematic Gram-positive pathogens of the *Enterococcus* and *Staphylococcus* genera) pathogens.[44,45] It is within this KAPE context, four Gram-negative genera that are increasingly resistant to antibiotics, and which possess cell-wall recycling pathways that (as we shall see) are intimately connected to their β-lactam resistance,[46] that is the theme of this review.

2.1. Mur Biosynthetic Pathway to Lipid II

De novo biosynthesis of lipid II occurs in the cytoplasm of the bacterium, with all but the last two steps catalyzed by soluble enzymes.[47–51] Lipid II assembly reflects its three distinct structural segments (Figure 3): the undecaprenol diphosphate, the GlcNAc-MurNAc disaccharide, and the pentapeptide stem attached to the *N*-acetylmuramic acid (MurNAc) saccharide. The union of these three parts occurs at the MurNAc saccharide. MurNAc is derived biosynthetically from *N*-acetylglucosamine (GlcNAc)-*O*-UDP (Scheme 3). The cytoplasmic Mur pathway[52–57] culminates in the synthesis of lipid II (Scheme 4). In the first committed steps of this pathway, a D-lactyl segment is appended to the C-3 hydroxyl of GlcNAc-*O*-UDP by the sequential actions of the MurA (phosphoenolpyruvate) transferase and MurB reductase. Added stepwise to the resulting MurNAc-*O*-UDP is the first amino acid of the peptide stem (L-Ala, catalyzed by MurC), the second (D-G1u, catalyzed by MurD), the third (*meso*-diaminopimelate, catalyzed by MurE), and last by MurF the addition of the fourth and fifth amino acids, incorporated as the D-Ala-D-Ala dipeptide. The glycopeptide of the resulting MurNAc-*O*-UDP-pentapeptide ("Park's nucleotide") is then transferred, in a reaction catalyzed by the MraY translocase, to the phosphate of the membrane-embedded undecaprenol phosphate, and with release of UMP.[58–64] This now membrane-bound reaction product (lipid I) is last acted upon by the MurG glycosyl transferase in the final step of Lipid II biosynthesis, wherein GlcNAc is added (using GlcNAc-*O*-UDP as the glycosyl-donor substrate) to the C-4 of its MurNAc saccharide.[65,66] Translocation of lipid II across the membrane[67–72] provides lipid II as a substrate for the initiation of peptidoglycan biosynthesis in the periplasm.

2.2. Cytoplasmic Muropeptide Recycling for Entry into the Mur Pathway

Whereas the pathway for lipid II biosynthesis delivers a single entity as the monomeric structure for initiating cell-wall biosynthesis, the recycling pathway, a pathway that likewise has distinct periplasmic and cytoplasmic enzymatic events, returns to the cytoplasm an ensemble of structures. These structures reflect the substrate specificity of the enzymes tasked in the periplasm and in the cytoplasm for the excision and degradation of the

peptidoglycan, the postpolymerization structural transformations made in the periplasm to the peptidoglycan, and the substrate specificity of the AmpG transporter. This latter criterion defines the gross structure of these muropeptides as derivatives of the GlcNAc-MurNAc disaccharide (**1**, Scheme 3). Evaluating both the breadth (in terms of structural modification to the disaccharide, and to the stem peptide of the disaccharide) and the relative abundance of the structures in this ensemble is accomplished only with great difficulty.[73] Nonetheless, it is evident that the ensemble of muropeptide structures brought into the periplasm is changed as a result of the presence of cell-wall-acting antibiotics (especially the *β*-lactams), and that Gram-negative bacteria use this change to initiate defensive and virulence pathways [5,28,30,31,74]

While the structures are not known, and the fullness of (and interconnectedness of) the defensive and virulence response is uncertain, the core substrate structure (the GlcNAc-MurNAc disaccharide with its stem oligopeptide) and core enzyme catalysts that act on this structure to comprise the cytoplasmic pathway for muropeptide recycling, are known. The initial four enzymes of the pathway, NagZ, LdcA, AmpD, and AnmK, are common to the Gram-negative bacteria. NagZ is the glycoside hydrolase that cleaves the GlcNAc-MurNAc disaccharide bond to give the monosaccharide pair.[75–83] LdcA is a serine-dependent L,D-carboxypeptidase that recognizes the tetrapeptide stem structure (but not the pentapeptide stem), and catalyzes the hydrolysis of the *meso*-diaminopimelate-D-Ala amide bond with release of D-Ala, to give the tripeptide.[84–86] LdcA acts on the tetrapeptide stems of MurNAc mono-saccharides, the tetrapeptide stems of the GlcNAc-MurNAc disaccharide (**1**, Scheme 3), and the free tetrapeptide (**3b**). In *Neisseria gonorrheae* LdcA contributes to muropeptide release from the bacterium as a periplasmic enzyme, in contrast to cytoplasmic localization of the enzyme in *E. coli* and *P. aeruginosa*, for the ultimate purpose of inducing an inflammatory response in the host inflammation.[87] AmpD is a zinc-dependent endopeptidase that cleaves the amide link between the lactyl moiety of the MurNAc saccharide and the first L-Ala amino acid of the stem.[88,89,90–98] The peptide segments released from the stem by AmpD are reused as substrates by the Mpl ligase,[99–101] that transforms UDP-GlcNAc to UDP-MurNAc-tripeptide. The stemless anhMurNAc product of AmpD is transformed by AnmK-catalyzed ATP-dependent ring opening of the anhydrous saccharide structure, to give MurNAc-6-phosphate.[102,103] The mechanism of this enzyme is open to speculation.[104,105] There is no evidence supporting the organization of the first three of these four enzymes as an ordered pathway. As we shall discuss, mutational loss of AmpD activity is a particular event that correlates directly with induction of *ampC* hyperexpression.

The remaining enzymes used in muropeptide cytoplasmic recycling differ between the *Enterobacteriaceae* (such as *E. coli* and *Citrobacter freundii*) and the *Pseudomonadaceae* (such as *P. aeruginosa*). In the *Enterobacteriaceae*, the catabolic activities of two enzymes, MurQ and NagA, transform the stemless MurNAc saccharide to glucosamine-6-phosphate (Gln-6-P), for entry into the de novo lipid II biosynthetic pathway (via GlmM and GlmU catalysis) as UDP-GlcNAc. MurQ etherase removes the lactyl moiety from the MurNAc saccharide.[102,106] The mechanism used by MurQ proceeds from the acyclic MurNAc aldehyde, via an E_{1cb} (retro-oxa-Michael) ejection of the lactate as its alkoxide, followed by oxa-Michael addition of water to the transient enal to complete turnover.[107,108] The product

of MurQ, GlcNAc-6-phosphate, is transformed by the zinc-dependent NagA deacetylase to GlcN-6-phosphate (GlcN for glucosamine).[109,111] In contrast, in the *Pseudomonadaceae* (which lack both MurQ and the GlcNAc kinase NagK), two different enzymes use an anabolic route to complete cytoplasmic muropeptide recycling.[42,43] MupP, the first enzyme, is a phosphatase that converts MurNAc-6-P to MurNAc. MurNAc is then phosphorylated on its anomeric hydroxyl in a reaction catalyzed by the AmgK kinase[112] to give MurNAc-1-P as a product. This product enters the lipid II biosynthetic route as a substrate for MurU (to give UDP-MurNAc). The biochemical rationale for the seeming paradox of sequential phosphatase and kinase activities (as opposed to the use of a single phosphomutase) is not obvious. Possible explanations suggested by Borisova et al. and by Fumeaux and Bernhardt are a physiological requirement (for unknown reasons) for the preservation of a steady-state MurNAc pool or a physiological reason (again, for unknown reasons) for suppression of an anhMurNAc pool.[42,43]

The two pathways offer a further point of critical difference. The anabolic muropeptide recycling route used by the *Pseudomonadaceae* provides a complementary pathway (to the de novo Mur pathway) to UDP-MurNAc. Accordingly, the therapeutic use of an antibiotic inhibitor of an enzyme in the Mur pathway (such as fosfomycin, an inhibitor of MurA) would be anticipated to result in diminished antibiotic efficacy in the *Pseudomonadaceae* as compared to the *Enterobacteriaceae*. This expectation is demonstrated.[42] Conversely, in the *Pseudomonadaceae*, the simultaneous blockade of both pathways to UDP-MurNAc, the Mur pathway and the cytoplasmic recycling pathway, by use of combination therapy would be anticipated to offer antibiotic potentiation, or even synergy. [113,114]

3. PERIPLASMIC EVENTS: COORDINATION OF CELL-WALL SYNTHESIS, REMODELING, AND DEGRADATION

As indicated earlier, the bacterial cell wall is an essential macromolecule that surrounds the cell. Although a single polymeric molecule, the PG that comprises the cell wall, shows astonishing structural complexity and structural dynamics. Its preservation requires the coordinated interplay of numerous enzymatic activities that control its shape, size, synthesis, remodeling, and overall integrity.[115–118] The cytoplasmic events of cell-wall biosynthesis culminate with the synthesis of lipid II as the key biosynthetic intermediate of PG biosynthesis. Upon the translocation of lipid II to the periplasm, it experiences polymerization by transglycosylases, which produce the nascent linear PG (Scheme 6). This nascent PG serves as substrate for certain transpeptidase members of the PBP family, serine-dependent enzymes that experience acylation and deacylation by the PG in their chemistry, which integrate the nascent PG with the cutting-edge of the growing cell wall by the cross-linking reaction at the peptide stems (Scheme 5). The transglycosylase and transpeptidase activities could be found at times in a single bifunctional protein.

3.1. Penicillin-Binding Proteins: Building the Cell-Wall

The biosynthesis of the cell wall in the periplasm is a monumental task that requires the coordinated participation, as enzyme catalysts, of both high-molecular-mass (HMM) and low-molecular-mass (LMM) PBPs. *E. coli*, for example, has 13 PBP enzymes (PBP1a,

PBP1b, PBP1c, PBP2, PBP3 (FtsI), PBP4, PBP5, PBP6a, PBP6b, PBP7, PBP4a, PBP4b, and AmpH) and *P. aeruginosa* has nine (PBP1a, PBP1b, PBP2, PBP3, PBP3a, PBP4, PBP5, PBP6, and PBP7).[1,11,31,119] These PBPs are believed to form supramolecular complexes with transmembrane proteins, scaffolding proteins, hydrolases, and other proteins of the periplasm. The final cell wall is composed of glycan strands consisting of a repeating saccharide pair, GlcNAc and MurNAc interconnected by a β-1,4-glycosidic bond. A cross-linked peptide stem joins adjacent glycan strands. As previously stated, in Gram-negative bacteria the full-length stem structure on the MurNAc saccharide is the pentapeptide L-Ala-γ-D-Glu-*meso*-diaminopimelate-D-Ala-D-Ala (**3c**). In Gram-positive bacteria L-lysine (often with additional amino-acid extensions on its ϵ-amine) replaces the *m*-DAP (*meso*-1,6-diaminopimelate) residue.[120] These peptide stems subsequently are cross-linked to form the three-dimensional web that encases the bacterium and so provides the structural integrity essential to the viability of the bacterium.[121–125] In the model Gram-negative organism *E. coli*, an estimated 44–60% of the total number of peptide stems are cross-linked.[126] Although numerous models have been proposed to describe the structure of the cell wall,[127–133] its three-dimensional structure remains unsolved. Moreover, while the cell wall is indeed a single macromolecule, it likely has four distinct substructures: the PG of the sidewall, of the sidewall to septal PG transition, and of the early and the late (mature) septal PG.

It is believed that the HMM PBPs of the Gram-negative bacterium catalyze PG synthesis, while the LMM PBPs catalyze (or control by their catalysis) PG maturation. HMM PBPs are assigned as either Class A or Class B enzymes.[10,72,134–140] The HMM PBPs are anchored to the outer-leaflet of the inner membrane by a noncleavable N-terminal peptide anchor. In addition to their β-lactam-sensitive transpeptidase domain, Class A HMM PBPs encode an N-terminal glycosyltransferase domain enabling catalysis of glycan-strand formation, and hence are termed bifunctional PBPs (having both glycosyl-transferase and transpeptidase active sites). Class A HMM PBPs include PBP1a, PBP1b, and PBP1c of *E. coli* and PBP1a and PBP1b of *P. aeruginosa*. Deletion of the PBP1c and PBP1a or PBP1b genes in *E. coli* is tolerated, while loss of both PBP1a and PBP1b is lethal.[141,142] The viability requirement for at least one Class A HMM PBP is common across most Gram-negative bacteria.[11] The glycosyltransferase domain of the *E. coli* MtgA also contributes to cell-wall elongation.[143] Class A HMM PBPs initiate glycan strand synthesis using two lipid II molecules, with one donating its GlcNAc-MurNAc disaccharide to the other as an acceptor. Glycan strand elongation then occurs by sequential addition of the GlcNAc-MurNAc disaccharide provided by additional lipid II molecules (Scheme 6). Each transfer of a GlcNAc-MurNAc disaccharide from lipid II to the C4 hydroxyl of the GlcNAc of the acceptor strand releases its undecaprenyl-pyrophosphate lipid segment. This undecaprenyl-pyrophosphate subsequently is either dephosphorylated or used as a phosphate donor in lipopolysaccharide biosynthesis.[144–146] The precise mechanisms for undecaprenyl phosphate recycling and reentry into the biosynthetic pathways of cell-envelope synthesis are uncertain.[147]

Class B HMM PBPs are solely transpeptidase (monofunctional) enzymes. The function of the N-terminal domain of Class B HMM PBPs that leads to the C-terminal transpeptidase active site provides a recognition domain for other proteins,[148] as well as acting as a spacer

to distance the transpeptidase domain from the membrane.[135,149] The N-terminal domain of Class B HMM PBPs likewise may provide the hydrophobic surface for interactions with partner proteins.[135] Moreover, Class B HMM PBPs are implicated in the determination of cell morphology. Class B HMM PBPs include PBP2 (elongation) and PBP3 (septation) of *E. coli* and *P. aeruginosa.* The transpeptidase active site, found in the C-terminal domain of both Class A and B HMM PBPs, transfers the penultimate acyl moiety of the D-Ala—D-Ala motif of the peptide stem to the distal amine of the *m*-DAP (in Gram-negative bacteria; to the L-Lys in Gram-positive bacteria) and releases the terminal D-Ala as a leaving group (Scheme 7). This acyl-transfer process uses the serine of the absolutely conserved (Ser-Xaa-Xaa-Lys) active-site catalytic motif as the initial acyl-acceptor, thus forming an acyl-enzyme intermediate.[150] This acyl intermediate is in turn the acyl-donor to the *m*-DAP/L-Lys amine, completing turnover. The resulting amide bond is termed a 4,3-cross-link as it connects the D-Ala (fourth amino acid of the donor stem) to the *m*-DAP/L-Lys (third amino acid of the acceptor stem). A 3,3-cross-link may form between two *m*-DAPs of adjacent glycan strands, although only as a minor product in *P. aeruginosa* and *E. coli*. The inner-membrane-bound lytic transglycosylase G (MltG) is believed to be the catalyst that determines glycan strand length, by termination of glycan polymerization.[151]

3.2. Elongasome: Providing Shape to the Rod-Shape Bacterium

The rod shape of two of the best-studied Gram-negative bacteria, *E. coli* and *P. aeruginosa*, results from cell-wall growth by lateral sidewall elongation, culminating in the initiation at the midcell of a septal PG (Figure 4). Progressive constriction of the two membranes[153] is coordinated with the completion of the PG of the septum. The final step is separation of this septal PG to form concomitant with cell division (release of the daughter bacterium) two new poles. Division and elongation each use separate multiprotein and multienzyme complexes: the divisome (Figure 4a) and elongasome (Figure 4b), respectively. [1,154,155] The cytoplasmic actin-like oligomeric protein MreB is the key platform protein of the elongasome.[156–161] MreB oligomerizes as an antiparallel filamentous strand in an ATP-dependent process and makes contact with the face of the inner leaflet of the cytoplasmic membrane.[162,163] The hypothesis that MreB provides a circumferential track for the ordered insertion of new PG into old PG has given way to a new model wherein nascent PG is inserted as discrete patches into old PG.[156–158,164–178] A perspective on MreB localization is given by Errington.[179] A simple understanding of the role of MreB in the organization of the elongasome is challenged by the finding that MreB is not necessary to the preservation of the rod shape of *E. coli*.[180] Regardless, the MreB scaffold is still visualized as the anchor for the numerous other protein components of the elongasome, including especially the transmembrane protein RodZ (Figure 4b).[180,181] RodZ encodes an N-terminal cytoplasmic domain (used to complex to the MreB scaffold), a transmembrane region spanning the inner membrane, and a C-terminal periplasmic domain.[182,183] The C-terminal domain of RodZ complexes with MreC and MreD, which serve as a scaffold for the proteins of the elongasome in the periplasm (Figure 4b).[184] No catalytic function has been assigned as of yet to RodZ. Biosynthesis of the lateral PG cell wall is performed primarily by PBP1a. PBP1a forms a complex with the outer-membrane-anchored lipoprotein LpoA. LpoA activates PBP1a for catalysis and thus is also an essential protein of PG biosynthesis.[185,186] RodA is a second (in addition to PBP1a) glycosyl transferase of the elongasome and a

member of the SEDS family of proteins.[187,188] The activity of RodA may pair with the transpeptidase activity of the monofunctional class B PBP2. These protein families interact since PBP2 forms a complex with the scaffold protein MreC.[148] In addition, RodA may participate in the translocation of lipid II across the inner membrane.[189] The identification of the lipid II flippase protein (from among RodA, FtsW, and MurJ) emerged from the collective studies from several laboratories. The current consensus recognizes MurJ, an inner membrane anchored member of both the elongasome and divisome, as the lipid II flippase.[67,71,190–192] Studies supporting MurJ assignment as the lipid II flippase include the observation that cytoplasmic PG precursors accumulate in MurJ-depleted cells, a consequence of which is ultimate cell lysis due to diminished PG biosynthesis.[190,193,194] Radiolabeled lipid II flipping in *E. coli* spheroplasts was MurJ-dependent.[67] Lastly, an X-ray structure of MurJ confirms lipid II binding as a putative substrate.[71]

3.3. Divisome: From One Parent Cell to Two Daughter Cells

Bacterial division is functionally independent of bacterial elongation (Figure 4).[195–198] The seminal work of Bi and Lutkenhaus introduced the divisome as a macromolecular complex of proteins that creates and closes the septum to separate the dividing cells (Figure 4a).[199] Septum formation involves the progressive constriction of the helical protein ring' the Z-ring, that is formed at the midcell of the maturing elongated bacterium. The midcell Z-ring is formed by the GTP-dependent polymerization of the FtsZ protein. FtsZ-led division is spatially and temporally coordinated with chromosome replication.[200–204] GTP maintains the FtsZ-ring as a tubulin-like treadmilling platform for the cell-division machinery.[205–215] Septum synthesis occurs in two steps. The first step initiates septal PG synthesis using an "early division protein" assembly and completes it using a "late division protein" assembly.[216,217] The Z-ring polymer of FtsZ proteins is anchored at midcell to the inner leaflet of the inner membrane by the protein FtsA. Additional proteins (ZipA, EzrA, and the Zap proteins) assist in the cytoplasmic assembly of the FtsZ/FtsA-formed proto- or Z-ring (Figure 4a). The FtsZ helical ring is discontinuous. It exists as separate filaments linked laterally by interactions of bundling proteins found at the beginning and end of each filament.[218] How these lateral interactions of linked filaments contribute to "treadmillling" (in vivo-depolymerizing from one end of the filament and polymerizing from the other end) is not clear.[197] Upon initiation of septum synthesis by the "early divisome protein" assembly, the "late division proteins" PBP3 (gene name *ftsI*, alternative protein name FtsI, a monofunctional transpeptidase) and FtsW assemble. The transition between catalysis by the two assemblies may involve a significant time delay of up to 20% of the growth and division life cycle. The transition to "late division protein" assembly is regulated by the proteins FtsE and FtsX, which activate FtsA. The paired proteins PBP3 and FtsW (a putative transglycosylase, as assessed by sequence similarity to RodA) are essential for septal PG formation.[219,220] Like RodA, FtsW is able to serve as a lipid II transporter protein for the divisome; however, as described previously, MurJ serves as the primary facilitator of this process.[189,221] The putative transglycosylase activity of FtsW is presumed to coordinate with PBP3. This symbiotic relationship between FtsW and PBP3 parallels the bifunctional catalysis of PBP1b, akin to RodA and PBP2 to PBP1a, respectively, of the elongasome. PBP1b encodes a glycosyl transferase domain, a transpeptidase domain, and a third noncatalytic UB2H domain.[222] Its glycosyltransferase activity contributes to de novo

sidewall PG synthesis, and its transpeptidase activity correlates directly to the rod shape of *E. coli*.[223] The UB2H domain is the docking site for the outer-membrane anchored lipoprotein LpoB,[224] the activating protein for PBP1b (akin to the regulation of PBP1a and its requisite partner LpoA).[185,186,225–230] A functionally similar protein to *E. coli* LpoB, LpoP, activates *P. aeruginosa* PBP1b.[231] In addition, proteins of unknown function, including FtsN, FtsQ, FtsL, and FtsB, are members of, or are functionally proximal to, the divisome.[197] FtsN coordinates the early and late division proteins by interacting with a postactivation FtsA and stabilizing a four-protein complex of FtsQ, FtsL, and FtsB.

3.4. Penicillin-Binding Proteins: Maintaining the Cell Wall

A careful balance between synthesis of the cell wall by HMM PBPs, coordinated by LMM PBPs, and degradation of the cell wall by amidases and lytic transglycosylases (LTs), maintains the cell wall. Most LMM PBPs are DD-carboxypeptidases and/or endopeptidases and are not critical to cell viability under standard laboratory growth conditions.[119,142,232] *E. coli* has five LMM PBPs: PBP4, PBP5, PBP6a, PBP6b (also known as DacD), and PBP7/8. PBP5, PBP6a, and PBP6b are monofunctional DD-carboxypeptidases and PBP7/8 (PBP8 is a proteolytic degradation product of PBP7) is a monofuntional DD-endopeptidase.[233–235] DD-Carboxypeptidases remove the terminal D-Ala from the nascent pentapeptide stem, and DD-endopeptidases cleave the cross-linked peptide stems of the mature PG macromolecule. The action of a DD-carboxypeptidase regulates the degree of cross-linking of the PG, since the 4,3-cross-links cannot be formed without the terminal D-Ala cleavage.[5,11] PBP5 is the most abundant PBP and is considered to be the workhorse DD-carboxypeptidase of *E. coli*.[236–238] However, at acidic pH (pH < 5), PBP6b replaces PBP5 as the workhorse DD-carboxypeptidase.[119] Like all other PBPs, PBP5 is located in the periplasm, but is uniquely anchored to the membrane[239] by an amphipathic helix that is required for its in vivo activity.[240] Notably, PBP5 is the only PBP known to participate in both the divisome and elongasome.[232] PBP5 knockouts show prominent morphological defects (branching and topological distortions), as do PBP5 hyper-expressing cells (osmotically stable spherical cells).[241,242] For these reasons, PBP5 has been the historical focus of the study of the DD-carboxypeptidase family. The active site motif of the N-terminal DD-carboxypeptidases domain of PBP5 (Ser-Xaa-Xaa-Lys, Ser-Xaa-Asn, and Lys-Thr-Gly) is conserved among members of the family. However, the C-terminal domain is not conserved. The DD-carboxypeptidase activity of PBP5 uses the nucleophilic serine of the catalytic tetrad (Ser-Xaa-Xaa-Lys) for acylation. A water molecule is recruited through hydrogen-bond coordination with the second active-site serine (Ser-Xaa-Asn). This water molecule then is activated by the active-site lysine (Lys-Thr-Gly) for hydrolytic deacylation.[243] This hydrolysis event yields a tetrapeptide stem product with release of the terminal D-alanine of the stem.[237]

3.5. Amidases: Modifying Cell-Wall Architecture

A family of zinc-dependent *N*-acetylmuramyl-L-alanine amidases resides in the periplasm and act as potent peptide-cleaving enzymes (Scheme 8). *E. coli* has four periplasmic amidases (AmiA–AmiD).[244] AmiA, AmiB, and AmiC function collaboratively in cell division. AmiB and AmiC are important members of the divisome. In their absence, the bacterium is incapable of the septal splitting that completes cell division.[245–248] While

AmiA–AmiC process the peptide stems of cross-linked PG, AmiD is anchored to the inner leaflet of the outer membrane and is activated against a wide range of substrates, notably including short-chain muropeptides and 1,6-anhydromuropeptides.[249] Comparison of the genome sequence of *E. coli* AmiD to the genome of *P. aeruginosa* identified two *P. aeruginosa* periplasmic amidases, AmpDh2 and AmpDh3.[90,93–98] Both AmpDh2 and AmpDh3 encode two domains, one a conserved amidase domain on the N-terminus and the other a C-terminal PG-binding domain. A coiled-coil/loop on the N-terminus and the C-terminal domain together facilitate oligomerization. AmpDh2 is a dimer anchored to the inner leaflet of the outer membrane and AmpDh3 is a tetramer that exists as a soluble protein complex in the periplasmic milieu.[96] The specificities of AmpDh2 and AmpDh3 are complementary. AmpDh3 prefers cleavage of the cross-linked peptide stems found in the insoluble fraction of the purified sacculus, whereas AmpDh2 prefers cleaving non-cross-linked peptide stems isolated from the soluble fraction of the sacculus.[94,95] These findings suggest that AmpDh2, and similarly AmiA–C, regulate the PG core structure, while AmpDh3 (and similarly AmiD) acts on the peripheral PG structure.[97]

3.6. Lytic Transglycosylases: Liberation of Cell-Wall Fragments

Removal of peptide stems from muropeptides in the periplasm is neither sufficient nor required to target mature PG for cytoplasmic recycling. Rather, the task of muropeptide processing is initiated by the large, structurally diverse superfamily of LTs. The LTs perform a nonhydrolytic transacetalization reaction that cleaves the glycosidic bond between MurNAc and GlcNAc, thus transforming the MurNAc of the substrate to a 1,6-anhMurNAc in the product (Scheme 9). LTs are classified structurally as members of the extensive glycoside hydrolase superfamily, although their catalytic activity is nonhydrolytic. The key catalytic residue of the LTs is either a Glu or Asp depending on the enzyme family (families 1–6).[250,251] This acid residue serves as a general-acid proton donor to the glycosidic oxygen of the β-1,4-linkage in promoting the glycosidic bond cleavage, and subsequently as a general-base residue with respect to the muramic 6-OH to complete catalysis by 1,6-anhMurNAc formation. The central high-energy species is an oxocarbenium cation centered on the anomeric carbon of the MurNAc. The final chemical transformation entails a conversion of the all-equatorial substituents of the MurNAc to all-axial positions in 1,6-anhMurNAc, which is believed to force the substrate out of the active site of the enzyme due to the conformational shift of the peptide stem.[252] The distinctive 1,6-anhMurNAc terminus of their muropeptide reaction products is a structural tag enabling the transport of these muropeptides across the inner membrane, via the 1,6-anhMurNAc-selective AmpG permease, to the cytoplasm for recycling. In addition to their role in cell-wall recycling during cell growth, the LTs play critical roles in the detection of cell-wall-acting antibiotics, the formation of macromolecular secretion systems and flagellar assemblies, cell division, and (in some species) bacterial virulence.[250,251,253–255] Knockout studies show that no single LT is critical to the in vitro viability of the bacterium. However, a viable pan-LT deletion mutant could not be obtained.[256] Notwithstanding that each LT might not be critical for survival, their individual functions would appear to be favored as their genes are conserved across most (>95%) *P. aeruginosa* genomes of known sequence. These findings imply functional redundancy among the LT superfamily. Indeed, in vitro studies show that with few exception, the catalytic reactions of LTs overlap.[257,258]

Most LTs are membrane-bound (designated as “Mlt” LTs) lipoproteins anchored to the inner leaflet of the outer membrane. At least one LT is anchored to the outer leaflet of the inner membrane (the MltG, which the reader recalls, regulates the nascent-glycan strand sizing). Some LTs are termed soluble (“Slt”) proteins, for lack of a membrane anchor, but this does not preclude their involvement in multiprotein complexes, which might effectively anchor them. The soluble LTs of *E. coli* and *P. aeruginosa* exhibit greater activity in vitro than the soluble recombinant forms of their membrane-anchored counterparts (lacking membrane anchor).[257,258] The roster of known LTs in *E. coli* now numbers nine: MltA, MltB/Slt35 (Slt35 is a proteolytic degradation product of MltB), MltC, MltD, MltE, MltF, MltG, RlpA, and Slt70. *P. aeruginosa* has 11 known LTs: MltA, MltB/Slt35, MltD, MltF, MltF2, MltG, RlpA, Slt, SltB1 (SltB), SltB2 (SltG), and SltB3 (SltH). Subfamily assignments and structural comparisons of the LTs were reviewed recently.[251] In the present report, we describe the activities of a few LTs. Soluble lytic transglycosylase 70-kDa (Slt70) is the only soluble LT of *E. coli*. In contrast *P. aeruginosa* has four soluble LTs. *E. coli* Slt70 is primarily an exolytic catalyst, acting at the terminus of a glycan strand to release a GlcNAc-MurNAc disaccharide, while capping the strand with a nonreducing 1,6-anhMurNAc saccharide terminus. The functional purpose of the unusual annular three-dimensional structure of the Slt70-type enzymes (as exemplified by the *E. coli* Slt70 enzyme,[259] the LtgA enzyme of *Neisseria meningitidis,*[260] and the Slt enzyme of *P. aeruginosa*[261]*)* is uncertain. Membrane-bound lytic transglycosylase E (MltE) is the primary endolytic LT of *E. coli*, cleaving a central MurNAc-GlcNAc glycosidic link of the glycan strands of the PG. MltE recently was implicated in the assembly of type IV secretion systems (T4SS) in enteroaggregative *E. coli* strains.[257,262] Rare lipoprotein A (RlpA) is the primary endolytic protein of *P. aeruginosa* and cleaves only glycan strands that previously have had their peptide stems removed (so-called “naked” glycan strands) by an amidase.[258,263] An RlpA knockout mutant is the only single *P. aeruginosa* LT mutant to exhibit microscopically a changed phenotype. In media without salt, **Δ**RlpA *P. aeruginosa* forms filamentous cells as a result of failed (incomplete) cell division.[263] These results suggest that RlpA may be a heretofore unassigned member of the divisome. LTs are regulated by binding of partner proteins and binding of ligands to secondary allosteric domains. The activity of *E. coli* MltE increases following complexation with its partner protein, TssM, to facilitate assembly of the type IV secretion system.[262,264–266] The activity of *P. aeruginosa* MltF is dependent on the binding of a tetrapeptide stem to its allosteric domain, which triggers a dramatic conformation change and activation.[267] The activity of *P. aeruginosa* SltB1 may be regulated by the formation of an inactive catenated homodimer,[268] which would require separation of the monomers for activation.[269] Other studies indicate that some LTs form macromolecular complexes with PBPs.[235,269–271]

3.7. Transporters: Pathway to the Cytoplasm

AmpG is the membrane permease that transports the 1,6-anhydromuropeptides to the cytoplasm, driven by the proton-motive force.[272,273] Topological predictions suggest that AmpG is an integral membrane-protein showing ten transmembrane segments.[274,275] The principle requirement for recognition for AmpG-mediated cytoplasmic transport is the GlcNAc-1,6-anhMurNAc disaccharide.[276–278] Both the “naked” disaccharide GlcNAc-1,6-anhMurNAc (product of amidases and LTs) and the disaccharide GlcNAc-MurNAc-peptides

(product of LTs) were transported, while free peptide stems (product of amidases), “naked” monosaccharide 1,6-anhMurNAc (product of periplasmic glucosaminidases, LTs and amidases), and the monosaccharide 1,6-anhMurNAc-peptides (**2**, Scheme 3) (product of periplasmic glucosaminidases and LTs) were not. A recent study expressed AmpG in *E. coli* spheroplasts and demonstrated transport of muropeptides across the membrane in vitro.[278] An AmpG homologue, AmpP, of *P. aeruginosa* was suggested to serve as an 1,6-anhydromuropeptide transport protein in *β*-lactam-stressed cells,[275] but this finding was not replicated for these *P. aeruginosa* proteins in *E. coli* spheroplasts[278] or in knockout studies measuring *ampC* transcription.[279,280] Other membrane permeases in *E. Coli* transport fragments of cell-wall degradation to the cytoplasm. An oligopeptide permease (Opp) transports the free tripeptide (**3a**) product of the periplasmic amidases to the cytoplasm for recycling. Existing models surmise that free tripeptide (**3a**) binds murein peptide permease A (MppA) and is transported into the cytoplasm via membrane-bound and cytoplasmic components OppB, OppC, OppD, and OppF.[281] For the purpose of transporting nonmurein peptides, the MppA homologue OppA, serves a similar role as MppA in crossmembrane transport. Notably *E. coli* mutants that lacked Opp had unimpeded muropeptide transport to the cytoplasm, implicating Opp as a minor permease in this regard.[273]

3.8. Cytoplasmic Amidases and Glucosaminidases: Final Steps

Anhydromuropeptides that evade periplasmic amidases are acted upon by the AmpD amidase in the cytoplasm. Akin to the periplasmic amidases, AmpD is a zinc-dependent *N*-acetylmur-amyl-L-alanine amidase having a single domain. It is highly selective for muropeptides that contain a 1,6-anhMurNAc moiety.[96,282] AmpD cleaves 1,6-anhMurNAc-tripeptide (**2a**, Scheme 3) 10000 times faster than MurNAc-peptides and UDP-MurNAc-pentapeptide (**4**, Scheme 3).[283] This difference prevents AmpD interference in the synthesis of cell-wall precursors.[283] In *ampD* knockout mutants, 1,6-anhMurNAc-tripeptide (**2a**, Scheme 3) accumulates in the cytoplasm. The pairing of this finding with the knowledge that AmpG funnels GlcNAc-1,6-anhMurNAc-peptides (**1**, Scheme 3) into the cytoplasm led to the assignment of a cytoplasmic glucosaminidase to the cell-wall recycling scheme.[284,285] Deletion of the *nagZ* gene encoding the cytoplasmic *β*-*N*-acetyl-glucosaminidase resulted in complete loss of cytoplasmic glucosaminidase activity. As expected, the double Δ*nagZ*/Δ*ampD* mutant accumulates GlcNAc-1,6-anhMurNAc-tripeptide (**1a**, Scheme 3) in the cytoplasm. Therefore, muropeptide catalysis by NagZ is understood to follow muropeptide transport to the cytoplasm and to likely precede amide hydrolysis by AmpD. The mechanism by which NagZ cleaves the glycosidic bond of the disaccharide-peptide (**1c**, Scheme 3), forming *N*-acetylglucosamine (GlcNAc or NAG) and 1,6-anhMurNAc-peptide (**2**, Scheme 3), was studied recently (Scheme 10).[79,82,286] NagZ uses an aspartic acid-histidine catalytic dyad for glycosidic hydrolysis, with interception of the positive charge that develops on the anomeric carbon of the GlcNAc by the aspartate, to give an ester intermediate. An active-site water molecule is activated by proton abstraction by the catalytic histidine and concurrently an oxocarbenium ion forms on C1 of the GlcNAc. The activated water molecule displaces the ester-linked intermediate to complete the turnover. The protein activity is regulated by a zinc ion that binds and represses catalytic function, in that zinc departure and subsequent conformational rearrangement is required for catalysis.

4. EVOLUTIONARY ADAPTION TO THE CHEMICAL IMPRINTING ON THE CELL WALL BY THE β-LACTAM ANTIBIOTIC

The discovery that Gram-negative pathogens embed a sensory mechanism to detect β-lactam antibiotics into their cell-wall recycling process so as to induce expression of the AmpC resistance enzyme is foundational to all subsequent studies on the cell-wall recycling process. AmpR transcriptional regulator control of this pathway was studied initially in *C. freundii* (a close relative of *E. coli*), as *E. coli* lacks AmpR and thus is incapable of inducing AmpC β-lactamase expression from its *ampC* gene. AmpR senses the disruption of the muropeptide pool, by the β-lactam at the molecular level with AmpC expression as a consequence. More recent studies on this important signaling pathway have focused on the clinical scourge *P. aeruginosa* and the emerging problematic bacterium *Stenotrophomonas maltophilia*.[92,287,288] The biological targets of the β-lactam antibiotics are the PBP enzymes, whose names derive from their role as the enzyme targets of the β-lactams. The seminal study by Tipper and Strominger proposed mimicry between the β-lactam structure and the acyl-D-Ala-D-Ala terminus of the peptide stems of the PG.[289] While this mimicry explained why β-lactams efficiently acylate the PBPs at the active-site serine, it did not account for why this acylation is functionally irreversible, nor did it identify the mechanistic link between cause (PBP inhibition) and effect (eventual cell lysis). The hypothesis that loss of PBP function sufficiently compromises the overall integrity of the PG macromolecule so as to culminate in its bactericidal rupture is supported by countless studies. Yet this explanation must be an oversimplification. If we understand that PBPs catalyze a diverse array of PG transformations, how actually does PBP inactivation cause cell lysis? This answer is paramount not only for understanding the bactericidal mechanism of a β-lactam antibiotic but also for understanding the resistance mechanisms against the β-lactams. An equally significant question is whether the bactericidal mechanism is the same across the extraordinary diversity of structure of the β-lactam antibiotics. Recent results suggest not. A study identifying the subset of the PBPs targeted by the clinically important β-lactam antibiotics used against Gram-negative bacteria demonstrates that the loss of multiple PBP functions, either singly or together, is bactericidal.[290] Therefore, may we assume that there is a single signaling pathway that results in the induction of the AmpC β-lactamase? Notwithstanding the abundant evidence that suggests a significant commonality within the resistance-signaling pathway, probably not.

4.1. Penicillin-Binding Protein 4: Source of the β-Lactam Imprint

The identification of the mutations that arise in pathogenic bacteria to bolster their resistance to antibiotics gives valuable insight into the biochemistry of the resistance pathways. Mutations within the AmpC-resistance pathway, as found in extensively β-lactam-resistant *P. aeruginosa* clinical isolates, have been particularly insightful. Among these isolates are those with mutation of the *dacB* gene (encoding the PBP4 enzyme) so as to inactivate this PBP.[291] The significance of PBP4 in the AmpC induction pathway was confirmed by the observation that a PBP4-knockout strain hyperexpressed AmpC β-lactamase.[292] PBP4 is a dimeric, low-molecular-mass bifunctional PBP with both DD-carboxypeptidase and DD-endopeptidase activities.[234,293–296] PBP4 is regarded as the “sentinel” enzyme that recognizes, through its loss in activity by β-lactams, their presence.[291,292,296] These findings

implicate a direct role for PBP4 in the alteration of the muropeptide pool that regulates, through effector structures (i.e., muropeptides), AmpR. Given our understanding of PBP4's activity in vitro, its inactivation would coincide with the enrichment of muropeptides containing a complete pentapeptide stem (loss of DD-carboxypeptidase activity) and/or a muropeptide containing an intact 4,3-cross-linked peptide stems (loss of DD-endopeptidase activity).[296] Differentiating between these two structural possibilities is challenging, especially when the identity of the relevant PBP4 substrate(s) is unknown. Are its important muropeptide substrates liberated from nascent PG or the mature PG macromolecule? Are these muropeptides substrates liberated from the elongasome, the early divisome, the late divisome, or the delicate cell wall that conjoins the lateral sidewall PG and the septal PG?

4.2. Lytic Transglycosylase: Recognition of the Cell-Wall Perturbation

In a tour de force study, Cho et al. demonstrated in *E. coli* that exposure to β-lactam antibiotics results in an accumulation, in the periplasm, of nascent pentapeptide-containing PG chains (Figure 5a).[297] Nonincorporation (or misincorporation) of accumulated nascent PG correlates to cell lysis (Figure 5b). Degradation (for the purpose of correction and recycling) of accumulating nascent PG is thought to be primarily a function of the workhorse LT, Slt70 (homologous to Slt of *P. aeruginosa)* (Figure 5, panels c–e). Slt70 has the ability to cleave PG by both endolytic (Figure 5c) and exolytic degradation (Figure 5d), and both activities are necessary for nascent PG degradation (as the nascent PG termini are tethered at one end to the mature PG, and at the other end to the membrane). These two complementary activities would enrich the periplasmic milieu with GlcNAc-1,6-anhMurNAc-pentapeptide (**2c**), a substrate for AmpG transport. Reconciliation of the observations of Cho et al.[297] and the consequences of loss of PBP4 function into a coherent pathway that culminates with AmpC hyperexpression has not been attempted previously.

4.3. Derivation of a Unique Chemical Imprint

We offer a proposal. Our key surmise is that the muropeptide effector of AmpR derives from the coordinated action of Slt and PBP4 (as a DD-carboxypeptidase) on the nascent aberrant PG of the generic structure [GlcNAc-MurNAc-pentapeptide]$_n$–lipid II postulated by Cho et al.[297] For PBP4 to be the primary DD-carboxypeptidase (cleaving the terminal D-Ala of Slt-liberated 1,6-anhydromuropeptides, and superseding PBP5, PBP6a, and PBP6b), some combination of restrictive partners, membrane-anchoring localization, gene regulation, and/or allosteric regulation must be in play. Several observations make *E. coli* PBP6a and PBP6b unlikely participants in the transformation of muropeptide-signaling muropeptides. PBP6a of *E. coli* has a counterintuitive protective (or safeguarding) effect that preserves in vitro the pentapeptide stems of PG, while the gene for PBP6b is upregulated at acidic pH so as to compensate for the loss of activity of other PBPs.[119] The source of the nascent PG is either the PG at the elongasome-divisome interface or the septum of the divisome. Three LMM PBPs of *E. coli* (PBP4, PBP5, and PBP7/8) localize to the divisome.[298] PBP7/8 acts as a DD-endopeptidase on the mature PG macromolecule. Liberated muropeptides are not thought to be PBP7/8 substrates[299] consistent with the inability of a *P. aeruginosa* PBP7/8 knockout to hyperexpress AmpR in the absence of an antibiotic.[292] The possible catalysts for the periplasmic conversion of the free GlcNAc-1,6-anhMurNAc-pentapeptide (**1c**) obtained from nascent PG, to GlcNAc-1,6-anhMurNAc-tetrapeptide (**1b**), are PBP4 and

PBP5. PBP4 is the more probable candidate. Although PBP5 is a DD-carboxypeptidase, the most abundant of the PBPs, and the PBP most resistant to β-lactam inactivation, its probable physiological focus is the regulation of the number of acyl-D-Ala-D-Ala termini available for cross-linking to form polymeric PG. At least 50% of the PG at the divisome is cross-linked as a result of PBP1b transpeptidation, and some of the non-cross-linked stems of this PG remain in pentapeptide form.[73,300] We propose that the AmpR signaling molecule originates from the >50% of nascent PG that retain the pentapeptide stem. This proposal is consistent with studies demonstrating that Slt-knockouts, in the presence of a β-lactam antibiotics, experience morphological deformation ("bulge formation") at midcell,[301] such as might arise from the inability to clear (as a result of the absence of Slt) accumulated nascent PG.

4.4. Constructing a Pathway to Resistance

The premise that the source of the AmpR signaling molecule is nascent PG of the elongasome-divisome interface (or of the septum of the divisome), and PBP4 is the DD-carboxypeptidase catalyst acting on the Slt-liberated muropeptides, leads to the following pathway. Exposure of the organism to the β-lactam antibiotic initiates formation of the linear non-cross-linked nascent PG (product of lipid II polymerization by transglycosylases). As a subset of the PBPs are inhibited, a negative chemical imprint of the exposure to the antibiotic is made onto the cell wall, which entails enrichment of the structure in pentapeptide stems. As this aberrant structure is accumulated, it is turned over by Slt (and possibly other LTs), which release muropeptides enriched in pentapeptide stems within the periplasmic space (Figure 6). The ultimate LT product, GlcNAc-1,6-anhMurNAc enriched in pentapeptide (**1c**, Figure 6), is a substrate for the AmpG permease (as this permease requires the presence of a GlcNAc-1,6-anhMurNAc (**1**, Figure 6) disaccharide moiety as a minimal substrate motif). *ampG*-Knockout strains show only low-level constitutive expression of AmpC β-lactamase, and this level is unchanged in the presence of β-lactams.[272,302] This finding fits several key observations. First, the signaling molecule for induction of AmpC β-lactamase arises from PG recycling, and the identity of the muropeptide effector that acts on AmpR to induce AmpC β-lactamase must coincide with the substrate specificity of AmpG. Therefore, the AmpC β-lactamase inducer must have a GlcNAc-1,6-anhMurNAc (**1**, Figure 6) peptide structure as it enters the cytoplasm. The *ampC* transcriptional regulator, AmpR, is likely cytoplasmic based on knowledge of other transcriptional regulators. However, a recent study proposes that in *Pseudomonas,* the effector-binding domain of AmpR is positioned in the periplasm and the DNA-binding domain of AmpR is positioned within the cytoplasm.[303] A cytoplasmic location for the DNA-binding domain is necessary, as this is the location of the DNA In the absence of explicit evidence, one presumes the effector-binding domain of AmpR is likely to be cytoplasmic, the location that is consistent with the behavior of the *ampG*-knockout strain, but further experimentation is called for.

4.5. Identifying the Muropeptide Key to Resistance

The initial steps in muropeptide recycling following muropeptide entry into the cytoplasm are catalyzed by the AmpD amidase and the NagZ glucosaminidase, as discussed above. Each of these two enzymes affects profoundly AmpC β-lactamase expression. *ampD*-Knockout strains occur frequently in the clinic and show constitutive hyperexpression of the AmpC β-lactamase even without a β-lactam inducer (as do *dacB*-knockout strains).[90,91,304]

In contrast, *nagZ*-knockout strains require a β-lactam inducer to show AmpC hyper-expression.[76,78,305] The level of AmpC hyper-expression in the *P. aeruginosa nagZ*-knockout strain in the presence of a β-lactam inducer is greater than the level of induced AmpC expression of the wild-type strain or of the *ampG*-knockout strain. In accordance with Zamorano et al., the *ampG*-knockout strain is incapable of β-lactam-induced AmpC hyperexpression.[280] NagZ inhibition achieves modest levels of synergy with β-lactams.[76,77,81,83,114,306–309]. These data suggest that induction of AmpC β-lactamase occurs both by a NagZ-precursor muropeptide and by a NagZ-derived muropeptide. The muropeptide structure(s) most consistent with the data for *C. freundii*, *E. clocae*, and *P. aeruginosa* is that the primary (or exclusive) AmpR-activating inducer molecule is the 1,6-anhMurNAc saccharide having a stem peptide.[272,283] The stem peptide is likely the full-length pentapeptide, which is enriched in the cell wall on exposure of the organism to β-lactam antibiotics.

The original work by Jacobs et al. identified muropeptide recycling as essential to the induction of the AmpC β-lactamase and the muropeptides from this recycling as effectors of AmpR. The authors proposed that binding of the lipid II-precursor molecule, UDP-MurNAc-pentapeptide (**4**, Figure 6), to the effector-binding domain of AmpR represses transcription of the *ampC* gene.[310] This molecule was confirmed subsequently as a ligand of the effector-binding domain of *C. freundii* AmpR by both nondenaturing mass spectrometry and X-ray crystallography.[311,312] The muropeptide that accumulates in the cytoplasm in the presence of an inducing β-lactam was suggested to be the 1,6-anhMurNAc-tripeptide (**2a**, Figure 6), wherein displacement of UDP-MurNAc-pentapeptide (**4**, Figure 6) from the effector-binding site of AmpR by this muropeptide would result in the derepression of transcription of *ampC.* In subsequent studies 1,6-anhMurNAc-tripeptide (**2a**, Figure 6), 1,6-anhMurNAc-tetrapeptide (**2b**, Figure 6), 1,6-anhMurNAc-pentapeptide (**2c**, Figure 6), and free pentapeptide (**3c**, Figure 6) were all also proposed as activators of AmpR.[41,276,288,310,313,314] Although it is highly probable that the muropeptide(s) that repress are structurally distinct from the muropeptide(s) that activate, there is no reason to believe that only a single muropeptide entity coincides with these respective activities. Nonetheless, for more than two decades the consensus interpretation identified UDP-MurNAc-pentapeptide (**4**, Figure 6) as the primary repressor muropeptide and 1.6-anhMurNAc-tripeptide (**2a**, Figure 6) as the primary derepressor (activator) muropeptide effector for regulation of AmpR. This consensus was countered, however, by the observation of bound UDP-MurNAc-pentapeptide (**4**, Figure 6) in the X-ray structure of *C.freundii* AmpR.[312] Moreover, the substructure of this molecule embedded in the effector-binding site of AmpR was the pentapeptide stem, and not the saccharide moiety, as had been modeled previously.[311] This binding mode challenged the existing dogma. The shorter tripeptide stem of the proposed AmpR-activating 1,6-anhMurNAc-tripeptide (**2a**, Figure 6) effector molecule would lack the electrostatic “anchor” contact that was observed between the acyl-D-Ala-D-Ala terminus of the UDP-MurNAc-pentapeptide (**4**, Figure 6) and the base of the effector-binding site.[312] Two subsequent studies validate a revised interpretation of the repressor and derepressor effector structures. Pentapeptide-containing 1,6-anhydromuropeptides accumulate in live *P. aeruginosa* cells upon exposure to β-lactam antibiotics.[73] Furthermore, the comparative assessment of the ability of four authentic synthetic[315,316] muropeptides to

bind to the effector-binding site of *P. aeruginosa* AmpR was consistent with the critical significance of the pentapeptide stem for AmpR recognition (Figure 6). UDP-MurNAc-pentapeptide (**4**, Figure 6), 1,6-anhMurNAc-pentapeptide (**2c**, Figure 6), and GlcNAc-1,6-anhMurNAc-pentapeptide (**1c**, Figure 6) bound to the AmpR effector-binding domain, whereas 1,6-anhMurNAc-tripeptide (**2a**, Figure 6) did not.[317] These recent data support AmpR recognition of pentapeptide-containing 1,6-anhydromuropeptides (and possibly free pentapeptide (**3c**, Figure 6). The relationship of the structural response of AmpR to these ligands, with respect to the repression or activation of *ampC* transcription, is not known.

An interpretive caution with respect to this conclusion is the likelihood that both AmpR and its functional role in β-lactamase induction might have evolved differently in other bacteria. This caution is made evident by studies with the emerging Gram-negative pathogen *Stenotrophomonas maltophilia*. This bacterium encodes two β-lactamases (termed L1 and L2), both under AmpR control.[318] In the absence of an inducer, AmpR activates L1 expression and represses that of L2, while in the presence of an inducer, expression of both genes is activated.[319] This activation is regulated through the muropeptide-recycling pathway, also initiated by LT activity.[320] β-Lactamase activation may occur as a consequence of (the presumably complementary) loss of activity (due to inhibition) of two PBPs, either its PBP1a encoded by the *mrcA* gene[321] or its PBP2 encoded by the *mrcB* gene.[322] To augment complexity, only one of the two *ampD* homologues in the cytoplasm participates[323] and loss of its activity causes β-lactamase hyperexpression.[288] Overall regulation of L1 and L2 expression through muropeptide recycling has both NagZ-dependent and NagZ-independent AmpR pathways.[80] Using an *mrdA* (PBP2) deletion mutant and LC/MS analysis of the resulting significant perturbation in the periplasmic and cytoplasmic muropeptide pools, Huang et al. suggest that the structure of the muropeptide inducer in *S. maltophilia* is the 1,6anhMurNAc-tetrapeptide (**2b**, Figure 6).[322]

5. AMPR: GATEKEEPER TO β-LACTAM ANTIBIOTIC RESISTANCE

A central focus in the regulation of AmpC expression is the transcriptional regulator AmpR.[27,324–326] AmpR is a classic LysR-type transcriptional regulator (LTTR). LTTRs are ubiquitous in both Gram-positive and Gram-negative bacteria, where different LTTRs regulate critical facets of bacterial function, including virulence factor expression, stress response, quorum sensing, motility, fixation of both CO_2 and N_2, the biosynthesis of amino acids, and the catabolism of aromatic compounds.[327–330] Notwithstanding their regulatory importance, the LTTR proteins challenge experimental study. The structural archetype and namesake of the LTTR family, LysR, is the transcriptional regulator of the *lysA* gene encoding the enzyme diaminopimelate decarboxylase.[331–334] The LysR structure is representative of the LTTR family of prokaryotic DNA-binding proteins. LysR comprises two linked domains of approximately 330 amino acids. The DNA-binding domain (DBD) of LTTR proteins is an N-terminal winged-helix-turn-helix (HTH) domain that is typical for HTH transcriptional repressors, in contrast to HTH transcriptional activators (non-LTTRs) that commonly encode a C-terminal HTH DNA-binding domain.[335] The HTH DNA-binding domain of LTTRs is 20–90 amino acids removed from the N-terminus. The C-terminus domain of the LTTR family is the binding domain for a regulatory coinducer (or alternatively, the “effector”, the term commonly used with respect to AmpR) small-molecule

structure. The effector-binding domain (EBD) is in turn composed of two subdomains, termed respectively the region of difference 1 (RD1) and region of difference 2 (RD2) subdomains. This subdomain nomenclature derives from the sequence differences among various coinducer binding domains, as these are less highly conserved compared to the HTH DNA-binding domains. The RD1 and RD2 subdomains each arrange in Rossmann-like folds (nine α-helices and nine β-strands) to form a cavity, the coinducer binding cleft, at their interface.[336] The RD1 and RD2 subdomains connect by a hinge of antiparallel β-strands that cross at the base of the binding cavity. The coinducer-binding cleft typically spans between residues 95 and 210 in LTTR proteins.[337–339] AmpR fits seamlessly into this classical LTTR architecture. Its N-terminal DNA-binding domain spans residues 1–67, and its C-terminal effector-binding domain spans residues 83–296. The AmpR protein of the clinically important *P. aeruginosa* has notable sequence homology to the better-studied *C. freundii* AmpR (58%) and *Enterobacter cloacae* AmpR (62%) proteins.[340]

5.1. Formation of a Large Assembly Is Required for Gene Regulation

The structures of 11 LTTR proteins are known to date. Ten structures assemble as tetramers (the exception is the octameric CrgA LTTR).[341] Each of the ten other full-length LTTR structures [AphB (3SZP),[342] ArgP (3ISP),[343] BenM (3K1N),[344] CbnR (1IXC),[345] CrgA (3HHG),[341] DntR (5AE5),[346] OxyR (4 × 6G),[347] TsaR (3FXR),[348] unknown (2ESN), and unknown (3FZV)] have a dimer of dimers (that is, tetrameric) assembly. The tetramer interface between each dimer is designated the α10–α10 region.[344] Three models, each with structural support, have been proposed to explain how the neighboring effector-binding domains interact within the tetramer (Figure 7).[344] Scheme I (Figure 7a) shows the CbnR LTTR wherein the dimer–dimer interaction is offset, resulting in a weak α10–α10 interaction. Scheme II (Figure 7b) shows the full-length structural arrangement of the ArgP, TsaR, and (the undesignated LTTR) 2ESN. There is no interaction between the α10–α10 interface for these three. Subtle, but potentially significant, difference exists within the scheme II classification with regard to the positioning of the DNA-binding domain. ArgP binds linear DNA, whereas 2ESN binds bent DNA. CrgA shows a scheme II-type formation with respect to its coinducer binding domains but with structural divergence as a result of a unique angular relationship between the coinducer domain and the linker-helix to the DNA-binding domain, which allow protein surface interactions that promote an octameric arrangement. Notwithstanding this variance, each scheme II structure reveals a broad gap between the two dimeric components of the full-length structures, resulting in no interactions between the α10–α10 regions. In contrast to the Scheme II LTTRs, Scheme III (Figure 7c) shows extensive interactions between the α10–α10 regions, as exemplified by the undesignated LTTR, 3FZV. This protein displays extensive protein surface contact at the dimer of dimers interface.

No full-length AmpR structure has been solved. Small-angle X-ray scattering (SAXS) analysis of the *C. freundii* AmpR protein led to the proposal that the structure of AmpR closely resembles that of ArgP, a Scheme II LTTR.[312,343] Structures of the isolated (that is, separated from its DNA-binding domain) effector-binding domains of both *C. freundii* and *P. aeruginosa* AmpR are homodimers, and the full-length *C. freundii* AmpR protein by mass spectrometry is a tetramer.[311,312,317] Identical homodimer interactions for each dimer of the

tetramer, as seen crystallographically for the dimeric effector domains themselves, are presumed.

5.2. Regulation of the Amp Operon

A divergent arrangement of the genes within the LTTR operon is a hallmark. This arrangement bestows an evolutionary advantage, as it imparts recombinant stability and minimizes genetic rearrangement. This stability arises in part from the internalization of the promoters between the genes, enabling regulation of the operon by a single transcriptional regulatory protein.[349] In many Gram-negative bacteria (including *C. freundii* and *P. aeruginosa),* the *ampR* gene is positioned upstream of the *ampC* gene.[350] In *P. aeruginosa*, the intercistronic regions (IR) between the *ampR* and *ampC* genes is 148 base pairs.[32,303] This gene motif (*ampR*-IR-*ampC*) is also conserved in other clinically important pathogens, including *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *E. cloacae*.[27,351,352] In Gram-negative bacteria that lack *ampR* (such as *E. coli*), *ampC* is positioned downstream of the fumarate reductase (*frd*) operon.[351,353,354] In the absence of AmpR in these bacteria, AmpC is expressed constitutively, although often at exceedingly low and thus clinically insignificant levels. In bacteria that use AmpR regulation of its operon, both AmpC inducibility and hyperexpression are characteristic of highly β-lactam-resistant strains. β-Lactam resistance as a result of AmpC β-lactamase expression rarely arises in bacteria lacking AmpR.[33,355–360] Experimental induction of *ampC* expression is interpreted in term of three levels: AmpR-activated *ampC* $\gg$ low-level constitutive (non-AmpR regulated) expression of *ampC* > AmpR-repressed *ampC*.

5.3. Sliding-Dimer Model

The HTH motif of the DNA-binding domain of LTTRs is conserved not just in all LTTRs, but in 95% of all prokaryotic DNA-binding proteins (the remaining 5% comprise the helix–loop–helix, zinc finger, and β-sheet-antiparallel domains).[361–363] The LTTR HTH slots into the major groove of DNA (11.6 Å width by 8.5 Å depth) in an interaction determined by DNA-sequence recognition. Hence, each monomer of the dimeric DNA-binding domains of the LTTR tetramer binds one-half-site of the symmetry-related DNA.[362–365] LTTR binding at two positions to the divergently transcribed operon blocks gene transcription. Broadly speaking (as the precise binding position of each LTTR varies), LTTRs bind at the site between the −35 and +20 nucleotides relative to the transcriptional start site (termed the activation-binding sites, ABS′ or ABS″) and at a second site further upstream (termed the repression-binding site, RBS) (Figure 8). Transcription of the *ampC* gene in Gram-negative rod-shaped bacteria is performed by the "housekeeping" sigma factor 70 (σ^{70}, RpoD). The characteristic polymerase-binding site of σ^{70} is centered at 10 and 35 nucleotide base pairs upstream of the transcriptional start site (−10 and −35). In the repressed state, AmpR is believed to exclude σ^{70} transcription by blocking the σ^{70} polymerase-binding site. This block is believed to result from the positioning of two DNA-binding domains at the *ampC* polymerase-binding site (−10 and −35) with low affinity, while the other two DNA-binding domains of the AmpR tetramer bind to the high-affinity RBS positioned in the AmpR "box". Accordingly, the −10 and −35 AmpR binding sites are named the low-affinity ABS's, or ABS′ and ABS″, respectively. A "sliding-dimer mechanism" supersedes the scheme I–III models with its proposal that LTTRs undergo a dramatic conformational change, from a

compact form when repressed to an expanded form when activated (derepressed) (Figure 8).[348,366] This mechanism posits that the previously assigned Scheme I LTTRs represent the compact repressed conformation, and the previously assigned Scheme II LTTRs represent the expanded activated conformation.[346] The homeostatic state of an LTTR in the repressed form binds to the RBS and ABS′ (Figure 8). Upon activation, the two DNA-binding domains bound to the ABS′ slide upstream to the ABS″ to allow gene transcription, while at the same time the binding of the two DNA-binding domains to the RBS are unperturbed (Figure 8). One LTTR structure, that of DNTR, may exemplify these two distinct conformational states. Effector binding transforms DNTR from a compact state (gene repression) to an extended ("open quaternary") conformation (Figure 8).[346] Biophysical experiments (including SAX data) support multiple conformational states for LTTRs and give credibility to the sliding-dimer model.[329,343,346,347,367,368] Further discussion of the DNA complex with the DNA-binding domain interaction of the LysR regulators is provided by Koentjoro et al. in the context of a recent crystal structure.[369] In the absence of X-ray structure evidence for AmpR, all models remain plausible. For instance, the AmpR tetramer may disassociate from the DNA or disassemble entirely concurrent with activator binding, thereby alleviating the operon of AmpR-mediated repression. Nonetheless, the "sliding-dimer mechanism" for LTTR regulation is congruent with all existing AmpR data available.

5.4. Effectors Trigger Conformational Rearrangement

The companion question with respect to the dynamic control of LTTR function is the structural identities of its specific coinducer(s) and how these entities when bound to the coinducer-binding domain of the LTTR determine the conformational state of the full-length LTTR. Given the profound challenges to the structural study of full-length LTTRs, numerous studies have explored this question with the coinducer domains separated from the full-length LTTR. The separated coinducer domains generally have much better aqueous solubility, and thus are much more easily studied experimentally. Nonetheless, these structural studies have yet to provide a definitive answer with respect to this conformational relationship. Indeed, recent studies examining the conformational change that accompanies the binding of a coinducer to its coinducer-binding domain can be interpreted in terms of a plurality of conformational mechanisms used by LTTR to control gene regulation. Quite possibly, the alternate conformational states that characterize a repressed LTTR and an activated LTTR, may be unique to each LTTR. While the central effector-binding site between RDI and RDII is widely conserved, some LTTRs possess secondary effector-binding sites of lower structural conservation. An extreme example of this phenomenon is seen in the full-length structure of TsaR, an LTTR that coordinates degradation of *p*-toluenesulfonate as the carbon source for *Comamonas testosterone*.[370,371] The structure of TSAR (3FXU) was solved in complex with its natural inducer, *p*-toluenesulfonate. In addition to residing in the primary coinducer-binding pocket, the coinducer was also present at several secondary-binding sites located around the entire protein surface, including the β-sheets comprising the winged portion of the DNA-binding domain.[348] The possibility that this secondary binding is nonspecific (that is, an artifact of the crystallization) cannot be excluded. Crystallographic evidence for specific binding of the coinducer to the primary coinducer binding site is provided by the structures for *cis,cis*-muconate binding to the CatM (2F7C) and to the BenM (2F78) LTTRs. However, overlay of these two structures reveals a

secondary-binding site on BenM that is occupied by benzoate. The benzoate-binding site notably was not seen in the structure of CatM.[372] The possibility that the benzoate binding was an artifact of crystallization was refuted by in vitro transcription studies that showed that both *cis,cis*-muconate and benzoate acted as BenM coinducers.[368] The crystal structure of DntR, a protein that regulates oxidative degradation of 2,4-dinitrotoluene, gives further evidence of functional secondary coinducer-binding sites.[346] The coinducer-bound structure of DNTR revealed the presence of salicylate both at the primary coinducer-binding site and also at a secondary-binding site. Binding of salicylate to its secondary coinducer-binding site of the DntR LTTR sufficed to trigger a dramatic conformational change. Importantly, the secondary-binding sites seen for AphB, BenM, and DntR are in different locations. This observation supports the idea that dual regulation may be a common event within the LTTR family.[366,372]

6. CONCLUSION

The search for the answers as to how Gram-negative bacteria use the AmpR system to mobilize resistance mechanisms in response to β-lactam-dependent compromise of their cell wall is a multidecade pilgrimage. Although the central answer now has been known for more than two decades, AmpR responds to a β-lactam-induced fluctuation in the composition of the muropeptides that enter the cytoplasm for recycling, embodying this answer in terms of structural changes effected by the enzymes of a surrounding pathway has been an arduous undertaking, in all aspects of its chemistry, biochemistry, and microbiology. The pilgrimage is unfinished. Its completion may be defined by answers to two questions. What is the muropeptide structure, or structures, that transform AmpR from a repressed to a derepressed state? And how might this transformation be blocked, as a means of preserving β-lactam clinical efficacy?

Although this conclusion cannot offer a scheme identifying the role of the muropeptide that transforms AmpR so as to predispose the *ampC* gene to transcription, our grasp of the pathway gives circumstantial evidence toward such identity. The essential structural pairing must be that of an anhMurNAc saccharide possessing a peptide stem.[46,272] The requirements for LT involvement in *ampC* induction[373] and the structural requirement for AmpG passage to the cytoplasm[277] sets the anhMurNAc structural character, and the prevalence of AmpD incapacitation (as a mutational means to forestall removal of the stem) in order to secure high-level *ampC* expression defines the second structural character. Additional structural clarity is possible. In *P. aeruginosa,* the contributing role of loss of PBP4 function[291,296] strongly implicates this PBP as the β-lactam sentinel in this bacterium. In the absence of a β-lactam antibiotic, the carboxypeptidase/endopeptidase catalytic activities of PBP4 ensure that the muropeptides returned to the cytoplasm lack a pentapeptide stem. In the presence of a β-lactam, PBP4 is inactivated. Consequently, the muropeptides returned to the cytoplasm are enriched in pentapeptide stems.[374] Both the composition of the muropeptide pool in *P. aeruginosa* under β-lactam stress,[73] and direct evaluation of the effector-binding domain of AmpR with muropeptides,[317] support the anhMurNAc-pentapeptide as sufficient structure for binding to AmpR in order to affect *ampC* expression.[276] Two provisos limit further clarification of this structure (toward specific muropeptide candidate structures). The first proviso is that there is one additional anhMurNAc candidate (in addition to anhMurNAc

itself) that fulfills this generic structural criterion. The incoming muropeptides to the cytoplasm are as GlcNAc-anhMurNAc(stem) disaccharides. Upon PBP4 inactivation, the stem of these disaccharides will be pentapeptides. Nonetheless, the several studies that show that NagZ inhibition improves incrementally β-lactam efficacy against *P. aeruginosa* suggest that the GlcNAc-anhMurNAc-pentapeptide disaccharide may be less effective than anhMurNAc-pentapeptide for AmpR derepression.[30,76,78,81,307,308] The counterbalancing structure to these derepressing effector structures may also possess the pentapeptide stem. The culminating intermediate in the cytoplasm for PG biosynthesis, UDP-MurNAc-pentapeptide (**4**, Figure 6), accumulates under β-lactam stress.[310,375] The coincidence of this accumulation with the observation that the absence of FtsZ prevents *ampC* induction (hence, implicating the process of septal PG synthesis as essential to *ampC* expression)[375,376] is consistent with this assignment for UDP-MurNAc-pentapeptide.

The second proviso is the difficulty in generalizing the circumstances for the operation of AmpR in *P. aeruginosa* as a nexus for β-lactam resistance, to other bacteria. As noted previously, *E. coli* lacks AmpR, and while *S. maltophilia* has AmpR, all evidence points to fundamental contrasts in comparison to *P. aeruginosa* with respect to its pathways for peptidoglycan recycling and AmpR-dependent β-lactamase(s) expression.[320,322] The first bacteria used for the study of the AmpR pathway were *E. cloacae* and *C. freundii*.[27] In these seminal studies, UDP-MurNAc-pentapeptide (**4**, Figure 6) was suggested to function as the AmpR repressor consistent with a decrease in its cytoplasmic concentration during β-lactam stress.[310,314] The possibility of the pentapeptide stem structure as a feature common to both AmpR repressor structures (UDP-MurNAc-pentapeptide **4**, Figure 6) and derepressor structures (such anhMurNAc-pentapeptide, **2c**) is discussed cogently in connection with the most recent *C. freundii* AmpR structural studies.[311,312] These same studies (and others in *P. aeruginosa*)[73,317] would appear to exclude a role for 1,6-anhMurNAc-tripeptide (**2a**, Figure 6) in AmpR regulation. Whether AmpR is regulated by the replete structural guise of the pentapeptide, or by a subtler competition in the cytoplasm for effector binding to its effector domain, remains an unsolved question. For example, a decrease in the concentration of **4** may allow its displacement from the effector-binding site of AmpR by the pentapeptide motif of either **1c** or **2c** (Figure 6). Accordingly, it is plausible (as was proposed originally) that AmpR activation results from a concentration-dependent displacement mechanism wherein loss of PBP function by the β-lactam effects simultaneously an increase in the cytoplasmic concentration of both **1c** or **2c** so as to allow these pentapeptides to displace **4** as its own concentration decreases (Figure 6). Alternatively, AmpR may exist without a bound effector in its repressed state and derepress upon the influx of pentapeptide structures following encounter with the β-lactam antibiotic.

The second question, is the AmpR nexus now understood sufficiently to consider its exploitation to support β-lactam clinical efficacy?, is both the more relevant and the more challenging question. Notwithstanding the enigmatic character of AmpR, the identity of all other members of the Gram-negative peptidoglycan recycling and biosynthetic pathways are known. Almost without exception, their functions are also known, and where we are not completely certain as to precise function within the pathways, for example, is PBP4 the solitary PBP sentinel used by *P. aeruginosa* to detect β-lactams?, and is the only result of its

inactivation a disproportionate influx of GlcNAc-MurNAc-pentapeptide muropeptides to the cytoplasm?, the surmises that may be made are sufficiently robust as to direct credible experimentation. Some conclusions are facile. The absolute importance of designing β-lactam structures that are selective for the HMM PBPs, and do not inactivate LMM PBPs, is a now nearly timeless medicinal chemistry touchstone.[377,378] It is not by accident that the antipseudomonal β-lactams (such as piperacillin and ceftazidime) possess this character. Likewise, inhibition of AmpD would be undesirable. The outstanding vulnerable proteins in the cytoplasm include the Mur pathway, NagZ, and AmpR; AmpG in the membrane; and in the periplasm, the lytic transglycosylases. In all of these latter examples, the conception is a synergistic combination of a new molecular entity, targeted to one of these proteins, and combined with a β-lactam antibiotic. Although the extraordinary value of combination antibacterial therapy has proven history as exemplified by the β-lactam/β-lactamase inhibitor combinations,[379,380] the challenge to devising an appropriate combination[381] capable of clinical efficacy cannot be underestimated.[382–384] Nonetheless, there can be little doubt that the inexorable expansion of multidrug-resistant bacteria will demand consideration of combination antibacterial therapy.

The obvious targets for pairing with the β-lactam antibiotic are discussed succinctly. The possible virtue of shutting down PG synthesis in *P. aeruginosa* by the depletion of the MurNAc pool, as a result of simultaneous inhibition of the Mur pathway (such as with fosfomycin)[385,386] and of PG recycling, was noted previously in this review.[113] Initial data with fosfomycin using *P. aeruginosa* mutants that are defective for recycling confirm this approach as promising.[83,114] Among the recycling-impaired mutants that demonstrated β-lactam-fosfomycin synergy was one with loss of NagZ activity. Moreover, synergy was preserved in PAO1 wild-type *P. aeruginosa* in the presence of high concentrations of a poor NagZ inhibitor. As none of the known NagZ inhibitors is potent (submicromolar), these observations support strongly efforts toward improved inhibitor potency. Muropeptide passage through AmpG is required for *ampC* induction.[272,280] A final cytoplasmic target is AmpR itself. Given the ease with which the effector domain of AmpR may be studied (in sharp distinction to the full-length protein) and with recognition of its seeming preference for engaging pentapeptide stems, there is reason to believe that the structure-based design of strongly binding pentapeptide analogs, or pentapeptides modified for affinity labeling, could be accomplished successfully. AmpG is required for muropeptide entry. A recent high-throughput screening effort against AmpG identified a series of AmpG inhibitors. Although all had modest potency, all are amenable to medicinal chemistry optimization.[387] A possible caution against AmpG as an antibacterial target is the possibility that its inhibition would result in a spillover of the accumulating muropeptides from the periplasm to the medium,[249] eliciting an inflammatory response as occurs with *N. gonorrheae* and *Bordetella pertussis.*[388,389] Nonetheless, it is evident that the full breadth of the roles of AmpG in muropeptide recycling, β-lactam susceptibility, and biofilm formation has yet to be determined.[390] A complementary approach to AmpG inhibition, given that this permease is driven by the proton-motive force (PMF),[277] is inhibition of this force. Indeed, this may be the mechanism of the structures identified through screening.[387] As PMF attenuation could disrupt additionally active efflux and flagellar motion, the potential value of PMF as a means for antibiotic synergy is recognized. [391–394]

The obvious periplasmic target(s) to attain β-lactam (and quite possibly other antibiotics) synergy are the lytic transglycosylases. The appearance within a bacterium of adjacent gene clusters[395] encoding the biosynthesis of an LT inhibitor (bulgecin)[396,397] and of a β-lactam antibiotic[398] implicitly affirms the functional synergy observed following the initial discovery of the bulgecins.[399] A strategy for LT inhibition faces two key barriers. Gram-negative bacteria possess a family of LTs, few of which have assigned functional roles. Nonetheless, it is evident from studies using LT gene knockouts in both *P. aeruginosa*[292,400] and *S. oneidensis*[401] that inhibitor selectivity within the family may be required. The second limitation is the absence of an appropriate high-throughput assay for the LT enzymes to measure both inhibitor selectivity and inhibitor potency. Last, bulgecin (an iminosaccharide-class inhibitor of LTs) is not at all easily sourced from its producing microorganism, and apart from bulgecin, there are exceedingly few other reported LT inhibitors. The synthesis of a practical yet potent LT inhibitor will likely require extensive medicinal chemistry effort. There are, however, exemplary protein structural data for numerous LT family members, as guidance in support of medicinal chemistry structure-based design.

A final discussion point remains. Understanding the genetic ensemble that confers advantage to a bacterium as a pathogen is extraordinarily difficult. For example, a dominant clonal strain of pathogenic *P. aeruginosa* will possess genes permissive for high level expression of AmpC (often, by AmpD loss, augmented by resistance mutations in AmpC itself) as well as for additional β-lactamases.[402,403] These events reflect directly the prominence of the β-lactams in *P. aeruginosa* chemotherapy. A facile conclusion in this circumstance might be that interfering with PG recycling in such a bacterium might not confer advantage. This conclusion awaits, however, experimental validation. Some plasmids with the *ampC* gene retain key proteins of the AmpR system.[404,405] Although genetic incapacitation of AmpD is frequently associated with high level AmpC expression, loss of function in *P. aeruginosa* of all three of its AmpD enzymes results in a significant loss of fitness.[25] The pathway confluence of the three AmpD enzymes in peptidoglycan recycling and in *ampC* expression is not straightforward.[93] This circumstance may well hold with respect to the other enzymes suggested above as possible points of intervention, to attain β-lactam synergy. What is evident at this point in time of our understanding of the relationship between peptidoglycan recycling, and β-lactam efficacy, is that the framework of the pathways is now sufficiently well-understood so as to enable credible experimental evaluation.

ACKNOWLEDGMENTS

The authors acknowledge the financial support of the National Institutes of Health (GM61629, S.M.) and the award of a predoctoral fellowship (D.A.D.) from the Chemistry-Biochemistry-Biology Interface Program (NIH Training Grant T32GM075762) and the ECK Institute of Global Health at the University of Notre Dame.

Biographies

David A. Dik is an alumnus of Hillsdale College. He is now working toward a doctoral degree in Chemical Biology from the University of Notre Dame under the mentorship of Prof. Shahriar Mobashery. He studies biochemical-signaling pathways that manifest bacterial resistance to antibiotics, enzymes repurposed by bacteria to engage resistance, and novel strategies to block resistance.

Jed F. Fisher has returned to the compelling enzymology of the resistance enzymes of the pathogenic bacteria four decades after defining (with Jeremy Knowles) the acyl-enzyme mechanism for catalysis by and the inactivation of the class A β-lactamases.

Shahriar Mobashery received his training from undergraduate to postdoctoral studies at the University of Southern California, the University of Chicago, and the Rockefeller University, respectively. He holds the Navari Chair in the Department of Chemistry and Biochemistry at the University of Notre Dame.

ABBREVIATIONS

PG	peptidoglycan
anhMurNAc	anhydro-*N*-acetylmuramic
GlcNAc	*N*-acetylglucosamine
MurNAc	*N*-acetylmuramic
PBP	penicillin-binding protein
LT	lytic transglycosylase

REFERENCES

(1). Höltje JV Growth of the Stress-bearing and Shape-maintaining Murein Sacculus of *Escherichia coli*. Microbiol. Mol. Biol. Rev 1998, 62, 181–203. [PubMed: 9529891]

(2). Zhao H; Patel V; Helmann JD; Dörr T Don't Let Sleeping Dogmas lie: New Views of Peptidoglycan Synthesis and its Regulation. Mol. Microbiol 2017, 106, 847–860. [PubMed: 28975672]

(3). van der Ploeg R; Verheul J; Vischer NOE; Alexeeva S; Hoogendoorn E; Postma M; Banzhaf M; Vollmer W; den Blaauwen T Colocalization and Interaction Between Elongasome and Divisome During a Preparative Cell Division Phase in *Escherichia coli*. Mol. Microbiol 2013, 87, 1074–1087. [PubMed: 23387922]

(4). Labischinski H; Goodell EW; Goodell A; Hochberg ML Direct Proof of a "More-than-single-layered" Peptidoglycan Architecture of *Escherichia coli* W7: A Neutron Small-angle Sattering Study. J. Bacteriol 1991, 173, 751–756. [PubMed: 1987162]

(5). Johnson JW; Fisher JF; Mobashery S Bacterial Cell-Wall Recycling. Ann. N. Y. Acad. Sci 2013, 1277, 54–75. [PubMed: 23163477]

(6). Sutcliffe IC A Phylum Level Perspective on Bacterial Cell Envelope Architecture. Trends Microbiol. 2010, 18, 464–470. [PubMed: 20637628]

(7). Davies JA; Anderson GK; Beveridge TJ; Clark HC Chemical Mechanism of the Gram stain and Synthesis of a New Electron-opaque Marker for Electron Microscopy which Rplaces the Iodine Mordant of the Stain. J. Bacteriol 1983, 156, 837–845. [PubMed: 6195147]

(8). Beveridge TJ Use of the Gram Stain in Microbiology. Biotech. Hstochem 2001, 76, 111–118.

(9). O'Toole GA Classic Spotlight: How the Gram stain works. J. Bacteriol 2016, 198, 3128. [PubMed: 27815540]

(10). Goffin C; Ghuysen JM Multimodular Penicillin-binding Proteins: An Enigmatic Family of Orthologs and Paralogs. Microbiol. Mol. Biol. Rev 1998, 62, 1079–1093. [PubMed: 9841666]

(11). Sauvage E; Kerff F; Terrak M; Ayala JA; Charlier P The Penicillin-binding Proteins: Structure and Role in Peptidoglycan Biosynthesis. FEMS Microbiol. Rev 2008, 32, 234–258. [PubMed: 18266856]

(12). Zapun A; Contreras-Martel C; Vernet T Penicillin-binding Proteins and β-Lactam Resistance. FEMS Microbiol. Rev 2008, 32, 361–385. [PubMed: 18248419]

(13). Zervosen A; Sauvage E; Frère JM; Charlier P; Luxen A Development of New Drugs for an Old Parget: the Penicillin-binding Proteins. Molecules 2012, 17, 12478–12505. [PubMed: 23095893]

(14). Krishnamoorthy G; Leus IV; Weeks JW; Wolloscheck D; Rybenkov VV; Zgurskaya HI Synergy Between Active Efflux and Outer Membrane Diffusion Defines Rules of Antibiotic Permeation into Gram-negative Bacteria. mBio 2017, 8, No. e01172-17.

(15). Rodríguez-Baño J; Gutiérrez-Gutiérrez B; Machuca I; Pascual A Treatment of Infections Caused by Extended-spectrum-β-Lactamase-, AmpC-, and Carbapenemase-producing *Enterobacteriaceae*. Clin. Microbiol. Rev 2018, 31, No. e00079-17.

(16). Wouters J; Bauvois C Structures of Class C β-Lactamases and Perspectives in Drug Design In Beta-Lactamases; Frère JM, Ed.; Nova Science Publishers, Inc: New York, 2012; pp 79–101.

(17). Skalweit MJ; Li M; Taracila MA Effect of Asparagine Substitutions in the YXN Loop of a Class C β-Lactamase of *Acinetobacter baumannii* on Substrate and Inhibitor Kinetics. Antimicrob. Agents Chemother 2015, 59, 1472–1477. [PubMed: 25534745]

(18). Powers RA Structural and Functional Aspects of Extended-spectrum AmpC Cephalosporinases. Curr. Drug Targets 2016, 17, 1051–1060. [PubMed: 26073861]

(19). Madec JY; Haenni M; Nordmann P; Poirel L Extended-spectrum β-Lactamase/AmpC- and Carbapenemase-producing *Enterobacteriaceae* in Animals: A Threat for Humans. Clin. Microbiol. Infect 2017, 23, 826–833. [PubMed: 28143782]

(20). Meroueh SO; Minasov G; Lee W; Shoichet BK; Mobashery S Structural Aspects for Evolution of β-Lactamases from Penicillin-binding Proteins. J. Am. Chem. Soc 2003, 125, 9612–9618. [PubMed: 12904027]

(21). Chen Y; Minasov G; Roth TA; Prati F; Shoichet BK The Deacylation Mechanism of AmpC β-Lactamase at Ultrahigh Resolution. J. Am. Chem. Soc 2006, 128, 2970–2976. [PubMed: 16506777]

(22). Chen Y; McReynolds A; Shoichet BK Re-examining the Role of Lys67 in Class C β-Lactamase Catalysis. Protein Sci. 2009, 18, 662–669. [PubMed: 19241376]

(23). Tripathi R; Nair NN Mechanism of the Acyl-enzyme Complex Formation from the Henry-Michaelis Complex of class C β-Lactamases with β-Lactam Antibiotics. J. Am. Chem. Soc 2013, 135, 14679–14690. [PubMed: 24010547]

(24). Awasthi S; Gupta S; Tripathi R; Nair NN Mechanism and Kinetics of Aztreonam Hydrolysis Catalyzed by Class-C β-Lactamase: A Temperature Accelerated Sliced Sampling Study. J. Phys. Chem. B 2018, 111, 4299–4308.

(25). Pérez-Gallego M; Torrens G; Castillo-Vera J; Moya B; Zamorano L; Cabot G; Hultenby K; Albertí S; Mellroth P; Henriques-Normark B; Normark S; Oliver A; Juan C Impact of AmpC Derepression on Fitness and Virulence: The Mechanism or the Pathway? mBio 2016, 7, No. e01783-16.

(26). Torrens G; Pérez-Gallego M; Moya B; Munar-Bestard M; Zamorano L; Cabot G; Blázquez J; Ayala JA; Oliver A; Juan C Targeting the Permeability Barrier and Peptidoglycan Recycling Pathways to Disarm *Pseudomonas aeruginosa* Against the Innate Immune System. PLoS One 2017, 12, e0181932. [PubMed: 28742861]

(27). Lindberg F; Westman L; Normark S Regulatory Components in *Citrobacter freundii ampC* β-Lactamase Induction. Proc. Natl. Acad. Sci. U. S. A 1985, 82, 4620–4624. [PubMed: 2991883]

(28). Mayer C Bacterial Cell Wall Recycling. eLS 2012, DOI: 10.1002/9780470015902.a0021974.

(29). Fisher JF; Mobashery S The Sentinel Role of Peptidoglycan Recycling in the β-Lactam Resistance of the Gram-negative *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Bioorg. Chem 2014, 56, 41–48. [PubMed: 24955547]

(30). Juan C; Torrens G; González-Nicolau M; Oliver A Diversity and Regulation of Intrinsic β-Lactamases from Non-fermenting and Other Gram-negative Opportunistic Pathogens. FEMS Microbiol. Rev 2017, 41, 781–815. [PubMed: 29029112]

(31). Dhar S; Kumari H; Balasubramanian D; Mathee K Cell-wall Recycling and Synthesis in *Escherichia coli* and *Pseudomonas aeruginosa*—Their Role in the Development of Resistance. J. Med. Microbiol 2018, 67, 1–21. [PubMed: 29185941]

(32). Kong KF; Jayawardena SR; Indulkar SD; Del Puerto A; Koh CL; Hoiby N; Mathee K *Pseudomonas aeruginosa* AmpR is a Global Transcriptional Factor that Regulates Expression of AmpC and PoxB β-Lactamases, Proteases, Quorum Sensing, and Other Virulence Factors. Antimicrob. Agents Chemother 2005, 49, 4567–4575. [PubMed: 16251297]

(33). Gifford DR; Furió V; Papkou A; Vogwill T; Oliver A; MacLean RC Identifying and Exploiting Genes that Potentiate the Evolution of Antibiotic Resistance. Nat. Ecol. Evol 2018, DOI: 10.1038/s41559-018-0547-x.

(34). Moynihan PJ; Sychantha D; Clarke AJ Chemical Biology of Peptidoglycan Acetylation and Deacetylation. Bioorg. Chem 2014, 54, 44–50. [PubMed: 24769153]

(35). Chang JD; Foster EE; Wallace AG; Kim SJ Peptidoglycan *O*-Acetylation Increases in Response to Vancomycin Treatment in Vancomycin-resistant *Enterococcus faecalis*. Sci. Rep 2017, 7, 46500. [PubMed: 28406232]

(36). Yahashiri A; Jorgenson MA; Weiss DS The SPOR Domain: A Widely-conserved Peptidoglycan Binding Domain that Targets Proteins to the Site of Cell Division. J. Bacteriol 2017, 199, No. e00118-17.

(37). Young KD Bacterial Shape: Two-dimensional Questions and Possibilities. Annu. Rev. Microbiol 2010, 64, 223–240. [PubMed: 20825347]

(38). Cesar S; Huang KC Thinking Big: The Tunability of Bacterial Cell Size. FEMS Microbiol. Rev 2017, 41, 672–678. [PubMed: 28961755]

(39). Willis L; Huang KC Sizing up the Bacterial Cell Cycle. Nat. Rev. Microbiol 2017, 15, 606–620. [PubMed: 28804128]

(40). Caccamo PD; Brun YV The Molecular Basis of Nncanonical Bacterial Morphology. Trends Microbiol. 2018, 26, 191–208. [PubMed: 29056293]

(41). Park JT; Uehara T How Bacteria Consume their own Exoskeletons (Turnover and Recycling of Cell Wall Peptidoglycan). Microbiol. Mol. Biol. Rev 2008, 72, 211–227. [PubMed: 18535144]

(42). Borisova M; Gisin J; Mayer C The *N*-Acetylmuramic acid 6-Phosphate Phosphatase MupP Completes the *Pseudomonas* Peptidoglycan Recycling Pathway Leading to Intrinsic Fosfomycin Resistance. mBio 2017, 8, No e00092–17. [PubMed: 28351914]

(43). Fumeaux C; Bernhardt TG Identification of MupP as a new Peptidoglycan Recycling Factor and Antibiotic Resistance Determinant in *Pseudomonas aeruginosa*. mBio 2017, 8, No. e00102-17.

(44). Boucher HW; Talbot GH; Bradley JS; Edwards JE; Gilbert D; Rice LB; Scheld M; Spellberg B; Bartlett J Bad Bugs, No Drugs: no ESKAPE! An Update from the Infectious Diseases Society of America. Clin. Infect. Dis 2009, 48, 1–12. [PubMed: 19035777]

(45). Pendleton JN; Gorman SP; Gilmore BF Clinical Relevance of the ESKAPE Pathogens. Expert Rev. Anti-Infect. Ther 2013, 11, 297–308. [PubMed: 23458769]

(46). Park JT The Convergence of Murein Recycling Research with β-Lactamase Research. Microb. Drug Resist 1996, 2, 105–112. [PubMed: 9158731]

(47). van Heijenoort J Recent Advances in the Formation of the Bacterial Peptidoglycan Monomer Unit. Nat. Prod. Rep 2001, 18, 503–519. [PubMed: 11699883]

(48). van Heijenoort J Lipid Intermediates in the Biosynthesis of Bacterial Peptidoglycan. Microbiol. Mol. Biol. Rev 2007, 71, 620–635. [PubMed: 18063720]

(49). Bouhss A; Trunkfield AE; Bugg TD; Mengin-Lecreulx D The Biosynthesis of Peptidoglycan Lipid-linked Intermediates. FEMS Microbiol. Rev 2008, 32, 208–233. [PubMed: 18081839]

(50). Teo ACK; Roper DI Core Steps of Membrane-bound Peptidoglycan Biosynthesis: Recent Advances, Insight and Opportunities. Antibiotics 2015, 4, 495–520. [PubMed: 27025638]

(51). Liu Y; Breukink E The Membrane Steps of Bacterial Cell Wall Synthesis as Antibiotic Targets. Antibiotics 2016, 5, 28.

(52). Kotnik M; Anderluh PS; Prezelj A Development of Novel Inhibitors Targeting Intracellular Steps of Peptidoglycan Biosynthesis. Curr. Pharm. Des 2007, 13, 2283–2309. [PubMed: 17692001]

(53). Barreteau H; Kovac A; Boniface A; Sova M; Gobec S; Blanot D Cytoplasmic Steps of Peptidoglycan Biosynthesis. FEMS Microbiol. Rev 2008, 32, 168–172. [PubMed: 18266853]

(54). Bugg TDH; Braddick D; Dowson CG; Roper DI Bacterial Cell Wall Assembly: Still an Attractive Antibacterial Target. Trends Biotechnol. 2011, 29, 167–173. [PubMed: 21232809]

(55). Hrast M; Sosič I; Šink R; Gobec S Inhibitors of the Peptidoglycan Biosynthesis Enzymes MurA–F. Bioorg. Chem 2014, 55, 2–15. [PubMed: 24755374]

(56). Kouidmi I; Levesque RC; Paradis-Bleau C The Biology of Mur Ligases as an Antibacterial Target. Mol. Microbiol 2014, 94, 242–253. [PubMed: 25130693]

(57). Squeglia F; Ruggiero A; Berisio R Chemistry of Peptidoglycan in *Mycobacterium tuberculosis* life style: An Off-the-wall Balance of Synthesis and Degradation. Chem. - Eur. J 2018, 24, 2533–2546. [PubMed: 28925518]

(58). Al-Dabbagh B; Henry X; El Ghachi M; Auger G; Blanot D; Parquet C; Mengin-Lecreulx D; Bouhss A Active Site Mapping of MraY, a Member of the Polyprenyl-phosphate *N*-Acetylhexosamine 1-phosphate Transferase Superfamily, Catalyzing the First Membrane Step of Peptidoglycan Biosynthesis. Biochemistry 2008, 47, 8919–8928. [PubMed: 18672909]

(59). Chung BC; Zhao J; Gillespie RA; Kwon DY; Guan Z; Hong J; Zhou P; Lee SY Crystal Structure of MraY, an Essential Membrane Enzyme for Bacterial Cell Wall Synthesis. Science 2013, 341, 1012–1016. [PubMed: 23990562]

(60). Al-Dabbagh B; Olatunji S; Crouvoisier M; El Ghachi M; Blanot D; Mengin-Lecreulx D; Bouhss A Catalytic Mechanism of MraY and WecA, two Paralogues of the Polyprenyl-phosphate *N*-acetylhexosamine 1-phosphate Transferase Superfamily. Biochimie 2016, 127, 249–257. [PubMed: 27312048]

(61). Henrich E; Ma Y; Engels I; Münch D; Otten C; Schneider T; Henrichfreise B; Sahl H-G; Dötsch V; Bernhard F Lipid Requirements for the Enzymatic Activity of MraY Translocases and *in Vitro* Reconstitution of Lipid II Synthesis Pathway. J. Biol. Chem 2016, 291, 2535–2546. [PubMed: 26620564]

(62). Koppermann S; Ducho C Natural Products at Work: Structural Insights into Inhibition of the Bacterial Membrane Protein MraY. Angew. Chem., Int. Ed 2016, 55, 11722–11724.

(63). Hakulinen JK; Hering J; Brändén G; Chen H; Snijder A; Ek M; Johansson P MraY-antibiotic Complex Reveals Details of Tunicamycin Mode of Action. Nat. Chem. Biol 2017, 13, 265–267. [PubMed: 28068312]

(64). Liu Y; Rodrigues JP; Bonvin AM; Zaal EA; Berkers CR; Heger M; Gawarecka K; Swiezewska E; Breukink E; Egmond MR New Insight into the Catalytic Mechanism of Bacterial MraY from Enzyme Kinetics and Docking studies. J. Biol. Chem 2016, 291, 15057–15068. [PubMed: 27226570]

(65). Chen L; Men H; Ha S; Ye XY; Brunner L; Hu Y; Walker S Intrinsic Lipid Preferences and Kinetic Mechanism of *Escherichia coli* MurG. Biochemistry 2002, 41, 6824–6833. [PubMed: 12022887]

(66). Crouvoisier M; Auger G; Blanot D; Mengin-Lecreulx D Role of the Amino Acid Invariants in the Active Site of MurG as Evaluated by Site-Directed Mutagenesis. Biochimie 2007, 89, 1498–1508. [PubMed: 17692452]

(67). Sham LT; Butler EK; Lebar MD; Kahne D; Bernhardt TG; Ruiz N Bacterial Cell Wall. MurJ is the Flippase of Lipid-linked Precursors for Peptidoglycan Biogenesis. Science 2014, 345, 220–222. [PubMed: 25013077]

(68). Young KD Microbiology. A Flipping Cell Wall Ferry. Science 2014, 345, 139–140. [PubMed: 25013047]

(69). Ruiz N Lipid Flippases for Bacterial Peptidoglycan Biosynthesis. Lipid Insights 2015, 8, 21–31. [PubMed: 26792999]

(70). Ruiz N Filling Holes in Peptidoglycan Biogenesis of *Escherichia coli*. Curr. Opin. Microbiol 2016, 34, 1–6. [PubMed: 27449418]

(71). Kuk ACY; Mashalidis EH; Lee SY Crystal Structure of the MOP Flippase MurJ in an Inward-facing Conformation. Nat. Struct. Mol. Biol 2017, 24, 171–176. [PubMed: 28024149]

(72). Leclercq S; Derouaux A; Olatunji S; Fraipont C; Egan AJ; Vollmer W; Breukink E; Terrak M Interplay between Penicillin-binding Proteins and SEDS Proteins Promotes Bacterial Cell Wall Synthesis. Sci Rep. 2017, 7, 43306. [PubMed: 28233869]

(73). Lee M; Dhar S; De Benedetti S; Hesek D; Boggess B; Blázquez B; Mathee K; Mobashery S Muropeptides in *Pseudomonas aeruginosa* and Their Role as Elicitors of β-Lactam-Antibiotic Resistance. Angew. Chem., Int. Ed 2016, 55, 6882–6886.

(74). Domínguez-Gil T; Molina R; Alcorlo M; Hermoso JA Renew or Die: The Molecular Mechanisms of Peptidoglycan Recycling and Antibiotic Resistance in Gram-negative Pathogens. Drug Resist. Updates 2016, 28, 91–104.

(75). Stubbs KA; Scaffidi A; Debowski AW; Mark BL; Stick RV; Vocadlo DJ Synthesis and Use of Mechanism-based Protein-profiling Probes for Retaining β-D-Glucosaminidases Facilitate Identification of *Pseudomonas aeruginosa* NagZ. J. Am. Chem. Soc 2008, 130, 327–335. [PubMed: 18067297]

(76). Asgarali A; Stubbs KA; Oliver A; Vocadlo DJ; Mark BL Inactivation of the Glycoside Hydrolase NagZ Attenuates anti-Pseudomonal β-Lactam Resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother 2009, 53, 2274–2282. [PubMed: 19273679]

(77). Balcewich MD; Stubbs KA; He Y; James TW; Davies GJ; Vocadlo DJ; Mark BL Insight into a Strategy for Attenuating AmpC-mediated β-Lactam resistance: Structural Basis for Slective Inhibition of the Glycoside Hdrolase NagZ. Protein Sci. 2009, 18, 1541–1551. [PubMed: 19499593]

(78). Zamorano L; Reeve TM; Deng L; Juan C; Moya B; Cabot G; Vocadlo DJ; Mark BL; Oliver A NagZ Inactivation Prevents and Reverts β-Lactam Resistance, driven by AmpD and PBP4Mutations, in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother 2010, 54, 3557–3563. [PubMed: 20566764]

(79). Bacik JP; Whitworth GE; Stubbs KA; Vocadlo DJ; Mark BL Active Site Plasticity Within the Gycoside Hydrolase NagZ Underlies a Dynamic Mechanism of Substrate Distortion. Chem. Biol 2012, 19, 1471–1482. [PubMed: 23177201]

(80). Huang Y-W; Hu R-M; Lin C-W; Chung T-C; Yang T-C NagZ-Dependent and NagZ-Independent Mechanisms for β-Lactamase Expression in *Stenotrophomonas maltophilia*. Antimicrob. Agents Chemother 2012, 56, 1936–1941. [PubMed: 22252801]

(81). Yamaguchi T; Blázquez B; Hesek D; Lee MK; Llarrull LI; Boggess B; Oliver AG; Fisher JF; Mobashery S Inhibitors for Bacterial Cell-Wall Recycling. ACS Med. Chem. Lett 2012, 3, 238–242.

(82). Acebrón I; Mahasenan KV; De Benedetti S; Lee M; Artola-Recolons C; Hesek D; Wang H; Hermoso JA; Mobashery S Catalytic Cycle of the *N*-Acetylglucosaminidase NagZ from *Pseudomonas aeruginosa*. J. Am. Chem. Soc 2017, 139, 6795–6798. [PubMed: 28482153]

(83). Vadlamani G; Stubbs KA; Désiré J; Blériot Y; Vocadlo DJ; Mark BL Conformational Flexibility of the Glycosidase NagZ Allows It to Bind Structurally Diverse Inhibitors to Suppress β-Lactam Antibiotic Resistance. Protein Sci. 2017, 26, 1161–1170. [PubMed: 28370529]

(84). Templin MF; Ursinus A; Höltje JV A Defect in Cell Wall Recycling Triggers Autolysis During the Stationary Growth Phase of *Escherichia coli*. EMBO J. 1999, 18, 4108–4117. [PubMed: 10428950]

(85). Das D; Hervé M; Elsliger M-A; Kadam RU; Grant JC; Chiu H-J; Knuth MW; Klock HE; Miller MD; Godzik A; Lesley SA; Deacon AM; Mengin-Lecreulx D; Wilson IA Structure and Function of a Novel LD-Carboxypeptidase A Involved in Peptidoglycan Recycling. J. Bacteriol 2013, 195, 5555–5566. [PubMed: 24123814]

(86). Meyer K; Addy C; Akashi S; Roper DI; Tame JRH The Crystal Structure and Oligomeric Form of *Escherichia coli* L,D-Carboxypeptidase A. Biochem. Biophys. Res. Commun 2018, 499, 594–599. [PubMed: 29601819]

(87). Lenz JD; Hackett KT; Dillard JP A Single Dual-function Enzyme Controls the Production of Inflammatory NOD Agonist Peptidoglycan Fragments by *Neisseria gonorrhoeae*. mBio 2017, 8, No. e01464-17.

(88). Juan C; Macia MD; Gutierrez O; Vidal C; Pérez JL; Oliver A Molecular Mechanisms of β-Lactam Resistance Mediated by AmpC Hyperproduction in *Pseudomonas aeruginosa* Clinical Strains. Antimicrob. Agents Chemother 2005, 49, 4733–4738. [PubMed: 16251318]

(89). Juan C; Moya B; Pérez JL; Oliver A Stepwise Upregulation of the *Pseudomonas aeruginosa* Chromosomal Cephalosporinase Conferring High-level β-Lactam Resistance involves Three AmpD Homologues. Antimicrob. Agents Chemother 2006, 50, 1780–1787. [PubMed: 16641450]

(90). Moya B; Juan C; Albertí S; Pérez JL; Oliver A Benefit of Having Multiple ampD genes for Acquiring β-Lactam Resistance without Losing Fitness and Virulence in Pseudomonas aeruginosa. Antimicrob. Agents Chemother 2008, 52, 3694–3700. [PubMed: 18644952]

(91). Zamorano L; Moyá B; Juan C; Oliver A Differential β-Lactam Resistance Response driven by *ampD* or *dacB* (PBP4) Inactivation in Genetically Diverse *Pseudomonas aeruginosa* Strains. J. Antimicrob. Chemother 2010, 65, 1540–1542. [PubMed: 20435778]

(92). Moya B; Beceiro A; Cabot G; Juan C; Zamorano L; Albertí S; Oliver A Pan-β-Lactam Resistance Development in *Pseudomonas aeruginosa* Clinical Strains: Molecular Mechanisms, PBP Profiles, and Binding Affinities. Antimicrob. Agents Chemother 2012, 56, 4771–4778. [PubMed: 22733064]

(93). Schmidtke AJ; Hanson ND Role of ampD Homologs in Overproduction of AmpC in Clinical Isolates of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother 2008, 52, 3922–3927. [PubMed: 18779353]

(94). Lee M; Artola-Recolons C; Carrasco-López C; Martínez-Caballero S; Hesek D; Spink EE; Lastochkin E; Zhang W; Hellman LM; Boggess B; Hermoso JA; Mobashery S Cell-wall Remodeling by the Zinc-protease AmpDh3 from *Pseudomonas aeruginosa*. J. Am. Chem. Soc 2013, 135, 12604–12607. [PubMed: 23931161]

(95). Martínez-Caballero S; Lee M; Artola-Recolons C; Carrasco-López C; Hesek D; Spink E; Lastochkin E; Zhang W; Hellman LM; Boggess B; Mobashery S; Hermoso JA Reaction Products and the X-ray Structure of AmpDh2, a Virulence Determinant of *Pseudomonas aeruginosa*. J. Am. Chem. Soc 2013, 135, 10318–10321. [PubMed: 23819763]

(96). Zhang W; Lee M; Hesek D; Lastochkin E; Boggess B; Mobashery S Reactions of the Three AmpD Enzymes of *Pseudomonas aeruginosa*. J. Am. Chem. Soc 2013, 135, 4950–4953. [PubMed: 23510438]

(97). Rivera I; Molina R; Lee M; Mobashery S; Hermoso JA Orthologous and Paralogous AmpD Peptidoglycan Amidases from Gram-negative Bacteria. Microb. Drug Resist 2016, 22, 470–476. [PubMed: 27326855]

(98). Lee M; Hesek D; Lastochkin E; Dik DA; Boggess B; Mobashery S Deciphering the Nature of Enzymatic Modifications of Bacterial Cell Walls. ChemBioChem 2017, 18, 1696–1702. [PubMed: 28591487]

(99). Hervé M; Boniface A; Gobec S; Blanot D; Mengin-Lecreulx D Biochemical Characterization and Physiological Proper ties of *Escherichia coli* UDP-*N*-acetylmuramate:L-alanyl-γ-D-glutamyl-meso-diaminopimelate Ligase. J. Bacteriol 2007, 189, 3987–3995. [PubMed: 17384195]

(100). Das D; Hervé M; Feuerhelm J; Farr CL; Chiu H-J; Elsliger M-A; Knuth MW; Klock HE; Miller MD; Godzik A; Lesley SA; Deacon AM; Mengin-Lecreulx D; Wilson IA Structure and Function of the First Full-length Murein Peptide Ligase (Mpl) Cell Wall Recycling Protein. PLoS One 2011, 6, e17624. [PubMed: 21445265]

(101). Hervé M; Kovac A; Cardoso C; Patin D; Brus B; Barreteau H; Mengin-Lecreulx D; Gobec S; Blanot D Synthetic Tripeptides as Alternate Substrates of Murein Peptide Ligase (Mpl). Biochimie 2013, 95, 1120–1126. [PubMed: 23270797]

(102). Uehara T; Suefuji K; Valbuena N; Meehan B; Donegan M; Park JT Recycling of the Anhydro-*N*-acetylmuramic Acid Derived from Cell Wall Murein Involves a Two-Step Conversion to *N*-Acetylglucosamine-phosphate. J. Bacteriol 2005, 187, 3643–3649. [PubMed: 15901686]

(103). Bacik JP; Whitworth GE; Stubbs KA; Yadav AK; Martin DR; Bailey-Elkin BA; Vocadlo DJ; Mark BL Molecular Basis of 1,6-Anhydro Bond Cleavage and Phosphoryl Transfer by *Pseudomonas aeruginosa* 1,6-Anhydro-*N*-acetylmuramic Acid Kinase. J. Biol. Chem 2011, 286, 12283–12291. [PubMed: 21288904]

(104). Bacik JP; Tavassoli M; Patel TR; McKenna SA; Vocadlo DJ; Khajehpour M; Mark BL Conformational Itinerary of *Pseudomonas aeruginosa* 1,6-Anhydro-*N*-acetylmuramic Acid Kinase During its Catalytic Cycle. J. Biol. Chem 2014, 289, 4504–4514. [PubMed: 24362022]

(105). Bacik JP; Jarboe LR Bioconversion of Anhydrosugars: Emerging Concepts and Strategies. IUBMB Life 2016, 68, 700–708. [PubMed: 27416973]

(106). Uehara T; Suefuji K; Jaeger T; Mayer C; Park JT MurQ etherase is Required by *Escherichia coli* in order to Metabolize Anhydro-*N*-acetylmuramic Acid Obtained Either from the Environment or from Its Own Cell Wall. J. Bacteriol 2006, 188, 1660–1662. [PubMed: 16452451]

(107). Hadi T; Dahl U; Mayer C; Tanner ME Mechanistic Studies on *N*-Acetylmuramic acid 6-phosphate Hydrolase (MurQ): An Etherase Involved in Peptidoglycan Recycling. Biochemistry 2008, 47, 11547–11558. [PubMed: 18837509]

(108). Hadi T; Hazra S; Tanner ME; Blanchard JS Structure of MurNAc 6-phosphate Hydrolase (MurQ) from *Haemophilus influenzae* with a Bound Inhibitor. Biochemistry 2013, 52, 9358–9366. [PubMed: 24251551]

(109). Hall RS; Xiang DF; Xu C; Raushel FM *N*-Acetyl-D-glucosamine-6-phosphate Deacetylase: Substrate Activation via a Single Divalent Metal Ion. Biochemistry 2007, 46, 7942–7952. [PubMed: 17567047]

(110). Hall RS; Brown S; Fedorov AA; Fedorov EV; Xu C; Babbitt PC; Almo SC; Raushel FM Structural Diversity within the Mononuclear and Binuclear Active Sites of *N*-Acetyl-D-glucosamine-6-phosphate Deacetylase. Biochemistry 2007, 46, 7953–7962. [PubMed: 17567048]

(111). Plumbridge J An Alternative Route for Recycling of *N*-Acetylglucosamine from Peptidoglycan Involves the *N*-Acetylglucosamine Phosphotransferase System in *Escherichia coli*. J. Bacteriol 2009, 191, 5641–5647. [PubMed: 19617367]

(112). Gisin J; Schneider A; Nagele B; Borisova M; Mayer C A Cell Wall Recycling Shortcut that Bypasses Peptidoglycan *de novo* Biosynthesis. Nat. Chem. Biol 2013, 9, 491–493. [PubMed: 23831760]

(113). Borisova M; Gisin J; Mayer C Blocking Peptidoglycan Recycling in *Pseudomonas aeruginosa* Attenuates Intrinsic Resistance to Fosfomycin. Microb. Drug Resist 2014, 20, 231–237. [PubMed: 24819062]

(114). Hamou-Segarra M; Zamorano L; Vadlamani G; Chu M; Sanchez-Diener I; Juan C; Blázquez J; Hattie M; Stubbs KA; Mark BL; Oliver A Synergistic Activity of Fosfomycin, β-Lactams and Peptidoglycan Recycling Inhibition Against *Pseudomonas aeruginosa*. J. Antimicrob. Chemother 2017, 72, 448–454. [PubMed: 27999022]

(115). van Heijenoort J Assembly of the Monomer unit of Bacterial Peptidoglycan. Cell. Mol. Life Sci 1998, 54, 300–304. [PubMed: 9614964]

(116). Silhavy TJ; Kahne D; Walker S The Bacterial Cell Envelope. Cold Spring Harbor Perspect. Biol 2010, 2, a000414.

(117). Dufresne K; Paradis-Bleau C Biology and Assembly of the Bacterial Envelope. Adv. Exp. Med. Biol 2015, 883, 41–76. [PubMed: 26621461]

(118). Kleanthous C; Armitage JP The Bacterial Cell Envelope. Philos. Trans. R. Soc. B 2015, 370, 20150019.

(119). Peters K; Kannan S; Rao VA; Biboy J; Vollmer D; Erickson SW; Lewis RJ; Young KD; Vollmer W The Redundancy of Peptidoglycan Carboxypeptidases Ensures Robust Cell Shape Maintenance in *Escherichia coli*. mBio 2016, 7, No. e00819-16.

(120). Schleifer KH; Kandler O Peptidoglycan Types of Bacterial Cell Walls and Their Taxonomic Implications. Bacteriol. Rev 1972, 36, 407–477. [PubMed: 4568761]

(121). Labischinski H; Barnickel G; Naumann D; Keller P Conformational and Topological Aspects of the Three-dimensional Architecture of Bacterial Peptidoglycan. Ann. Inst. Pasteur/Microbiol 1985, 136, 45–50.

(122). Koch AL Simulation of the Conformation of the Murein Fabric: the Oligoglycan, Penta-muropeptide, and Cross-linked Nona-muropeptide. Arch. Microbiol 2000, 174, 429–439. [PubMed: 11195099]

(123). Koch AL Length Distribution of the Peptidoglycan Chains in the Sacculus of *Escherichia coli*. J. Theor. Biol 2000, 204, 533–541. [PubMed: 10833354]

(124). Meroueh SO; Bencze KZ; Hesek D; Lee M; Fisher JF; Stemmler TL; Mobashery S Three-dimensional Structure of the Bacterial Cell Wall Peptidoglycan. Proc. Natl. Acad. Sci. U. S. A 2006, 103, 4404–4409. [PubMed: 16537437]

(125). Lovering AL; Safadi SS; Strynadka NCJ Structural Perspective of Peptidoglycan Biosynthesis and Assembly. Annu. Rev. Biochem 2012, 81, 451–478. [PubMed: 22663080]

(126). Glauner B; Höltje JV; Schwarz U The Composition of the Murein of *Escherichia coli*. J. Biol. Chem 1988, 263, 10088–10095. [PubMed: 3292521]

(127). Demchick P; Koch AL The Permeability of the Wall Fabric of *Escherichia coli* and Bacillus subtilis. J. Bacteriol 1996, 178, 768–773. [PubMed: 8550511]

(128). Koch AL The Three-for-one model for Gram-negative Wall Growth: A Problem and a Possible Solution. FEMS Microbiol. Lett 1998, 162, 127–134. [PubMed: 9595673]

(129). Koch AL Orientation of the Peptidoglycan Chains in the Sacculus of *Escherichia coli*. Res. Microbiol 1998, 149, 689–701. [PubMed: 9921576]

(130). Turner RD; Hurd AF; Cadby A; Hobbs JK; Foster SJ Cell Wall Elongation Mode in Gram-negative Bacteria is Determined by Peptidoglycan Architecture. Nat. Commun 2013, 4, 1496. [PubMed: 23422664]

(131). Kim SJ; Chang J; Singh M Peptidoglycan Architecture of Gram-positive Bacteria by Solid-state NMR. Biochim. Biophys. Acta, Biomembr 2015, 1848, 350–362.

(132). Singh M; Kim SJ; Sharif S; Preobrazhenskaya M; Schaefer J REDOR Constraints on the Peptidoglycan Lattice Architecture of Staphylococcus aureus and its FemA Mutant. Biochim. Biophys. Acta, Biomembr 2015, 1848, 363–368.

(133). Yang H; Singh M; Kim SJ; Schaefer J Characterization of the Tertiary Sructure of the Peptidoglycan of *Enterococcus faecalis*. Biochim. Biophys. Acta, Biomembr 2017, 1859, 2171–2180. [PubMed: 28784459]

(134). Ghuysen JM Serine β-Lactamases and Penicillin-binding Proteins. Annu. Rev. Microbiol 1991, 45, 37–67. [PubMed: 1741619]

(135). Macheboeuf P; Contreras-Martel C; Job V; Dideberg O; Dessen A Penicillin-binding Proteins: Key Players in Bacterial Cell Cycle and Drug Resistance Processes. FEMS Microbiol. Rev 2006, 30, 673–691. [PubMed: 16911039]

(136). Frère JM; Page MGP Penicillin-binding Proteins: Evergreen Drug Targets. Curr. Opin. Pharmacol 2014, 18, 112–119. [PubMed: 25450065]

(137). Cho H; Wivagg CN; Kapoor M; Barry Z; Rohs PDA; Suh H; Marto JA; Garner EC; Bernhardt TG Bacterial Cell Wall Biogenesis is Mediated by SEDS and PBP Polymerase Families Functioning Semi-autonomously. Nat. Microbiol 2016, 1, 16172. [PubMed: 27643381]

(138). de Boer PA Classic Spotlight: Cellular Sites of Peptidoglycan Synthesis Revealed. J. Bacteriol 2016, 198, 1795. [PubMed: 27298280]

(139). Sauvage E; Terrak M Glycosyltransferases and Trans-peptidases/PBPs: Valuble Targets for New Antibacterials. Antibiotics 2016, 5, 12.

(140). Pazos M; Peters K; Vollmer W Robust Peptidoglycan Growth by Dynamic and Variable Multi-protein Complexes. Curr. Opin. Microbiol 2017, 36, 55–61. [PubMed: 28214390]

(141). Yousif SY; Broome-Smith JK; Spratt BG Lysis of *Escherichia coli* by β-Lactam Antibiotics: Deletion Analysis of the Role of Penicillin-binding Proteins 1A and 1B. Microbiology 1985, 131, 2839–2845.

(142). Denome SA; Elf PK; Henderson TA; Nelson DE; Young KD *Escherichia coli* Mutants Lacking All Possible Combinations of Eight PBPs: Viability, Characteristics, and Implications for Peptidoglycan Synthesis. J. Bacteriol 1999, 181, 3981–3993. [PubMed: 10383966]

(143). Di Berardino M; Dijkstra A; Stuber D; Keck W; Gubler M The Monofunctional Glycosyltransferase of *Escherichia coli* is a Member of a New Class of Peptidoglycan-Synthesising enzymes. FEBS Lett 1996, 392, 184–188. [PubMed: 8772200]

(144). Touzé T; Tran AX; Hankins JV; Mengin-Lecreulx D; Trent MS Periplasmic Phosphorylation of Lipid A is Lnked to the Synthesis of Undecaprenyl Phosphate. Mol. Microbiol 2008, 67, 264–277. [PubMed: 18047581]

(145). Valvano MA Undecaprenyl Phosphate Recycling Comes Out of Age. Mol. Microbiol 2008, 67, 232–235. [PubMed: 18086187]

(146). Jorgenson MA; Young KD Interrupting Biosynthesis of O-antigen or the Lipopolysaccharide Core Produces Morphological Defects in *Escherichia coli* by Sequestering Undecaprenyl Phosphate. J. Bacteriol 2016, 198, 3070–3079. [PubMed: 27573014]

(147). Jorgenson MA; Kannan S; Laubacher ME; Young KD Dead-end Intermediates in the Enterobacterial Common Antigen Pathway induce Morphological Defects in *Escherichia coli* by Competing for Undecaprenyl Phosphate. Mol. Microbiol 2016, 100, 1–14. [PubMed: 26593043]

(148). Contreras-Martel C; Martins A; Ecobichon C; Trindade DM; Mattel PJ; Hicham S; Hardouin P; Ghachi ME; Boneca IG; Dessen A Molecular Architecture of the PBP2-MreC core Bacterial Cell Wall Synthesis Complex. Nat. Commun 2017, 8, 776. [PubMed: 28974686]

(149). Goffin C; Fraipont C; Ayala J; Terrak M; Nguyen-Disteche M; Ghuysen JM The Non-Penicillin-binding Module of the Tripartite Penicillin-binding Protein 3 of *Escherichia coli* is Required for Folding and/or Stability of the Penicillin-binding Module and the Membrane-anchoring Module Confers Cell Septation Activity on the Folded Structure. J. Bacterial 1996, 178, 5402–5409.

(150). Pratt RF β-Lactamases: Why and How. J. Med. Chem 2016, 59, 8207–8220. [PubMed: 27232275]

(151). Yunck R; Cho H; Bernhardt TG Identification of MltG as a Potential Terminase for Peptidoglycan Polymerization in Bacteria. Mol. Microbiol 2016, 99, 700–718. [PubMed: 26507882]

(152). Hugonnet J-E; Mengin-Lecreulx D; Monton A; den Blaauwen T; Carbonnelle E; Veckerle C; Bran YV; van Nieuwenhze M; Bouchier C; Tu K; Rice LB; Arthur M Factors Essential for L,D-transpeptidase-mediated Peptidoglycan Cross-linking and β-Lactam Resistance in *Escherichia coli*. eLife 2016, 5, No. e19469.

(153). Egan AJF Bacterial Outer Membrane Constriction. Mol. Microbiol 2018, 107, 676–687. [PubMed: 29315884]

(154). Vollmer W; Bertsche U Murein (Peptidoglycan) Structure, Architecture and Biosynthesis in *Escherichia coli*. Biochim. Biophys. Acta, Biomembr 2008, 1778, 1714–1734.

(155). Good MC; Zalatan JG; Lim WA Scaffold Proteins: Hubs for Controlling the Flow of Cellular Information. Science 2011, 332, 680–686. [PubMed: 21551057]

(156). Garner EC; Bernard R; Wang W; Zhuang X; Rudner DZ; Mitchison T Coupled, Circumferential Motions of the Cell Wall Synthesis Machinery and MreB Filaments in B. subtilis. Science 2011, 333, 222–225. [PubMed: 21636745]

(157). Dominguez-Escobar J; Chastanet A; Crevenna AH; Fromion V; Wedlich-Soldner R; Carballido-Lopez R Processive Movement of MreB-associated Cell Wall Biosynthetic Complexes in Bacteria. Science 2011, 333, 225–228. [PubMed: 21636744]

(158). van Teeffelen S; Wang S; Furchtgott L; Huang KC; Wingreen NS; Shaevitz JW; Gitai Z The Bacterial Actin MreB Rotates, and Rotation Depends on Cell-Wall Assembly. Proc. Natl. Acad. Sci. U. S. A 2011, 108, 15822–15827. [PubMed: 21903929]

(159). Schirner K; Eun YJ; Dion M; Luo Y; Helmann JD; Garner EC; Walker S Lipid-linked Cell Wall Precursors Regulate Membrane Association of Bacterial Actin MreB. Nat. Chem. Biol 2015, 11, 38–45. [PubMed: 25402772]

(160). Fu G; Bandaria JN; Le Gall AV; Fan X; Yildiz A; Mignot T; Zusman DR; Nan B MotAB-like Machinery drives the Movement of MreB Filaments during Bacterial Gliding Motility. Proc. Natl. Acad. Sci. U. S. A 2018, 115, 2484–2489. [PubMed: 29463706]

(161). Hussain S; Wivagg CN; Szwedziak P; Wong F; Schaefer K; Izoré T; Renner LD; Holmes MJ; Sun Y; Bisson-Filho AW; Walker S; Amir A; Löwe J; Garner EC MreB Filaments Align Along Greatest Principal Membrane Curvature to Orient Cell Wall Synthesis. eLife 2018, 7, No. e32471.

(162). Salje J; van den Ent F; de Boer P; Löwe J Direct Membrane Binding by Bacterial Actin MreB. Mol. Cell 2011, 43, 478–487. [PubMed: 21816350]

(163). van den Ent F; Izoré T; Bharat TA; Johnson CM; Löwe J Bacterial Actin MreB forms Antiparallel Double Filaments. eLife 2014, 3, No. e02634.

(164). Jones LJ; Carballido-Lopez R; Errington J Control of Cell Shape in Bacteria: Helical, Actin-like Filaments in Bacillus subtilis. Cell 2001, 104, 913–922. [PubMed: 11290328]

(165). Carballido-Lopez R; Formstone A; Li Y; Ehrlich SD; Noirot P; Errington J Actin Homolog MreBH Governs Cell Morphogenesis by Localization of the Cell Wall Hydrolase LytE. Dev. Cell 2006, 11, 399–409. [PubMed: 16950129]

(166). Espeli O; Nurse P; Levine C; Lee C; Marians KJ SetB: An integral Membrane Protein that Affects Chromosome Segregation in *Escherichia coli*. Mol. Microbiol 2003, 50, 495–509. [PubMed: 14617174]

(167). Kruse T; Moller-Jensen J; Lobner-Olesen A; Gerdes K Dysfunctional MreB inhibits chromosome segregation in *Escherichia coli*. EMBO J. 2003, 22, 5283–5292. [PubMed: 14517265]

(168). Shih YL; Le T; Rothfield L Division Site Selection in *Escherichia coli* Involves Dynamic Redistribution of Min Proteins Within Coiled Structures that Extend Between the Two Cell Poles. Proc. Natl. Acad. Sci. U. S. A 2003, 100, 7865–7870. [PubMed: 12766229]

(169). Defeu Soufo HJ; Graumann PL Dynamic movement of actin-like proteins within bacterial cells. EMBO Rep. 2004, 5, 789–794. [PubMed: 15272301]

(170). Figge RM; Divakaruni AV; Gober JW MreB, the Cell Shape-determining Bacterial Actin Homologue, Co-ordinates Cell Wall Morphogenesis in *Caulobacter crescentus*. Mol. Microbiol 2004, 51, 1321–1332. [PubMed: 14982627]

(171). Gitai Z; Dye N; Shapiro L An Actin-like Gene Can Determine Cell Polarity in Bacteria. Proc. Natl. Acad. Sci. U. S. A 2004, 101, 8643–8648. [PubMed: 15159537]

(172). Campo N; Tjalsma H; Buist G; Stepniak D; Meijer M; Veenhuis M; Westermann M; Müller JP; Bron S; Kok J; Kuipers OP; Jongbloed JD Subcellular Sites for Bacterial Protein Export. Mol. Microbiol 2004, 53, 1583–1599. [PubMed: 15341641]

(173). Leaver M; Errington J Roles for MreC and MreD Proteins in Helical Growth of the Cylindrical Cell Wall in *Bacillus subtilis*. Mol. Microbiol 2005, 57, 1196–1209. [PubMed: 16101995]

(174). Vats P; Rothfield L Duplication and Segregation of the Actin (MreB) Cytoskeleton During the Prokaryotic Cell Cycle. Proc. Natl. Acad. Sci. U. S. A 2007, 104, 17795–17800. [PubMed: 17978175]

(175). Kawai Y; Daniel RA; Errington J Regulation of Cell Wall Morphogenesis in *Bacillus subtilis* by Recruitment of PBP1 to the MreB Helix. Mol. Microbiol 2009, 71, 1131–1144. [PubMed: 19192185]

(176). Foulquier E; Pompeo F; Bernadac A; Espinosa L; Galinier A The YvcK Protein is Required for Morphogenesis via Localization of PBP1 under Gluconeogenic Growth Conditions in *Bacillus subtilis*. Mol. Microbiol 2011, 80, 309–318. [PubMed: 21320184]

(177). Swulius MT; Chen S; Ding HJ; Li Z; Briegel A; Pilhofer M; Tocheva EI; Lybarger SR; Johnson TL; Sandkvist M; Jensen GJ Long Helical Filaments are not seen Encircling Cells in Electron Cryotomograms of Rod-shaped Bacteria. Biochem. Biophys. Res. Commun 2011, 407, 650–655. [PubMed: 21419100]

(178). van Teeffelen S; Gitai Z Rotate into shape: MreB and bacterial morphogenesis. EMBO J. 2011, 30, 4856–4857. [PubMed: 22166997]

(179). Errington J Bacterial Morphogenesis and the Enigmatic MreB Helix. Nat. Rev. Microbiol 2015, 13, 241–248. [PubMed: 25578957]

(180). Morgenstein RM; Bratton BP; Nguyen JP; Ouzounov N; Shaevitz JW; Gitai Z RodZ links MreB to Cell Wall Synthesis to Mediate MreB Rotation and Robust Morphogenesis. Proc. Natl. Acad. Sci. U. S. A 2015, 112, 12510–12515. [PubMed: 26396257]

(181). Shi H; Bratton BP; Gitai Z; Huang KC How to Build a Bacterial Cell: MreB as the Foreman of *E. coli* Construction. Cell 2018, 172, 1294–1305. [PubMed: 29522748]

(182). van den Ent F; Johnson CM; Persons L; de Boer P; Löwe J Bacterial actin MreB assembles in complex with cell shape protein RodZ. EMBO J. 2010, 29, 1081–1090. [PubMed: 20168300]

(183). Colavin A; Shi H; Huang KC RodZ Modulates Geometric Localization of the Bacterial Actin MreB to Regulate Cell Shape. Nat. Commun 2018, 9, 1280. [PubMed: 29599448]

(184). Divakaruni AV; Baida C; White CL; Gober JW The Cell Shape Proteins MreB and MreC Control Cell Morphogenesis by Positioning Cell Wall Synthetic Complexes. Mol. Microbiol 2007, 66, 174–188. [PubMed: 17880425]

(185). Typas A; Banzhaf M; van den Berg van Saparoea B; Verheul J; Biboy J; Nichols RJ; Zietek M; Beilharz K; Kannenberg K; von Rechenberg M; Breukink E; den Blaauwen T; Gross CA; Vollmer W Regulation of Peptidoglycan Synthesis by Outer-Membrane Proteins. Cell 2010, 143, 1097–1109. [PubMed: 21183073]

(186). Paradis-Bleau C; Markovski M; Uehara T; Lupoli TJ; Walker S; Kahne DE; Bernhardt TG Lipoprotein Cofactors Located in the Outer Membrane Activate Bacterial Cell Wall Polymerases. Cell 2010, 143, 1110–1120. [PubMed: 21183074]

(187). Meeske AJ; Riley EP; Robins WP; Uehara T; Mekalanos JJ; Kahne D; Walker S; Kruse AC; Bernhardt TG; Rudner DZ SEDS Proteins are a Widespread Family of Bacterial Cell Wall Polymerases. Nature 2016, 537, 634–638. [PubMed: 27525505]

(188). Sjodt M; Brock K; Dobihal G; Rohs PDA; Green AG; Hopf TA; Meeske AJ; Srisuknimit V; Kahne D; Walker S; Marks DS; Bernhardt TG; Rudner DZ; Kruse AC Structure of the Peptidoglycan Polymerase RodA Resolved by Evolutionary Coupling Analysis. Nature 2018, 556, 118–121. [PubMed: 29590088]

(189). Mohammadi T; van Dam V; Sijbrandi R; Vernet T; Zapun A; Bouhss A; Diepeveen-de Bruin M; Nguyen-Disteche M; de Kruijff B; Breukink E Identification of FtsW as a transporter of lipid-linked cell wall precursors across the membrane. EMBO J. 2011, 30, 1425–1432. [PubMed: 21386816]

(190). Ruiz N; Gronenberg LS; Kahne D; Silhavy TJ Identification of Two Inner-Membrane Proteins Required for the Transport of Lipopolysaccharide to the Outer Membrane of *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. A 2008, 105, 5537–5542. [PubMed: 18375759]

(191). Meeske AJ; Sham LT; Kimsey H; Koo BM; Gross CA; Bernhardt TG; Rudner DZ MurJ and a Novel Lipid II Flippase are Required for Cell Wall Biogenesis in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U. S. A 2015, 112, 6437–6442. [PubMed: 25918422]

(192). Rubino FA; Kumar S; Ruiz N; Walker S; Kahne D Membrane Potential is Required for MurJ Function. J. Am. Chem. Soc 2018, 140, 4481–4484. [PubMed: 29558128]

(193). Inoue A; Murata Y; Takahashi H; Tsuji N; Fujisaki S; Kato J Involvement of an Essential Gene, *mviN*, in Murein Synthesis in *Escherichia coli*. J. Bacteriol 2008, 190, 7298–7301. [PubMed: 18708495]

(194). Mohamed YF; Valvano MA A *Burkholderia cenocepacia* MurJ (MviN) Homolog is Essential for Cell Wall Peptidoglycan Synthesis and Bacterial Viability. Glycobiology 2014, 24, 564–576. [PubMed: 24688094]

(195). Egan AJ; Vollmer W The Physiology of Bacterial Cell Division. Ann. N. Y. Acad. Sci 2013, 1277, 8–28. [PubMed: 23215820]

(196). Egan AJF; Cleverley RM; Peters K; Lewis RJ; Vollmer W Regulation of Bacterial Cell Wall Growth. FEBS J. 2017, 284, 851–867. [PubMed: 27862967]

(197). den Blaauwen T; Hamoen LW; Levin PA The Divisome at 25: The Road Ahead. Curr. Opin. Microbiol 2017, 36, 85–94. [PubMed: 28254403]

(198). Dewachter L; Verstraeten N; Fauvart M; Michiels J An Integrative View of Cell Cycl Control in *Escherichia coli*. FEMS Microbiol. Rev 2018, 42, 116–136. [PubMed: 29365084]

(199). Bi EF; Lutkenhaus J FtsZ ring Structure Associated with Division in *Escherichia coli*. Nature 1991, 354, 161–164. [PubMed: 1944597]

(200). Levin PA; Kurtser IG; Grossman AD Identification and Characterization of a Negative Regulator of FtsZ Ring Formation in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U. S. A 1999, 96, 9642–9647. [PubMed: 10449747]

(201). Raskin DM; de Boer PA Rapid Pole-to-Pole Oscillation of a Protein Required for Directing Division to the Middle of *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. A 1999, 96, 4971–4976. [PubMed: 10220403]

(202). Wu LJ; Errington J Coordination of Cell Division and Chromosome Segregation by a Nucleoid Occlusion Protein in *Bacillus subtilis*. Cell 2004, 117, 915–925. [PubMed: 15210112]

(203). Bernhardt TG; de Boer PA SlmA, a Nucleoid-associated, FtsZ Binding Protein Required for Blocking Septal Ring Assembly over Chromosomes in *E. coli*. Mol. Cell 2005, 18, 555–564. [PubMed: 15916962]

(204). Thanbichler M; Shapiro L MipZ, a Spatial Regulator Coordinating Chromosome Segregation with Cell Division in *Caulobacter*. Cell 2006, 126, 147–162. [PubMed: 16839883]

(205). Löwe J; Amos LA Crystal Structure of the Bacterial Cell-division Protein FtsZ. Nature 1998, 391 , 203–206. [PubMed: 9428770]

(206). Nogales E A Structural View of Microtubule Dynamics. Cell. Mol Life Sci 1999, 56, 133–142. [PubMed: 11213253]

(207). Nogales E; Whittaker M; Milligan RA; Downing KH High-Resolution Model of the Microtubule. Cell 1999, 96, 79–88. [PubMed: 9989499]

(208). Schwille P Bacterial Cell Division: A Swirling Ring to Rule Them All? Curr. Biol 2014, 24, R157–9. [PubMed: 24556438]

(209). Huecas S; Ramírez-Aportela E; Vergoñós A; Núñez-Ramírez R; Llorca O; Díaz JF; Juan-Rodríguez D; Oliva MA; Castellen P; Andreu JM Self-organization of FtsZ Polymers in Solution Reveals Spacer Role of the Disordered C-terminal Tail. Biophys. J 2017, 113, 1831–1844. [PubMed: 29045877]

(210). Bisson-Filho AW; Hsu YP; Squyres GR; Kuru E; Wu F; Jukes C; Sun Y; Dekker C; Holden S; VanNieuwenhze MS; Brun YV; Garner EC Treadmilling by FtsZ Filaments Drives Peptidoglycan Synthesis and Bacterial Cell Division. Science 2017, 355, 739–743. [PubMed: 28209898]

(211). Schoenemann KM; Margolin W Bacterial Division: FtsZ Treadmills to Build a Beautiful Wall. Curr. Biol 2017, 27, R301–R303. [PubMed: 28441562]

(212). Yang X; Lyu Z; Miguel A; McQuillen R; Huang KC; Xiao J GTPase Activity-coupled Treadmilling of the Bacterial Tubulin FtsZ Organizes Septal Cell Wall Synthesis. Science 2017, 355, 744–747. [PubMed: 28209899]

(213). Wagstaff JM; Tsim M; Oliva MA; García-Sanchez A; Kureisaite-Ciziene D; Andreu JM; Löwe J A Polymerization-associated Structural Switch in FtsZ that Enables Treadmilling of Model Filaments. mBio 2017, 8, No. e00254-17.

(214). Monteiro JM; Pereira AR; Reichmann NT; Saraiva BM; Fernandes PB; Veiga H; Tavares AC; Santos M; Ferreira MT; Macário V; VanNieuwenhze MS; Filipe SR; Pinho MG Peptidoglycan Synthesis Drives an FtsZ-Treadmilling-independent Step of Cytokinesis. Nature 2018, 554, 528–532. [PubMed: 29443967]

(215). Wagstaff J; Löwe J Prokaryotic Cytoskeletons: Protein Filaments Organizing Small Cells. Nat. Rev. Microbiol 2018, 16, 187. [PubMed: 29355854]

(216). Aarsman MEG; Piette A; Fraipont C; Vinkenvleugel TMF; Nguyen-Distèche M; den Blaauwen T Maturation of the *Escherichia coli* Divisome Occurs in Two Steps. Mol. Microbiol 2005, 55, 1631–1645. [PubMed: 15752189]

(217). Gamba P; Veening JW; Saunders NJ; Hamoen LW; Daniel RA Two-step Assembly Dynamics of the *Bacillus subtilis* Divisome. J. Bacteriol 2009, 191, 4186–4194. [PubMed: 19429628]

(218). Huang K-H; Durand-Heredia J; Janakiraman A FtsZ Ring Stability: of Bundles, Tubules, Cosslinks, and Curves. J. Bacteriol 2013, 195, 1859–1868. [PubMed: 23457247]

(219). Pogliano J; Pogliano K; Weiss DS; Losick R; Beckwith J Inactivation of FtsI Inhibits Constriction of the FtsZ Cytokinetic Ring and Delays the Assembly of FtsZ Rings at Potential Division Sites. Proc. Natl. Acad. Sci. U. S. A 1997, 94, 559–564. [PubMed: 9012823]

(220). Wang L; Khattar MK; Donachie WD; Lutkenhaus J FtsI and FtsW are Localized to the Septum in *Escherichia coli*. J. Bacteriol 1998, 180, 2810–2816. [PubMed: 9603865]

(221). Liu X; Meiressonne NY; Bouhss A; den Blaauwen T The Combined Activities of FtsW and MurJ Are Required for Lipid II Translocation During Cell Division in *Escherichia coli*. bioRxiv 2017, 10, 1–31.

(222). Sung MT; Lai YT; Huang CY; Chou LY; Shih HW; Cheng WC; Wong CH; Ma C Crystal Structure of the Membrane-bound Bifunctional Transglycosylase PBP1b from *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. A 2009, 106, 8824–8829. [PubMed: 19458048]

(223). Ranjit DK; Jorgenson MA; Young KD PBP1B Glycosyltransferase and Transpeptidase Activities Play Different Essential Roles During the *De Novo* Regeneration of Rod-shaped Morphology in *Escherichia coli*. J. Bacteriol 2017, 199, e00612–16. [PubMed: 28096447]

(224). Arthur M Regulation of Bacterial Peptidoglycan Polymerization. Trends Microbiol 2016, 24, 519–521. [PubMed: 27236859]

(225). Young KD New Ways to Make Old Walls: Bacterial Surprises. Cell 2010, 143, 1042–1044. [PubMed: 21183069]

(226). King DT; Lameignere E; Strynadka NCJ Structural Insights into the Lipoprotein Outer Membrane Regulator of Penicillinbinding Protein 1B. J. Biol. Chem 2014, 289, 19245–19253. [PubMed: 24808177]

(227). Lupoli TJ; Lebar MD; Markovski M; Bernhardt T; Kahne D; Walker S Lipoprotein Activators Stimulate *Escherichia coli* Penicillin-binding Proteins by Different Mechanisms. J. Am. Chem. Soc 2014, 136, 52–55. [PubMed: 24341982]

(228). Egan AJF; Biboy J; van't Veer I; Breukink E; Vollmer W Activities and Regulation of Peptidoglycan Synthases. Philos. Trans. R. Soc., B 2015, 370, 20150031.

(229). Gray AN; Egan AJF; van't Veer IL; Verheul J; Colavin A; Koumoutsi A; Biboy J; Altelaar AFM; Damen MJ; Huang KC; Simorre JP; Breukink E; den Blaauwen T; Typas A; Gross CA; Vollmer W Coordination of Peptidoglycan Synthesis and Outer Membrane Constriction during *Escherichia coli* Cell Division. eLife 2015, 4, No. e07118.

(230). Markovski M; Bohrhunter JL; Lupoli TJ; Uehara T; Walker S; Kahne DE; Bernhardt TG Cofactor Bypass Variants Reveal a Conformational Control Mechanism Governing Cell Wall Polymerase Activity. Proc. Natl. Acad. Sci. U. S. A 2016, 113, 4788–4793. [PubMed: 27071112]

(231). Greene NG; Fumeaux C; Bernhardt TG Conserved Mechanism of Cell-Wall Synthase Regulation Revealed by the Identification of a New PBP Activator in *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. U. S. A 2018, 115, 3150–3155. [PubMed: 29507210]

(232). Potluri LP; de Pedro MA; Young KD *Escherichia coli* Low-molecular-weight Penicillin-binding Proteins help Orient Septal FtsZ, and their Absence Leads to Asymmetric Cell Division and Branching. Mol. Microbiol 2012, 84, 203–224. [PubMed: 22390731]

(233). Nelson DE; Young KD Contributions of PBP 5 and DD-Carboxypeptidase PBPs to Maintenance of Cell Shape in *Escherichia coli*. J. Bacteriol. 2001, 183, 3055–3064. [PubMed: 11325933]

(234). Meberg BM; Paulson AL; Priyadarshini R; Young KD Endopeptidase PBP 4 and 7 Play Auxiliary Roles in Determining Uniform Morphology of *Escherichia coli*. J. Bacteriol 2004, 186, 8326–8336. [PubMed: 15576782]

(235). Romeis T; Höltje JV Specific Interaction of Penicillinbinding Proteins 3 and 7/8 with Soluble Lytic Transglycosylase in *Escherichia coli*. J. Biol. Chem 1994, 269, 21603–21607. [PubMed: 8063800]

(236). Spratt BG Properties of the Penicillin-binding Proteins of *Escherichia coli* K12. Eur. J. Biochem 1977, 72, 341–352. [PubMed: 319999]

(237). Hesek D; Suvorov M; Morio K; Lee M; Brown S; Vakulenko SB; Mobashery S Synthetic Peptidoglycan Substrates for Penicillin-binding Protein 5 of Gram-negative Bacteria. J. Org. Chem 2004, 69, 778–784. [PubMed: 14750804]

(238). Zhang W; Shi Q; Meroueh SO; Vakulenko SB; Mobashery S Catalytic Mechanism of Penicillin-binding Protein 5 of *Escherichia coli*. Biochemistry 2007, 46, 10113–10121. [PubMed: 17685588]

(239). Nelson DE; Ghosh AS; Paulson AL; Young KD Contribution of Membrane-binding and Enzymatic Domains of PBP 5 to Maintenance of Uniform Cellular Morphology of *Escherichia coli*. J. Bacteriol 2002, 184, 3630–3639. [PubMed: 12057958]

(240). O'Daniel PI; Zajicek J; Zhang W; Shi Q; Fisher JF; Mobashery S Elucidation of the Structure of the Membrane Anchor of Penicillin-binding Protein 5 of *Escherichia coli*. J. Am. Chem. Soc 2010, 132, 4110–4118. [PubMed: 20192190]

(241). Santos JM; Lobo M; Matos AP; De Pedro MA; Arraiano CM The Gene *bolA* Regulates *dacA* (PBP5), *dacC* (PBP6) and *ampC* (AmpC), Promoting Normal Morphology in *Escherichia coli*. Mol. Microbiol 2002, 45, 1729–1740. [PubMed: 12354237]

(242). Varma A; Young KD FtsZ collaborates with Penicillinbinding Proteins to Generate Bacterial Cell Shape in *Escherichia coli*. J. Bacteriol 2004, 186, 6768–6774. [PubMed: 15466028]

(243). Shi Q; Meroueh SO; Fisher JF; Mobashery S Investigation of the Mechanism of the Cell Wall DD-carboxypeptidase Reaction of Penicillin-Binding Protein 5 of *Escherichia coli* by QM/MM Calculations. J. Am. Chem. Soc 2008, 130, 9293–9303. [PubMed: 18576637]

(244). van Heijenoort J Peptidoglycan Hydrolases of *Escherichia coli*. Microbiol. Mol. Biol. Rev 2011, 75, 636–663. [PubMed: 22126997]

(245). Uehara T; Dinh T; Bernhardt TG LytM-domain Factors are Required for Daughter Cell Separation and Rapid Ampicillin-induced Lysis in *Escherichia coli*. J. Bacteriol 2009, 191, 5094–5107. [PubMed: 19525345]

(246). Uehara T; Parzych KR; Dinh T; Bernhardt TG Daughter cell separation is controlled by cytokinetic ring-activated cell wall hydrolysis. EMBO J. 2010, 29, 1412–1422. [PubMed: 20300061]

(247). Peters NT; Dinh T; Bernhardt TG A Fail-safe Mechanism in the Septal Ring Assembly Pathway Generated by the Sequential Recruitment of Cell Separation Amidases and their Activators. J. Bacteriol 2011, 193, 4973–4983. [PubMed: 21764913]

(248). Lenz JD; Stohl EA; Robertson RM; Hackett KT; Fisher K; Xiong K; Lee M; Hesek D; Mobashery S; Seifert HS; Davies C; Dillard JP Amidase Activity of AmiC Controls Cell Separation and Stem Peptide Release and Is Enhanced by NlpD in *Neisseria gonorrhoeae*. J. Biol. Chem 2016, 291, 10916–10933. [PubMed: 26984407]

(249). Uehara T; Park JT An Anhydro-*N*-acetylmuramyl-L-alanine Amidase with Broad Specificity Tethered to the Outer Membrane of *Escherichia coli*. J. Bacteriol 2007, 189, 5634–5641. [PubMed: 17526703]

(250). Herlihey FA; Clarke AJ Controlling Autolysis during Flagella Insertion in Gram-negative Bacteria. Adv. Exp. Med. Biol 2016, 925, 41–56.

(251). Dik DA; Marous DR; Fisher JF; Mobashery S Lytic Transglycosylases: Concinnity in Concision of the Bacterial Cell Wall. Crit. Rev. Biochem. Mol. Biol 2017, 52, 503–542. [PubMed: 28644060]

(252). Byun B; Mahasenan KV; Dik DA; Marous DR; Speri E; Kumarasiri M; Fisher JF; Hermoso JA; Mobashery S Mechanism of the *Escherichia coli* MltE Lytic Transglycosylase, the Cell-Wall-Penetrating Enzyme for Type VI Secretion System Assembly. Sci. Rep 2018, 8, 4110. [PubMed: 29515200]

(253). Blackburn NT; Clarke AJ Identification of Four Families of Peptidoglycan Lytic Transglycosylases. J. Mol. Evol 2001, 52, 78–84. [PubMed: 11139297]

(254). Scheurwater E; Reid CW; Clarke AJ Lytic Transglycosylases: Bacterial Space-making Autolysins. Int. J. Biochem. Cell Biol 2008, 40, 586–591. [PubMed: 17468031]

(255). Chan YA; Hackett KT; Dillard JP The Lytic Transglycosylases of *Neisseria gonorrhoeae*. Microb. Drug Resist 2012, 18, 271–279. [PubMed: 22432703]

(256). Scheurwater EM; Clarke AJ The C-terminal Domain of *Escherichia coli* YfhD Functions as a Lytic Transglycosylase. J. Biol. Chem 2008, 283, 8363–8373. [PubMed: 18234673]

(257). Lee M; Hesek D; Llarrull LI; Lastochkin E; Pi H; Boggess B; Mobashery S Reactions of all *Escherichia coli* Lytic Transglycosylases with Bacterial Cell Wall. J. Am. Chem. Soc 2013, 135, 3311–3314. [PubMed: 23421439]

(258). Lee M; Hesek D; Dik DA; Fishovitz J; Lastochkin E; Boggess B; Fisher JF; Mobashery S From Genome to Proteome to Elucidation of Reactions for all Eleven Known Lytic Transglycosylases from *Pseudomonas aeruginosa*. Angew. Chem., Int. Ed 2017, 56, 2735–2739.

(259). Thunnissen AM; Isaacs NW; Dijkstral BW The Catalytic Domain of a Bacterial Lytic Transglycosylase Defines a Novel Class of Lysozymes. Proteins: Struct., Fund., Genet 1995, 22, 245–258.

(260). Williams AH; Wheeler R; Rateau L; Malosse C; Chamot-Rooke J; Haouz A; Taha MK; Boneca IG A Step-by-step in crystallo guide to Bond Cleavage and 1,6-Anhydro Sugar Product Synthesis by a Peptidoglycan Degrading Lytic Transglycosylase. J. Biol. Chem 2018, 293, 6000–6010. [PubMed: 29483188]

(261). Lee M; Batuecas MT; Tomoshige S; Domínguez-Gil T; Mahasenan KV; Dik DA; Hesek D; Millán C; Usón I; Lastochkin E; Hermoso JA; Mobashery S Exolytic and Endolytic Turnover of Peptidoglycan by Lytic Transglycosylase Slt of *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. U. S. A 2018, 115, 4393–4398. [PubMed: 29632171]

(262). Santin YG; Cascales E Domestication of a housekeeping transglycosylase for assembly of a Type VI secretion system. EMBO Rep. 2017, 18, 138–149. [PubMed: 27920034]

(263). Jorgenson MA; Chen Y; Yahashiri A; Popham DL; Weiss DS The Bacterial Septal Ring Protein RlpA is a Lytic Transglycosylase that Contributes to Rod Shape and Daughter Cell Separation in *Pseudomonas aeruginosa*. Mol. Microbiol 2014, 93, 113–128. [PubMed: 24806796]

(264). Artola-Recolons C; Carrasco-López C; Llarrull LI; Kumarasiri M; Lastochkin E; Martinez de Ilarduya I; Meindl K; Usón I; Mobashery S; Hermoso JA High-Resolution Crystal Structure of an Outer Membrane-anchored Endolytic Peptidoglycan Lytic Transglycosylase (MltE) from *Escherichia coli*. Biochemistry 2011, 50, 2384–2386. [PubMed: 21341761]

(265). Fibriansah G; Gliubich FI; Thunnissen AM On the Mechanism of Peptidoglycan Binding and Cleavage by the endo-Specific Lytic Transglycosylase MltE from *Escherichia coli*. Biochemistry 2012, 51, 9164–9177. [PubMed: 23075328]

(266). Durand E; Nguyen VS; Zoued A; Logger L; Pehau-Arnaudet G; Aschtgen MS; Spinelli S; Desmyter A; Bardiaux B; Dujeancourt A; Roussel A; Cambillau C; Cascales E; Fronzes R Biogenesis and Structure of a Type VI Secretion Membrane Core Complex. Nature 2015, 523, 555–560. [PubMed: 26200339]

(267). Domínguez-Gil T; Lee M; Acebrón-Avalos I; Mahasenan KV; Hesek D; Dik DA; Byun B; Lastochkin E; Fisher JF; Mobashery S; Hermoso JA Activation by Allostery in Cell-wall Remodeling by a Modular Membrane-bound Lytic Transglycosylase from *Pseudomonas aeruginosa*. Structure 2016, 24, 1729–1741. [PubMed: 27618662]

(268). Domínguez-Gil T; Molina R; Dik DA; Spink E; Mobashery S; Hermoso JA X-ray Structure of Catenated Lytic Transglycosylase SltB1. Biochemistry 2017, 56, 6317–6320. [PubMed: 29131935]

(269). Nikolaidis I; Izoré T; Job V; Thielens N; Breukink E; Dessen A Calcium-dependent Complex Formation between PBP2 and Lytic Transglycosylase SltB1 of *Pseudomonas aeruginosa*. Microb. Drug Resist 2012, 18, 298–305. [PubMed: 22432706]

(270). Vollmer W; von Rechenberg M; Höltje JV Demonstration of Molecular Interactions Between the Murein Polymerase PBP1B, the Lytic Transglycosylase MltA, and the Scaffolding Protein MipA of *Escherichia coli*. J. Biol. Chem 1999, 274, 6726–6734. [PubMed: 10037771]

(271). Legaree BA; Clarke AJ Interaction of Penicillin-binding Protein 2 with Soluble Lytic Transglycosylase B1 in *Pseudomonas aeruginosa*. J. Bacteriol 2008, 190, 6922–6926. [PubMed: 18708507]

(272). Jacobs C; Huang LJ; Bartowsky E; Normark S; Park JT Bacterial Cell Wall Recycling Provides Cytosolic Muropeptides as Effectors for β-Lactamase Induction. EMBO J. 1994, 13, 4684–4694. [PubMed: 7925310]

(273). Park JT Turnover and Recycling of the Murein Sacculus in Oligopeptide Permease-negative strains of *Escherichia coli*: Indirect Evidence for an Alternative Permease System and for a Monolayered Sacculus. J. Bacteriol 1993, 175, 7–11. [PubMed: 8416911]

(274). Chahboune A; Decaffmeyer M; Brasseur R; Joris B Membrane Topology of the *Escherichia coli* AmpG Permease Required for Recycling of Cell Wall Anhydromuropeptides and AmpC β-Lactamase Induction. Antimicrob. Agents Chemother 2005, 49, 1145–1149. [PubMed: 15728916]

(275). Kong KF; Aguila A; Schneper L; Mathee K *Pseudomonas aeruginosa* β-Lactamase Induction Requires Two Permeases, AmpG and AmpP. BMC Microbiol. 2010, 10, 328. [PubMed: 21192796]

(276). Dietz H; Pfeifle D; Wiedemann B The Signal Molecule for β-Lactamase Induction in *Enterobacter cloacae* is the Anhydromuramyl-pentapeptide. Antimicrob. Agents Chemother 1997, 41, 2113–2120. [PubMed: 9333034]

(277). Cheng Q; Park JT Substrate Specificity of the AmpG Permease Required for Recycling of Cell Wall Anhydro-muropeptides. J. Bacteriol 2002, 184, 6434–6436. [PubMed: 12426329]

(278). Perley-Robertson GE; Yadav AK; Winogrodzki JL; Stubbs KA; Mark BL; Vocadlo DJ A Fluorescent Transport Assay Enables Studying AmpG Permeases Involved in Peptidoglycan Recycling and Antibiotic Resistance. ACS Chem. Biol 2016, 11, 2626–2635. [PubMed: 27442597]

(279). Zhang Y; Bao Q; Gagnon LA; Huletsky A; Oliver A; Jin S; Langaee T *ampG* gene of *Pseudomonas aeruginosa* and its Role in β-Lactamase Expression. Antimicrob. Agents Chemother 2010, 54, 4772–4779. [PubMed: 20713660]

(280). Zamorano L; Reeve TM; Juan C; Moya B; Cabot G; Vocadlo DJ; Mark BL; Oliver A AmpG Inactivation Restores Susceptibility of panβ-Lactam-Resistant *Pseudomonas aeruginosa* Clinical Strains. Antimicrob. Agents Chemother 2011, 55, 1990–1996. [PubMed: 21357303]

(281). Park JT; Raychaudhuri D; Li H; Normark S; Mengin-Lecreulx D MppA, a Periplasmic Binding Protein Essential for Import of the Bacterial Cell Wall Peptide L-Alanyl-γ-D-glutamyl-meso-diaminopimelate. J. Bacteriol 1998, 180, 1215–1223. [PubMed: 9495761]

(282). Höltje JV; Kopp U; Ursinus A; Wiedemann B The Negative Regulator of β-Lactamase Induction AmpD is a *N*-Acetyl-anhydromuramyl-L-alanine Amidase. FEMS Microbiol. Lett 1994, 122, 159–164. [PubMed: 7958768]

(283). Jacobs C; Joris B; Jamin M; Klarsov K; Van Beeumen J; Mengin-Lecreulx D; van Heijenoort J; Park JT; Normark S; Frère JM AmpD, Essential for Both β-Lactamase Regulation and Cell Wall Recycling, is a Novel Cytosolic *N*-Acetylmuramyl-L-alanine Amidase. Mol. Microbiol 1995, 15, 553–559. [PubMed: 7783625]

(284). Yem DW; Wu HC Purification and Properties of β-*N*-Acetylglucosaminidase from *Escherichia coli*. J. Bacteriol 1976, 125, 324–331. [PubMed: 1377]

(285). Cheng Q; Li H; Merdek K; Park JT Molecular Characterization of the β-*N*-Acetylglucosaminidase of *Escherichia coli* and its Role in Cell Wall Recycling. J. Bacteriol 2000, 182, 4836–4840. [PubMed: 10940025]

(286). Vocadlo DJ; Mayer C; He S; Withers SG Mechanism of Action and Identification of Asp242 as the Catalytic Nucleophile of *Vibrio furnisii N*-Acetylβ-D-Glucosaminidase using 2-Acetamido-2-deoxy-5-fluoro-α-L-Idopyranosyl fluoride. Biochemistry 2000, 39, 117–126. [PubMed: 10625486]

(287). Brooke JS *Stenotrophomonas maltophilia*: An Emerging Global Opportunistic Pathogen. Clin. Microbiol. Rev 2012, 25, 2–41. [PubMed: 22232370]

(288). Talfan A; Mounsey O; Charman M; Townsend E; Avison MB Involvement of Mutation in *ampD* I, *mrcA*, and at Least One Additional Gene in β-Lactamase Hyperproduction in *Stenotrophomonas maltophilia*. Antimicrob. Agents Chemother 2013, 57, 5486–5491. [PubMed: 23979761]

(289). Tipper DJ; Strominger JL Mechanism of Action of Penicillins: A Proposal Bsed on their Structural Similarity to Acyl-D-alanyl-D-alanine. Proc. Natl. Acad. Sci. U. S. A 1965, 54, 1133–1141. [PubMed: 5219821]

(290). Kocaoglu O; Carlson EE Profiling of β-Lactam Selectivity for Penicillin-binding Proteins in *Escherichia coli* strain DC2. Antimicrob. Agents Chemother 2015, 59, 2785–2790. [PubMed: 25733506]

(291). Moya B; Dotsch A; Juan C; Blázquez J; Zamorano L; Haussler S; Oliver A β-Lactam Resistance Response Triggered by Inactivation of a Nonessential Penicillin-binding Protein. PLoS Pathog 2009, 5, e1000353. [PubMed: 19325877]

(292). Cavallari JF; Lamers RP; Scheurwater EM; Matos AL; Burrows LL Changes to Its Peptidoglycan-Remodeling Enzyme Repertoire Modulate β-Lactam Resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother 2013, 57, 3078–3084. [PubMed: 23612194]

(293). Korat B; Mottl H; Keck W Penicillin-binding Protein 4 of *Escherichia coli*: Molecular Cloning of the *dacB* Gene, Controlled Overexpression, and Alterations in Murein Composition. Mol. Microbiol 1991, 5, 675–684. [PubMed: 2046551]

(294). Kishida H; Unzai S; Roper DI; Lloyd A; Park SY; Tame JR Crystal Structure of Penicillin-binding Protein 4 (*dacB*) from *Escherichia coli*, both in the Native Form and Covalently Linked to Various Antibiotics. Biochemistry 2006, 45, 783–792. [PubMed: 16411754]

(295). Clarke TB; Kawai F; Park SY; Tame JR; Dowson CG; Roper DI Mutational Analysis of the Substrate Specificity of *Escherichia coli* PBP 4. Biochemistry 2009, 48, 2675–2683. [PubMed: 19209901]

(296). Lee M; Hesek D; Blázquez B; Lastochkin E; Boggess B; Fisher JF; Mobashery S Catalytic Spectrum of the Penicillinbinding Protein 4 of *Pseudomonas aeruginosa*, a Nexus for the

Induction of β-Lactam Antibiotic Resistance. J. Am. Chem. Soc 2015, 137, 190–200. [PubMed: 25495032]

(297). Cho H; Uehara T; Bernhardt TG β-Lactam Antibiotics Induce a Lethal Malfunctioning of the Bacterial Cell Wall Synthesis Machinery. Cell 2014, 159, 1300–1311. [PubMed: 25480295]

(298). Potluri L; Karczmarek A; Verheul J; Piette A; Wilkin JM; Werth N; Banzhaf M; Vollmer W; Young KD; Nguyen-Disteche M; den Blaauwen T Septal and Lateral Wall Localization of PBP5, the Major D,D-Carboxypeptidase of *Escherichia coli*, Requires Substrate Recognition and Membrane Attachment. Mol. Microbiol 2010, 77, 300–323. [PubMed: 20545860]

(299). Romeis T; Höltje JV Penicillin-binding Protein 7/8 of *Escherichia coli* is a DD-Endopeptidase. Eur. J. Biochem 1994, 224, 597–604. [PubMed: 7925376]

(300). Bertsche U; Breukink E; Kast T; Vollmer W In vitro Murein Peptidoglycan Synthesis by Dimers of the Bifunctional Transglycosylase-Transpeptidase PBP1B from *Escherichia coli*. J. Biol. Chem 2005, 280, 38096–38101. [PubMed: 16154998]

(301). Templin MF; Edwards DH; Höltje JV A Murein Hydrolase is the Specific Target of Bulgecin in *Escherichia coli*. J. Biol. Chem 1992, 267, 20039–20043. [PubMed: 1400320]

(302). Korfmann G; Sanders CC ampG is Essential for High-Level Expression of AmpC β-Lactamase in Enterobacter cloacae. Antimicrob. Agents Chemother 1989, 33, 1946–1951. [PubMed: 2692514]

(303). Caille O; Zincke D; Merighi M; Balasubramanian D; Kumari H; Kong KF; Silva-Herzog E; Narasimhan G; Schneper L ; Lory S; Mathee K Structural and Functional Characterization of *Pseudomonas aeruginosa* Global Regulator AmpR. J. Bacteriol 2014, 196, 3890–3902. [PubMed: 25182487]

(304). Lindquist S; Galleni M; Lindberg F; Normark S Signalling Proteins in Enterobacterial AmpC β-Lactamase Regulation. Mol. Microbiol 1989, 3, 1091–1102. [PubMed: 2691840]

(305). Lindberg F; Lindquist S; Normark S Inactivation of the ampD Gene Causes Semiconstitutive Overproduction of the Inducible *Citrobacter freundii* β-Lactamase. J. Bacteriol 1987, 169, 1923–1928. [PubMed: 3032901]

(306). Stubbs KA; Balcewich M; Mark BL; Vocadlo DJ Small Molecule Inhibitors of a Glycoside Hydrolase Attenuate Inducible AmpC-mediated β-Lactam Resistance. J. Biol. Chem 2007, 282, 21382–21391. [PubMed: 17439950]

(307). Mondon M; Hur S; Vadlamani G; Rodrigues P; Tsybina P; Oliver A; Mark BL; Vocadlo DJ; Blériot Y Selective Trihydroxyazepane NagZ Inhibitors Increase Sensitivity of *Pseudomonas aeruginosa* to β-Lactams. Chem. Commun 2013, 49, 10983–10985.

(308). Stubbs KA; Bacik JP; Perley-Robertson GE; Whitworth GE; Gloster TM; Vocadlo DJ; Mark BL The Development of Selective Inhibitors of NagZ: Increased Susceptibility of Gram-negative Bacteria to β-Lactams. ChemBioChem 2013, 14, 1973–1981. [PubMed: 24009110]

(309). Bouquet J; King DT; Vadlamani G; Benzie GR; Iorga B ; Ide D; Adachi I; Kato A; Vocadlo DJ; Mark BL; Blériot Y; Désiré J Selective Trihydroxylated Azepane Inhibitors of NagZ, a Glycosidase Involved in *Pseudomonas aeruginosa* Resistance to β-Lactam Antibiotics. Org. Biomol. Chem 2017, 15, 4609–4619. [PubMed: 28513749]

(310). Jacobs C; Fmre JM; Normark S Cytosolic Intermediates for Cell Wall Biosynthesis and Degradation Control Inducible β-Lactam resistance in Gram-negative Bacteria. Cell 1997, 88, 823–832. [PubMed: 9118225]

(311). Balcewich MD; Reeve TM; Orlikow EA; Donald LJ; Vocadlo DJ; Mark BL Crystal Structure of the AmpR Effector Binding Domain Provides Insight into the Molecular Regulation of Inducible *ampC* β-Lactamase. J. Mol. Biol 2010, 400, 998–1010. [PubMed: 20594961]

(312). Vadlamani G; Thomas MD; Patel TR; Donald LJ; Reeve TM; Stetefeld J; Standing KG; Vocadlo DJ; Mark BL The β-Lactamase Gene Regulator AmpR Is a Tetramer that Recognizes and Binds the D-Ala-D-Ala Motif of Its Repressor UDP-*N*-acetylmuramic Acid (MurNAc)-pentapeptide. J. Biol. Chem 2015, 290, 2630–2643. [PubMed: 25480792]

(313). Dietz H; Pfeifle D; Wiedemann B Location of Nacetylmuramyl-L-alanyl-D-glutamyl-*meso*-diaminopimelic acid, Presumed Signal Molecule for β-Lactamase Induction, in the Bacterial Cell. Antimicrob. Agents Chemother 1996, 40, 2173–2177. [PubMed: 8878601]

(314). Wiedemann B; Dietz H; Pfeifle D Induction of β-Lactamase in *Enterobacter cloacae*. Clin. Infect. Dis 1998, 27, S42–S47. [PubMed: 9710670]

(315). Suvorov M; Lee M; Hesek D; Boggess B; Mobashery S Lytic Transglycosylase MltB of *Escherichia coli* and its Role in Recycling of Peptidoglycan Strands of Bacterial Cell Wall. J. Am. Chem. Soc 2008, 130, 11878–11879. [PubMed: 18700763]

(316). Hesek D; Lee M; Zhang W; Noll BC; Mobashery S Total Synthesis of *N*-acetylglucosamine-1,6-Anhydro-*N*-acetylmuramylpentapeptide and Evaluation of Its Turnover by AmpD from *Escherichia coli*. J. Am. Chem. Soc 2009, 131, 5187–5193. [PubMed: 19309146]

(317). Dik DA; Domínguez-Gil T; Lee M; Hesek D; Byun B; Fishovitz J; Boggess B; Hellman LM; Fisher JF; Hermoso JA; Mobashery S Muropeptide Binding and the X-ray Structure of the Effector Domain of the Transcriptional Regulator AmpR of *Pseudomonas aeruginosa*. J. Am. Chem. Soc 2017, 139, 1448–1451. [PubMed: 28079369]

(318). Okazaki A; Avison MB Induction of L1 and L2 β-Lactamase Production in *Stenotrophomonas maltophilia* Is Dependent on an AmpR-type Regulator. Antimicrob. Agents Chemother 2008, 52, 1525–1528. [PubMed: 18212097]

(319). Lin CW; Huang YW; Hu RM; Chiang KH; Yang TC The Role of AmpR in Regulation of L1 and L2 β-Lactamases in *Stenotrophomonas maltophilia*. Res. Microbiol 2009, 160, 152–158. [PubMed: 19071216]

(320). Huang Y-W; Wu C-J; Hu R-M; Lin Y-T; Yang T-C Interplay among Membrane-Bound Lytic Transglycosylase D1, the CreBC Two-component Regulatory System, the AmpNG-AmpDINagZ-AmpR Regulatory Circuit, and L1/L2 β-Lactamase Expression in *Stenotrophomonas maltophilia*. Antimicrob. Agents Chemother 2015,59, 6866–6872. [PubMed: 26282431]

(321). Lin CW; Lin HC; Huang YW; Chung TC; Yang TC Inactivation of mrcA gene Derepresses the Basal-level Expression of L1 and L2 β-Lactamases in *Stenotrophomonas maltophilia*. J. Antimicrob. Chemother 2011, 66, 2033–2037. [PubMed: 21719470]

(322). Huang YW; Wang Y; Lin Y; Lin C; Lin YT; Hsu CC; Yang TC Impacts of Penicillin-binding Protein 2 Inactivation on β-Lactamase Expression and Muropeptide Profile in *Stenotrophomonas maltophilia*. mSystems 2017, 2, No. e00077-17.

(323). Yang TC; Huang YW; Hu RM; Huang SC; Lin YT AmpDI is Involved in Expression of the Chromosomal L1 and L2 β-Lactamases of *Stenotrophomonas maltophilia*. Antimicrob. Agents Chemother 2009, 53, 2902–2907. [PubMed: 19414581]

(324). Lindberg F; Normark S Contribution of Chromosomal β-Lactamases to β-Lactam Resistance in Enterobacteria. Clin. Infect. Dis 1986, 8, S292–S304.

(325). Normark S; Lindquist S; Lindberg F Chromosomal β-Lactam Resistance in Enterobacteria. Scand. J. Infect. Dis. Suppl 1986, 49, 38–45. [PubMed: 3547624]

(326). Honoré N; Nicolas MH; Cole ST Inducible Cephalosporinase Production in Clinical Isolates of *Enterobacter cloacae* is Controlled by a Regulatory Gene that has been deleted from *Escherichia coli*. EMBO J. 1986, 5, 3709–3714. [PubMed: 3030737]

(327). Schell MA Molecular Biology of the LysR family of Transcriptional Regulators. Annu. Rev. Microbiol 1993, 47, 597–626. [PubMed: 8257110]

(328). Gerischer U Specific and Global Regulation of Genes Associated with the Degradation of Aromatic Compounds in Bacteria. J. Mol. Microbiol. Biotechnol 2002, 4, 111–121. [PubMed: 11873906]

(329). Tropel D; van der Meer JR Bacterial Transcriptional Regulators for Degradation Pathways of Aromatic Compounds. Microbiol. Mol. Biol. Rev 2004, 68, 474–500. [PubMed: 15353566]

(330). Maddocks SE; Oyston PCF Structure and Function of the LysR-type Transcriptional Regulator (LTTR) Family Proteins. Microbiology 2008, 154, 3609–3623. [PubMed: 19047729]

(331). Stragier P; Richaud F; Borne F; Patte JC Regulation of Diaminopimelate Decarboxylase Synthesis in *Escherichia coli*. I. Identification of a *lysR* Gene Encoding an Activator of the *lysA* Gene. J. Mol. Biol 1983, 168, 307–320. [PubMed: 6411928]

(332). Stragier P; Danos O; Patte JC Regulation of Diaminopimelate Decarboxylase Synthesis in *Escherichia coli*. II. Nucleotide Sequence of the *lysA* Fene and its Regulatory Region. J. Mol. Biol 1983, 168, 321–331. [PubMed: 6350601]

(333). Stragier P; Patte JC Regulation of Diaminopimelate Decarboxylase Synthesis in *Escherichia coli*. III. Nucleotide Sequence and Regulation of the *lysR* gene. J. Mol. Biol 1983, 168, 333–350. [PubMed: 6350602]

(334). Stragier P; Borne F; Richaud F; Richaud C; Patte JC Regulatory Pattern of the *Escherichia coli lysA* Gene: Expression of Chromosomal *lysA-lacZ* Fusions. J. Bacteriol 1983, 156, 1198–1203. [PubMed: 6417111]

(335). Pérez-Rueda E; Collado-Vides J The Repertoire of DNA binding Transcriptional Regulators in *Escherichia coli* K-12. Nucleic Acids Res 2000, 28, 1838–1847. [PubMed: 10734204]

(336). Neidle EL; Hartnett C; Ornston LN Characterization of *Acinetobacter calcoaceticus catM*, a Repressor Gene Hhomologous in Sequence to Transcriptional Activator Genes. J. Bacteriol 1989, 171, 5410–5421. [PubMed: 2793826]

(337). Burn JE; Hamilton WD; Wootton JC; Johnston AW Single and Multiple Mutations Affecting Properties of the Regulatory Gene *nodD* of *Rhizobium*. Mol. Microbiol 1989, 3, 1567–1577. [PubMed: 2615655]

(338). Cebolla A; Sousa C; de Lorenzo V Effector Specificity Mutants of the Transcriptional Activator NahR of Naphthalene Degrading *Pseudomonas* Define Protein Sites Involved in Binding of Aromatic Inducers. J. Biol. Chem 1997, 272, 3986–3992. [PubMed: 9020104]

(339). Jørgensen C; Dandanell G Isolation and Characterization of Mutations in the *Escherichia coli* Regulatory Protein XapR. J. Bacteriol 1999, 181, 4397–4403. [PubMed: 10400599]

(340). Lodge JM; Minchin SD; Piddock LJV; Busby SJW Cloning, Sequencing and Analysis of the Structural Gne and Regulatory Region of the *Pseudomonas aeruginosa* Chromosomal *ampC* β-Lactamase. Biochem. J 1990, 272, 627–631. [PubMed: 2125210]

(341). Sainsbury S; Lane LA; Ren J; Gilbert RJ; Saunders NJ; Robinson CV; Stuart DI; Owens RJ The Structure of CrgA from *Neisseria meningitidis* Reveals a New Octameric Assembly State for LysR Transcriptional Regulators. Nucleic Acids Res 2009, 37, 4545–4558. [PubMed: 19474343]

(342). Taylor JL; De Silva RS; Kovacikova G; Lin W; Taylor RK; Skorupski K; Kull FJ The Crystal Structure of AphB, a Virulence Gene Activator from *Vibrio cholerae*, Reveals Residues that Influence its Response to Oxygen and pH. Mol. Microbiol 2012, 83, 457–470. [PubMed: 22053934]

(343). Zhou X; Lou Z; Fu S; Yang A; Shen H; Li Z; Feng Y; Bartlam M; Wang H; Rao Z Crystal Structure of ArgP from *Mycobacterium tuberculosis* confirms two distinct conformations of Full-length LysR Transcriptional Regulators and Reveals its Function in DNA Binding and Transcriptional Regulation. J. Mol. Biol 2010, 396, 1012–1024. [PubMed: 20036253]

(344). Ruangprasert A; Craven SH; Neidle EL; Momany C Full-length Structures of BenM and Two Variants Reveal Different Oligomerization Schemes for LysR-type Transcriptional Regulators. J. Mol. Biol 2010, 404, 568–586. [PubMed: 20932977]

(345). Muraoka S; Okumura R; Ogawa N; Nonaka T; Miyashita K; Senda T Crystal structure of a Full-length LysR-type Transcriptional Regulator, CbnR: Unusual Combination of Two Subunit Forms and Molecular Bases for Causing and Changing DNA Bend. J. Mol. Biol 2003, 328, 555–566. [PubMed: 12706716]

(346). Lerche M; Dian C; Round A; Lönneborg R; Brzezinski P; Leonard GA The Solution Configurations of Inactive and Activated DntR have Implications for the Sliding Dimer Mechanism of LysR Transcription Factors. Sci. Rep 2016, 6, 19988. [PubMed: 26817994]

(347). Jo I; Chung IY; Bae HW; Kim JS; Song S; Cho YH; Ha NC Structural Details of the OxyR Peroxide-sensing Mechanism. Proc. Natl. Acad. Sci. U. S. A 2015, 112, 6443–6448. [PubMed: 25931525]

(348). Monferrer D; Tralau T; Kertesz MA; Dix I; Solà M; Usón I Structural Studies on the Full-length LysR-type Regulator TsaR from *Comamonas testosteroni* T-2 Reveal a Novel Open Conformation of the Tetrameric LTTR Fold. Mol. Microbiol 2010, 75, 1199–1214. [PubMed: 20059681]

(349). Beck CF; Warren RA Divergent Promoters, a Common Form of Gene Organization. Microbiol. Rev 1988, 52, 318–326. [PubMed: 3054465]

(350). Lodge J; Busby S; Piddock L Investigation of the *Pseudomonas aeruginosa ampR* Gene and its Role at the Chromosomal *ampC* β-Lactamase Promoter. FEMS Microbiol. Lett 1993, 111, 315–320. [PubMed: 8405939]

(351). Cole ST; Nicolas MH β-Lactam Resistance Mechanisms in Gram-negative Bacteria. Microbiol. Sci 1986, 3, 334–339. [PubMed: 2856616]

(352). Nicolas MH; Honore N; Jarlier V; Philippon A; Cole ST Molecular Genetic Analysis of Cephalosporinase Production and its Role in β-Lactam Resistance in Clinical Isolates of Enterobacter cloacae. Antimicrob. Agents Chemother 1987, 31, 295–299. [PubMed: 3032094]

(353). Grundström T; Jaurin B Overlap between *ampC* and *frd* Operons on the *Escherichia coli* Chromosome. Proc. Natl. Acad. Sci. U. S. A 1982, 79, 1111–1115. [PubMed: 7041115]

(354). Bergström S; Lindberg FP; Olsson O; Normark S Comparison of the Overlapping *frd* and *ampC* Operons of *Escherichia coli* with the Corresponding DNA sequences in Other Gram-negative Bacteria. J. Bacteriol 1983, 155, 1297–1305. [PubMed: 6350266]

(355). Normark S; Edlund T; Grundström T; Bergström S; Wolf-Watz H *Escherichia coli* K-12 Mutants Hyperproducing Chromosomal β-Lactamase by Gene Repetitions. J. Bacteriol 1977, 132, 912–922. [PubMed: 336612]

(356). Normark S; Burman LG Resistance of *Escherichia coli* to Penicillins: Fine-structure Mapping and Dominance of Chromosomal β-Lactamase Mutations. J. Bacteriol 1977, 132, 1–7. [PubMed: 334718]

(357). Cole ST; Guest JR Production of a Soluble Form of Fumarate Reductase by Multiple Gene Duplication in *Escherichia coli* K12. Eur. J. Biochem 1979, 102, 65–71. [PubMed: 391567]

(358). Edlund T; Grundström T; Normark S Isolation and Characterization of DNA Repetitions Carrying the Chromosomal β-Lactamase Gene of *Escherichia coli* K-12. Mol. Gen. Genet 1979, 173, 115–125. [PubMed: 386030]

(359). Jaurin B; Grundström T; Normark S Sequence Elements Determining *ampC* Promoter Strength in *E. coli*. EMBO J 1982, 1,875–881. [PubMed: 6329713]

(360). Olsson O; Bergström S; Normark S Identification of a Novel ampC β-Lactamase Promoter in a Clinical Isolate of *Escherichia coli*. EMBO J 1982, 1, 1411–1416. [PubMed: 6765197]

(361). Pérez-Rueda E; Collado-Vides J Common History at the Origin of the Position-function Correlation in Transcriptional Regulators in Archaea and Bacteria. J. Mol. Evol 2001, 53, 172–179. [PubMed: 11523004]

(362). Huffman JL; Brennan RG Prokaryotic Transcription Regulators: More than Just the Helix-Turn-Helix Motif. Curr. Opin. Struct. Biol 2002, 12, 98–106. [PubMed: 11839496]

(363). Aravind L; Anantharaman V; Balaji S; Babu MM; Iyer LM The Many Faces of the Helix-Turn-Helix Domain: Transcription Regulation and Beyond. FEMS Microbiol. Rev 2005, 29, 231–262. [PubMed: 15808743]

(364). Brennan RG; Matthews BW Structural Basis of DNA protein Recognition. Trends Biochem. Sci 1989, 14, 286–290. [PubMed: 2672451]

(365). Brennan RG; Matthews BW The Helix-Turn-Helix DNA Binding Motif. J. Biol. Chem 1989, 264, 1903–1906. [PubMed: 2644244]

(366). Devesse L; Smirnova I; Lonneborg R; Kapp U; Brzezinski P; Leonard GA; Dian C Crystal Structures of DntR Inducer Binding Domains in Complex with Salicylate Offer Insights into the Activation of LysR-type Transcriptional Regulators. Mol. Microbiol 2011, 81, 354–367. [PubMed: 21692874]

(367). Porrúa O; García-Jaramillo M; Santero E; Govantes F The LysR-Type Regulator AtzR binding site: DNA sequences Involved in Activation, Repression and Cyanuric Acid-dependent Repositioning. Mol. Microbiol 2007, 66, 410–427. [PubMed: 17854404]

(368). Bundy BM; Collier LS; Hoover TR; Neidle EL Synergistic Transcriptional Activation by One Regulatory Protein in Response to Two Metabolites. Proc. Natl. Acad. Sci. U. S. A 2002, 99, 7693–7698. [PubMed: 12032345]

(369). Koentjoro MP; Adachi N; Senda M; Ogawa N; Senda T Crystal Structure of the DNA-binding Domain of the LysR-type Transcriptional Regulator CbnR in Complex with a DNA Fragment of the Recognition-binding Site in the Promoter Region. FEBS J. 2018, 285, 977–989. [PubMed: 29323785]

(370). Cook AM; Laue H; Junker F Microbial Desulfonation. FEMS Microbiol. Rev 1998, 22, 399–419. [PubMed: 9990724]

(371). Tralau T; Mampel J; Cook AM; Ruff J Characterization of TsaR, an Oxygen-sensitive LysR-type Regulator for the Degradation of *p*-Toluenesulfonate in *Comamonas testosteroni* T-2. Appl Environ. Microbiol 2003, 69, 2298–2305. [PubMed: 12676713]

(372). Ezezika OC; Haddad S; Clark TJ; Neidle EL; Momany C Distinct Effector-binding Stes Enable Synergistic Transcriptional Activation by BenM, a LysR-type Regulator. J. Mol. Biol 2007, 367, 616–629. [PubMed: 17291527]

(373). Korsak D; Liebscher S; Vollmer W Susceptibility to Antibiotics and β-Lactamase Induction in Murein Hydrolase Mutants of *Escherichia coli*. Antimicrob. Agents Chemother 2005, 49, 1404–1409. [PubMed: 15793119]

(374). Pfeifle D; Janas E; Wiedemann B Role of Penicillin-binding Proteins in the Initiation of the AmpC β-Lactamase Expression in Enterobacter cloacae. Antimicrob. Agents Chemother 2000, 44, 169–172. [PubMed: 10602741]

(375). Uehara T; Park JT Role of the Murein Precursor UDP-*N*-acetylmuramyl-L-Ala-γ-D-Glu-meso-diaminopimelic acid-D-Ala-D-Ala in Repression of β-Lactamase Induction in Cell Division Mutants. J. Bacteriol 2002, 184, 4233–4239. [PubMed: 12107141]

(376). Ottolenghi AC; Ayala JA Induction of a Class I β-Lactamase from *Citrobacter freundii* in *Escherichia coli* Requires Active *ftsZ* but not *ftsA* or *ftsQ* Products. Antimicrob. Agents Chemother 1991, 35, 2359–2365. [PubMed: 1804009]

(377). Schurek KN; Wiebe R; Karlowsky JA; Rubinstein E; Hoban DJ; Zhanel GG Faropenem: Review of a New Oral Penem. Expert Rev. Anti-Infect. Ther 2007, 5, 185–198. [PubMed: 17402834]

(378). Testero SA; Fisher JF; Mobashery S β-Lactam Antibiotics In Burger's Medicinal Chemistry, Drug Discovery and Development, 7th ed.; John Wiley & Sons, 2010; Vol. 7, pp 259–404.

(379). Ambrose PG; Lomovskaya O; Griffith DC; Dudley MN; VanScoy B β-Lactamase Inhibitors: What You Really Need to Know. Curr. Opin. Pharmacol 2017, 36, 86–93. [PubMed: 29096172]

(380). Bush K Game Changers: New β-Lactamase Inhibitor Combinations Targeting Antibiotic Resistance in Gram-Negative Bacteria. ACS Infect. Dis 2018, 4, 84–87. [PubMed: 29232103]

(381). Bush K Synergistic Antibiotic Combinations In Topics in Medicinal Chemistry; Springer: Berlin, 2018; Vol. 25, pp 69–88.

(382). Bush K; Page MGP What We May Expect from Novel Antibacterial Agents in the Pipeline with Respect to Resistance and Pharmacodynamic Principles. J. Pharmacokinet. Pharmacodyn 2017, 44, 113–132. [PubMed: 28161807]

(383). El Chakhtoura NG; Saade E; Iovleva A; Yasmin M; Wilson B; Pérez F; Bonomo RA Therapies for Multidrug Resistant and Extensively Drug-resistant Non-fermenting Gram-negative Bacteria Causing Nosocomial Infections: A Perilous Journey toward 'Molecularly Targeted' Therapy. Expert Rev. Anti-Infect. Ther 2018, 16, 89–110. [PubMed: 29310479]

(384). Shlaes DM The Clinical Development of Antibacterial Drugs: A Guide for the Discovery Scientist In Topics in Medicinal Chemistry; Springer: Berline, 2018; Vol. 25, pp 149–164.

(385). Dijkmans AC; Zacarias NVO; Burggraaf J; Mouton JW; Wilms EB; van Nieuwkoop C; Touw DJ; Stevens J; Kamerling IMC Fosfomycin: Pharmacological, Clinical and Future Perspectives. Antibiotics 2017, 6, 24.

(386). Silver LL Fosfomycin: Mechanism and Resistance. Cold Spring Harbor Perspect. Med 2017, 7, a025262.

(387). Collia D; Bannister TD; Tan H; Jin S; Langaee T; Shumate J; Scampavia L; Spicer TP A Rapid Phenotypic Wholecell Screening Approach for the Identification of Small-molecule Inhibitors that Counter β-Lactamase Resistance in *Pseudomonas aeruginosa*. SLAS Discovery 2018, 23, 55–64. [PubMed: 28850797]

(388). Chan JM; Dillard JP Attention Seeker: Production, Modification, and Release of Inflammatory Peptidoglycan Fragments in *Neisseria* Species. J. Bacteriol 2017, 199, No. e00354-17.

(389). Magalhaes JG; Philpott DJ; Nahori MA; Jehanno M; Fritz J; Le Bourhis L; Viala J; Hugot JP; Giovannini M; Bertin J; et al. Murine Nod1 but Not Its Human Orthologue Mediates Innate Immune Detection of Tracheal Cytotoxin. EMBO Rep 2005, 6, 1201–1207. [PubMed: 16211083]

(390). Mallik D; Pal S; Ghosh AS Involvement of AmpG in Mediating a Dynamic Relationship between Serine β-Lactamase Induction and Biofilm Forming Ability of *Escherichia coli*. FEMS Microbiol. Lett 2018, 365, 1 DOI: 10.1093/femsle/fny065.

(391). Farha MA; Verschoor CP; Bowdish D; Brown ED Collapsing the Proton Motive Force to Identify Synergistic Combinations against *Staphylococcus aureus*. Chem. Biol 2013, 20, 1168–1178. [PubMed: 23972939]

(392). Feng X; Zhu W; Schurig-Briccio LA; Lindert S; Shoen C; Hitchings R; Li J; Wang Y; Baig N; Zhou T; Kim BK; Crick DC; Cynamon M; McCammon JA; Gennis RB; Oldfield E Antiinfectives Targeting Enzymes and the Proton Motive Force. Proc. Natl. Acad. Sci. U. S. A 2015, 112, E7073–E7082. [PubMed: 26644565]

(393). Gorityala BK; Guchhait G; Goswami S; Fernando DM; Kumar A; Zhanel GG; Schweizer F Hybrid Antibiotic Overcomes Resistance in *P. aeruginosa* by Enhancing Outer Membrane Penetration and Reducing Efflux. J. Med. Chem 2016, 59, 8441–8455. [PubMed: 27524179]

(394). Farha MA; French S; Stokes JM; Brown ED Bicarbonate Alters Bacterial Susceptibility to Antibiotics by Targeting the Proton Motive Force. ACS Infect. Dis 2018, 4, 382–390. [PubMed: 29264917]

(395). Horsman ME; Marous DR; Li R; Oliver RA; Byun B; Emrich SJ; Boggess B; Townsend CA; Mobashery S Whole-Genome Shotgun Sequencing of Two β-Proteobacterial Species in Search of the Bulgecin Biosynthetic Cluster. ACS Chem. Biol 2017, 12, 2552–2557. [PubMed: 28937735]

(396). Skalweit MJ; Li M Bulgecin A as a β-Lactam Enhancer for Carbapenem-resistant *Pseudomonas aeruginosa* and Carbapenem-resistant *Acinetobacter baumannii* Clinical Isolates Containing Various Resistance Mechanisms. Drug Des. Dev. Ther 2016, 10, 3013–3020.

(397). Williams AH; Wheeler R; Thiriau C; Haouz A; Taha MK; Boneca IG Bulgecin A: The Key to a Broad-Spectrum Inhibitor that Targets Lytic Transglycosylases. Antibiotics 2017, 6, 8.

(398). Oliver RA; Li R; Townsend CA Monobactam Formation in Sulfazecin by a Nonribosomal Peptide Synthetase Thioesterase. Nat. Chem. Biol 2017, 14, 5–7. [PubMed: 29155429]

(399). Imada A; Kintaka K; Nakao M; Shinagawa S Bulgecin, a Bacterial Metabolite which in Concert with β-Lactam Antibiotics Causes Bulge Formation. J. Antibiot 1982, 35, 1400–1403. [PubMed: 6757229]

(400). Lamers RP; Nguyen UT; Nguyen Y; Buensuceso RN; Burrows LL Loss of Membrane-bound Lytic Transglycosylases Increases Outer Membrane Permeability and β-Lactam Sensitivity in *Pseudomonas aeruginosa*. MicrobiologyOpen 2015, 4, 879–895. [PubMed: 26374494]

(401). Yin J; Sun Y; Sun Y; Yu Z; Qiu J; Gao H Deletion of Lytic Transglycosylases Increases β-Lactam Resistance in Shewanella oneidensis. Front. Microbiol 2018, 9, 13. [PubMed: 29403465]

(402). Fraile-Ribot PA; Cabot G; Mulet X; Perianez L; Martin-Pena ML; Juan C; Pérez JL; Oliver A Mechanisms Leading to in vivo Ceftolozane/Tazobactam Resistance Development During the Treatment of Infections caused by MDR *Pseudomonas aeruginosa*. J. Antimicrob. Chemother 2018, 73, 658–683. [PubMed: 29149337]

(403). Treepong P; Kos VN; Guyeux C; Blanc DS; Bertrand X; Valot B; Hocquet D Global Emergence of the Widespread *Pseudomonas aeruginosa* ST235 Clone. Clin. Microbiol. Infect 2018, 24, 258–266. [PubMed: 28648860]

(404). Luan Y; Li GL; Duo LB; Wang WP; Wang CY; Zhang HG; He F; He X; Chen SJ; Luo DT DHA-1 Plasmid-mediated AmpC β-Lactamase Expression and Regulation of *Klebsiella pneumoniae* Isolates. Mol. Med. Rep 2015, 11, 3069–3077. [PubMed: 25483576]

(405). Nakano R; Nakano A; Yano H; Okamoto R Role of AmpR in the High Expression of the Plasmid-Encoded AmpC β-Lactamase CFE-1. mSphere 2017, 2, e00192–17. [PubMed: 28808689]

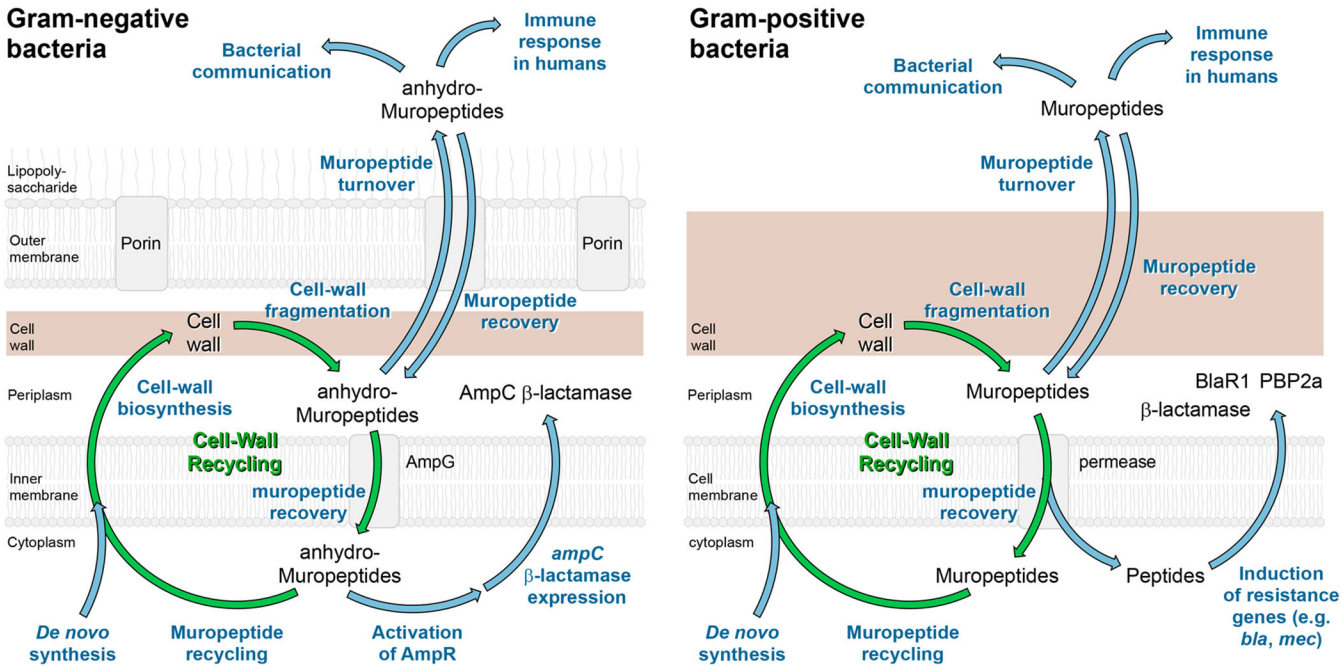


Figure 1.

A comparison of Gram-negative and Gram-positive cell-wall flux. The events of cell-wall biosynthesis, recycling, and turnover are depicted. In rod-shaped Gram-negative bacteria, the PG of the cell wall is found within the periplasm. It has perhaps a single monolayer thickness in its sidewall, and a bi or trilayer thickness at the septa. In contrast, the Gram-positive PG is a multilayered exoskeleton.[4] Together, de novo cell wall and recycled cell wall are manufactured in the cytoplasm and in final form as lipid II are translocated to the periplasm, where they experience a diverse array of structural modification to accommodate many biological pathways. In both types of bacteria, although less frequent in Gram-positive bacteria, the cell wall is recycled. Adapted with permission from ref 5. Copyright 2013 New York Academy of Sciences.

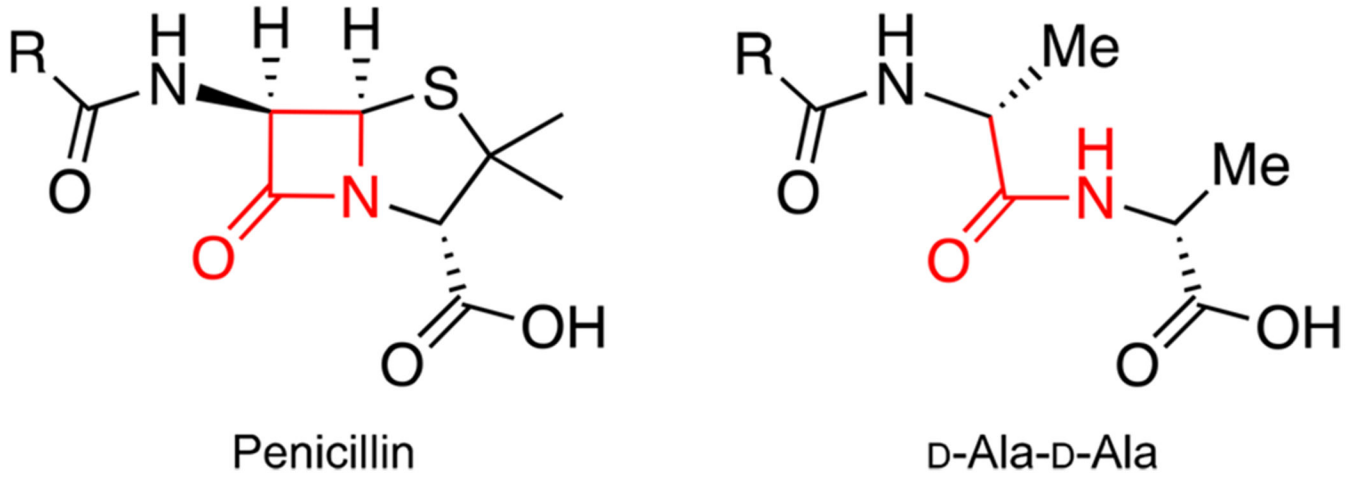


Figure 2.

β-Lactam antibiotics mimic the native acyl-D-alanine-D-alanine substrate of the PBP enzymes. The *β*-lactam ring of the penicillin (left) and the central amide bond of the D-Ala-D-Ala peptide (right) are shown in red.

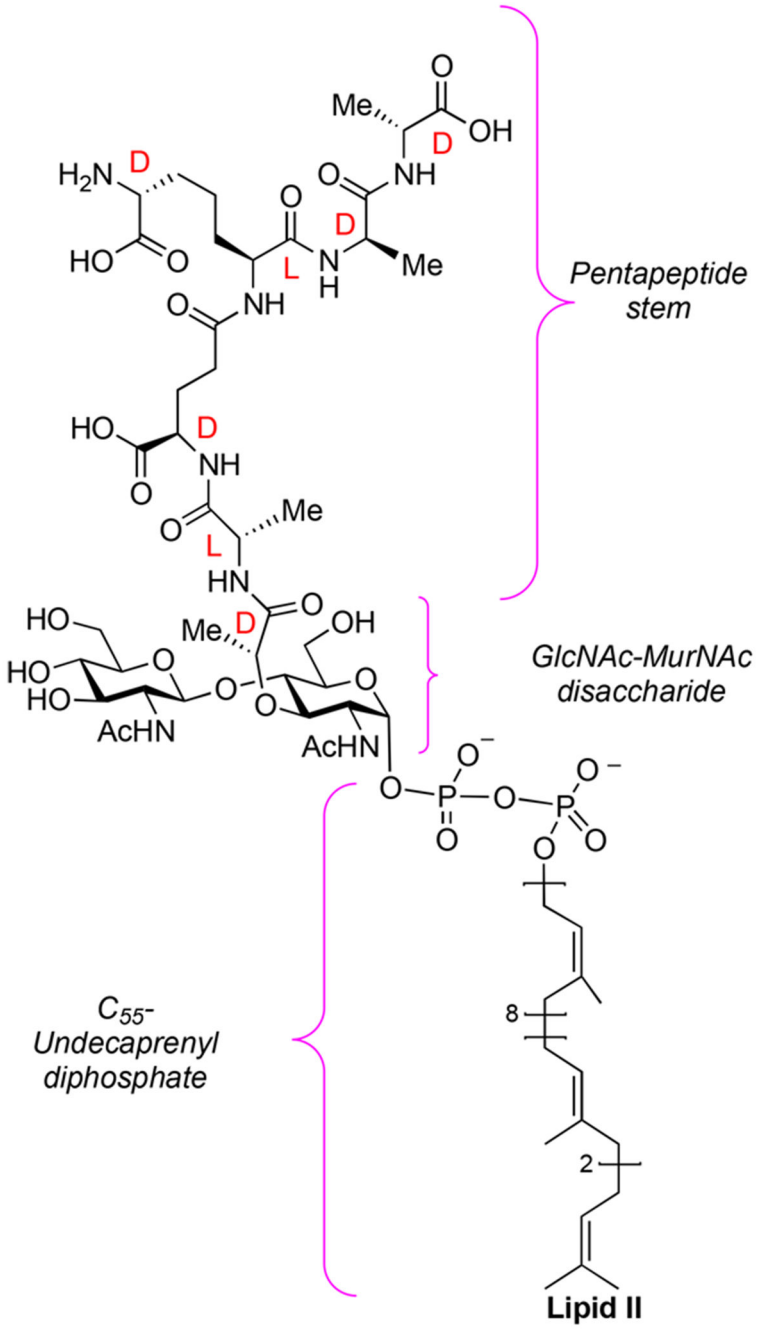


Figure 3.

Structure of lipid II (undecaprenyl diphosphate-MurNAc-pentapeptide-GlcNAc) of the Gram-negative bacterium. The stereochemistry of the lactyl moiety of the MurNAc saccharide, and of each of the stereogenic carbons of the amino acids of the peptide stem of the MurNAc (L or D), is shown by the red-colored labels.

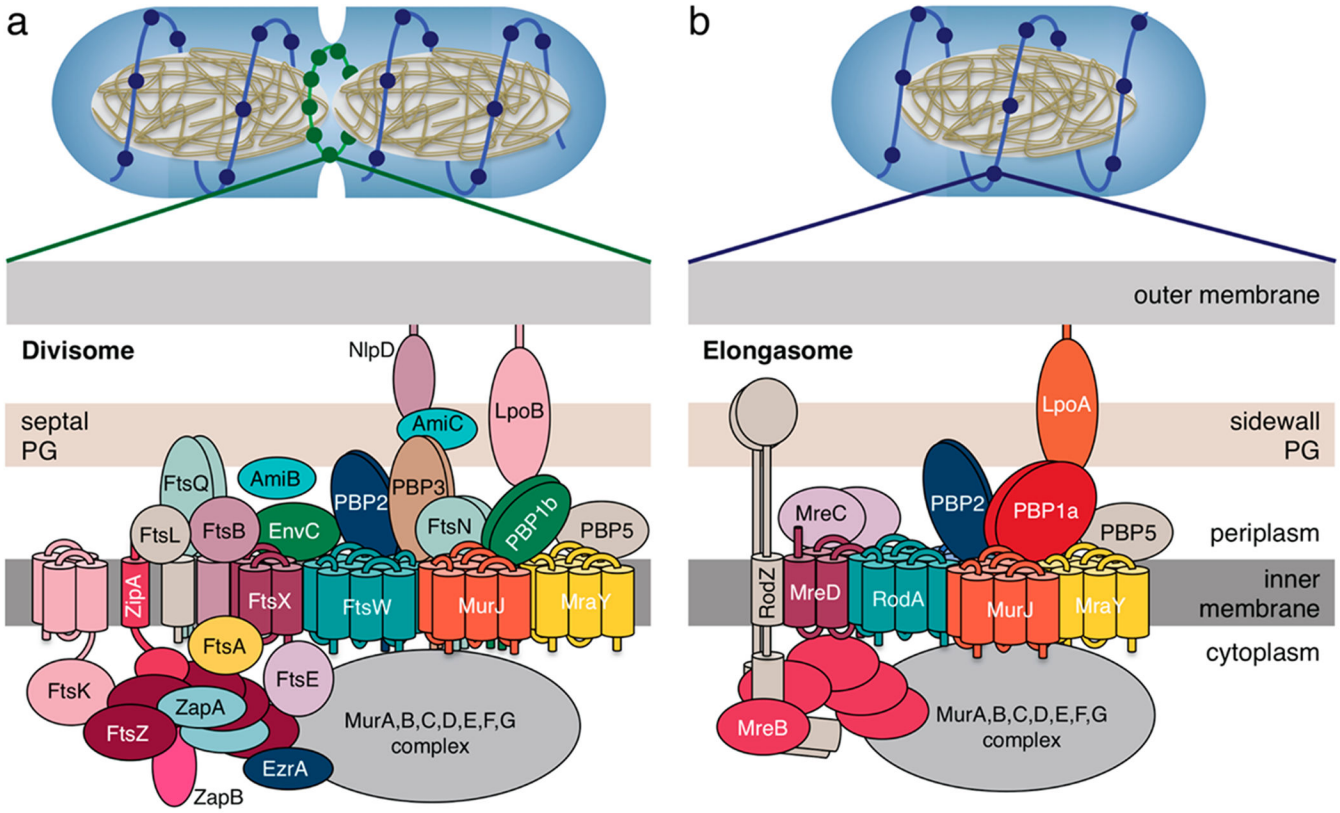


Figure 4.

(a) The divisome protein complex forms at the midcell and facilitates septation. The divisome tracks along the midcell (see green line). (b) The elongasome protein complex forms at the lateral sidewalls and facilitates sidewall expansion. The elongasome tracks along the lateral sidewall (see blue line). Notably absent from modern interpretations of these systems are the lytic transglycosylases, whose participation, although certain, remains undefined. Adapted with permission from ref 152. Copyright 2016 eLife Sciences Publications (https://creativecommons.org/licenses/by/4.0/).

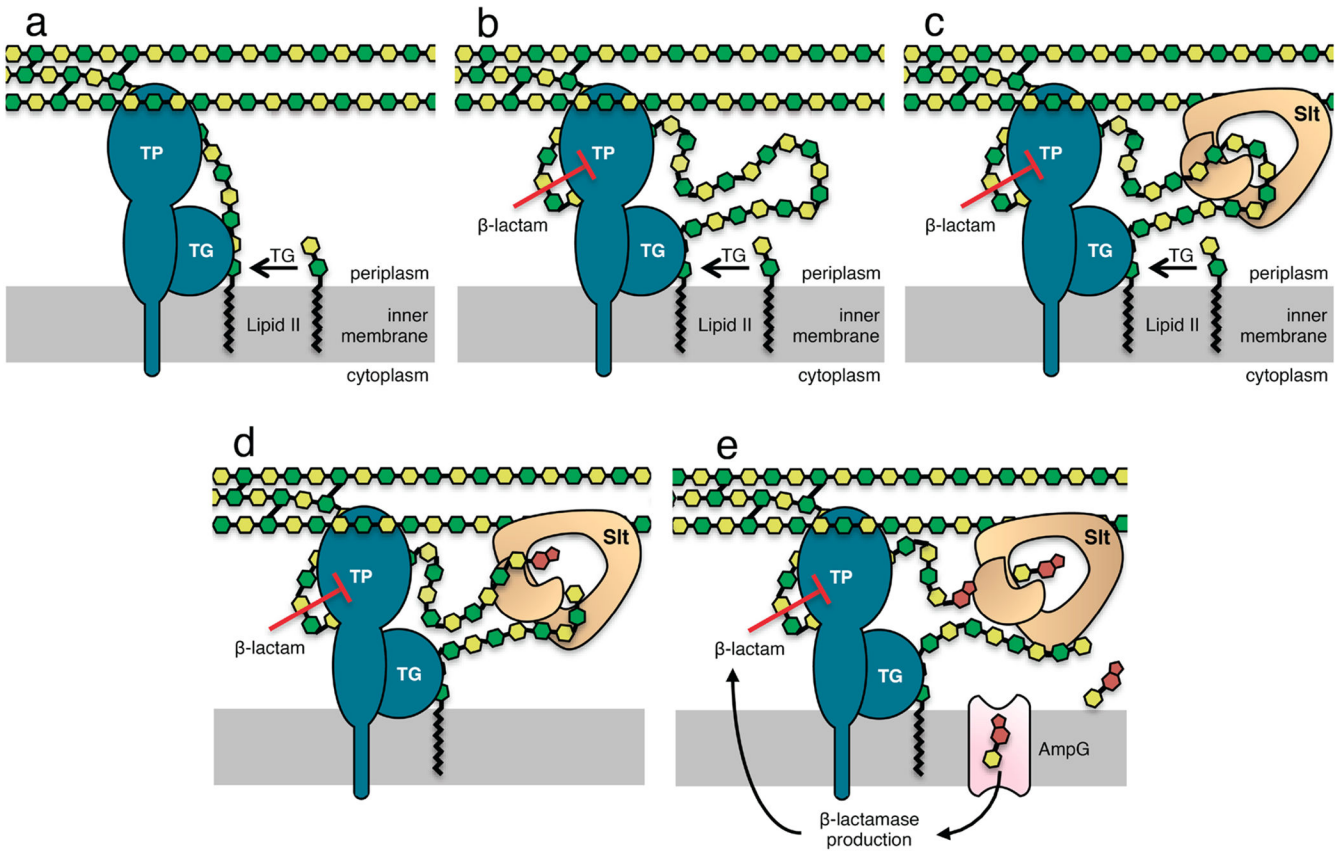


Figure 5.

(a) PBPs elongate nascent PG (TG, transglycosylase domain) and incorporate it into the PG macromolecule (TP, transpeptidase domain). (b) *β*-Lactam antibiotics inhibit the incorporation of nascent PG (GlcNAc, yellow hexagon; MurNAc, green hexagon; anhMurNAc, red hexagon) into the cell wall, resulting in an aberrant form shown. (c–e) The aberrant PG might be misincorporated, resulting in the inevitable death of the bacterium. Slt degrades the nascent aberrant PG in an attempt at onset of repair to protect the bacterium.[297] Adapted with permission from ref 261. Copyright 2018 United States National Academy of Sciences.

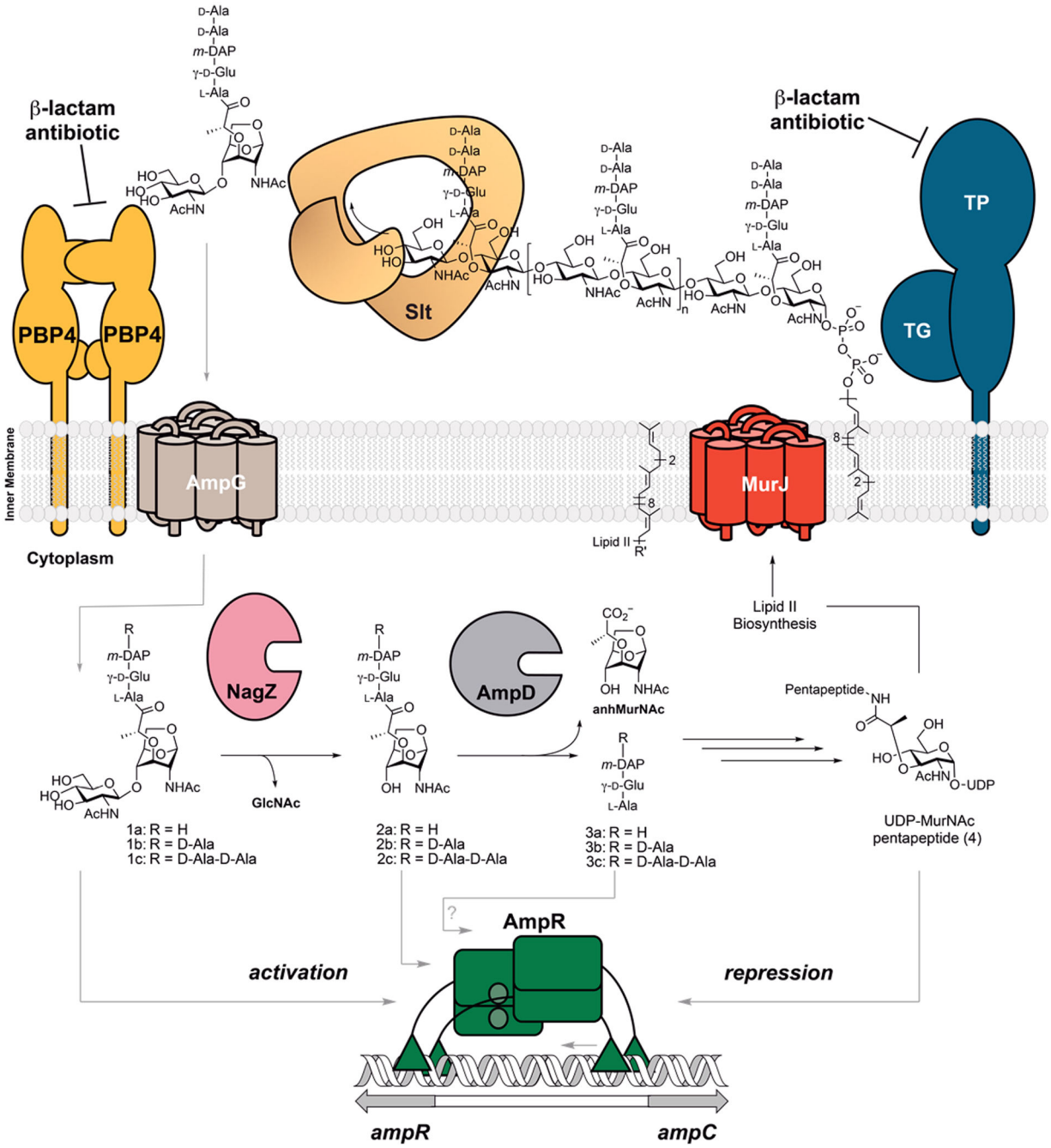


Figure 6.

β-Lactam antibiotics inhibit the transpeptidase (TP) domain of the PBPs. The unhindered transglycosylase (TG) domain of bifunctional HMM PBPs continues the lengthening of the nascent PG chain. Nascent PG retains a pentapeptide stem. Slt cleaves the accumulated nascent PG as an effort toward repair. Slt-liberated PG is transported to the cytoplasm by the AmpG permease, where in current form as GlcNAc-1,6-anhMurNAc-pentapeptide (**1c**) it activates AmpR. NagZ recognizes compound **1c** as a substrate and catalyzes the formation of 1,6-anhMurNAc (**2c**), which also functions to activate AmpR. Both AmpR activators are

substrates for AmpD, which catalyzes the formation of recycling intermediates GlcNAc and free pentapeptide (**3c**). GlcNAc and **3c** are recycled by the bacterium in an effort to make new cell wall. Cell-wall precursor UDP-MurNAc-pentapeptides (**4**) is the AmpR repressor and exists in both homeostasis and β-lactam challenge. The increased concentration of **1c** or **2c** sufficiently displaces homeostatic concentration of **4** during β-lactam challenge, triggering AmpR activation and β-lactamase transcription.

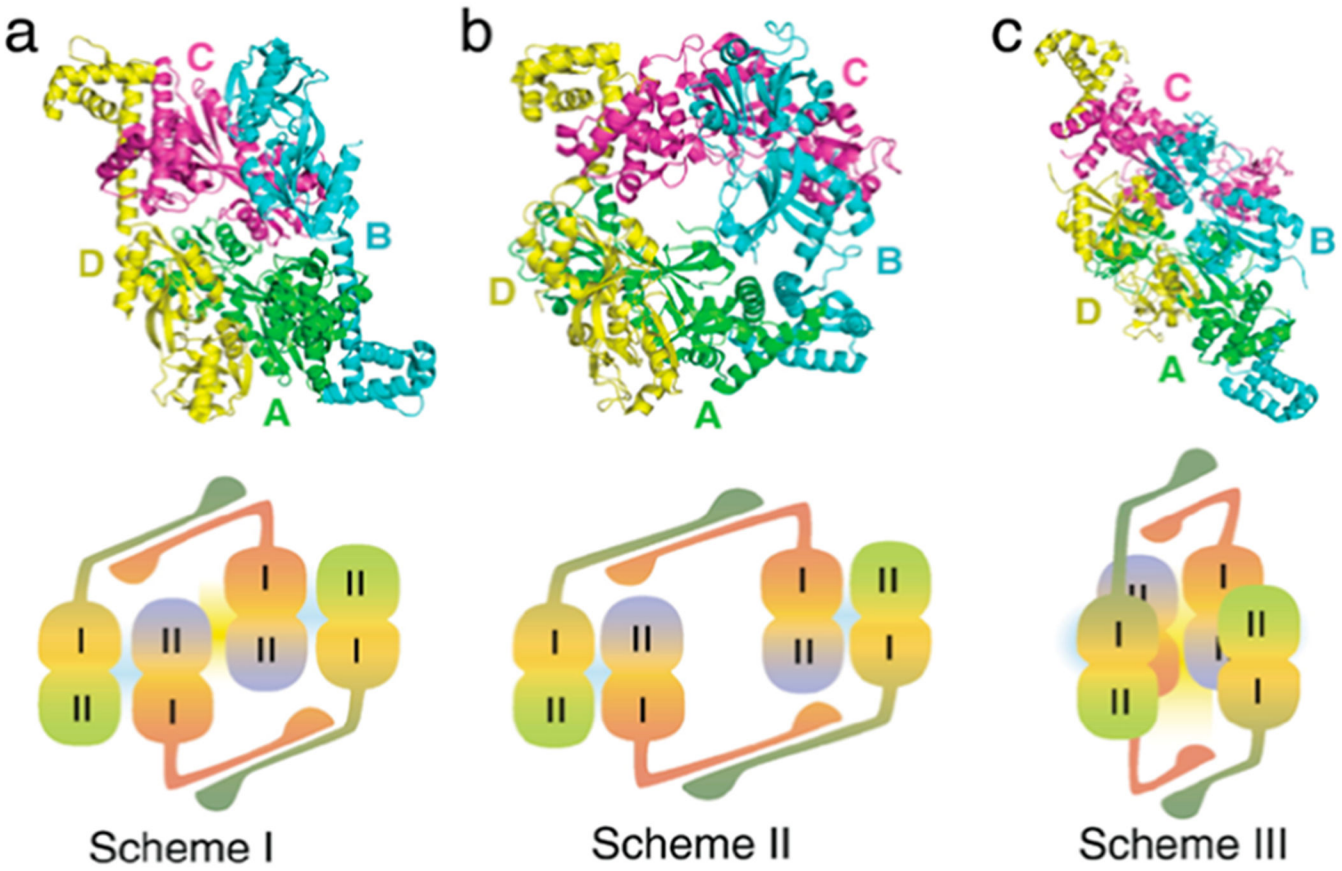


Figure 7.

Architecture of LTTR oligomers, as revealed in X-ray structure determinations, adopts distinct conformations, which are defined by a three-scheme classification. Each full-length LTTR comprises a DNA-binding domain, a region of difference I effector-binding domain subdomain (I), and a region of difference II effectorbinding domain subdomain (II). (a) Scheme I identifies LTTRs with weak interactions of the α10–α10 (yellow highlight) region as a result of an offset arrangement of the effector-binding domains. (b) Scheme II identifies LTTRs with separated α10–α10 regions. (c) Scheme III identifies LTTRs with strong interactions of the α10–α10 region (yellow highlight) as a result of extensive surface interactions of the effector-binding domains. Reproduced with permission from ref 330.

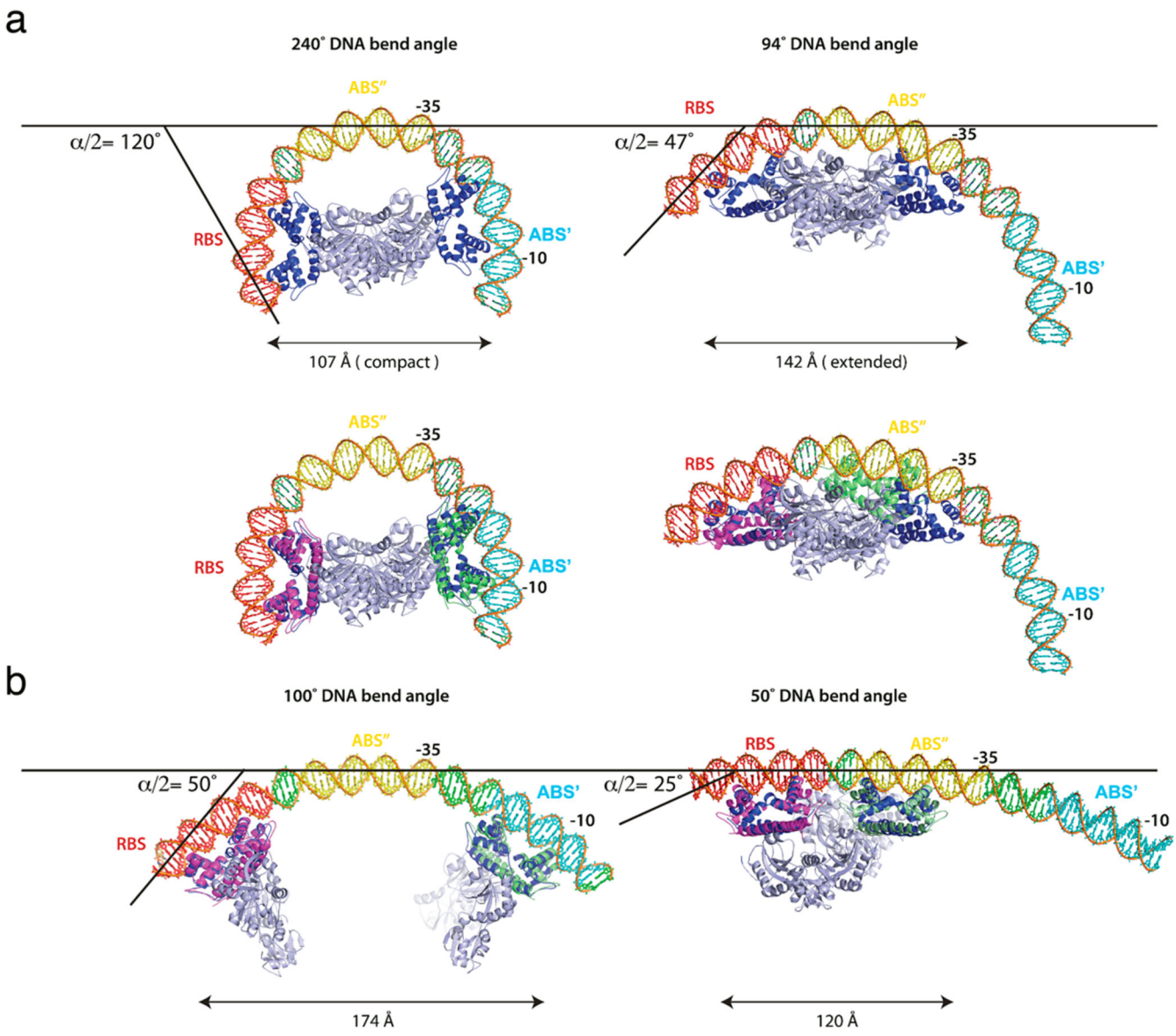
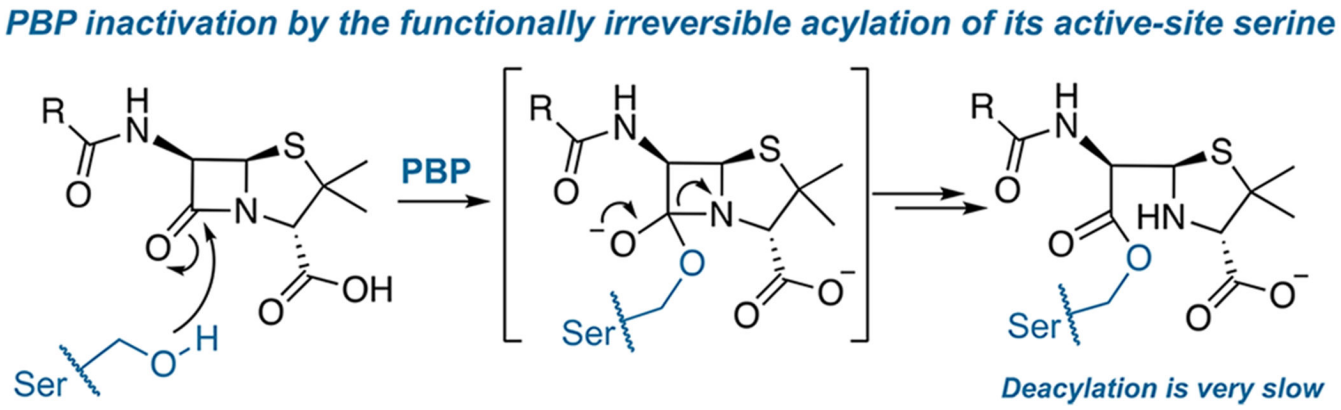


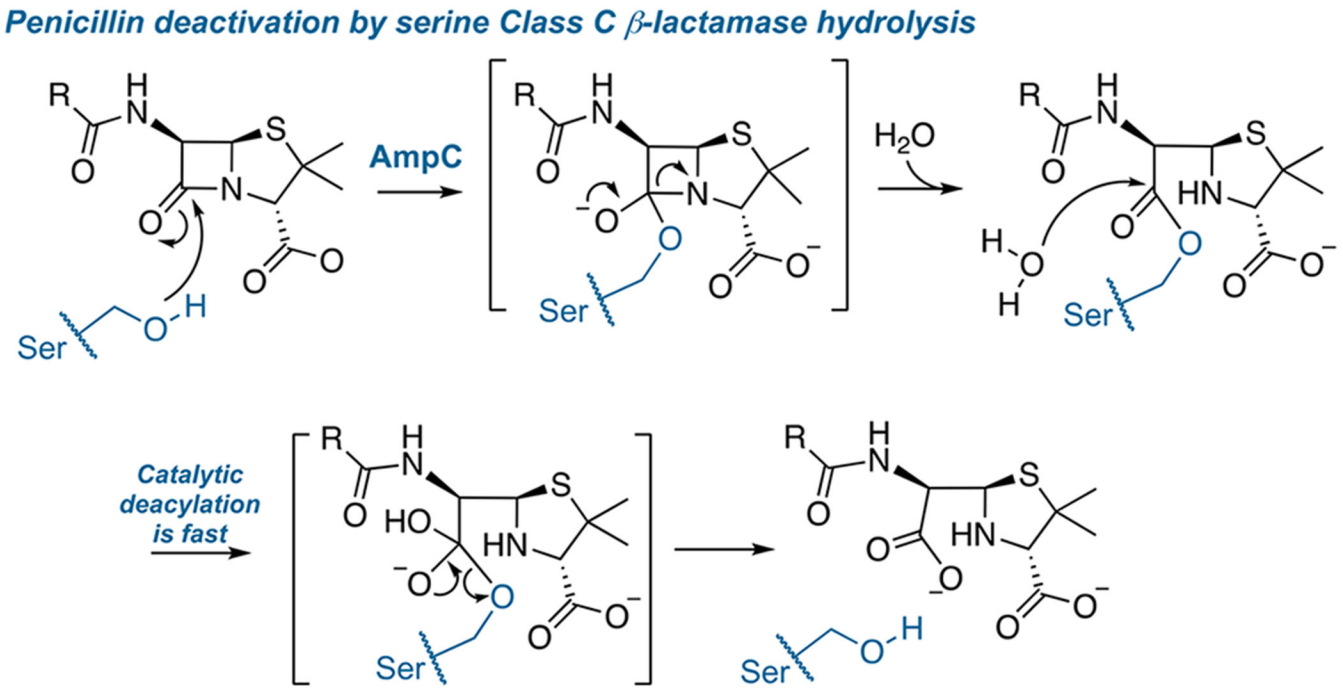
Figure 8.

Full-length structure of DNTR was solved by small-angle X-ray scattering (SAX) and a model was proposed by which DNTR could bind its requisite DNA In a repressed compact conformation, DNTR binds to the RBS and ABS′. In an activated extended conformation, DNTR binds RBS and ABS″. (a) The model of DNTR is depicted, whereby the requisite DNA bends at a 240° angle in the repressed conformation and is relaxed to a 94° angle in the active conformation. Importantly it has been surmised that not all LTTRs bend DNA to the same degree. (b) A second model is shown that depicts how an LTTR could bind to its requisite DNA, if the DNA was bent to a lesser degree. In this model, the repressed conformation bends DNA at a 100° angle, and the active conformation bends DNA at a 50° angle. This model stipulates that the dimers of the LTTR tetramer cannot interact in the repressed conformation. Reproduced with permission from ref 346. (https://creativecommons.org/licenses/by/4.0/). Image courtesy of Prof. Gordon A. Leonard.



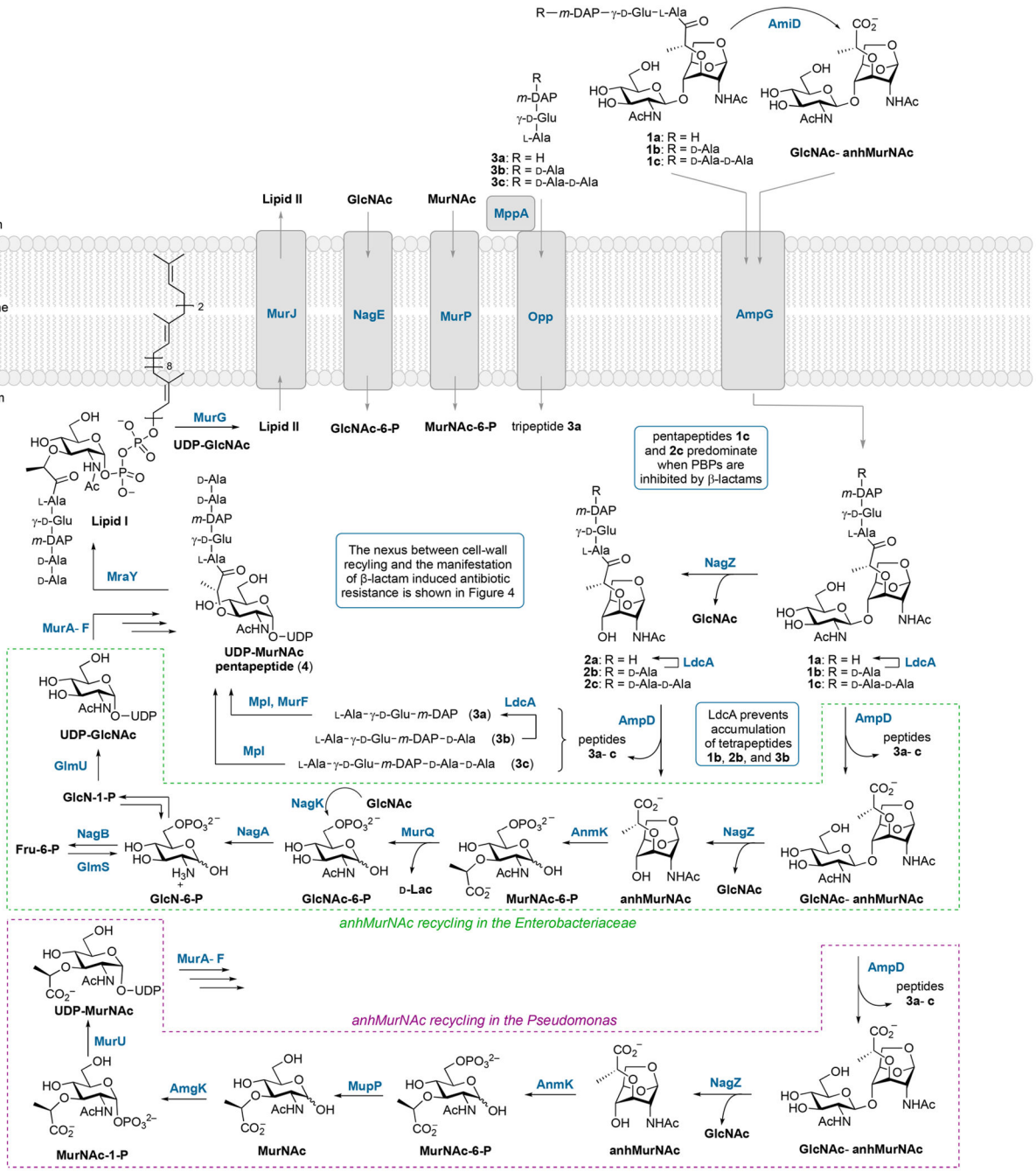
Scheme 1. PBP Inactivation by β-Lactam Acylation of the Catalytic Serine Residue of the PBP[a]

[a] Although the resulting acyl-enzyme eventually could undergo hydrolysis, restoring the active PBP, the time scale for this hydrolysis well exceeds that of the viability of the bacterium.



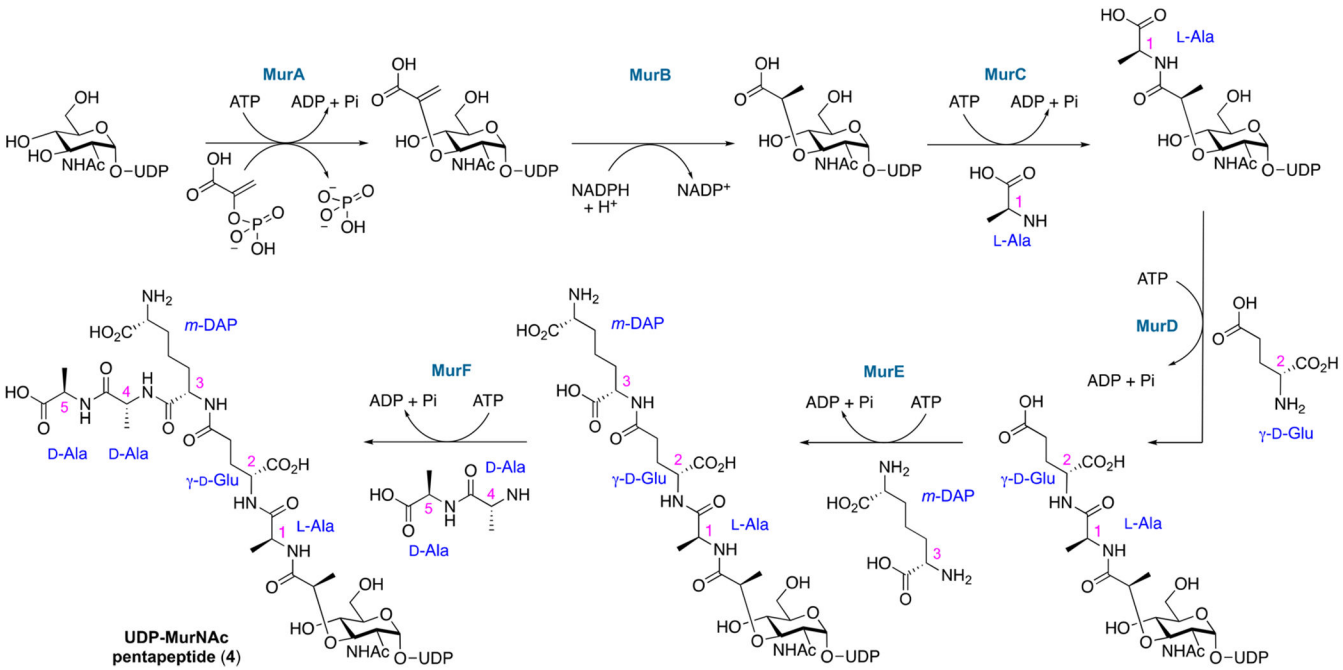
Scheme 2.

Catalytic Deactivation of the β-Lactam Antibiotic (Here Represented As a Penicillin) by β-Lactamase (Here Represented As a Class C Serine-Dependent Enzyme) by Hydrolytic Opening of Its β-Lactam Ring.



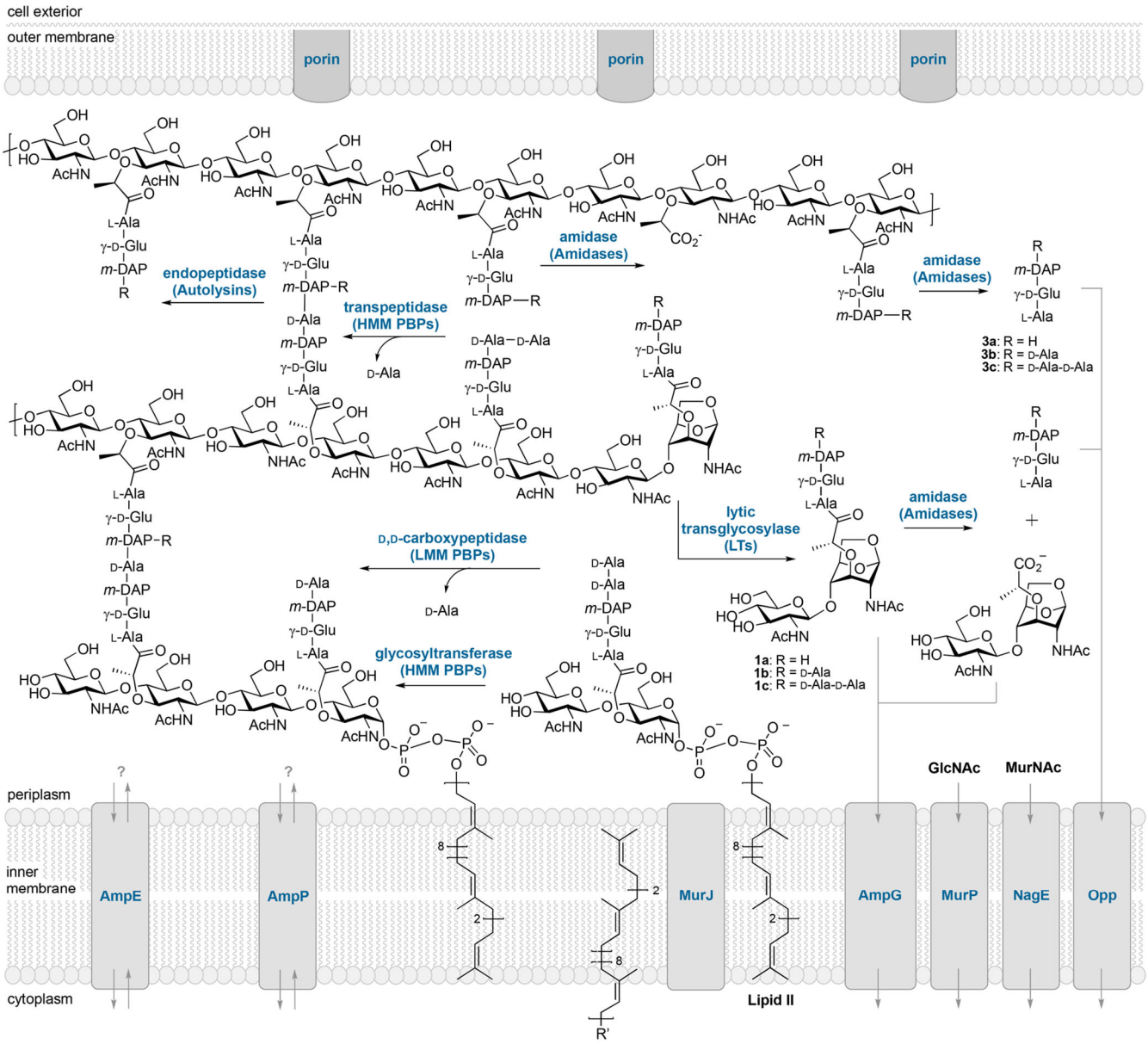
Scheme 3. Turnover of the Gram-Negative Cell-Wall Muropeptides Is Shown[a]

[a]The disaccharide is disassembled by a NagZ glucosaminidase, and the peptide stem is separated by the AmpD amidase. Turnover of cell-wall saccharides is an independent pathway from that of peptide turnover. The resulting saccharide pool, coupled with de novo synthesized saccharides, undergo biosynthesis culminating in the cell-wall precursor lipid II. Notably, the lipid II precursor UDP-MurNAc pentapeptide (**4**) and the cell-wall recycled muropeptides GlcNAc-1,6-anhMurNAc pentapeptide (**1c**) and 1,6-anhMurNAc pentapeptide (**2c**) serve as important effectors in the regulation of antibiotic resistance.



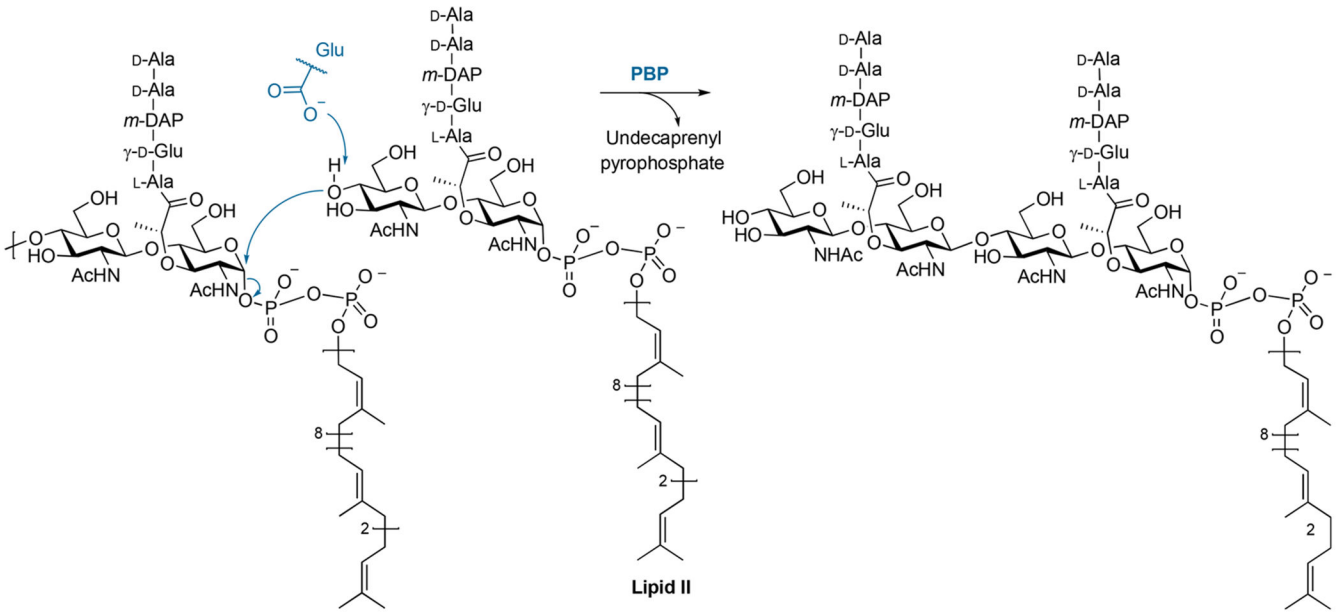
Scheme 4.

Biosynthetic Transformations (MurA–MurF) in the Mur Pathway Leading from UDP-GlcNAc to UDP-MurNAc-pentapeptide (4)



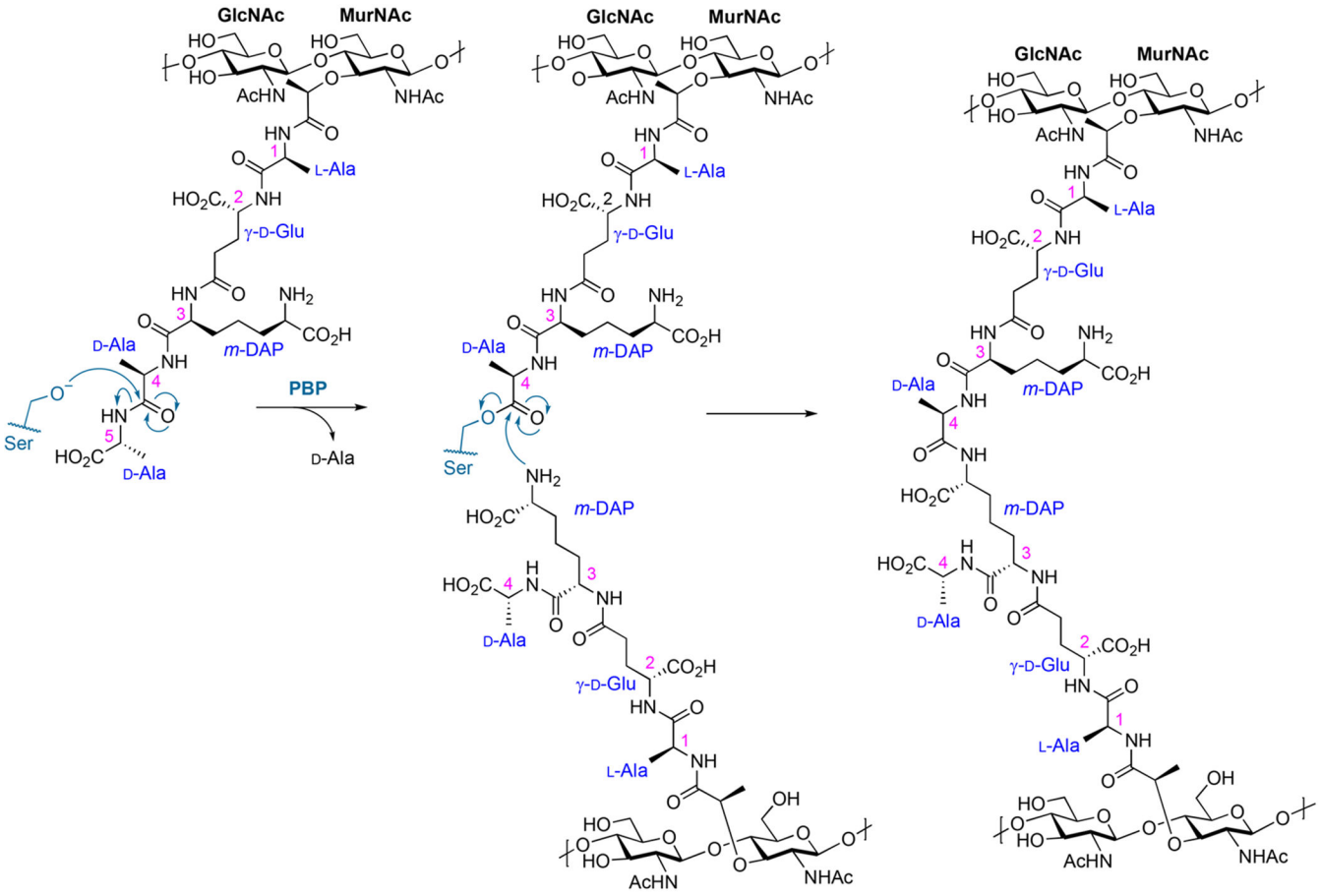
Scheme 5. Biosynthesis, Modifications, and Degradation of the Gram-Negative Cell-Wall Macromolecule[a]

[a]PBP glycosyltransferases assemble Lipid II by accretion. The nascent PG chain exists in pentapeptide form. PBP carboxypeptidases cleave the terminal D-Ala of the peptide stem in an effort to regulate the degree of cell-wall crosslinking. PBP transpeptidases crosslink nascent PG to the cell-wall macromolecule in a process that allows for selective incorporation of new PG at specific sites. PBPs and amidases modify the PG to accommodate various biological events including pili or flagellum formation, secretion systems assembly, elongation, and division. The lytic transglycosylases cleave PG in a unique reaction that forms a 1,6-anhydromuropeptide. These muropeptides are substrates for the AmpG permease, which transports muropeptides to the cytoplasm for recycling.



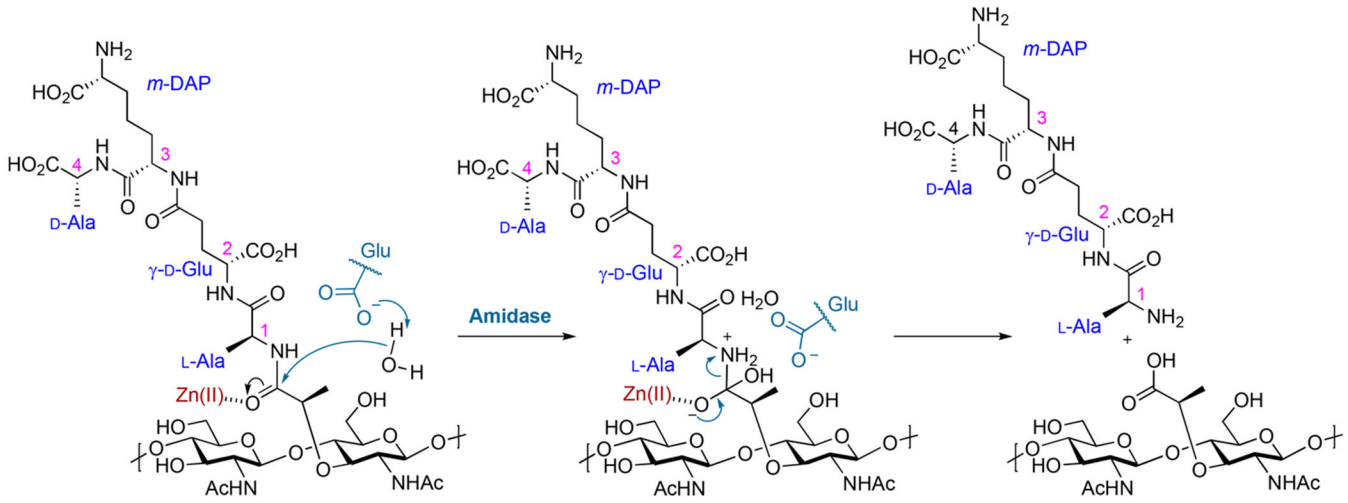
Scheme 6. Glycosyltransferase Reaction Whereby Nascent PG Chain Is Formed by Catalysis of Adjacent Lipid II Molecules[a]

[a]Bifunctional HMM PBPs, RodA, and FtsW perform this reaction, which subsequently releases the lipid II acceptor strand from the membrane. The chemistry likely involves the formation of a reactive oxocarbenium species at the anomeric carbon of MurNAc (not depicted), as either an intermediate or transition state.



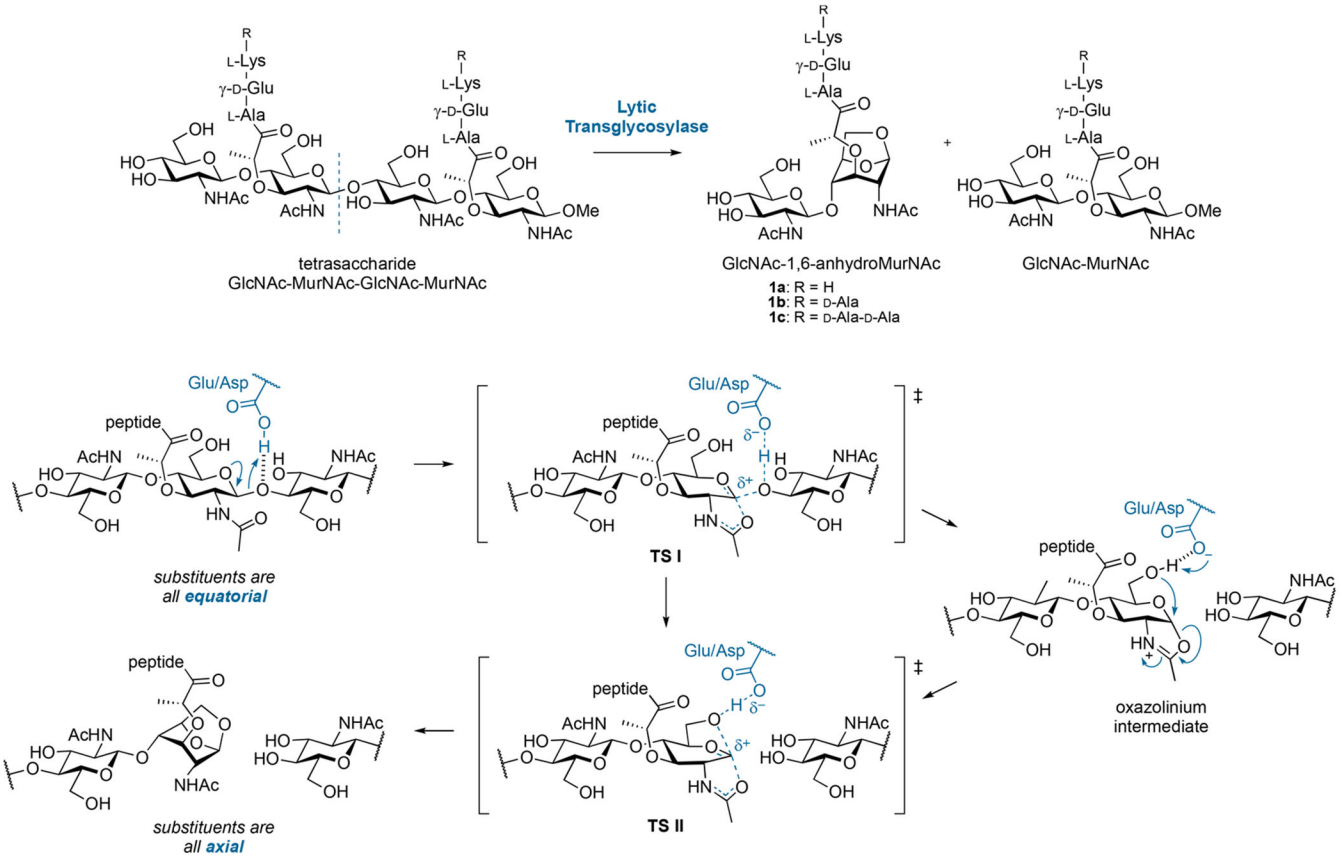
Scheme 7. Crosslinking of the Cell-Wall Macromolecule Is an Essential Step in Cell-Wall Biosynthesis[a]

[a]Inhibition of this process, by the β-lactam antibiotic, leads to lysis of the bacterium. The crosslinking reaction shown is a 4,3-crosslink, although 3,3-crosslinks also form. Monofunctional and bifunctional HMM PBPs crosslink the cell-wall polymer.



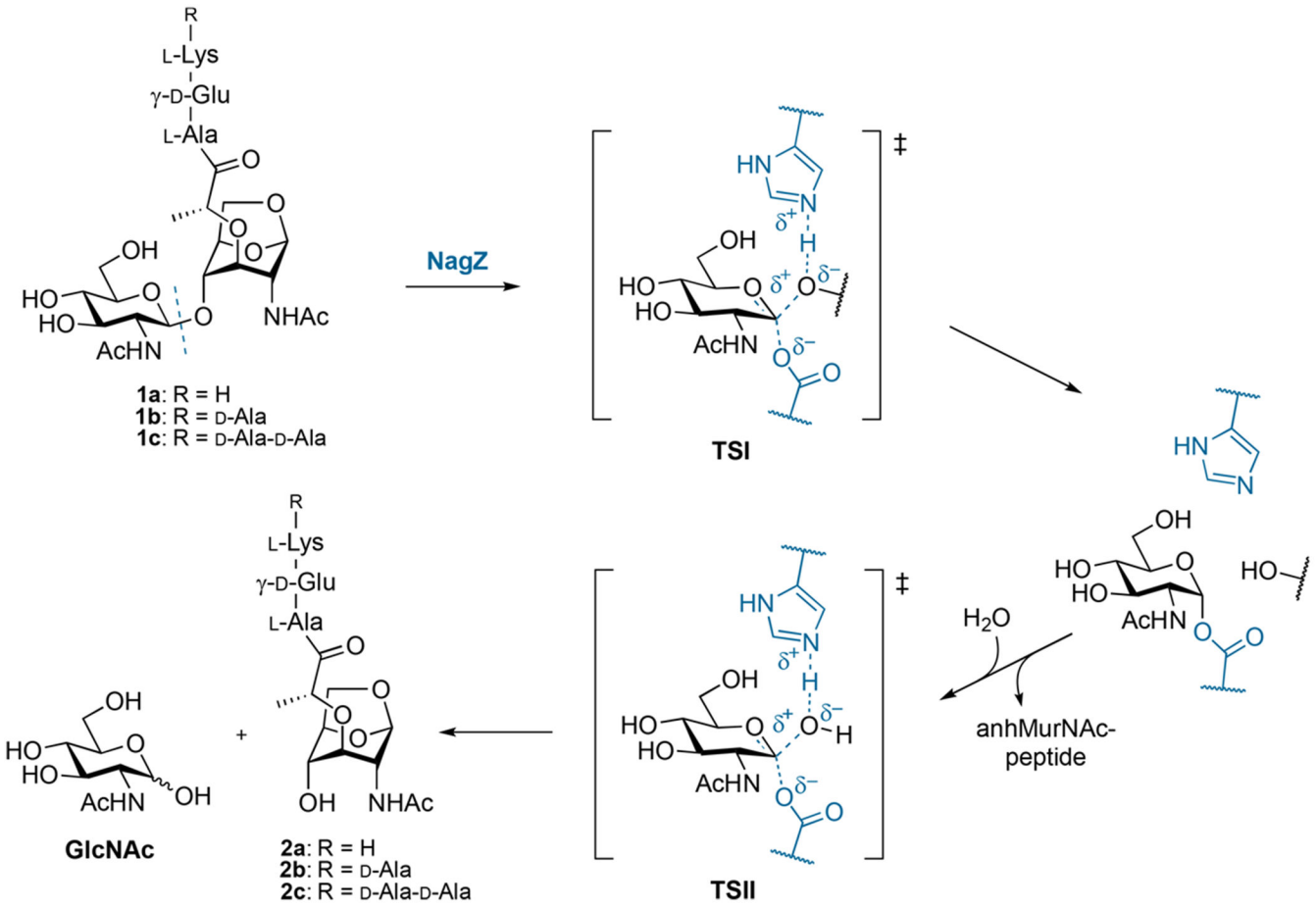
Scheme 8. Amidases Catalyze Hydrolysis of the Stem Peptide of the Muropeptides, and Produce Free Peptide and Denuded (or "Naked") Glycan As Products[a]

[a]Denuded cell wall in the periplasm at the septum is an important component of cell division. While in the cytoplasm, amide hydrolysis is an essential step in muropeptide turnover.



Scheme 9. LTs Catalyze a Unique Non-Hydrolytic Transacetalization Reaction That Cleaves the MurNAc-GlcNAc β-(1,4)-Glycosidic Bond and Converts MurNAc to 1,6-anhMurNAc[a]

[a]The products of LT reactions are specific substrates for the AmpG permease, which allows for cytoplasmic transport and subsequent recycling. The formation of the discrete oxazolinium intermediate might not be relevant for all LTs.[252]



Scheme 10. Turnover of GlcNAc-anhMurNAc Involves an Aspartic Acid Covalent Intermediate, Two Transition State Species Each Invoking an Oxocarbenium, and a Dramatic Conformational Protein Rearrangement[a]

[a] The reaction results in the hydrolysis of the GlcNAc-MurNAc β-(1,4)-glycosidic bond.