Chapter 5
Peptidoglycan

Manuel Pazos and Katharina Peters

Abstract The peptidoglycan sacculus is a net-like polymer that surrounds the cytoplasmic membrane in most bacteria. It is essential to maintain the bacterial cell shape and protect from turgor. The peptidoglycan has a basic composition, common to all bacteria, with species-specific variations that can modify its biophysical properties or the pathogenicity of the bacteria. The synthesis of peptidoglycan starts in the cytoplasm and the precursor lipid II is flipped across the cytoplasmic membrane. The new peptidoglycan strands are synthesised and incorporated into the pre-existing sacculus by the coordinated activities of peptidoglycan synthases and hydrolases. In the model organism *Escherichia coli* there are two complexes required for the elongation and division. Each of them is regulated by different proteins from both the cytoplasmic and periplasmic sides that ensure the well-coordinated synthesis of new peptidoglycan.

Keywords Peptidoglycan · Sacculus · Lipid II · PBPs · LDTs · TPase · GTase · Glycan strands · Cross-linking · Elongation · Septation

Introduction

The peptidoglycan (PG) sacculus is an elastic and net-like polymer that surrounds the cytoplasmic membrane in most bacteria. It is rigid enough to maintain the species-specific bacterial cell shape, serving as a scaffold to attach proteins and other polymers, but also porous enough to allow the diffusion of chemical signals, nutrients and virulence factors. The PG protects the cell from bursting due to its turgor, which pushes the cytoplasmic membrane towards the cell wall. In Gram-negative bacteria, a thin and single PG layer is located in the periplasm surrounding the cytoplasmic

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membrane. In contrast, the PG in Gram-positive bacteria is thicker and multi-layered with covalently attached cell wall compounds like capsular polysaccharides, cell surface proteins and wall teichoic acids (Fig. 5.1). Bacteria belonging to Rickettsiaceae, Anaplasmataceae and Mycoplasmataceae families do not have PG. The presence of PG in Chlamydiaceae has been proven (Pilhofer et al. 2013; Packiam et al. 2015; Liechti et al. 2016). In order to preserve the structural integrity of the cell envelope during cell growth and division, the synthetic and hydrolytic PG enzymes must be coordinated to enlarge and divide the sacculi. According to the current model this coordination is achieved by multi-enzyme complexes that extend from the cytoplasm to the outer membrane.

This chapter is mainly focused on the model organism *Escherichia coli*, but the reader will find references to other organisms along the different sections. After describing the PG composition, modifications and its biophysical properties, the different enzymatic activities and their regulation are presented in the cellular context. The importance of the fluorescent D-amino acids for the field is highlighted, and a detailed description of the current understanding of the PG substrate flippase candidates and the unusual LD-transpeptidases (LDTs) is included.

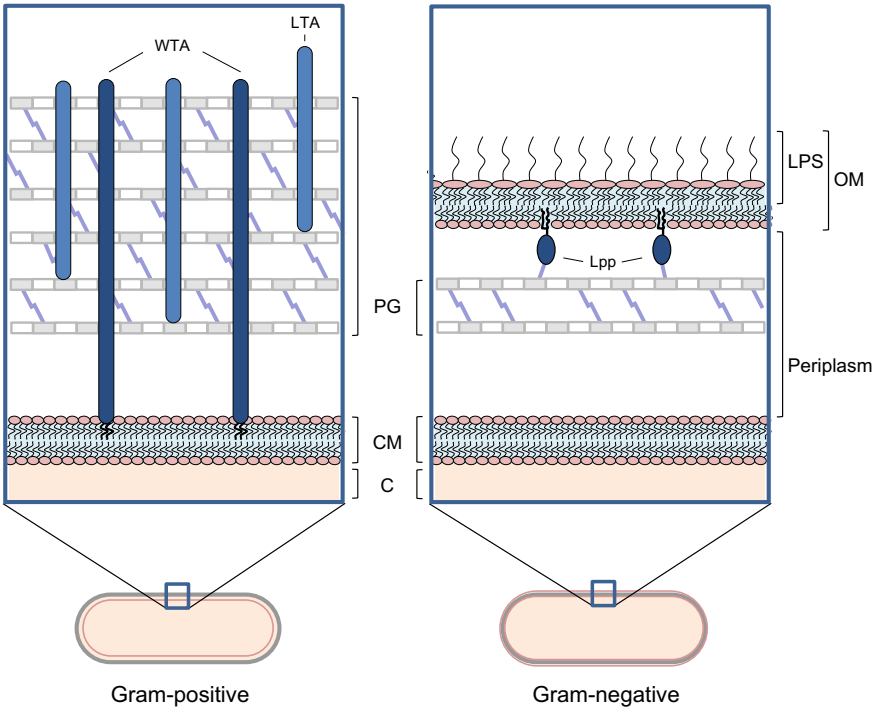

Fig. 5.1 Schematic structure of the cell wall in Gram-positive and Gram-negative bacteria. C, cytoplasm; CM, cytoplasmic membrane; PG, peptidoglycan; OM, outer membrane; LPS, lipopolysaccharide; LTA, lipoteichoic acid; WTA, wall teichoic acid; Lpp, Braun's lipoprotein

The Peptidoglycan Composition, Its Modifications and Their Function

The PG sacculus is an essential macromolecule exclusively found in bacteria. Its basic structure comprises linear glycan strands that are cross-linked by short peptides (Schleifer and Kandler 1972). The glycan strands, whose length varies between species, are made out of alternating β-1,4-connected *N*-acetylglucosamine (Glc*N*Ac) and *N*-acetylmuramic acid (Mur*N*Ac) residues. Mur*N*Ac is a lactic acid ether derivative of Glc*N*Ac, and the stem pentapeptides are attached to its D-lactyl moiety at position C3. The stem pentapeptides contain L- and D-amino acids, whereby the latter is characteristic of bacterial PG. The PG structure shows high diversity between different species, but it is well conserved in bacteria belonging to the same species. Modifications in the PG can enhance the bacterial fitness and virulence, providing resistance against environmental stresses, hydrolytic host enzymes and antimicrobial agents.

Variations in the Stem Peptide

The amino acid composition of the pentapeptide shows species-specific variations. Some examples are shown in Fig. 5.2 and described in the following lines. In Gram-negative bacteria like *E. coli* the pentapeptide in nascent PG consists of L-Ala-D-iGlu-mDAP-D-Ala-D-Ala (mDAP: *meso*-diaminopimelic acid). Two adjacent peptides are more frequently cross-linked via an amide bond between the carboxyl group of the D-Ala at position four of one peptide and the ε-amino group of mDAP at position three of another one. The D-Ala-D-Ala motif of the pentapeptide is the universal substrate of the PG cross-linking enzymes. The terminal D-Ala is released during the cross-linking reaction.

In *Mycobacterium leprae* the first amino acid of the stem peptide is Gly instead of L-Ala. This modification is suggested to be due to the growth environment (Mahapatra et al. 2000). In the Gram-positive pathogens *Streptococcus pneumoniae* and *Staphylococcus aureus*, and in *Mycobacterium tuberculosis* the α-carboxyl group of the D-iso-glutamate on position two of the stem peptide is amidated into D-iso-glutamine by the essential amidotransferase complex MurT/GatD (Zapun et al. 2013; Morlot et al. 2018; Münch et al. 2012; Figueiredo et al. 2012). In *S. pneumoniae* unamidated glutamate is predominantly found in uncross-linked PG monomers (Bui et al. 2012). In vitro assays using recombinant pneumococcal PBPs revealed that the amidation of the stem peptides is needed for an efficient cross-linking reaction (Zapun et al. 2013). In *S. aureus* the lack of amidation correlates with a decrease in cross-linking and with a higher susceptibility to antibiotics (Stranden et al. 1997; Boyle-Vavra et al. 2001; Figueiredo et al. 2012). Thus, understanding the molecular mechanism of the amidation reaction may help in the development of new therapeutics to target these important pathogens. Recent studies provide biochemical and structural

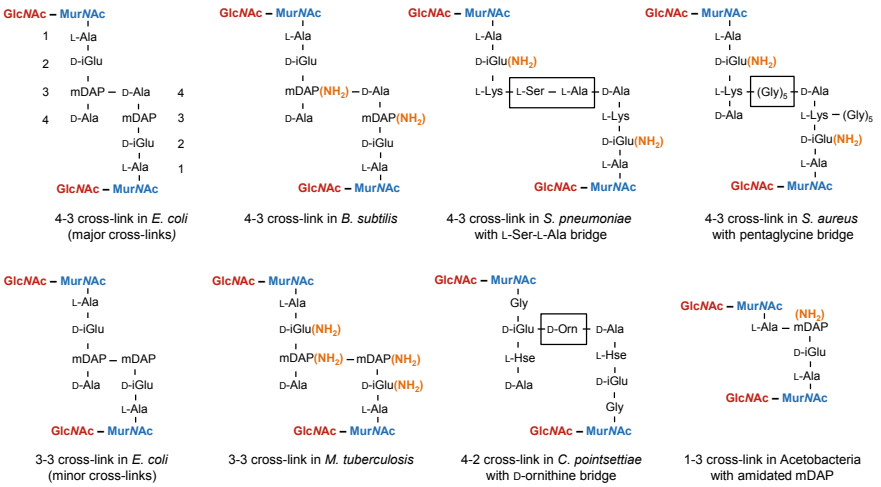

Fig. 5.2 Examples of peptides and cross-link types in the peptidoglycan of different species. Amidation of residues is depicted in orange. Interpeptide bridges are framed with a square

insights about the MurT/GatD complex of *S. pneumoniae* (Morlot et al. 2018) and *S. aureus* (Noldeke et al. 2018). The amino acid at position three of the stem peptide shows the greatest variation. Most Gram-negative bacteria, Mycobacteria and Bacilli contain an mDAP residue at this position. *Bacillus subtilis* has an amidated mDAP (Atrih et al. 1999), due to the action of the amidotransferase AsnB (Dajkovic et al. 2017). In *M. tuberculosis* AsnB also amidates the mDAP residue, and this modification is essential for cell growth (Ngadjeua et al. 2018). The amidotransferase LtsA performs the amidation of the mDAP residue in the PG of *Corynebacterium glutamicum* (Levefaudes et al. 2015). Spirochetes, such as Borrelia or Treponema, have an ornithine residue instead of mDAP (Schleifer and Kandler 1972; Yanagihara et al. 1984). Other species contain at position three different diamino acids like *meso*-lanthionine (*Fusobacterium nucleatum*) and D-Lys (*Thermatoga maritima*), or monoamino acids like L-Ala, L-Glu or L-homoserine (reviewed in Vollmer et al. 2008a). However, most Gram-positive bacteria have an L-Lys at position three, which often carries a linear peptide branch linked to its ε-amino group. These interpeptide bridges show a great diversity, with sizes varying from two to seven residues and a wide range of amino acids. In case of *S. aureus* the FemXAB peptidyltransferases catalyse the addition of a characteristic Gly_5-interpeptide bridge (Schleifer and Kandler 1972). *S. pneumoniae* contains branched stem peptides with an L-Ser-L-Ala or L-Ala-L-Ala dipeptide linked to the ε-amino group of L-Lys. This modification is added by MurM and MurN (Filipe and Tomasz 2000). The degree of branching and cross-linking of PG varies between strains. While most pneumococcal strains contain a small percentage of branched peptides, the PG of resistant strains is highly branched and cross-linked (Garcia-Bustos and Tomasz 1990; Severin et al. 1996). Most bacteria contain D-Ala-D-Ala at positions four and five of the stem peptide.

This motif is recognized by vancomycin and other glycopeptide antibiotics that form a complex with the PG precursors, preventing their incorporation into the sacculus of Gram-positive bacteria. The replacement of the D-Ala at position five by D-Lac or D-Ser prevents the binding of the antibiotic to the precursor and mediates resistance of enterococci (Arthur et al. 1993, 1996).

Variations in the Peptide Cross-Links

The type and extent of the peptide cross-links is different between species. The most abundant cross-links connect the D-Ala at position four of one stem peptide with the mDAP (or L-Lys) at position three of another one (4-3 cross-links). They are synthesized by DD-transpeptidases (DD-TPases). Less frequent are the 4-2 cross-links, found in Corynebacteria, that connect the D-Ala at position four and the D-iGlu at position two of adjacent stem peptides. *Corynebacterium pointsettiae* contains an L-homoserine at position three of the stem peptide, which is non-reactive to form cross-links. Therefore, the cross-link starts at D-iGlu and is created via a D-ornithine bridge (Fig. 5.2) (Schleifer and Kandler 1972). Some bacteria contain a small amount of 3-3 cross-links, while pathogens like *Clostridium difficile* and *M. tuberculosis* contain predominantly 3-3 cross-links in the PG. These cross-links are made by LD-transpeptidases (LDTs) that connect two mDAP residues of adjacent stem peptides (see section "Peptidoglycan Synthesis" for further details on the cross-linking reactions).

Recently, novel PG structures have been described in Acetobacteria, which proliferate at low pH and produce acetic acid. These modifications include the amidation of the α-(L)-carboxyl group of mDAP and a novel LD (1-3) cross-link, which connects the L-Ala residue of one stem peptide with the mDAP of another one (Fig. 5.2) (Espaillat et al. 2016). The enzymes catalyzing both modifications are unknown.

Modifications of the Glycan Strands

The glycan strands can be modified by *N*-deacetylation and *O*-acetylation on either one or both Glc*N*Ac and Mur*N*Ac subunits, and by *N*-glycolylation on Mur*N*Ac (Fig. 5.3) (reviewed in Vollmer 2008; Yadav et al. 2018). The *N*-glycolylation reaction occurs in the cytoplasm during the synthesis of the PG precursors. The *N*-deacetylation and *O*-acetylation reactions take place outside the cytoplasm, once the glycan strands have been synthesized. In pathogenic species these modifications contribute to the survival in the host organism by increasing the resistance to PG degrading enzymes, preventing cell lysis and the release of bacterial products that could be detected by the host immune system.

In many Gram-positive and Gram-negative bacteria the PG chains contain an extra acetyl group linked to the C6–OH group of some Mur*N*Ac (*O*-acetylation). The

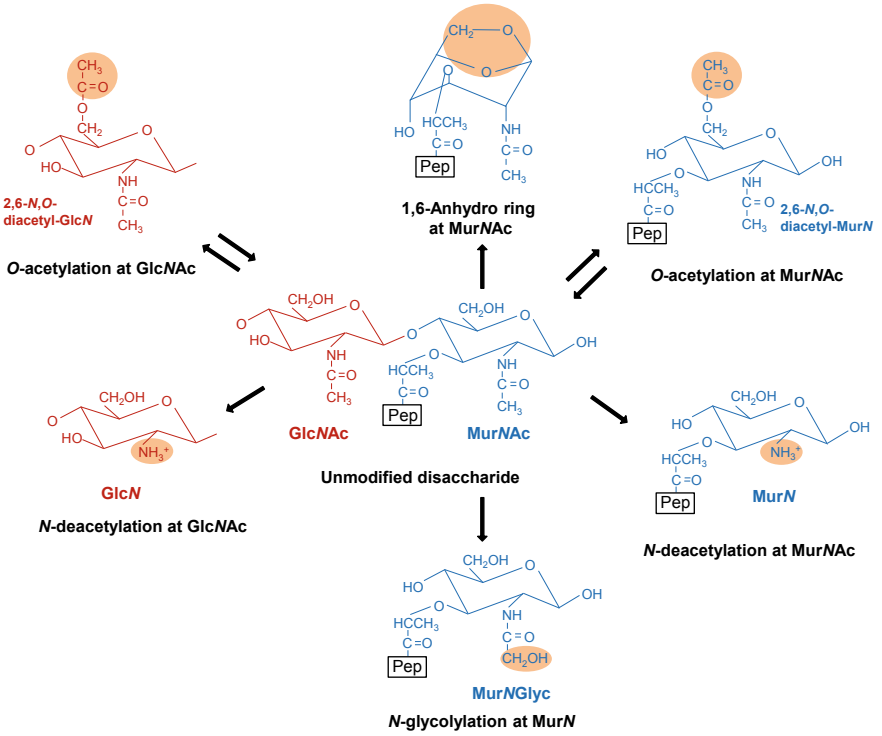

Fig. 5.3 Summary of modifications in the PG glycan strands. The structure of the unmodified Glc*N*Ac-Mur*N*Ac disaccharide (middle) and of selected modifications in the Glc*N*Ac (red) and Mur*N*Ac (blue) are shown. Modifications are highlighted in orange. The *O*-acetylation of Glc*N*Ac or Mur*N*Ac is reversible. Pep, peptide linked to Mur*N*Ac. Modified from Vollmer (2008) and Yadav et al. (2018)

degree of *O*-acetylation varies between <20 and 70% depending on the species and growth conditions. The bulky acetyl group represents a steric hindrance and prevents the binding of the muramidase lysozyme (Pushkaran et al. 2015), which is secreted by the host immune system cells to hydrolyse the glycan strands between Mur*N*Ac and Glc*N*Ac. Therefore, PG *O*-acetylation is a major virulence factor in pathogenic bacteria. For the *O*-acetylation of Mur*N*Ac, the acetyl moiety is translocated from a cytoplasmic donor molecule to the periplasm or extracellular space and transferred to the Mur*N*Ac subunit. In Gram-positive bacteria the *O*-acetyltransferases of the OatA-type perform both processes (Bera et al. 2005; Bernard et al. 2012), while Gram-negative bacteria require the coordinated action of multiple enzymes of the Pat or Pac family (Weadge et al. 2005; Moynihan and Clarke 2010; Dillard and Hackett 2005). In *S. aureus* OatA provides resistance to lysozyme (Bera et al. 2005), protects against killing by macrophages (Shimada et al. 2010), reduces the induction of pro-inflammatory cytokines and permits reinfection (Sanchez et al. 2017). OatA homologs have been identified in different Gram-positive species. In *S. pneumoniae*

the OatA homolog Adr catalyses the *O*-acetylation of Mur*N*Ac and protects dividing cells from cleavage by the pneumococcal autolysin LytA. Adr localizes at the septa and shows mislocalization in some cell division mutants, suggesting an important role in pneumococcal cell division (Bonnet et al. 2017). In vancomycin-resistant *Enterococcus faecalis* the vancomycin treatment increases the levels of *O*-acetylation, which leads to lysozyme resistance and to an increase in virulence (Chang et al. 2017). *O*-acetylation of Mur*N*Ac blocks the function of the lytic transglycosylases (LTs), which have the same substrate specificity as lysozyme but catalyse a transglycosylation reaction resulting in the formation of 1,6-anhydro-Mur*N*Ac (see section "Peptidoglycan Hydrolysis and Remodelling" for further information).

In Gram-negative bacteria the level of *O*-acetylation regulates the activities of the LTs (Weadge et al. 2005; Weadge and Clarke 2006). Glycan strands can be *O*-deacetylated at the Mur*N*Ac *O*-acetyl subunit. The *O*-acetylesterase Ape reverts the *O*-acetylation of Mur*N*Ac, and Ape homologs have been found in Gram-positive and Gram-negative bacteria (Weadge et al. 2005). In *Campylobacter jejuni* the deletion of *ape1* results in the accumulation of *O*-acetylated PG, which impairs the cellular fitness and leads to defects in morphology, motility, biofilm formation and virulence (Ha et al. 2016). This highlights the importance of regulating the degree of *O*-acetylation via the activity of *O*-acetyltransferases and *O*-acetylesterases for the bacterial cell. In contrast, the *O*-acetylation of Glc*N*Ac has been only described in *Lactobacillus plantarum*, where it inhibits the major autolysin Acm2 (Bernard et al. 2011).

N-deacetylation of Glc*N*Ac occurs mostly in Gram-positive bacteria (Vollmer and Tomasz 2000; Boneca et al. 2007; Peltier et al. 2011; Benachour et al. 2012) but also in some Gram-negative like *Shigella flexneri* (Kaoukab-Raji et al. 2012). The PG deacetylase A enzyme (PgdA), identified for the first time in *S. pneumonia*, removes the acetyl group at position C2 of the Glc*N*Ac. PgdA mutants are more susceptible to lysozyme and less virulent (Vollmer and Tomasz 2000). In *Listeria monocytogenes* 50% of the Glc*N*Ac residues are deacetylated (Boneca et al. 2007) and ~23% of the Mur*N*Ac residues are *O*-acetylated at position C6 (Aubry et al. 2011), whereby both modifications enhance synergistically the resistance against lysozyme. In this organism, PgdA activity is regulated by the cell division protein GpsB and the PG synthase PBP A1 (Rismondo et al. 2018). The absence of GpsB increases the lysozyme resistance due to a rise in *N*-deacetylated muropeptides, and the absence of PBP A1 reverses this phenotype. This regulation is supported by in vitro studies suggesting that all three proteins form a complex. In *Lactococcus lactis* YvhB catalyses the *O*-acetylation of Mur*N*Ac, and XynD performs the *N*-deacetylation of Glc*N*Ac. Both modifications increase the cross-linkage and the cell wall integrity, leading to acid resistance and to the production of the polycyclic antibacterial peptide nisin (Cao et al. 2018). In the predatory bacteria *Bdellovibrio bacteriovorus*, the PgdA homologs Bd0468 and Bd3279 deacetylate the Glc*N*Ac residues of the prey PG during predation, making it more susceptible for destruction at the end of predation (Lambert et al. 2016).

The *N*-glycolylation of Mur*N*Ac subunits exists in Mycobacteria and related Actinomycetales. This modification is added by the cytoplasmic UDP-Mur*N*Ac hydroxylase NamH during the synthesis of the UDP-linked precursors, where the *N*-acetyl group at the C2 of the muramyl dipeptides is hydroxylated to an *N*-glycolyl group. The degree of *N*-glycolylation varies depending on the bacterial species, and it changes in response to antibiotics. The deletion of *namH* increases the susceptibility to lysozyme and β-lactams (Raymond et al. 2005). *N*-glycolylated Mur*N*Ac activates the innate immunity more than *N*-acetylated Mur*N*Ac, underlining its contribution to the unusual immunogenicity of Mycobacteria (Coulombe et al. 2009) but not to the pathogenicity of *M. tuberculosis* infection (Hansen et al. 2014).

Biophysical Properties of Peptidoglycan

The PG sacculus has essential stress-bearing functions in the cell, which include maintaining the cellular shape during cell growth and division within changing environmental conditions, and constraining the cell volume under turgor. In order to fulfil these functions, the PG is elastic and stiff at the same time, but also porous to allow diffusion of small proteins.

Thickness of Peptidoglycan

The osmotic pressure in Gram-negative bacteria like *E. coli* is up to 3 atm (Cayley et al. 2000) and in Gram-positive bacteria such as *B. subtilis* and *S. aureus* is significantly higher (up to 20 atm) (Whatmore and Reed 1990). The PG provides mechanical strength to counteract the osmotic pressure in the cell (Höltje 1998; Koch 1988). The thickness of the PG, which can vary depending on the species, contributes to cell stiffness. Consistent with that, Gram-positive bacteria contain a multi-layered and thick PG. The PG of Gram-negative bacteria is a thin and single layer located between the inner and the outer membranes (Fig. 5.1). A recent study suggests that mechanical loads are balanced between the outer membrane and the PG, and postulates a stress-bearing function for the outer membrane during osmotic changes (Rojas et al. 2018).

Isolated PG sacculi maintain the shape and the size of intact cells. Using atomic force microscopy (AFM) dried sacculi from *E. coli* are 3 nm thick, while rehydrated sacculi are 6 nm thick (Yao et al. 1999). In the same study rehydrated sacculi of *Pseudomonas aeruginosa* are only 3 nm thick. These data are in agreement with measurements performed by cryo-transmission electron microscopy (cryo-TEM) of frozen-hydrated sections, which preserves native structures (*E. coli*: 6.35 ± 0.53 nm; *P. aeruginosa*: 2.41 ± 0.54 nm) (Matias et al. 2003).

Cryo-TEM analysis of frozen-hydrated sections of *B. subtilis* cells revealed two distinct zones: a 22 nm thick low-density zone surrounding the cytoplasmic membrane and a 33 nm thick high-density zone surrounding the previous one. The high density zone contains PG, teichoic acids and associated proteins (Matias and Beveridge 2005). A thinner *B. subtilis* Δ*ponA* mutant (lacking the bifunctional PG synthase PBP1) shows a 30 nm thick PG (Beeby et al. 2013).

Strength, Stiffness and Elasticity of Peptidoglycan

The mechanical properties of PG have been analysed using different techniques. The cellular stiffness can be described by the Young's modulus, also known as the modulus of elasticity. To determine it, a physical force is applied to a sample (either cells or isolated sacculi) and the degree by which it can be stretched is measured. If the sample can withstand the force, it will return to the preloaded size after removing the force. If the load is too high for the elastic limits of the sample, it will irreversibly deform and rupture. The elasticity of isolated *E. coli* sacculi, measured by stretching them with the tip of the AFM, varies on the hydration state as air dried sacculi are more rigid than rehydrated ones. Differences in the elastic anisotropy between the long and the short axis of the sacculus suggests that the more flexible peptide cross-links are longitudinally orientated and the more rigid glycan strands are circumferentially orientated (Yao et al. 1999). The proposed architecture is supported by the fact that changes in the volume of osmotically shocked *E. coli* cells are mainly due to changes in cell length but not in cell diameter (van den Bogaart et al. 2007). Recent analysis of individual *E. coli* glycan chains by AFM have shown long and circumferentially orientated glycan chains (200 nm) under normal conditions and shorter and disordered glycan chains when a spheroid cell shape is induced (Turner et al. 2018).

Another technique to measure the mechanical properties of bacterial cells is the Cell Length Analysis of Mechanical Properties (CLAMP), which enables a high throughput screening. Using this technique the Young's moduli of Gram-negative and Gram-positive rod-shaped bacteria are estimated on the basis of initial growth rates of hydrogel-encapsulated living cells with varying stiffness (Tuson et al. 2012). A decrease in relative cell elongation over time is observed with increasing stiffness of the hydrogels. The estimated Young's modulus for *E. coli* is fourfold higher than the result obtained with AFM for hydrated *E. coli* sacculi (Yao et al. 1999). This difference can be explained by the fact that the outer membrane also contributes to the cell wall stiffness. Using the CLAMP technique the Young's moduli of the cell envelopes of *P. aeruginosa* and *B. subtilis* are similar to *E. coli*. Several genes affecting cell stiffness have been identified by measuring the cell growth of hydrogel-encapsulated mutant cells from the entire KEIO-collection (*E. coli* single-gene deletion mutants). These genes have roles in diverse processes, suggesting that cell stiffness is affected by different cellular functions beside the cell wall-related ones (Auer et al. 2016).

In addition to the thickness, other structural parameters as the amount of cross-links and the glycan strands length influence the mechanical properties of the PG. Compared to exponentially growing cells, stationary cells of *E. coli* contain slightly more cross-links, higher level of LD cross-links (see section "LD-Transpeptidases") and more cross-links between the outer membrane-anchored protein Lpp and the PG, which might strengthen the cell envelope and presumably enhance the cellular stiffness (Vollmer and Bertsche 2008). The cell division process of *S. aureus* changes the mechanical properties of the cell wall, as the newly formed septum is stiffer than the surrounding mature cell wall, most likely due to a higher level of cross-links (Bailey et al. 2014b). A decrease in the cross-linkage of *S. aureus* PG, by the absence of PBP4, results in a small reduction of cell wall stiffness (Loskill et al. 2014). The absence of glucosaminidases, which hydrolyse the bonds between Glc*N*Ac and Mur*N*Ac, increases the cellular stiffness in *S. aureus*, increasing the glycan chains length (Wheeler et al. 2015).

Bacteria from diverse phyla produce non-canonical D-amino acids that are incorporated into the PG and modify its strength and flexibility. In *Vibrio cholerae* the racemase BsrV produces these amino acids (mainly D-Met and D-Leu) during stationary growth and LDTs catalyse their incorporation into the PG. The *bsrV* mutant cells have shorter glycan strands and both, *bsrV* and *ldtA ldtB* (encoding for LDTs) mutants are significantly more sensitive to osmotic shock and accumulate an excess of PG during stationary phase (Lam et al. 2009; Cava et al. 2011).

Porosity of Peptidoglycan

In early work the pore sizes in *B. subtilis* and *Bacillus licheniformis* PG were estimated (radius of 2.5 nm) by measuring the diffusion of different sized proteins out of the cells, after *n*-butanol treatment to permeabilize the lipid bilayers (Hughes et al. 1975). A more recent study using isolated *E. coli* and *B. subtilis* sacculi and fluorescein-labelled dextrans of known molecular weights, determined a similar pore radius for both sacculi (2.06 nm in *E. coli*, 2.12 nm in *B. subtilis*) (Demchick and Koch 1996). On the basis of these values it was estimated that a globular, hydrophilic protein with a molecular weight of 25 kDa can freely pass through the relaxed sacculi, while globular proteins of about 50 kDa should be able to diffuse through stretched sacculi (Demchick and Koch 1996). A homeostatic mechanism has been postulated for γ-proteobacteria like *E. coli*, in which the PG synthesis rate is coupled to the growth rate of the cell via the PG pores size (Typas et al. 2010).

Peptidoglycan Precursor Synthesis

The synthesis of the PG precursor starts with the formation of both nucleotide sugar-linked precursor UDP-Glc*N*Ac and UDP-Mur*N*Ac in the cytoplasm (Fig. 5.4) (Barreteau et al. 2008). UDP-Glc*N*Ac is synthesised from fructose 6-phosphate by the

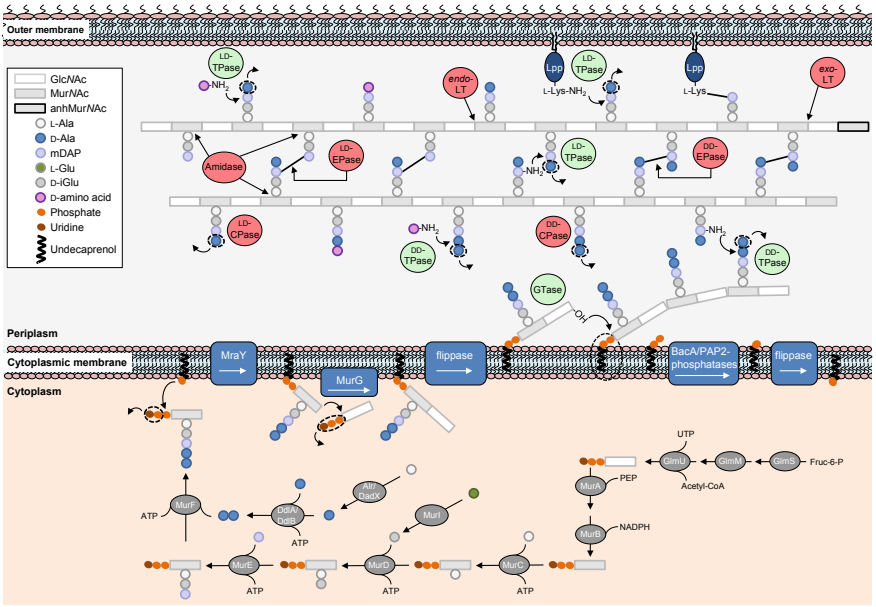

Fig. 5.4 Synthetic and hydrolytic reactions occurring during the synthesis and incorporation of new peptidoglycan in *E. coli*. Modified from Typas et al. (2011)

enzymes GlmS, GlmM and GlmU (the last reaction requires acetyl-coenzyme A and uridine triphosphate). UDP-Mur*N*Ac is synthesised by the addition of enolpyruvate to UDP-Glc*N*Ac (by MurA) and the following reduction (by MurB). The pentapeptide moiety is added to the UDP-Mur*N*Ac by MurC, MurD, MurE and MurF enzymes in a sequence of ATP-dependent reactions that add L-Ala, D-iGlu, mDAP and D-Ala-D-Ala, respectively. The racemases generate the D-Ala (Alr or DadX) and D-iGlu (MurI) enantiomers, and the ligases DdlA and DdlB) synthesize the D-Ala-D-Ala dipeptide required. The first three amino acids (L-Ala-D-iGlu-mDAP) can also be added as a tripeptide by the ligase Mpl through the "recycling pathway". Phospho-Mur*N*Ac-pentapeptide is then transferred onto the lipid carrier undecaprenyl phosphate by MraY, which crystal structure has been recently solved (Hakulinen et al. 2017), resulting in the formation of undecaprenyl pyrophosphate Mur*N*Ac pentapeptide (lipid I) and releasing uridine monophosphate (UMP). The subsequent transfer of Glc*N*Ac to the lipid I by MurG results in the formation of undecaprenyl pyrophosphate Mur*N*Ac(Glc*N*Ac) pentapeptide (lipid II) and the release of uridine diphosphate (UDP). In a final step lipid II is translocated (or flipped) from the inner to the outer leaflet of the cytoplasmic membrane where it is used as substrate for the synthesis of new PG, releasing the lipid carrier undecaprenol pyrophosphate. Due to the essentiality of the process, these reaction steps are potential targets for antibiotics (Hrast et al. 2014; Kouidmi et al. 2014; Liu and Breukink 2016).

Lipid II Flippase(s)

The identity of the lipid II flippase(s) is a controversial topic in the field, and two main candidates to catalyse this reaction has been proposed: FtsW and MurJ.

FtsW is a widely conserved integral membrane protein, member of the SEDS (shape, elongation, division and sporulation) family, and originally identified from a filamentous temperature sensitive mutant, showing its essential role for cell division (Ishino et al. 1989; Khattar et al. 1994). Different studies have dissected its 10 transmembrane segments, identifying several essential residues required for the functionality of the protein (Lara and Ayala 2002; Pastoret et al. 2004; Mohammadi et al. 2014) and its direct interaction with other cell division proteins as FtsN, FtsQ, PBP3 or PBP1B (Di Lallo et al. 2003; Karimova et al. 2005; Alexeeva et al. 2010; Fraipont et al. 2011; Leclercq et al. 2017). In vitro experiments have shown that FtsW binds lipid II (Mohammadi et al. 2011; Leclercq et al. 2017) although with low affinity (Bolla et al. 2018), and is able to flip fluorescently labelled lipid II in liposomes and *E. coli* vesicles (Mohammadi et al. 2011, 2014). However in vivo experiments do not support its lipid II flippase activity, showing no accumulation of lipid II at the inner leaflet of the cytoplasmic membrane in FtsW-depleted cells (Lara et al. 2005; Sham et al. 2014).

MurJ is an integral membrane protein that belongs to the MOP (multidrug/oligosaccharidyl-lipid/polysaccharide) exporter superfamily of proteins. It was identified by bioinformatic analysis and isolated from a temperature sensitive mutant (Ruiz 2008; Inoue et al. 2008). It contains 14 transmembrane segments that adopt a V-shaped structure with a central solvent-exposed cavity (Kuk et al. 2017; Zheng et al. 2018). Several residues are necessary for the correct functionality of the protein, including some of the charged residues located in the central cavity (Butler et al. 2013, 2014; Zheng et al. 2018). The inactivation of MurJ causes cell shape defects and lysis (Ruiz 2008; Inoue et al. 2008) and, supporting its flippase activity, an accumulation of lipid II intermediates in the cell (Sham et al. 2014; Qiao et al. 2017; Chamakura et al. 2017; Rubino et al. 2018). Its essential role can be substituted by other transporters from different species as *B. subtilis* (MurJ and Amj) (Meeske et al. 2015) or *Streptococcus pyogenes* (YtgP) (Ruiz 2009), or by transporters with a more relaxed substrate specificity (Elhenawy et al. 2016; Sham et al. 2018). In vitro evidences do not support its lipid II flippase activity in proteoliposomes and *E. coli* vesicles (Mohammadi et al. 2011) and show contrary results on lipid II binding (Leclercq et al. 2017; Bolla et al. 2018).

Lipid Carrier

Undecaprenyl phosphate is the essential and unique lipid carrier used for the synthesis of the bacterial extracellular cell wall polymers as PG, teichoic acids and O-antigen. It is produced by dephosphorylation of the undecaprenyl pyrophosphate,

either from de novo synthesis or from the recycling pathway, by membrane embedded phosphatases (BacA or PAP2-type phosphatases) (Manat et al. 2014). In the de novo synthesis pathway, UppS uses farnesyl pyrophosphate and eight isopentenyl pyrophosphate molecules to produce undecaprenyl pyrophosphate. In the recycling pathway, the undecaprenyl pyrophosphate is released after the glycosyltransferase reaction performed by the PG synthases to incorporate the disaccharide pentapeptide moiety of the lipid II substrate into the PG glycan chains. In both cases undecaprenyl phosphate is generated in the outer leaflet of the cytoplasmic membrane, as the active sites of BacA and the PAP2-type phosphatases PgpB, YbjG and LpxT are oriented towards the periplasm (Touze et al. 2008; Fan et al. 2014; Tatar et al. 2007; Manat et al. 2015; El Ghachi et al. 2018; Workman et al. 2018). The lipid carrier has to be flipped across the membrane, as the synthesis of the PG precursor takes place in the inner leaflet, but the required protein has not yet been identified.

Peptidoglycan Synthesis

The synthesis of new PG into the sacculus comprises the polymerization of glycan chains by glycosyltransferases (GTases) and their incorporation to new or pre-existing chains through cross-linkage of the stem peptides by transpeptidases (TPases). There are different TPase reactions depending on the donor muropeptide (Fig. 5.4). The most abundant reaction occurs when a pentapeptide donor cross-links the D-Ala at position four with the mDAP at position three of the acceptor muropeptide (DD-TPase reaction, 4-3 cross-link). The DD-TPases are named penicillin binding proteins (PBPs), as β-lactams covalently bind to the catalytic site serine residue, block the access to the donor muropeptide and therefore inhibit the enzymatic reaction (see Sauvage et al. 2008 for a detailed description of PBPs). In a less frequent reaction two muropeptides are cross-linked by their mDAP residues at position three (LD-TPase reaction, 3-3 cross-link).

The best characterised PG GTases belong to the GT51 family. They contain a conserved sequence with five motifs, a structure mainly composed of α-helices and a catalytic glutamate residue. The phosphoglycolipid antibiotic moenomycin binds to the active site, competing with the newly synthesised glycan chain for the donor site. *E. coli* contains four GT51 GTases: a monofunctional GTase (MtgA) and three bifunctional class A PBPs (encoding also DD-TPase domain) known as PBP1A, PBP1B and PBP1C (Sauvage et al. 2008). PBP1A and PBP1B are the most important class A PBPs and both contain a non-catalytic domain (ODD in PBP1A and UB2H in PBP1B) involved in the regulation of the GTase and TPase activities (Typas et al. 2010). Either PBP1A or PBP1B is required for cell viability (Yousif et al. 1985; Kato et al. 1985). PBP1A is preferentially involved in the synthesis of PG during cell elongation and PBP1B during septation. Both proteins are functionally semi-redundant, meaning that the cell can compensate the absence of each other although not under all tested conditions (Garcia del Portillo and de Pedro 1990, 1991; Denome et al. 1999; Pepper et al. 2006; Ranjit and Young 2013). Several in vitro studies have

characterised the activities of both proteins either alone (Barrett et al. 2004; Bertsche et al. 2005; Born et al. 2006) or in presence of different interacting partners, as in case of PBP1A and PBP2 (Banzhaf et al. 2012) or LpoA (Typas et al. 2010; Lupoli et al. 2014), and PBP1B and FtsN (Müller et al. 2007) or LpoB (Typas et al. 2010; Egan et al. 2014, 2018; Lupoli et al. 2014). The crystal structure of *E. coli* PBP1B has been described in complex with different antibiotics (Sung et al. 2009; King et al. 2017). Crystal structures of other bifunctional PBPs from different bacteria are also available (Jeong et al. 2013; Lovering et al. 2007; Yuan et al. 2007).

E. coli has two essential monofunctional DD-TPases or class B PBPs, PBP2 and PBP3. Whereas PBP2 is required for cell elongation and its inactivation produces a spherical phenotype, PBP3 is needed for septation and its inactivation generates long filamented cells. In both cases cells eventually lyse. Crystal structures of *E. coli* PBP3 and *H. pylori* PBP2 are solved (Sauvage et al. 2014; Contreras-Martel et al. 2017) (see section "Regulation of Peptidoglycan Growth" for further details on the elongation and septation complexes).

Recently the SEDS family protein RodA has been described as a GTase. It does not belong to the known GT51 family and is not inhibited by the antibiotic moenomycin. The crystal structure of RodA from *Thermus thermophilus* has been solved (Meeske et al. 2016; Cho et al. 2016; Emami et al. 2017; Sjodt et al. 2018). Future work will show if the SEDS protein FtsW, essential for cell division, is also a GTase (Taguchi et al. 2018).

LD-*Transpeptidases*

In *E. coli* only 5–15% of the PG cross-links are 3-3 (Glauner et al. 1988). This transpeptidase reaction (LD-TPase) is catalysed by the non-essential LD-transpeptidases (LDTs) (Magnet et al. 2008) that use a disaccharide-tetrapeptide as acyl donor and form cross-links between two mDAP residues of adjacent stem peptides. LDTs contain a catalytic cysteine residue (Mainardi et al. 2005) and are insensitive to β-lactams, with the exception of carbapenems that also target PBPs (Mainardi et al. 2007). Even though the 3-3 are the minor cross-links in the PG of most bacteria, the LDTs are promising targets for new antimicrobial drugs against important pathogens, and therefore they will be discussed in more detail.

Ldt_{fm}, from an ampicillin-resistant *Enterococcus faecium* strain, was the first discovered LDT with documented LD-TPase activity. In the presence of the antibiotic *E. faecium* cells produce exclusively 3-3 cross-links (Mainardi et al. 2000, 2002, 2005). This alternative cross-linkage enables the bypass of PBPs and confers resistance to β-lactam antibiotics, although a DD-CPase reaction is needed to produce the tetrapeptides that can be used by the LDTs. Since then, Ldt homologues with a catalytic YkuD like domain (PFAM 03744) have been found and described among pathogen and non-pathogen Gram-positive and Gram-negative bacteria.

E. coli has six periplasmic LDTs (LdtA-F) with different functions. LdtC and LdtE contain an N-terminal PG-binding lysin motif (LysM) domain, which is found

in many cell wall hydrolases (Buist et al. 2008), suggesting that these enzymes may be active when bound to PG. LdtA, LdtB and LdtC (ErfK, YbiS and YcfS) catalyse the covalent attachment of the outer membrane lipoprotein Lpp (Braun's lipoprotein) to PG, stabilizing the cell envelope (Magnet et al. 2007). These enzymes link the ε-amino group of the C-terminal lysine of Lpp to the α-carboxyl group of the mDAP residue in the stem peptide of the PG (Fig. 5.4). Lpp is a small α-helical protein that with 1 million molecules per cell is the most abundant protein in *E. coli* (Li et al. 2014). Lpp is anchored to the outer membrane by a lipid moiety composed of acyl chains attached to the cysteine residue at its N-terminus. Under normal growth conditions, about one third of the Lpp molecules are linked to PG (Inouye et al. 1972). LdtD and LdtE (YcbB and YnhG) form 3-3 cross-links (Fig. 5.4) (Magnet et al. 2008). The expression of *ldtD* is regulated by the Cpx pathway, which mediates adaption to cell envelope stress (Bernal-Cabas et al. 2015). In response to Cpx-activated conditions the production of LdtD is upregulated resulting in increased 3-3 cross-linkage, suggesting a role for LdtD under cellular stress conditions (Bernal-Cabas et al. 2015; Delhaye et al. 2016). Furthermore, LdtD is twice the size of the other *E. coli* LDTs and shares lower sequence similarity with them (Sanders and Pavelka 2013). LdtF (YafK) is an Ldt homolog protein with yet unknown enzymic function, although a role in biofilm formation has been suggested in pathogenic *E. coli* (Sheikh et al. 2001). The deletion of all six *ldt* genes leads to a lack of 3-3 cross-links and a reduction in Lpp-PG attachment, indicating the non-essentiality of these enzymes (Peters et al. 2018). Remarkably, the ampicillin resistant *E. coli* strain M1 shows elevated levels of the alarmone (p)ppGpp and is able to replace the DD-TPase activity of the PBPs by production of the β-lactam-insensitive LdtD (Hugonnet et al. 2016). This finding shows a new mode of PG polymerization in *E. coli* requiring the GTase activity of PBP1B, the DD-CPase activity of PBP5 and the LD-TPase activity of LdtD (Hugonnet et al. 2016).

LDTs play important roles in different pathogenic bacteria. The formation of 3-3 cross-links is essential for the virulence of the pathogen *Salmonella enterica* serovar Typhi. In this organism the secretion of the typhoid toxin depends on the activity of the *N*-acetyl-β-D-muramidase TtsA, which hydrolyses the 3-3 cross-links synthesised by the LDT YcbB (Geiger et al. 2018). LDTs from *E. coli* and *E. faecium* are inhibited by copper ions at sub-millimolar concentrations, likely through the binding to the thiol group of the catalytic cysteine residue and its activity inhibition. The resulting lack of 3-3 cross-links and the decrease in the Lpp-PG attachment impair the robustness of the cell envelope (Peters et al. 2018). Additionally this inhibition counteracts the LDT-mediated β-lactam resistance of *E. coli* and *E. faecium* strains (Peters et al. 2018). The PG of *C. difficile* and *M. tuberculosis* is predominantly 3-3 cross-linked, with several LDTs encoded in each organism, suggesting an important role for these enzymes (Peltier et al. 2011; Lavollay et al. 2008, 2011; Sutterlin et al. 2018). The deletion of two of the three LDTs in *C. difficile* leads to a significant decrease in 3-3 cross-links, partially compensated by an increase in 4-3 cross-links by PBPs, and a less cross-linked PG (Peltier et al. 2011). Attempts to inactivate the third LDT have been unsuccessful. Out of the five LDT homologues in *M. tuberculosis* (Ldt_{Mt1}–Ldt_{Mt5}), Ldt_{Mt2} is the dominant one, as its inactivation causes altered colony

morphology, loss of virulence and increased susceptibility to amoxicillin (Gupta et al. 2010). The LD-TPase activity of Ldt_{Mt2} requires an amidated mDAP in the stem peptide, catalysed by the amidotransferase AsnB (Ngadjeua et al. 2018). Ldt_{Mt1}, Ldt_{Mt3}, Ldt_{Mt4} and Ldt_{Mt5} are all of similar size and share an amino acid sequence identity of 28–36% with Ldt_{Mt2}. The crystal structure of Ldt_{Mt5} shows structural and functional differences to the other LDTs, since it contains a different catalytic site and a proline rich C-terminal domain. Cells lacking a functional Ldt_{Mt5} displays aberrant growth and increased sensitivity to osmotic shock and certain carbapenems, indicating its critical role in maintaining the cell wall integrity (Brammer et al. 2015).

Peptidoglycan Hydrolysis and Remodelling

E. coli cells coordinate the synthesis of PG with the cleavage of the pre-existing PG material by dedicated hydrolases to incorporate new PG into the sacculi. PG hydrolases are also involved in autolysis, maturation, turnover and recycling of PG, showing substrate and PG-linkage specificity. According to the cleavage site they can be classified as *N*-acetylmuramoyl-L-Alanine amidases (amidases), carboxypeptidases, endopeptidases and lytic transglycosylases (van Heijenoort 2011; Vollmer et al. 2008b) (Fig. 5.4). Further information about the PG-recycling and β-lactamase induction pathways can be found in different reviews (Park and Uehara 2008; Juan et al. 2017; Dik et al. 2018).

*N-Acetylmuramoyl-*L*-Alanine Amidases*

Amidases specifically cleave the amide bond linking the L-Ala residue of the stem peptide to the Mur*N*Ac subunit of the muropeptide. *E. coli* contains five different amidases, which are grouped in two superfamilies based on the amino acid sequence similarity: AmiA, AmiB and AmiC (pfam: amidase 3) and AmpD and AmiD (pfam: amidase 2). AmiA, AmiB and AmiC exhibit specificity for Mur*N*Ac substrates, AmpD for 1,6-anhydro-Mur*N*Ac, and AmiD cleave both Mur*N*Ac and 1,6-anhydro-Mur*N*Ac substrates.

AmiA, AmiB and AmiC are exported to the periplasm via the Tat system (AmiA and AmiC) and through the Sec translocon (AmiB) (Ize et al. 2003; Bernhardt and de Boer 2003). Although AmiB and AmiC are recruited to the septal position during cell division, and AmiA remains dispersed throughout the cell periplasm (Bernhardt and de Boer 2003; Peters et al. 2011), all of them perform redundant reactions that compensate the absence of the others. The inactivation of all three amidases results in cell chains, in which daughter cells are not separated from each other. Single and double mutants have milder phenotypes, showing shorter cell chains (Heidrich et al. 2001). Genetic evidences indicate a partially redundant role of the Rcs and Cpx stress responses in supporting the growth and viability of the triple mutant

(Yakhnina et al. 2015). The activity of the amidases is dependent on the presence of two LytM-domain containing proteins, EnvC and NlpD, as the inactivation of both genes shows the same cell chain phenotype than the triple *amiABC* mutant. Their LytM-domains do not contain the residues required for the coordination of the catalytic Zn^{2+} ion and lack the ion itself (Uehara et al. 2009, 2010; Peters et al. 2013), typical from the metallo-peptidase family M23 (Pfam: Peptidase_M23). EnvC activates AmiA and AmiB in the outer-leaflet of the cytoplasmic membrane, and the outer membrane-anchored lipoprotein NlpD activates AmiC (Uehara et al. 2010). The structure of AmiC (Rocaboy et al. 2013) and studies on AmiB show the presence of a conserved α-helix blocking the access to the catalytic site (Yang et al. 2012). Conformational changes induced by the interaction with the activator enable the open access to the active site (Yang et al. 2012) (see section "Regulation of Peptidoglycan Growth" for further details on EnvC and NlpD functioning).

AmpD is a cytoplasmic Zn^{2+}-dependent amidase required for the PG-recycling process during bacterial cell growth. The specificity for 1,6-anhydro-Mur*N*Ac containing substrates (Jacobs et al. 1995) prevents AmpD of interfering with the synthesis of cell wall precursors. The inactivation of AmpD leads to the accumulation of its substrate 1,6-anhydro-Mur*N*Ac-L-Ala-D-iGlu-mDAP (Jacobs et al. 1994).

AmiD is an outer membrane-anchored lipoprotein with Zn^{2+}-dependent amidase activity. It cleaves the amide bond of intact PG or soluble fragments containing tri-, tetra- or pentapeptides, regardless of the presence or absence of 1,6-anhydro-Mur*N*Ac (Uehara and Park 2007; Pennartz et al. 2009). AmpD and AmiD are the only proteins with specificity for 1,6-anhydro-Mur*N*Ac substrates (Uehara and Park 2007). A reaction mechanism has been proposed based on the crystal structures of AmiD, alone and in complex with either the 1,6-anhydro-Mur*N*Ac-tripeptide or the tripeptide (Kerff et al. 2010). AmiD is not required for cell separation (Uehara and Park 2007).

Lytic Transglycosylases

Lytic transglycosylases (LTs) are periplasmic *N*-acetylmuramidases that catalyse the cleavage of the β-1,4 glycosidic bond between Mur*N*Ac and Glc*N*Ac, generating a 1,6-anhydro bond in the Mur*N*Ac. The resultant muropeptides containing 1,6-anhydro-Mur*N*Ac are transported from periplasm to cytoplasm, via the transmembrane protein AmpG. The cleavage reaction performed by LTs can take place at the end of the glycan chains (exolytic activity) or within them (endolytic activity). *E. coli* contains eight LTs (MltA-MltG and Slt). LTs are functionally redundant and the absence of six of them (MltA-E and Slt) does not affect cell viability although generates cell chain phenotype (Heidrich et al. 2002). RlpA, recently described as an interacting partner of the cell division protein FtsK in *E. coli* (Berezuk et al. 2018), is considered a LT based on its activity in *P. aeruginosa* (Jorgenson et al. 2014). Slt is a periplasmic LT, MltA-F are outer membrane-anchored lipoproteins, and MltG is anchored to the outer-leaflet of the cytoplasmic membrane. LTs are classified based on their

domain architecture, belonging to family 1 (Slt, family 1A; MltC, family 1B; MltE, family 1C; MltD, family 1D; MltF, family 1E), family 2 (MltA), family 3 (MltB) and YceG-family (MltG). Slt is described in more detail in the following lines, and further information about LTs can be found in different reviews (van Heijenoort 2011; Dik et al. 2017; Alcorlo et al. 2017).

Slt has a main exolytic activity performed on non cross-linked muropeptides containing stem peptides, although a low endolytic activity has been described (Lee et al. 2013, 2018). It has a "doughnut-shaped" structure, as shown in complex with the specific inhibitor bulgecin or the 1,6-anhydro-muropeptide (Thunnissen et al. 1994, 1995; van Asselt et al. 1999). Recent works with *P. aeruginosa* Slt and *Neisseria meningitidis* LtgA suggest that conformational rearrangements on the active site take place during the hydrolytic reactions (Lee et al. 2018; Williams et al. 2018). In *E. coli* Slt interacts with the PG synthases PBP1B, PBP1C, PBP2 and PBP3, and with the PG endopeptidase PBP7 (Romeis and Höltje 1994b; von Rechenberg et al. 1996). In the absence of *slt*, or protein inactivation by addition of bulgecin, cells do not show different phenotype or alter the PG composition but modified sensitivity to certain β-lactams. Inactivation of PBP3 in *slt* mutant cells produces bulges and rapid lysis (Templin et al. 1992), instead of cell filamentation and lysis as in WT cells (Spratt 1975). The sensitivity to the PBP2-specific inhibitor mecillinam in *slt* cells depends on the FtsZ protein levels, being more sensitive at WT levels (Templin et al. 1992) and more resistant at high levels (Vinella et al. 1993). The hypersensitivity to mecillinam has been described as a consequence of the accumulation of newly synthesised PG glycan chains and its misincorporation into the sacculus by LDTs. In WT cells the non cross-linked new PG glycan chains are removed by Slt, contributing to the increased PG turnover by the β-lactam mecillinam stress response (Cho et al. 2014).

Endopeptidases

Endopeptidases (EPases) catalyse the cleavage of the amide bonds between amino acids from different stem peptides. According to the substrate specificity, they are classified as DD-endopeptidases (DD-EPases) and LD-endopeptidases (LD-EPases). DD-EPases cleave the bond formed by the 4-3 cross-links, in *E. coli* between D-Ala at position four from one stem peptide and mDAP at position three from a different stem peptide. LD-EPases show specificity for the bond formed by the 3-3 cross-links, in *E. coli* between the mDAP residues at position three of different stem peptides. *E. coli* contain seven proteins encoding for DD-EPase activity, three of them are sensitive to β-lactams (the class C PBPs PBP4, PBP7 and AmpH) and four are insensitive to β-lactams (MepA, MepH, MepM and MepS). The absence of each single protein does not change the cell phenotype, indicating functional redundancy between them. Recently it has been shown that the overproduction of MepA, MepM, PBP7 or MepS confers resistance to mecillinam through the stimulation of the PG synthesis by PBP1B (Lai et al. 2017).

PBP4 is a periplasmic protein that shows partial association with the cytoplasmic membrane (Korat et al. 1991; Jacoby and Young 1988; Leidenix et al. 1989). In addition to the DD-EPase activity, PBP4 also shows DD-carboxypeptidase (DD-CPase) activity (see section "Carboxypeptidases"), using either soluble muropeptides or isolated PG as substrate (Korat et al. 1991; Li et al. 2004; Clarke et al. 2009). The crystal structure of the protein alone and bound to different antibiotics shows three different domains and the formation of a soluble dimer (Kishida et al. 2006). Residues close to the catalytic serine encoded in the domain I are required for the accommodation of the mDAP residue of the stem peptide substrate (Clarke et al. 2009). In vivo studies based on the combination of gene deletions suggest a role for PBP4 in different cell processes including cell morphology, separation of daughter cells and biofilm formation (Meberg et al. 2004; Priyadarshini et al. 2006; Gallant et al. 2005).

The periplasmic protein PBP7 shows a loose association with the cytoplasmic membrane, which can be abolished by high salt treatment (Romeis and Höltje 1994a). A C-terminal truncated variant, identified as PBP8, led to confusion in the past (Henderson et al. 1994). PBP7 shows DD-EPase activity when isolated PG is used as substrate, but not in case of purified dimeric muropeptides (Romeis and Höltje 1994a). It interacts with Slt, which enhances its LTase activity (Romeis and Höltje 1994b). The inactivation or overproduction of PBP7 does not generate any cellular defect (Henderson et al. 1995). Combined inactivation of PBP7, PBP4 and PBP5 enhances the morphological defects caused by the absence of PBP5 (main DD-CPase in *E. coli*) (Meberg et al. 2004), and the additional inactivation of AmpH activates the Rcs phosphorelay and Cpx stress responses (Evans et al. 2013).

AmpH is a periplasmic protein associated to the cytoplasmic membrane, despite the lack of any membrane anchoring domain. In contrast to PBP4 and PBP7, high salt treatment does not dissociate the protein from the membrane fraction, requiring detergent for that purpose. AmpH displays DD-EPase and DD-CPase activities using both intact sacculi and isolated dimeric muropeptides as substrates, and weak β-lactamase activity (Gonzalez-Leiza et al. 2011). Inactivation of AmpH does not cause any defect in the cell, unless it is combined with other mutations producing morphological changes (Henderson et al. 1997) or stress response activation (Evans et al. 2013). A role in PG recycling and remodelling has been suggested based on the wide range of substrates used by AmpH.

MepA is a periplasmic protein belonging to the LAS metallopeptidase family (lysostaphin-type enzymes, D-alanyl-D-alanine CPase, and sonic hedgehog protein), which is characterised for the presence of a Zn^{2+}-binding site in the catalytic domain. It shows DD-EPase activity on intact sacculi and isolated muropeptides (Keck and Schwarz 1979; Firczuk and Bochtler 2007) and LD-EPase activity on intact sacculi (Engel et al. 1992). The conserved metal ligands are required for the correct folding and catalytic activity of the protein (Firczuk and Bochtler 2007), which can be inhibited by DNA, lipoteichoic acid and metal-chelating agents (Tomioka and Matsuhashi 1978; Keck and Schwarz 1979). Inactivation or overproduction of MepA does not cause any change in the PG composition or cell phenotype

(Iida et al. 1983; Keck et al. 1990). The combined inactivation of PBP4, PBP7 and MepA does not cause any defect in the cell, but enhances the cell chain phenotype due to the absence of amidases and LTs (Heidrich et al. 2002).

MepH, MepM and MepS, in contrast to the previously described EPases, are redundantly essential as their absence cause cell lysis (Singh et al. 2012). MepH is a periplasmic protein of the NlpC/P60 peptidase superfamily with the conserved Cys-His-His catalytic triad (Aramini et al. 2008). MepM is a bitopic cytoplasmic membrane protein of the metallopeptidase family M23, containing the characteristic LytM domain and the catalytic Zn^{2+}-binding site. Both proteins show DD-EPase activity against isolated muropeptides and, partially, intact sacculi (Singh et al. 2012). MepS is an outer membrane-anchored lipoprotein belonging to the NlpC/P60 peptidase superfamily, which shows DD-EPase and weak LD-CPase activity against isolated muropeptides but not against intact sacculi (Singh et al. 2012). MepS protein levels are higher during exponential phase, being modulated by the proteolytic system NlpI-Prc (Singh et al. 2015). NlpI is an outer membrane-anchored lipoprotein containing tetratricopeptide-repeats that facilitates the interaction between MepS and the periplasmic protease Prc (Singh et al. 2015; Su et al. 2017). A reaction mechanism for the degradation of MepS has been proposed based on the crystal structure and biophysical and mutational analyses of the NlpI-Prc complex (Su et al. 2017). The impairment of this proteolytic system alters the morphology of the cells at high-salinity growth conditions (Kerr et al. 2014), changes the PG dynamics in stationary phase, decreases the Lpp-PG attachment and increases the formation of outer membrane vesicles (Schwechheimer et al. 2015).

Carboxypeptidases

Carboxypeptidases (CPases) remove the terminal residues from stem peptides. *E. coli* encodes for six non-essential DD-CPases (PBP4, PBP4b, PBP5, PBP6, PBP6b and AmpH) that remove the D-Ala residue from pentapeptides, and one cytoplasmic LD-CPase (LdcA) that removes the D-Ala residue from tetrapeptides. PBP4 and AmpH also show DD-EPase activity and were described in the section "Endopeptidases".

PBP4b is a non-essential protein, showing sequence homology to AmpH and weak DD-CPase activity on artificial substrate (Vega and Ayala 2006) but not on natural isolated muropeptides (J. Ayala, unpublished results mentioned in Sauvage et al. 2008).

PBP5 is one of the best characterised and most abundant PBP in the cell. It is a membrane protein anchored to the outer leaflet of the cytoplasmic membrane by its C-terminal amphipathic helix. It shows sequence similarities with PBP4, PBP6 and certain β-lactamases, and has been recently described to dimerise (Meiresonne et al. 2017). The crystal structure of a soluble form of PBP5 shows the presence of an N-terminal domain (domain I) including the catalytic site and a C-terminal domain (domain II) containing a hydrophobic surface and the mentioned membrane-binding helix. The domain I contains a Ω-loop-like region, similar to the one found in certain

β-lactamases, which is essential for keeping the β-lactam resistance and the cell shape (Dutta et al. 2015; Kar et al. 2018). The function of the domain II is not well known, although it seems to be required for the stabilization and full function of the protein. It shows DD-CPase activity against soluble muropeptides and cross-linked and uncross-linked PG. Although non-essential, the loss of PBP5 activity causes cell shape defects and an increase in the pentapeptide content in the PG. These shape defects are enhanced and lead to the formation of branches when combined with the absence of other PBPs. Variations in the level of the cell division protein FtsZ and in the formation of the FtsZ-ring structure also enhance the cell shape defects (Varma and Young 2004; Varma et al. 2007; Potluri et al. 2012a). PBP5 localizes to areas of ongoing PG synthesis, meaning along the lateral cell wall and at division sites before septation starts (preseptal sites) (Potluri et al. 2010). The absence of the membrane binding helix does not affect the catalytic activity of the protein, although the resultant soluble PBP5 does not localize at preseptal sites or restore the phenotype in mutant cells, as also observed for inactive PBP5 variants (Nelson and Young 2000; Nelson et al. 2002; Potluri et al. 2010). Overproduction of PBP5 causes non-viable spherical cells (Markiewicz et al. 1982), suggesting a role for PBP5 in regulation of the pentapeptide subunits required for the formation of cross-links by DD-TPase. The combination of the DD-CPase activity of PBP5, the LD-TPase activity of LdtD and the GTase activity of PBP1B has been described to bypass the DD-TPase activity of the PBPs (Hugonnet et al. 2016).

PBP6 and PBP6b have strong sequence and structural similarities with PBP5, including the C-terminal membrane anchor that can substitute the one from PBP5 (Nelson et al. 2002). PBP6 shows weaker activity than PBP5 against similar substrates (Amanuma and Strominger 1980). In the absence of PBP5, the main DD-CPase activity is performed by PBP4 at neutral pH, and by PBP6b at low pH. The role of PBP6b at low pH is also supported by its ability to maintain the WT cell shape in the absence of PBP5 (Peters et al. 2016).

Regulation of Peptidoglycan Growth

During the *E. coli* cell cycle the PG sacculus is enlarged and split through the enzymatic activities encoded in the protein complexes named elongasome and divisome, which facilitate cell elongation and cell division, respectively. Both, synthetic and hydrolytic activities are required for the incorporation of the new PG material, as proposed in the 3-for-1 growth model (Höltje 1998). The control of these activities, from the cytoplasm by the cytoskeletal proteins and from the periplasmic side by outer membrane and periplasmic proteins, ensures the precise and coordinated synthesis of new PG. The composition and functioning of both elongasome and divisome are largely unknown. However, the protein interactions described in the literature support the formation of these multienzymatic complexes, although it is unlikely that all the interactions take place at the same time (Fig. 5.5) (Typas et al. 2011; Egan and Vollmer 2013). The presence of large sets of seemingly redundant enzymes, under

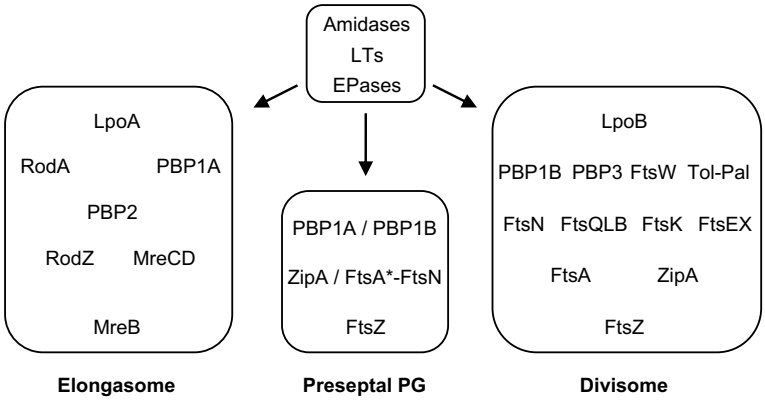

Fig. 5.5 Components of the multienzymatic complexes suggested for new PG incorporation during cell growth and division in *E. coli*. The hydrolytic enzymes (amidases, LTs and EPases) are not specified, as their coordination with the synthetic activities remains largely unknown in each case. LTs, lytic transglycosylases; EPases, endopeptidases

laboratory standard conditions, may be required to ensure a robust peptidoglycan growth under the different environmental conditions (Pazos et al. 2017).

Regulation of Peptidoglycan Synthesis by the Cytoskeletal Proteins

The elongasome is required for the elongation and shape maintenance of rod-shape cells. It contains the synthetic activities of RodA (GTase), PBP2 (TPase) and PBP1A (GTase and TPase). The activity of the elongasome is regulated by the cytoskeletal protein MreB and the membrane-associated MreC, MreD and RodZ proteins. MreB is an actin homolog that polymerizes in an ATP-dependent manner into dynamic double antiparallel filaments (van den Ent et al. 2014), which localize on the inner-leaflet of the cytoplasmic membrane in a curvature-dependent way and rotate along the short cell axis as patches (Garner et al. 2011). MreB binds directly to the membrane through its N-terminal amphiphatic helix (Salje et al. 2011). The disruption of these filaments has an impact on the cell shape, including the eventual spherical phenotype obtained after inactivation of MreB (Kruse et al. 2005). The MreB inhibitor A22 competes for the ATP binding pocket of MreB, which is not able to polymerize and remains homogeneously localized in the cytoplasm. The presence of A22-blocked MreB monomers reduces the characteristic growth heterogeneity and generates cell wall growth at the poles (Ursell et al. 2014), which were previously suggested to be inert (de Pedro et al. 1997). Cell poles are enriched in cardiolipin and phosphatidylglycerol, which prevent the localization of MreB filaments but not of MreB monomers (Kawazura et al. 2017). The fact that MreB motion requires an

active elongasome, and the elongasome can function in the absence of an active MreB, suggests a passive role of MreB in guiding the elongasome (van Teeffelen et al. 2011; Ursell et al. 2014). Single particle tracking data showed that these protein complexes are very dynamic, as observed by the different motion of PBP2 and MreB (Lee et al. 2014), the presence of MreB-like slower subpopulations of RodA and PBP2 (Cho et al. 2016) or the fast and diffusive behaviour of the class A PBPs (Cho et al. 2016; Lee et al. 2016), which can be modified either by specific inhibitors or activators (Lee et al. 2016). MreB localization requires a functional Sec-translocon system for the correct insertion of RodZ into the membrane (Govindarajan and Amster-Choder 2017; Rawat et al. 2015). RodZ is required for the attachment of MreB filaments to the cytoplasmic membrane, modulating their localization in the cell (Colavin et al. 2018; Bratton et al. 2018). Further information about MreB and associated proteins can be found in reviews (Errington 2015; Shi et al. 2018).

Despite the essentiality of MreB and the elongasome, the overproduction of the tubulin homolog FtsZ is able to restore cell viability, but not rod-shape, upon deletion of any of them (Bendezu and de Boer 2008). Although the mechanism of this FtsZ-mediated rescue is not clear, it is known that both MreB and FtsZ interact directly at the cell division site and that this interaction is essential for cell division but not for rod-shape maintenance. An impaired interaction is lethal for the cell, which shows neither preseptal nor septal PG synthesis (Fenton and Gerdes 2013). Preseptal PG is synthesised at the future division site before any constriction is visible, in a PBP3-independent manner. It is considered as a transition stage between cell elongation and cell division, or an early cell division stage (Nanninga 1991; de Pedro et al. 1997). So far, in *E. coli* only the cell division proteins FtsZ and ZipA, and either PBP1A or PBP1B are described as essential proteins for preseptal PG synthesis. Several proteins from the elongasome or divisome are not required, e.g. RodA, FtsA, FtsEX, FtsK or FtsQ (Potluri et al. 2012b). ZipA links the cytosolic Z-ring and the PG synthases PBP1A or PBP1B, an essential role that can be compensated by the mutant FtsA* presumably bound to FtsN (Fig. 5.5) (Pazos et al. 2018). In the absence of both ZipA and FtsN, the cells are not viable and do not synthesise preseptal PG. Further work is required to study the essentiality of the preseptal PG synthesis for the correct cell septation.

The divisome is involved in the septation and separation of the two daughter cells. Several proteins are required for the correct assembly and functioning of the divisome and many described interactions support the hypothesis of a multiprotein complex (Fig. 5.5) (reviewed in Egan and Vollmer 2013). The synthesis of the septal PG is carried out by PBP1B (GTase and TPase) and PBP3 (TPase). Controversial results about the SEDS family protein FtsW propose that either it is a lipid II flippase or a GTase. Future work will clarify its role. The cell division protein FtsZ is the scaffold in which the divisome is build up. FtsZ polymerizes into a ring-like structure, termed the Z-ring. FtsZ polymerization and depolymerization requires the binding and hydrolysis of GTP, respectively. These filaments are bound to the inner-leaflet of the cytoplasmic membrane by the membrane-attached proteins FtsA and ZipA. The GTP hydrolysis generates conformational changes in the FtsZ filaments that adopt a curved shape (Lu et al. 2000), which has been proposed to supply the constriction

force in liposomes (Osawa et al. 2008; Osawa and Erickson 2013). The cellular localization of the Z-ring is regulated by the Min system, the nucleoid occlusion, and the Ter region of the chromosome (reviewed in Schumacher 2017; Wettmann and Kruse 2018; Bailey et al. 2014a). The placement of the Z-ring at mid-cell is essential to generate two identical daughter cells after septum synthesis and cleavage. Septal PG synthesis is guided and driven by the dynamic FtsZ polymers, which move around the ring structure by treadmilling (Yang et al. 2017; Bisson-Filho et al. 2017). Remarkably, in the round-shaped bacteria *S. aureus* the recruitment of the proposed lipid II flippase MurJ to the divisome speeds up the synthesis of septal PG and makes it independent of FtsZ-treadmilling (Monteiro et al. 2018). In *E. coli*, FtsZ is not present at the division site during the whole process of septation, as it moves to the future cell division sites before constriction is finished (Soderstrom et al. 2014, 2016). Instead of that, the cell division proteins FtsN and PBP3, both spatially separated from the FtsZ ring, remain at division site until septum synthesis is completed (Soderstrom et al. 2016, 2018). PBP3 activity, but not FtsZ, has been shown to be the main regulator of septum closure (Coltharp et al. 2016), leading to different models about the source of constrictive force (Xiao and Goley 2016; Schoenemann and Margolin 2017; Holden 2018).

Regulation of Peptidoglycan Synthesis from the Periplasmic Side

E. coli contains periplasmic proteins that regulate the activities of the PG multienzymatic complexes. LpoA and LpoB are two outer membrane-anchored lipoproteins that activates the synthetic activities of PBP1A and PBP1B, respectively. The presence of one of these proteins is essential for cell viability, in a similar way to PBP1A and PBP1B, suggesting that the Lpo's are required for the proper functioning of the PBPs (Typas et al. 2010; Paradis-Bleau et al. 2010). The regions involved in the interaction with their cognate PBPs are identified (Jean et al. 2014; Egan et al. 2014; King et al. 2014; Sathiyamoorthy et al. 2017). In vitro studies have shown that LpoA stimulates the TPase activity of PBP1A, and LpoB stimulates both GTase and TPase activities of PBP1B (Lupoli et al. 2014; Egan et al. 2014, 2018). The identification of PBP1B mutants able to bypass the in vivo requirement of LpoB support the role of LpoB in PBP1B activation (Markovski et al. 2016). It has been hypothesised that the activation by Lpo's could regulate the PG enlargement depending on the sacculus pores size, which might be different depending on the cellular growth rate (Typas et al. 2010).

During cell division several proteins have been shown to regulate and coordinate the activity of the PG synthases and hydrolases. In the next lines some recent results are briefly mentioned, including the Tol-Pal complex, FtsN and PG amidases. The Tol-Pal complex is a well conserved system in Gram negative bacteria and includes cytoplasmic membrane, periplasmic and outer membrane proteins. These proteins

interact with each other and, in the presence of proton motive force, maintain the stability of the outer membrane (Cascales et al. 2000, 2001, 2002). TolA and CpoB interact directly with PBP1B and regulate its synthetic activities. TolA enhances the GTase activity of PBP1B, either in presence or absence of LpoB, and CpoB decreases the TPase activity of PBP1B in presence of LpoB without interfering with the GTase activation (Gray et al. 2015; Egan et al. 2018). The CpoB effect can be reverted by TolA (Gray et al. 2015).

FtsN is considered the septation trigger, as it is mainly recruited at late stages of cell division just before constriction starts. This recruitment is due to the binding of its periplasmic SPOR (sporulation-related repeat) domain to PG glycan chains (Ursinus et al. 2004) lacking the stem peptides, suggesting that it follows the action of amidases (Yahashiri et al. 2015). The cytoplasmic region of FtsN is required for an earlier recruitment of FtsN to midcell by the interaction with FtsA (Busiek et al. 2012; Busiek and Margolin 2014; Pichoff et al. 2015). Whereas favouring the FtsA-FtsN interaction the cell bypasses different impairments in other cell division proteins (Pichoff et al. 2018), point mutations in FtsA abolish the essentiality of FtsN (Bernard et al. 2007). The essentiality of FtsN seems to be encoded in a small peptide from the periplasmic domain (Gerding et al. 2009; Liu et al. 2015), which would be involved in the eventual activation of septation. FtsN interacts with different cell division proteins including the PG synthases PBP3 and PBP1B, and the potential PG synthase FtsW (Di Lallo et al. 2003; Müller et al. 2007; Alexeeva et al. 2010), and in vitro experiments have shown that FtsN enhances both GTase and TPase activities of PBP1B (Müller et al. 2007). Due to the non-essentiality of PBP1B, the activation of PBP3 and/or FtsW is likely to be the final target of FtsN. Genetic evidences have shown that the conserved cell division proteins FtsQLB are involved in this activation pathway (Liu et al. 2015; Tsang and Bernhardt 2015).

E. coli contains three PG amidases (AmiA, AmiB and AmiC) required for splitting the PG sacculi of two daughter cells during septation, although only AmiB and AmiC are recruited to the division site. Their catalytic activities are regulated by protein activators: AmiA and AmiB by EnvC, and AmiC by NlpD. The correct localization of both activators is essential for the temporal and spatial regulation of the amidase activities. EnvC is localized at preseptal positions (Peters et al. 2011) by the cell division ATP-binding cassette complex FtsEX, in which FtsE is the cytoplasmic nucleotide-binding protein and FtsX is the integral membrane component that binds to EnvC (de Leeuw et al. 1999; Yang et al. 2011). Conformational changes in FtsX, driven by the ATPase activity of FtsE, are essential for the amidase activation by EnvC (Yang et al. 2011). NlpD, as EnvC, it is located at the division site before septal PG is synthesised (Peters et al. 2011). Additional proteins, as the Tol-Pal complex and YraP, are required for the activation of AmiC by NlpD (Tsang et al. 2017).

Fluorescent D-Amino Acids as Tools

The recent development of new fluorescent D-amino acids (FDAAs), combined with the continuous improvement of microscopic techniques, enables the visualization of PG synthesis, remodelling and dynamics in high resolution. These powerful tools are used to study PG synthesis processes during cell elongation and division in diverse Gram-positive and Gram-negative bacteria. The FDAAs designed by VanNieuwenhze's and Brun's labs mimic the acyl acceptor during the PG synthesis reaction and are thought to be incorporated into the stem peptides through a D-amino-exchange reaction performed by either DD-TPase (Lupoli et al. 2011) or DD-CPase and LD-TPase activities (Cava et al. 2011; Hsu et al. 2017) (Fig. 5.4). Based on their specificity, FDAAs efficiently label the active PG synthesis sites with minimal cell toxicities (Kuru et al. 2012). FDAAs have been successfully used in many PG studies of diverse species, providing new insights about PG synthesis, recycling and turnover (for a detailed review about FDAAs, including an updated list of studies using them (see Radkov et al. 2018)).

Concluding Remarks

In the last years we have gained insight into many different aspects of the bacterial cell wall, but we are still far from understand this complex cellular structure. The introduction of new tools and technologies (as FDAAs, high resolution microscopy or high-throughput genetic screenings), combined with the multidisciplinary contribution to the field, is currently boosting the knowledge of the field. Besides the new biophysical and structural approaches, the better understanding of the essential proteins roles might lead to the identification of new antimicrobial drug targets, for example the LDTs. New antimicrobial drugs are required to reduce the dramatic spread of multidrug resistant bacteria. In terms of basic knowledge, the formation and coordination of the elongasome and divisome complexes remain elusive. The further characterisation of the transition between both elongation and division, and the following septum formation and cell constriction will aid the comprehension of this complex but exciting macromolecule, the peptidoglycan.

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References

Alcorlo M, Martinez-Caballero S, Molina R, Hermoso JA (2017) Carbohydrate recognition and lysis by bacterial peptidoglycan hydrolases. Curr Opin Struct Biol 44:87–100

Alexeeva S, Gadella Tw Jr, Verheul J, Verhoeven GS, Den Blaauwen T (2010) Direct interactions of early and late assembling division proteins in *Escherichia coli* cells resolved by FRET. Mol Microbiol 77:384–389

Amanuma H, Strominger JL (1980) Purification and properties of penicillin-binding proteins 5 and 6 from *Escherichia coli* membranes. J Biol Chem 255:11173–11180

Aramini JM, Rossi P, Huang YJ, Zhao L, Jiang M, Maglaqui M, Xiao R, Locke J, Nair R, Rost B, Acton TB, Inouye M, Montelione GT (2008) Solution NMR structure of the NlpC/P60 domain of lipoprotein Spr from *Escherichia coli*: structural evidence for a novel cysteine peptidase catalytic triad. Biochemistry 47:9715–9717

Arthur M, Molinas C, Depardieu F, Courvalin P (1993) Characterization of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. J Bacteriol 175:117–127

Arthur M, Reynolds P, Courvalin P (1996) Glycopeptide resistance in *enterococci*. Trends Microbiol 4:401–407

Atrih A, Bacher G, Allmaier G, Williamson MP, Foster SJ (1999) Analysis of peptidoglycan structure from vegetative cells of *Bacillus subtilis* 168 and role of PBP 5 in peptidoglycan maturation. J Bacteriol 181:3956–3966

Aubry C, Goulard C, Nahori MA, Cayet N, Decalf J, Sachse M, Boneca IG, Cossart P, Dussurget O (2011) OatA, a peptidoglycan O-acetyltransferase involved in *Listeria monocytogenes* immune escape, is critical for virulence. J Infect Dis 204:731–740

Auer GK, Lee TK, Rajendram M, Cesar S, Miguel A, Huang KC, Weibel DB (2016) Mechanical genomics identifies diverse modulators of bacterial cell stiffness. Cell Syst 2:402–411

Bailey MW, Bisicchia P, Warren BT, Sherratt DJ, Mannik J (2014a) Evidence for divisome localization mechanisms independent of the min system and SlmA in *Escherichia coli*. PLoS Genet 10:e1004504

Bailey RG, Turner RD, Mullin N, Clarke N, Foster SJ, Hobbs JK (2014b) The interplay between cell wall mechanical properties and the cell cycle in *Staphylococcus aureus*. Biophys J 107:2538–2545

Banzhaf M, van den berg van saparoea B, Terrak M, Fraipont C, Egan A, Philippe J, Zapun A, Breukink E, Nguyen-Disteche M, den Blaauwen T, Vollmer W (2012) Cooperativity of peptidoglycan synthases active in bacterial cell elongation. Mol Microbiol 85:179–194

Barreteau H, Kovac A, Boniface A, Sova M, Gobec S, Blanot D (2008) Cytoplasmic steps of peptidoglycan biosynthesis. FEMS Microbiol Rev 32:168–207

Barrett DS, Chen L, Litterman NK, Walker S (2004) Expression and characterization of the isolated glycosyltransferase module of *Escherichia coli* PBP1b. Biochemistry 43:12375–12381

Beeby M, Gumbart JC, Roux B, Jensen GJ (2013) Architecture and assembly of the Gram-positive cell wall. Mol Microbiol 88:664–672

Benachour A, Ladjouzi R, le Jeune A, Hebert L, Thorpe S, Courtin P, Chapot-Chartier MP, Prajsnar TK, Foster SJ, Mesnage S (2012) The lysozyme-induced peptidoglycan *N*-acetylglucosamine deacetylase PgdA (EF1843) is required for *Enterococcus faecalis* virulence. J Bacteriol 194:6066–6073

Bendezu FO, de Boer PA (2008) Conditional lethality, division defects, membrane involution, and endocytosis in mre and mrd shape mutants of *Escherichia coli*. J Bacteriol 190:1792–1811

Bera A, Herbert S, Jakob A, Vollmer W, Gotz F (2005) Why are pathogenic staphylococci so lysozyme resistant? the peptidoglycan *O*-acetyltransferase OatA is the major determinant for lysozyme resistance of *Staphylococcus aureus*. Mol Microbiol 55:778–787

Berezuk AM, Glavota S, Roach EJ, Goodyear MC, Krieger JR, Khursigara CM (2018) Outer membrane lipoprotein RlpA is a novel periplasmic interaction partner of the cell division protein FtsK in *Escherichia coli*. Sci Rep 8:12933

Bernal-Cabas M, Ayala JA, Raivio TL (2015) The Cpx envelope stress response modifies peptidoglycan cross-linking via the LD-transpeptidase LdtD and the novel protein YgaU. J Bacteriol 197:603–614

Bernard CS, Sadasivam M, Shiomi D, Margolin W (2007) An altered FtsA can compensate for the loss of essential cell division protein FtsN in *Escherichia coli*. Mol Microbiol 64:1289–1305

Bernard E, Rolain T, Courtin P, Guillot A, Langella P, Hols P, Chapot-Chartier MP (2011) Characterization of *O*-acetylation of *N*-acetylglucosamine: a novel structural variation of bacterial peptidoglycan. J Biol Chem 286:23950–23958

Bernard E, Rolain T, David B, Andre G, Dupres V, Dufrene YF, Hallet B, Chapot-Chartier MP, Hols P (2012) Dual role for the *O*-acetyltransferase OatA in peptidoglycan modification and control of cell septation in *Lactobacillus plantarum*. PLoS ONE 7:e47893

Bernhardt TG, de Boer PA (2003) The *Escherichia coli* amidase AmiC is a periplasmic septal ring component exported via the twin-arginine transport pathway. Mol Microbiol 48:1171–1182

Bertsche U, Breukink E, Kast T, Vollmer W (2005) *In vitro* murein peptidoglycan synthesis by dimers of the bifunctional transglycosylase-transpeptidase PBP1B from *Escherichia coli*. J Biol Chem 280:38096–38101

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 (2017) Treadmilling by FtsZ filaments drives peptidoglycan synthesis and bacterial cell division. Science 355:739–743

Bolla JR, Sauer JB, Wu D, Mehmood S, Allison TM, Robinson CV (2018) Direct observation of the influence of cardiolipin and antibiotics on lipid II binding to MurJ. Nat Chem 10:363–371

Boneca IG, Dussurget O, Cabanes D, Nahori MA, Sousa S, Lecuit M, Psylinakis E, Bouriotis V, Hugot JP, Giovannini M, Coyle A, Bertin J, Namane A, Rousselle JC, Cayet N, Prevost MC, Balloy V, Chignard M, Philpott DJ, Cossart P, Girardin SE (2007) A critical role for peptidoglycan *N*-deacetylation in Listeria evasion from the host innate immune system. Proc Natl Acad Sci USA 104:997–1002

Bonnet J, Durmort C, Jacq M, Mortier-Barriere I, Campo N, Vannieuwenhze MS, Brun YV, Arthaud C, Gallet B, Moriscot C, Morlot C, Vernet T, di Guilmi AM (2017) Peptidoglycan *O*-acetylation is functionally related to cell wall biosynthesis and cell division in *Streptococcus pneumoniae*. Mol Microbiol 106:832–846

Born P, Breukink E, Vollmer W (2006) *In vitro* synthesis of cross-linked murein and its attachment to sacculi by PBP1A from *Escherichia coli*. J Biol Chem 281:26985–26993

Boyle-Vavra S, Labischinski H, Ebert CC, Ehlert K, Daum RS (2001) A spectrum of changes occurs in peptidoglycan composition of glycopeptide-intermediate clinical *Staphylococcus aureus* isolates. Antimicrob Agents Chemother 45:280–287

Brammer LAB, Ghosh A, Pan Y, Jakoncic J, Lloyd EP, Townsend CA, Lamichhane G, Bianchet MA (2015) Loss of a functionally and structurally distinct LD-transpeptidase, LdtMt5, compromises cell wall integrity in *Mycobacterium tuberculosis*. J Biol Chem 290:25670–25685

Bratton BP, Shaevitz JW, Gitai Z, Morgenstein RM (2018) MreB polymers and curvature localization are enhanced by RodZ and predict *E. coli*'s cylindrical uniformity. Nat Commun 9:2797

Bui NK, Eberhardt A, Vollmer D, Kern T, Bougault C, Tomasz A, Simorre JP, Vollmer W (2012) Isolation and analysis of cell wall components from *Streptococcus pneumoniae*. Anal Biochem 421:657–666

Buist G, Steen A, Kok J, Kuipers OP (2008) LysM, a widely distributed protein motif for binding to (peptido)glycans. Mol Microbiol 68:838–847

Busiek KK, Eraso JM, Wang Y, Margolin W (2012) The early divisome protein FtsA interacts directly through its 1c subdomain with the cytoplasmic domain of the late divisome protein FtsN. J Bacteriol 194:1989–2000

Busiek KK, Margolin W (2014) A role for FtsA in SPOR-independent localization of the essential *Escherichia coli* cell division protein FtsN. Mol Microbiol 92:1212–1226

Butler EK, Davis RM, Bari V, Nicholson PA, Ruiz N (2013) Structure-function analysis of MurJ reveals a solvent-exposed cavity containing residues essential for peptidoglycan biogenesis in *Escherichia coli*. J Bacteriol 195:4639–4649

Butler EK, Tan WB, Joseph H, Ruiz N (2014) Charge requirements of lipid II flippase activity in *Escherichia coli*. J Bacteriol 196:4111–4119

Cao L, Liang D, Hao P, Song Q, Xue E, Caiyin Q, Cheng Z, Qiao J (2018) The increase of O-acetylation and N-deacetylation in cell wall promotes acid resistance and nisin production through improving cell wall integrity in *Lactococcus lactis*. J Ind Microbiol Biotechnol

Cascales E, Bernadac A, Gavioli M, Lazzaroni JC, Lloubes R (2002) Pal lipoprotein of *Escherichia coli* plays a major role in outer membrane integrity. J Bacteriol 184:754–759

Cascales E, Gavioli M, Sturgis JN, Lloubes R (2000) Proton motive force drives the interaction of the inner membrane TolA and outer membrane pal proteins in *Escherichia coli*. Mol Microbiol 38:904–915

Cascales E, Lloubes R, Sturgis JN (2001) The TolQ-TolR proteins energize TolA and share homologies with the flagellar motor proteins MotA-MotB. Mol Microbiol 42:795–807

Cava F, de Pedro MA, Lam H, Davis BM, Waldor MK (2011) Distinct pathways for modification of the bacterial cell wall by non-canonical D-amino acids. EMBO J 30:3442–3453

Cayley DS, Guttman HJ, Record MT Jr (2000) Biophysical characterization of changes in amounts and activity of *Escherichia coli* cell and compartment water and turgor pressure in response to osmotic stress. Biophys J 78:1748–1764

Chamakura KR, Sham LT, Davis RM, Min L, Cho H, Ruiz N, Bernhardt TG, Young R (2017) A viral protein antibiotic inhibits lipid II flippase activity. Nat Microbiol 2:1480–1484

Chang JD, Foster EE, Wallace AG, Kim SJ (2017) Peptidoglycan *O*-acetylation increases in response to vancomycin treatment in vancomycin-resistant *Enterococcus faecalis*. Sci Rep 7:46500

Cho H, Uehara T, Bernhardt TG (2014) Beta-lactam antibiotics induce a lethal malfunctioning of the bacterial cell wall synthesis machinery. Cell 159:1300–1311

Cho H, Wivagg CN, Kapoor M, Barry Z, Rohs PD, Suh H, Marto JA, Garner EC, Bernhardt TG (2016). Bacterial cell wall biogenesis is mediated by SEDS and PBP polymerase families functioning semi-autonomously. Nat Microbiol 16172

Clarke TB, Kawai F, Park SY, Tame JR, Dowson CG, Roper DI (2009) Mutational analysis of the substrate specificity of *Escherichia coli* penicillin binding protein 4. Biochemistry 48:2675–2683

Colavin A, Shi H, Huang KC (2018) RodZ modulates geometric localization of the bacterial actin MreB to regulate cell shape. Nat Commun 9:1280

Coltharp C, Buss J, Plumer TM, Xiao J (2016) Defining the rate-limiting processes of bacterial cytokinesis. Proc Natl Acad Sci USA 113:E1044–E1053

Contreras-Martel C, Martins A, Ecobichon C, Trindade DM, Mattei PJ, Hicham S, Hardouin P, Ghachi ME, Boneca IG, Dessen A (2017) Molecular architecture of the PBP2-MreC core bacterial cell wall synthesis complex. Nat Commun 8:776

Coulombe F, Divangahi M, Veyrier F, de Leseleuc L, Gleason JL, Yang Y, Kelliher MA, Pandey AK, Sassetti CM, Reed MB, Behr MA (2009) Increased NOD2-mediated recognition of N-glycolyl muramyl dipeptide. J Exp Med 206:1709–1716

Dajkovic A, Tesson B, Chauhan S, Courtin P, Keary R, Flores P, Marliere C, Filipe SR, Chapot-Chartier MP, Carballido-Lopez R (2017) Hydrolysis of peptidoglycan is modulated by amidation of meso-diaminopimelic acid and Mg^{2+} in *Bacillus subtilis*. Mol Microbiol 104:972–988

de Leeuw E, Graham B, Phillips GJ, Ten Hagen-Jongman CM, Oudega B, Luirink J (1999) Molecular characterization of *Escherichia coli* FtsE and FtsX. Mol Microbiol 31:983–993

de Pedro MA, Quintela JC, Höltje JV, Schwarz H (1997) Murein segregation in *Escherichia coli*. J Bacteriol 179:2823–2834

Delhaye A, Collet JF, Laloux G (2016) Fine-tuning of the Cpx envelope stress response is required for cell wall homeostasis in *Escherichia coli*. MBio 7:e00047–16

Demchick P, Koch AL (1996) The permeability of the wall fabric of *Escherichia coli* and *Bacillus subtilis*. J Bacteriol 178:768–773

Denome SA, Elf PK, Henderson TA, Nelson DE, Young KD (1999) *Escherichia coli* mutants lacking all possible combinations of eight penicillin binding proteins: viability, characteristics, and implications for peptidoglycan synthesis. J Bacteriol 181:3981–3993

di Lallo G, Fagioli M, Barionovi D, Ghelardini P, Paolozzi L (2003) Use of a two-hybrid assay to study the assembly of a complex multicomponent protein machinery: bacterial septosome differentiation. Microbiology 149:3353–3359

Dik DA, Fisher JF, Mobashery S (2018) Cell-wall recycling of the Gram-negative bacteria and the nexus to antibiotic resistance. Chem Rev 118:5952–5984

Dik DA, Marous DR, Fisher JF, Mobashery S (2017) Lytic transglycosylases: concinnity in concision of the bacterial cell wall. Crit Rev Biochem Mol Biol 52:503–542

Dillard JP, Hackett KT (2005) Mutations affecting peptidoglycan acetylation in *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Infect Immun 73:5697–5705

Dutta M, Kar D, Bansal A, Chakraborty S, Ghosh AS (2015) A single amino acid substitution in the Omega-like loop of *E. coli* PBP5 disrupts its ability to maintain cell shape and intrinsic beta-lactam resistance. Microbiology 161:895–902

Egan AJ, Jean NL, Koumoutsi A, Bougault CM, Biboy J, Sassine J, Solovyova AS, Breukink E, Typas A, Vollmer W, Simorre JP (2014) Outer-membrane lipoprotein LpoB spans the periplasm to stimulate the peptidoglycan synthase PBP1B. Proc Natl Acad Sci USA 111:8197–8202

Egan AJ, Vollmer W (2013) The physiology of bacterial cell division. Ann N Y Acad Sci 1277:8–28

Egan AJF, Maya-Martinez R, Ayala I, Bougault CM, Banzhaf M, Breukink E, Vollmer W, Simorre JP (2018) Induced conformational changes activate the peptidoglycan synthase PBP1B. Mol Microbiol 110:335–356

el Ghachi M, Howe N, Huang CY, Olieric V, Warshamanage R, Touze T, Weichert D, Stansfeld PJ, Wang M, Kerff F, Caffrey M (2018) Crystal structure of undecaprenyl-pyrophosphate phosphatase and its role in peptidoglycan biosynthesis. Nat Commun 9:1078

Elhenawy W, Davis RM, Fero J, Salama NR, Felman MF, Ruiz N (2016) The O-antigen flippase Wzk can substitute for MurJ in peptidoglycan synthesis in *Helicobacter pylori* and *Escherichia coli*. PLoS ONE 11:e0161587

Emami K, Guyet A, Kawai Y, Devi J, Wu LJ, Allenby N, Daniel RA, Errington J (2017) RodA as the missing glycosyltransferase in *Bacillus subtilis* and antibiotic discovery for the peptidoglycan polymerase pathway. Nat Microbiol 2:16253

Engel H, van Leeuwen A, Dijkstra A, Keck W (1992) Enzymatic preparation of 1,6-anhydromuropeptides by immobilized murein hydrolases from *Escherichia coli* fused to staphylococcal protein A. Appl Microbiol Biotechnol 37:772–783

Errington J (2015) Bacterial morphogenesis and the enigmatic MreB helix. Nat Rev Microbiol 13:241–248

Espaillat A, Forsmo O, el Biari K, Bjork R, Lemaitre B, Trygg J, Canada FJ, de Pedro MA, Cava F (2016) Chemometric analysis of bacterial peptidoglycan reveals atypical modifications that empower the cell wall against predatory enzymes and fly innate immunity. J Am Chem Soc 138:9193–9204

Evans KL, Kannan S, Li G, de Pedro MA, Young KD (2013) Eliminating a set of four penicillin binding proteins triggers the Rcs phosphorelay and Cpx stress responses in *Escherichia coli*. J Bacteriol 195:4415–4424

Fan J, Jiang D, Zhao Y, Liu J, Zhang XC (2014) Crystal structure of lipid phosphatase *Escherichia coli* phosphatidylglycerophosphate phosphatase B. Proc Natl Acad Sci USA 111:7636–7640

Fenton AK, Gerdes K (2013) Direct interaction of FtsZ and MreB is required for septum synthesis and cell division in *Escherichia coli*. EMBO J 32:1953–1965

Figueiredo TA, Sobral RG, Ludovice AM, Almeida JM, Bui NK, Vollmer W, de Lencastre H, Tomasz A (2012) Identification of genetic determinants and enzymes involved with the amidation of glutamic acid residues in the peptidoglycan of *Staphylococcus aureus*. PLoS Pathog 8:e1002508

Filipe SR, Tomasz A (2000) Inhibition of the expression of penicillin resistance in *Streptococcus pneumoniae* by inactivation of cell wall muropeptide branching genes. Proc Natl Acad Sci USA 97:4891–4896

Firczuk M, Bochtler M (2007) Mutational analysis of peptidoglycan amidase MepA. Biochemistry 46:120–128

Fraipont C, Alexeeva S, Wolf B, van der Ploeg R, Schloesser M, den Blaauwen T, Nguyen-Disteche M (2011) The integral membrane FtsW protein and peptidoglycan synthase PBP3 form a subcomplex in *Escherichia coli*. Microbiology 157:251–259

Gallant CV, Daniels C, Leung JM, Ghosh AS, Young KD, Kotra LP, Burrows LL (2005) Common beta-lactamases inhibit bacterial biofilm formation. Mol Microbiol 58:1012–1024

Garcia-Bustos J, Tomasz A (1990) A biological price of antibiotic resistance: major changes in the peptidoglycan structure of penicillin-resistant pneumococci. Proc Natl Acad Sci USA 87:5415–5419

Garcia Del Portillo F, De Pedro MA (1990) Differential effect of mutational impairment of penicillin-binding proteins 1A and 1B on *Escherichia coli* strains harboring thermosensitive mutations in the cell division genes *ftsA*, *ftsQ*, *ftsZ*, and *pbpB*. J Bacteriol 172:5863–5870

Garcia Del Portillo F, De Pedro MA (1991) Penicillin-binding protein 2 is essential for the integrity of growing cells of *Escherichia coli ponB* strains. J Bacteriol 173:4530–4532

Garner EC, Bernard R, Wang W, Zhuang X, Rudner DZ, Mitchison T (2011) Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in *B. subtilis*. Science 333:222–225

Geiger T, Pazos M, Lara-Tejero M, Vollmer W, Galan JE (2018). Peptidoglycan editing by a specific LD-transpeptidase controls the muramidase-dependent secretion of typhoid toxin. Nat Microbiol

Gerding MA, Liu B, Bendezu FO, Hale CA, Bernhardt TG, de Boer PA (2009) Self-enhanced accumulation of FtsN at division sites and roles for other proteins with a SPOR domain (DamX, DedD, and RlpA) in *Escherichia coli* cell constriction. J Bacteriol 191:7383–7401

Glauner B, Höltje JV, Schwarz U (1988) The composition of the murein of *Escherichia coli*. J Biol Chem 263:10088–10095

Gonzalez-Leiza SM, de Pedro MA, Ayala JA (2011) AmpH, a bifunctional DD-endopeptidase and DD-carboxypeptidase of *Escherichia coli*. J Bacteriol 193:6887–6894

Govindarajan S, Amster-Choder O (2017) The bacterial Sec system is required for the organization and function of the MreB cytoskeleton. PLoS Genet 13:e1007017

Gray AN, Egan AJ, Van't Veer IL, Verheul J, Colavin A, Koumoutsi A, Biboy J, Altelaar AF, Damen MJ, Huang KC, Simorre JP, Breukink E, Den Blaauwen T, Typas A, Gross CA, Vollmer W (2015) Coordination of peptidoglycan synthesis and outer membrane constriction during *Escherichia coli* cell division. Elife 4

Gupta R, Lavollay M, Mainardi JL, Arthur M, Bishai WR, Lamichhane G (2010) The *Mycobacterium tuberculosis* protein LdtMt2 is a nonclassical transpeptidase required for virulence and resistance to amoxicillin. Nat Med 16:466–469

Ha R, Frirdich E, Sychantha D, Biboy J, Taveirne ME, Johnson JG, Dirita VJ, Vollmer W, Clarke AJ, Gaynor EC (2016) Accumulation of peptidoglycan *O*-acetylation leads to altered cell wall biochemistry and negatively impacts pathogenesis factors of *Campylobacter jejuni*. J Biol Chem 291:22686–22702

Hakulinen JK, Hering J, Branden G, Chen H, Snijder A, Ek M, Johansson P (2017) MraY-antibiotic complex reveals details of tunicamycin mode of action. Nat Chem Biol 13:265–267

Hansen JM, Golchin SA, Veyrier FJ, Domenech P, Boneca IG, Azad AK, Rajaram MV, Schlesinger LS, Divangahi M, Reed MB, Behr MA (2014) *N*-glycolylated peptidoglycan contributes to the immunogenicity but not pathogenicity of *Mycobacterium tuberculosis*. J Infect Dis 209:1045–1054

Heidrich C, Templin MF, Ursinus A, Merdanovic M, Berger J, Schwarz H, de Pedro MA, Höltje JV (2001) Involvement of *N*-acetylmuramyl-L-alanine amidases in cell separation and antibiotic-induced autolysis of *Escherichia coli*. Mol Microbiol 41:167–178

Heidrich C, Ursinus A, Berger J, Schwarz H, Höltje JV (2002) Effects of multiple deletions of murein hydrolases on viability, septum cleavage, and sensitivity to large toxic molecules in *Escherichia coli*. J Bacteriol 184:6093–6099

Henderson TA, Dombrosky PM, Young KD (1994) Artifactual processing of penicillin-binding proteins 7 and 1b by the OmpT protease of *Escherichia coli*. J Bacteriol 176:256–259

Henderson TA, Templin M, Young KD (1995) Identification and cloning of the gene encoding penicillin-binding protein 7 of *Escherichia coli*. J Bacteriol 177:2074–2079

Henderson TA, Young KD, Denome SA, Elf PK (1997) AmpC and AmpH, proteins related to the class C beta-lactamases, bind penicillin and contribute to the normal morphology of *Escherichia coli*. J Bacteriol 179:6112–6121

Holden S (2018) Probing the mechanistic principles of bacterial cell division with super-resolution microscopy. Curr Opin Microbiol 43:84–91

Höltje JV (1998) Growth of the stress-bearing and shape-maintaining murein sacculus of *Escherichia coli*. Microbiol Mol Biol Rev 62:181–203

Hrast M, Sosic I, Sink R, Gobec S (2014) Inhibitors of the peptidoglycan biosynthesis enzymes MurA-F. Bioorg Chem 55:2–15

Hsu YP, Rittichier J, Kuru E, Yablonowski J, Pasciak E, Tekkam S, Hall E, Murphy B, Lee TK, Garner EC, Huang KC, Brun YV, Vannieuwenhze MS (2017) Full color palette of fluorescent D-amino acids for *in situ* labeling of bacterial cell walls. Chem Sci 8:6313–6321

Hughes RC, Thurman PF, Stokes E (1975) Estimates of the porosity of *Bacillus licheniformis* and *Bacillus subtilis* cell walls. Z Immunitatsforsch Exp Klin Immunol 149:126–135

Hugonnet JE, Mengin-Lecreulx D, Monton A, Den Blaauwen T, Carbonnelle E, Veckerle C, Brun YV, Van Nieuwenhze M, Bouchier C, Tu K, Rice LB, Arthur M (2016). Factors essential for LD-transpeptidase-mediated peptidoglycan cross-linking and beta-lactam resistance in *Escherichia coli*. Elife 5

Iida K, Hirota Y, Schwarz U (1983) Mutants of *Escherichia coli* defective in penicillin-insensitive murein DD-endopeptidase. Mol Gen Genet 189:215–221

Inoue A, Murata Y, Takahashi H, Tsuji N, Fujisaki S, Kato J (2008) Involvement of an essential gene, *mviN*, in murein synthesis in *Escherichia coli*. J Bacteriol 190:7298–7301

Inouye M, Shaw J, Shen C (1972) The assembly of a structural lipoprotein in the envelope of *Escherichia coli*. J Biol Chem 247:8154–8159

Ishino F, Jung HK, Ikeda M, Doi M, Wachi M, Matsuhashi M (1989) New mutations *fts-36*, *lts-33*, and *ftsW* clustered in the *mra* region of the *Escherichia coli* chromosome induce thermosensitive cell growth and division. J Bacteriol 171:5523–5530

Ize B, Stanley NR, Buchanan G, Palmer T (2003) Role of the *Escherichia coli* Tat pathway in outer membrane integrity. Mol Microbiol 48:1183–1193

Jacobs C, Huang LJ, Bartowsky E, Normark S, Park JT (1994) Bacterial cell wall recycling provides cytosolic muropeptides as effectors for beta-lactamase induction. EMBO J 13:4684–4694

Jacobs C, Joris B, Jamin M, Klarsov K, van Beeumen J, Mengin-Lecreulx D, van Heijenoort J, Park JT, Normark S, Frere JM (1995) AmpD, essential for both beta-lactamase regulation and cell wall recycling, is a novel cytosolic *N*-acetylmuramyl-L-alanine amidase. Mol Microbiol 15:553–559

Jacoby GH, Young KD (1988) Unequal distribution of penicillin-binding proteins among inner membrane vesicles of *Escherichia coli*. J Bacteriol 170:3660–3667

Jean NL, Bougault CM, Lodge A, Derouaux A, Callens G, Egan AJ, Ayala I, Lewis RJ, Vollmer W, Simorre JP (2014) Elongated structure of the outer-membrane activator of peptidoglycan synthesis LpoA: implications for PBP1A stimulation. Structure 22:1047–1054

Jeong JH, Kim YS, Rojviriya C, Ha SC, Kang BS, Kim YG (2013) Crystal structures of bifunctional penicillin-binding protein 4 from *Listeria monocytogenes*. Antimicrob Agents Chemother 57:3507–3512

Jorgenson MA, Chen Y, Yahashiri A, Popham DL, Weiss DS (2014) The bacterial septal ring protein RlpA is a lytic transglycosylase that contributes to rod shape and daughter cell separation in *Pseudomonas aeruginosa*. Mol Microbiol 93:113–128

Juan C, Torrens G, Gonzalez-Nicolau M, Oliver A (2017) Diversity and regulation of intrinsic beta-lactamases from non-fermenting and other Gram-negative opportunistic pathogens. FEMS Microbiol Rev 41:781–815

Kaoukab-Raji A, Biskri L, Bernardini ML, Allaoui A (2012) Characterization of SfPgdA, a *Shigella flexneri* peptidoglycan deacetylase required for bacterial persistence within polymorphonuclear neutrophils. Microbes Infect 14:619–627

Kar D, Pandey SD, Mallick S, Dutta M, Ghosh AS (2018) Substitution of alanine at position 184 with glutamic acid in *Escherichia coli* PBP5 omega-like loop introduces a moderate cephalosporinase activity. Protein J 37:122–131

Karimova G, Dautin N, Ladant D (2005) Interaction network among *Escherichia coli* membrane proteins involved in cell division as revealed by bacterial two-hybrid analysis. J Bacteriol 187:2233–2243

Kato J, Suzuki H, Hirota Y (1985) Dispensability of either penicillin-binding protein-1a or -1b involved in the essential process for cell elongation in *Escherichia coli*. Mol Gen Genet 200:272–277

Kawazura T, Matsumoto K, Kojima K, Kato F, Kanai T, Niki H, Shiomi D (2017) Exclusion of assembled MreB by anionic phospholipids at cell poles confers cell polarity for bidirectional growth. Mol Microbiol 104:472–486

Keck W, Schwarz U (1979) *Escherichia coli* murein-DD-endopeptidase insensitive to beta-lactam antibiotics. J Bacteriol 139:770–774

Keck W, van Leeuwen AM, Huber M, Goodell EW (1990) Cloning and characterization of *mepA*, the structural gene of the penicillin-insensitive murein endopeptidase from *Escherichia coli*. Mol Microbiol 4:209–219

Kerff F, Petrella S, Mercier F, Sauvage E, Herman R, Pennartz A, Zervosen A, Luxen A, Frere JM, Joris B, Charlier P (2010) Specific structural features of the *N*-acetylmuramoyl-L-alanine amidase AmiD from *Escherichia coli* and mechanistic implications for enzymes of this family. J Mol Biol 397:249–259

Kerr CH, Culham DE, Marom D, Wood JM (2014) Salinity-dependent impacts of ProQ, Prc, and Spr deficiencies on *Escherichia coli* cell structure. J Bacteriol 196:1286–1296

Khattar MM, Begg KJ, Donachie WD (1994) Identification of FtsW and characterization of a new *ftsW* division mutant of *Escherichia coli*. J Bacteriol 176:7140–7147

King DT, Lameignere E, Strynadka NC (2014) Structural insights into the lipoprotein outer membrane regulator of penicillin-binding protein 1B. J Biol Chem 289:19245–19253

King DT, Wasney GA, Nosella M, Fong A, Strynadka NC (2017) Structural insights into inhibition of *Escherichia coli* penicillin-binding protein 1B. J Biol Chem 292:979–993

Kishida H, Unzai S, Roper DI, Lloyd A, Park SY, Tame JR (2006) Crystal structure of penicillin binding protein 4 (dacB) from *Escherichia coli*, both in the native form and covalently linked to various antibiotics. Biochemistry 45:783–792

Koch AL (1988) Biophysics of bacterial walls viewed as stress-bearing fabric. Microbiol Rev 52:337–353

Korat B, Mottl H, Keck W (1991) Penicillin-binding protein 4 of *Escherichia coli*: molecular cloning of the *dacB* gene, controlled overexpression, and alterations in murein composition. Mol Microbiol 5:675–684

Kouidmi I, Levesque RC, Paradis-Bleau C (2014) The biology of Mur ligases as an antibacterial target. Mol Microbiol 94:242–253

Kruse T, Bork-Jensen J, Gerdes K (2005) The morphogenetic MreBCD proteins of *Escherichia coli* form an essential membrane-bound complex. Mol Microbiol 55:78–89

Kuk AC, Mashalidis EH, Lee SY (2017) Crystal structure of the MOP flippase MurJ in an inward-facing conformation. Nat Struct Mol Biol 24:171–176

Kuru E, Hughes HV, Brown PJ, Hall E, Tekkam S, Cava F, de Pedro MA, Brun YV, Vannieuwenhze MS (2012) *In situ* probing of newly synthesized peptidoglycan in live bacteria with fluorescent D-amino acids. Angew Chem Int Ed Engl 51:12519–12523

Lai GC, Cho H, Bernhardt TG (2017) The mecillinam resistome reveals a role for peptidoglycan endopeptidases in stimulating cell wall synthesis in *Escherichia coli*. PLoS Genet 13:e1006934

Lam H, Oh DC, Cava F, Takacs CN, Clardy J, de Pedro MA, Waldor MK (2009) D-amino acids govern stationary phase cell wall remodeling in bacteria. Science 325:1552–1555

Lambert C, Lerner TR, Bui NK, Somers H, Aizawa S, Liddell S, Clark A, Vollmer W, Lovering AL, Sockett RE (2016) Interrupting peptidoglycan deacetylation during Bdellovibrio predator-

prey interaction prevents ultimate destruction of prey wall, liberating bacterial-ghosts. Sci Rep 6:26010
Lara B, Ayala JA (2002) Topological characterization of the essential *Escherichia coli* cell division protein FtsW. FEMS Microbiol Lett 216:23–32
Lara B, Mengin-Lecreulx D, Ayala JA, van Heijenoort J (2005) Peptidoglycan precursor pools associated with MraY and FtsW deficiencies or antibiotic treatments. FEMS Microbiol Lett 250:195–200
Lavollay M, Arthur M, Fourgeaud M, Dubost L, Marie A, Veziris N, Blanot D, Gutmann L, Mainardi JL (2008) The peptidoglycan of stationary-phase *Mycobacterium tuberculosis* predominantly contains cross-links generated by LD-transpeptidation. J Bacteriol 190:4360–4366
Lavollay M, Fourgeaud M, Herrmann JL, Dubost L, Marie A, Gutmann L, Arthur M, Mainardi JL (2011) The peptidoglycan of *Mycobacterium abscessus* is predominantly cross-linked by LD-transpeptidases. J Bacteriol 193:778–782
Leclercq S, Derouaux A, Olatunji S, Fraipont C, Egan AJ, Vollmer W, Breukink E, Terrak M (2017) Interplay between penicillin-binding proteins and SEDS proteins promotes bacterial cell wall synthesis. Sci Rep 7:43306
Lee M, Batuecas MT, Tomoshige S, Dominguez-Gil T, Mahasenan KV, Dik DA, Hesek D, Millan C, Uson I, Lastochkin E, Hermoso JA, Mobashery S (2018) Exolytic and endolytic turnover of peptidoglycan by lytic transglycosylase Slt of *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA 115:4393–4398
Lee M, Hesek D, Llarrull LI, Lastochkin E, Pi H, Boggess B, Mobashery S (2013) Reactions of all *Escherichia coli* lytic transglycosylases with bacterial cell wall. J Am Chem Soc 135:3311–3314
Lee TK, Meng K, Shi H, Huang KC (2016) Single-molecule imaging reveals modulation of cell wall synthesis dynamics in live bacterial cells. Nat Commun 7:13170
Lee TK, Tropini C, Hsin J, Desmarais SM, Ursell TS, Gong E, Gitai Z, Monds RD, Huang KC (2014) A dynamically assembled cell wall synthesis machinery buffers cell growth. Proc Natl Acad Sci USA 111:4554–4559
Leidenix MJ, Jacoby GH, Henderson TA, Young KD (1989) Separation of *Escherichia coli* penicillin-binding proteins into different membrane vesicles by agarose electrophoresis and sizing chromatography. J Bacteriol 171:5680–5686
Levefaudes M, Patin D, De Sousa-D'auria C, Chami M, Blanot D, Herve M, Arthur M, Houssin C, Mengin-Lecreulx D (2015) Diaminopimelic acid amidation in corynebacteriales: new insights into the role of LtsA in peptidoglycan modification. J Biol Chem 290:13079–13094
Li GW, Burkhardt D, Gross C, Weissman JS (2014) Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources. Cell 157:624–635
Li SY, Höltje JV, Young KD (2004) Comparison of high-performance liquid chromatography and fluorophore-assisted carbohydrate electrophoresis methods for analyzing peptidoglycan composition of *Escherichia coli*. Anal Biochem 326:1–12
Liechti G, Kuru E, Packiam M, Hsu YP, Tekkam S, Hall E, Rittichier JT, Vannieuwenhze M, Brun YV, Maurelli AT (2016) Pathogenic chlamydia lack a classical sacculus but synthesize a narrow, mid-cell peptidoglycan ring, regulated by MreB, for cell division. PLoS Pathog 12:e1005590
Liu B, Persons L, Lee L, de Boer PA (2015) Roles for both FtsA and the FtsBLQ subcomplex in FtsN-stimulated cell constriction in *Escherichia coli*. Mol Microbiol 95:945–970
Liu Y, Breukink E (2016) The membrane steps of bacterial cell wall synthesis as antibiotic targets. Antibiotics (Basel) 5
Loskill P, Pereira PM, Jung P, Bischoff M, Herrmann M, Pinho MG, Jacobs K (2014) Reduction of the peptidoglycan crosslinking causes a decrease in stiffness of the *Staphylococcus aureus* cell envelope. Biophys J 107:1082–1089
Lovering AL, de Castro LH, Lim D, Strynadka NC (2007) Structural insight into the transglycosylation step of bacterial cell-wall biosynthesis. Science 315:1402–1405
Lu C, Reedy M, Erickson HP (2000) Straight and curved conformations of FtsZ are regulated by GTP hydrolysis. J Bacteriol 182:164–170

Lupoli TJ, Lebar MD, Markovski M, Bernhardt T, Kahne D, Walker S (2014) Lipoprotein activators stimulate *Escherichia coli* penicillin-binding proteins by different mechanisms. J Am Chem Soc 136:52–55

Lupoli TJ, Tsukamoto H, Doud EH, Wang TS, Walker S, Kahne D (2011) Transpeptidase-mediated incorporation of D-amino acids into bacterial peptidoglycan. J Am Chem Soc 133:10748–10751

Magnet S, Bellais S, Dubost L, Fourgeaud M, Mainardi JL, Petit-Frere S, Marie A, Mengin-Lecreulx D, Arthur M, Gutmann L (2007) Identification of the LD-transpeptidases responsible for attachment of the Braun lipoprotein to *Escherichia coli* peptidoglycan. J Bacteriol 189:3927–3931

Magnet S, Dubost L, Marie A, Arthur M, Gutmann L (2008) Identification of the LD-transpeptidases for peptidoglycan cross-linking in *Escherichia coli*. J Bacteriol 190:4782–4785

Mahapatra S, Crick DC, Brennan PJ (2000) Comparison of the UDP-*N*-acetylmuramate:L-alanine ligase enzymes from *Mycobacterium tuberculosis* and *Mycobacterium leprae*. J Bacteriol 182:6827–6830

Mainardi JL, Fourgeaud M, Hugonnet JE, Dubost L, Brouard JP, Ouazzani J, Rice LB, Gutmann L, Arthur M (2005) A novel peptidoglycan cross-linking enzyme for a beta-lactam-resistant transpeptidation pathway. J Biol Chem 280:38146–38152

Mainardi JL, Hugonnet JE, Rusconi F, Fourgeaud M, Dubost L, Moumi AN, Delfosse V, Mayer C, Gutmann L, Rice LB, Arthur M (2007) Unexpected inhibition of peptidoglycan LD-transpeptidase from *Enterococcus faecium* by the beta-lactam imipenem. J Biol Chem 282:30414–30422

Mainardi JL, Legrand R, Arthur M, Schoot B, van Heijenoort J, Gutmann L (2000) Novel mechanism of beta-lactam resistance due to bypass of DD-transpeptidation in *Enterococcus faecium*. J Biol Chem 275:16490–16496

Mainardi JL, Morel V, Fourgeaud M, Cremniter J, Blanot D, Legrand R, Frehel C, Arthur M, van Heijenoort J, Gutmann L (2002) Balance between two transpeptidation mechanisms determines the expression of beta-lactam resistance in *Enterococcus faecium*. J Biol Chem 277:35801–35807

Manat G, el Ghachi M, Auger R, Baouche K, Olatunji S, Kerff F, Touze T, Mengin-Lecreulx D, Bouhss A (2015) Membrane topology and biochemical characterization of the *Escherichia coli* BacA undecaprenyl-pyrophosphate phosphatase. PLoS ONE 10:e0142870

Manat G, Roure S, Auger R, Bouhss A, Barreteau H, Mengin-Lecreulx D, Touze T (2014) Deciphering the metabolism of undecaprenyl-phosphate: the bacterial cell-wall unit carrier at the membrane frontier. Microb Drug Resist 20:199–214

Markiewicz Z, Broome-Smith JK, Schwarz U, Spratt BG (1982) Spherical *E. coli* due to elevated levels of D-alanine carboxypeptidase. Nature 297:702–704

Markovski M, Bohrhunter JL, Lupoli TJ, Uehara T, Walker S, Kahne DE, Bernhardt TG (2016) Cofactor bypass variants reveal a conformational control mechanism governing cell wall polymerase activity. Proc Natl Acad Sci USA 113:4788–4793

Matias VR, Al-Amoudi A, Dubochet J, Beveridge TJ (2003) Cryo-transmission electron microscopy of frozen-hydrated sections of *Escherichia coli* and *Pseudomonas aeruginosa*. J Bacteriol 185:6112–6118

Matias VR, Beveridge TJ (2005) Cryo-electron microscopy reveals native polymeric cell wall structure in *Bacillus subtilis* 168 and the existence of a periplasmic space. Mol Microbiol 56:240–251

Meberg BM, Paulson AL, Priyadarshini R, Young KD (2004) Endopeptidase penicillin-binding proteins 4 and 7 play auxiliary roles in determining uniform morphology of *Escherichia coli*. J Bacteriol 186:8326–8336

Meeske AJ, Riley EP, Robins WP, Uehara T, Mekalanos JJ, Kahne D, Walker S, Kruse AC, Bernhardt TG, Rudner DZ (2016) SEDS proteins are a widespread family of bacterial cell wall polymerases. Nature 537:634–638

Meeske AJ, Sham LT, Kimsey H, Koo BM, Gross CA, Bernhardt TG, Rudner DZ (2015) MurJ and a novel lipid II flippase are required for cell wall biogenesis in *Bacillus subtilis*. Proc Natl Acad Sci USA 112:6437–6442

Meiresonne NY, Van Der Ploeg R, Hink MA, Den Blaauwen (2017) Activity-related conformational changes in DD-carboxypeptidases revealed by *in vivo* periplasmic forster resonance energy transfer assay in *Escherichia coli*. MBio 8

Mohammadi T, Sijbrandi R, Lutters M, Verheul J, Martin NI, den Blaauwen T, de Kruijff B, Breukink E (2014) Specificity of the transport of lipid II by FtsW in *Escherichia coli*. J Biol Chem 289:14707–14718

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 (2011) Identification of FtsW as a transporter of lipid-linked cell wall precursors across the membrane. EMBO J 30:1425–1432

Monteiro JM, Pereira AR, Reichmann NT, Saraiva BM, Fernandes PB, Veiga H, Tavares AC, Santos M, Ferreira MT, Macario V, Vannieuwenhze MS, Filipe SR, Pinho MG (2018) Peptidoglycan synthesis drives an FtsZ-treadmilling-independent step of cytokinesis. Nature 554:528–532

Morlot C, Straume D, Peters K, Hegnar OA, Simon N, Villard AM, Contreras-Martel C, Leisico F, Breukink E, Gravier-Pelletier C, le Corre L, Vollmer W, Pietrancosta N, Havarstein LS, Zapun A (2018) Structure of the essential peptidoglycan amidotransferase MurT/GatD complex from *Streptococcus pneumoniae*. Nat Commun 9:3180

Moynihan PJ, Clarke AJ (2010) *O*-acetylation of peptidoglycan in gram-negative bacteria: identification and characterization of peptidoglycan *O*-acetyltransferase in *Neisseria gonorrhoeae*. J Biol Chem 285:13264–13273

Müller P, Ewers C, Bertsche U, Anstett M, Kallis T, Breukink E, Fraipont C, Terrak M, Nguyen-Disteche M, Vollmer W (2007) The essential cell division protein FtsN interacts with the murein (peptidoglycan) synthase PBP1B in *Escherichia coli*. J Biol Chem 282:36394–36402

Münch D, Roemer T, Lee SH, Engeser M, Sahl HG, Schneider T (2012) Identification and *in vitro* analysis of the GatD/MurT enzyme-complex catalyzing lipid II amidation in *Staphylococcus aureus*. PLoS Pathog 8:e1002509

Nanninga N (1991) Cell division and peptidoglycan assembly in *Escherichia coli*. Mol Microbiol 5:791–795

Nelson DE, Ghosh AS, Paulson AL, Young KD (2002) Contribution of membrane-binding and enzymatic domains of penicillin binding protein 5 to maintenance of uniform cellular morphology of *Escherichia coli*. J Bacteriol 184:3630–3639

Nelson DE, Young KD (2000) Penicillin binding protein 5 affects cell diameter, contour, and morphology of *Escherichia coli*. J Bacteriol 182:1714–1721

Ngadjeua F, Braud E, Saidjalolov S, Iannazzo L, Schnappinger D, Ehrt S, Hugonnet JE, Mengin-Lecreulx D, Patin D, Etheve-Quelquejeu M, Fonvielle M, Arthur M (2018) Critical impact of peptidoglycan precursor amidation on the activity of LD-transpeptidases from *Enterococcus faecium* and *Mycobacterium tuberculosis*. Chemistry 24:5743–5747

Noldeke ER, Muckenfuss LM, Niemann V, Müller A, Stork E, Zocher G, Schneider T, Stehle T (2018) Structural basis of cell wall peptidoglycan amidation by the GatD/MurT complex of *Staphylococcus aureus*. Sci Rep 8:12953

Osawa M, Anderson DE, Erickson HP (2008) Reconstitution of contractile FtsZ rings in liposomes. Science 320:792–794

Osawa M, Erickson HP (2013) Liposome division by a simple bacterial division machinery. Proc Natl Acad Sci USA 110:11000–11004

Packiam M, Weinrick B, Jacobs WR Jr, Maurelli AT (2015) Structural characterization of muropeptides from *Chlamydia trachomatis* peptidoglycan by mass spectrometry resolves "chlamydial anomaly". Proc Natl Acad Sci USA 112:11660–11665

Paradis-Bleau C, Markovski M, Uehara T, Lupoli TJ, Walker S, Kahne DE, Bernhardt TG (2010) Lipoprotein cofactors located in the outer membrane activate bacterial cell wall polymerases. Cell 143:1110–1120

Park JT, Uehara T (2008) How bacteria consume their own exoskeletons (turnover and recycling of cell wall peptidoglycan). Microbiol Mol Biol Rev 72:211–227 (table of contents)

Pastoret S, Fraipont C, den Blaauwen T, Wolf B, Aarsman ME, Piette A, Thomas A, Brasseur R, Nguyen-Disteche M (2004) Functional analysis of the cell division protein FtsW of *Escherichia coli*. J Bacteriol 186:8370–8379

Pazos M, Peters K, Casanova M, Palacios P, Vannieuwenhze M, Breukink E, Vicente M, Vollmer W (2018) Z-ring membrane anchors associate with cell wall synthases to initiate bacterial cell division. Nat Commun 9:5090

Pazos M, Peters K, Vollmer W (2017) Robust peptidoglycan growth by dynamic and variable multi-protein complexes. Curr Opin Microbiol 36:55–61

Peltier J, Courtin P, el Meouche I, Lemee L, Chapot-Chartier MP, Pons JL (2011) *Clostridium difficile* has an original peptidoglycan structure with a high level of *N*-acetylglucosamine deacetylation and mainly 3-3 cross-links. J Biol Chem 286:29053–29062

Pennartz A, Genereux C, Parquet C, Mengin-Lecreulx D, Joris B (2009) Substrate-induced inactivation of the *Escherichia coli* AmiD *N*-acetylmuramoyl-L-alanine amidase highlights a new strategy to inhibit this class of enzyme. Antimicrob Agents Chemother 53:2991–2997

Pepper ED, Farrell MJ, Finkel SE (2006) Role of penicillin-binding protein 1b in competitive stationary-phase survival of *Escherichia coli*. FEMS Microbiol Lett 263:61–67

Peters K, Kannan S, Rao VA, Biboy J, Vollmer D, Erickson SW, Lewis RJ, Young KD, Vollmer W (2016) The redundancy of peptidoglycan carboxypeptidases ensures robust cell shape maintenance in *Escherichia coli*. MBio 7:e00819-16

Peters K, Pazos M, Edoo Z, Hugonnet JE, Martorana AM, Polissi A, Vannieuwenhze MS, Arthur M, Vollmer W (2018) Copper inhibits peptidoglycan LD-transpeptidases suppressing beta-lactam resistance due to bypass of penicillin-binding proteins. Proc Natl Acad Sci USA 115:10786–10791

Peters NT, Dinh T, Bernhardt TG (2011) A fail-safe mechanism in the septal ring assembly pathway generated by the sequential recruitment of cell separation amidases and their activators. J Bacteriol 193:4973–4983

Peters NT, Morlot C, Yang DC, Uehara T, Vernet T, Bernhardt TG (2013) Structure-function analysis of the LytM domain of EnvC, an activator of cell wall remodelling at the *Escherichia coli* division site. Mol Microbiol 89:690–701

Pichoff S, Du S, Lutkenhaus J (2015) The bypass of ZipA by overexpression of FtsN requires a previously unknown conserved FtsN motif essential for FtsA-FtsN interaction supporting a model in which FtsA monomers recruit late cell division proteins to the Z ring. Mol Microbiol 95:971–987

Pichoff S, Du S, Lutkenhaus J (2018) Disruption of divisome assembly rescued by FtsN-FtsA interaction in *Escherichia coli*. Proc Natl Acad Sci USA 115:E6855–E6862

Pilhofer M, Aistleitner K, Biboy J, Gray J, Kuru E, Hall E, Brun YV, Vannieuwenhze MS, Vollmer W, Horn M, Jensen GJ (2013) Discovery of chlamydial peptidoglycan reveals bacteria with murein sacculi but without FtsZ. Nat Commun 4:2856

Potluri L, Karczmarek A, Verheul J, Piette A, Wilkin JM, Werth N, Banzhaf M, Vollmer W, Young KD, Nguyen-Disteche M, den Blaauwen T (2010) Septal and lateral wall localization of PBP5, the major DD-carboxypeptidase of *Escherichia coli*, requires substrate recognition and membrane attachment. Mol Microbiol 77:300–323

Potluri LP, de Pedro MA, Young KD (2012a) *Escherichia coli* low-molecular-weight penicillin-binding proteins help orient septal FtsZ, and their absence leads to asymmetric cell division and branching. Mol Microbiol 84:203–224

Potluri LP, Kannan S, Young KD (2012b) ZipA is required for FtsZ-dependent preseptal peptidoglycan synthesis prior to invagination during cell division. J Bacteriol 194:5334–5342

Priyadarshini R, Popham DL, Young KD (2006) Daughter cell separation by penicillin-binding proteins and peptidoglycan amidases in *Escherichia coli*. J Bacteriol 188:5345–5355

Pushkaran AC, Nataraj N, Nair N, Gotz F, Biswas R, Mohan CG (2015) Understanding the structure-function relationship of lysozyme resistance in *Staphylococcus aureus* by peptidoglycan *O*-acetylation using molecular docking, dynamics, and lysis assay. J Chem Inf Model 55:760–770

Qiao Y, Srisuknimit V, Rubino F, Schaefer K, Ruiz N, Walker S, Kahne D (2017) Lipid II overproduction allows direct assay of transpeptidase inhibition by beta-lactams. Nat Chem Biol 13:793–798

Radkov AD, Hsu YP, Booher G, Vannieuwenhze MS (2018) Imaging bacterial cell wall biosynthesis. Annu Rev Biochem 87:991–1014

Ranjit DK, Young KD (2013) The Rcs stress response and accessory envelope proteins are required for *de novo* generation of cell shape in *Escherichia coli*. J Bacteriol 195:2452–2462

Rawat S, Zhu L, Lindner E, Dalbey RE, White SH (2015) SecA drives transmembrane insertion of RodZ, an unusual single-span membrane protein. J Mol Biol 427:1023–1037

Raymond JB, Mahapatra S, Crick DC, Pavelka MS Jr (2005) Identification of the *namH* gene, encoding the hydroxylase responsible for the *N*-glycolylation of the mycobacterial peptidoglycan. J Biol Chem 280:326–333

Rismondo J, Wamp S, Aldridge C, Vollmer W, Halbedel S (2018) Stimulation of PgdA-dependent peptidoglycan *N*-deacetylation by GpsB-PBP A1 in *Listeria monocytogenes*. Mol Microbiol 107:472–487

Rocaboy M, Herman R, Sauvage E, Remaut H, Moonens K, Terrak M, Charlier P, Kerff F (2013) The crystal structure of the cell division amidase AmiC reveals the fold of the AMIN domain, a new peptidoglycan binding domain. Mol Microbiol 90:267–277

Rojas ER, Billings G, Odermatt PD, Auer GK, Zhu L, Miguel A, Chang F, Weibel DB, Theriot JA, Huang KC (2018) The outer membrane is an essential load-bearing element in Gram-negative bacteria. Nature 559:617–621

Romeis T, Höltje JV (1994a) Penicillin-binding protein 7/8 of *Escherichia coli* is a DD-endopeptidase. Eur J Biochem 224:597–604

Romeis T, Höltje JV (1994b) Specific interaction of penicillin-binding proteins 3 and 7/8 with soluble lytic transglycosylase in *Escherichia coli*. J Biol Chem 269:21603–21607

Rubino FA, Kumar S, Ruiz N, Walker S, Kahne DE (2018) Membrane potential is required for MurJ function. J Am Chem Soc 140:4481–4484

Ruiz N (2008) Bioinformatics identification of MurJ (MviN) as the peptidoglycan lipid II flippase in *Escherichia coli*. Proc Natl Acad Sci USA 105:15553–15557

Ruiz N (2009) *Streptococcus pyogenes* YtgP (Spy_0390) complements *Escherichia coli* strains depleted of the putative peptidoglycan flippase MurJ. Antimicrob Agents Chemother 53:3604–3605

Salje J, van den Ent F, de Boer P, Lowe J (2011) Direct membrane binding by bacterial actin MreB. Mol Cell 43:478–487

Sanchez M, Kolar SL, Müller S, Reyes CN, Wolf AJ, Ogawa C, Singhania R, de Carvalho DD, Arditi M, Underhill DM, Martins GA, Liu GY (2017) *O*-acetylation of peptidoglycan limits helper T cell priming and permits *Staphylococcus aureus* reinfection. Cell Host Microbe 22(543–551):e4

Sanders AN, Pavelka MS (2013) Phenotypic analysis of *Eschericia coli* mutants lacking LD-transpeptidases. Microbiology 159:1842–1852

Sathiyamoorthy K, Vijayalakshmi J, Tirupati B, Fan L, Saper MA (2017) Structural analyses of the *Haemophilus influenzae* peptidoglycan synthase activator LpoA suggest multiple conformations in solution. J Biol Chem 292:17626–17642

Sauvage E, Derouaux A, Fraipont C, Joris M, Herman R, Rocaboy M, Schloesser M, Dumas J, Kerff F, Nguyen-Disteche M, Charlier P (2014) Crystal structure of penicillin-binding protein 3 (PBP3) from *Escherichia coli*. PLoS ONE 9:e98042

Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P (2008) The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. FEMS Microbiol Rev 32:234–258

Schleifer KH, Kandler O (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol Rev 36:407–477

Schoenemann KM, Margolin W (2017) Bacterial division: FtsZ treadmills to build a beautiful wall. Curr Biol 27:R301–R303

Schumacher MA (2017) Bacterial nucleoid occlusion: multiple mechanisms for preventing chromosome bisection during cell division. Subcell Biochem 84:267–298

Schwechheimer C, Rodriguez DL, Kuehn MJ (2015) NlpI-mediated modulation of outer membrane vesicle production through peptidoglycan dynamics in *Escherichia coli*. Microbiologyopen 4:375–389

Severin A, Figueiredo AM, Tomasz A (1996) Separation of abnormal cell wall composition from penicillin resistance through genetic transformation of *Streptococcus pneumoniae*. J Bacteriol 178:1788–1792

Sham LT, Butler EK, Lebar MD, Kahne D, Bernhardt TG, Ruiz N (2014) Bacterial cell wall. MurJ is the flippase of lipid-linked precursors for peptidoglycan biogenesis. Science 345:220–222

Sham LT, Zheng S, Yakhnina AA, Kruse AC, Bernhardt TG (2018) Loss of specificity variants of WzxC suggest that substrate recognition is coupled with transporter opening in MOP-family flippases. Mol Microbiol 109:633–641

Sheikh J, Hicks S, Dall'Agnol M, Phillips AD, Nataro JP (2001) Roles for Fis and YafK in biofilm formation by enteroaggregative *Escherichia coli*. Mol Microbiol 41:983–997

Shi H, Bratton BP, Gitai Z, Huang KC (2018) How to build a bacterial cell: MreB as the foreman of *E. coli* construction. Cell 172:1294–1305

Shimada T, Park BG, Wolf AJ, Brikos C, Goodridge HS, Becker CA, Reyes CN, Miao EA, Aderem A, Gotz F, Liu GY, Underhill DM (2010) *Staphylococcus aureus* evades lysozyme-based peptidoglycan digestion that links phagocytosis, inflammasome activation, and IL-1beta secretion. Cell Host Microbe 7:38–49

Singh SK, Parveen S, Saisree L, Reddy M (2015) Regulated proteolysis of a cross-link-specific peptidoglycan hydrolase contributes to bacterial morphogenesis. Proc Natl Acad Sci USA 112:10956–10961

Singh SK, Saisree L, Amrutha RN, Reddy M (2012) Three redundant murein endopeptidases catalyse an essential cleavage step in peptidoglycan synthesis of *Escherichia coli* K12. Mol Microbiol 86:1036–1051

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 (2018) Structure of the peptidoglycan polymerase RodA resolved by evolutionary coupling analysis. Nature 556:118–121

Soderstrom B, Chan H, Shilling PJ, Skoglund U, Daley DO (2018) Spatial separation of FtsZ and FtsN during cell division. Mol Microbiol 107:387–401

Soderstrom B, Mirzadeh K, Toddo S, von Heijne G, Skoglund U, Daley DO (2016) Coordinated disassembly of the divisome complex in *Escherichia coli*. Mol Microbiol 101:425–438

Soderstrom B, Skoog K, Blom H, Weiss DS, von Heijne G, Daley DO (2014) Disassembly of the divisome in *Escherichia coli*: evidence that FtsZ dissociates before compartmentalization. Mol Microbiol 92:1–9

Spratt BG (1975) Distinct penicillin binding proteins involved in the division, elongation, and shape of *Escherichia coli* K12. Proc Natl Acad Sci USA 72:2999–3003

Stranden AM, Ehlert K, Labischinski H, Berger-Bachi B (1997) Cell wall monoglycine cross-bridges and methicillin hypersusceptibility in a *femAB* null mutant of methicillin-resistant *Staphylococcus aureus*. J Bacteriol 179:9–16

Su MY, Som N, Wu CY, Su SC, Kuo YT, Ke LC, Ho MR, Tzeng SR, Teng CH, Mengin-Lecreulx D, Reddy M, Chang CI (2017) Structural basis of adaptor-mediated protein degradation by the tail-specific PDZ-protease Prc. Nat Commun 8:1516

Sung MT, Lai YT, Huang CY, Chou LY, Shih HW, Cheng WC, Wong CH, Ma C (2009) Crystal structure of the membrane-bound bifunctional transglycosylase PBP1b from *Escherichia coli*. Proc Natl Acad Sci USA 106:8824–8829

Sutterlin L, Edoo Z, Hugonnet JE, Mainardi JL, Arthur M (2018) Peptidoglycan cross-linking activity of LD-transpeptidases from *Clostridium difficile* and inactivation of these enzymes by beta-lactams. Antimicrob Agents Chemother 62

Taguchi A, Welsh MA, Marmont LS, Lee W, Kahne D, Bernhardt TG, Walker S (2018) FtsW is a peptidoglycan polymerase that is activated by its cognate penicillin-binding protein. Preprint at. https://www.biorxiv.org/content/early/2018/06/29/358663

Tatar LD, Marolda CL, Polischuk AN, van Leeuwen D, Valvano MA (2007) An *Escherichia coli* undecaprenyl-pyrophosphate phosphatase implicated in undecaprenyl phosphate recycling. Microbiology 153:2518–2529

Templin MF, Edwards DH, Höltje JV (1992) A murein hydrolase is the specific target of bulgecin in *Escherichia coli*. J Biol Chem 267:20039–20043

Thunnissen AM, Dijkstra AJ, Kalk KH, Rozeboom HJ, Engel H, Keck W, Dijkstra BW (1994) Doughnut-shaped structure of a bacterial muramidase revealed by X-ray crystallography. Nature 367:750–753

Thunnissen AM, Rozeboom HJ, Kalk KH, Dijkstra BW (1995) Structure of the 70-kDa soluble lytic transglycosylase complexed with bulgecin A. Implications for the enzymatic mechanism. Biochemistry 34:12729–12737

Tomioka S, Matsuhashi M (1978) Purification of penicillin-insensitive DD-endopeptidase, a new cell wall peptidoglycan-hydrolyzing enzyme in *Escherichia coli*, and its inhibition by deoxyribonucleic acids. Biochem Biophys Res Commun 84:978–984

Touze T, Blanot D, Mengin-Lecreulx D (2008) Substrate specificity and membrane topology of *Escherichia coli* PgpB, an undecaprenyl pyrophosphate phosphatase. J Biol Chem 283:16573–16583

Tsang MJ, Bernhardt TG (2015) A role for the FtsQLB complex in cytokinetic ring activation revealed by an *ftsL* allele that accelerates division. Mol Microbiol 95:925–944

Tsang MJ, Yakhnina AA, Bernhardt TG (2017) NlpD links cell wall remodeling and outer membrane invagination during cytokinesis in *Escherichia coli*. PLoS Genet 13:e1006888

Turner RD, Mesnage S, Hobbs JK, Foster SJ (2018) Molecular imaging of glycan chains couples cell-wall polysaccharide architecture to bacterial cell morphology. Nat Commun 9:1263

Tuson HH, Auer GK, Renner LD, Hasebe M, Tropini C, Salick M, Crone WC, Gopinathan A, Huang KC, Weibel DB (2012) Measuring the stiffness of bacterial cells from growth rates in hydrogels of tunable elasticity. Mol Microbiol 84:874–891

Typas A, Banzhaf M, Gross CA, Vollmer W (2011) From the regulation of peptidoglycan synthesis to bacterial growth and morphology. Nat Rev Microbiol 10:123–136

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 (2010). Regulation of peptidoglycan synthesis by outer-membrane proteins. Cell 143:1097–1109

Uehara T, Dinh T, Bernhardt TG (2009) LytM-domain factors are required for daughter cell separation and rapid ampicillin-induced lysis in *Escherichia coli*. J Bacteriol 191:5094–5107

Uehara T, Park JT (2007) An anhydro-*N*-acetylmuramyl-L-alanine amidase with broad specificity tethered to the outer membrane of *Escherichia coli*. J Bacteriol 189:5634–5641

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

Ursell TS, Nguyen J, Monds RD, Colavin A, Billings G, Ouzounov N, Gitai Z, Shaevitz JW, Huang KC (2014) Rod-like bacterial shape is maintained by feedback between cell curvature and cytoskeletal localization. Proc Natl Acad Sci USA 111:E1025–E1034

Ursinus A, van den Ent F, Brechtel S, de Pedro M, Höltje JV, Lowe J, Vollmer W (2004) Murein (peptidoglycan) binding property of the essential cell division protein FtsN from *Escherichia coli*. J Bacteriol 186:6728–6737

van Asselt EJ, Thunnissen AM, Dijkstra BW (1999) High resolution crystal structures of the *Escherichia coli* lytic transglycosylase Slt70 and its complex with a peptidoglycan fragment. J Mol Biol 291:877–898

van den Bogaart G, Hermans N, Krasnikov V, Poolman B (2007) Protein mobility and diffusive barriers in *Escherichia coli*: consequences of osmotic stress. Mol Microbiol 64:858–871

van den Ent F, Izore T, Bharat TA, Johnson CM, Lowe J (2014) Bacterial actin MreB forms antiparallel double filaments. Elife 3:e02634

van Heijenoort J (2011) Peptidoglycan hydrolases of *Escherichia coli*. Microbiol Mol Biol Rev 75:636–663

van Teeffelen S, Wang S, Furchtgott L, Huang KC, Wingreen NS, Shaevitz JW, Gitai Z (2011) The bacterial actin MreB rotates, and rotation depends on cell-wall assembly. Proc Natl Acad Sci USA 108:15822–15827

Varma A, de Pedro MA, Young KD (2007) FtsZ directs a second mode of peptidoglycan synthesis in *Escherichia coli*. J Bacteriol 189:5692–5704
Varma A, Young KD (2004) FtsZ collaborates with penicillin binding proteins to generate bacterial cell shape in *Escherichia coli*. J Bacteriol 186:6768–6774
Vega D, Ayala JA (2006) The DD-carboxypeptidase activity encoded by *pbp4B* is not essential for the cell growth of *Escherichia coli*. Arch Microbiol 185:23–27
Vinella D, Joseleau-Petit D, Thevenet D, Bouloc P, D'Ari R (1993) Penicillin-binding protein 2 inactivation in *Escherichia coli* results in cell division inhibition, which is relieved by FtsZ overexpression. J Bacteriol 175:6704–6710
Vollmer W (2008) Structural variation in the glycan strands of bacterial peptidoglycan. FEMS Microbiol Rev 32:287–306
Vollmer W, Bertsche U (2008) Murein (peptidoglycan) structure, architecture and biosynthesis in *Escherichia coli*. Biochim Biophys Acta 1778:1714–1734
Vollmer W, Blanot D, de Pedro MA (2008a) Peptidoglycan structure and architecture. FEMS Microbiol Rev 32:149–167
Vollmer W, Joris B, Charlier P, Foster S (2008b) Bacterial peptidoglycan (murein) hydrolases. FEMS Microbiol Rev 32:259–286
Vollmer W, Tomasz A (2000) The *pgdA* gene encodes for a peptidoglycan *N*-acetylglucosamine deacetylase in *Streptococcus pneumoniae*. J Biol Chem 275:20496–20501
von Rechenberg M, Ursinus A, Höltje JV (1996) Affinity chromatography as a means to study multienzyme complexes involved in murein synthesis. Microb Drug Resist 2:155–157
Weadge JT, Clarke AJ (2006) Identification and characterization of *O*-acetylpeptidoglycan esterase: a novel enzyme discovered in *Neisseria gonorrhoeae*. Biochemistry 45:839–851
Weadge JT, Pfeffer JM, Clarke AJ (2005) Identification of a new family of enzymes with potential *O*-acetylpeptidoglycan esterase activity in both Gram-positive and Gram-negative bacteria. BMC Microbiol 5:49
Wettmann L, Kruse K (2018) The Min-protein oscillations in *Escherichia coli*: an example of self-organized cellular protein waves. Philos Trans R Soc Lond B Biol Sci 373
Whatmore AM, Reed RH (1990) Determination of turgor pressure in *Bacillus subtilis*: a possible role for K+ in turgor regulation. J Gen Microbiol 136:2521–2526
Wheeler R, Turner RD, Bailey RG, Salamaga B, Mesnage S, Mohamad SA, Hayhurst EJ, Horsburgh M, Hobbs JK, Foster SJ (2015) Bacterial cell enlargement requires control of cell wall stiffness mediated by peptidoglycan hydrolases. MBio 6:e00660
Williams AH, Wheeler R, Rateau L, Malosse C, Chamot-Rooke J, Haouz A, Taha MK, Boneca IG (2018) 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 293:6000–6010
Workman SD, Worrall LJ, Strynadka NCJ (2018) Crystal structure of an intramembranal phosphatase central to bacterial cell-wall peptidoglycan biosynthesis and lipid recycling. Nat Commun 9:1159
Xiao J, Goley ED (2016) Redefining the roles of the FtsZ-ring in bacterial cytokinesis. Curr Opin Microbiol 34:90–96
Yadav AK, Espaillat A, Cava F (2018) Bacterial strategies to preserve cell wall integrity against environmental threats. Front Microbiol 9:2064
Yahashiri A, Jorgenson MA, Weiss DS (2015) Bacterial SPOR domains are recruited to septal peptidoglycan by binding to glycan strands that lack stem peptides. Proc Natl Acad Sci USA 112:11347–11352
Yakhnina AA, McManus HR, Bernhardt TG (2015) The cell wall amidase AmiB is essential for *Pseudomonas aeruginosa* cell division, drug resistance and viability. Mol Microbiol 97:957–973
Yanagihara Y, Kamisango K, Yasuda S, Kobayashi S, Mifuchi I, Azuma I, Yamamura Y, Johnson RC (1984) Chemical compositions of cell walls and polysaccharide fractions of spirochetes. Microbiol Immunol 28:535–544

Yang DC, Peters NT, Parzych KR, Uehara T, Markovski M, Bernhardt TG (2011) An ATP-binding cassette transporter-like complex governs cell-wall hydrolysis at the bacterial cytokinetic ring. Proc Natl Acad Sci USA 108:E1052–E1060

Yang DC, Tan K, Joachimiak A, Bernhardt TG (2012) A conformational switch controls cell wall-remodelling enzymes required for bacterial cell division. Mol Microbiol 85:768–781

Yang X, Lyu Z, Miguel A, McQuillen R, Huang KC, Xiao J (2017) GTPase activity-coupled treadmilling of the bacterial tubulin FtsZ organizes septal cell wall synthesis. Science 355:744–747

Yao X, Jericho M, Pink D, Beveridge T (1999) Thickness and elasticity of gram-negative murein sacculi measured by atomic force microscopy. J Bacteriol 181:6865–6875

Yousif SY, Broome-Smith JK, Spratt BG (1985) Lysis of *Escherichia coli* by beta-lactam antibiotics: deletion analysis of the role of penicillin-binding proteins 1A and 1B. J Gen Microbiol 131:2839–2845

Yuan Y, Barrett D, Zhang Y, Kahne D, Sliz P, Walker S (2007) Crystal structure of a peptidoglycan glycosyltransferase suggests a model for processive glycan chain synthesis. Proc Natl Acad Sci USA 104:5348–5353

Zapun A, Philippe J, Abrahams KA, Signor L, Roper DI, Breukink E, Vernet T (2013) *In vitro* reconstitution of peptidoglycan assembly from the Gram-positive pathogen *Streptococcus pneumoniae*. ACS Chem Biol 8:2688–2696

Zheng S, Sham LT, Rubino FA, Brock KP, Robins WP, Mekalanos JJ, Marks DS, Bernhardt TG, Kruse AC (2018) Structure and mutagenic analysis of the lipid II flippase MurJ from *Escherichia coli*. Proc Natl Acad Sci USA 115:6709–6714