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The physiology of bacterial cell division

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Bacterial cell division is facilitated by the divisome, a dynamic multiprotein assembly localizing at mid-cell to synthesize the stress-bearing peptidoglycan and to constrict all cell envelope layers. Divisome assembly occurs in two steps and involves multiple interactions between more than 20 essential and accessory cell division proteins. Well before constriction and while the cell is still elongating, the tubulin-like FtsZ and early cell division proteins form a ring-like structure at mid-cell. Cell division starts once certain peptidoglycan enzymes and their activators have moved to the FtsZ-ring. Gram-negative bacteria like *Escherichia coli* simultaneously synthesize and cleave the septum peptidoglycan during division leading to a constriction. The outer membrane constricts together with the peptidoglycan layer with the help of the transenvelope spanning Tol–Pal system.

Keywords: cell division; divisome; peptidoglycan; penicillin-binding protein; peptidoglycan hydrolyase; outer membrane; Tol–Pal

Introduction

Propagating bacteria undergo cycles of growth and cell division during which all cellular compounds are synthesized in a regulated manner and they become distributed to the daughter cells. A bacterial cell has only two components that are inherited as a single molecule, the chromosome and the cell wall peptidoglycan (murein) sacculus. Obviously, the single copy number, huge size (compared to the cell size), and essentiality of both components necessitate safe mechanisms for their replication/growth and distribution to the daughter cells. The sacculus, an integral component of the cell envelope, encases the cytoplasmic membrane to form a net-like and continuous layer[1,2] that is required to maintain cell shape and osmotic stability against the turgor. In Gram-negative bacteria the peptidoglycan is 3–6 nm thick and likely a single layer, whereas in Gram-positive species it is thicker than 10–20 nm and contains covalently attached secondary cell wall polymers such as teichoic acids and capsular polysaccharides.[3]

The chemical composition of peptidoglycan is well known. It is composed of glycan chains made of alternating *N*-acetylmuramic acid (Mur*N*Ac) and *N*-acetyglucosamine (Glc*N*Ac) residues that are connected by short peptides.[4] These contain both L- and D-amino acids and, in Gram-negative bacteria such as *Escherichia coli*, are synthesized as pentapeptides with the following sequence: L-Ala-D-iGlu-*m*-Dap-D-Ala-D-Ala (*m*-Dap, *meso*-diaminopimelic acid). Two or more peptides protruding from neighboring glycan chains can be connected to form dimeric or multimeric cross-links. Moreover, in some Gram-negative species, peptides can serve as attachment sites for Lpp (Braun's lipoprotein), which anchors with its lipid modification in the outer membrane and ensures a firm connection between the outer membrane and the peptidoglycan.[5] Many Gram-positive species anchor cell wall proteins to the peptides in peptidoglycan by the enzyme sortase.[6] In Gram-positive bacteria there is considerable variation in the structure of the peptides, and many species structurally modify the glycan chains (for example,

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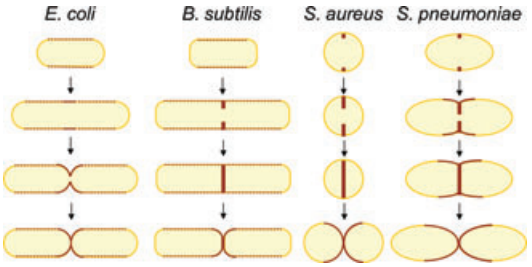

Figure 1. Sites of peptidoglycan synthesis during growth and cell division in different bacteria. The rod-shaped *E. coli* and *B. subtilis* elongate by insertion of new peptidoglycan (red dots) into the lateral cell wall. Coccal *S. aureus* cells lack an elongation phase, and the ovococcus *S. pneumoniae* elongates from a growth zone at mid-cell. The dark red lines indicate sites of zonal peptidoglycan synthesis. Gram-negative bacteria synthesize and split the septum simultaneously, resulting in a constriction. By contrast, Gram-positive bacteria synthesize a complete septum before cell separation.

by *N*-deacetylation or *O*-acetylation)[7] and/or the peptides (by amidation).[8,9]

The molecular architecture of the mainly single-layered sacculus from Gram-negative bacteria has been debated over the last decades.[10] The current model is based on the enhanced elasticity of isolated, rod-shaped *E. coli* sacculi in the direction of the long axis, their thickness of 3–6 nm, and cryo-electrontomography imaging. Accordingly, peptidoglycan adopts a single-layered architecture with glycan strands oriented mainly perpendicular to the long axis of the cell. The more flexible peptide cross-links are oriented mainly in the direction of the long axis. Cryo-electrontomography images suggest some degree of disorder in the layer,[11] consistent with the glycan strands being much shorter than the circumference of the cell and the chemical heterogeneity of the peptides.[12,13] These images have been produced from isolated, that is, relaxed, sacculi. Presumably, in the cell, where the sacculus is stretched by the turgor, the glycan chains, and peptides adopt a more ordered configuration that facilitates the activities of peptidoglycan synthetic and hydrolytic enzymes.[3]

The molecular mechanisms by which the peptidoglycan layer grows during cell elongation and division are largely unknown. According to a current model, membrane-bound multienzyme complexes made of peptidoglycan synthases and hydrolases are positioned and/or regulated from inside the cell by cytoskeletal proteins and their interaction partners, many of which are membrane bound.[14,15] The actin-like MreB is required for maintaining rod shape in many species, except those which exhibit polar growth, for example actinobacteria and certain α-proteobacteria.[14,16] MreB and associated proteins localize on a helical path along the length of the cell,[14] and recent high-resolution techniques showed them moving in dynamic patches around the cell, depending on ongoing peptidoglycan synthesis.[17–19] It appears that cytoskeletal and peptidoglycan proteins act interdependently to ensure maintaining the diameter of the cell by a yet unknown mechanism.

Cell division is orchestrated by FtsZ and more than 12 other essential cell division proteins (Fts proteins).[20] They form the divisome complex to generate the new poles of the daughter cells. Between elongation and cell division, *Caulobacter crescentus* and to lesser extent *E. coli* employ a preseptal phase of elongation in which the cells elongate by cell envelope growth at mid-cell dependent on FtsZ.[21,22] In *E. coli* and presumably other Gram-negative bacteria, growth of the sacculus is also regulated from the outside by recently identified outer membrane lipoproteins, LpoA and LpoB.[23,24] However, while many players for sacculus growth have been identified, there is little knowledge about the molecular details driving and regulating cell elongation and division.

Gram-negative bacteria employ a constrictive mode of cell division, that is, the synthesis of the new septum, its splitting, and the invagination of the outer membrane occur simultaneously. By contrast, most Gram-positive bacteria first completely synthesize the septal cross-wall before splitting it for cell separation (Fig. 1).

Antibiotic inhibition of bacterial cell division and peptidoglycan synthesis has been covered by recent articles.[25–28] In the present review we focus mainly on the physiology of cell division in Gram-negative bacteria because most available data were obtained from the model bacterium *E. coli*. Unless otherwise noted any data and/or conclusions discussed, particularly pertaining to interaction and activity data, are derived from work on this organism. We briefly summarize the peptidoglycan biosynthetic pathway, which is the target of important antibiotics, and present the peptidoglycan enzymes and their activities and interactions with an emphasis on peptidoglycan synthesis during cell division. We also discuss cell division proteins and

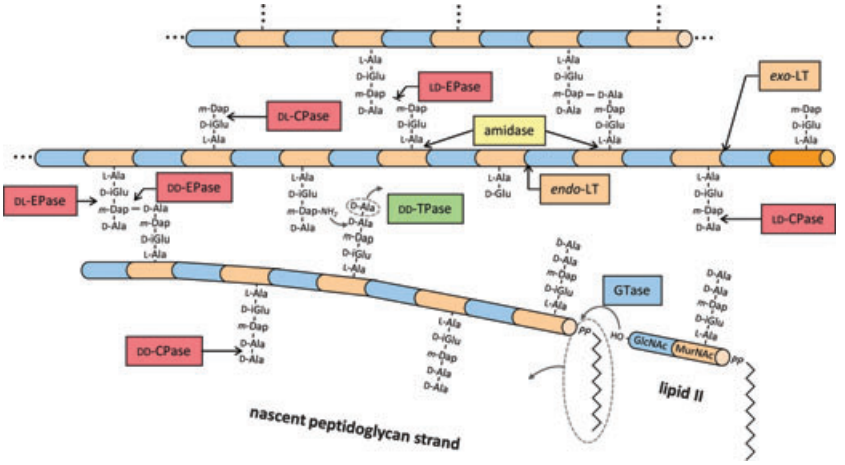

Figure 2. Peptidoglycan synthesis and hydrolysis. A nascent peptidoglycan strand is synthesized from the lipid II precursor by glycosyltransferase (GTase) reactions and is attached to peptidoglycan by a DD-transpeptidase (DD-TPase) cross-linking two peptides. Peptidoglycan is remodeled and hydrolyzed by various hydrolases: DD- or LD-carboxypeptidases (CPases) remove a terminal amino acid from the peptides, endopeptidases (EPases) hydrolyse DD- or LD-cross-links, lytic transglycosylases (LTs) cleave within the glycan strands producing 1,6-anhydro-*N*-acetylmuramic acid-containing muropeptides, and amidases hydrolyzes the amide bond between MurNAc and L-Ala. Light brown, *N*-acetylmuramic acid; blue rod, *N*-acetylglucosamine; dark orange rod, 1,6-anhydro-*N*-acetylmuramic acid.

summarize the current knowledge on the regulation of septum-splitting peptidoglycan hydrolases and on the process of outer membrane invagination during constriction.

Peptidoglycan synthesis and hydrolysis

The biosynthesis of peptidoglycan begins in the cytoplasm with the synthesis of the nucleotide-activated precursors UDP-Glc*N*Ac and UDP-Mur*N*Ac. The latter is synthesized from UDP-Glc*N*Ac by the enzymes MurA and MurB. The amino acid ligases MurC, MurD, MurE, and MurF catalyze the sequential ligation of L-Ala, D-Glu, *m*-Dap, and D-Ala-D-Ala to UDP-Mur*N*Ac.[29] D-amino acids are converted from the L-enantiomers by racemases, and the D-Ala-D-Ala dipeptide is synthesized by the DdlA/B ligases. The next steps occur at the inner face of the cytoplasmic membrane.[30] The transferase MraY forms undecaprenyl-pyrophosphoryl-Mur*N*Ac(pentapeptide) (lipid I) and then the glycosyltransferase MurG transfers a Glc*N*Ac residue from UDP-Glc*N*Ac to lipid I, forming undecaprenyl-pyrophosphoryl-Mur*N*Ac(pentapeptide)-Glc*N*Ac (lipid II). Many Gram-positive bacteria, but not *E. coli*, modify lipid II or nascent peptidoglycan by the addition of amino acids to position 3 of the peptide by Fem-transferases,[31] by the amidation of carboxylic groups in the peptide,[8,9] and/or by *O*-acetylation/*N*-deacetylation of glycan residues.[7] Lipid II is transported across the cytoplasmic membrane by FtsW/RodA flippases[32] where it is used as substrate of the glycosyltransferase (GTase) reactions to produce new glycan strands at the periplasmic face of the cytoplasmic membrane (Fig. 2).[33] Biochemical data and crystal structures indicate that GTases act processively.[34] The growing glycan strand is the donor and lipid II the acceptor in the reaction, which releases the undecaprenol pyrophosphate moiety from the growing glycan strand.[34–36] Peptides protruding from different glycan strands are cross-linked by transpeptidase (TPase) reactions to produce the net-like peptidoglycan polymer (Fig. 2).[37] TPases use a pentapeptide as donor and a tri-, tetra-, or pentapeptide as acceptor. During the reaction the terminal D-alanine residue of the donor peptide is released.

Synthesis of new peptidoglycan and its incorporation into the sacculus is accompanied by the removal of old material by hydrolases (Mur*N*Ac-L-alanine amidases, DD-endopeptidases and lytic transglycosylases), a process called peptidoglycan turnover.[38] The soluble peptidoglycan turnover products are transported into the cytoplasm where they are recycled for *de novo* peptidoglycan synthesis.[39]

The precise molecular mechanisms by which the peptidoglycan layer grows during cell elongation and division are not known. There are several models. According to Burman and Park, DD-endopeptidases cleave peptide cross-links in the sacculus to allow the insertion of two newly synthesized glycan strands.[40] The "three for one" model by Höltje assumes that three new glycan strands are synthesized and attached underneath a single "docking" strand in the existing peptidoglycan. Simultaneous removal of the docking strand by hydrolysis allows the insertion of the three new strands into the peptidoglycan layer.[41] This mechanism explains the observed peptidoglycan turnover as the removal of the docking stands. Höltje also proposed that peptidoglycan synthases and hydrolases form multienzyme complexes in which all activities required for a safe peptidoglycan growth mechanism are coordinated.[15] Although an active peptidoglycan enlargement complex has not yet been isolated from cells, recent research has identified several interactions between different peptidoglycan synthases, peptidoglycan synthases and hydrolases, and peptidoglycan enzymes and regulatory proteins (see below).

Activities and interactions of peptidoglycan synthases

Peptidoglycan synthases fall into three categories; bifunctional GTase/TPase (class A penicillin-binding proteins (PBPs)), monofunctional TPase (class B PBPs), and monofunctional GTase.[37] *E. coli* has three bifunctional PBPs (PBP1A, PBP1B, and PBP1C). PBP1A and PBP1B have major and semiredundant roles in peptidoglycan synthesis. PBP2 and PBP3 are monofunctional TPases essential for cell elongation and cell division, respectively. The functions of the nonessential PBP1C and the monofunctional GTase MtgA are not known, although the latter localizes to the cell division site.[42] All peptidoglycan synthases are anchored to the cytoplasmic membrane, with their catalytic sites residing outside the cytoplasm.

The activities of class A PBPs from *E. coli* have been demonstrated *in vitro* with their natural substrate. PBP1A is capable of polymerizing lipid II to form a cross-linked peptidoglycan product that contains glycan strands with an average length of ~20 disaccharide units and with ~18–26% of the peptides present in cross-links.[43] The catalytic amino acids Glu-94 and Ser-473 are essential for GTase and TPase activity, respectively. Remarkably, in the presence of purified peptidoglycan sacculi, PBP1A is capable of attaching a fraction of the newly made peptidoglycan to the sacculi via transpeptidation reactions.[43] Transpeptidation-mediated attachment of nascent peptidoglycan chains occurs in the cell during peptidoglycan growth.[40,44]

PBP1B exists as two main isoforms, PBP1Bα and PBP1Bγ, that differ in the length of their short cytoplasmic parts. PBP1Bγ is a truncated version originating from a translational start at Met-46 of PBP1Bα.[45] A third version, PBP1Bβ, is generated by an artificial cleavage of PBP1Bα by the outer membrane protease OmpT during cell fractionation.[46] PBP1B dimerizes both in the cell and *in vitro* with a K_D of 0.13 μM.[47,48] It exhibits GTase and TPase activities with the native lipid II substrate *in vitro*. The enzyme is most efficient at conditions favoring its dimerization, suggesting that it might be active as dimer in the cell. *In vitro*, PBP1B produced a peptidoglycan product with an average glycan strand length of >25 disaccharide units, with up to 50% of the peptides being part of cross-links.[47] The catalytic amino acid residues Glu-233 and Ser-510 are essential for the GTase and TPase activities, respectively.[47,49] Other conserved residues within the GTase domain of PBP1B (Asp-234, Phe-237, His-240, Thr-267, and Gln-271) are essential for both GTase activity and *in vivo* functioning.[50] PBP1B is among the few class A PBPs with a known crystal structure, which includes the transmembrane helix and, next to the GTase and TPase domains, a small UvrB-like domain situated between both catalytic domains called UB2H.[36]

Bifunctional PBPs coordinate their GTase and TPase activities. Both, PBP1A and PBP1B require ongoing GTase reactions for efficient cross-linking TPase activity.[43,47] Hence, it is possible that the growing glycan strands produced by the GTase site delivers a pentapeptide donor peptide into the active site of the TPase for the formation of a cross-link; this hypothesis is supported by the crystal structure of PBP1B showing that the GTase donor site for binding of the growing glycan chain and the TPase site are located on the same face of the molecule.[36] The GTase activity can be affected by other factors: the GTase activity of PBP1B is stimulated by LpoB and FtsN[23,47] and that of PBP1A by PBP2,[51] whereas full-length *S. pneumoniae* PBP2a produced longer

glycan strands than the truncated version lacking the transmembrane anchor.[52]

Class B PBPs have an N-terminal membrane anchor followed by a noncatalytic domain and a TPase domain. The noncatalytic domain might be required for the correct folding of the protein, as isolated TP domains of class B PBPs are intrinsically unstable.[53] In addition, the noncatalytic domain might act as a "pedestal" to optimally position the TPase domain to interacting proteins and the peptidoglycan layer.[54] The TPase activity of a class B PBP had not been demonstrated until recently for *E. coli* PBP2, which is active in the presence of PBP1A and peptidoglycan sacculi.[51] Under these conditions PBP2 contributes to the attachment of newly made peptidoglycan chains to sacculi by TPase reactions. Cells with a temperature-sensitive PBP2 version grown at elevated temperature or cells treated with the PBP2-specific β-lactam mecillinam grow into spheres and eventually lyse, indicating the essential role of PBP2 in cell elongation. PBP2 localizes to the lateral wall and to mid-cell at an early stage of septation, but it leaves mid-cell before completion of cell division, indicating a role in cell elongation and early septation.[55] In *C. cresentus* but not *E. coli* a mild osmotic upshift causes the relocation of PBP2 to mid-cell, indicating that changing environmental conditions may affect growth modes in bacteria.[56] *H. pylori* PBP2 interacts with the cell elongation protein MreC,[57] which, in *E. coli*, forms a complex with MreB and MreD.[58] PBP3 (also called FtsI) localizes to the septum and its activity is essential for cell division.[59] Although purified PBP3 binds a range of β-lactams and is capable of transferring the donor moiety of an artificial thioester substrate to a D-amino acid acceptor,[60] its activity with a natural pentapeptide donor and a peptidoglycan acceptor has yet to be established. The interactions of PBP3 with cell division proteins are discussed below.

Several interactions between different peptidoglycan synthases and between peptidoglycan synthases and other proteins have been detected.[61] *Pseudomonas aeruginosa* PBP2 interacts with a Ca^{2+}-binding EF-hand motif of the peptidoglcyan hydrolase SltB1.[62] *E. coli* PBP1B interacts with PBP3 *in vitro* with a K_D of 0.4 μM, and the two proteins can be cross-linked in the cell.[63] The interaction was confirmed by bacterial two-hybrid system, which further showed that the first 56 amino acids of PBP3 are sufficient for the interaction with PBP1B. An interaction between PBP1B and PBP3 in the cell is also consistent with the following two observations: (i) a fraction of the cellular PBP1B pool of wild-type cells localizes to mid-cell, depending on the presence, but not activity, of PBP3,[63] and (ii) overexpression of PBP1B suppresses the thermosensitive growth of *pbpB*2158 (Ts) mutant cells, presumably by stabilizing the labile PBP3 version by direct protein–protein interaction.[64] Hence, PBP1B and PBP3 likely provide the main peptidoglycan synthesis activity for cell division, while PBP1A and PBP2 are likely most active during cell elongation.

Outer membrane lipoprotein activators of PBPs

In *E. coli* and presumably other Gram-negative bacteria peptidoglycan synthesis is controlled not only from inside the cell by cytoskeletal structures ultimately linked to MreB and FtsZ, but also by recently discovered outer membrane lipoproteins LpoA and LpoB. These proteins are essential for activating, from *outside* the sacculus, their cognate peptidoglycan synthase PBP1A and PBP1B, respectively.[23,24] The cell requires either PBP1A-LpoA or PBP1B-LpoB for growth. Hence, the depletion of one of the *lpo* genes in the absence of the other, or in the absence of a noncognate PBP, results in the lysis of the cell.

The Lpo proteins interact with small, noncatalytic regions in their cognate PBP; for LpoB the noncatalytic region is the UB2H domain between the PBP1B GTase and TPase domains.[24] Interestingly, both Lpo proteins stimulate *in vitro* the TPase activity of their cognate, but not noncognate, PBP.[24] LpoB also stimulates the GTase activity of PBP1B.[23] It appears that PBP1A and PBP1B activity require stimulation by LpoA and LpoB, respectively, for proper functioning in the cell, which explains the lytic phenotype of *lpoA/lpoB*-depleted cells. Lpo proteins localize independently of, but with the same preference for the side wall or mid-cell position as, their cognate PBP. LpoA has been shown to localize predominantly to the lateral wall and LpoB to the lateral wall and mid-cell.[24] The septal localization of LpoB depends on the activity of PBP3, with LpoB mid-cell localization being diminished after inhibition of PBP3 with aztreonam. Presumably, LpoB requires ongoing septal peptidoglycan synthesis for mid-cell localization.[24]

Why are peptidoglycan synthases activated by outer membrane proteins? The following hypothesis is based on the crystal structure of PBP1B that shows the LpoB-interacting UB2H domain to locate not further than ~60 Å away from the cytoplasmic membrane.[24,65] Hence, with a distance from the cytoplasmic membrane to the peptidoglycan layer of ~90 Å, the PBP1B-LpoB interaction site would be located in the space between the cytoplasmic membrane and the peptidoglycan, making it necessary for LpoB to reach from the outer membrane through the pores in the peptidoglycan to activate PBP1B. According to this hypothesis, the activation of PBPs by Lpo proteins becomes responsive to the state of the pores in the peptidoglycan network. The Lpo-mediated activation of PBPs would be more efficient if the peptidoglycan were stetched and the pores were wider. Conversely, the activation of PBPs would be less efficient if the peptidoglycan were more relaxed and the pores were smaller. Hence, peptidoglycan growth is activated when required, that is, when it is stretched. This homeostatic regulation would allow the cell to maintain constant peptidoglycan surface density and thickness, and would contribute to the adjustment of peptidoglycan synthesis rate to the overall growth rate of the cell. However, most likely there are further mechanisms contributing to the regulation of peptidoglycan growth.[65]

Components of the divisome and their interactions

The cell division complex (divisome) is assembled at mid-cell to synthesize and cleave the septum and to separate the cell into two daughters. Herein we consider a protein as a divisome component if it localizes to mid-cell in an FtsZ-dependent manner and if it participates in the process of cell division, whether or not it is essential. The assembly of the divisome is initiated by the GTP-dependent polymerization of the tubulin-like FtsZ in a head-to-tail association.[66–68] Individual subunits form filaments and arches that combine to a ring-structure—termed *Z-ring*—proximal to the inner face of the cytoplasmic membrane at the prospective division site.[69,70] The *Z*-ring is highly dynamic and FtsZ subunits constantly exchange with free, cytosolic FtsZ molecules in a time scale of a few seconds.[71] *In vitro*, rings of FtsZ can form and spontaneously open and depolymerize, as observed by atomic force microscopy.[72] FtsZ is stabilized at mid-cell position by several positive regulators (see below). In addition, there are at least two mechanisms that, together, prevent *Z*-ring formation and cell division away from mid-cell. The MinC/MinD/MinE proteins prevent *Z*-ring assembly near the poles and their absence causes polar divisions, leading to DNA-free minicells. In *E. coli* the MinC/MinD inhibitor complex of *Z*-ring formation oscillates from pole-to-pole, driven by the membrane-bound ATPase MinE, resulting in the average concentration of MinC/MinD being smallest at mid-cell, thus allowing *Z*-ring assembly.[73,74] The crystal structure of MinD–MinE complexes are consistent with a model of MinE moving between membrane-bound MinD molecules driven by induced conformational changes.[75]

Another negative regulator of FtsZ assembly, the *B. subtilis* nucleoid occlusion factor Noc and its *E. coli* functional analog SlmA prevent cell division near the chromosomal DNA to avoid the closing of the septum through the nucleoid, which would be lethal.[76,77] Recent structural and mechanistic data suggest that SlmA dimers bind to specific sites on the chromosome and interact with FtsZ to disrupt its polymerization.[78,79] The *B. subtilis* Noc protein has ~70 binding sites on the chromosome and coordinates chromosome segregation with division.[80] Noc might not interact directly with FtsZ, indicating its mechanism of action is different from that of SlmA, possibly involving downstream components of the divisome.[81] The nucleoid occlusion mechanism and Min proteins also ensure the remarkably high precision by which *E. coli* cells define their mid-cell position, leading to daughter cells with an average length deviation of only a few percent.[82,83] The division site selection is robust and works even in *E. coli* cells grown in nanofabricated channels to irregular cell shape.[84]

Cell division in Gram-negative bacteria

The divisome proteins of *E. coli* (Table 1) assemble at mid-cell in two steps (Fig. 3):[85] FtsZ, FtsA, ZipA, ZapA, ZapB, ZapC, ZapD, and FtsEX assemble early at the future division site and well before a constriction is visible. Their localization coincides with the phase of preseptal elongation, and it has been suggested that once assembled the early cell division proteins control cell wall elongation complexes containing PBP1A and PBP2.[61] Immediately before constriction the divisome matures by incorporating FtsK, FtsQ, FtsL, FtsB, FtsW, PBP3 (FtsI)-PBP1B,

Table 1. Divisome proteins of *E. coli*

Function/category	Protein (gene)[a]	Role/remarks[b]
Cytoskeletal protein	•FtsZ (***ftsZ***)	• tubulin-like, polymerizes with GTP, forms the Z-ring at mid-cell
Membrane attachment of FtsZ and regulation of Z-ring dynamics	• FtsA (***ftsA***), ZipA (***zipA***), ZapA (*zapA*), ZapB (*zapB*), ZapC (*zapC*), ZapD (*yacF*/*zapD*)	• Membrane attachment of FtsZ polymers (FtsA, ZipA) • Stabilization of Z-ring and regulation of its dynamics (ZapA, ZapB, ZapC, ZapD)
Divisome maturation and stability, PG-binding	• FtsK (***ftsK***), FtsQ (***ftsQ***), FtsL (***ftsL***), FtsB (***ftsB***)	• Recruitment of downstream divisome proteins (FtsK, FtsQLB) and DNA transport (FtsK)
	• FtsW (***ftsW***)	• Lipid II flippase (FtsW)
	• FtsN (***ftsN***), DamX (*damX*), DedD (*dedD*), RlpA (*rlpA*)	• PG binding (FtsN, DamX, DedD, RlpA) and divisome stability (FtsN)
PG synthesis (and its regulation)	• PBP1B (*mrcB*), PBP3 (***ftsI***), MtgA (*mtgA*)	• Synthesis of PG (PBP1B, PBP3, MtgA)
	• LpoB (*lpoB*)	• Activation of PBP1B (LpoB)
PG hydrolysis (and its regulation)	• AmiA (*amiA*), AmiB (*amiB*), AmiC (*amiC*)	• Septal PG cleavage for daughter cell separation (AmiA, AmiB, AmiC)
	• FtsE (***ftsE***[c]), FtsX (***ftsX***[c]), EnvC (*envC*), NlpD (*nlpD*)	• Control of septal PG cleavage (FtsEX, EnvC, NlpD)
OM invagination	• Pal (*pal*), TolA (*tolA*), TolB (*tolB*), TolQ (*tolQ*), TolR (*tolR*)	• OM invagination and stability during division (TolA,TolB, TolQ, TolR, Pal) • PG binding (Pal)

[a]Genes essential for cell division are written in bold.
[b]See the text for detailed discussion and references.
[c]Not essential at high osmolarity of the growth medium.

and FtsN, while PBP2 leaves the cell division site.[55,85] How the steps in divisome assembly are temporally controlled is largely unknown but likely involves multiple protein–protein interactions between its components. The interactions of relevant divisome proteins are summarized in Figure 4. The timing of mid-cell arrival of cell division proteins is similar in *C. crescentus,* with some differences, for example the relatively late arrival of FtsW and FtsB.[86]

ZipA and the actin-like FtsA are essential for cell division. They bind to the same C-terminal region in FtsZ, stabilize the *Z*-ring, and anchor it to the cytoplasmic membrane.[87,88] FtsA polymerizes bi-directionally and forms membrane-attached protofilaments.[89,90] The membrane potential stimulates the attachment of FtsA and other cell morphogenesis proteins like MreB and MinD to the membranes, which explains why compounds affecting the membrane potential delocalize these proteins and cause growth and cell division defects.[91] Other cytoplasmic *Z*-ring associated proteins, ZapA, ZapB, ZapC, and ZapD, are dispensable for cell division. ZapA and ZapD interact with FtsZ, stimulating protofilament association and stabilizing the *Z*-ring.[92–94] Cells with reduced FtsZ level cannot divide when they lack ZapA, and a double mutant lacking *zapA* and *zapD* has an increased cell length, consistent with a slight division defect.[92,93] ZapB interacts with ZapA and forms spontaneous filaments *in vitro*.[95,96] ZapB-mCherry localizes inside the *Z*-ring and presumably stabilizes the *Z*-ring via ZapA.[97] ZapA and ZapB are required for mid-cell anchoring of MatP, a protein that structures the chromosomal terminus region

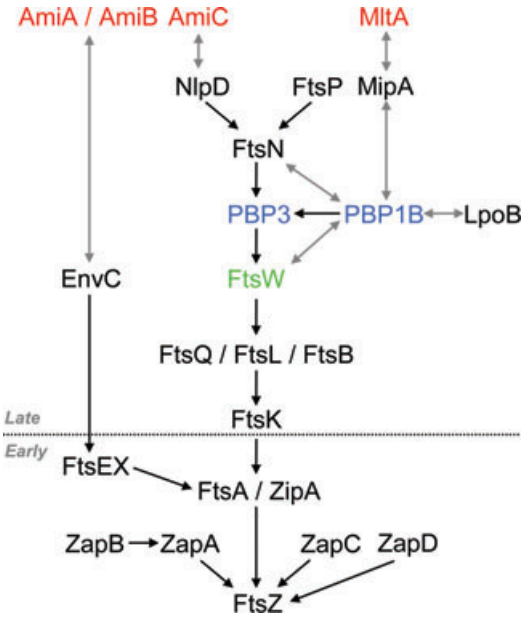

Figure 3. Hierarchical recruitment of cell division proteins. The divisome is build from inside the cell, with FtsZ and the early cell division proteins localizing well before septation starts. The black arrows indicate dependency on mid-cell localization mediated in most cases by direct protein–protein interaction. The gray arrows show further direct interactions involving peptidoglycan enzymes. The late cell division proteins include the lipid II flippase FtsW (green), peptidoglycan synthases (blue), and peptidoglycan hydrolases (red). PBP1B-LpoB are not essential for cell division, as their function can be taken over by PBP1A-LpoA. Peptidoglycan hydrolases including the amidases are not required for cell division but for separation of daughter cells.

into a macrodomain.[98] The relocation of the terminus macrodomain from the cell pole to the division site via MatP–ZapA/ZapB interactions occurs prior to the replication of the terminus region and is required for proper nucleoid segregation.[98] ZapC interacts with FtsZ and localizes to mid-cell where it stabilizes the *Z*-ring by promoting FtsZ polymer bundling and by suppressing the GTPase activity of FtsZ.[99,100] In bacteria other than *E. coli* there are different combinations of FtsZ-ring stabilizing proteins.[101]

FtsEX is an ATP-binding cassette (ABC) transporter homolog that binds to FtsZ via the ATP-binding protein FtsE.[102–104] Mid-cell localization of FtsE depends on the presence of FtsZ and ZipA. FtsE has been shown to be essential for cell division when cells grow in low-osmolarity growth medium;[105,106] its function in cell division is to recruit and regulate peptidoglycan hydrolases (see below).

In order to start septation, a number of essential "late" cell division proteins, FtsK, FtsQ, FtsL, FtsB, FtsW, PBP3, and FtsN, assemble to the *Z*-ring almost simultaneously and in an interdependent fashion (Fig. 3).[85,107] Several other proteins not essential for septation also localize to mid-cell depending on Fts proteins, including the peptidoglycan synthase PBP1B[63] and the hydrolases AmiB and AmiC[108,109] and their regulators LpoB,[24] EnvC, and NlpD.[110] Presumably, these peptidoglycan enzymes are not essential for cell division/cell separation because their function can be taken over by redundant enzymes. For example, in the absence of the bi-functional peptidolgycan synthase PBP1B, the homologous PBP1A becomes essential and shows enhanced localization at mid-cell, where it presumably takes over PBP1B's role in septal peptidoglycan synthesis.[51]

FtsK is a multifunctional protein involved in both chromosome segregation and cell division. In bacterial two hybrid assays FtsK interacts with FtsZ, FtsQ, FtsL, and PBP3.[111–113] The cytoplasmic domain of FtsK forms hexamers to directionally transport DNA, required for decatenation of sister chromosomes, together with the Xer recombinases.[114,115] This function of FtsK is only required in the fraction of cells with catenated chromosomes. The membrane/periplasmic part of FtsK is essential for cell division, presumably by stabilizing late-cell division proteins at the septum, which explains the existence of mutations in *ftsA* and *ftsQ* that allow for septation in the absence of FtsK.[112,116,117]

FtsK is required for septal recruitment of a preformed FtsQ–FtsL–FtsB complex.[107,118] Each of these proteins is a bitopic membrane protein with a small cytoplasmic part and periplasmic domains. The crystal structure of the periplasmic domains of FtsQ identified two distinct regions: (i) a POTRA (polypeptide transport associated) domain, of which the second β-strand is essential for FtsQ mid-cell localization, and (ii) the C-terminal β-domain of FtsQ that is essential for the recruitment of FtsL, FtsB, and FtsW.[119,120] The C-terminal domain of the periplasmic part of FtsL is required for its mid-cell localization via interaction with FtsQ.[121] FtsQ-FtsL-FtsB 1:1:1 or 2:2:2 complexes have been modeled,[122] the latter being consistent with the crystal structure of an FtsQ dimer.[120] Small angle

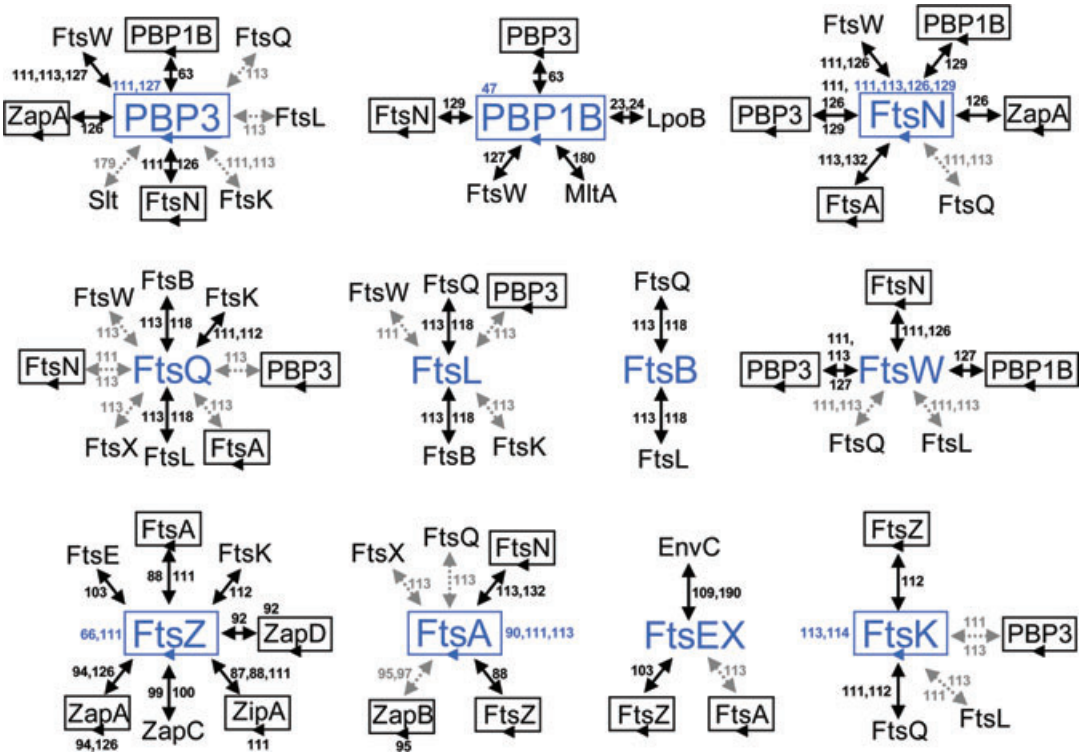

Figure 4. Protein–protein interactions of divisome proteins. Interactions are shown individually. Solid black lines represent direct interactions identified *in vitro* and in the cell; dashed gray lines represent interactions shown solely by bacterial two-hybrid assays. Rectangular arrows enclosing proteins indicate homodimerization or multimerization. Numbers refer to reference numbers in the reference list. The referencing is not exhaustive for well-studied interactions (like FtsZ-FtsZ or FtsZ-FtsA).

X-ray scattering of a homologous DivIB(FtsQ)-FtsL-DivIC(FtsB) complex from the Gram-positive *Streptococcus pneumoniae* is consistent with a 1:1:1 complex.[123] In *E. coli* FtsQ has been shown to interact with FtsW and PBP3 by bacterial two-hybrid analysis.[111,113] The requirement for FtsQ can be bypassed by expressing fusion proteins of ZapA-FtsL and ZapA-FtsB, either of which is sufficient for the recruitment of FtsW and PBP3.[124]

FtsW is member of the SEDS (shape, elongation, division, and sporulation) family of integral membrane proteins that also includes the essential cell elongation protein RodA and the sporulation protein SpoVE of the Gram-positive *B. subtilis*.[125] FtsW, an integral transmembrane protein with 10 transmembrane regions, has recently been identified as the long-searched transporter (flippase) for the lipid II precursor.[32] Consistent with this function, FtsW interacts with both PG synthases, PBP3 and PBP1B, and is required for recruiting PBP3 to the divisome, presumably together with PBP1B.[63,126–128] The TPase PBP3 also interacts with FtsL,[113] FtsQ,[113] PBP1B,[63] and FtsN.[129] PBP3 can be recruited to the divisome independently of the FtsQLB complex via an FtsW-ZapA fusion, and targeting a FtsW-PBP3 fusion to mid-cell restored the recruitment of FtsQLB in cells depleted of FtsA.[124] These data suggest that FtsW-PBP3 and FtsQLB complexes form independently of one another before recruitment to the divisome, which is consistent with interaction studies using Förster Resonance Energy Transfer (FRET) and co-immunoprecipitation.[126,127] In *C. crescentus*, PBP3's early localization to the *Z*-ring relies largely on a functional TPase domain, indicating that TPase substrates, or an active state of the enzyme, might be required.[130]

FtsN is an essential cell division protein that has been thought to be restricted to enterobacteria. However, recent work in *C. crescentus* showed that FtsN-like proteins are more widely distributed among the proteobacteria.[131] In *E. coli*, FtsN interacts with both early and late cell division

proteins—FtsA, ZapA, PBP3, FtsW, and PBP1B—as shown by various *in vitro* (surface plasmon resonance, affinity chromatography) and *in vivo* (co-immunoprecipitation, FRET) techniques.[126,127,129,132] Bacterial two-hybrid analysis shows additional interactions with FtsQ.[111,113] FtsN is a bitopic membrane protein with a short cytoplasmic part and one transmembrane region. The periplasmic part contains three α-helices followed by a proline/glutamate-rich unstructured region and a globular C-terminal SPOR peptidoglycan binding domain.[133] While the SPOR domain has been shown to interact with peptidoglycan *in vitro* it is not essential for cell division.[134] Presumably, the peptidoglycan-binding function of FtsN's SPOR domain can be taken over by the SPOR domain-containing proteins DamX, DedD, and RlpA, which localize to cell division site, as mutants lacking multiple SPOR domain proteins show cell division defects.[135,136] The precise role of FtsN during cell division is not known. Its periplasmic region, comprising the three partially formed α-helices (amino acids 62–67, 80–93, and 117–123), is essential for cell division for unknown reasons. FtsN interacts with both PBP1B and PBP3[63,129] and stimulates the activity of PBP1B, presumably by stabilizing the dimeric form of PBP1B[129] consistent with the hypothesis that a main functional role for FtsN in cell division is to coordinate the peptidoglycan synthases active during septation. Additional functions of FtsN have also been suggested. For example, FtsN could provide a signal for completion of divisome assembly to the cytoskeletal components, a role supported by its cytoplasmic interaction with the 1c subdomain of FtsA[132] and the existence of mutants with altered FtsA that can divide without FtsN.[137] FtsN has also been implicated in a divisome stabilizing function, which is supported by the observation that FtsN-depletion leads to the disassembly of the already assembled divisome components, including the early proteins.[138]

FtsP (SufI) is a recently characterized soluble periplasmic protein involved in cell division, although its precise role is unknown. It localizes to the division sites dependent on the presence of FtsZ, FtsQ, FtsL, and FtsN.[139] FtsP is dispensable under normal growth conditions but is required for divisome stability when cells grow at various stress conditions, including oxidative stress and DNA damage.[140] The crystal structure of FtsP shows structural similarity to the multicopper oxidase protein family, but does not bind the metal ion.[139]

Cell division proteins specific to Gram-positive bacteria

In this section we highlight several recent findings specific to Gram-positive bacteria. These have a thick septum peptidoglycan with distinct zones of high and low electron densities, as visualized by cryo-electrontomography,[141,142] and they complete septal cross-wall synthesis before daughter cell separation (Fig. 1). Gram-positive species contain most of the essential division proteins discussed above, with a few exceptions such as FtsK, FtsP, and FtsN.

Conserved eukaryotic-type Ser/Thr protein kinases regulate various cellular processes including cell division in Gram-positive species. The *Streptococcus pneumoniae* protein kinase StkP is required for proper septal cell wall synthesis by yet unknown mechanisms.[143,144] It contains several PASTA domains, which are also present in some PBPs and have been suggested to bind to peptidoglycan fragments and β-lactams.[145] StkP phosphorylates the cell division protein DivIVA, which is found in many Gram-positive species that undergo elongation and division modes of PG synthesis, and both proteins localize to mid-cell and the poles. In *Bacillus subtilis* DivIVA regulates the septation site by positioning and stabilizing the FtsZ inhibitor MinC/MinD at the cell poles. Mutants lacking DivIVA lose the topological control over MinC/MinD and form minicells derived from aberrant septation events at the poles.[146] DivIVA localizes to the new poles immediately after daughter cell separation and independent of divisome proteins to prevent aberrant *Z*-ring assembly after division.[147,148] Interestingly, DivIVA has an amphiphatic alpha-helix for membrane attachment, and finds the new cell pole localization by virtue of its affinity to curved membranes, a feature that explains its polar localization in a number of heterologous organisms like *E. coli* and yeast.[149]

The cytoplasmic cell division protein EzrA is present in *B. subtilis* and *S. aureus*, but EzrA depletion causes a severe cell division phenotype and an increase in cell diameter only in the latter spherical-shaped species.[150–152] EzrA has a N-terminal membrane anchor and interacts with FtsZ. It acts as a negative regulator of *Z*-ring assembly by decreasing GTP binding affinity and increasing

the GTPase activity of FtsZ, thus increasing FtsZ-depolymerization.[151,153,154] Its localization to the divisome during division suggested that it regulates *Z*-ring dynamics during constriction.[153] EzrA has also been shown to play a role in the switch from lateral to septal cell wall synthesis in *B. subtilis,* together with GpsB.[155] This is achieved by positioning the class A PBP1 (encoded by the *ponA* gene), the major PG synthase of *B. subtilis*, through direct protein–protein interactions. EzrA and GpsB recruit PBP1 to the divisome, and GpsB is responsible for the removal of PBP1 from the mature cell pole after division making it available for cell elongation.[155]

Another recently identified member of the *B. subtilis* divisome, not found in Gram-negative bacteria, is SepF. Cells lacking SepF form grossly distorted division septa.[156] SepF is recruited to the divisome by direct interaction with FtsZ. It negatively regulates the GTPase activity of FtsZ and thus stabilizes FtsZ filaments.[156,157] Interestingly, *in vitro* SepF forms relatively large rings with a diameter of 50 nm and it is able to bundle FtsZ filaments into long, regular structures which resemble microtubules. Therefore, it has been proposed that SepF is required for the regular arrangement and bundling of FtsZ filaments for proper septum placement.[158]

Peptidoglycan hydrolases for growth regulation and cell separation

Peptidoglycan hydrolases are ubiquitous enzymes.[159] Some of them lyse target bacteria as part of the host defense or in bacterial interactions.[160,161] Others are produced by bacteria to remodel or turn over their own peptidoglycan and to facilitate cell separation during or after cell division.

DD-Carboxypeptidases trim pentapeptides in newly made peptidoglycan to tetrapeptides, which can be further shortened to tripeptides by LD-carboxypeptidases and to dipeptides to DL-carboxypeptidases (Fig. 2). The activities of these enzymes might spatiotemporally regulate the incorporation of new peptidoglycan into the sacculus by controlling the amount of donor and acceptor peptides for transpeptidation, perhaps explaining the observed shape defects in carboxypeptidase mutants of *E. coli*, *Helicobacter pylori*, and *Campylobacter jejuni.*[162–164] In *E. coli* DD-carboxypeptidases help to properly orient the *Z*-ring in the cell division plane, and their absence causes cells to form branched cell shapes.[165] PBP5, the major DD-carboxypeptidase in *E. coli*, contributes to the maintenance of the cell shape and diameter.[166] It localizes to foci in the lateral wall and to the cell division site presumably by recognizing active areas of PG synthesis.[167]

Lytic transglycosylases, DD-endopeptidases and *N*-acetylmuramyl-L-alanine amidases (amidases) are responsible for peptidoglycan turnover and separation of daughter cells. Their cleavage sites in peptidoglycan are shown in Figure 2. The *E. coli* DD-endopeptidases PBP4 and PBP7 hydrolyze DD cross-links,[168] whereas MepA is capable of cleaving both DD and LD cross-links.[169] The *E. coli* DD-endopeptidases have auxiliary roles in cell morphogenesis,[170,171] in contrast to the three *H. pylori* DD-endopeptidases Csd1, Csd2, and Csd3, which contribute to the generation of helical cell shape presumably by localized relaxation of peptide cross-links.[172]

E. coli has seven lytic transglycosylases, including the periplasmic Slt70 and the outer membrane lipoproteins MltA, MltB, MltC, MltD, MltE, and MltF. These muramidases cleave the β-1,4 glycosidic bond between Mur*N*Ac and Glc*N*Ac, forming a 1,6-anhydro ring on the Mur*N*Ac residue.[173] There are some differences in the specificity of lytic transglycosylases. Slt70 only cleaves glycan strands with peptides but not glycan strands lacking these, whereas MltA is able cleave either type.[174] A number of crystal structures of Slt70 and soluble versions of MltA, MltB, and MltE have allowed deduction of the mechanism of the glycan strand cleavage. They possess a catalytic glutamate residue and lack the conserved catalytic aspartate present in lysozyme, which, unlike lytic transglycosylases, *hydrolyzes* the glycosidic bond between GlcNAc and MurNAc.[175–178] Lytic transglycosylases interact with PBPs suggesting that they are components of peptidoglycan synthesis complexes.[179,180]

E. coli has four amidases, the soluble periplasmic AmiA, AmiB, and AmiC[181] and the outer membrane lipoprotein AmiD.[182,183] They hydrolyze the amide bond between Mur*N*Ac and L-Ala and therefore release the peptides from the glycan strand. AmiB and AmiC localize to the division site, whereas AmiA localizes more diffuse within the periplasm.[108,109] The latter is a zinc metalloenzyme active on polymeric peptidoglycan and requiring at least tetrasaccharide sized fragments.[184] AmiA, AmiB, and AmiC have major roles in the cleavage of the septum

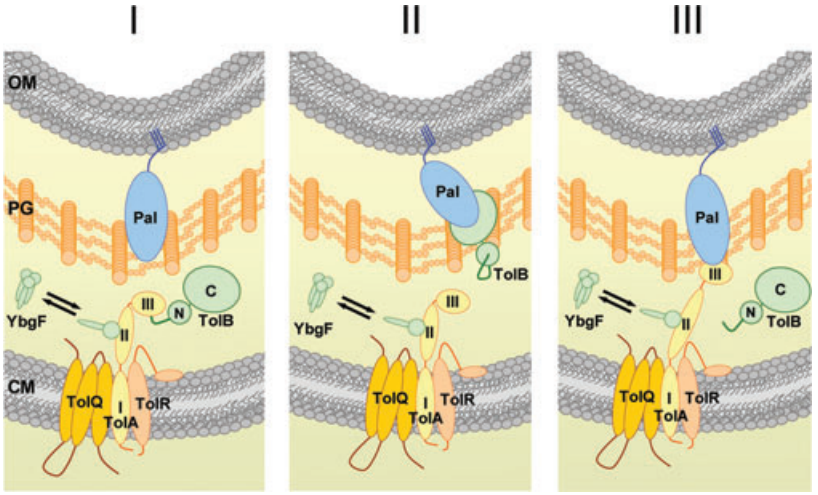

Figure 5. Different presumed interaction states of Tol–Pal proteins. The cartoon shows the proteins of the Tol–Pal complex and their domains as linked ovals or spheres. The three panels show the proposed interaction states of Tol–Pal proteins between the cytoplasmic membrane (CM) and outer membrane (OM). Panel I shows Pal bound to the peptidoglycan (PG) sacculus and TolB binding with its N-terminal loop region (shown as a green line) to the IM-anchored TolA, which forms a complex with TolQ and TolR membrane proteins. In panel II Pal binds to the C-terminal domain of TolB after their respective dissociation from PG and TolA. Panel III shows Pal bound to domain III of TolA after dissociation from TolB. It is not yet clear if TolB interacts with TolA and/or Pal to form a trimeric Pal–TolA–TolB complex, and the existence of a TolA–Pal interaction is disputed. *In vitro* data support the states depicted in panels I and II, while *in vivo* cross-linking data suggest that all three states are possible. Thus, either a transient trans-envelope complex links the OM, PG, and IM (state III) or the cycling between states I and II maintains sufficient contact between the envelope layers for proper invagination. The role of YbgF is not yet known. Alone, YbgF forms a homotrimer, though its interaction with domain II of TolA via its C-terminal domain has a 1:1 stoichiometry.

during cell division to allow the separation of daughter cells.[181] Mutants lacking two or more amidases form chains of nonseparated cells and have increased outer membrane permeability. Both phenotypes become more severe in amidase mutants additionally lacking DD-endopeptidases and/or lytic transglycosylases.[185,186] Remarkably, about 1/3 of the newly made septal peptidoglycan is immediately removed during septum synthesis presumably by the septum-splitting hydrolases.[187] This is consistent with the finding that AmiC is capable of removing fluorescent peptides incorporated into the peptidoglycan during growth from the division site.[188]

Regulation of septum cleaving amidases

How the potentially dangerous peptidoglycan hydrolases are regulated in the cell is not well understood. Recent work from the Bernhardt laboratory has provided significant understanding of the regulation of septum cleaving amidases. These data suggest that the amidases require specific activator proteins, EnvC or NlpD, that are themselves recruited to the divisome and are activated by FtsN or FtsEX.[109] The periplasmic EnvC activates AmiA and AmiB, and the outer membrane lipoprotein NlpD activates AmiC.[109] Importantly, the expression of a mis-localized EnvC causes cell lysis, presumably by activating AmiA and AmiB at inappropriate sites away from mid-cell.[109] FtsN is required for mid-cell localization of AmiB but not of EnvC, which is recruited earlier by FtsEX. FtsN is also required for mid-cell localization of AmiC and its activator NlpD.[189] Importantly, the recruitment and activation of the amidases to the divisome requires active PBP3. EnvC and NlpD, but not the amidases, localize in cephalexin-treated cells with blocked PBP3, indicating that PBP3-catalyzed septal PG synthesis precedes the recruitment and activation of the amidases.[189]

Both EnvC and NlpD possess a catalytically inactive C-terminal LytM domain. EnvC has a coiled–coil region that is essential for its recruitment to the divisome,[110] which also requires the large periplasmic region in FtsX. Interestingly, FtsE's ATPase function is required for EnvC-mediated activation of AmiA and AmiB, suggesting that hydrolysis of bonds in PG for septum cleavage is coupled to hydrolysis of ATP.[190] The activation of AmiB requires

a conformational change to remove an α-helix to open the active site cleft for substrate binding, a mechanism that appears to be conserved between septum-cleaving amidases.[191]

Outer membrane constriction during cell division

The outer membrane protects Gram-negative bacteria against many antibiotics and antibacterial enzymes by preventing access to their periplasmic or intracellular target.[192] In *E. coli* the outer membrane is firmly attached to the peptidoglycan by the highly abundant outer membrane lipoprotein Lpp (Braun's lipoprotein), a fraction of which is covalently linked to peptidoglycan.[5,193] Other abundant outer membrane proteins, such as OmpA and the lipoprotein Pal, interact noncovalently with the peptidoglycan layer. The deletion of *pal* or *lpp* genes results in reduced outer membrane integrity and increased release of outer membrane vesicles into the growth medium.[194,195] Overexpression of *pal* rescues *lpp* null mutants, but an overexpression of *lpp* cannot rescue *pal* deletion strains, indicating that Pal has a more specific role than simply tethering the OM to the peptidoglycan.[195] Indeed, Pal has been implicated with members of the Tol–Pal system in aiding outer membrane constriction during cell division. Cells deficient in this function typically form short chains in low osmotic growth medium and have a reduced outer membrane stability, leading to periplasmic leakage and the formation of outer membrane blebs at division sites.[196]

The Tol–Pal system is conserved in Gram-negative species and consists of the integral cytoplasmic membrane proteins TolQ, TolR, and TolA, the soluble periplasmic protein TolB, and the outer membrane protein Pal.[197] Each of these proteins localize to mid-cell during division dependent on FtsN, with TolQ and TolA localizing independently from the other Tol–Pal components.[196] TolA is able to localize to mid-cell in *ftsA* mutant cells that divide without FtsN, indicating that ongoing septation might be sufficient for its localization.[137] Protein–protein interactions between Tol–Pal proteins can potentially connect the cytoplasmic membrane, peptidoglycan, and outer membrane.[196] TolQ contains two larger cytoplasmic and three transmembrane regions; TolR and TolA are both anchored in the cytoplasmic membrane by a single transmembrane region near the N-terminus with

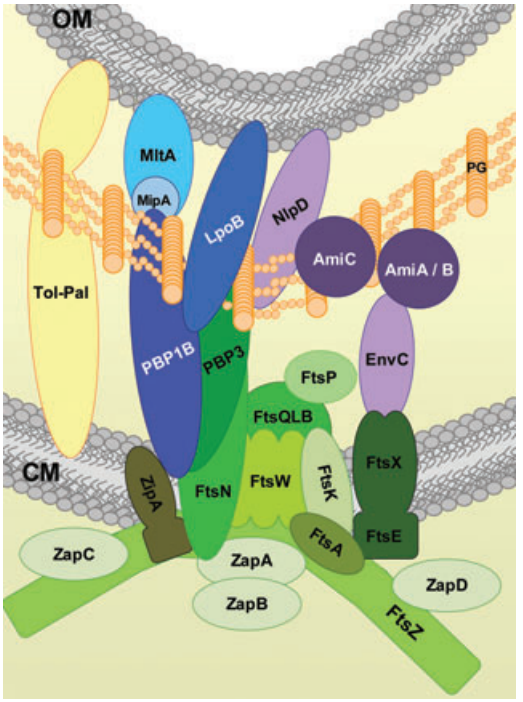

Figure 6. Cartoon of the divisome. The proteins of the divisome are shown as ovals, spheres, or as filament (FtsZ). Their localization in the cytoplasmic membrane (CM), periplasm, or outer membrane (OM) are indicated. Interactions between proteins within the complex are roughly represented. It is not known if MipA–MltA, which interact with PBP1B, localize to mid-cell.

most of the protein locating in the periplasm.[198] The periplasmic part of TolR includes a C-terminal amphiphatic helix that is proposed to associate with the periplasmic leaflet of the cytoplasmic membrane.[199] The periplasmic region of TolA contains the elongated, mainly α-helical, domain II and the globular C-terminal domain III.[200,201] The crystal structure of TolB shows a C-terminal six-bladed β-propeller domain, along with a globular N-terminal domain that features a flexible 12 amino acid N-terminus that binds into a cleft between the N- and C-terminal domains when TolB interacts with Pal.[202,203]

TolQ, TolR, and TolA form a cytoplasmic membrane complex through interactions via their transmembrane regions.[204,205] Domain II of TolA also interacts with YbgF, a nonessential protein of unknown function encoded by the last gene in the *tol–pal* operon.[206] Presumably, TolA undergoes conformational changes in its periplasmic domains driven by TolQ/TolR, changes that are energized by the membrane's proton motive force (pmf). The mechanism is thought to provide the energy for

outer membrane constriction during cell division, but most details of the process are not understood. Likely, there are different states of interactions between the Tol–Pal proteins in the cell envelope (Fig. 5).[196,207] TolA was cross-linked to Pal via domain III, and subsequent *in vivo* cross-linking mapped separate interaction sites of Pal with TolB and TolA.[208,209] However, studies with the purified proteins could not confirm an interaction between TolA and Pal or the formation of a ternary TolA–Pal–TolB complex.[203] Gerding *et al*. proposed that pmf-dependent conformational changes in TolA produce cycles of Pal-binding and -dissociation and, consequently, facilitate transient interactions between the outer membrane and PG and the outer membrane and the inner membrane, respectively.[196] In addition, TolB might cycle between a Pal-bound and Pal-unbound form whereby only the latter allows for interaction with TolA via its flexible 12 amino acid N-terminus (Fig. 5).[203]

The PBP1B–LpoB complex spans from the cytoplasmic membrane to the outer membrane and thus could contribute to the constriction of the outer membrane during cell division. This hypothesis is supported by the observation that cells lacking both a functional Tol–Pal system (*pal*$^-$) and LpoB show a severe lysis phenotype, while the single mutants are viable and do not lyse.[24] In a *pal*$^-$ background, LpoB mislocalization to the inner membrane is as detrimental to the cell as is the deletion of *lpoB*.[24] Therefore, correct outer membrane localization of LpoB is required to support outer membrane constriction in the absence of a functional Tol–Pal system. For unknown reasons PBP1A–LpoA cannot take over this specific function of PBP1B–LpoB. In summary, in *E. coli* it is presumably the combined activities of the Tol–Pal system and the peptidoglycan synthesis complex for cell division (with PBP1B and LpoB) that facilitate outer membrane constriction during cell division. Tol–Pal proteins are essential in *C. crescentus*, which lacks PBP1B and LpoB.[210] In *C. crescentus*, the LytM-domain protein DipM is required for constriction. A *dipM* mutant also has thicker peptidoglycan and loses the integrity of the outer membrane, presumably by mal-functioning of the Tol–Pal system.[211–213]

Concluding remarks

Much progress has been made over the last decades toward understanding the physiology of bacterial cell division. The divisome consists of more than 20 proteins, including novel ones recently identified (Fig. 6). The crystal structures of some of the key proteins and some important interactions are now known, as are their interdependency for localization and the timing of their arrival at the division site. However, the molecular mechanisms and dynamics underlying the synthesis and cleavage of the division septum have remained largely unknown. Many cell division proteins lack an enzymatic activity and might act in coordinating cytokinesis, peptidoglycan synthesis and cleavage, and outer membrane invagination. We expect that recent technological advances, including *in vitro* reconstruction of peptidoglycan synthesis and divisomal protein assembly, superresolution microscopy, and high-throughput chemical genomics, will help bring a more complete understanding of bacterial cell division.

Acknowledgments

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Conflicts of interest

The authors declare no conflicts of interest.

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