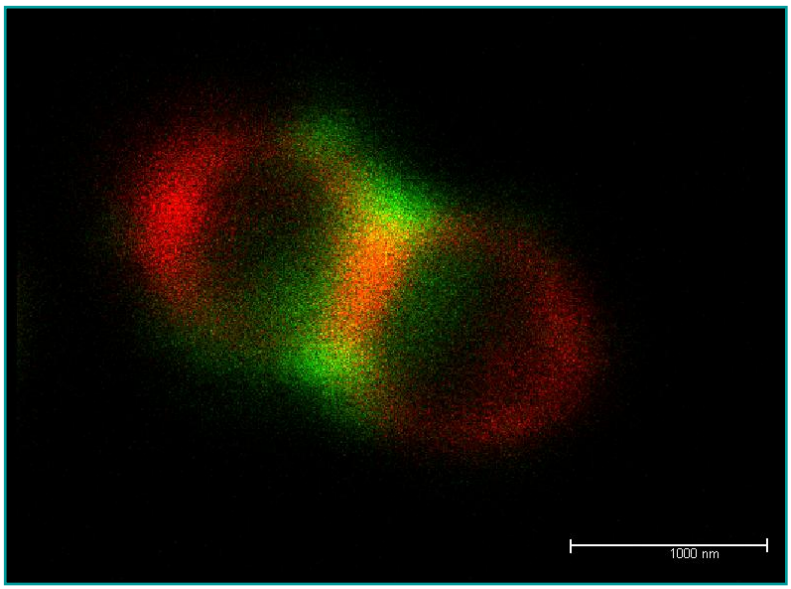
Mechanisms of bacterial cell division; implications for deposition of the complement membrane attack complex



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Microscopic photograph title page: Courtesy of Evelien Berends.
MAC deposition on dividing *Streptococcus pyogenes* cell.
Green = antibody against the MAC
Red = membrane dye

Contents

Chapter 1: Introduction

This thesis discusses the cell wall structure of Gram-positive bacteria during the division cycle and aims to correlate this to site-specific binding of the membrane attack complex (MAC or C5b-9) of the human complement system. Experimental results demonstrating binding of fully assembled MAC to selected Gram-positive bacteria provided the basis for this review (E. Berends, unpublished results). Since Gram-positive bacteria are considered to be resistant to MAC-mediated lysis due to their thick peptidoglycan layer, this association was highly unexpected. More striking was that the MAC was deposited on specific sites on the cell surface, i.e. the divisional septum (*Streptococcus pyogenes*) or the cell poles (*Bacillus subtilis*). As the central complement protein C3b was found to cover the entire bacterial surface, the terminal pathway leading to MAC deposition could be initiated dispersedly (E. Berends, unpublished results). Nevertheless, the MAC only remained at these specific sites.

To be able to better understand why the MAC is deposited on Gram-positive bacteria and what determines its location, this thesis will cover cell wall structure during division and sporulation of Gram-positive bacteria. This thesis aims to give a compact overview of the mechanisms of cell division of three important Gram-positive species: *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus pyogenes* (Group A *Streptococcus*). Cell division of *Escherichia coli* will be discussed to highlight similarities and differences between Gram-positive and Gram-negative bacteria. Finally, I will discuss the implications for binding of the complement MAC to these Gram-positive bacteria.

Chapter 1a: Introduction to the bacterial cell wall

Gram-positive versus Gram-negative bacteria

The inner contents and the cytoplasmic membrane are similar in all bacteria. Based on what is on the outside of the cell membrane, bacteria can be divided into two major classes. The Gram staining, published in 1844 by Hans Christian Gram, classifies almost all bacterial species as either Gram-negative or Gram-positive. Bacteria from both classes own an extracellular layer of peptidoglycan polymers, which is essential for replication, shape and survival. Peptidoglycan (PG) consists of polysaccharides linked by peptides, and is only found in bacterial species. In Gram-negative bacteria, the

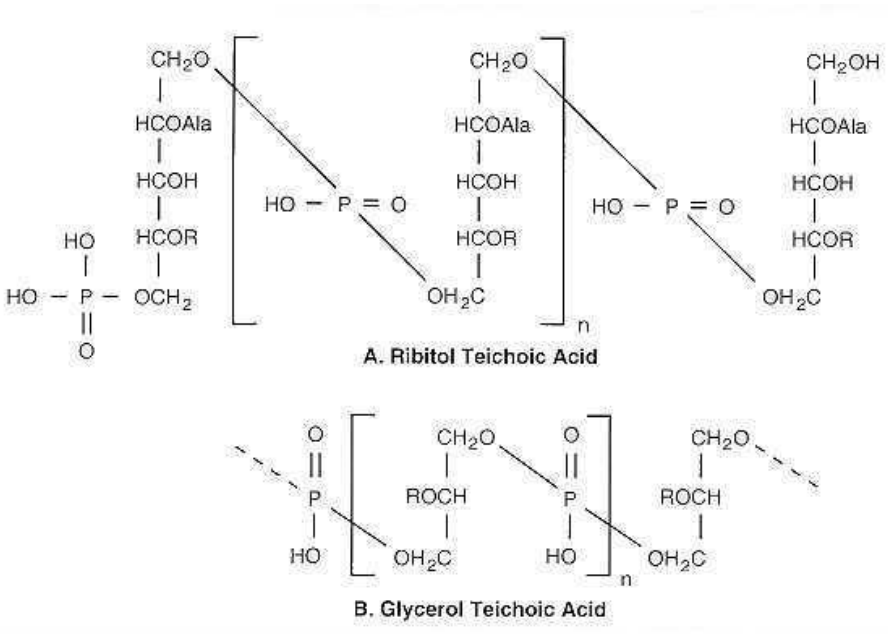

Figure 1: General structure of teichoic acids. The brackets denote one monomer. **(A)** Repeating ribitol backbone with 5 hydroxyl groups. **(B)** Repeating glycerol backbone with 3 hydroxylgroups. Ala = D-alanine side group. R denotes a range of different side groups. Baron, 1996

peptidoglycan layer is much thinner than in Gram-positive species, although the biochemical structure is very similar (Scheffers and Pinho, 2005; Schleifer and Kandler, 1972). Gram-negative bacteria possess an extra lipid membrane that encloses the peptidoglycan layer, termed the outer membrane. The peptidoglycan layer is covalently attached to the outer membrane via lipoprotein (Lpp or Braun's lipoprotein) (Braun, 1975). The most important component of the outer membrane is Lipopolysaccharide (LPS). Transmembrane proteins perform additional functions such as transport of metabolites. Gram-negative bacteria never contain teichoic acids.

In Gram-positive bacteria the peptidoglycan forms a thick, multilayered meshwork. Cell wall proteins are covalently or non-covalently attached to the peptidoglycan. Most Gram-positive species also contain complex polysaccharides (C polysaccharides) and teichoic acids in their cell wall (Coley *et al.*, 1972). The backbone of teichoic acids (TAs) consists of multiple glycerol (3 hydroxyl groups) or ribitol (5 hydroxyl groups) residues linked by phosphates (Fig. 1) (Baron, 1996). The choice between glycerol or ribitol depends on the species. Whereas *S. pyogenes* only has glycerophosphate TAs, *B. subtilis* produces both glycerophosphate and ribitolphosphate TAs (Esko *et al.*, 2009). The side groups attached at the site of the hydroxyl groups (e.g. D-alanine or monosaccharides) define different species and serotypes (Esko *et al.*, 2009). The TAs are often covalently linked to the PG layer and are

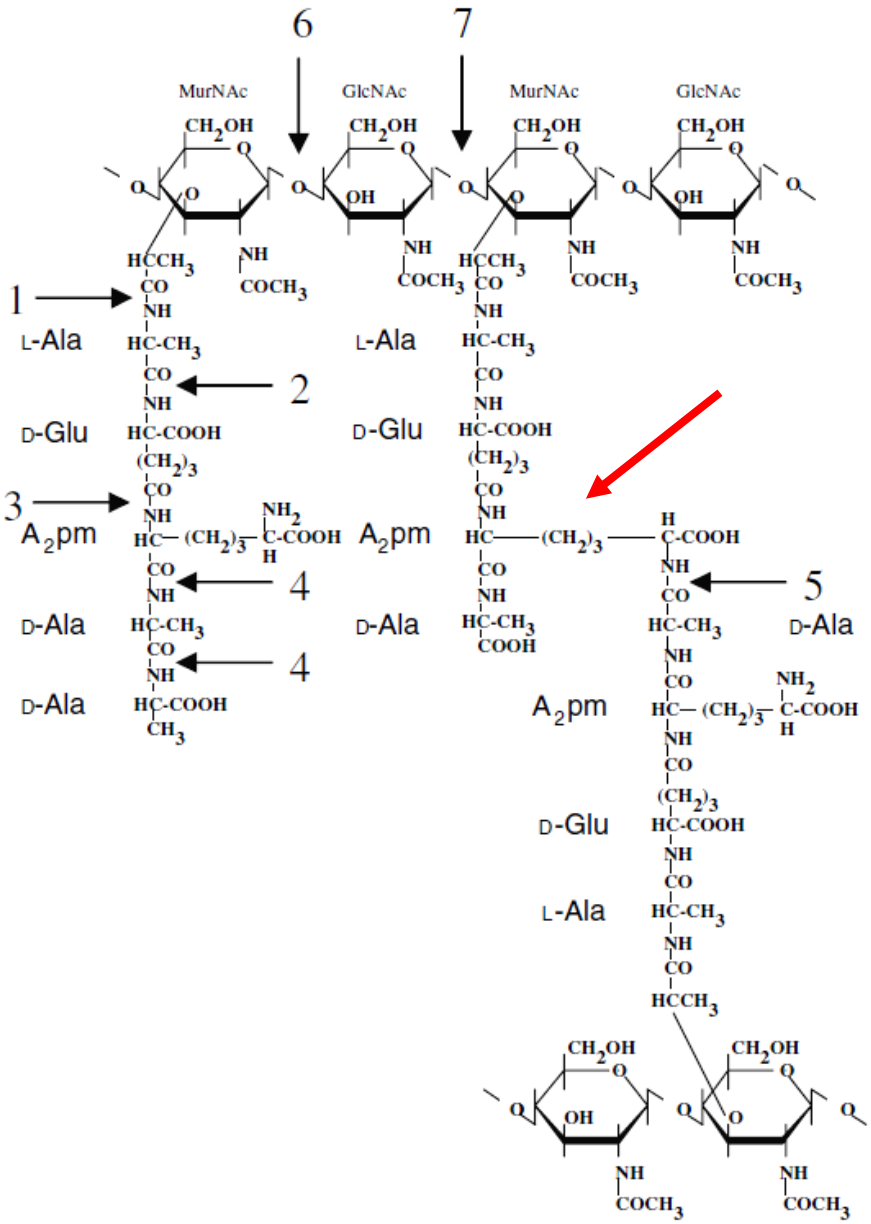

Figure 2: Structure of typical *B. subtilis* peptidoglycan. The red arrow indicates the covalent attachment of two linker-peptides. A_2pm = diamino acid. MurNAc = *N*-acetyl-D-muramic acid. GlcNAc = *N*-acetylglucosamine. The black arrows indicate hydrolytic bonds attacked by cell wall hydrolases: 1, *N*-acetylmuramoyl-L-alanine amidase; 2, LD-endopeptidase; 3, DL-endopeptidase; 4, carboxypeptidase; 5, DD-endopeptidase; 6, muramidase and lytic transglycosylase; 7, *N*-acetylglucosaminidase. Fukushima *et al.*, 2007

then called wall teichoic acids (WTAs). When the acid is instead covalently linked to a membrane lipid, it is called a lipoteichoic acid (LTA) (Esko *et al.*, 2009). Both WTAs and LTAs extend beyond the wall surface. TAs are important antigens that facilitate adherence and determine virulence (Carruthers and Kabat, 1983). They enhance bacterial resistance to environmental stresses (Vergara-Irigaray *et al.*, 2008; Oku *et al.*, 2009), antibiotics (Peschel *et al.*, 2000) and antimicrobial peptides (Peschel *et al.*, 1999). In addition, they can initiate the alternative activation pathway of the complement system or serve as a binding site for bacteriophages (Chatterjee, 1969; Winkelstein and Tomasz, 1978).

Peptidoglycan structure

Peptidoglycan consists of multiple glycans, polysaccharide chains of the alternating sugars N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc). The sugars are linked by glycosidic bonds, which are the target of human lysozyme (Rupley, 1967). The glycan chains can not branch and need to be crosslinked to obtain a solid cell wall structure. Pentapeptides that are attached to the glycans as side chains serve as crosslinks of species-specific sequence. The sequence L-alanine – D-glutamic acid – diaminopimelic acid (DAP) – D-alanine – D-alanine is most commonly found in Gram-negative bacteria (Vollmer, 2008). Most Gram-positive cocci have exchanged DAP for lysine on the third place (Vollmer, 2008). After enzymatic removal of the terminal D-alanine of one pentapeptide, two peptides of different glycan chains are connected by the protein family of penicillin-binding proteins (PBPs). The D-alanine at position 4 is covalently attached to the third amino acid of the other linker-peptide (Fig. 2, red arrow). Inevitably, this should be a diamino amino acid, meaning it has two amino groups. Many Gram-positive species are characterized by an extra "interpeptide bridge" of varying amino acid composition between the two linker-peptides. For example, *Staphylococcus aureus* has an interbridge of 5 glycines (Maidhof *et al.*, 1991) (Fig. 3 and see § Interspecies differences in peptidoglycan structure).

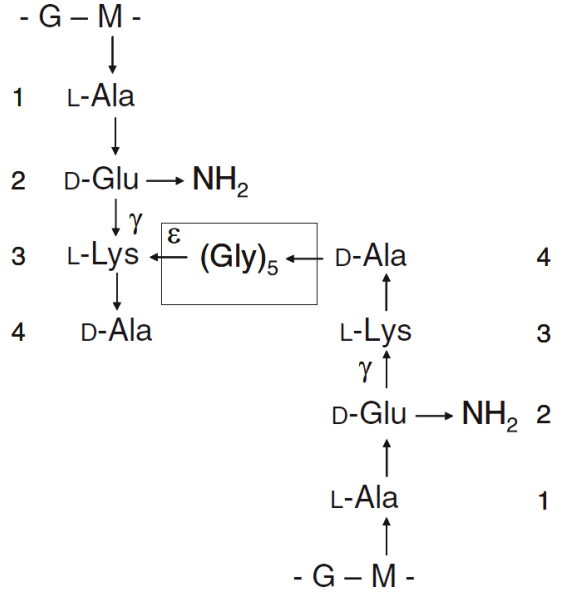

Figure 3: Position of the pentaglycine interbridge in *S. aureus*. The glycine chain connects the 3rd amino acid of one linker-peptide with the D-Ala at the 4th position of another linker-peptide. G = GlcNac, M = MurNAc.
Adapted from Vollmer, 2008.

In general, the arrangement of peptidoglycan macromolecules in the bacterial cell wall is still not elucidated. Competing models exist in which peptidoglycan chains are proposed to lie either parallel or perpendicular to the cell membrane (Vollmer and Holtje, 2004).

Peptidoglycan synthesis

The synthesis of peptidoglycan mainly takes place in the cytoplasm. A whole range of enzymes are involved in producing the sugars (reviewed in (Barreteau *et al.*, 2008). The family of Mur ligases is responsible for generating the pentapeptides which are covalently attached to the sugars. These enzymes are proposed to assemble in a complex positioned on the cytoskeletal protein MreB (White *et al.*, 2010). A MurNAc monosaccharide with peptide is transferred to bactoprenol, a carrier lipid in

the membrane, resulting in an intermediate called Lipid I. When a GlcNAc monosaccharide is coupled to Lipid I, the intermediate is called Lipid II and is ready for transfer across the membrane. The enzyme responsible for flipping Lipid II to the outer membrane leaflet is a member of the SEDS (shape, elongation, division, sporulation) family (van Dam *et al.*, 2007). Multiple studies postulate the *E. coli* proteins RodA and FtsW or their homologues as the flippases (Ishino and Matsuhashi, 1981; Ishino *et al.*, 1986; Ehlert and Holtje, 1996; Errington *et al.*, 2003). After transfer and detachment from bactoprenol, the PG precursors polymerize catalyzed by different penicillin-binding proteins (PBPs). The PBPs are divided in three classes, low molecular weight (LMW), high molecular weight (HMW, subdivided into class A and B) PBPs, and β-lactamases (Ghuysen, 1990). Via transglycosylation and transpeptidation steps, the disaccharides form chains.

Besides PG synthases, enzymes degrading peptidoglycan are also indispensable to shape the cell wall. United under the term peptidoglycan hydrolases, this group of enzymes contains many different types specialized in degrading for example a type of peptide or glycoside bond. Overall, more than 30 peptidoglycan hydrolases have been identified, which can be divided into families based on their sequence similarities (Smith *et al.*, 2000). The name of the hydrolase is derived from its substrate (Fig. 2, numbers 1-7). Glucosaminidases and muramidases cleave the sugar bonds in the glycan backbone. Endopeptidases cleave at specific sites in the linker-peptides, whereas amidases have a more general ability to cleave peptide bonds (amide groups). Their transcription is regulated by σ factors expressed in specific cell cycle stages, generally corresponding to their putative functions in the cell (Smith *et al.*, 2000).

Table 1: Amino acid variations in the linker peptide. Position 1 denotes the amino acid connected to the glycan strand (Vollmer, 2008)

Position	Residue encountered	Examples
1	L-Ala	Most species
	Gly	*Mycobacterium leprae, Brevibacterium imperiale*
	L-Ser	*Butyribacterium rettgeri*
2	D-Isoglutamate	Most Gram-negative species
	D-Isoglutamine*	Most Gram-positive species, Mycobacteria
	threo-3-Hydroxyglutamate*	*Microbacterium lacticum*
3	*meso*-A_2pm	Most Gram-negative species, Bacilli, Mycobacteria
	L-Lys	Most Gram-positive species
	L-Orn	Spirochetes, *Thermus thermophilus*
	L-Lys/L-Orn	*Bifidobacterium globosum*
	L-Lys/D-Lys	*Thermotoga maritima*
	LL-A_2pm	*Streptomyces albus*, *Propionibacterium petersonii*
	meso-Lanthionine	*Fusobacterium nucleatum*
	L-2,4-Diaminobutyrate	*Corynebacterium aquaticum*
	L-Homoserine	*Corynebacterium poinsettiae*
	L-Ala	*Erysipelothrix rhusiopathiae*
	L-Glu	*Arthrobacter* J. 39
	Amidated *meso*-A_2pm*	*Bacillus subtilis*
	2,6-Diamino-3-hydroxypimelate†	*Ampuraliella regularis*
	L-5-Hydroxylysine†	*Streptococcus pyogenes*‡
	N^{γ}-Acetyl-L-2,4-diaminobutyrate*	*Corynebacterium insidiosum*
4	D-Ala	All bacteria
5	D-Ala	Most bacteria
	D-Ser	*Enterococcus gallinarum*
	D-Lac	*Lactobacillus casei*, Enterococci with acquired resistance to vancomycin

*These residues result from reactions occurring posterior to the action of Mur ligases.

†The process of formation of these residues (direct incorporation by MurE or subsequent hydroxylation of the nonhydroxylated residue) is unclear (Muñoz *et al.*, 1966; Perkins, 1969).

‡In this organism, a 10 : 1 ratio of lysine to hydroxylysine was found Muñoz *et al.* (1966).

Interspecies differences in peptidoglycan structure

As mentioned, the amino acid sequence in cross-linking peptides can deviate from the default sequence. Table 1 displays other possible amino acids for each position. However, most interspecies variation is found in the interpeptide bridge, which is between 1 and 7 amino acids long. Inter bridges are generated by the so-called 'branching enzymes' (summarized in (Vollmer, 2008).

Milder modifications of the glycan chains or peptidoglycan architecture are also possible (Vollmer, 2008). The polymer length, density and degree of cross-linking vary dependent on species and growth conditions. Whereas in *S. aureus* most glycan strands consist of 3-10 disaccharide units (Boneca *et al.*, 2000), the *B. subtilis* chains can extend to 250 disaccharides (Ward, 1973). Taken together, more than 100 different types of peptidoglycan polymers have been isolated. The density, the amount of peptidoglycan per surface unit, is of major interest because this affects the resistance to e.g. antibiotics (Prats and de Pedro, 1989). How density is regulated during cell growth is currently unknown. The thickness of the peptidoglycan layer is not correlated to the length of the peptidoglycan chains. The degree of cross-linking reflects the percentage of engaged linker-peptides. Although information is scarce, literature suggests a wide distribution in degree of cross-linking. Depending on environmental conditions, it ranges from 44 to 60% in *E. coli* to 93% in *S. aureus* (Glauner *et al.*, 1988; Rogers, 1979).

Visualization of peptidoglycan synthesis

The above mentioned factors may not only differ with species or environmental conditions, but also with the age of the macromolecules. Newly synthesized peptidoglycan can often be distinguished from "old" peptidoglycan, although this difference gradually disappears over time. Characteristics as composition of the peptide, degree of cross-linking, chain length and the amount of incorporated lipoproteins may distinguish new from old peptidoglycan (Prats and de Pedro, 1989; de Pedro and Schwarz, 1981; Fordham and Gilvarg, 1974; Burman and Park, 1983; Glauner and Holtje, 1990).

The first methods to visualize peptidoglycan biosynthesis were based on following subunit incorporation in time. Different amino acids have been radioactively labeled to track their location during cell wall synthesis (de Pedro *et al.*, 1997; De Pedro *et al.*, 2003; Woldringh *et al.*, 1985). Sometimes the labeled amino acid was added in a pulse-chase fashion, or combined with immune-detection. Later, a more subtle method was developed by adding a fluorescent group to vancomycin (Van-FL) (Daniel and Errington, 2003). This antibiotic recognizes the D-Ala-D-Ala end of the nascent pentapeptide. Because the terminal D-Ala is cut off or occupied in time, vancomycin only binds peptido(Daniel and Errington, 2003)glycan that was recently synthesized. A similar image is obtained with fluorescent ramoplanin, which binds diphospho-MurNAc (Tiyanont *et al.*, 2006). Both antibiotics have been shown to solely bind PG precursors and the ends of growing glycan strands. Fluorescent telavancin recognizes Lipid II, also labeling sites of active wall synthesis (Lunde *et al.*, 2010).

Chapter 1b: Introduction to the human complement system

The complement system is an essential part of the immune system. It prevents and counteracts specific bacterial, viral, fungal and parasitic infections and some tumor cells. The system includes various factors, referred to by numbers (e.g. C1, C2) or by capitals (e.g. Factor D), that are abundantly present in the serum and tissue fluids. Complement factors reside in an inactive conformation, until they are activated by pathogens or other non-self molecules. There are three different pathways initiating the complement cascade, all leading to opsonization of micro-organisms for phagocytosis

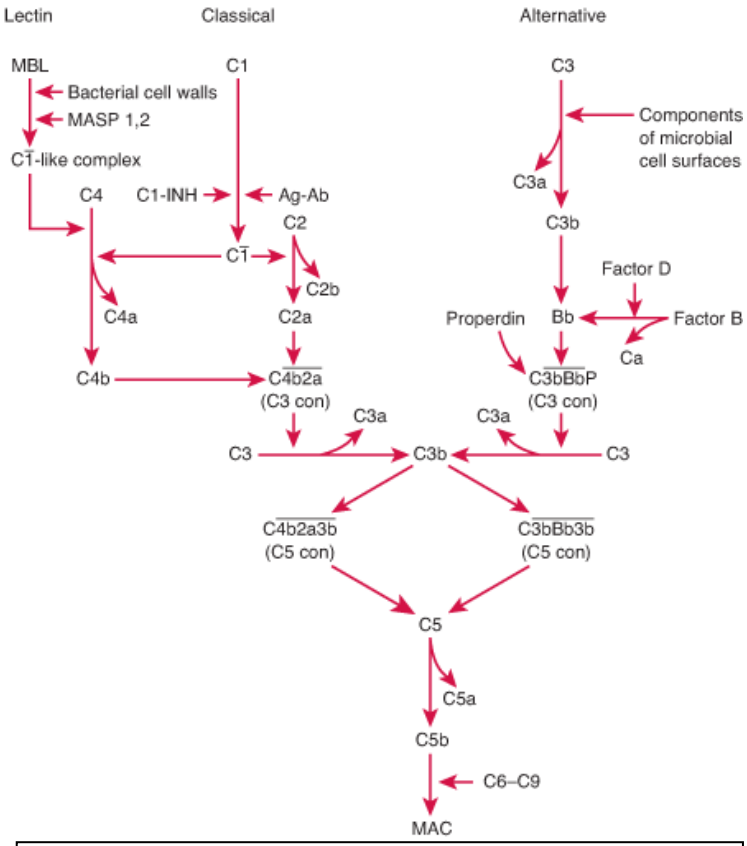

Figure 4: An overview of the three pathways of the complement system.
Taken from merckmanuals.com.

(C3b), recruitment of phagocytes to the site of infection (C5a), and direct killing by formation of the membrane attack complex (MAC), consisting of factors C5b, C6, C7, C8 and C9 (C5b-9; Fig. 4).

The classical pathway requires the adaptive immune system, because the activation signal is found on antigen-antibody complexes (Daha *et al.*, 2011). These complexes can either be deposited on pathogenic surfaces or soluble in the serum. Upon antigen binding, either IgM or IgG subclasses (IgG1, 2 and 3 in humans) are capable of activating complement factor C1 due to conformational changes in the constant part. After activation and cleavage of C1, C1a cleaves C4 and C2, leading to the formation of the C4b2a complex. This complex functions as a C3 convertase, cleaving C3 into C3a and C3b. By binding of an additional C3b molecule to the C3 convertase, it gets substrate-specificity for C5, resulting in the splitting of C5 in C5a and C5b. Then, C5b initiates the formation of the membrane attack complex.

The alternative pathway is completely part of innate immunity, as activation is independent of antibodies (Zipfel *et al.*, 2007). At a low rate, C3 is spontaneously hydrolyzed in the serum. The C3b product disappears rapidly because it has a very short half-life. But when C3b encounters a susceptible surface, it is sufficiently stabilized to initiate the alternative complement cascade. C3b mediates the cleavage of factor B by factor D. The cleavage product Bb forms together with C3b a C3 convertase. Additional C3b production results in C5 convertase complexes (C3bBb3b).

The mannose-binding lectin (MBL) pathway or lectin pathway is similar to the classical pathway, only the activation signal differs (Matsushita, 1996). When mannose sugar groups on the surface of a micro-organism are recognized, MBL or the plasma protein ficolin forms an active complex resembling C1 function (Endo *et al.*, 2007). This complex mediates the cleavage of C2 and C4, thereby entering the classical pathway with use of the same C3 and C5 convertases.

MAC assembly starts when C5b binds C6 and C7, either on a cell surface or on an immune complex. C7 undergoes a conformational change, enabling it to integrate into a lipid bilayer. If a lipid membrane is not readily available, the complex is also able to migrate to a nearby cell. This may lead to “innocent bystander killing” of nonpathogenic cells, although healthy host cells have inhibitory mechanisms to prevent this (Schonermark *et al.*, 1991). Subsequently, C5b67 recruits C8, which integrates in the membrane and induces binding and polymerization of 10-17 C9 molecules. The concurrent conformational change enables the C9 polymer to integrate into the membrane, where it

forms a large pore (DiScipio, 1991). Due to uncontrolled entry of water and loss of ions, the target cell swells and dies.

According to general consensus, the MAC can not reach the membrane of Gram-positive bacteria due to the thick PG layer(Joiner *et al.*, 1983; Frank, 2001). Hence, Gram-positive bacteria are resistant to MAC-mediated killing (Joiner *et al.*, 1983; Frank, 2001). Still, the complement system is essential in the host innate immune response against Gram-positive bacteria, e.g. through opsonization by C3b deposition and chemotaxis by C5a. Paradoxically, *S. pyogenes* does produce a MAC-inhibitory protein, Streptococcal inhibitor of complement (SIC) (Fernie-King *et al.*, 2001). SIC binds the C5b-7 complex, thereby preventing formation of the MAC. However, the relevance of this effect is under debate, as SIC also has a role in inhibiting antimicrobial peptides (Fernie-King *et al.*, 2004).

Chapter 2: *Bacillus subtilis*

The Gram-positive, non-pathogenic *Bacillus subtilis* is the most widely used model for research on the cell wall of Gram-positive bacteria. Upon cell division, this rod-shaped bacterium forms long strings of daughter cells. In confocal microscopy, MAC binding was mostly observed at the cell poles (E. Berends, unpublished results).

Bacterial shape and orientation of the division plane

The rod-shape of *Bacillus subtilis* is maintained by the bacterial cytoskeleton. The most important cytoskeletal protein is MreB, but *B. subtilis* also expresses the MreB-homologues Mbl and MreBH. All three proteins resemble the tertiary structure and function of the eukaryotic cytoskeletal protein actin (van den Ent *et al.*, 2001; Jones *et al.*, 2001). Localization of MreB and Mbl is suggested to depend on membrane proteins MreC and MreD (Leaver and Errington, 2005). Generally it is thought that MreB is located just beneath the membrane in a broad helical pattern, maintaining shape and withstanding mechanical stress (Jones *et al.*, 2001; Shih *et al.*, 2003; Carballido-Lopez and Errington, 2003). However, three recent studies challenge this view by stating that MreB and associated proteins form moving patches (Dominguez-Escobar *et al.*, 2011; Garner *et al.*, 2011; Swulius *et al.*, 2011), leaving it undecided which model fits best. In any case, the cytoskeleton functions as a scaffold for assembly of protein complexes, both intracellular and extracellular (Daniel and Errington, 2003; Jones *et al.*, 2001). MreB or its homologues are found in many other bacterial species, with the notable exception of spherical bacteria (Jones *et al.*, 2001). These species do not need a cytoskeleton to maintain their shape.

Intrinsic to its shape, *B. subtilis* only has one divisional plane. By elongation and medial division, its rod-shape is preserved. The division septum will form the future poles of the two daughter cells. Normally, newly divided bacteria form a long string and detach based on environmental conditions. Interestingly, if genes responsible for septum synthesis are knocked out, the division cycle (including elongation of the lateral wall) continues, forming a long rod with multiple genome copies (Marston *et al.*, 1998). This indicates that the elongation machinery requires different proteins than the septal wall synthesis machinery.

Lateral cell wall synthesis

Elongation, the production of lat(Marston *et al.*, 1998)eral cell wall, requires the synthesis of new peptidoglycan. By using fluorescently labeled vancomycin (Van-FL), this process was visualized (Daniel and Errington, 2003). Remarkably, at the lateral wall Van-FL staining is observed in a helical pattern, dependent on the presence of Mbl (Fig. 5) (Daniel and Errington, 2003; Tiyanont *et al.*, 2006). This observation led to the hypothesis that the peptidoglycan synthesis machinery assembles using the cytoskeleton as a scaffold (Daniel and Errington, 2003; Jones *et al.*, 2001). Intracellularly, the enzymes responsible for production of the Lipid II precursor are proposed to assemble based on MreB (den Blaauwen *et al.*, 2008; Mohammadi *et al.*, 2007; Divakaruni *et al.*, 2007). These include the soluble Mur ligases and the membrane bound MraY and MurG. MraY is required to produce Lipid I, after which MurG couples the sugar-pentapeptide group to it to yield Lipid II (Bouhss *et al.*, 2004; Miyao *et al.*,

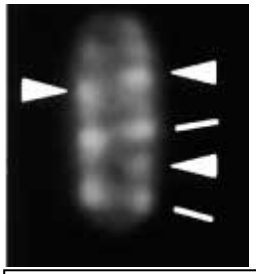

Figure 5: Helical pattern of Van-FL staining in *B. subtilis*. Lines and arrowheads indicate tilted bands and peripheral dots, respectively, that are characteristic of a helical mode of staining. Adapted from Daniel and Errington, 2003.

1992). In addition, a lipid flippase is part of the intracellular synthesis machinery. After transfer of Lipid II to the outer lipid layer, PBPs complete the peptidoglycan synthesis. Extracellular complexes containing PBPs are suggested to associate to the location of cytoskeletal proteins, although evidence for this hypothesis is limited in *B. subtilis* (Kawai *et al.*, 2009; Simon, M.J. & Day, R.A., 2000). Some PBPs have transmembrane tails that may directly interact with intracellular MreB (Kawai *et al.*, 2009). MreC, a transmembrane protein, could also provide a link between extracellular and cytoplasmic enzymes, as direct interaction with PBPs via its extracellular domain and MreB via its intracellular domain, has been demonstrated (Leaver and Errington, 2005; van den Ent *et al.*, 2006). MreC and MreD are shown to be indispensable for the lateral wall staining by Van-FL (Leaver and Errington, 2005), corresponding to their function in positioning the cytoskeleton. In conclusion, the intracellular MreBCD complex seems to be essential for lateral cell wall synthesis.

By gene knock-out experiments, lateral and septal wall synthesis are shown to be executed by different enzymes (Yanouri *et al.*, 1993; Wei *et al.*, 2003; Scheffers *et al.*, 2004). Accordingly, most PBP types display a preferred localization to the lateral cell wall, the division site, or both. PBP3, 4a, 5 and possibly 4 are responsible for lateral peptidoglycan synthesis (Scheffers *et al.*, 2004).

Incorporation of new peptidoglycan macromolecules requires the coordinated action of synthases and hydrolases. *B. subtilis* is the model organism Koch based his model on of “inside-to-outside” growth of the cell wall of Gram-positive bacteria (Koch and Doyle, 1985). This unsurpassed model claims that new peptidoglycan is added on the membrane side of the cell wall, where the wall does not yet have to withstand the large pressure exerted by the cell’s turgor. When additional peptidoglycan is synthesized, all peptidoglycan layers shift further to the surface, where the peptidoglycan acquires its final extended structure. These layers yield the high integrity of the cell wall. The outermost layers of peptidoglycan eventually tear and are degraded, but due to the underlying closed layers the cell wall will remain intact (Koch and Doyle, 1985).

Chromosome segregation

Contrary to the eukaryotic cell cycle, bacterial duplication is characterized by overlapping processes. Simultaneous with cell wall elongation, the *B. subtilis* DNA is replicated and segregated. Nevertheless, the bacillus has to reach a certain length before replication is initiated, presumably to be able to fit two genome copies in the mother cell (Weart *et al.*, 2007; Mattei *et al.*, 2010). Although a nuclear membrane is lacking, the bacterial genome is rather organized in a folded structure called the nucleoid. The Structural Maintenance of Chromosomes (SMC) complex, consisting of an SMC dimer and two interacting proteins ScpA and ScpB, is responsible for organizing the DNA (Mascarenhas *et al.*, 2002; Volkov *et al.*, 2003).

The *B. subtilis* genome consists of one circular chromosome with a single origin of replication (oriC). When the ATPase DnaA separates the DNA strands starting from the oriC, DNA polymerase III and accessory proteins can bind and start replication in two directions (Johnson and O'Donnell, 2005; Mott and Berger, 2007). The two oriC copies subsequently move to opposite poles of the mother cell, pulling along the rest of the DNA while it is being synthesized (Berkmen and Grossman, 2006; Lemon and Grossman, 2001). Although not known how, the MreB and Mbl cytoskeleton enables this transport (Soufo and Graumann, 2003). The DNA passes through the replication machinery as opposed to a moving replication complex. Reaching the termination site (terC), the replication machinery is detached by specific termination proteins (Bussiere and Bastia, 1999). The two separate nucleoids get segregated further by unknown mechanisms.

Z ring formation and regulation

In almost all bacterial species, the future division site can be recognized by the construction of a ring-shaped structure, the "Z ring". The ring is built from FtsZ, a protein with a tubulin-like structure (Erickson, 1995; Bi and Lutkenhaus, 1991). Following a yet unknown signal, the FtsZ subunits assemble in mid-cell just underneath the membrane (Bi and Lutkenhaus, 1991). FtsA attaches the Z ring to the cell membrane, thereby stabilizing the interactions (Jensen *et al.*, 2005). The proper ratio for normal cell division is 1:5 for FtsA:FtsZ (Feucht *et al.*, 2001). The Z ring is the most important part of the division machinery, as it functions as a scaffold for all necessary proteins for division and separation (Gamba *et al.*, July 1, 2009). To prevent the formation of asymmetric daughter cells, the timing and placement of the Z ring should be carefully regulated. Multiple mechanisms execute this regulation.

To determine the proper moment in time to initiate Z ring assembly, nutrient availability and replication status should be decisive factors. *B. subtilis*' UgtP, a protein of a conserved metabolic pathway, is used as a sensor for nutrient availability during the division cycle (Weart *et al.*, 2007). UgtP stability and function depend on glucose levels. When these are too low, UgtP interacts directly with FtsZ to prevent assembly (Weart *et al.*, 2007).

To investigate the interplay between DNA replication and Z ring regulation, multiple *B. subtilis* mutants were studied. When initiation of replication is disabled, Z rings cease to form at mid-cell but still develop asymmetrically next to the nucleoid (Regamey *et al.*, 2000; Harry *et al.*, ; Harry *et al.*, 1999). When the initiation phase was allowed to occur but further replication was blocked, Z rings would form over the centrally located, unreplicated nucleoid (Regamey *et al.*, 2000; Harry *et al.*, ; Harry *et al.*, 1999). In conclusion, merely the initial stages of DNA replication generate an indispensable signal for Z ring positioning. However, replication status is not involved in Z ring timing.

Besides timing, localization of the Z ring is essential. Two mechanisms determine the cellular location; nucleoid occlusion and the Min system. The "nucleoid occlusion model" states that the nucleoid prevents Z ring formation by inhibitory signals, preventing any cell division cutting through DNA material (Woldringh *et al.*, 1991). This is exerted by the protein Noc, which provides an inhibitory signal upon binding specific DNA sequences (Wu *et al.*, 2009; Wu and Errington, 2004). When two new nucleoids segregate, the inhibition disappears in between, allowing Z ring formation and cell division (Rothfield *et al.*, 2005). It has been suggested that the translocation of chromosomes away from the mid-cell reveals a binding site for FtsZ assembly later in the division cycle (Regamey *et al.*, 2000; Harry *et al.*, 1999), although little evidence supports this model.

To prevent formation of septa at the nucleoid-free cell poles, the Min system complements the nucleoid occlusion. The name of the system was derived from the fact that mutants lacking the *min* genes produce "mini cells" without genomic material, due to the placement of division septa close to the poles (Jaffe *et al.*, 1988). In *B. subtilis*, the proteins MinC, MinD, MinJ and DivIVA comprise the system. DivIVA is a functional homologue of *E. coli* MinE and is together with MinJ responsible for specific concentration of MinC and MinD at the poles (Marston *et al.*, 1998; Marston and Errington, 1999; Patrick and Kearns, 2008). The recognition signal for DivIVA is unknown, but is likely to be deduced from a conserved component or structure of the poles as DivIVA from *B. subtilis* can also recognize poles of other bacterial species (Edwards *et al.*, 2000). MinD is a membrane protein that anchors MinC and supports its activation (Marston *et al.*, 1998). MinC directly prevents FtsZ complex formation (Marston *et al.*, 1998; Marston and Errington, 1999). When division progresses, the Min proteins locate to the nascent cell poles, attracted by proteins of the division machinery (Marston *et al.*, 1998; Marston and Errington, 1999; Edwards and Errington, 1997; Cha

and Stewart, 1997). Therefore they will immediately be present to suppress Z ring formation in the daughter cell.

Although these two mechanisms are indispensable, they can not fully explain the positioning of the Z ring. Other proteins promote (ZapA, SepF) or inhibit (EzrA, ClpX) Z ring formation and stability, as reviewed by Adams and Errington (Adams and Errington, 2009). These mechanisms come into play in case of DNA damage or an unfavorable environment.

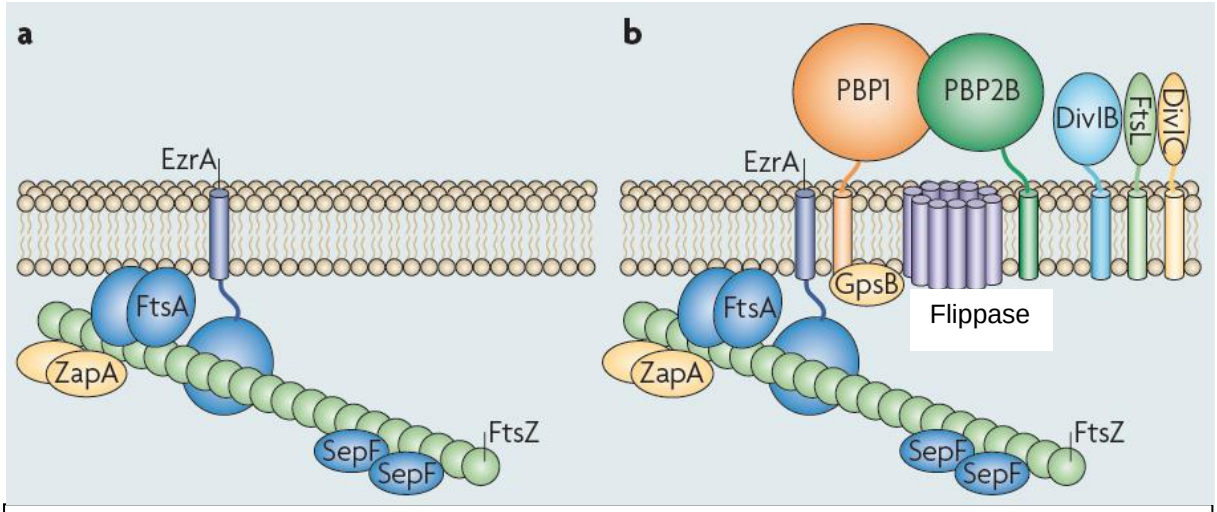

Figure 6: The two steps of assembly of the divisome in *B. subtilis*. Upon Z ring formation, the divisome assembles. **(a)** In the first step, proteins directly associate with FtsZ. **(b)** The second group of proteins assembles cooperatively after a substantial delay in time. The identity of the flippase enzyme has not been confirmed.
Adapted from Adams and Errington, 2009.

Assembly of the division machinery

Once the Z ring is formed, it serves as a scaffold for assembling the proteins needed for cell division, collectively named the "divisome". This cytokinetic ring consists of at least 10 proteins, as reviewed by Errington *et al.* (2003), which form a complex in two steps (Fig. 6) (Gamba *et al.*, July 1, 2009). In the first step, proteins are directly recruited to FtsZ (Fig. 6a) (Gamba *et al.*, July 1, 2009). FtsA is required to attach the Z ring to the membrane (Jensen *et al.*, 2005), whereas ZapA promotes Z ring assembly (Gueiros-Filho and Losick, 2002). EzrA (extra Z rings A) recruits the mainly extracellular PBP1, an essential PBP for division, directing peptidoglycan synthesis to the septal wall (Scheffers *et al.*, 2004; Claessen *et al.*, 2008). In addition, EzrA is a negative regulator of Z ring assembly (Levin *et al.*, 1999). SepF was recently proposed to be crucial for regularly arranging FtsZ filaments (Gundogdu *et al.*, 2011; Small *et al.*, 2007; Singh *et al.*, 2008).

After a substantial delay in time, the second group of proteins is engaged in a cooperative way (Fig. 6b). These proteins are recruited by other signals than the first set and localization to the divisome is dependent on each other (Errington *et al.*, 2003). GpsB (guiding PBP1-shuttling protein B) cooperates with EzrA (Claessen *et al.*, 2008). PBP2B, DivIC, DivIB, and FtsL are transmembrane proteins that predominantly operate through their large extracellular domain (Errington *et al.*, 2003). PBP2B is involved in septal wall biosynthesis (Daniel *et al.*, 2000). DivIC stabilizes FtsL, which is suggested to be an essential regulator of *B. subtilis* cell division (Daniel *et al.*, 2000; Bramkamp *et al.*, 2006; Wadenpohl and Bramkamp, 2010)(Daniel and Errington, 2000). Together, these proteins exert all necessary activities for cell division.

Formation of the division septum

When the divisome is completed, the Z ring starts to constrict. As the ring constricts, the cell membranes of the two future daughter cells are gradually severed. A new patch of cell wall must be produced to cover the new poles. Constituents such as peptidoglycan and teichoic acids are fabricated at the division site, and the so-called septum or septal wall invaginates into the cell. The existing lateral cell wall does not constrict in *B. subtilis*. When the membranes of the daughter cells are completely disconnected, the septum is finished. PBP1 and PBP2B, recruited to the divisome, are specifically localized to the septal wall (Scheffers *et al.*, 2004; Daniel *et al.*, 2000; Pedersen *et al.*, 1999) and thus directly involved in the biosynthesis (Scheffers *et al.*, 2004; Pedersen *et al.*, 1999)(Scheffers *et al.*, 2004; Pedersen *et al.*, 1999; Pedersen *et al.*, 1999; Pinho and Errington, 2005). Also PbpX is localized exclusively to the division septum, dependent on FtsZ (Scheffers *et al.*, 2004). These three PBP types are division-specific and are not found in elongation complexes (Yanouri *et al.*, 1993; Wei *et al.*, 2003; Scheffers *et al.*, 2004). Additional enzymes are partially equal to the elongation machinery, as are some non-specific PBPs. Although transmembrane proteins provide specific anchors for the extracellular complexes, substrate recognition provides additional signals. These are important during cytokinetic constriction, when the PBP2B remains spread over the entire septum despite movement of the divisome (Daniel *et al.*, 2000).

By Van-FL staining it was shown that the rate of peptidoglycan synthesis at the site of division is higher than in the lateral cell wall (Daniel and Errington, 2003). (Scheffers *et al.*, 2004)Van-FL binding was absent at the cell poles, corresponding to previous studies. The peptidoglycan at the poles is generally considered “inert”, as the rate of degradation and synthesis is much lower than in the rest of the cell wall (Mobley *et al.*, 1984; Schlaeppi *et al.*, 1982).

Separation

After completion, the septum needs to be separated into two polar cell wall patches, primarily mediated by peptidoglycan hydrolases. At this point, the hydrolases, which are indispensable for normal cell shape, need to be specifically directed to the septum. In *B. subtilis*, LytC, LytD, LytE, LytG, LytH, LytF and CwlS (YojL) are all associated with cell separation, although they are functionally redundant (Ohnishi *et al.*, 1999; Ishikawa *et al.*, 1998; Blackman *et al.*, 1998; Horsburgh *et al.*, 2003b; Horsburgh *et al.*, 2003a; Fukushima *et al.*, 2006). By means of fluorescent tagging, LytE and LytF were proven to locate to separation sites and cell poles (Yamamoto *et al.*, 2003). This specificity suggests the existence of certain receptors in the septal wall, although nothing is known about potential candidates. Interaction with the divisome may also play a role, as LytE depends on PBP2B for septal localization (Carballido-López *et al.*, 2006). Besides that, LytE is involved in lateral wall hydrolysis, and is then proposed to locate based on MreBH position (Carballido-López *et al.*, 2006). This interplay was inferred from protein interaction in a yeast two-hybrid screen, and the helical pattern of LytE immune-fluorescence. The resulting model states that after translation, LytE accumulates by binding MreBH. MreBH supports LytE transport through the membrane, leading to the extracellular accumulation of LytE enzymes on specific spots. At the same time, Mbl recruits PBPs. As a result, synthases and hydrolases are combined to ensure tight regulation of the peptidoglycan layers (Carballido-López *et al.*, 2006). Although controversial, this model could also apply to septal wall localization of hydrolases.

Only after the complete septal wall is formed, the autolytic enzymes start to cleave the peptidoglycan layer in two (Fig. 7). This is in contrast to *E. coli*, where septal wall synthesis and cleavage occur almost simultaneously (see Chapter 5), leading to a characteristic furrow in the cell

wall seen at the division site (Fig. 7). After complete separation, the daughter cells are ready to enter a new cycle of cell division.

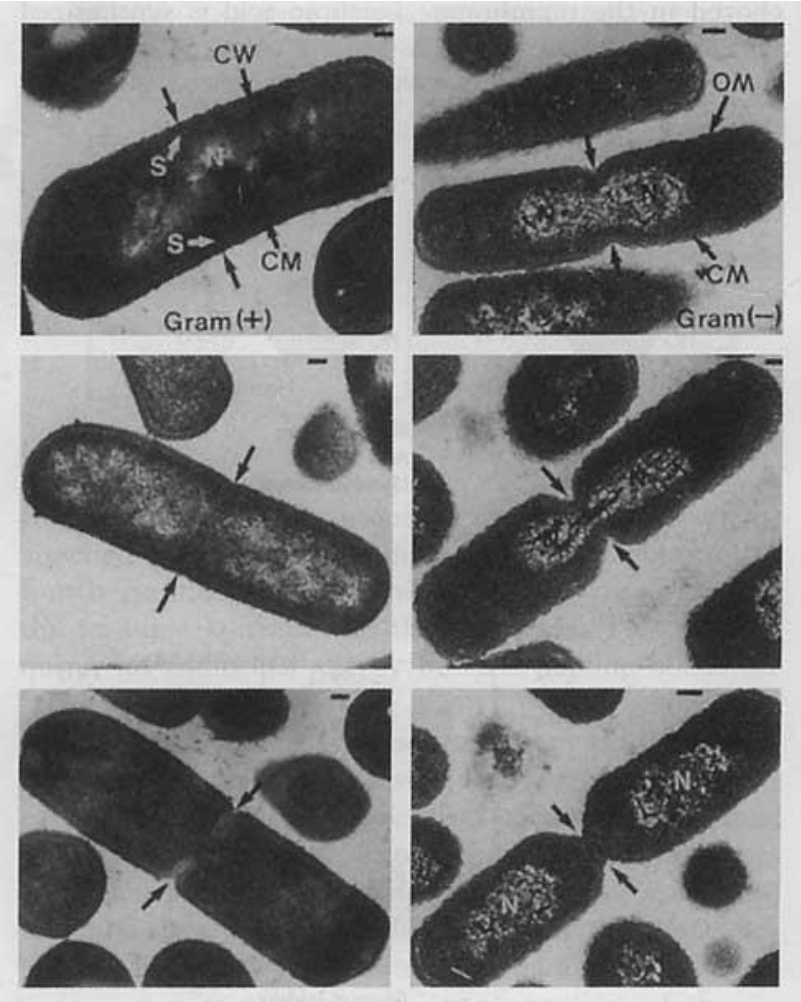

Figure 7: Electron microscopic photographs of division of *B. subtilis* (left) and *E. coli* (right). CW = cell wall; CM = cytoplasmic membrane; S = septum; N = nucleoid; OM = outer membrane. Bar = 0.2 um.
(Murray *et al.*, 2002)

Chapter 3: *Staphylococcus aureus*

Staphylococcus aureus is a spherical species, truly conforming to the name "cocci". Daughter cells are observed in three-dimensional clusters, from where the species obtains its name; the Greek word "staphyle" means grape cluster.

Bacterial shape and orientation of the division plane

In contrast to *B. subtilis*, *Staphylococcus aureus* misses the genes for the cytoskeletal proteins MreB and Mbl (Jones *et al.*, 2001). While it does express MreC and MreD, these proteins are not essential for *S. aureus* survival (Chaudhuri *et al.*, 2009). Apparently, a cytoskeleton is not needed to maintain the energetically favorable spherical shape. Additionally, for organizing PG synthesis machinery a cytoskeleton is dismissible, as elongation synthesis does not occur in *S. aureus*. In *S. aureus* only one type of cell wall synthesis occurs. All new PG synthesis takes place at the division septum, as shown by radiolabeling (Briles and Tomasz, 1970; Giesbrecht *et al.*, 1976) and Van-FL staining (Pinho and Errington, 2003). There it relies on the FtsZ scaffold to assemble the synthesis machinery. In addition, some inside-to-outside growth and recycling of old cell wall material is proposed to occur during the vegetative state (Boneca *et al.*, 2000; Giesbrecht *et al.*, 1998).

In theory, *S. aureus* has an indefinite number of division planes stretching the full diameter of the cell due to its shape. However, *S. aureus* divides in three dimensions, by alternating division planes perpendicular to the previous (Tzagoloff and Novick, 1977). The division plane is also characterized by formation of a Z ring.

Chromosome segregation and Z ring formation and regulation

How positioning of the division plane is regulated remains largely unknown. Little knowledge exists on regulation of Z ring formation and chromosome segregation. It has been suggested that a Z ring would only be stable when it forms at the maximum diameter of the cell, thereby guiding the Z ring to the proper location (Zapun *et al.*, 2008). In *S. aureus*, FtsZ is also anchored to the membrane via interaction with FtsA (Yan *et al.*, 2000). The question remains how the Z ring forms carefully between the nucleoids, in a plane that differs upon each division.

It is known that nucleoid occlusion inhibits Z ring formation over the nucleoids (Veiga *et al.*, 2011). *S. aureus* Noc protein accumulates at the DNA, essential in preventing random cutting of the DNA. In Noc mutants, many cells showed Z ring formation over the nucleoid or multiple Z rings. Like in *B. subtilis*, Noc-mediated inhibition is relieved in between the nucleoids upon segregation. Accordingly, nucleoid segregation occurs prior to Z ring formation in *S. aureus* (Veiga *et al.*, 2011). Interestingly, this means that chromosomal segregation has the lead in determining the alternating division planes.

Veiga *et al.* proposed a model to explain nucleoid segregation patterns resulting in perpendicular division planes (Fig. 8). In this model, the two oriC copies determine the direction of the chromosomes segregation. The first division plane is chosen randomly. At the beginning of the second division cycle, the daughter cell is momentarily not perfectly spherical but has a long and short axis (Fig. 8A). This provides a recognition signal for the oriC, which pull the DNA along the long axis of the daughter cell. Subsequently, in the third division cycle, the oriC are attracted to two specific sites in the cell membrane, which seems to be where the two previous division planes crossed each other (Fig. 8B). At this cross-point, an unidentified oriC binding protein is thought to accumulate (Veiga *et al.*, 2011). Another study supports this model by showing that cell division

leaves “scars” on the *S. aureus* cell (Yamada *et al.*, 1996). These ring-shaped scars could supply a recognition site for an oriC binding protein, leading to a maximal concentration at the cross point (Veiga *et al.*, 2011). When the oriC move to these cross points, the nucleoids would segregate in such a way to create a third perpendicular division plane. Although this model is yet to be confirmed, it explains mechanisms of nucleoid segregation and divisional plane positioning. Additional mechanisms of Z ring positioning might exist, although *S. aureus* holds no Min genes (Margolin, 2001; Flardh, 2003).

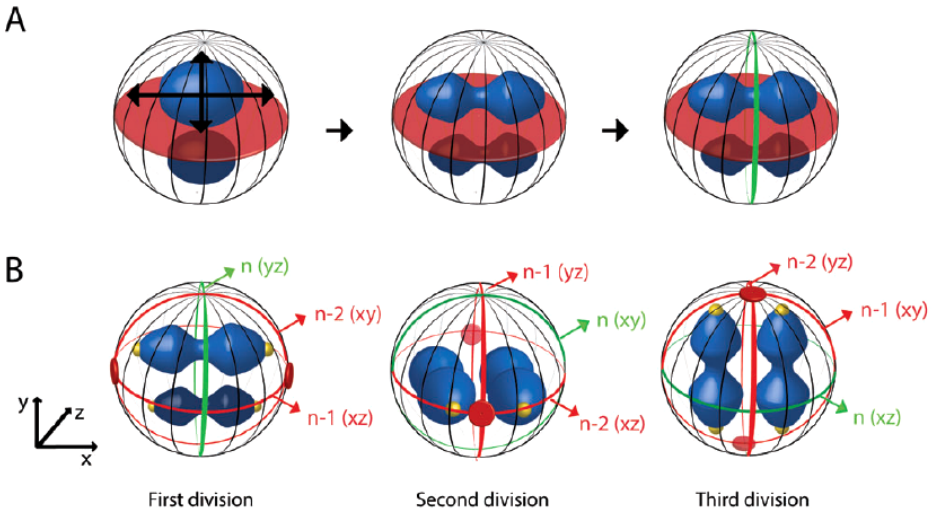

Figure 8: Model for determination of three perpendicular division planes in *S. aureus*. (A) The first round of division is denoted by the red division plane. After division, the asymmetric daughter cell has a long and short axis (black arrows). When the nucleoids (blue) segregate along the axis, Noc inhibition is relieved in the middle. Z ring formation determines the next division plane (green), which is perpendicular to the previous. **(B)** The division plane of two division cycles ago (n-2) and the previous division plane (n-1) form cross-points (small red circles). The two oriC copies (yellow dots) are attracted to the cross-points and thereby determine the new plane of division (green). An unidentified oriC-binding protein might accumulate at the cross-points. Upon each division, one hemisphere is generated of new material, but the cross-points remain.
(Veiga *et al.*, 2011)

As predicted from homology with proteins in *E. coli* and *B. subtilis*, the proteins SMC and spoIIIE are involved in DNA movement during chromosome segregation (Yu *et al.*, 2010). SMC was already known for its role in chromosome organization. Now it was demonstrated that SMC or spoIIIE single knock out mutants have defects in chromosome segregation, resulting in low percentages of anucleated cells or cells with an abnormal amount of DNA (Yu *et al.*, 2010). However, proteins other than these two play a role, since in double SMC$^-$spoIIIE$^-$ mutants, a relatively high number of daughter cells inherits the normal amount of DNA (Yu *et al.*, 2010). It has to be mentioned that *S. aureus* cells have a rather small volume, which is after DNA replication almost completely filled by DNA. Consequently, it is difficult for DNA to escape enclosure in a future daughter cell. Nonetheless, random cutting of chromosomes by septal closure also does not occur very often in these double mutants. The gene deficiency more severely affects chromosomal organization than segregation (Yu *et al.*, 2010).

Assembly of the division machinery

As mentioned, positioning of the septal PG synthesis machinery depends on FtsZ. If FtsZ is absent, cell wall synthesis spreads over the entire cell surface and continues without any subsequent cell division (Pinho and Errington, 2003). The cell obtains up to 8 times its normal volume before it lyses.

To identify divisome subunits, conserved *S. aureus* homologues of known divisome subunits of *B. subtilis* were tested in a bacterial two hybrid screen (Steele *et al.*, 2011). This resulted in many putative divisome interactions (Fig. 9), similar to the interaction webs found in *E. coli* and *S. pneumoniae*. EzrA is also expressed in *S. aureus*, and a GFP-EzrA fusion protein was shown to localize to the division septum in a ring-shape (Steele *et al.*, 2011). When FtsZ was knocked down, the ring-like positioning of EzrA strongly decreased. These data indicate that localization of EzrA depends on the Z ring, similar to *B. subtilis* EzrA. Correspondingly, EzrA is in *S. aureus* also involved in recruitment of a PBP. Using inducible *ezra*-mutants, it was shown that EzrA is essential for localization of GpsB and PBP2 to the Z ring, possibly via direct interaction. The absence of EzrA strongly reduces septal Van-FL staining. In *B. subtilis*, EzrA is mostly recognized as a negative regulator of division, but this view should maybe be more refined. Overall, in *S. aureus*, EzrA is crucial for bacterial division (Steele *et al.*, 2011).

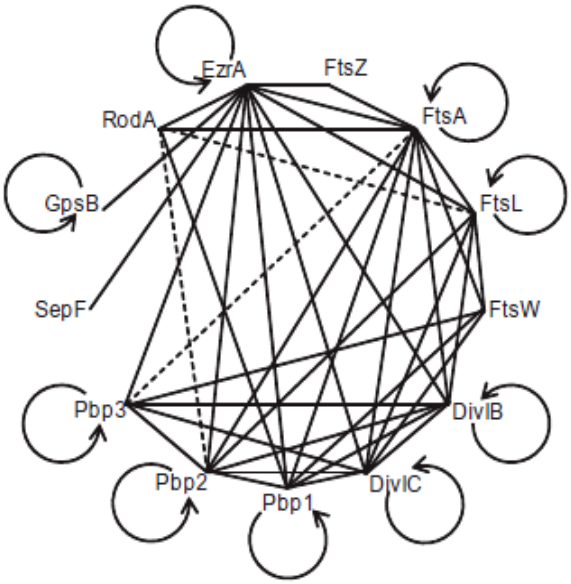

Figure 9: Web of interactions between putative divisome subunits in *S. aureus*. These proteins were chosen for their homology to *B. subtilis* divisome subunits. Interactions were determined by means of a bacterial two-hybrid screen. Positive interactions are denoted with a solid line, putative interactions with a dotted line.
(Steele *et al.*, 2011)

To assemble the rest of the divisome, a temporal system plays a role in *S. aureus* similar to *B. subtilis*. After Z ring formation, the divisome is recruited in a sequential manner. The functions of the other potential divisome subunits (FtsZ, FtsA, EzrA, GpsB, SepF, Pbp1, Pbp2, Pbp3, DivIB, DivIC, FtsL, FtsW, RodA) require further research. It is not known why both putative flippases FtsW and RodA are conserved in *S. aureus*, as only one synthesis machinery exists. DivIVA is also expressed in *S. aureus*, where it localizes to the septum instead of the cell poles (Pinho and Errington, 2004). However it has no vital function, as *DivIVA* knockouts do not show any defects during the division cycle (Pinho and Errington, 2004).

Formation of the division septum

Where data exists, cytoplasmic production of PG precursors in *S. aureus* is largely similar to *B. subtilis.* The extracellular polymerization of PG differs, as *S. aureus* has only 4 PBPs, type 1 to 4, as opposed to 16 in *B. subtilis*. PBP1 localizes to the septum independent of substrate recognition (Pereira *et al.*, 2009). It is essential for viability and functions in septation and cell separation.PBP2 is essential for viability (Pinho and Errington, 2003), whereas PBP3 and 4 are not (Scheffers, 2005). PBP4 is responsible for the unusually high degree of PG cross-linking in *S. aureus (Memmi et al., 2008)*. PBP4 localizes specifically to the septum, where it matures the nascent PG by cross-linking (Memmi *et al.*, 2008).

As mentioned in Chapter 1, *S. aureus* has an extraordinary linker-peptide with a 5 glycines addition. This enables cross-linking of glycan strands that would otherwise be too far apart (Lapidot and Irving, 1979; Lapidot and Irving, 1977), resulting in a high coverage of cross-linking of 80 to 90%

(Gally and Archibald, 1993; Snowden *et al.*, 1989). Mainly PBP4 is responsible for this high cross-linking, which contributes to a higher resistance to antibiotics (Memmi *et al.*, 2008; Leski and Tomasz, 2005). Methicillin-resistant *S. aureus* (MRSA) has a fifth subtype, PBP2a, which is resistant to β-lactam antibiotics (Lim and Strynadka, 2002). This type originates from another bacterial species.

When the Z ring constricts, the septum peptidoglycan layer is formed by centripetal growth. Centripetal growth can be described as the closing movement of a camera diaphragm. During closure, PBPs remain dispersed over the entire septum, to complete and mature the PG layer (Pinho and Errington, 2005). Simultaneously, teichoic acids and proteins are integrated. The septum is sufficient to create two cell wall patches, which will form hemispheres probably due to osmotic pressure.

Transmission electron microscopy of frozen-hydrated thin sections of *S. aureus* showed that in the septum, two high-density regions are sandwiched by three layers of lower density (Matias and Beveridge, 2007) (Fig. 10). The two outer low-density layers represent the periplasmic space, similar to the one in Gram-negative bacteria (Matias and Beveridge, 2006). The middle low-density layer represents ongoing degradation of the middle of the septum. It develops shortly after initiation of septum growth, indicating that PG synthesis and hydrolysis happen simultaneously (Matias and Beveridge, 2007). Eventually this will lead to separation of the two daughter cells. Because lateral cell wall is retained during septal invagination (Fig. 10), the lateral membrane is not allowed to constrict simultaneously. Therefore, if the septum is closed, the cell still looks like a single sphere (Fig. 11). Only after the entire septum is formed, the lateral cell wall at the periphery of the septal wall gradually thins and disappears, allowing full separation (Matias and Beveridge, 2007).

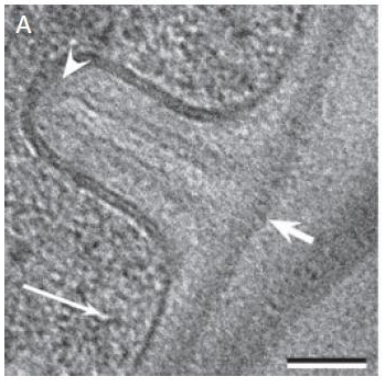

Figure 10: Two high-density regions sandwiched by three layers of lower density in the invaginating septum. Transmission electron microscopic photograph of frozen-hydrated section of *S. aureus* septum. A patch of lateral cell wall is retained during septal invagination (short arrow). Adapted from Matias and Beveridge, 2007.

In *S. aureus*, convincing mathematical evidence is obtained that supports the so-called "scaffold" model of PG arrangement (Dmitriev *et al.*, 2004). This model states that the PG glycan strands lie perpendicular to the cell membrane, with the cross-linking peptides arranged parallel to the membrane. Mathematically, this model would fit best to explain the high degree of cross-linking, the relatively short PG chains and sequential alteration of the division plane (Dmitriev *et al.*, 2004). Also centripetal growth can be accounted for in this model. Atomic Force Microscopy is performed to visualize peptidoglycan architecture, but individual glycan strands could not be distinguished (Touhami *et al.*, 2004). No complete evidence is provided in recent years to establish a new dogma.

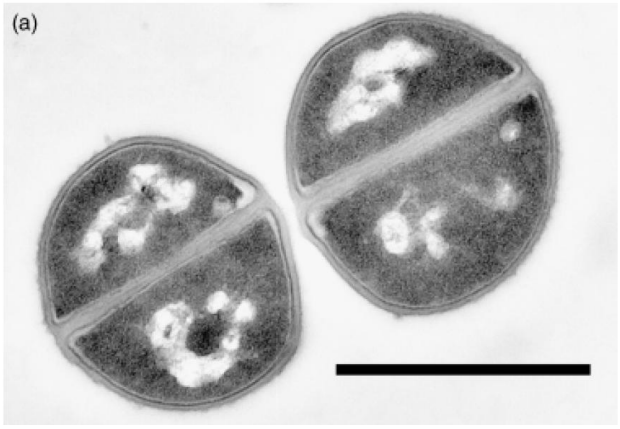

Figure 11: Electron microscopic photograph of two *S. aureus* cells harboring a completed division septum. Adapted from Zapun *et al.*, 2008.

Separation

To obtain full cell separation, PG hydrolases must be activated. Atl (autolysin) is a *S. aureus* protein that is important for cell separation, as *atl* mutants form large cell clusters that do not detach properly (Sugai *et al.*, 1995). Atl is first translated as a large precursor protein (pro-Atl), which already has PG hydrolase activity (Oshida *et al.*, 1995). Its R1, R2 and R3 repeat domains cause specific transportation to the equatorial ring, the surface rim of the septum (Baba and Schneewind, 1998). Each of these domains is sufficient to generate the transportation signal. Extracellulary, the pro-Atl gets cleaved twice, resulting in two autolytic enzymes, an amidase and a glucosaminidase, and a propeptide of unknown function (Oshida *et al.*, 1995). The amidase possesses the R1 and R2 domains, the glucosaminidase R3. The R1 and R2 domains are involved in direct binding of amidase to the peptidoglycan. Both enzymes remain bound to the equatorial ring (Yamada *et al.*, 1996).

A second hydrolase identified in *S. aureus* is Sle1 (Kajimura *et al.*, 2005). This is a *N*-acetylmuramyl-L-alanine amidase, meaning it cleaves *N*-acetylmuramyl-L-alanine bonds in the linker-peptides of peptidoglycan. From the phenotype of a *sle1* mutant its role in cell separation is obvious, but whether it specifically localizes to the site of division has not been visualized yet.

Based on DNA sequence, *S. aureus* is predicted to have six more hydrolase genes, termed LytX, LytY, LytZ, LytN, LytA and LytM (Wang *et al.*, 1991; Ramadurai and Jayaswal, 1997; Baba *et al.*, 2008). Whether they are involved in septum splitting is unknown, as only LytN has been characterized to date. Induced expression of a LytN-mCherry protein did show fluorescent signal at the septum (Frankel *et al.*, 2011). LytN mutants interfere with the normal structure of the septal wall, confirming its crucial function in septum autolysis. Interestingly, the precursor of LytN contains a YSIRK/GS motif, which was previously characterized as a signaling motif for secretion at the septal wall (DeDent *et al.*, 2008). Although recombinant mature LytN can bind to the entire cell wall after exogenous administration, the signaling peptide causes the precursor LytN to be solely secreted at the septum. There it directly binds its PG substrate and stays (Frankel *et al.*, 2011).

Besides substrates, membrane proteins can function as anchors for autolytic enzymes. Very recently the membrane proteins MsrR, SA0908 and SA2103 were suggested to play a role in septum formation and cell separation (Over *et al.*, 2011).

To explain the partial degradation of the lateral PG layer during septum invagination, Giesbrecht *et al.* proposed the existence of murosomes. They described these as vesicular structures at the equatorial ring, resulting in a ring of holes in the cell wall (Giesbrecht *et al.*, 1998; Touhami *et al.*, 2004; Giesbrecht *et al.*, 1985). In these extracellular organelles, autolytic activity is supposed to be increased, leading to a hole in the peptidoglycan layer. Touhami *et al.* showed that with progression of autolysis, the size of the holes increases until they merge. The degradation activities would also spread towards the inside, progressively splitting the septum to achieve separation. This is in agreement with the idea of lateral cell wall bridges that only gradually disappear after septum completion as described above. However, the existence of murosomes is speculative and has not been confirmed in later studies yet.

Because *S. aureus* divides in three perpendicular planes, the 8 daughter cells would in theory be found in cubic arrangements. However, as observed by microscopy, *S. aureus* appear in irregular groups, also referred to as grape clusters. This might be the result of lytic enzymes effecting cell movement after separation (Koyama *et al.*, 1977).

Chapter 4: *Streptococcus pyogenes*

In contrast to the cell division mechanisms of rod-shaped and spherical bacteria, *Streptococci* use a different machinery. This relates to the cell shape of these species that is neither a rod nor a sphere but approaches a more ovoid shape, also referred to as ovococci.

Group A Streptococci (GAS) officially include all types of streptococci displaying group A antigen, though GAS is often synonymous with *Streptococcus pyogenes*. This species is the only group A member that can infect humans. Because it is an important human pathogen, a considerable amount of literature has been published on infection characteristics, immune evasion and vaccine candidates. However, little attention has been paid to the fundamentals of division in this bacterium. In this chapter I will recap the existing knowledge.

Bacterial shape and orientation of the division plane

Although *Streptococcus pyogenes* is classified as an ovococcus, the cells often have a less elongated, more spherical shape than other streptococci (Zapun *et al.*, 2008). Similar to rod-shaped bacteria their division plane lies perpendicular to the longitudinal axis of the cell, resulting in linear chains of daughter cells. Like *S. aureus*, *S. pyogenes* does not possess the MreB and Mbl genes, so it does not have an actin-like cytoskeleton to maintain shape (Jones *et al.*, 2001). *S. pyogenes* is an outsider within the ovococci, together with *Streptococcus agalactiae*, because they have additionally lost the MreC, MreD and RodA genes (Zapun *et al.*, 2008; Noirclerc-Savoye *et al.*, 2003). Surprisingly, the gene for the MreB-anchoring protein DivIVA is conserved, although its function is not clarified (Beres *et al.*, 2002).

Lateral cell wall synthesis

From *S. pyogenes*' ovoid shape, it would be expected that the cell wall slightly elongates prior to division to maintain the ovoid shape. Indeed, in another ovococcus *Streptococcus pneumoniae*, a distinctive site and machinery is distinguished for elongation synthesis. Lateral cell wall synthesis was observed at a band around the equatorial ring in *S. pneumoniae* and *S. faecalis*, by radiolabeling and Van-FL staining (Daniel and Errington, 2003; TOMASZ *et al.*, 1964; Higgins and Shockman, 1970). This peripheral synthesis could be separated from centripetal septal growth (Fig. 12). However, it has not been clarified whether *S. pyogenes* has two mechanisms of cell wall synthesis too. In the landmark paper on *S. pyogenes* division by Cole *et al.* (1962) this seemed to hold true, but no studies have reexamined this issue. Moreover, it remains questionable whether PG synthesis of *S. pyogenes* can be compared to PG synthesis in *S. pneumoniae*. Some differences exist in the machinery subunits, for example in the classification of PBP types. Additionally, *S. pneumoniae* is dependent on the MreCD complex to direct peripheral synthesis machinery (Land and Winkler, 2011), whereas *S. pyogenes* has lost these genes. Taken together, it is plausible that *S. pyogenes* only has a septal mode of cell wall synthesis, similar to a few other *Streptococcus* species (Lleo *et al.*, 1990).

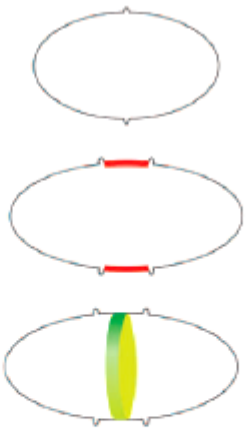

Figure 12: Two different modes of peptidoglycan synthesis at the equatorial ring of *S. pneumoniae*. Depicted in red is the peripheral synthesis at the equatorial ring, resulting in cell elongation. The centripetal synthesis of the division septum is depicted in green. Adapted from Scheffers *et al.*, 2005.

The *S. pyogenes* PBPs are homologous to *B. subtilis* PBPs but the number is lower. *S. pyogenes* has homologues to PBP1a, 1b, 2a, 2x and 3 but

a striking absence of a PBP2B homologue (Zapun *et al.*, 2008). Instead, it has two PBPs that are not yet characterized (Zapun *et al.*, 2008). This exemplifies the literature on *S. pyogenes* cell division. Inferences are made based on genomic sequences and phenotypes of knock-out mutants, but the underlying molecular mechanisms have not been elucidated yet.

Z ring formation and regulation

In streptococci, the Min system is absent. A functional nucleoid occlusion system has also not been identified. It has been proposed that the Z ring can form at the largest cell perimeter, which is similar to *S. aureus* (Zapun *et al.*, 2008).() However, how the Z ring is regulated to form perpendicular to the long axis has not been clarified. In *S. pneumoniae,* the Z ring and divisome are suggested to form prior to nucleoid segregation (Morlot *et al.*, 2004; Morlot *et al.*, 2005; Morlot *et al.*, 2003).

Formation of the division septum

It is plausible that FtsZ functions as a scaffold for the divisome and peptidoglycan synthesis machinery in *S. pyogenes* too. Subsequently, the septal wall is produced by centripetal growth. Cole *et al.* (1962) visualized cell wall synthesis by initial staining of the existing wall of *S. pyogenes* with fluorescent antibodies. During a cycle of vegetative growth, newly formed cell wall appeared as a non-fluorescent patch (COLE and HAHN, 1962). These patches originated from the septum and extended both peripherally and centripetally (COLE and HAHN, 1962).

Separation

No definitive evidence exists on the function of any PG hydrolase during *S. pyogenes* cell separation at the end of cell division. The PG hydrolase CdhA (CHAP-domain-containing and chain-forming cell wall hydrolase from Group A *Streptococcus*) localizes to the septum, where it probably has a role in daughter cell separation (Pancholi *et al.*, 2010). In addition, *S. pyogenes* expresses a homologue of PcsB, which is in *S. pneumoniae* recognized as a PG hydrolase (Ng *et al.*, 2003). Putatively, it has a similar function in *S. pyogenes*, although its indispensability for survival remains to be verified (Liu *et al.*, 2006a).

Furthermore, *S. pyogenes* possesses an open reading frame with a similar sequence to the *S. aureus* gene encoding the PG hydrolase Sle1 (Kajimura *et al.*, 2005). To my knowledge, the *S. pyogenes* gene product has not been studied.

Chapter 5: *Escherichia coli*, the Gram-negative model

Gram-positive and Gram-negative bacteria have a significantly different cell wall structure. Therefore, it is interesting to compare these two classes in the mechanisms of cell division and cell wall synthesis. *Escherichia coli* is the most studied Gram-negative species, accordingly considered as a model organism for division. It is a rod-shaped bacterium with a medial division plane perpendicular to the long axis, similar to *B. subtilis*. In this chapter I will highlight the similarities and differences between *E. coli* and *B. subtilis* (Fig. 13).

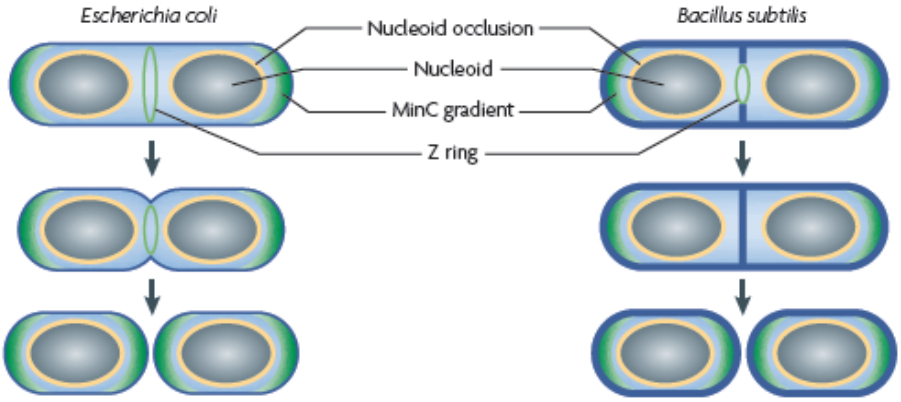

Figure 13: Schematic representation of major differences and similarities between cell division in *E. coli* and *B. subtilis*. Adapted from Adams and Errington, 2009.

Elongation of the cell wall

Intrinsic to its rod-shape, *E. coli* has separate mechanisms for elongation and septal wall synthesis. Van-FL staining showed a helical pattern of lateral PG synthesis (Varma *et al.*, 2007). *E. coli* also has a helical arranged cytoskeleton based on MreB, C and D (Kruse *et al.*, 2005). Conceivably, the elongation complex associates based on the MreBCD protein complex (Fig. 14). Although the doubts about the helical arrangement in *B. subtilis*, as opposed to moving patches, may also apply to *E. coli* (Dominguez-Escobar *et al.*, 2011; Garner *et al.*, 2011; Swulius *et al.*, 2011).

Intracellularly, MraY and MurG are responsible for generating Lipid II and are most likely integrated in the elongation complex (Mohammadi *et al.*, 2007; Mengin-Lecreulx *et al.*, 1991; Boyle and Donachie, 1998; Bouhss *et al.*, 1999; Brandish *et al.*, 1996; Ikeda *et al.*, 1991). Two proteins with 30% sequence homology are candidates for the Lipid II flippase. RodA is proposed to act as a flippase in the elongation complex, whereas FtsW is expected to act during septum formation (den Blaauwen *et al.*, 2008). PBP2 is an elongation-specific PBP and associates to the elongation complex on the periplasmic side (Den Blaauwen *et al.*, 2003). PBP2-GFP is observed in a helical pattern similar to the MreBCD complex (Den Blaauwen *et al.*, 2003). Nevertheless, PBP2 can also locate to the septal wall where it helps to maintain the diameter of the new cell poles (Den Blaauwen *et al.*, 2003). PBP1A and 1B are functional in both elongation and septum synthesis (Scheffers and Pinho, 2005; Broome-Smith

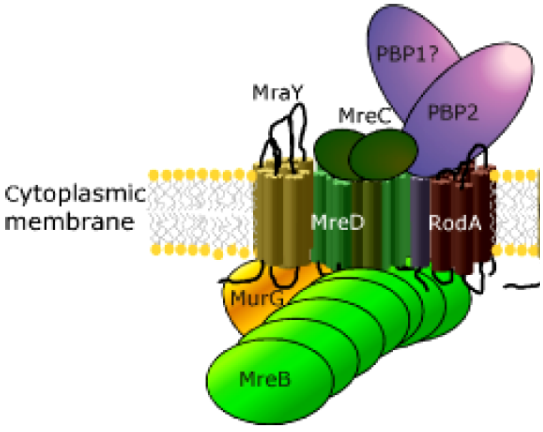

Figure 14: Hypothetical arrangement of the elongation complex of *E. coli*. The MreBCD cytoskeleton probably serves as a scaffold for assembling all proteins needed for lateral peptidoglycan synthesis.
Adapted from den Blaauwen *et al.*, 2008.

et al., 1985). PBP1C depends on binding to PBP1B, 2 or 3 to exert its function (Scheffers and Pinho, 2005; Schiffer and Holtje, 1999). Interestingly, also lipoproteins in the outer membrane are implicated in regulation of lateral PG synthesis (Typas *et al.*, 2010).

Although the PG layer is much thinner in Gram-negative bacteria, the molecular structure is similar. However, contrary to the inside-to-outside model of *B. subtilis*, an alternative model is proposed for incorporation of new peptidoglycan strands in the existing layer (Fig. 15). The "three-for-one" model states that three new, connected glycan strands are incorporated replacing one old glycan strand, the "docking strand" (Holtje, 1998; Holtje, 1996). While the new strands are attached via peptide bridges, the old strand is removed. Accuracy of this model, as well as the three-dimensional arrangement of the PG polymers, remains to be elucidated. The scaffold model by Dmitriev *et al.* (2004; see Chapter 3) is less plausible when applied to *E. coli*. As Vollmer en Höltje (2004) pointed out, the amount of PG measured would not sufficiently cover the *E. coli* surface if it is arranged perpendicular to the membrane.

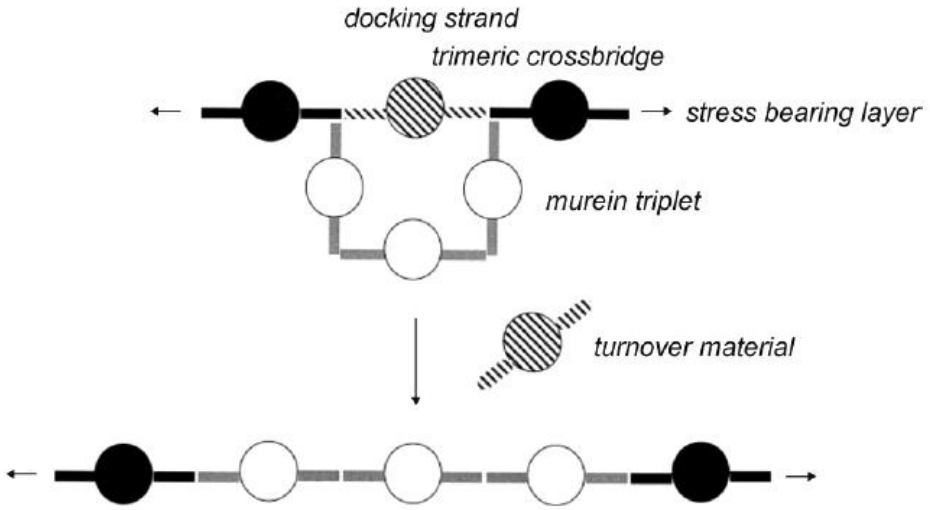

Figure 15: Three-for-one model of peptidoglycan incorporation in *E. coli* cell wall. Three new, connected glycan strands are incorporated replacing one old glycan strand, the "docking strand". As soon as the new strands are connected, the old strand is removed.
Adapted from Scheffers *et al.*, 2005.

Chromosome segregation

The *E. coli* genome consists of one circular chromosome with a single oriC and terC. Segregation of the two condensed nucleoids is a largely similar process as for *B. subtilis*. The *E. coli* SMC complex is called the MukBEF complex (Danilova *et al.*, 2007). DnaA unwinds the DNA helix and during replication the two oriC pull along the DNA copies toward the poles. Also in *E. coli*, the MreB cytoskeleton is essential for chromosomal movement (Kruse and Gerdes, 2005). In addition, *E. coli* expresses FtsK, a protein that actively aids in segregation (Begg *et al.*, 1995). Six FtsK proteins insert in the cell membrane as a complex and transport DNA away from the site of membrane constriction (Massey *et al.*, 2006; Saleh *et al.*, 2004).

Z ring formation and regulation

Some proteins involved in *E. coli* Z ring formation are known in *B. subtilis* (see Chapter 3), others are specific for *E. coli*. For example, the Z ring is tethered to the inner cell membrane via FtsA, but also via ZipA (Hale and de Boer, 1997). Both proteins directly interact with FtsZ, functioning like Z ring

promoters (Pichoff and Lutkenhaus, 2002; Hale and de Boer, 1999). Similar to *B. subtilis*, proper cell division depends on a 1:5 ratio of FtsA to FtsZ (Rueda *et al.*, 2003). ZipA is unique for Gammaproteobacteria. Also the ZipA transmembrane domain may be involved in anchoring proteins (Hale *et al.*, 2000).

No regulatory mechanism dependent on nutrient availability has been identified yet, as *E. coli* lacks UgtP. However, *E. coli* has a similar glucose-dependent metabolic pathway, for which a function in Z ring timing may be discovered in the future (Weart *et al.*, 2007).

To regulate Z ring positioning, *E. coli* also exhibits nucleoid occlusion and the Min system. Similarly to *B. subtilis* Noc, the analogous protein SlmA (synthetic lethal with a defective Min) binds the DNA via specific binding sequences (Cho *et al.*, 2011). SlmA inhibits Z ring assembly by interfering with FtsZ polymerization.

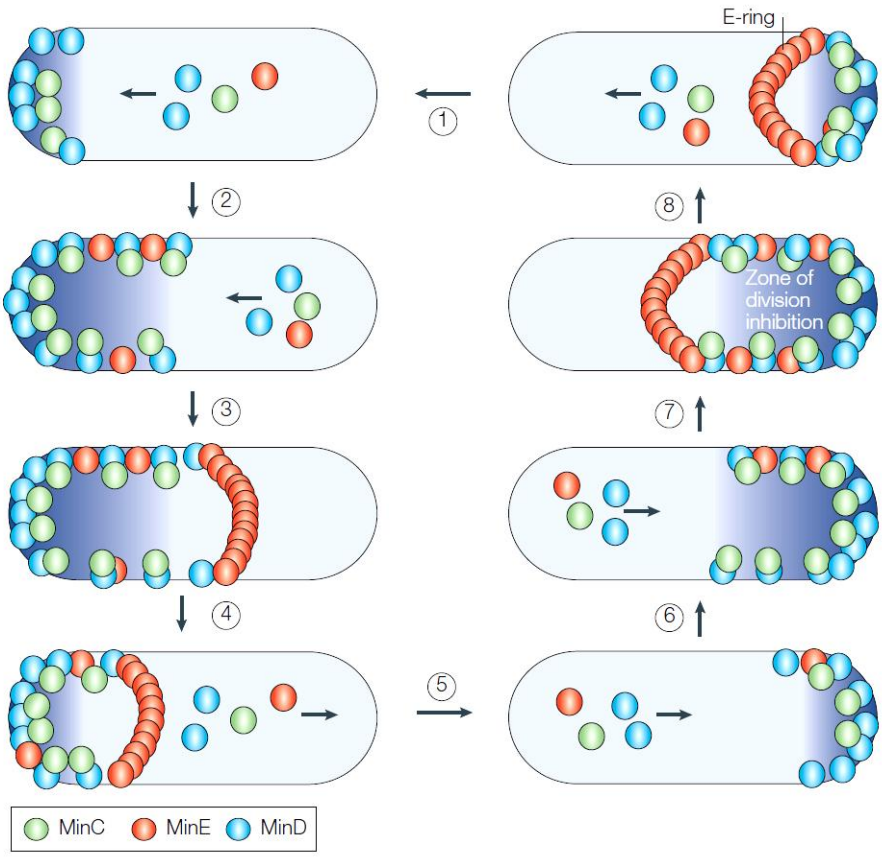

Figure 16: Oscillation cycle of MinCDE proteins in *E. coli*. The cycle starts with MinD binding to the poles. There it recruits more MinD subunits and MinC and MinE to the complex (2). MinE proteins form an E ring capping the pole complex (3), which stimulates disassembly of the pole complex (4). The complex then moves toward the other pole where the cycle starts over (5). Dark blue zones indicate zones of Z ring inhibition by MinCDE. (Rothfield *et al.*, 2005)

At the cell poles, the Min system consists of MinC, D and E. But characteristic for *E. coli*, the Min proteins only assemble at one of the poles. To provide Z ring inhibition at both poles, the proteins oscillate from one pole to the other (Fig. 16) (Raskin and de Boer, 1999). The process starts with MinD binding ATP, whereupon it associates with the membrane and recruits further MinD-ATP to generate long polymers (Suefuji *et al.*, 2002; de Boer, 2010; Hu *et al.*, 2003). Also homodimers of MinC and MinE are recruited. Growth of the polymers toward the mid-cell is halted by the formation of an E ring, a ring-like structure composed of MinE capping the pole complex (Fu *et al.*, 2001; Shih *et al.*, 2002). Subsequently, the protein complex contracts toward the pole because the E ring stimulates MinC release and the inherent ATPase function of MinD (Lackner *et al.*, 2003). ATP hydrolysis causes the MinD polymers to dissociate. The individual subunits then move toward the other pole and there the assembly of the complex starts again. How this movement is established and how MinD recognizes the poles has not been elucidated. The MinCDE complex inhibits Z ring

formation by competing with FtsA and ZipA for binding FtsZ and by interfering with polymer interactions (Dajkovic *et al.*, 2008a; Shen and Lutkenhaus, 2010; Shen and Lutkenhaus, 2009). Due to the fast oscillation, Z ring assembly is effectively prevented at both cell poles.

Other Z ring promoters and inhibitors have been identified. Similar to *B. subtilis, E. coli* ZapA promotes Z ring formation, probably by direct binding to FtsZ (Gueiros-Filho and Losick, 2002; Small *et al.*, 2007; Low *et al.*, 2004). Additionally, *E. coli* expresses ZapB and ZapC, which play redundant roles in Z ring formation (Ebersbach *et al.*, 2008; Hale *et al.*, 2011). Specific for gammaproteobacteria is the cell division inhibitor SulA, which directly inhibits FtsZ polymerization (Dajkovic *et al.*, 2008b). The earlier mentioned SepF and EzrA are only expressed in Gram-positive bacteria (Adams and Errington, 2009).

Assembly of the division machinery

The Z ring functions as the scaffold for the assembly of the divisome, consisting of FtsK, FtsQ, FtsL, FtsB, FtsW, PBP3 (also called FtsI) and FtsN (Fig. 17). The putative functions of the divisome sununits were recently reviewed by Den Blaauwen *et al.* (2008). The *E. coli* divisome is proposed to assemble in three sequential sub-complexes (Goehring *et al.*, 2006).

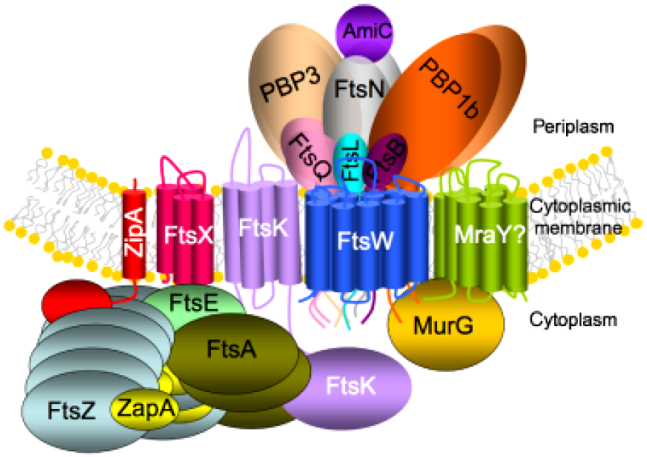

Figure 17: Hypothetical arrangement of the *E. coli* divisome. (den Blaauwen *et al.*, 2008)

Formation of the division septum and membrane constriction

Upon completion of the divisome, Z ring constriction is initiated by FtsN (Addinall *et al.*, 1997; Moll and Thanbichler, 2009). As the inner membrane constricts, the division septum is simultaneously synthesized. Septum-specific PBP3, the equivalent of *B. subtilis* PBP2B, is attracted to the septum by interaction with FtsW and FtsQ (den Blaauwen *et al.*, 2008; Spratt, 1975; Weiss *et al.*, 1999). PBP1B directly interacts with PBP3 (Bertsche *et al.*, 2006). As mentioned, PBP2 regulates the pole diameter. PBP5 translocates to sites of active PG synthesis depending on its substrate, thus also to the septum (Potluri *et al.*, 2010). For other PBPs (e.g. 4, 6, 7) of *E. coli* localization data are not available.

During septum synthesis, the septal cell wall is simultaneously divided in two pole patches. Therefore, the outer membrane concurrently invaginates with the growing septum. This cooperative movement is effected by the Tol-Pal system that connects the inner and outer membrane (Gerding *et al.*, 2007). The complex consists of TolA, TolQ and TolR in the inner membrane, TolB in the periplasmic space and Pal anchored in the outer membrane. These proteins interact in the division septum, depending on the presence of FtsN (Gerding *et al.*, 2007). In this way, the divisome mediates constriction of the outer membrane. In microscopic images, the invagination of the total cell envelope is seen as a clear furrow separating the two daughter cells (Fig. 7). The required energy could be supplied by Z ring constriction or the inwards growth of the PG layer.

Separation

The three amidases AmiA, AmiB and AmiC (N-acetyl-muramyl-L-alanine amidases) are the most important PG hydrolases for septum separation in *E. coli* (Heidrich *et al.*, 2001; Priyadarshini *et al.*, 2006; Uehara *et al.*, 2010). Specific localization to the division septum in constricting cells was proven

for AmiC, using a GFP-fusion protein. AmiC accumulates in the periplasm based on FtsN localization (Bernhardt and de Boer, 2003). Additional proteins are needed to activate these amidases. The periplasmic endopeptidase EnvC fulfills another function by activating AmiA and AmiB (Uehara *et al.*, 2010). The outer membrane lipoprotein NlpD activates AmiC. EnvC and NlpD localize specifically to the division septum where they aid in cell separation (Bernhardt and de Boer, 2004; Uehara *et al.*, 2009). EnvC binds to a membrane complex of FtsE and FtsX that functions as an ABC transporter system (Yang *et al.*, 2011) (Fig. 18). The FtsEX ATPase activity is essential for EnvC-mediated activation of AmiA and AmiB (Yang *et al.*, 2011). The FtsEX-EnvC interaction facilitates an essential conformational change in EnvC, enabling it to activate the amidase. Because FtsE interacts with FtsZ as well, this mechanism couples Z ring constriction and initiation of septum autolysis (Corbin *et al.*, 2007).

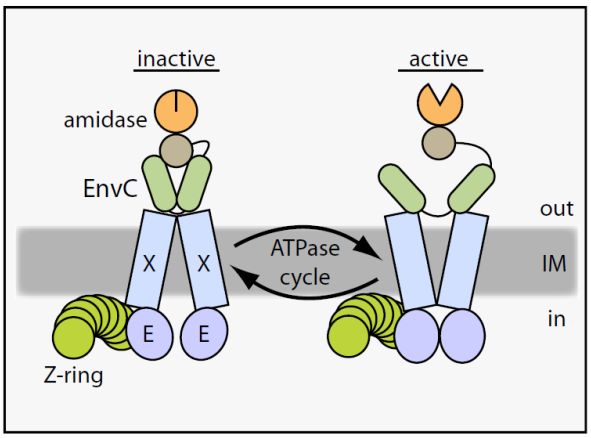

Figure 18: Model of the putative FtsEX complex in the *E. coli* inner membrane. The ATPase activity of FtsE causes a conformational change in the FtsEX that is transmitted to EnvC. EnvC then activates the amidase.
E = FtsE, X = FtsX.
(Yang *et al.*, 2011)

Chapter 6: Sporulation

Besides normal vegetative growth, *B. subtilis* has a second mode of cell division. When the environment is unfavorable, *B. subtilis* can engage in asymmetric cell division creating endospores. Endospores are compacted cells with a double membrane and a protein coat. They are extremely resistant against e.g. UV light, heat and desiccation. This spore formation, called sporulation, ensures survival of the bacteria. When circumstances become favorable, the spores can germinate and engage in vegetative cell growth. Sporulation has been a major research interest in the past years, mostly using *B. subtilis* as the model organism. Because MAC deposited on *B. subtilis* cell poles, sporulation is of interest for this thesis. In this chapter I compare sporulation with normal cell growth and look at the implications for cell wall composition during division.

Replication and Z ring formation during sporulation

The process of sporulation is initiated by phosphorylation of one major transcription regulator, Spo0A. External and internal signals integrate in a signal transduction pathway that leads to the activation of a set of kinases (summarized in (Piggot and Hilbert, 2004). When the level of phosphorylated Spo0A is above a certain threshold, it promotes expression of the *spoIIA*, *spoIIE* and *spoIIG* loci (Piggot and Hilbert, 2004). Eventually, Spo0A initiates asymmetric septation and affects chromosome positioning and prespore-specific gene expression (Fujita and Losick, 2003). The ABC transporter complex FtsEX, which I discussed for its function in *E. coli* autolysis (see Chapter 5), can delay the onset of sporulation in *B. subtilis* (Garti-Levi *et al.*, 2008). Possibly, FtsEX transports extracellular signals over the membrane, integrating them in the Spo0A pathway.

Sporulation starts with DNA replication, exactly the same as in vegetative cell division. The activation status of DnaA is regulated by the ratio between sporulation proteins Spo0J and Soj (Scholefield *et al.*, 2011). The two oriC bind to the cell poles and pull along the chromosome copies. The protein RacA binds the oriC by recognizing specific DNA sequences adjacent to the oriC (Ben-Yehuda *et al.*, 2005). RacA is recruited to the cell poles, presumably by DivIVA (Ben-Yehuda *et al.*, 2003; Wu and Errington, 2003). Independent of other division proteins, DivIVA localizes to the pole (Quisel *et al.*, 1999). Possibly, by recognizing the negative curvature of the pole membranes (Lenarcic *et al.*, 2009; Ramamurthi *et al.*, 2009).

During sporulation, the chromosomes do not form compact nucleoids, but are stretched along the full axis. This conformation is referred to as the axial filament, clearly visible by electron microscopy (Kay and Warren, 1968). When the axial filament is positioned, FtsZ subunits translocate from mid-cell toward the cell poles via a helical transport pattern (Ben-Yehuda and Losick, 2002). This helical route was also demonstrated for FtsA and EzrA localization at both Z rings (Ben-Yehuda and Losick, 2002). The mechanisms positioning the Z rings are not entirely known. The Min system does not play a role in this process (Levin *et al.*, 1992; Lee and Price, 1993). It is shown that the protein SpoIIE is involved in positioning and stabilizing the Z ring, possibly via membrane tethering analogous to ZipA and FtsA (Ben-Yehuda and Losick, 2002). SpoIIE arranges in a ring shape in the cell and can directly interact with FtsZ (Lucet *et al.*, 2000). In the end, only one of the Z rings persists. However, the factors blocking maturation of the other division site remain to be identified.

Formation of the sporulation septum

After localization of SpoIIE and FtsZ, the septum synthesis machinery is recruited to the definite Z ring. Although the machinery is almost the same as in the regular septum, it produces a much thinner

septum PG layer. PpbX, PBP1, PBP2C, PBP2B and PBP2D have been shown to localize to the sporulation septum (Scheffers *et al.*, 2004; Daniel *et al.*, 2000; Scheffers, 2005). PbpX is transported to both Z rings, following the same helical pattern as FtsZ (Scheffers, 2005)(Scheffers *et al.*, 2004) (Scheffers *et al.*, 2004)
(Scheffers *et al.*, 2004)
. Since *pbpX* knockout mutants display no defects in sporulation or growth, PbpX is not essential for septal synthesis (Scheffers, 2005). PBP1 and 2B are recruited only to the definite septum and are crucial for proper sporulation (Scheffers *et al.*, 2004; Daniel *et al.*, 2000). PBP2C and 2D have redundant functions during sporulation (McPherson *et al.*, 2001). The recognition signals for the various PBPs are unknown. Possibly, specific PG substrates play a role.

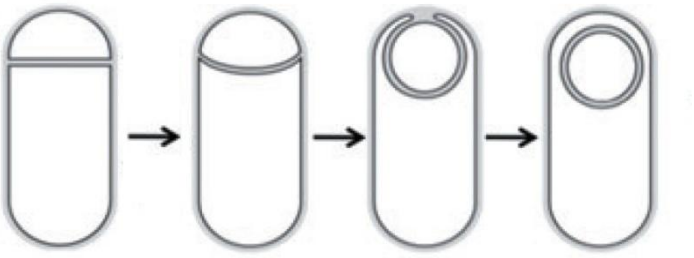

Figure 19: Simplified representation of the engulfment process. The septum divides the cell in two asymmetric daughter cells (left panel). The septum curves toward the cell pole and continues to engulf the entire prespore. In the end, the prespore is surrounded by a double membrane.
Adapted from Meyer *et al.*, 2010.

The resulting sporulation septum divides the cell in two asymmetric daughter cells (Fig. 19). The smallest cell is called the prespore or forespore, the larger cell is called the mother cell. When the septum is formed, the prespore contains only one third of its chromosome (Dworkin, 2003). Nonetheless, the plasma membrane and the septum constrict, enclosing the axial filament in a narrow channel.

Chromosome segregation

The endospore must receive a full copy of the bacterial chromosome. Therefore, the remaining two thirds of the chromosome must be transported through the septum. This transport is effected by SpoIIIE, a sporulation-specific addition to the divisome (Ramamurthi *et al.*, 2009). SpoIIIE is a transmembrane protein from the FtsK family that has a DNA translocase function similar to *E. coli* FtsK (see Chapter 5). SpoIIIE complexes, consisting of six monomers, insert in the membrane at the mother cell side of the septum (Sharp and Pogliano, 2002). After complete membrane constriction, the SpoIIIE hexamer shapes the connecting channel containing the chromosome. As the chromosome is transported, two channels are needed to enclose the two chromosomal branches (Wu and Errington, 1998; Burton *et al.*, 2007). The SpoIIIE complex actively transports the rest of the chromosome into the prespore (Wu and Errington, 2004). The transport is guided by binding of the complex to SpoIIIE recognition sequences (SRS) in the DNA (Ptacin *et al.*, 2008). Once in the prespore, small acid-soluble proteins (SASPs) compact the chromosome and protects it to exogenous factors.

Engulfment

After DNA translocation, the mother cell starts to engulf the prespore. The septum curves toward the nearest cell pole (Fig. 19). By the continuous advancement of the septum ends, the entire prespore is engulfed. The core, containing the DNA, will get enveloped by a double membrane with a PG layer in between (Fig. 19). The two membranes bordering the septum now form the inner and outer prespore membrane. The movement of the membranes is thought to be mediated by three different mechanisms: the DMP complex, the SpoIIQ-SpoIIIAH protein pair and peptidoglycan synthesis (Fig. 20).

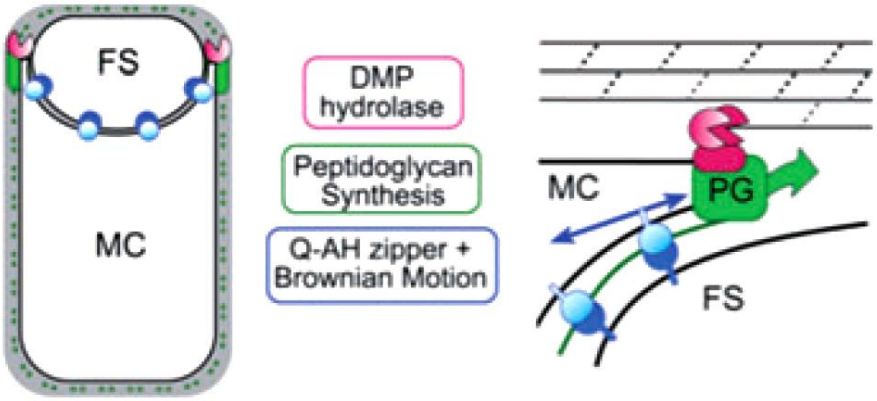

Figure 20: Model for membrane movement during engulfment. The DMP complex hydrolyses the existing PG layer. The SpoIIQ-SpoIIIAH complex connects the two forespore membranes. The peptidoglycan of the spore cortex is synthesized in between the two membranes. For further information see the text.
MC = mother cell, FS = forespore, PG = peptidoglycan. Adapted from Meyer *et al.*, 2010.

The DMP complex, consisting of SpoIIM and the PG hydrolases SpoIID and SpoIIP, is inserted in the future outer membrane (Abanes-De Mello *et al.*, 2002; Morlot *et al.*, 2010). At the corner where the septum invaginated, the septal PG connects to the PG layer of the peripheral cell wall. This connection persists at the tip of the membrane bulge that engulfs the prespore. To allow further growth, the PG layer needs to open up. SpoIID and SpoIIP mediate this PG lysis, allowing movement of the engulfing mother cell membrane (Abanes-De Mello *et al.*, 2002).

The two transmembrane proteins SpoIIQ and SpoIIIAH form a membrane anchor (Broder and Pogliano, 2006). After translation in the prespore, SpoIIQ inserts in the inner forespore membrane. SpoIIIAH is produced in the mother cell and specifically migrates to the future outer membrane. The extracellular domains connect the two membranes enclosing the septal PG. These bridging complexes translocate along the engulfing membrane and function in membrane movement. When the PG layer is absent, the DMP complex loses its function. Then the SpoIIQ-SpoIIIAH complex is sufficient to effect engulfment (Broder and Pogliano, 2006). It is still unclear whether these mechanisms work simultaneously or sequentially in *B. subtilis* (Higgins and Dworkin, 2012).

Formation of the spore cortex

The remnant of the septal wall, a thin layer of PG, surrounds the inner forespore membrane (Fig. 21). This is called the germ cell wall, as it will later form the cell wall of the germinating spore. However, to render the spore highly resistant to environmental factors, an additional, thick layer of PG is formed in between the two membranes. This layer is termed the spore cortex (Fig. 21). Cortex synthesis is already initiated during engulfment, mediated by proteins produced in the mother cell. From the cytoplasmic membrane of the mother cell, the transmembrane protein SpoVE translocates specifically to the outer forespore membrane (Real *et al.*, 2008). Here it recruits SpoVD, a sporulation specific PBP, to aid in cortex synthesis (Daniel *et al.*, 1994; Fay *et al.*, 2010). A *spoVD* or *spoVE* mutant produces spores without a cortex. The SEDS protein SpoVE acts, like its homologues FtsW and RodA, as a lipid II translocase (Fay *et al.*, 2010). It is thought that the mechanical strain built up by glycan polymerization, has a role as driving force of engulfment (Fig. 20) (Meyer *et al.*, 2010). The cortex PG is similar to the cell wall PG, although the

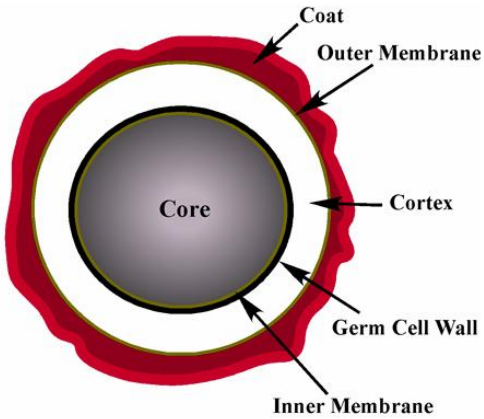

Figure 21: The different parts of the mature endospore.
Taken from http://micro.cornell.edu.

number of peptide side chains is lower, resulting in a lower degree of cross-linking (Popham, 2002). PG cross-linking in the spore is partially regulated by the carboxypeptidases 5*, DacB and DacF (Popham *et al.*, 1999; Popham *et al.*, 1995).

Membrane fusion and separation

When the two ends of the former septum engulf the prespore, they eventually meet and fuse to complete engulfment. Surprisingly, the DNA translocase SpoIIIE is necessary for this membrane fusion (Liu *et al.*, 2006b; Sharp and Pogliano, 2003; Sharp and Pogliano, 1999). *spoIIIE* mutants are not able to complete membrane fusion. During engulfment, SpoIIIE constitutively localizes to the septum ends. Originating from the middle of the septum, it migrates along with the engulfing membrane after DNA translocation has been completed. In accordance with its dual function, membrane fusion is mediated by the transmembrane domain of SpoIIIE, whereas its cytoplasmic domain functions in DNA translocation (Sharp and Pogliano, 2003; Becker and Pogliano, 2007). A patch of newly synthesized PG simultaneously closes the cortex (Meyer *et al.*, 2010).

Formation of the spore coat

Surrounding the double membrane, each *B. subtilis* endospore has a coat for extra protection. The spore coat consists of more than 70 different proteins, which are specific for each bacterial species. It has four distinct layers: the basement layer surrounding the membrane, the inner coat, outer coat and crust. Each layer is produced under regulation of its own major transcriptional regulator: SpoIVA, SafA, CotE and CotZ, respectively. The first regulatory proteins already localize during engulfment (Pogliano *et al.*, 1995; Webb *et al.*, 1995). When engulfment is finished, the coat production starts at one side of the prespore. In multiple waves the layers spread over the entire endospore surface (McKenney and Eichenberger, 2011; Wang *et al.*, 2009). It was recently demonstrated that SpoIIQ and SpoIIIAH have crucial roles in coat formation (McKenney and Eichenberger, 2011).

Chapter 7: Implications for MAC deposition

Little has been published about the distinctive features of the bacterial cell wall at certain spots or cell cycle stages. Multiple studies have proposed that the septal wall contains some receptor to explain the septal localization of their protein of interest. Nevertheless, no single receptor has been undeniably identified in any bacterial species. In addition, due to resistance against MAC-mediated lysis, the binding of complement factors of the MAC has never been studied in Gram-positive bacteria. For these two reasons, we can only speculate about what causes the site-specific MAC deposition on the investigated Gram-positive species. Confocal microscopy data indicate specific binding of the MAC at the division septum. In *S. pyogenes* at the symmetric septum, in *B. subtilis* at the cell pole.

My first hypothesis to explain site-specific MAC deposition on Gram-positive bacteria is that the terminal complement components get access to the bacterial membrane. Possibly, insufficient maturation of the septal peptidoglycan causes the cell wall to "leak" at this site, allowing complement factors to reach the underlying cell membrane. Alternatively, site-specific activation of PG hydrolases, meant to separate the daughter cells, might cause such leaks when the activity is ill-balanced. In support of the idea that these leaks develop specifically at sites of active cell wall synthesis, protein secretion systems (i.e. the Sec machinery) in the membrane of Gram-positive bacteria often localize to these active sites (Buist *et al.*, 2006). A porous cell wall could be preferable for large secreted proteins that need to cross it. Only small proteins (<25 kDa) are able to translocate across the cell wall at any location (Demchick and Koch, 1996). Conceivably, complement components are able to reach the membrane via the same route as secreted proteins. However, if the complement components do assemble on the membrane, it remains unclear why Gram-positive bacteria are not susceptible to MAC-mediated lysis. In *S. pyogenes* the inhibitor SIC could play a role in this.

On the other hand, the MAC may assemble outside a cell membrane, contrary to the current consensus. Antibody-coated beads were shown to bind fully assembled MACs, rendering a lipid bilayer non-essential (E. Berends, unpublished results). Therefore, bacterial surface proteins on the cell wall of the septum or poles could be involved in MAC localization. Surface proteins might either induce membrane-independent assembly of the MAC or function as a specific binding-site for the MAC. Especially hydrophobic binding domains within surface proteins are potential candidates, as conformational changes in the complement factors expose hydrophobic domains. Sites of PG synthesis are characterized by other synthesis pathways such as those producing teichoic acids or lipoproteins.

In relation to these two hypotheses, I will discuss existing knowledge on localization patterns in the selected species in order to find target candidates for MAC deposition. For *B. subtilis* I will deal with factors localizing to the sporulation septum and cell poles, whereas for *S. aureus* and *S. pyogenes* I will focus on localization to the symmetric septum.

Bacillus subtilis

During *B. subtilis* elongation in the vegetative growth cycle, peptidoglycan is not synthesized at the poles of the mother cell. But during sporulation, PG hydrolysis and synthesis cooperate in engulfment of the prespore. The transforming septum could be susceptible to breaches in the PG layer, as the existing germ cell wall around the prespore is thin and the spore cortex is under construction.

However, it remains to be elucidated whether sporulation had occurred during the confocal microscopy experiments.

Independent of division or sporulation, other factors distinguish the cell poles. McpB (methyl-accepting chemotaxis protein B) and TlpA (Transducer-like protein A) are transmembrane proteins mainly present at the cell poles (Kirby *et al.*, 2000; Meile *et al.*, 2006). They are asparagine receptors mediating chemotaxis (Hanlon and Ordal, 1994). In addition, a secretion system is located at the cell poles. The Tat system contains two separate translocase complexes, TatAdTatCd and TatAyTatCy, and an individual protein, TatAc. TatAd and TatCd are the transmembrane subunits. These translocases are responsible for transporting folded proteins over the cell membrane. TatCy, TatAd and TatAc are clearly visualized in concentrated foci on the cell membrane, most prominently at the cell poles (Meile *et al.*, 2006; Ridder *et al.*, 2009). Although fluorescence was too low to visualize TatCd and TatAy, the presence of TatCd or TatAy was essential for TatAd localization. This indicates that complete complexes are formed at these foci at the poles (Ridder *et al.*, 2009). The signals targeting these transmembrane proteins to the poles might be found in the content of site-specific lipid rafts. Particularly cardiolipin is enriched in lipid rafts at the *B. subtilis* poles and septum (Kawai *et al.*, 2004).

As the division septum becomes two new poles, septal localization might lead to polar localization in the nascent daughter cell. Factors remaining from division would be found in a single pole. For example, the lipoprotein YerB localizes both to the poles and the division septum. Currently, its function is unknown (Meile *et al.*, 2006).

Other processes may cause temporal breaches of the PG layer. For example conjugation, when a pilus is built on the cell membrane. Proteins of the plasmid conjugation machinery (VirD2, VirB4, VirD4, VirB1 and VirB11) were found to concentrate at a single cell pole, although this localization was not exclusive (Bauer *et al.*, 2011). The localization disappears when the cell enters a vegetative growth cycle. A second example is the uptake of naked DNA from the environment. This state of “natural competence” is mediated by the family of Com (Competence) proteins, which also localize specifically at the poles (Hahn *et al.*, 2009). Potentially one of these mechanisms provides an opportunity for MAC entry into the periplasmic space.

Staphylococcus aureus

As mentioned previously, PBPs, Atl and LytN are specifically targeted to the septum (Scheffers and Pinho, 2005; Yamada *et al.*, 1996; Frankel *et al.*, 2011). Consistent with synthesis of new PG at the septum, a PBP2-GFP fusion protein shows localization to the septum (Pinho and Errington, 2005; Pinho and Errington, 2003). At the beginning of septum formation, PBP2-GFP is observed as two dots, indicating it localizes in a ring around the division plane. When the septum forms, PBP2 travels along to the interior of the cell, visualized as a line across the cell. Adding the β-lactam antibiotic oxacillin to the bacterial culture inhibits this localization, causing PBP2 to disperse over the cell surface (Pinho and Errington, 2005). Oxacillin irreversibly blocks the active site of PBP2 as it closely resembles the PBP2 substrate, the D-Ala-D-Ala terminus of linker-peptides. Antibiotics modifying (D-cycloserine) or blocking (vancomycin) the PBP2 substrate have the same effect on PBP2 localization (Pinho and Errington, 2005). In conclusion, PBP2 depends on substrate recognition to localize to the septum (Pinho and Errington, 2005). However, when PBP2a (only in MRSA) is simultaneously expressed, it counteracts the ocaxillin-induced dispersion of PBP2 (Pinho and Errington, 2005). Thus protein-protein interaction with PBP2a plays a subordinate role in localizing PBP2.

PBP4 also localizes specifically to the septum. In contrast, this is not dependent on substrate recognition. Its location is based on the synthesis of wall teichoic acids (Atilano *et al.*, 2010), which is coupled to PG synthesis in *S. aureus* (Campbell *et al.*, 2011). Wall teichoic acids are important in regulation of cell wall growth, since their absence results in irregular and displaced septa (Campbell *et al.*, 2011). Absence of functional TagO, the first enzyme in the WTA synthesis pathway, leads to PBP4 delocalization (Atilano *et al.*, 2010). Atilano *et al.* proposed a model in which PBP4 specifically recognizes immature WTAs present at the septum, whereas mature WTAs are found in the rest of the cell wall.

To explain the localization of the Atl autolytic enzymes, it has been proposed that the three R domains recognize a specific receptor or PG modification at the septal wall (Baba and Schneewind, 1998). Recently it was discovered that the septal localization depends on WTAs, though in a different manner than for PBP4. Normally, mature WTAs are spread over the entire surface of the cell wall, but relatively low on the equatorial ring (Schlag *et al.*, 2010). A *S. aureus* mutant without WTA expression demonstrated dispersed, but higher Atl binding (Schlag *et al.*, 2010). Atl is evenly secreted over the cell surface, but this experiment indicates that Atl binding is inhibited at sites of high WTA content. Schlag *et al.* suggested that mature WTAs inhibit Atl binding. As a result, the lateral cell wall is protected against the lytic effect of Atl, in contrast to the septal PG layer. Atl also targets to the site of the septum on protoplasts, bacteria stripped of their PG cell wall, suggesting that it binds a target in the plasma membrane (Yamada *et al.*, 1996). Therefore, recognition of immature WTAs, as proposed for PBP4, would be an implausible explanation for Atl localization. In conclusion, Atl localization to the septal membrane is determined by the absence of mature WTAs, instead of the presence of immature WTAs.

In addition, the secreted protein IsaA (immunodominant staphylococcal antigen A) is also observed specifically at the septal wall (Sakata *et al.*, 2005). This housekeeping protein is presumed to function as a lytic transglycosylase of PG, due to its sequence homology with the lytic transglycolases of Gram-negative bacteria (Sakata *et al.*, 2005; Mushegian *et al.*, 1996). In combination with its location, this implicates IsaA has a function in cell wall hydrolysis. The mechanism of septal localization is unknown. Site-specific secretion might play a role, although a YSIRK/GS motif has not been identified yet in IsaA (Buist *et al.*, 2006).

Besides LytN, there are other *S. aureus* proteins with the YSIRK/GS motif (see Chapter 3), which mediates septal secretion. These are ClfA (clumping factor A), Spa (Staphylococcal protein A), FnbpB (fibronectin-binding protein B), SdrC and SdrD (serine-aspartate repeat protein C and D) (DeDent *et al.*, 2008). ClfA causes clotting of *S. aureus* in blood by interaction with fibrinogen, which shields the bacteria from the immune system (Josefsson *et al.*, 2008). FnbpB is a multifunctional adhesin that can bind to fibronectin, fibrinogen and elastin in order to promote colonization of the host and internalization of bacteria into endothelial cells (Burke *et al.*, 2011). Protein A is well-known for binding the Fc region of immunoglobulins, preventing opsonization (Sjoquist and Wadso, 1971). SdrC and SdrD play an undefined role in adherence to host cells (Corrigan *et al.*, 2009; Barbu *et al.*, 2010). These proteins all have in common that they recognize host factors. This indicates that, around the septum, interaction with the host occurs. Interference with host factors at that site is relevant for the bacterium, although the proteins might spread to other sites on the cell surface after secretion. Conceivably, *S. aureus* might express complement-inhibitory proteins with a YSIRK/GS motif. These would protect the bacterium against the complement system at the vulnerable site of new PG synthesis.

Streptococcus pyogenes

In addition to PG synthesis, Cole *et al.* investigated protein synthesis by radiolabeling (COLE and HAHN, 1962). Alike peptidoglycan, synthesis of new transmembrane proteins appears to occur solely at the division septum. To the best of my knowledge, more recent data on *S. pyogenes* protein synthesis is non-existent.

Rosch *et al.* postulated that the Sec secretion system of *S. pyogenes* exclusively concentrates in a single central point on the cell membrane, the so-called ExPortal (Rosch *et al.*, 2007; Rosch and Caparon, 2005). However, this view has been challenged by Carlsson *et al.* (2006), who showed that the SecA subunit is dispersed on the cell surface. Some *S. pyogenes* proteins have signal sequences that direct their site-specific secretion (Carlsson *et al.*, 2006). The M protein signal sequence targets to the septum (Carlsson *et al.*, 2006). This sequence contains a YSIRK-like motif, namely YSLRK, although this motif is dismissible for the function of the signal sequence.

Lastly, the protein sortase A is seen in foci around the division septum (Raz and Fischetti, 2008). Sortase A mediates the covalent linking of secreted proteins to the cell wall constituents. Thus the site-specific secreted proteins could be specifically retained at the septum. However, the mechanisms behind sortase A localization are completely unknown.

Physiological relevance of MAC deposition on Gram-positive bacteria

Based on this literature study, we can conclude that the factors mediating site-specific binding of the MAC to Gram-positive bacteria remain unknown. A complicating factor is that the physiological relevance of this binding remains unclear. The host does not seem to benefit from MAC deposition, as the bacteria prove to be resistant against MAC-mediated killing. Also for the bacteria, no beneficial effect of MAC binding has been found so far. Would MAC binding to specific sites result in specific advantages or disadvantages for the bacteria, as opposed to equal spreading over the cell wall? As long as effects of MAC deposition are unidentified, this question will be difficult to answer. Based on the current knowledge, several possible explanations remain.

Protection against MAC insertion might be only relevant at sites of new cell wall synthesis, because of the temporal porosity of the cell wall proposed in this chapter. A defense mechanism might have evolved in which constituents of the septal wall capture the MAC before it can reach the membrane. The cell wall might also be able to capture only complement subunits. We have to bear in mind that the fluorescent MAC-antibody recognizes polymerized C9. Although C9 is known to only polymerize when binding to the C5b678 complex, this view is part of the same consensus stating that the MAC can only form when inserted in a lipid bilayer. Therefore, we have to consider the possibility that a surface constituent is able to mediate C9 polymerization without the other MAC subunits.

Another possibility is that the developing septal cell wall does not yet contain a complement inhibitor like the older cell wall. Little attention has been paid to MAC inhibitors of Gram-positive bacteria, rendering it possible that these remain to be identified in the future.

References

- Abanes-De Mello, A., Y.L. Sun, S. Aung, and K. Pogliano. 2002. A cytoskeleton-like role for the bacterial cell wall during engulfment of the Bacillus subtilis forespore. *Genes Dev.* 16:3253-3264. doi: 10.1101/gad.1039902.
- Adams, D.W., and J. Errington. 2009. Bacterial cell division: assembly, maintenance and disassembly of the Z ring. *Nat.Rev.Microbiol.* 7:642-653. doi: 10.1038/nrmicro2198.
- Addinall, S.G., C. Cao, and J. Lutkenhaus. 1997. FtsN, a late recruit to the septum in Escherichia coli. *Mol.Microbiol.* 25:303-309.
- Atilano, M.L., P.M. Pereira, J. Yates, P. Reed, H. Veiga, M.G. Pinho, and S.R. Filipe. 2010. Teichoic acids are temporal and spatial regulators of peptidoglycan cross-linking in Staphylococcus aureus. *Proc.Natl.Acad.Sci.U.S.A.* 107:18991-18996. doi: 10.1073/pnas.1004304107.
- Baba, T., T. Bae, O. Schneewind, F. Takeuchi, and K. Hiramatsu. 2008. Genome sequence of Staphylococcus aureus strain Newman and comparative analysis of staphylococcal genomes: polymorphism and evolution of two major pathogenicity islands. *J.Bacteriol.* 190:300-310. doi: 10.1128/JB.01000-07.
- Baba, T., and O. Schneewind. 1998. Targeting of muralytic enzymes to the cell division site of Gram-positive bacteria: repeat domains direct autolysin to the equatorial surface ring of Staphylococcus aureus. *EMBO J.* 17:4639-4646. doi: 10.1093/emboj/17.16.4639.
- Barbu, E.M., V.K. Ganesh, S. Gurusiddappa, R.C. Mackenzie, T.J. Foster, T.C. Sudhof, and M. Hook. 2010. beta-Neurexin is a ligand for the Staphylococcus aureus MSCRAMM SdrC. *PLoS Pathog.* 6:e1000726. doi: 10.1371/journal.ppat.1000726.
- Baron, S. 1996. Medical Microbiology. 4th edition.
- Barreteau, H., A. Kovac, A. Boniface, M. Sova, S. Gobec, and D. Blanot. 2008. Cytoplasmic steps of peptidoglycan biosynthesis. *FEMS Microbiol.Rev.* 32:168-207. doi: 10.1111/j.1574-6976.2008.00104.x.
- Bauer, T., T. Rosch, M. Itaya, and P.L. Graumann. 2011. Localization pattern of conjugation machinery in a Gram-positive bacterium. *J.Bacteriol.* 193:6244-6256. doi: 10.1128/JB.00175-11.
- Becker, E.C., and K. Pogliano. 2007. Cell-specific SpoIIIE assembly and DNA translocation polarity are dictated by chromosome orientation. *Mol.Microbiol.* 66:1066-1079. doi: 10.1111/j.1365-2958.2007.05992.x.
- Begg, K.J., S.J. Dewar, and W.D. Donachie. 1995. A new Escherichia coli cell division gene, ftsK. *J.Bacteriol.* 177:6211-6222.
- Ben-Yehuda, S., M. Fujita, X.S. Liu, B. Gorbatyuk, D. Skoko, J. Yan, J.F. Marko, J.S. Liu, P. Eichenberger, D.Z. Rudner, and R. Losick. 2005. Defining a centromere-like element in Bacillus subtilis by Identifying the binding sites for the chromosome-anchoring protein RacA. *Mol.Cell.* 17:773-782. doi: 10.1016/j.molcel.2005.02.023.
- Ben-Yehuda, S., and R. Losick. 2002. Asymmetric cell division in B. subtilis involves a spiral-like intermediate of the cytokinetic protein FtsZ. *Cell.* 109:257-266.
- Ben-Yehuda, S., D.Z. Rudner, and R. Losick. 2003. RacA, a bacterial protein that anchors chromosomes to the cell poles. *Science.* 299:532-536. doi: 10.1126/science.1079914.
- Beres, S.B., G.L. Sylva, K.D. Barbian, B. Lei, J.S. Hoff, N.D. Mammarella, M.Y. Liu, J.C. Smoot, S.F. Porcella, L.D. Parkins, D.S. Campbell, T.M. Smith, J.K. McCormick, D.Y. Leung, P.M. Schlievert, and J.M. Musser. 2002. Genome sequence of a serotype M3 strain of group A Streptococcus: phage-encoded toxins, the high-virulence phenotype, and clone emergence. *Proc.Natl.Acad.Sci.U.S.A.* 99:10078-10083. doi: 10.1073/pnas.152298499.
- Berkmen, M.B., and A.D. Grossman. 2006. Spatial and temporal organization of the Bacillus subtilis replication cycle. *Mol.Microbiol.* 62:57-71. doi: 10.1111/j.1365-2958.2006.05356.x.
- Bernhardt, T.G., and P.A. de Boer. 2004. Screening for synthetic lethal mutants in Escherichia coli and identification of EnvC (YibP) as a periplasmic septal ring factor with murein hydrolase activity. *Mol.Microbiol.* 52:1255-1269. doi: 10.1111/j.1365-2958.2004.04063.x.
- Bernhardt, T.G., and P.A. de Boer. 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., T. Kast, B. Wolf, C. Fraipont, M.E. Aarsman, K. Kannenberg, M. von Rechenberg, M. Nguyen-Disteche, T. den Blaauwen, J.V. Holtje, and W. Vollmer. 2006. Interaction between two murein (peptidoglycan) synthases, PBP3 and PBP1B, in Escherichia coli. *Mol.Microbiol.* 61:675-690. doi: 10.1111/j.1365-2958.2006.05280.x.
- Bi, E.F., and J. Lutkenhaus. 1991. FtsZ ring structure associated with division in Escherichia coli. *Nature.* 354:161-164. doi: 10.1038/354161a0.
- Blackman, S.A., T.J. Smith, and S.J. Foster. 1998. The role of autolysins during vegetative growth of Bacillus subtilis 168. *Microbiology.* 144 (Pt 1):73-82.
- Boneca, I.G., Z.H. Huang, D.A. Gage, and A. Tomasz. 2000. Characterization of Staphylococcus aureus cell wall glycan strands, evidence for a new beta-N-acetylglucosaminidase activity. *J.Biol.Chem.* 275:9910-9918.
- Bouhss, A., D. Mengin-Lecreulx, D. Le Beller, and J. Van Heijenoort. 1999. Topological analysis of the MraY protein catalysing the first membrane step of peptidoglycan synthesis. *Mol.Microbiol.* 34:576-585.
- Bouhss, A., M. Crouvoisier, D. Blanot, and D. Mengin-Lecreulx. 2004. Purification and Characterization of the Bacterial MraY Translocase Catalyzing the First Membrane Step of Peptidoglycan Biosynthesis. *Journal of Biological Chemistry.* 279:29974-29980. doi: 10.1074/jbc.M314165200.
- Boyle, D.S., and W.D. Donachie. 1998. mraY is an essential gene for cell growth in Escherichia coli. *J.Bacteriol.* 180:6429-6432.
- Bramkamp, M., L. Weston, R.A. Daniel, and J. Errington. 2006. Regulated intramembrane proteolysis of FtsL protein and the control of cell division in Bacillus subtilis. *Mol.Microbiol.* 62:580-591. doi: 10.1111/j.1365-2958.2006.05402.x.
- Brandish, P.E., K.I. Kimura, M. Inukai, R. Southgate, J.T. Lonsdale, and T.D. Bugg. 1996. Modes of action of tunicamycin, liposidomycin B, and mureidomycin A: inhibition of phospho-N-acetylmuramyl-pentapeptide translocase from Escherichia coli. *Antimicrob.Agents Chemother.* 40:1640-1644.
- Braun, V. 1975. Covalent lipoprotein from the outer membrane of Escherichia coli. *Biochim.Biophys.Acta.* 415:335-377.
- Briles, E.B., and A. Tomasz. 1970. Radioautographic evidence for equatorial wall growth in a gram-positive bacterium. Segregation of choline-3H-labeled teichoic acid. *J.Cell Biol.* 47:786-790.
- Broder, D.H., and K. Pogliano. 2006. Forespore engulfment mediated by a ratchet-like mechanism. *Cell.* 126:917-928. doi: 10.1016/j.cell.2006.06.053.

- Broome-Smith, J.K., A. Edelman, S. Yousif, and B.G. Spratt. 1985. The nucleotide sequences of the ponA and ponB genes encoding penicillin-binding protein 1A and 1B of Escherichia coli K12. *Eur.J.Biochem.* 147:437-446.
- Buist, G., A.N. Ridder, J. Kok, and O.P. Kuipers. 2006. Different subcellular locations of secretome components of Gram-positive bacteria. *Microbiology.* 152:2867-2874. doi: 10.1099/mic.0.29113-0.
- Burke, F.M., A. Di Poto, P. Speziale, and T.J. Foster. 2011. The A domain of fibronectin-binding protein B of Staphylococcus aureus contains a novel fibronectin binding site. *FEBS J.* 278:2359-2371. doi: 10.1111/j.1742-4658.2011.08159.x; 10.1111/j.1742-4658.2011.08159.x.
- Burman, L.G., and J.T. Park. 1983. Changes in the composition of Escherichia coli murein as it ages during exponential growth. *J.Bacteriol.* 155:447-453.
- Burton, B.M., K.A. Marquis, N.L. Sullivan, T.A. Rapoport, and D.Z. Rudner. 2007. The ATPase SpoIIIE transports DNA across fused septal membranes during sporulation in Bacillus subtilis. *Cell.* 131:1301-1312. doi: 10.1016/j.cell.2007.11.009.
- Bussiere, D.E., and D. Bastia. 1999. Termination of DNA replication of bacterial and plasmid chromosomes. *Mol.Microbiol.* 31:1611-1618.
- Campbell, J., A.K. Singh, J.P. Santa Maria Jr, Y. Kim, S. Brown, J.G. Swoboda, E. Mylonakis, B.J. Wilkinson, and S. Walker. 2011. Synthetic lethal compound combinations reveal a fundamental connection between wall teichoic acid and peptidoglycan biosyntheses in Staphylococcus aureus. *ACS Chem.Biol.* 6:106-116. doi: 10.1021/cb100269f.
- Carballido-Lopez, R., and J. Errington. 2003. The bacterial cytoskeleton: in vivo dynamics of the actin-like protein Mbl of Bacillus subtilis. *Dev.Cell.* 4:19-28.
- Carballido-López, R., A. Formstone, Y. Li, S.D. Ehrlich, P. Noirot, and J. Errington. 2006. Actin Homolog MreBH Governs Cell Morphogenesis by Localization of the Cell Wall Hydrolase LytE. *Developmental Cell.* 11:399-409. doi: 10.1016/j.devcel.2006.07.017.
- Carlsson, F., M. Stalhammar-Carlemalm, K. Flardh, C. Sandin, E. Carlemalm, and G. Lindahl. 2006. Signal sequence directs localized secretion of bacterial surface proteins. *Nature.* 442:943-946. doi: 10.1038/nature05021.
- Carruthers, M.M., and W.J. Kabat. 1983. Mediation of staphylococcal adherence to mucosal cells by lipoteichoic acid. *Infect.Immun.* 40:444-446.
- Cha, J.H., and G.C. Stewart. 1997. The divIVA minicell locus of Bacillus subtilis. *J.Bacteriol.* 179:1671-1683.
- Chatterjee, A.N. 1969. Use of bacteriophage-resistant mutants to study the nature of the bacteriophage receptor site of Staphylococcus aureus. *J.Bacteriol.* 98:519-527.
- Chaudhuri, R.R., A.G. Allen, P.J. Owen, G. Shalom, K. Stone, M. Harrison, T.A. Burgis, M. Lockyer, J. Garcia-Lara, S.J. Foster, S.J. Pleasance, S.E. Peters, D.J. Maskell, and I.G. Charles. 2009. Comprehensive identification of essential Staphylococcus aureus genes using Transposon-Mediated Differential Hybridisation (TMDH). *BMC Genomics.* 10:291. doi: 10.1186/1471-2164-10-291.
- Cho, H., H.R. McManus, S.L. Dove, and T.G. Bernhardt. 2011. Nucleoid occlusion factor SlmA is a DNA-activated FtsZ polymerization antagonist. *Proc.Natl.Acad.Sci.U.S.A.* 108:3773-3778. doi: 10.1073/pnas.1018674108.
- Claessen, D., R. Emmins, L.W. Hamoen, R.A. Daniel, J. Errington, and D.H. Edwards. 2008. Control of the cell elongation-division cycle by shuttling of PBP1 protein in Bacillus subtilis. *Mol.Microbiol.* 68:1029-1046. doi: 10.1111/j.1365-2958.2008.06210.x.
- COLE, R.M., and J.J. HAHN. 1962. Cell wall replication in Streptococcus pyogenes. *Science.* 135:722-724.
- Coley, J., M. Duckworth, and J. Baddiley. 1972. The occurrence of lipoteichoic acids in the membranes of gram-positive bacteria. *J.Gen.Microbiol.* 73:587-591.
- Corbin, B.D., Y. Wang, T.K. Beuria, and W. Margolin. 2007. Interaction between cell division proteins FtsE and FtsZ. *J.Bacteriol.* 189:3026-3035. doi: 10.1128/JB.01581-06.
- Corrigan, R.M., H. Miajlovic, and T.J. Foster. 2009. Surface proteins that promote adherence of Staphylococcus aureus to human desquamated nasal epithelial cells. *BMC Microbiol.* 9:22. doi: 10.1186/1471-2180-9-22.
- Daha, N.A., N.K. Banda, A. Roos, F.J. Beurskens, J.M. Bakker, M.R. Daha, and L.A. Trouw. 2011. Complement activation by (auto-) antibodies. *Mol.Immunol.* 48:1656-1665. doi: 10.1016/j.molimm.2011.04.024.
- Dajkovic, A., G. Lan, S.X. Sun, D. Wirtz, and J. Lutkenhaus. 2008a. MinC spatially controls bacterial cytokinesis by antagonizing the scaffolding function of FtsZ. *Curr.Biol.* 18:235-244. doi: 10.1016/j.cub.2008.01.042.
- Dajkovic, A., A. Mukherjee, and J. Lutkenhaus. 2008b. Investigation of regulation of FtsZ assembly by SulA and development of a model for FtsZ polymerization. *J.Bacteriol.* 190:2513-2526. doi: 10.1128/JB.01612-07.
- Daniel, R.A., S. Drake, C.E. Buchanan, R. Scholle, and J. Errington. 1994. The Bacillus subtilis spoVD gene encodes a mother-cell-specific penicillin-binding protein required for spore morphogenesis. *J.Mol.Biol.* 235:209-220.
- Daniel, R.A., and J. Errington. 2000. Intrinsic instability of the essential cell division protein FtsL of Bacillus subtilis and a role for DivIB protein in FtsL turnover. *Mol.Microbiol.* 36:278-289.
- Daniel, R.A., E.J. Harry, and J. Errington. 2000. Role of penicillin-binding protein PBP 2B in assembly and functioning of the division machinery of Bacillus subtilis. *Mol.Microbiol.* 35:299-311.
- Daniel, R.A., and J. Errington. 2003. Control of Cell Morphogenesis in Bacteria: Two Distinct Ways to Make a Rod-Shaped Cell. *Cell.* 113:767-776. doi: 10.1016/S0092-8674(03)00421-5.
- Danilova, O., R. Reyes-Lamothe, M. Pinskaya, D. Sherratt, and C. Possoz. 2007. MukB colocalizes with the oriC region and is required for organization of the two Escherichia coli chromosome arms into separate cell halves. *Mol.Microbiol.* 65:1485-1492. doi: 10.1111/j.1365-2958.2007.05881.x.
- de Boer, P.A. 2010. Advances in understanding E. coli cell fission. *Curr.Opin.Microbiol.* 13:730-737. doi: 10.1016/j.mib.2010.09.015.
- de Pedro, M.A., J.C. Quintela, J.V. Holtje, and H. Schwarz. 1997. Murein segregation in Escherichia coli. *J.Bacteriol.* 179:2823-2834.
- De Pedro, M.A., H. Schwarz, and A.L. Koch. 2003. Patchiness of murein insertion into the sidewall of Escherichia coli. *Microbiology.* 149:1753-1761.
- de Pedro, M.A., and U. Schwarz. 1981. Heterogeneity of newly inserted and preexisting murein in the sacculus of Escherichia coli. *Proc.Natl.Acad.Sci.U.S.A.* 78:5856-5860.
- DeDent, A., T. Bae, D.M. Missiakas, and O. Schneewind. 2008. Signal peptides direct surface proteins to two distinct envelope locations of Staphylococcus aureus. *EMBO J.* 27:2656-2668. doi: 10.1038/emboj.2008.185.

- Demchick, P., and A.L. Koch. 1996. The permeability of the wall fabric of Escherichia coli and Bacillus subtilis. *J.Bacteriol.* 178:768-773.
- Den Blaauwen, T., M.E. Aarsman, N.O. Vischer, and N. Nanninga. 2003. Penicillin-binding protein PBP2 of Escherichia coli localizes preferentially in the lateral wall and at mid-cell in comparison with the old cell pole. *Mol.Microbiol.* 47:539-547.
- den Blaauwen, T., M.A. de Pedro, M. Nguyen-Disteche, and J.A. Ayala. 2008. Morphogenesis of rod-shaped sacculi. *FEMS Microbiol.Rev.* 32:321-344. doi: 10.1111/j.1574-6976.2007.00090.x.
- DiScipio, R.G. 1991. The relationship between polymerization of complement component C9 and membrane channel formation. *J.Immunol.* 147:4239-4247.
- Divakaruni, A.V., C. Baida, C.L. White, and J.W. Gober. 2007. The cell shape proteins MreB and MreC control cell morphogenesis by positioning cell wall synthetic complexes. *Mol.Microbiol.* 66:174-188. doi: 10.1111/j.1365-2958.2007.05910.x.
- Dmitriev, B.A., F.V. Toukach, O. Holst, E.T. Rietschel, and S. Ehlers. 2004. Tertiary structure of Staphylococcus aureus cell wall murein. *J.Bacteriol.* 186:7141-7148. doi: 10.1128/JB.186.21.7141-7148.2004.
- Dominguez-Escobar, J., A. Chastanet, A.H. Crevenna, V. Fromion, R. Wedlich-Soldner, and R. Carballido-Lopez. 2011. Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria. *Science.* 333:225-228. doi: 10.1126/science.1203466.
- Dworkin, J. 2003. Transient genetic asymmetry and cell fate in a bacterium. *Trends Genet.* 19:107-112.
- Ebersbach, G., E. Galli, J. Moller-Jensen, J. Lowe, and K. Gerdes. 2008. Novel coiled-coil cell division factor ZapB stimulates Z ring assembly and cell division. *Mol.Microbiol.* 68:720-735. doi: 10.1111/j.1365-2958.2008.06190.x.
- Edwards, D.H., and J. Errington. 1997. The Bacillus subtilis DivIVA protein targets to the division septum and controls the site specificity of cell division. *Mol.Microbiol.* 24:905-915.
- Edwards, D.H., H.B. Thomaides, and J. Errington. 2000. Promiscuous targeting of Bacillus subtilis cell division protein DivIVA to division sites in Escherichia coli and fission yeast. *EMBO J.* 19:2719-2727. doi: 10.1093/emboj/19.11.2719.
- Ehlert, K., and J.V. Holtje. 1996. Role of precursor translocation in coordination of murein and phospholipid synthesis in Escherichia coli. *J.Bacteriol.* 178:6766-6771.
- Endo, Y., M. Matsushita, and T. Fujita. 2007. Role of ficolin in innate immunity and its molecular basis. *Immunobiology.* 212:371-379. doi: 10.1016/j.imbio.2006.11.014.
- Erickson, H.P. 1995. FtsZ, a prokaryotic homolog of tubulin? *Cell.* 80:367-370.
- Errington, J., R.A. Daniel, and D. Scheffers. 2003. Cytokinesis in Bacteria. *Microbiology and Molecular Biology Reviews.* 67:52-65. doi: 10.1128/MMBR.67.1.52-65.2003.
- Esko, J.D., T.L. Doering, and C.R.H. Raetz. 2009. Eubacteria and Archaea. *In* Essentials of Glycobiology. A. Varki, R.D. Cummings, J.D. Esko, H.H. Freeze, P. Stanley, C.R. Bertozzi, G.W. Hart and M.E. Etzler, editors. The Consortium of Glycobiology Editors, La Jolla, California, Cold Spring Harbor (NY).
- Fay, A., P. Meyer, and J. Dworkin. 2010. Interactions between late-acting proteins required for peptidoglycan synthesis during sporulation. *J.Mol.Biol.* 399:547-561. doi: 10.1016/j.jmb.2010.04.036.
- Fernie-King, B.A., D.J. Seilly, and P.J. Lachmann. 2004. The interaction of streptococcal inhibitor of complement (SIC) and its proteolytic fragments with the human beta defensins. *Immunology.* 111:444-452. doi: 10.1111/j.0019-2805.2004.01837.x.
- Fernie-King, B.A., D.J. Seilly, C. Willers, R. Wurzner, A. Davies, and P.J. Lachmann. 2001. Streptococcal inhibitor of complement (SIC) inhibits the membrane attack complex by preventing uptake of C567 onto cell membranes. *Immunology.* 103:390-398.
- Feucht, A., I. Lucet, M.D. Yudkin, and J. Errington. 2001. Cytological and biochemical characterization of the FtsA cell division protein of Bacillus subtilis. *Mol.Microbiol.* 40:115-125.
- Flardh, K. 2003. Growth polarity and cell division in Streptomyces. *Curr.Opin.Microbiol.* 6:564-571.
- Fordham, W.D., and C. Gilvarg. 1974. Kinetics of cross-linking of peptidoglycan in Bacillus megaterium. *J.Biol.Chem.* 249:2478-2482.
- Frank, M.M. 2001. Annihilating host defense. *Nat.Med.* 7:1285-1286. doi: 10.1038/nm1201-1285.
- Frankel, M.B., A.P. Hendrickx, D.M. Missiakas, and O. Schneewind. 2011. LytN, a murein hydrolase in the cross-wall compartment of Staphylococcus aureus, is involved in proper bacterial growth and envelope assembly. *J.Biol.Chem.* 286:32593-32605. doi: 10.1074/jbc.M111.258863.
- Fu, X., Y.L. Shih, Y. Zhang, and L.I. Rothfield. 2001. The MinE ring required for proper placement of the division site is a mobile structure that changes its cellular location during the Escherichia coli division cycle. *Proc.Natl.Acad.Sci.U.S.A.* 98:980-985. doi: 10.1073/pnas.031549298.
- Fujita, M., and R. Losick. 2003. The master regulator for entry into sporulation in Bacillus subtilis becomes a cell-specific transcription factor after asymmetric division. *Genes Dev.* 17:1166-1174. doi: 10.1101/gad.1078303.
- Fukushima, T., A. Afkham, S. Kurosawa, T. Tanabe, H. Yamamoto, and J. Sekiguchi. 2006. A new D,L-endopeptidase gene product, YojL (renamed CwlS), plays a role in cell separation with LytE and LytF in Bacillus subtilis. *J.Bacteriol.* 188:5541-5550. doi: 10.1128/JB.00188-06.
- Gally, D., and A.R. Archibald. 1993. Cell wall assembly in Staphylococcus aureus: proposed absence of secondary crosslinking reactions. *J.Gen.Microbiol.* 139:1907-1913.
- Gamba, P., J. Veening, N.J. Saunders, L.W. Hamoen, and R.A. Daniel. July 1, 2009. Two-Step Assembly Dynamics of the Bacillus subtilis Divisome. *Journal of Bacteriology.* 191:4186-4194. doi: 10.1128/JB.01758-08.
- Garner, E.C., R. Bernard, W. Wang, X. Zhuang, D.Z. Rudner, and T. Mitchison. 2011. Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in B. subtilis. *Science.* 333:222-225. doi: 10.1126/science.1203285.
- Garti-Levi, S., R. Hazan, J. Kain, M. Fujita, and S. Ben-Yehuda. 2008. The FtsEX ABC transporter directs cellular differentiation in Bacillus subtilis. *Mol.Microbiol.* 69:1018-1028. doi: 10.1111/j.1365-2958.2008.06340.x.
- Gerding, M.A., Y. Ogata, N.D. Pecora, H. Niki, and P.A. de Boer. 2007. The trans-envelope Tol-Pal complex is part of the cell division machinery and required for proper outer-membrane invagination during cell constriction in E. coli. *Mol.Microbiol.* 63:1008-1025. doi: 10.1111/j.1365-2958.2006.05571.x.
- Ghuysen, J.M. 1990. Membrane topology, structure, and functions of the penicillin-interactive proteins. *Biotechnol.Appl.Biochem.* 12:468-472.
- Giesbrecht, P., T. Kersten, H. Maidhof, and J. Wecke. 1998. Staphylococcal cell wall: morphogenesis and fatal variations in the presence of penicillin. *Microbiol.Mol.Biol.Rev.* 62:1371-1414.

- Giesbrecht, P., H. Labischinski, and J. Wecke. 1985. A special morphogenetic wall defect and the subsequent activity of "murosomes" as the very reason for penicillin-induced bacteriolysis in staphylococci. *Arch.Microbiol.* 141:315-324.
- Giesbrecht, P., J. Wecke, and B. Reinicke. 1976. On the morphogenesis of the cell wall of staphylococci. *Int.Rev.Cytol.* 44:225-318.
- Glauner, B., and J.V. Holtje. 1990. Growth pattern of the murein sacculus of Escherichia coli. *J.Biol.Chem.* 265:18988-18996.
- Glauner, B., J.V. Holtje, and U. Schwarz. 1988. The composition of the murein of Escherichia coli. *J.Biol.Chem.* 263:10088-10095.
- Goehring, N.W., M.D. Gonzalez, and J. Beckwith. 2006. Premature targeting of cell division proteins to midcell reveals hierarchies of protein interactions involved in divisome assembly. *Mol.Microbiol.* 61:33-45. doi: 10.1111/j.1365-2958.2006.05206.x.
- Gueiros-Filho, F.J., and R. Losick. 2002. A widely conserved bacterial cell division protein that promotes assembly of the tubulin-like protein FtsZ. *Genes Dev.* 16:2544-2556. doi: 10.1101/gad.1014102.
- Gundogdu, M.E., Y. Kawai, N. Pavlendova, N. Ogasawara, J. Errington, D.J. Scheffers, and L.W. Hamoen. 2011. Large ring polymers align FtsZ polymers for normal septum formation. *EMBO J.* 30:617-626. doi: 10.1038/emboj.2010.345.
- Hahn, J., N. Kramer, K. Briley Jr, and D. Dubnau. 2009. McsA and B mediate the delocalization of competence proteins from the cell poles of Bacillus subtilis. *Mol.Microbiol.* 72:202-215. doi: 10.1111/j.1365-2958.2009.06636.x.
- Hale, C.A., and P.A. de Boer. 1999. Recruitment of ZipA to the septal ring of Escherichia coli is dependent on FtsZ and independent of FtsA. *J.Bacteriol.* 181:167-176.
- Hale, C.A., and P.A. de Boer. 1997. Direct binding of FtsZ to ZipA, an essential component of the septal ring structure that mediates cell division in E. coli. *Cell.* 88:175-185.
- Hale, C.A., A.C. Rhee, and P.A. de Boer. 2000. ZipA-induced bundling of FtsZ polymers mediated by an interaction between C-terminal domains. *J.Bacteriol.* 182:5153-5166.
- Hale, C.A., D. Shiomi, B. Liu, T.G. Bernhardt, W. Margolin, H. Niki, and P.A. de Boer. 2011. Identification of Escherichia coli ZapC (YcbW) as a component of the division apparatus that binds and bundles FtsZ polymers. *J.Bacteriol.* 193:1393-1404. doi: 10.1128/JB.01245-10.
- Hanlon, D.W., and G.W. Ordal. 1994. Cloning and characterization of genes encoding methyl-accepting chemotaxis proteins in Bacillus subtilis. *J.Biol.Chem.* 269:14038-14046.
- Harry, E.J., J. Rodwell, and R.G. Wake. 1999. Co-ordinating DNA replication with cell division in bacteria: a link between the early stages of a round of replication and mid-cell Z ring assembly. *Mol.Microbiol.* 33:33-40.
- Harry, E., L. Monahan, and L. Thompson. Bacterial Cell Division: The Mechanism and Its Precison. *In* International Review of Cytology. Academic Press. 27-94.
- Heidrich, C., M.F. Templin, A. Ursinus, M. Merdanovic, J. Berger, H. Schwarz, M.A. de Pedro, and J.V. Holtje. 2001. Involvement of N-acetylmuramyl-L-alanine amidases in cell separation and antibiotic-induced autolysis of Escherichia coli. *Mol.Microbiol.* 41:167-178.
- Higgins, D., and J. Dworkin. 2012. Recent progress in Bacillus subtilis sporulation. *FEMS Microbiol.Rev.* 36:131-148. doi: 10.1111/j.1574-6976.2011.00310.x; 10.1111/j.1574-6976.2011.00310.x.
- Higgins, M.L., and G.D. Shockman. 1970. Model for cell wall growth of Streptococcus faecalis. *J.Bacteriol.* 101:643-648.
- Holtje, J.V. 1998. Growth of the stress-bearing and shape-maintaining murein sacculus of Escherichia coli. *Microbiol.Mol.Biol.Rev.* 62:181-203.
- Holtje, J.V. 1996. Molecular interplay of murein synthases and murein hydrolases in Escherichia coli. *Microb.Drug Resist.* 2:99-103.
- Horsburgh, G.J., A. Atrih, and S.J. Foster. 2003a. Characterization of LytH, a differentiation-associated peptidoglycan hydrolase of Bacillus subtilis involved in endospore cortex maturation. *J.Bacteriol.* 185:3813-3820.
- Horsburgh, G.J., A. Atrih, M.P. Williamson, and S.J. Foster. 2003b. LytG of Bacillus subtilis is a novel peptidoglycan hydrolase: the major active glucosaminidase. *Biochemistry.* 42:257-264. doi: 10.1021/bi020498c.
- Hu, Z., C. Saez, and J. Lutkenhaus. 2003. Recruitment of MinC, an inhibitor of Z-ring formation, to the membrane in Escherichia coli: role of MinD and MinE. *J.Bacteriol.* 185:196-203.
- Ikeda, M., M. Wachi, H.K. Jung, F. Ishino, and M. Matsuhashi. 1991. The Escherichia coli mraY gene encoding UDP-N-acetylmuramoyl-pentapeptide: undecaprenyl-phosphate phospho-N-acetylmuramoyl-pentapeptide transferase. *J.Bacteriol.* 173:1021-1026.
- Ishikawa, S., Y. Hara, R. Ohnishi, and J. Sekiguchi. 1998. Regulation of a new cell wall hydrolase gene, cwlF, which affects cell separation in Bacillus subtilis. *J.Bacteriol.* 180:2549-2555.
- Ishino, F., and M. Matsuhashi. 1981. Peptidoglycan synthetic enzyme activities of highly purified penicillin-binding protein 3 in Escherichia coli: a septum-forming reaction sequence. *Biochem.Biophys.Res.Commun.* 101:905-911.
- Ishino, F., W. Park, S. Tomioka, S. Tamaki, I. Takase, K. Kunugita, H. Matsuzawa, S. Asoh, T. Ohta, and B.G. Spratt. 1986. Peptidoglycan synthetic activities in membranes of Escherichia coli caused by overproduction of penicillin-binding protein 2 and rodA protein. *J.Biol.Chem.* 261:7024-7031.
- Jaffe, A., R. D'Ari, and S. Hiraga. 1988. Minicell-forming mutants of Escherichia coli: production of minicells and anucleate rods. *J.Bacteriol.* 170:3094-3101.
- Jensen, S.O., L.S. Thompson, and E.J. Harry. 2005. Cell division in Bacillus subtilis: FtsZ and FtsA association is Z-ring independent, and FtsA is required for efficient midcell Z-Ring assembly. *J.Bacteriol.* 187:6536-6544. doi: 10.1128/JB.187.18.6536-6544.2005.
- Johnson, A., and M. O'Donnell. 2005. Cellular DNA replicases: components and dynamics at the replication fork. *Annu.Rev.Biochem.* 74:283-315. doi: 10.1146/annurev.biochem.73.011303.073859.
- Joiner, K., E. Brown, C. Hammer, K. Warren, and M. Frank. 1983. Studies on the mechanism of bacterial resistance to complement-mediated killing. III. C5b-9 deposits stably on rough and type 7 S. pneumoniae without causing bacterial killing. *J.Immunol.* 130:845-849.
- Jones, L.J., R. Carballido-Lopez, and J. Errington. 2001. Control of cell shape in bacteria: helical, actin-like filaments in Bacillus subtilis. *Cell.* 104:913-922.
- Josefsson, E., J. Higgins, T.J. Foster, and A. Tarkowski. 2008. Fibrinogen binding sites P336 and Y338 of clumping factor A are crucial for Staphylococcus aureus virulence. *PLoS One.* 3:e2206. doi: 10.1371/journal.pone.0002206.

- Kajimura, J., T. Fujiwara, S. Yamada, Y. Suzawa, T. Nishida, Y. Oyamada, I. Hayashi, J. Yamagishi, H. Komatsuzawa, and M. Sugai. 2005. Identification and molecular characterization of an N-acetylmuramyl-l-alanine amidase Sle1 involved in cell separation of Staphylococcus aureus. *Mol.Microbiol.* 58:1087-1101. doi: 10.1111/j.1365-2958.2005.04881.x.
- Kawai, F., M. Shoda, R. Harashima, Y. Sadaie, H. Hara, and K. Matsumoto. 2004. Cardiolipin domains in Bacillus subtilis marburg membranes. *J.Bacteriol.* 186:1475-1483.
- Kawai, Y., R.A. Daniel, and J. Errington. 2009. Regulation of cell wall morphogenesis in Bacillus subtilis by recruitment of PBP1 to the MreB helix. *Mol.Microbiol.* 71:1131-1144. doi: 10.1111/j.1365-2958.2009.06601.x.
- Kay, D., and S.C. Warren. 1968. Sporulation in Bacillus subtilis. Morphological changes. *Biochem.J.* 109:819-824.
- Kirby, J.R., T.B. Niewold, S. Maloy, and G.W. Ordal. 2000. CheB is required for behavioural responses to negative stimuli during chemotaxis in Bacillus subtilis. *Mol.Microbiol.* 35:44-57. doi: 10.1046/j.1365-2958.2000.01676.x.
- Koch, A.L., and R.J. Doyle. 1985. Inside-to-outside growth and turnover of the wall of gram-positive rods. *J.Theor.Biol.* 117:137-157. doi: 10.1016/S0022-5193(85)80169-7.
- Koyama, T., M. Yamada, and M. Matsuhashi. 1977. Formation of regular packets of Staphylococcus aureus cells. *J.Bacteriol.* 129:1518-1523.
- Kruse, T., J. Bork-Jensen, and K. Gerdes. 2005. The morphogenetic MreBCD proteins of Escherichia coli form an essential membrane-bound complex. *Mol.Microbiol.* 55:78-89. doi: 10.1111/j.1365-2958.2004.04367.x.
- Kruse, T., and K. Gerdes. 2005. Bacterial DNA segregation by the actin-like MreB protein. *Trends Cell Biol.* 15:343-345. doi: 10.1016/j.tcb.2005.05.002.
- Lackner, L.L., D.M. Raskin, and P.A. de Boer. 2003. ATP-dependent interactions between Escherichia coli Min proteins and the phospholipid membrane in vitro. *J.Bacteriol.* 185:735-749.
- Land, A.D., and M.E. Winkler. 2011. The requirement for pneumococcal MreC and MreD is relieved by inactivation of the gene encoding PBP1a. *J.Bacteriol.* 193:4166-4179. doi: 10.1128/JB.05245-11.
- Lapidot, A., and C.S. Irving. 1979. Nitrogen-15 and carbon-13 dynamic nuclear magnetic resonance study of chain segmental motion of the peptidoglycan pentaglycine chain of 15N-Gly- and 13C2-Gly-labeled Staphylococcus aureus cells and isolated cell walls. *Biochemistry.* 18:1788-1796.
- Lapidot, A., and C.S. Irving. 1977. Dynamic structure of whole cells probed by nuclear Overhauser enhanced nitrogen-15 nuclear magnetic resonance spectroscopy. *Proc.Natl.Acad.Sci.U.S.A.* 74:1988-1992.
- Leaver, M., and J. Errington. 2005. Roles for MreC and MreD proteins in helical growth of the cylindrical cell wall in Bacillus subtilis. *Mol.Microbiol.* 57:1196-1209. doi: 10.1111/j.1365-2958.2005.04736.x.
- Lee, S., and C.W. Price. 1993. The minCD locus of Bacillus subtilis lacks the minE determinant that provides topological specificity to cell division. *Mol.Microbiol.* 7:601-610.
- Lemon, K.P., and A.D. Grossman. 2001. The extrusion-capture model for chromosome partitioning in bacteria. *Genes Dev.* 15:2031-2041. doi: 10.1101/gad.913301.
- Lenarcic, R., S. Halbedel, L. Visser, M. Shaw, L.J. Wu, J. Errington, D. Marenduzzo, and L.W. Hamoen. 2009. Localisation of DivIVA by targeting to negatively curved membranes. *EMBO J.* 28:2272-2282. doi: 10.1038/emboj.2009.129.
- Leski, T.A., and A. Tomasz. 2005. Role of penicillin-binding protein 2 (PBP2) in the antibiotic susceptibility and cell wall cross-linking of Staphylococcus aureus: evidence for the cooperative functioning of PBP2, PBP4, and PBP2A. *J.Bacteriol.* 187:1815-1824. doi: 10.1128/JB.187.5.1815-1824.2005.
- Levin, P.A., I.G. Kurtser, and A.D. Grossman. 1999. Identification and characterization of a negative regulator of FtsZ ring formation in Bacillus subtilis. *Proc.Natl.Acad.Sci.U.S.A.* 96:9642-9647.
- Levin, P.A., P.S. Margolis, P. Setlow, R. Losick, and D. Sun. 1992. Identification of Bacillus subtilis genes for septum placement and shape determination. *J.Bacteriol.* 174:6717-6728.
- Lim, D., and N.C. Strynadka. 2002. Structural basis for the beta lactam resistance of PBP2a from methicillin-resistant Staphylococcus aureus. *Nat.Struct.Biol.* 9:870-876. doi: 10.1038/nsb858.
- Liu, M., T.S. Hanks, J. Zhang, M.J. McClure, D.W. Siemsen, J.L. Elser, M.T. Quinn, and B. Lei. 2006a. Defects in ex vivo and in vivo growth and sensitivity to osmotic stress of group A Streptococcus caused by interruption of response regulator gene vicR. *Microbiology.* 152:967-978. doi: 10.1099/mic.0.28706-0.
- Liu, N.J., R.J. Dutton, and K. Pogliano. 2006b. Evidence that the SpoIIIE DNA translocase participates in membrane fusion during cytokinesis and engulfment. *Mol.Microbiol.* 59:1097-1113. doi: 10.1111/j.1365-2958.2005.05004.x.
- Lleo, M.M., P. Canepari, and G. Satta. 1990. Bacterial cell shape regulation: testing of additional predictions unique to the two-competing-sites model for peptidoglycan assembly and isolation of conditional rod-shaped mutants from some wild-type cocci. *J.Bacteriol.* 172:3758-3771.
- Low, H.H., M.C. Moncrieffe, and J. Lowe. 2004. The crystal structure of ZapA and its modulation of FtsZ polymerisation. *J.Mol.Biol.* 341:839-852. doi: 10.1016/j.jmb.2004.05.031.
- Lucet, I., A. Feucht, M.D. Yudkin, and J. Errington. 2000. Direct interaction between the cell division protein FtsZ and the cell differentiation protein SpoIIE. *EMBO J.* 19:1467-1475. doi: 10.1093/emboj/19.7.1467.
- Lunde, C.S., C.H. Rexer, S.R. Hartouni, S. Axt, and B.M. Benton. 2010. Fluorescence microscopy demonstrates enhanced targeting of telavancin to the division septum of Staphylococcus aureus. *Antimicrob.Agents Chemother.* 54:2198-2200. doi: 10.1128/AAC.01609-09.
- Maidhof, H., B. Reinicke, P. Blumel, B. Berger-Bachi, and H. Labischinski. 1991. femA, which encodes a factor essential for expression of methicillin resistance, affects glycine content of peptidoglycan in methicillin-resistant and methicillin-susceptible Staphylococcus aureus strains. *J.Bacteriol.* 173:3507-3513.
- Margolin, W. 2001. Spatial regulation of cytokinesis in bacteria. *Curr.Opin.Microbiol.* 4:647-652.
- Marston, A.L., and J. Errington. 1999. Selection of the midcell division site in Bacillus subtilis through MinD-dependent polar localization and activation of MinC. *Mol.Microbiol.* 33:84-96.
- Marston, A.L., H.B. Thomaides, D.H. Edwards, M.E. Sharpe, and J. Errington. 1998. Polar localization of the MinD protein of Bacillus subtilis and its role in selection of the mid-cell division site. *Genes Dev.* 12:3419-3430.
- Mascarenhas, J., J. Soppa, A.V. Strunnikov, and P.L. Graumann. 2002. Cell cycle-dependent localization of two novel prokaryotic chromosome segregation and condensation proteins in Bacillus subtilis that interact with SMC protein. *EMBO J.* 21:3108-3118. doi: 10.1093/emboj/cdf314.

- Massey, T.H., C.P. Mercogliano, J. Yates, D.J. Sherratt, and J. Lowe. 2006. Double-stranded DNA translocation: structure and mechanism of hexameric FtsK. *Mol.Cell.* 23:457-469. doi: 10.1016/j.molcel.2006.06.019.
- Matias, V.R., and T.J. Beveridge. 2007. Cryo-electron microscopy of cell division in Staphylococcus aureus reveals a mid-zone between nascent cross walls. *Mol.Microbiol.* 64:195-206. doi: 10.1111/j.1365-2958.2007.05634.x.
- Matias, V.R., and T.J. Beveridge. 2006. Native cell wall organization shown by cryo-electron microscopy confirms the existence of a periplasmic space in Staphylococcus aureus. *J.Bacteriol.* 188:1011-1021. doi: 10.1128/JB.188.3.1011-1021.2006.
- Matsushita, M. 1996. The lectin pathway of the complement system. *Microbiol.Immunol.* 40:887-893.
- Mattei, P.J., D. Neves, and A. Dessen. 2010. Bridging cell wall biosynthesis and bacterial morphogenesis. *Curr.Opin.Struct.Biol.* 20:749-755. doi: 10.1016/j.sbi.2010.09.014.
- McKenney, P.T., and P. Eichenberger. 2011. Dynamics of spore coat morphogenesis in Bacillus subtilis. *Mol.Microbiol.* doi: 10.1111/j.1365-2958.2011.07936.x; 10.1111/j.1365-2958.2011.07936.x.
- McPherson, D.C., A. Driks, and D.L. Popham. 2001. Two class A high-molecular-weight penicillin-binding proteins of Bacillus subtilis play redundant roles in sporulation. *J.Bacteriol.* 183:6046-6053. doi: 10.1128/JB.183.20.6046-6053.2001.
- Meile, J., L.J. Wu, S.D. Ehrlich, J. Errington, and P. Noirot. 2006. Systematic localisation of proteins fused to the green fluorescent protein in Bacillus subtilis: Identification of new proteins at the DNA replication factory. *Proteomics.* 6:2135-2146. doi: 10.1002/pmic.200500512.
- Memmi, G., S.R. Filipe, M.G. Pinho, Z. Fu, and A. Cheung. 2008. Staphylococcus aureus PBP4 is essential for beta-lactam resistance in community-acquired methicillin-resistant strains. *Antimicrob.Agents Chemother.* 52:3955-3966. doi: 10.1128/AAC.00049-08.
- Mengin-Lecreulx, D., L. Texier, M. Rousseau, and J. van Heijenoort. 1991. The murG gene of Escherichia coli codes for the UDP-N-acetylglucosamine: N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase involved in the membrane steps of peptidoglycan synthesis. *J.Bacteriol.* 173:4625-4636.
- Meyer, P., J. Gutierrez, K. Pogliano, and J. Dworkin. 2010. Cell wall synthesis is necessary for membrane dynamics during sporulation of Bacillus subtilis. *Mol.Microbiol.* 76:956-970. doi: 10.1111/j.1365-2958.2010.07155.x.
- Miyao, A., A. Yoshimura, T. Sato, T. Yamamoto, G. Theeragool, and Y. Kobayashi. 1992. Sequence of the Bacillus subtilis homolog of the Escherichia coli cell-division gene murG. *Gene.* 118:147-148.
- Mobley, H.L., A.L. Koch, R.J. Doyle, and U.N. Streips. 1984. Insertion and fate of the cell wall in Bacillus subtilis. *J.Bacteriol.* 158:169-179.
- Mohammadi, T., A. Karczmarek, M. Crouvoisier, A. Bouhss, D. Mengin-Lecreulx, and T. den Blaauwen. 2007. The essential peptidoglycan glycosyltransferase MurG forms a complex with proteins involved in lateral envelope growth as well as with proteins involved in cell division in Escherichia coli. *Mol.Microbiol.* 65:1106-1121. doi: 10.1111/j.1365-2958.2007.05851.x.
- Moll, A., and M. Thanbichler. 2009. FtsN-like proteins are conserved components of the cell division machinery in proteobacteria. *Mol.Microbiol.* 72:1037-1053. doi: 10.1111/j.1365-2958.2009.06706.x.
- Morlot, C., M. Noirclerc-Savoye, A. Zapun, O. Dideberg, and T. Vernet. 2004. The D,D-carboxypeptidase PBP3 organizes the division process of Streptococcus pneumoniae. *Mol.Microbiol.* 51:1641-1648.
- Morlot, C., L. Pernot, A. Le Gouellec, A.M. Di Guilmi, T. Vernet, O. Dideberg, and A. Dessen. 2005. Crystal structure of a peptidoglycan synthesis regulatory factor (PBP3) from Streptococcus pneumoniae. *J.Biol.Chem.* 280:15984-15991. doi: 10.1074/jbc.M408446200.
- Morlot, C., T. Uehara, K.A. Marquis, T.G. Bernhardt, and D.Z. Rudner. 2010. A highly coordinated cell wall degradation machine governs spore morphogenesis in Bacillus subtilis. *Genes Dev.* 24:411-422. doi: 10.1101/gad.1878110.
- Morlot, C., A. Zapun, O. Dideberg, and T. Vernet. 2003. Growth and division of Streptococcus pneumoniae: localization of the high molecular weight penicillin-binding proteins during the cell cycle. *Mol.Microbiol.* 50:845-855.
- Mott, M.L., and J.M. Berger. 2007. DNA replication initiation: mechanisms and regulation in bacteria. *Nat.Rev.Microbiol.* 5:343-354. doi: 10.1038/nrmicro1640.
- Mushegian, A.R., K.J. Fullner, E.V. Koonin, and E.W. Nester. 1996. A family of lysozyme-like virulence factors in bacterial pathogens of plants and animals. *Proc.Natl.Acad.Sci.U.S.A.* 93:7321-7326.
- Ng, W.L., G.T. Robertson, K.M. Kazmierczak, J. Zhao, R. Gilmour, and M.E. Winkler. 2003. Constitutive expression of PcsB suppresses the requirement for the essential VicR (YycF) response regulator in Streptococcus pneumoniae R6. *Mol.Microbiol.* 50:1647-1663.
- Noirclerc-Savoye, M., C. Morlot, P. Gérard, T. Vernet, and A. Zapun. 2003. Expression and purification of FtsW and RodA from Streptococcus pneumoniae, two membrane proteins involved in cell division and cell growth, respectively. *Protein Expr.Purif.* 30:18-25. doi: 10.1016/S1046-5928(03)00051-2.
- Ohnishi, R., S. Ishikawa, and J. Sekiguchi. 1999. Peptidoglycan hydrolase LytF plays a role in cell separation with CwlF during vegetative growth of Bacillus subtilis. *J.Bacteriol.* 181:3178-3184.
- Oku, Y., K. Kurokawa, M. Matsuo, S. Yamada, B.L. Lee, and K. Sekimizu. 2009. Pleiotropic roles of polyglycerolphosphate synthase of lipoteichoic acid in growth of Staphylococcus aureus cells. *J.Bacteriol.* 191:141-151. doi: 10.1128/JB.01221-08.
- Oshida, T., M. Sugai, H. Komatsuzawa, Y.M. Hong, H. Suginaka, and A. Tomasz. 1995. A Staphylococcus aureus autolysin that has an N-acetylmuramoyl-L-alanine amidase domain and an endo-beta-N-acetylglucosaminidase domain: cloning, sequence analysis, and characterization. *Proc.Natl.Acad.Sci.U.S.A.* 92:285-289.
- Over, B., R. Heusser, N. McCallum, B. Schulthess, P. Kupferschmied, J.M. Gaiani, C.D. Sifri, B. Berger-Bachi, and P. Stutzmann Meier. 2011. LytR-CpsA-Psr proteins in Staphylococcus aureus display partial functional redundancy and the deletion of all three severely impairs septum placement and cell separation. *FEMS Microbiol.Lett.* 320:142-151. doi: 10.1111/j.1574-6968.2011.02303.x; 10.1111/j.1574-6968.2011.02303.x.
- Pancholi, V., G. Boel, and H. Jin. 2010. Streptococcus pyogenes Ser/Thr kinase-regulated cell wall hydrolase is a cell division plane-recognizing and chain-forming virulence factor. *J.Biol.Chem.* 285:30861-30874. doi: 10.1074/jbc.M110.153825.
- Patrick, J.E., and D.B. Kearns. 2008. MinJ (YvjD) is a topological determinant of cell division in Bacillus subtilis. *Mol.Microbiol.* 70:1166-1179. doi: 10.1111/j.1365-2958.2008.06469.x.
- Pedersen, L.B., E.R. Angert, and P. Setlow. 1999. Septal localization of penicillin-binding protein 1 in Bacillus subtilis. *J.Bacteriol.* 181:3201-3211.

- Pereira, S.F., A.O. Henriques, M.G. Pinho, H. de Lencastre, and A. Tomasz. 2009. Evidence for a dual role of PBP1 in the cell division and cell separation of Staphylococcus aureus. *Mol.Microbiol.* 72:895-904. doi: 10.1111/j.1365-2958.2009.06687.x.
- Peschel, A., M. Otto, R.W. Jack, H. Kalbacher, G. Jung, and F. Gotz. 1999. Inactivation of the dlt operon in Staphylococcus aureus confers sensitivity to defensins, protegrins, and other antimicrobial peptides. *J.Biol.Chem.* 274:8405-8410.
- Peschel, A., C. Vuong, M. Otto, and F. Gotz. 2000. The D-alanine residues of Staphylococcus aureus teichoic acids alter the susceptibility to vancomycin and the activity of autolytic enzymes. *Antimicrob.Agents Chemother.* 44:2845-2847.
- Pichoff, S., and J. Lutkenhaus. 2002. Unique and overlapping roles for ZipA and FtsA in septal ring assembly in Escherichia coli. *EMBO J.* 21:685-693.
- Piggot, P.J., and D.W. Hilbert. 2004. Sporulation of Bacillus subtilis. *Curr.Opin.Microbiol.* 7:579-586. doi: 10.1016/j.mib.2004.10.001.
- Pinho, M.G., and J. Errington. 2004. A divIVA null mutant of Staphylococcus aureus undergoes normal cell division. *FEMS Microbiol.Lett.* 240:145-149. doi: 10.1016/j.femsle.2004.09.038.
- Pinho, M.G., and J. Errington. 2003. Dispersed mode of Staphylococcus aureus cell wall synthesis in the absence of the division machinery. *Mol.Microbiol.* 50:871-881.
- Pinho, M.G., and J. Errington. 2005. Recruitment of penicillin-binding protein PBP2 to the division site of Staphylococcus aureus is dependent on its transpeptidation substrates. *Mol.Microbiol.* 55:799-807. doi: 10.1111/j.1365-2958.2004.04420.x.
- Pogliano, K., E. Harry, and R. Losick. 1995. Visualization of the subcellular location of sporulation proteins in Bacillus subtilis using immunofluorescence microscopy. *Mol.Microbiol.* 18:459-470.
- Popham, D.L. 2002. Specialized peptidoglycan of the bacterial endospore: the inner wall of the lockbox. *Cell Mol.Life Sci.* 59:426-433.
- Popham, D.L., M.E. Gilmore, and P. Setlow. 1999. Roles of low-molecular-weight penicillin-binding proteins in Bacillus subtilis spore peptidoglycan synthesis and spore properties. *J.Bacteriol.* 181:126-132.
- Popham, D.L., B. Illades-Aguiar, and P. Setlow. 1995. The Bacillus subtilis dacB gene, encoding penicillin-binding protein 5*, is part of a three-gene operon required for proper spore cortex synthesis and spore core dehydration. *J.Bacteriol.* 177:4721-4729.
- Potluri, L., A. Karczmarek, J. Verheul, A. Piette, J.M. Wilkin, N. Werth, M. Banzhaf, W. Vollmer, K.D. Young, M. Nguyen-Disteche, and T. den Blaauwen. 2010. Septal and lateral wall localization of PBP5, the major D,D-carboxypeptidase of Escherichia coli, requires substrate recognition and membrane attachment. *Mol.Microbiol.* 77:300-323. doi: 10.1111/j.1365-2958.2010.07205.x.
- Prats, R., and M.A. de Pedro. 1989. Normal growth and division of Escherichia coli with a reduced amount of murein. *J.Bacteriol.* 171:3740-3745.
- Priyadarshini, R., D.L. Popham, and K.D. Young. 2006. Daughter cell separation by penicillin-binding proteins and peptidoglycan amidases in Escherichia coli. *J.Bacteriol.* 188:5345-5355. doi: 10.1128/JB.00476-06.
- Ptacin, J.L., M. Nollmann, E.C. Becker, N.R. Cozzarelli, K. Pogliano, and C. Bustamante. 2008. Sequence-directed DNA export guides chromosome translocation during sporulation in Bacillus subtilis. *Nat.Struct.Mol.Biol.* 15:485-493. doi: 10.1038/nsmb.1412.
- Quisel, J.D., D.C. Lin, and A.D. Grossman. 1999. Control of development by altered localization of a transcription factor in B. subtilis. *Mol.Cell.* 4:665-672.
- Ramadurai, L., and R.K. Jayaswal. 1997. Molecular cloning, sequencing, and expression of lytM, a unique autolytic gene of Staphylococcus aureus. *J.Bacteriol.* 179:3625-3631.
- Ramamurthi, K.S., S. Lecuyer, H.A. Stone, and R. Losick. 2009. Geometric cue for protein localization in a bacterium. *Science.* 323:1354-1357. doi: 10.1126/science.1169218.
- Raskin, D.M., and P.A. de Boer. 1999. MinDE-dependent pole-to-pole oscillation of division inhibitor MinC in Escherichia coli. *J.Bacteriol.* 181:6419-6424.
- Raz, A., and V.A. Fischetti. 2008. Sortase A localizes to distinct foci on the Streptococcus pyogenes membrane. *Proc.Natl.Acad.Sci.U.S.A.* 105:18549-18554. doi: 10.1073/pnas.0808301105.
- Real, G., A. Fay, A. Eldar, S.M. Pinto, A.O. Henriques, and J. Dworkin. 2008. Determinants for the subcellular localization and function of a nonessential SEDS protein. *J.Bacteriol.* 190:363-376. doi: 10.1128/JB.01482-07.
- Regamey, A., E.J. Harry, and R.G. Wake. 2000. Mid-cell Z ring assembly in the absence of entry into the elongation phase of the round of replication in bacteria: co-ordinating chromosome replication with cell division. *Mol.Microbiol.* 38:423-434.
- Ridder, A.N., E.J. de Jong, J.D. Jongbloed, and O.P. Kuipers. 2009. Subcellular localization of TatAd of Bacillus subtilis depends on the presence of TatCd or TatCy. *J.Bacteriol.* 191:4410-4418. doi: 10.1128/JB.00215-09.
- Rogers, H.J. 1979. Biogenesis of the wall in bacterial morphogenesis. *Adv.Microb.Physiol.* 19:1-62.
- Rosch, J.W., and M.G. Caparon. 2005. The ExPortal: an organelle dedicated to the biogenesis of secreted proteins in Streptococcus pyogenes. *Mol.Microbiol.* 58:959-968. doi: 10.1111/j.1365-2958.2005.04887.x.
- Rosch, J.W., F.F. Hsu, and M.G. Caparon. 2007. Anionic lipids enriched at the ExPortal of Streptococcus pyogenes. *J.Bacteriol.* 189:801-806. doi: 10.1128/JB.01549-06.
- Rothfield, L., A. Taghbalout, and Y.L. Shih. 2005. Spatial control of bacterial division-site placement. *Nat.Rev.Microbiol.* 3:959-968. doi: 10.1038/nrmicro1290.
- Rueda, S., M. Vicente, and J. Mingorance. 2003. Concentration and assembly of the division ring proteins FtsZ, FtsA, and ZipA during the Escherichia coli cell cycle. *J.Bacteriol.* 185:3344-3351.
- Rupley, J.A. 1967. The binding and cleavage by lysozyme of N-acetylglucosamine oligosaccharides. *Proc.R.Soc.Lond.B.Biol.Sci.* 167:416-428.
- Sakata, N., S. Terakubo, and T. Mukai. 2005. Subcellular location of the soluble lytic transglycosylase homologue in Staphylococcus aureus. *Curr.Microbiol.* 50:47-51. doi: 10.1007/s00284-004-4381-9.
- Saleh, O.A., C. Perals, F.X. Barre, and J.F. Allemand. 2004. Fast, DNA-sequence independent translocation by FtsK in a single-molecule experiment. *EMBO J.* 23:2430-2439. doi: 10.1038/sj.emboj.7600242.
- Scheffers, D.J. 2005. Dynamic localization of penicillin-binding proteins during spore development in Bacillus subtilis. *Microbiology.* 151:999-1012. doi: 10.1099/mic.0.27692-0.
- Scheffers, D.J., L.J. Jones, and J. Errington. 2004. Several distinct localization patterns for penicillin-binding proteins in Bacillus subtilis. *Mol.Microbiol.* 51:749-764.

- Scheffers, D.J., and M.G. Pinho. 2005. Bacterial cell wall synthesis: new insights from localization studies. *Microbiol.Mol.Biol.Rev.* 69:585-607. doi: 10.1128/MMBR.69.4.585-607.2005.
- Schiffer, G., and J.V. Holtje. 1999. Cloning and characterization of PBP 1C, a third member of the multimodular class A penicillin-binding proteins of Escherichia coli. *J.Biol.Chem.* 274:32031-32039.
- Schlaeppi, J.M., H.M. Pooley, and D. Karamata. 1982. Identification of cell wall subunits in bacillus subtilis and analysis of their segregation during growth. *J.Bacteriol.* 149:329-337.
- Schlag, M., R. Biswas, B. Krismer, T. Kohler, S. Zoll, W. Yu, H. Schwarz, A. Peschel, and F. Gotz. 2010. Role of staphylococcal wall teichoic acid in targeting the major autolysin Atl. *Mol.Microbiol.* 75:864-873. doi: 10.1111/j.1365-2958.2009.07007.x.
- Schleifer, K.H., and O. Kandler. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol.Rev.* 36:407-477.
- Scholefield, G., R. Whiting, J. Errington, and H. Murray. 2011. Spo0J regulates the oligomeric state of Soj to trigger its switch from an activator to an inhibitor of DNA replication initiation. *Mol.Microbiol.* 79:1089-1100. doi: 10.1111/j.1365-2958.2010.07507.x; 10.1111/j.1365-2958.2010.07507.x.
- Schonermark, M., R. Deppisch, G. Riedasch, K. Rother, and G.M. Hansch. 1991. Induction of mediator release from human glomerular mesangial cells by the terminal complement components C5b-9. *Int.Arch.Allergy Appl.Immunol.* 96:331-337.
- Sharp, M.D., and K. Pogliano. 2003. The membrane domain of SpoIIIE is required for membrane fusion during Bacillus subtilis sporulation. *J.Bacteriol.* 185:2005-2008.
- Sharp, M.D., and K. Pogliano. 2002. Role of cell-specific SpoIIIE assembly in polarity of DNA transfer. *Science.* 295:137-139. doi: 10.1126/science.1066274.
- Sharp, M.D., and K. Pogliano. 1999. An in vivo membrane fusion assay implicates SpoIIIE in the final stages of engulfment during Bacillus subtilis sporulation. *Proc.Natl.Acad.Sci.U.S.A.* 96:14553-14558.
- Shen, B., and J. Lutkenhaus. 2010. Examination of the interaction between FtsZ and MinCN in E. coli suggests how MinC disrupts Z rings. *Mol.Microbiol.* 75:1285-1298. doi: 10.1111/j.1365-2958.2010.07055.x.
- Shen, B., and J. Lutkenhaus. 2009. The conserved C-terminal tail of FtsZ is required for the septal localization and division inhibitory activity of MinC(C)/MinD. *Mol.Microbiol.* 72:410-424.
- Shih, Y.L., X. Fu, G.F. King, T. Le, and L. Rothfield. 2002. Division site placement in E.coli: mutations that prevent formation of the MinE ring lead to loss of the normal midcell arrest of growth of polar MinD membrane domains. *EMBO J.* 21:3347-3357. doi: 10.1093/emboj/cdf323.
- Shih, Y.L., T. Le, and L. Rothfield. 2003. Division site selection in Escherichia coli involves dynamic redistribution of Min proteins within coiled structures that extend between the two cell poles. *Proc.Natl.Acad.Sci.U.S.A.* 100:7865-7870. doi: 10.1073/pnas.1232225100.
- Simon, M.J. & Day, R.A. 2000. Improved Resolution of Hydrophobic Penicillin-binding Proteins and Their Covalently Linked Complexes on a Modified C18 Reversed Phase Column. *Analytical Letters.* 33:5:861-867.
- Singh, J.K., R.D. Makde, V. Kumar, and D. Panda. 2008. SepF increases the assembly and bundling of FtsZ polymers and stabilizes FtsZ protofilaments by binding along its length. *J.Biol.Chem.* 283:31116-31124. doi: 10.1074/jbc.M805910200.
- Sjoquist, J., and I.I. Wadso. 1971. A thermochemical study of the reaction between protein A from S. aureus and fragment Fc from immunoglobulin G. *FEBS Lett.* 14:254-256.
- Small, E., R. Marrington, A. Rodger, D.J. Scott, K. Sloan, D. Roper, T.R. Dafforn, and S.G. Addinall. 2007. FtsZ polymer-bundling by the Escherichia coli ZapA orthologue, YgfE, involves a conformational change in bound GTP. *J.Mol.Biol.* 369:210-221. doi: 10.1016/j.jmb.2007.03.025.
- Smith, T.J., S.A. Blackman, and S.J. Foster. 2000. Autolysins of Bacillus subtilis: multiple enzymes with multiple functions. *Microbiology.* 146:249-262.
- Snowden, M.A., H.R. Perkins, A.W. Wyke, M.V. Hayes, and J.B. Ward. 1989. Cross-linking and O-acetylation of newly synthesized peptidoglycan in Staphylococcus aureus H. *J.Gen.Microbiol.* 135:3015-3022.
- Soufo, H.J.D., and P.L. Graumann. 2003. Actin-like Proteins MreB and Mbl from Bacillus subtilis Are Required for Bipolar Positioning of Replication Origins. *Current Biology.* 13:1916-1920. doi: 10.1016/j.cub.2003.10.024.
- Spratt, B.G. 1975. Distinct penicillin binding proteins involved in the division, elongation, and shape of Escherichia coli K12. *Proc.Natl.Acad.Sci.U.S.A.* 72:2999-3003.
- Steele, V.R., A.L. Bottomley, J. Garcia-Lara, J. Kasturiarachchi, and S.J. Foster. 2011. Multiple essential roles for EzrA in cell division of Staphylococcus aureus. *Mol.Microbiol.* 80:542-555. doi: 10.1111/j.1365-2958.2011.07591.x; 10.1111/j.1365-2958.2011.07591.x.
- Suefuji, K., R. Valluzzi, and D. RayChaudhuri. 2002. Dynamic assembly of MinD into filament bundles modulated by ATP, phospholipids, and MinE. *Proc.Natl.Acad.Sci.U.S.A.* 99:16776-16781. doi: 10.1073/pnas.262671699.
- Sugai, M., H. Komatsuzawa, T. Akiyama, Y.M. Hong, T. Oshida, Y. Miyake, T. Yamaguchi, and H. Suginaka. 1995. Identification of endo-beta-N-acetylglucosaminidase and N-acetylmuramyl-L-alanine amidase as cluster-dispersing enzymes in Staphylococcus aureus. *J.Bacteriol.* 177:1491-1496.
- Swulius, M.T., S. Chen, H. Jane Ding, Z. Li, A. Briegel, M. Pilhofer, E.I. Tocheva, S.R. Lybarger, T.L. Johnson, M. Sandkvist, and G.J. Jensen. 2011. Long helical filaments are not seen encircling cells in electron cryotomograms of rod-shaped bacteria. *Biochem.Biophys.Res.Commun.* 407:650-655. doi: 10.1016/j.bbrc.2011.03.062.
- Tiyanont, K., T. Doan, M.B. Lazarus, X. Fang, D.Z. Rudner, and S. Walker. 2006. Imaging peptidoglycan biosynthesis in Bacillus subtilis with fluorescent antibiotics. *Proc.Natl.Acad.Sci.U.S.A.* 103:11033-11038. doi: 10.1073/pnas.0600829103.
- TOMASZ, A., J.D. JAMIESON, and E. OTTOLENGHI. 1964. The Fine Structure of Diplococcus Pneumoniae. *J.Cell Biol.* 22:453-467.
- Touhami, A., M.H. Jericho, and T.J. Beveridge. 2004. Atomic force microscopy of cell growth and division in Staphylococcus aureus. *J.Bacteriol.* 186:3286-3295. doi: 10.1128/JB.186.11.3286-3295.2004.
- Typas, A., M. Banzhaf, van den Berg van Saparoea,B., J. Verheul, J. Biboy, R.J. Nichols, M. Zietek, K. Beilharz, K. Kannenberg, M. von Rechenberg, E. Breukink, T. den Blaauwen, C.A. Gross, and W. Vollmer. 2010. Regulation of peptidoglycan synthesis by outer-membrane proteins. *Cell.* 143:1097-1109. doi: 10.1016/j.cell.2010.11.038.
- Tzagoloff, H., and R. Novick. 1977. Geometry of cell division in Staphylococcus aureus. *J.Bacteriol.* 129:343-350.
- Uehara, T., T. Dinh, and T.G. Bernhardt. 2009. LytM-domain factors are required for daughter cell separation and rapid ampicillin-induced lysis in Escherichia coli. *J.Bacteriol.* 191:5094-5107. doi: 10.1128/JB.00505-09.

- Uehara, T., K.R. Parzych, T. Dinh, and T.G. Bernhardt. 2010. Daughter cell separation is controlled by cytokinetic ring-activated cell wall hydrolysis. *EMBO J.* 29:1412-1422. doi: 10.1038/emboj.2010.36.
- van Dam, V., R. Sijbrandi, M. Kol, E. Swiezewska, B. de Kruijff, and E. Breukink. 2007. Transmembrane transport of peptidoglycan precursors across model and bacterial membranes. *Mol.Microbiol.* 64:1105-1114. doi: 10.1111/j.1365-2958.2007.05722.x.
- van den Ent, F., L.A. Amos, and J. Lowe. 2001. Prokaryotic origin of the actin cytoskeleton. *Nature.* 413:39-44. doi: 10.1038/35092500.
- van den Ent, F., M. Leaver, F. Bendezu, J. Errington, P. de Boer, and J. Lowe. 2006. Dimeric structure of the cell shape protein MreC and its functional implications. *Mol.Microbiol.* 62:1631-1642.
- Varma, A., M.A. de Pedro, and K.D. Young. 2007. FtsZ directs a second mode of peptidoglycan synthesis in Escherichia coli. *J.Bacteriol.* 189:5692-5704. doi: 10.1128/JB.00455-07.
- Veiga, H., A.M. Jorge, and M.G. Pinho. 2011. Absence of nucleoid occlusion effector Noc impairs formation of orthogonal FtsZ rings during Staphylococcus aureus cell division. *Mol.Microbiol.* 80:1366-1380. doi: 10.1111/j.1365-2958.2011.07651.x.
- Vergara-Irigaray, M., T. Maira-Litran, N. Merino, G.B. Pier, J.R. Penades, and I. Lasa. 2008. Wall teichoic acids are dispensable for anchoring the PNAG exopolysaccharide to the Staphylococcus aureus cell surface. *Microbiology.* 154:865-877. doi: 10.1099/mic.0.2007/013292-0.
- Volkov, A., J. Mascarenhas, C. Andrei-Selmer, H.D. Ulrich, and P.L. Graumann. 2003. A prokaryotic condensin/cohesin-like complex can actively compact chromosomes from a single position on the nucleoid and binds to DNA as a ring-like structure. *Mol.Cell.Biol.* 23:5638-5650.
- Vollmer, W. 2008. Structural variation in the glycan strands of bacterial peptidoglycan. *FEMS Microbiol.Rev.* 32:287-306. doi: 10.1111/j.1574-6976.2007.00088.x.
- Vollmer, W., and J.V. Holtje. 2004. The architecture of the murein (peptidoglycan) in gram-negative bacteria: vertical scaffold or horizontal layer(s)? *J.Bacteriol.* 186:5978-5987. doi: 10.1128/JB.186.18.5978-5987.2004.
- Wadenpohl, I., and M. Bramkamp. 2010. DivIC stabilizes FtsL against RasP cleavage. *J.Bacteriol.* 192:5260-5263. doi: 10.1128/JB.00287-10.
- Wang, K.H., A.L. Isidro, L. Domingues, H.A. Eskandarian, P.T. McKenney, K. Drew, P. Grabowski, M.H. Chua, S.N. Barry, M. Guan, R. Bonneau, A.O. Henriques, and P. Eichenberger. 2009. The coat morphogenetic protein SpoVID is necessary for spore encasement in Bacillus subtilis. *Mol.Microbiol.* 74:634-649. doi: 10.1111/j.1365-2958.2009.06886.x.
- Wang, X., B.J. Wilkinson, and R.K. Jayaswal. 1991. Sequence analysis of a Staphylococcus aureus gene encoding a peptidoglycan hydrolase activity. *Gene.* 102:105-109.
- Ward, J.B. 1973. The chain length of the glycans in bacterial cell walls. *Biochem.J.* 133:395-398.
- Weart, R.B., A.H. Lee, A.C. Chien, D.P. Haeusser, N.S. Hill, and P.A. Levin. 2007. A metabolic sensor governing cell size in bacteria. *Cell.* 130:335-347. doi: 10.1016/j.cell.2007.05.043.
- Webb, C.D., A. Decatur, A. Teleman, and R. Losick. 1995. Use of green fluorescent protein for visualization of cell-specific gene expression and subcellular protein localization during sporulation in Bacillus subtilis. *J.Bacteriol.* 177:5906-5911.
- Wei, Y., T. Havasy, D.C. McPherson, and D.L. Popham. 2003. Rod shape determination by the Bacillus subtilis class B penicillin-binding proteins encoded by pbpA and pbpH. *J.Bacteriol.* 185:4717-4726.
- Weiss, D.S., J.C. Chen, J.M. Ghigo, D. Boyd, and J. Beckwith. 1999. Localization of FtsI (PBP3) to the septal ring requires its membrane anchor, the Z ring, FtsA, FtsQ, and FtsL. *J.Bacteriol.* 181:508-520.
- White, C.L., A. Kitich, and J.W. Gober. 2010. Positioning cell wall synthetic complexes by the bacterial morphogenetic proteins MreB and MreD. *Mol.Microbiol.* 76:616-633. doi: 10.1111/j.1365-2958.2010.07108.x.
- Winkelstein, J.A., and A. Tomasz. 1978. Activation of the alternative complement pathway by pneumococcal cell wall teichoic acid. *J.Immunol.* 120:174-178.
- Woldringh, C.L., E. Mulder, P.G. Huls, and N. Vischer. 1991. Toporegulation of bacterial division according to the nucleoid occlusion model. *Res.Microbiol.* 142:309-320.
- Woldringh, C.L., J.A. Valkendurg, E. Pas, P.E. Taschner, P. Huls, and F.B. Wientjes. 1985. Physiological and geometrical conditions for cell division in Escherichia coli. *Ann.Inst.Pasteur Microbiol.* 136A:131-138.
- Wu, L.J., and J. Errington. 2004. Coordination of cell division and chromosome segregation by a nucleoid occlusion protein in Bacillus subtilis. *Cell.* 117:915-925. doi: 10.1016/j.cell.2004.06.002.
- Wu, L.J., and J. Errington. 2003. RacA and the Soj-Spo0J system combine to effect polar chromosome segregation in sporulating Bacillus subtilis. *Mol.Microbiol.* 49:1463-1475.
- Wu, L.J., and J. Errington. 1998. Use of asymmetric cell division and spoIIIE mutants to probe chromosome orientation and organization in Bacillus subtilis. *Mol.Microbiol.* 27:777-786.
- Wu, L.J., S. Ishikawa, Y. Kawai, T. Oshima, N. Ogasawara, and J. Errington. 2009. Noc protein binds to specific DNA sequences to coordinate cell division with chromosome segregation. *EMBO J.* 28:1940-1952. doi: 10.1038/emboj.2009.144.
- Yamada, S., M. Sugai, H. Komatsuzawa, S. Nakashima, T. Oshida, A. Matsumoto, and H. Suginaka. 1996. An autolysin ring associated with cell separation of Staphylococcus aureus. *J.Bacteriol.* 178:1565-1571.
- Yamamoto, H., S. Kurosawa, and J. Sekiguchi. 2003. Localization of the vegetative cell wall hydrolases LytC, LytE, and LytF on the Bacillus subtilis cell surface and stability of these enzymes to cell wall-bound or extracellular proteases. *J.Bacteriol.* 185:6666-6677.
- Yan, K., K.H. Pearce, and D.J. Payne. 2000. A conserved residue at the extreme C-terminus of FtsZ is critical for the FtsA-FtsZ interaction in Staphylococcus aureus. *Biochem.Biophys.Res.Commun.* 270:387-392. doi: 10.1006/bbrc.2000.2439.
- Yang, D.C., N.T. Peters, K.R. Parzych, T. Uehara, M. Markovski, and T.G. Bernhardt. 2011. An ATP-binding cassette transporter-like complex governs cell-wall hydrolysis at the bacterial cytokinetic ring. *Proc.Natl.Acad.Sci.U.S.A.* 108:E1052-60.
- Yanouri, A., R.A. Daniel, J. Errington, and C.E. Buchanan. 1993. Cloning and sequencing of the cell division gene pbpB, which encodes penicillin-binding protein 2B in Bacillus subtilis. *J.Bacteriol.* 175:7604-7616.
- Yu, W., S. Herbert, P.L. Graumann, and F. Gotz. 2010. Contribution of SMC (structural maintenance of chromosomes) and SpoIIIE to chromosome segregation in Staphylococci. *J.Bacteriol.* 192:4067-4073. doi: 10.1128/JB.00010-10.
- Zapun, A., T. Vernet, and M.G. Pinho. 2008. The different shapes of cocci. *FEMS Microbiol.Rev.* 32:345-360.
- Zipfel, P.F., M. Mihlan, and C. Skerka. 2007. The alternative pathway of complement: a pattern recognition system. *Adv.Exp.Med.Biol.* 598:80-92. doi: 10.1007/978-0-387-71767-8_7.