The minimalistic divisome reveals power of the cell division machinery

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Life cycle of *Bacillus subtilis*

*Bacillus subtilis* is a saprophytic rod-shaped Gram-positive endospore forming bacterium of the phylum Firmicutes that is commonly found in soil, water, and air, but also resides in the gastrointestinal tract of insects, animals and humans (Nicholson, 2002). *B. subtilis* has developed sophisticated strategies to survive in the extreme conditions it may live in. These strategies are tightly connected to the cell cycle of *B. subtilis* (Fig. 1), in which the *B. subtilis* cell undergoes replication and duplication of its genetic content, divides and ensures that both daughter cells harbour a full copy of the replicated and duplicated genetic information prior to cell division. The life cycle of *B. subtilis* comprises of vegetative growth and sporulation (Fig. 1). The striking phenotypic difference of the vegetative and sporulation life cycles of *B. subtilis* is that during vegetative growth cell division occurs symmetrical, producing two daughter cells of equal geometry, while sporulating cells divide asymmetrically, producing two cells of unequal sizes; a large mother cell and a smaller prespore (Fig. 1). Both cells will harbour a full copy of the ancestral genome and the smaller cell (prespore) will develop into a mature spore after which the larger cell will lyse. Sporulation is generally triggered by starvation that leads to cell death but survival of spores. This overview will mainly focus on cell division, the possible relation with chromosome replication and segregation in the Gram-positive model organism *B. subtilis*. However, where information is limited, and when
possible, a comparison will be made with the well characterized Gram-negative bacterium *Escherichia coli*.

Fig. 1. The cell cycle of *B. subtilis*

The left side represents the vegetative cell cycle of *B. subtilis* where a daughter cell first elongates (growth in length), while the chromosome is replicated, duplicated and segregated. The FtsZ then forms a ring like structure (Z-ring) between the segregated chromosomes and marks the nascent division site. The Z-ring then recruits an array of proteins that are required to form the cell division machinery that hereafter synthesizes the new septum to split the cell in two daughter cells of identical size and the divisome machinery dissolves and reassembles at the next cell division site. In nutrient poor conditions *B. subtilis* undergoes sporulation in which two Z-rings are formed adjacent to each cell poles. Only one of these Z-rings will form a septum over the chromosome that results in asymmetrical cell division. Completion of this division results in a large mother cell and a small prespore or fore spore. Specific transcriptional regulations of gene expression in the mother cell ($\sigma^F$) and the prespore ($\sigma^L$) ensure engulfment and maturation of the fore spore. Once matured, the mother cell lyses and releases a highly resistant and dormant spore that will enter the vegetative cell cycle again when nutrient conditions are favourable. The figure is adapted from (Jeff Errington and Wu, 2017).

**Assembly of the divisome**

Generally, the rod-shaped model organisms *Bacillus subtilis* and *Escherichia coli* divide symmetrically to produce two daughter cells of equal geometry each with a complete copy of the mother chromosome.
To ensure success of this critical process, both *B. subtilis* and *E. coli* must tightly regulate and link together chromosome replication, chromosome segregation, cell growth and cell division.

Cell division is orchestrated by the multi protein complex collectively known as the divisome. From the assembly of the divisome to the synthesis of the nascent inward growing septum, the process can be divided into three stages and each stage is tightly controlled (Aarsman *et al.*, 2005; Adams and Jeff Errington, 2009; Egan and Vollmer, 2013). The first step of divisome assembly is the polymerization of the bacterial tubulin homologue FtsZ (Fig. 2) (Lutkenhaus *et al.*, 1980). FtsZ uses GTP to polymerize in a head-to-tail orientation (Bi and Lutkenhaus, 1991; RayChaudhuri and James T Park, 1992; de Boer *et al.*, 1992; Mukherjee *et al.*, 1993; Ma *et al.*, 1996), and subsequently FtsZ polymers form a ring-like structure called the FtsZ-ring (or Z-ring) that is positioned at the middle of the cell and marks the new cell division site (Bi and Lutkenhaus, 1991). Biochemical studies have shown that FtsZ polymer formation is a cooperative process (Romberg *et al.*, 2001; Miraldi *et al.*, 2008), and many proteins regulate this process. The widely conserved and regulatory protein ZapA (FtsZ ring associated protein A) consists of two alpha/beta domains and exists as dimers and tetramers in solution (Low *et al.*, 2004). ZapA interacts with FtsZ, to drive FtsZ polymerization and to promote FtsZ polymers bundling (Gueiros-Filho and Losick, 2002), thereby contributing to the spatio-temporal tuning of the Z-ring. In *E. coli*, there are more functionally redundant Zap proteins (ZapA, ZapB, ZapC, ZapD and ZapE) that regulate Z-ring formation (Gueiros-Filho and
Losick, 2002; Ebersbach et al., 2008; Durand-Heredia et al., 2011; Hale et al., 2011; Durand-Heredia et al., 2012). ZapA recruits ZapB to the Z-ring where it enhances ZapA-FtsZ polymer interactions, thereby creating a ring-like ZapB structure inside the FtsZ-ring (Galli and Gerdes, 2010). The ZapA-ZapB complex connects Z-ring with chromosome replication and segregation via interaction with the terminus binding macro domain positioning protein MatP (Mercier et al., 2008; Espéli et al., 2012; Buss et al., 2015; Buss et al., 2017). The function of the ATPase ZapE in cell division is unclear, however, genetic and biochemical experiments showed that the requirement of ZapE for cell division becomes apparent at higher ZapE levels, in the absence of oxygen and at temperatures over 37 °C (Marteyn et al., 2014).

The Z-ring is tethered to the membrane by the structural proteins FtsA and SepF in B. subtilis, and FtsA and ZipA in E. coli (Pichoff and Lutkenhaus, 2002; Jensen et al., 2005; Duman et al., 2013). The conserved cell division protein FtsA belongs to the actin family of ATPases (Kabsch and Holmes, 1995) and utilises a conserved amphipathic helix at its C-terminus to tether to the cytoplasmic membrane (PLA et al., 1990). FtsA directly interacts with the conserved C-terminal domain of FtsZ through residues at its extreme C-terminus that are essential for its biological functions (Yim et al., 2000). Both E. coli and B. subtilis FtsA polymerizes to form polymers in vitro and in vivo, and this polymerization of FtsA is important for its function in cell division (Szwedziak et al., 2012; Krupka et al., 2017; Conti et al., 2017). In both bacteria, the cellular localization of FtsA is FtsZ dependent (Addinall and
Lutkenhaus, 1996; Ma et al., 1996; Feucht et al., 2001) and the deletion of ftsA severely affects cell division and blocks sporulation in B. subtilis while deletion is lethal in E. coli (Beall and Lutkenhaus, 1992; Kemp et al., 2002). For a long time, it was unclear how the FtsA and FtsZ filaments affect each other. Recent work has shown that, at least in vitro, aside from recruiting FtsZ to the cell membrane, FtsA also regulates the dynamics of the Z-ring (Loose and Mitchison, 2014).

In E. coli, FtsA shares some functions with the transmembrane protein ZipA (Pichoff and Lutkenhaus, 2002). Both proteins are interacting with FtsZ independently of each other, and interact with the conserved C-terminus of FtsZ (Hale and de Boer, 1997). Both FtsA and ZipA promote and stabilize FtsZ filaments in vitro (Hale et al., 2000) and organize these FtsZ filaments into higher order structures (RayChaudhuri, 1999). However, a recent report concluded that at physiological concentrations ZipA promotes FtsZ filamentation but not bundling of FtsZ filaments (Krupka et al., 2018). The first six residues of ZipA are sticking into the periplasmic space, followed by a transmembrane domain that anchors the protein to the cytoplasmic membrane. Unlike the membrane targeting sequence of FtsA, the transmembrane anchor of ZipA is irreplaceable, and both the transmembrane and the soluble cytoplasmic C-terminus domain are important for the interaction with FtsZ (Hale et al., 2000). Either FtsA or ZipA is required to form a membrane-anchored Z-ring, but completion of septation requires both proteins (Pichoff and Lutkenhaus, 2002), even though, ZipA alone is sufficient to recruit the late cell division proteins.
FtsQ, FtsL, and FtsN to the cell division site (Hale and de Boer, 2002). A gain of function mutant of FtsA (FtsA-R286W) or a mutation in FtsZ (FtsZ-L169R) (Haeusser et al., 2015) have been shown to abolish the necessity of ZipA for cell division suggesting that the FtsZ ring complex is sufficient for recruiting the other cell division proteins in *E. coli* (Geissler et al., 2003).

In *B. subtilis* the protein SepF has a comparable function as FtsA and directly interacts with the conserved C-terminus of FtsZ, stimulating bundling FtsZ polymers by reducing the GTPase activity of FtsZ (Singh et al., 2008). Furthermore, overexpression of SepF overcomes the severe cell division defect of the *ftsA* deletion mutant, and deletion of *sepF* in *B. subtilis* cells depleted for FtsA causes a complete block in Z-ring formation and cell division (Hamoen et al., 2006; Shu Ishikawa et al., 2006). Additionally, genetic experiments showed that a *sepF* null mutant is severely defective in septum synthesis supporting its importance for divisome formation (Hamoen et al., 2006). These findings illustrate the overlapping functions of FtsA and SepF, however, it was argued that the main function of SepF is to form a proper septum and not the initiation of divisome assembly (Hamoen et al., 2006). SepF is a highly conserved cell division protein in Gram-positive bacteria including *Bacilli* and cyanobacteria (Marston et al., 1998; Duman et al., 2013; Gola et al., 2015). *In vitro*, purified *B. subtilis* SepF forms rings of about 50 nm in width that align FtsZ polymers into tubules (Gündoğdu et al., 2011). In-depth structural and genetic analyses of *B. subtilis* SepF, showed that (i) SepF has an amphipathic helix at its N-terminus to target it to the lipid
membrane, and (ii) SepF possesses a globular domain at its C-terminus through which it interacts with FtsZ, and (iii) SepF can serve as a sole membrane anchor of the Z-ring (Duman et al., 2013). Based on this work, a model was postulated in which SepF forms arcs onto the nascent septa, leading the inward growing septa by assembling FtsZ polymers on top of these arcs (Duman et al., 2013).

Another key cell division protein that regulates the Z-ring in *B. subtilis* is EzrA (Levin et al., 1999). EzrA has a transmembrane anchor at its N-terminus (Haeusser et al., 2007) and its cytoplasmic domain binds to FtsZ. However, genetic studies showed that EzrA cannot take over the Z-ring anchoring role from either FtsA or SepF (Levin et al., 1999). Similarly to FtsA and SepF, EzrA directly interacts with the conserved C-terminus tail of FtsZ (Singh et al., 2007) to modulate Z-ring formation (Levin et al., 1999; Haeusser et al., 2004; Haeusser et al., 2007). EzrA comprises of five linear repeats of an unusual triple helical bundle, homologous to eukaryotic spectrin (Cleverley et al., 2013). Spectrin connects actin filaments to the membrane. EzrA forms a head-to-tail dimer that is bent into a semicircle (arc) (Cleverley et al., 2013). It is thought that such arcs cover membrane-attached FtsZ polymers, thereby
Fig. 2. Core components of the divisomes of *E. coli* and *B. subtilis*

The key components of the divisome complex in *E. coli* and *B. subtilis* are depicted. (A) In *E. coli*, FtsZ filaments are tethered to the inner membrane by FtsA and ZipA via interaction at the conserved C-terminus of FtsZ. ZapA stimulates FtsZ filamentation to form the FtsZ ring. Subsequently, the regulator of septal peptidoglycan (PG) synthesis and hydrolysis FtsX and FtsE are recruited. The midcell DNA translocase FtsK is also recruited early to the cell division site, followed by the FtsQLB complex (regulator of divisome activation). Hereafter, the septal PG synthases FtsW and FtsI are recruited and finally FtsN is recruited to trigger septal PG synthesis. (B) In *B. subtilis*, FtsZ polymers are bound to the cell membrane by the early cell division proteins SepF and FtsA. ZapA stimulates FtsZ bundling. The spectrin homologue EzrA forms a dimeric arc on the inward growing septum and modulates Z-ring formation. After formation of the Z-ring, a second group of proteins including Pbp2B, FtsL, DivIB, DivIC and FtsW are recruited to the Z-ring. This complex then synthesizes and guide the formation of the new inward growing septum and finalizes cell division.

restricting the formation of multiple Z-rings (Haeusser et al., 2004; Land et al., 2014).
Recruitment of the late cell division proteins and synthesis of the nascent septum

When the Z-ring is formed and positioned at midcell, after a temporal delay by an unknown mechanism, a second group of essential proteins are recruited to the divisome to complete the divisome complex and to initiate septum synthesis, the so called late cell division proteins (Aarsman et al., 2005; Gamba et al., 2009). In *E. coli*, after formation and placement of the Z-ring, the cell division proteins FtsEX, FtsK, FtsQLB, FtsW, FtsI and FtsN are sequentially localized to the Z-ring, and the preceding protein is required for the next recruit (Fig. 2A) (Buddelmeijer and Beckwith, 2002). FtsK is a motor protein that uses ATP hydrolysis to push away chromosomes trapped by the nascent septum (Stouf et al., 2013; Männik et al., 2017). In addition, the protein activates the recombinases XerD and XerC to resolve dimeric chromosomes after replication (Aussel et al., 2002; Bigot et al., 2004), and interacts with Topo IV to stimulate decatenation of chromosomes (Espéli et al., 2003; Bigot and Marians, 2010). However, the essential function of FtsK in cell division is the recruitment of FtsQ, FtsL, and FtsI (Chen and Beckwith, 2001), thereby connecting the Z-ring to the septal peptidoglycan synthesizing enzymes (Dubarry et al., 2010). The bitopic proteins FtsQ, FtsL and FtsB form a subcomplex (Fig. 2A) that serves as a scaffold to recruit downstream cell division proteins (Buddelmeijer and Beckwith, 2004; Glas et al., 2015). This FtsQLB subcomplex is required to recruit the integral protein FtsW (Lara and Ayala, 2002) that functions as the
glycosyltransferase in peptidoglycan synthesis (Meeske et al., 2016; Cho et al., 2016; Emami et al., 2017). FtsI (PBP3) serves as the transpeptidase for peptidoglycan synthesis at midcell (Botta and J T Park, 1981) and its recruitment to the cell division site requires the earlier proteins FtsZ, FtsA, FtsQ, FtsL and FtsW (Weiss et al., 1999). FtsN is the last essential cell division protein known to arrive at the divisome (Addinall et al., 1997). Generally, the arrival of FtsN at midcell is assumed to be the trigger for cell constriction (Gerding et al., 2009; Daley et al., 2016). After constriction, FtsEX, an ATP-binding (FtsE) and membrane binding (FtsX) transporter-like protein complex (Schmidt et al., 2004), utilizes ATP hydrolysis to act in cell separation by controlling cell wall hydrolysis through regulation of amidases in the periplasm (Yang et al., 2011; Du et al., 2016).

In *B. subtilis*, the main late cell division proteins comprise the conserved proteins, GpsB, FtsL, DivIB, Pbp2B, FtsW, and DivIVA (Fig. 2B) (Gamba et al., 2009). FtsW and Pbp2B are the key enzymes in synthesizing the septal cell wall (Gamba et al., 2009). FtsW is proposed to also act as an additional flipase for lipid II that translocase the cytoplasmic synthesized peptidoglycan precursor across the cell membrane (Mohammadi et al., 2011; Meeske et al., 2015). Pbp2B (FtsI in *E. coli*) crosslinks septal peptidoglycan (Nguyen-Distèche et al., 1998; Daniel et al., 2000), and GpsB together with EzrA promotes recruitment of the major bifunctional transglucosylase/transpeptidase Pbp1A/B (also known as PonA) from the lateral wall to the division site (Claessen et al., 2008). Like in *E. coli*, the bitopic membrane proteins FtsL, DivIC (FtsB in
*E. coli*, and DivIB (FtsQ in *E. coli*) form an interdependent subcomplex assumed to regulate the assembly of late proteins (Scheffers *et al.*, 2003). FtsL is inherently instable due to the activity of the transmembrane protease RasP (Bramkamp *et al.*, 2006). Only in a complex with DivICB the protein is stabilized (Daniel and J Errington, 2000; Wadenpohl and Bramkamp, 2010). Another key division protein is DivIVA, which is widely distributed amongst Gram-positive bacteria where it serves as a landmark protein. The protein is absent in Gram-negative bacteria. DivIVA interacts directly with the membrane and senses and binds to the strong negatively curved membrane area created by the inwardly growing nascent septum (Ramamurthi *et al.*, 2009; Lenarcic *et al.*, 2009). In *B. subtilis*, DivIVA also localizes to the cell poles and recruits the negative regulator of cell division; the Min system (see below) (Edwards and J Errington, 1997). Although not clearly understood yet, DivIVA is modulated through phosphorylation in bacteria such as *Streptomyces* spp., *Mycobacteria*, and *Corynebacterium* spp. where the protein is implicated in polar growth (Flärdh, 2003; Kang *et al.*, 2005; Hempel *et al.*, 2012; Saalbach *et al.*, 2013).

The striking difference in the assembly of the cell division machinery of *E. coli* and *B. subtilis* is that the recruitment of the cell division proteins is a hierarchical process in *E. coli* (Aarsman *et al.*, 2005), while in *B. subtilis* the essential late proteins localize in a highly cooperative fashion (Gamba *et al.*, 2009; Gamba *et al.*, 2016).
Positioning of the FtsZ ring by the Min system and Nucleoid occlusion

The concentration of FtsZ remains essentially unchanged during the cell cycle, and only a fraction is sufficient to form a Z ring (Weart and Levin, 2003; Erickson et al., 2010; Lutkenhaus et al., 2012). Thus, where and when Z-rings are formed must be strictly controlled. Two well characterised systems that prevent aberrant Z-ring formation at cell poles and over the nucleoid are the Min system and the nucleoid occlusion system, respectively.

The Min system is conserved in bacteria, archaea and eukaryotic mitochondria (Rothfield et al., 2005; Leger et al., 2015). Loss of the Min system causes minicells devoid of nucleoids as a result of aberrant cell division (Adler et al., 1967; Reeve et al., 1973). The Min system in *E. coli* consists of the FtsZ inhibitor MinC, the membrane binding Walker box ATPase MinD (de Boer et al., 1991) and MinE that organizes the topology of the MinCD complex in the cell (de Boer et al., 1989). In this bacteria, MinCD dynamically oscillates from pole-to-pole to result in a gradient with the lowest concentration at midcell and highest concentration at the cell poles (Raskin and de Boer, 1999a; Raskin and de Boer, 1999b). MinC interacts with MinD and is recruited to the membrane by MinD. This membrane-bound complex inhibits Z-ring formation due to the interaction between MinC and the conserved C-terminus of FtsZ, thereby interfering with FtsZ polymerization (Hu and Lutkenhaus, 2000; Dajkovic et al., 2008; Shen and Lutkenhaus, 2009). MinE controls the inhibitory activity of MinCD by disassembling the MinCD complex. MinE switches between two different conformations; freely diffusible cytoplasmic
MinE, in which the MinD interacting residues and the membrane targeting residues are kept together in a β-sheet, and a membrane bound form that is triggered by binding to membrane anchored-MinD, which frees the membrane targeting domain of MinE (Kyung-Tae Park et al., 2011). Binding of MinE to the MinCD complex releases MinC and causes the MinD dimer to undergo ATP hydrolysis and become monomeric, thereby weakening the interaction with the membrane. MinC is not required for the dynamic oscillation of MinD and MinE along the *E. coli* cell. The reciprocal interaction between MinD and MinE creates a reaction-diffusion couple that is responsible for the oscillation of the Min proteins between cell poles (Loose et al., 2011).

The Min system in *B. subtilis* is similar to that of *E. coli* except that it is not oscillating and the polar localization gradient is determined by the curvature sensing protein DivIVA and the integral protein MinJ. DivIVA recruits MinJ to cell division sites and cell poles where it forms a membrane tethered complex (Edwards and J Errington, 1997; Marston et al., 1998; Bramkamp et al., 2008; Patrick and Kearns, 2008). MinJ then recruits MinD where it acts as an adaptor for MinC (Marston et al., 1998). The DivIVA-MinJ-MinCD complex prevents formation of new Z-rings next to the emerging septum. In addition, this complex destabilizes the Z-ring after division has been completed (Marston et al., 1998; Bramkamp et al., 2008; Gregory et al., 2008; van Baarle and Bramkamp, 2009). Recently, it was suggested that in *B. subtilis* only a small fraction of MinD is recruited by DivIVA-MinJ, and that the remaining fraction of MinD is recruited to the septa by its C-terminal amphipathic α-helix (Kazuki
Ishikawa et al., 2017). Clearly, more work is needed to solve the exact mode of action of the Min system in *B. subtilis*.

The nucleoid occlusion system inhibits FtsZ polymerization over the nucleoid. The nucleoid occlusion factor of *B. subtilis*, Noc, binds to a 14-bp inverted repeat consensus sequence (Noc binding sequence, 5’-ATTTCCCGGAAAT-3’) that is found 74 times scattered over the genome, except for the Ter region (Wu et al., 2009). In the absence of Noc, nucleoid occlusion is impaired, and when initiation of DNA replication is blocked, cell division occurs over the nucleoid. Despite intensive attempts, neither direct interaction between Noc and FtsZ nor interaction between Noc and FtsZ-interacting proteins have been found (Wu and Jeff Errington, 2004; Wu et al., 2009; Adams et al., 2015). However, Noc utilizes a weak amphipathic helix at its N-terminus to bind to the cell membrane and can recruit chromosomal DNA to the membrane (Adams et al., 2015). It is assumed that the nucleoprotein complexes formed close to the cell membrane by Noc interferes with FtsZ polymerization (Wu et al., 2009; Adams et al., 2015). SlmA, the counter part of Noc in *E. coli*, also binds to DNA, but this protein interacts directly with FtsZ to inhibit its polymerization (Bernhardt and de Boer, 2005). Similarly to Noc, SlmA binds to a specific palindromic sequence (SlmA binding sequence, 5’-GTGAGTACTCAC-3’) that is spread over the chromosome with exception of the Ter region (Tonthat et al., 2011; Cho et al., 2011).
**Chromosome replication and segregation are linked to cell division**

Aside of the nucleoid occlusion system, in *E. coli* the essential cell division protein FtsK plays a role in proper chromosome segregation prior to cell division (Yu *et al.*, 1998; Liu *et al.*, 1998; Steiner *et al.*, 1999; Kennedy *et al.*, 2008). FtsK utilizes polarity of FtsK-binding sites on the chromosome (from origin to terminus) to translocate any chromosomal DNA trapped by the nascent septum into both daughter cells (Sivanathan *et al.*, 2006). The newly replicated chromosomes are catenated and must be unlinked by type II topoisomerase Topo IV (consisting of ParC and ParE) (Kato *et al.*, 1990; Peng and Marians, 1993; Ullsperger and Cozzarelli, 1996). FtsK also interacts with ParC thereby stimulating unlinking of the chromosomes by TopoIV (Espéli *et al.*, 2003). Chromosome dimers can also be resolved by the site specific recombinase XerC and XerD at so called *dif* sites on the chromosomes, and FtsK facilitate this process (Blakely *et al.*, 1993; Steiner *et al.*, 1999; Recchia and Sherratt, 1999). Another protein, MatP, binds at *matS* sites within the terminus macrodomain and this association protects the *dif* sites from early segregation (Mercier *et al.*, 2008). MatP also forms a complex with the cell division proteins ZapA and ZapB and by doing so it connects the macrodomain to cell division (Buss *et al.*, 2015).

*B. subtilis* does not contain a MatP homologue but it possess two FtsK homologues, SpoIIIIE and SftA (Kaimer *et al.*, 2009; Biller and Burkholder, 2009). SpoIIIIE acts primarily during sporulation and translocates chromosome trapped by the prespore septum (Panzer *et al.*, 1989; Wu and Jeffery Errington, 1994). On the other hand, SftA
appears to function primarily during vegetative growth to clear the midcell plain form chromosomes prior to cell division (Kaimer et al., 2009; Biller and Burkholder, 2009). After replication of the chromosome, RipX and CodV (the homologues of XerC and XerD in *E. coli*, respectively) separate the intertwined dimeric chromosome at the *dif* sites (Subramanya et al., 1997; Sciochetti et al., 1999; Sciochetti et al., 2001). It is assumed that both SpoIIIE and SftA positively affect dimer resolution by RipX and CodV, like their counter part FtsK in *E. coli*, however, it is not yet known by which mechanism (Kaimer et al., 2011).

**The scope of this thesis**

Despite the fact that most, if not all, proteins involved in bacterial cell division in *E. coli* and *B. subtilis* have been identified, it is still not clear how cell division is exactly executed in these model organisms. The aim of my PhD research was to gain more insight into the cell division process in *B. subtilis* and to determine how cell division can guide proteins to the cell poles in *E. coli*.

**Chapter 2** describes the construction of a minimal *B. subtilis* divisome. Cell division is carried out by a multitude of proteins, however, none of the proteins that regulate the formation and positioning of the Z-ring are essential. This complicates the analyses of their function as it seems to indicate a functional redundancy. In fact, several studies have shown that deleting two Z-ring regulatory genes simultaneously is lethal or results in sick cells. In chapter 2, I describe the attempt to remove as
many early cell division genes as possible. In the end, we were able to delete the genes of 8 conserved FtsZ-interacting proteins: zapA, minC, ugtP, minJ, ezrA, clpX, noc, ezrA, and ftsA, using a marker free method. Only SepF was required to anchor FtsZ to the membrane and to form active Z-rings. Next generation sequencing was used to confirm mutations that likely bypassed any synthetic lethal effect caused by the multiple gene deletions, and several mutations in cell division related genes were identified (in ponA, spoVG and sftA). One mutation was located in the gene braB adjacent to ezrA. This gene codes for a transporter for branched chain amino acids. Inactivation if this gene stimulated cell division and made cells more resilient to cell division inhibitors, indicating that braB codes for a new cell division related protein.

Chapter 3 describes a search for SepF interacting proteins using a genome-wide yeast-two-hybrid screen. Surprisingly, this revealed SftA as a potential interaction partner. An extensive genetic, microscopic and biochemical analysis was used to determine whether this interaction is real in vivo and whether SepF affects the activity SftA, or the other way around.

Chapter 4 revisited the determinants for polar localization of chemoreceptor in E. coli. Since the landmark report on the polar localization of chemoreceptors in E. coli, many models have been postulated, including stochastic nucleation, polar curvature, nucleoid
occlusion and Tol-Pal protein complex binding, to explain this polar localization mechanism. In Chapter 4 I provide evidence for membrane curvature as the main determinant for polar accumulation of chemoreceptors in *E. coli*. Importantly, the same mechanism is also active in *B. subtilis*. 
Literature cited


