BACTERIAL CELL DIVISION - STRUCTURE AND FUNCTION OF PROTEINS INVOLVED

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Abstract
Bacterial cell division in *Escherichia coli* and *Bacillus subtilis* is a well studied process where specific proteins assemble into highly ordered protein complexes at the proper time of the cell cycle. We know most of the protein players involved in this process, their localization, hierarchy of their assembly, some of the protein-protein contacts and the crystal structure some of these proteins. This short review summarizes the recent development in this field with emphasis to structure and function of proteins involved.

Bacterial cell division

The basic process of cell division is conceptually similar in eukaryotic and prokaryotic cells. This process is characterized by creating the division septum between the duplicated chromosomes. There are several advantages of studying cell division in prokaryotes. In general, it is still more simple process in bacteria than in eukaryotic cells and there are several outstanding models as *Escherichia coli* and *Bacillus subtilis*. The process of cell division is intensively studied on molecular level for decades but there are still many unanswered basic questions. Probably most is known about the mechanism of cell division of rod shaped bacteria, mainly Gram-negative *Escherichia coli* and Gram-positive *B. subtilis.* Cell division, often called septation, consists of invagination of cytoplasmatic membrane and peptidoglycan synthesis. Although many players in this process are well known, the mechanism of where, how and when cells form the division septum with high fidelity is often postulated in fully different models. Probably the most controversial question in cell division of rod shaped bacteria is where to divide, in other words, how the position of the division site is determined.

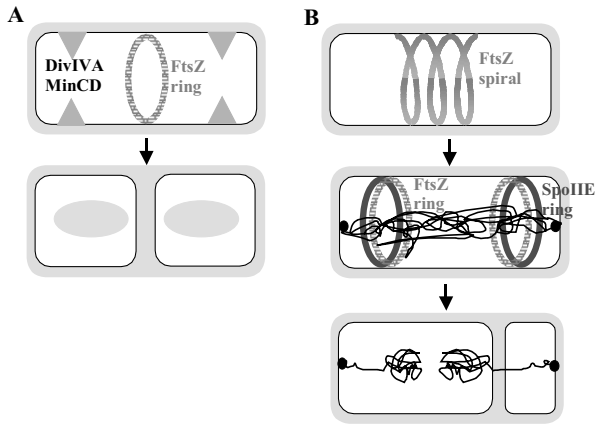

Figure 1. Vegetative (A) and asymmetric (B) cell division in *B. subtilis.*

The earliest event in the cell division cycle is the formation of the FtsZ ring at the future septum site (Fig. 1). FtsZ is highly conserved GTPase with high degree of similarity with the tubulins, eukaryotic cytoskeletal proteins. During vegetative growth the FtsZ ring forms at midcell and cell divides at this site (Fig. 1A). At least two distinct mechanisms are involved in accurate placement of the division machinery: the Min system and nucleoid occlusion.

Vegetative cell division in *Escherichia coli* and *Bacillus subtilis*

FtsZ is the most highly conserved of the known cell division proteins. It is present in the majority of prokaryotic species examined to date as well as it is present in lower and higher plants. A structural role for FtsZ was initially proposed because of its abundance in the cell and by its localization by immunogold labeling to a ring structure, so called Z ring, at the future site of division [1]. FtsZ is a homologue of tubulin, the eukarytic cytoskeletal protein involved in many essential processes including mitosis [2]. The solved crystal structures of FtsZ (Fig. 2A) and tubulin show extensive structural homology throughout the protein despite only limited primary sequence homology [3]. FtsZ and tubulin bind and hydrolyze GTP and assemble into protofilaments that have structures similar to those within microtubules. The next division protein that assembles at the division site is FtsA. FtsZ and FtsA proteins are the only cell division proteins that lack the clear membrane-spanning sequences. The crystal structure of FtsA from *Thermotoga maritima* was solved (Fig. 2B) [4]. *B. subtilis* FtsA was shown to dimerize and hydrolyse ATP but it does not appear to polymerise in vitro [5]. E. coli ZipA protein was shown to interact with FtsZ and to stabilize the Z ring immediately after it is assembled. ZipA is not particularly conserved among bacteria and *B. subtilis* has no such homologue. Crystal structure of ZipA was recently solved (Fig. 2C)[6]. The other division proteins in E. coli assembles in linear manner: FtsK, FtsQ, FtsL, YgbQ, FtsW, FtsI and FtsN. This is in contrast with *B. subtilis* where the equivalent division proteins are recruited in a much more concerted manner (reviewed in [7]). DivIB, DivIC, FtsL, PBP-2B and probably FtsW are all completely interdependent for assembly at the division site.

The main question that arose from the beginning of study of cell division process in rod-shaped bacteria was:

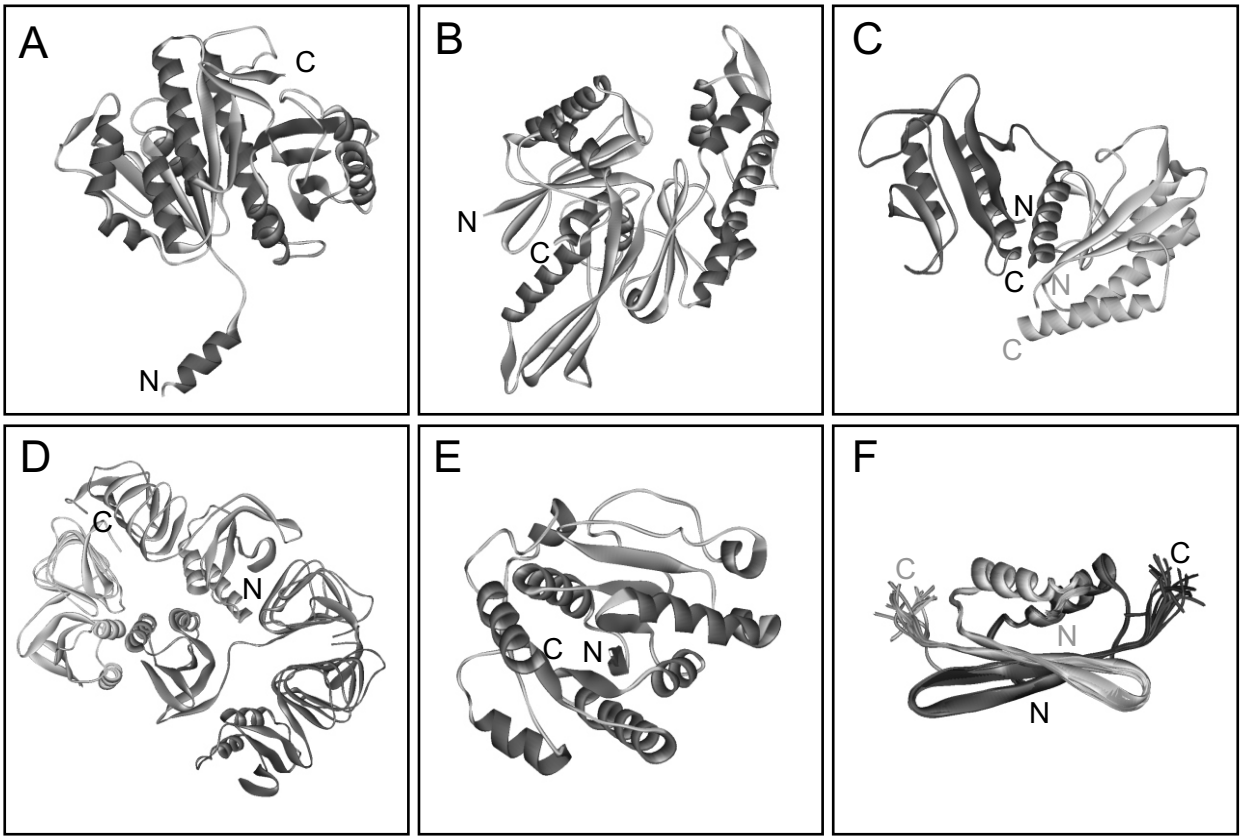

Figure 2. Crystal and NMR structures of proteins involved in bacterial cell division. A. FtsZ from *Methanococcus jannaschii* (PDB ID: 1FSZ). B. FtsA from *Thermotoga maritima* (PDB ID: 1E4G). C. ZipA dimer from *Escherichia coli* (PDB ID: 1F46). D. MinC tetramer from *Thermotoga maritima* (PDB ID: 1HF42). E. MinD from *Archeoglobus fulgidus* (PDB ID: 1HYQ). F. 15 NMR structures of MinE dimer from *Escherichia coli* (PDB ID: 1EV0).

'How do the bacteria recognize the site of septation with high fidelity at the midcell position?' It is becoming apparent that the Min system and nucleoid occlusion influence the positioning of the division site in *E. coli* and *B. subtilis* (reviewed in [8]).

The Min system functions mainly to prevent the possibility of division at the cell poles. In *E. coli* the Min system consists of MinC, MinD and MinE proteins. MinD activates MinC inhibition function and MinE is a topological factor allowing relief of division inhibition in the central region of the cell [9]. The recent localization experiments in living cells revealed that MinD, which is a membrane-associated ATPase [10], oscillates from pole to pole in dividing cells [11, 12]. This amazing oscillation repeats about every 10-20 seconds. The division inhibitor MinC can not oscillate on its own, but it binds to MinD and co-oscillates with the same pattern as MinD [12,13]. The MinE was originally shown to form the ring like structure at the mid-cell site [14]. However, the recent studies have shown that in living cells the localization of MinE protein undergoes also a rapid oscillation what is coupled to MinD oscillation [15]. This oscillating movement of MinD and MinE is co-dependent because lack of MinE causes uniform distribution of MinD around the cytoplasmatic membrane [16]. This view of Min protein dynamics has now been refined by the work of Shih et al. [17] who showed that MinCDE proteins are in fact organized into extended spirals that wind around the cell. Taken together, all these results raise important question - how does the oscillation work? Although, the crystal structures of MinC, MinD and MinE have been solved recently, it did not help to explain this unusual oscillation phenomenon of these proteins (Fig. 2D-F) [18,19]. Although, there are few models explaining this protein oscillation process [20,21], further genetic, localization and structural studies will be required for more complete understanding of this process.

B. subtilis, similarly as E. coli and wide variety of other prokaryotes, has MinC and MinD homologues, and these are important for the prevention of asymmetric septation during vegetative growth. However, *B. subtilis* lacks MinE and no MinC and MinD oscillation was observed [22,23]. In *B. subtilis*, DivIVA protein serves as the topological factor of mid-cell division [24,25]. However, DivIVA is not homologuoes with MinE and forms higher oligomers in comparison to MinE dimer [26,27,]. DivIVA functions differently in comparison with MinE. DivIVA requires FtsZ and other cell division proteins for its localization to the division sites late in their maturation [22,25,28]. Unlike most of other division proteins, it is then retained at the completed cell poles. The MinCD complex is also recruited to division sites, partly independently of DivIVA, but DivIVA is needed to bind the complex at the poles to block the asymmetric division in newly formed daughter cells [22,23]. The DivIVA/MinCD division system appears to have no direct role in initiation of FtsZ ring formation at the mid-cell site but rather it inhibits the division at the polar sites. DivIVA seems to have a second function during sporulation process, specifically at the stage of prespore

chromosome segregation [29]. Possibly, the most important factor of mid-cell division site selection in *B. subtilis* is the position of the nucleoid. Recent studies of cell division in outgrowing spores indicated the crucial role of nucleoid position for FtsZ ring formation [30]. DivIVA/MinCD system seems to be involved in inhibition of division at the polar nucleoid-free region sites and it is not crucial for mid-cell site selection [8]. The proposed nucleoid occlusion mechanism [31] is very attractive but still poorly defined model. This model states that the nucleoid had a negative effect on division wherever it occupies space in the cell. Thus, the mid-cell site appears and disappears cyclically during vegetative growth with rounds of chromosome replication [7].

Asymmetric cell division during sporulation in *Bacillus subtilis*

Fully different type of cell division is observed in rod-shaped spore forming *Bacilli* and *Clostridia* species during sporulation process. The first clear morphological feature of such sporulation process is the formation of an asymmetric septum (Fig. 1B) that bisects the bacterial cell into two unequally sized compartments, the larger mother cell and the smaller forespore. The proper positioning of this sporulation septum is dependent on Spo0A activity, which mediates the assembly of division proteins near the cell pole instead of at mid-cell. Spo0A controls the switch of FtsZ ring from midcell position to the polar positions during sporulation through a spiral intermediate [32]. Such a positional switch could be partially triggered through the activity of the sporulation specific protein SpoIIE, also a component of the sporulation septum, that is expressed as a result of Spo0A activity [33, 34, 35]. The central domain of SpoIIE is involved in oligomerization of the protein and is responsible for interaction with FtsZ, the protein essential for cell division [36, 37]. In *B. subtilis,* the division septum is a relatively thick structure containing a substantial amount of peptidoglycan (PG) that separates the two daughter cells at cytokinesis. In contrast, the asymmetric septum formed during sporulation is much thinner and most of the PG separating the two lipid bilayers that comprise the septal structure is removed soon after septation is complete. The pliable septal structure then migrates toward one pole of the cell, eventually engulfing the forespore compartment, which will mature into a dormant endospore. Based on electron micrographs of sporulating cells, it has been suggested that localized regions of lipid bilayer fusion might occur within the septal structure following removal of PG. This event might be a key to communication or transmission of a signal between two sporangium compartments [38].

Western blot analysis of fractionated cell extracts from mother cell and forespore, using polyclonal antibody against the globular part of *SpoIIE*, showed the possibility to target this protein specifically into asymmetric septa [39]. These results were surprising, considering the fact that spoIIE expression starts before the asymmetric sporulation septum begins to form and SpoIIE should be detected in membrane fraction of forespore and mother cell. Further localization experiments, with SpoIIE-GFP fusions in *B. subtilis* [40] and *B. megaterium* [39] using fluorescent microscopy, clearly showed the localization of SpoIIE in the asymmetric sporulation septum. There are at least two possibilities to explain these results. SpoIIE protein can specifically recognize the septation site or it can be built specifically into already formed septa. The second event is not very likely in view of electron-microscopy studies of SpoIIE mutant cells [33,41,33]. Some of these mutants (*spoIIE20*, *spoIIE21*, *spoIIE60* and other) were noted, with thick asymmetric septa similar to vegetative septa. It seems, that presence of SpoIIE is necessary at the beginning of the formation of the sporulation septa. Some results support the proposal that asymmetric septation is a modified form of vegetative septation and uses the same basic machinery, including proteins as FtsZ, FtsA and others. SpoIIE protein is the only one known sporulation specific protein which deletion or mutation change the ultrustructural feature of asymmetric septa.

What is known about the activation of asymmetric division during sporulation? Spo0A and ^{H} proteins are clearly involved in the shift of cell division to polar sites [42,32]. It was previously suggested that the switch in FtsZ ring localization is effected by a mechanism which blocks formation of such ring at mid-cell and releases the polar sites inhibition [42,43,]. The asymmetric division occurs at about the time normal medial division would occur. Therefore, in the cell with two complete chromosomes where sporulation was initiated, the medial division has to be blocked. Mutant cells affected in Spo0A, divide at mid-cell site without FtsZ ring relocalization effect [42]. However, recently it was shown that the switch from medial to polar Z rings is accomplished by spiral like structure of FtsZ that grows from mid-cell outward toward the cell poles, where it is converted into bipolar rings (Fig. 1B) [32]. Interestingly, the process is reversible and both FtsA and EzrA were shown to co-localize with the FtsZ. SpoIIE plays a crucial role in this process, possibly by activating the bipolar Z rings formation and by stabilizing of such structures.

The next event, after the FtsZ and SpoIIE rings formation, involves repositioning of the entire division machinery to the cell poles. Interesting characteristic of this process is that potential division sites are actually at both cell poles and they are used for septa formation in the disporic mutants, as observed in *spoIIE*, *spoIIAA*, *spoIIAC* (coding ^{F}), *spoIIGA* and *spoIIGB* (coding ^{E}) mutants. It is crucial, that in wild type cell the division occurs at only one of the sites. It is known that ^{E} -dependent genes block the maturation of the second polar division site [44].

Although, the asymmetric cell division during sporulation resembles the vegetative division, we do not know the mechanisms what are involved in accurate placement of the division machinery. Firstly, it appears that it does not involve the Min system because mutations in *minC* and *minD* have little effect on the sporulation frequency [24, 25, 43, 45]. However, it is not possible fully exclude the partial role of Min system during sporulation in light of the fact that in small proportion of *minD* mutant cells sporulation-like septum is misplaced from its normal polar site [29, 46]. Secondly, the polar septum during sporulation overcomes nucleoid occlusion and constricts around the nucleoid. The nature of the effector that over-

comes or eliminates the spatial veto exerted by the nucleoid is not known.

Although, the spatial regulation of vegetative and sporulation cell division significantly differ, both processes use essentially the same protein machinery, except SpoIIE protein what is specific component only of the sporulation septum. *B. subtilis* has homologues of most *E. coli* division proteins, including FtsZ, FtsA, FtsL, FtsQ (DivIB in B. *subtilis*), FtsW (YlaO in *B. subtilis*) and PBP3 (PBP 2B in *B. subtilis*) [46]. The hierarchy of assembly of mid-cell and sporulation division appears to be similar for both processes (again except SpoIIE).

Chromosome partitioning during sporulation

Generally, the cell must coordinate the cell division with the faithful segregation of the newly duplicated chromosomes to each daughter cell. This is followed in vegetatively growing *B. subtilis* cell by complex mechanism. The switch to polar cell division during sporulation has an interesting consequence for chromosome segregation. Strikingly, at the beginning of sporulation, instead of splitting two chromosomes, they form an elongated structure known as axial filament. Localization experiments of *oriC* by using Lac repressor (targeted to a *lacO* gene copies placed near *oriC*) and chromosome segregation protein Spo0J by means of GFP fusion revealed that axial filament formation is accompanied by migration of the *oriC* regions towards opposite poles of the cell [47]. Thus, the chromosomes in this predivisional sporangium are oriented with their replication origin regions to opposite poles of the cell. Interestingly, the anchoring sites of chromosomes are in region lying about 150-300 kbp away from *oriC*. One protein candidate involved in recruiting the chromosomes to this sites is the DivIVA protein [29], which forms an oligomer like structures [48]. The movement of two chromosomes is under the control of the phosphorelay system [49]. There are three known DNA-binding proteins involved in chromosome segregation during sporulation - Spo0J, RacA and Soj. Soj has an ability to undergo co-operative relocation from nucleoid to nucleoid [50] or pole to pole [51] and this movement requires Spo0J protein, which binds to condensation regions at the chromosome near the oriC. RacA was shown that also binds at the chromosome and is a part of the mechanism that attaches the two chromosomes to the poles, likely contacting DivIVA protein already localized at the cell pole (Fig. 3) [52,53].

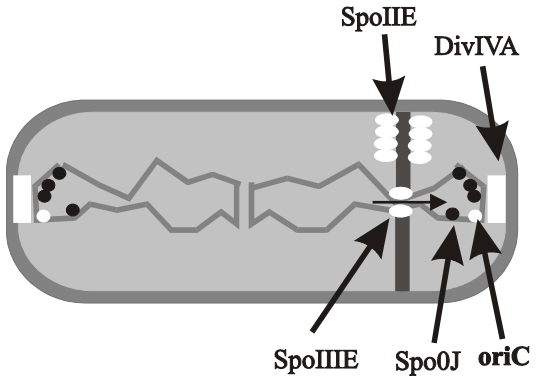

Figure 3. Outline of chromosome segregation during *Bacillus subtilis* sporulation.

Sporulation septum bisects the axial filament leaving only one third of one chromosome in the forespore, creating the transient genetic asymmetry [54]. The remaining two-thirds of the chromosome is then transferred, after 10-20 minutes, from the mother cell into the forespore via conjugation-like mechanism directed by SpoIIIE partitioning protein [55]. The SpoIIIE protein is targeted to the septum [55] and has ATP-dependent DNA-tracking activity with direct role in DNA transfer [56]. SpoIIIE hydrophobic amino-terminal domain is involved in targeting of the protein to the sporulation septum [56]. Additional function of SpoIIIE is in possible membrane-fusion process, later during forespore engulfment [57].

Conclusion and directions

Cell division as a fundamental cellular process still holds many secrets that are waiting to be unraveled. The major challenges now lie in understanding of assembly and disassembly of the protein complexes at the site of division. To understand the molecular mechanisms of these processes would require state of art experimental methods to solve the structure not only particular proteins but rather the protein complexes and their proper interpretation to explain such phenomena as asymmetry of protein localization, protein oscillation, protein spiral formation and other.

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