

Protein Localisation and Protein-protein Contact during Asymmetric Cell Division in *Bacillus subtilis*

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Key words: sporulation, asymmetric cell division, *Bacillus subtilis*

Bacillus subtilis is a gram-positive endospore forming bacterium used for decades as a model organism for studying cell differentiation and morphogenesis. This microorganism decides to sporulate by integrating a wide range of environmental and physiological signals. The signal kinases (e.g., KinA, KinB) initiate the flow of phosphate down the phosphorylay, leading to the phosphorylation of a key transcriptional regulatory protein Spo0A. Once activated by phosphorylation, Spo0A regulates, in addition to its own expression and the phosphorelay loop, the transcription at least seven genes that govern entry into sporulation. A hallmark of sporulation is the formation of the polar septum, which partitions cell into two, different sized compartments – a bigger mother cell and a smaller forespore.

B. subtilis when grown vegetatively, divides by precise placement of a septum at mid-cell. It is not known how the cell finds its middle. Two general models have been proposed to describe these phenomenon in *Escherichia coli*. One is exemplified by the periseptal annulus model (Cook et al 1987) and the second one is the nucleoid occlusion model (Mulder and Woldringh 1993). According first model, the cell determines where to place the new septum. In the second model, the site of division is not predetermined and septation can occur anywhere along the length of the cell with the exception of region that are close to the nucleoid and regions close to the poles. During sporulation the generation of two cells with different development fates is preceded by an asymmetric division. The asymmetric sporulation septum clearly has properties which distinguish it in structural and functional terms from the mid-cell division septum. There are four main differences between these two kinds of septa. First difference is the location of both septa. Second, the mid-cell division septum is a participant in cytokinesis, with the peptidoglycan component splitting bilaterally as daughter cells separate from one another. In contrast, the asymmetrically positioned sporulation septum is a participant in the engulfment process, during which most or all of the peptidoglycan is removed. Third, the division septum is a relatively thick structure containing a substantial amount of peptidoglycan which separates the two daughter cells at cytokinesis. The asymmetric septum formed during sporulation is much thinner. Fourth, even these two septation events are carried out by the same division machinery involving proteins such as FtsZ, FtsA, DivIB, DivIC, FtsL, PBP2B and other proteins, there are also proteins which are specifically associated only with the asymmetric septum, as SpoIIE, SpoIIIE, SpoIIGA and SpoIIGB.

MinC and MinD proteins are inhibitors of septation at medial and polar sites in both micro-organisms, in *E. coli* and *B. subtilis* and their absence leads to minicell formation during vegetative growth. MinE protein is an activator of septation in *E. coli* but no homologous counterpart was found in *B. subtilis*. The MinE protein appears to play a role in topological specificity in *E. coli* (Rothfield and Zhao 1996). Because of the similarity

between minicell formation and asymmetric septation during sporulation it was proposed that there were two MinE like *B. subtilis* proteins, with different topological properties (Rothfield and Zhao 1996). The vegetative specific MinE protein would activate septation at mid-cell whereas sporulation specific MinE would allow division at the polar position and simultaneously allow the division inhibitor to block septation at mid cell. This model also predicates the existence of two different topological properties for these MinE proteins. The vegetative MinE would counteract the action of the MinCD division inhibitor at mid-cell but not at the cell poles. In contrast, sporulation specific MinE would act against the division inhibitor at the polar sites and would block the mid cell site of septation. The search for MinE-like proteins in *Bacillus subtilis* led to the discovery of DivIVA protein which has some characteristics allowing it to carry out the mentioned process (Cha and Stewart 1997, Edwards and Errington 1997). Although genetic evidence supports the hypothesis that DivIVA has an analogous function to MinE in *E. coli* there are important differences between these two proteins. Cha and Stewart (1997) also proposed that DivIVA is involved in activation of polar septation sites during the sporulation process.

FtsZ are tubulin like proteins which create the rings at the freed sites of septation and thus appear to play a pivotal role in cell division in both *E. coli* and *B. subtilis* (B1 and Lutkenhaus 1990). It has been shown also that at the onset of sporulation assembly of FtsZ ring shifts from mid-cell to the two potential polar sites and this switch is dependent on the transcription factor Spo0A (Levin and Losick 1996).

Asymmetric septation at stage II of sporulation coincides with and may actively initiate the establishment of separate programmes of gene expression in two compartments: mother cell and forespore (Losick and Stragier 1992). SpoIIE protein plays a crucial role in activation of first compartment specific sigma factor σ^F during sporulation in *Bacillus subtilis* (Duncan et al 1995). SpoIIE possesses a serine phosphatase activity capable of dephosphorylating and therefore affecting the function of the regulatory protein SpoIIAA which in turn controls the activity of σ^F . This establishes a direct role for SpoIIE in initiating compartment-specific gene expression during sporulation. Other recent work suggests a σ^F independent role for SpoIIE in septum assembly (Barak and Youngman 1996, Feucht et al 1996). We have shown that *spoIIE* mutations fall into two distinct phenotypic classes. The *spoIIE* null mutants exhibit the thick-septum phenotype and missense mutants *spoIIE64* and *spoIIE71* display a strikingly different phenotype characterised by the presence of only thin asymmetric septa. Despite the formation of apparently normal asymmetric septa *spoIIE64* and *spoIIE71* mutants are fully defective in activation of σ^F -dependent gene expression.

Our localisation experiments with SpoIIE GFP fusion in *Bacillus subtilis* and *Bacillus megaterium* clearly showed that this fusion is localised to the sites of asymmetric sporulation septum assembly (Barak et al 1996). We speculate that SpoIIE plays a role in assembling the sporulation septum perhaps determining the special properties of the structure that permit inter compartment signaling during development.

We have placed expression of the *spoIIE* gene under control of the IPTG-inducible P_{spac} promoter and observed morphological changes of septa as well as time course of these changes. We also have investigated the influence of early expression of SpoIIE on septum formation in different mutant background (*spo0A*⁻, *spo0H*⁻ and *spoIIAC*⁻). Our results revealed importance of SpoIIE for initiation and proper asymmetric septum formation (Barak et al 1997). Several other factors must be present for this process. Some of these factors are sporulation specific as Spo0A and Spo0H. On the other hand, σ^F controlled gene products do not specify the sporulation septa formation.

The role of MinCD protein complex in repression of asymmetric septation were ques-

tioned Our study reports septation events in *minCD* mutants during sporulation, as well as the localisation of SpoIIE protein in such constructs Electron-microscopic studies revealed unusual septation at the mid-cell position in the absence of active MinCD complex We have proposed the role of the MinCD complex in asymmetric septum formation during sporulation of *B. subtilis* cells

Sporulation of *Bacillus subtilis* is one of the simplest and also the best understood differentiation process which involve more than 150 sporulation specific proteins in addition to some division and germination proteins More detailed insight into this process ask for using structural studies of the key sporulation and division proteins There are a few sporulation and division proteins whose structure were already solved as SinI-SinR complex (Lewis et al, in press and in this supplement), Spo0B (Zhou et al 1997), Spo0F (Madhusudan et al 1996), FtsZ (Lowe and Amos 1998) and also recently the two structural domains of Spo0A protein have been crystallised (Muchová et al, this supplement)

Acknowledgements. This work has been supported by the grant 2027 from the Slovak Academy of Sciences

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Extending Protein Families through Structural Studies

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Key words: protein structures, evolution

Structural biology is in an exciting era. Improved methods of protein production, crystallisation, X-ray data collection and analysis have greatly reduced the time and effort required to generate 3-D structures of proteins. The number of known structures now forms a useful database to search for relatedness of form and function. Fuelled by the explosion of primary sequences available from genome DNA sequencing projects, protein structures are revealing biochemical and evolutionary links.

Two examples are described. The structure of a DNA helicase suggests how the large number (currently over 300) of known proteins with a characteristic sequence motif "signature" may have evolved different activities and substrate specificity.

In contrast, the emerging Ntn (N-Terminal Nucleophile) hydrolase family is used as an example of protein structures revealing an evolutionary relatedness between proteins which have diverged to such an extent that no sequence homology can be detected, even including their active sites.

The apparent ease with which protein structures are determined is due to parallel advances in both the molecular and structural biology techniques involved. Recombinant protein production and purification is now more reliable, leading to greater chances of success for protein crystallisation. The dissection of large structures into their constituent domains has also proved to be a useful route to building up a native protein structure. Improved methods of data collection, notably crystal freezing to minimise damage from increasingly brilliant X-ray beams and detection by CCD optimises the use of synchrotron time. These advances mean that the biochemists can produce protein in which methionine residues are replaced by selenomethionine, and structural biologists can use Multiwavelength Anomalous Dispersion phasing within a reasonable time-frame to solve the protein structure, thus avoiding the requirement for heavy atom derivatives and concomitant problems with non-isomorphism.

The two structures described here exemplify the wealth of information which can be gleaned from protein structure. The first describes how the structure of one member of a protein super-family not only reveals biochemical and functional data, but also gives clues about the family evolution and acts as a guide to understanding how different activities and functionalities can be derived from elaborations of a common structural framework. DNA helicase from *Bacillus stearothermophilus* was the first member of the helicase family whose structure was solved (Subramanya *et al* 1996). The helicases unwind nucleic