Protein Targeting during *Bacillus subtilis* Sporulation

JONATHAN DWORKEIN

Department of Microbiology & Immunology, College of Physicians and Surgeons, Columbia University, New York, NY 10032

**ABSTRACT** The Gram-positive bacterium *Bacillus subtilis* initiates the formation of an endospore in response to conditions of nutrient limitation. The morphological differentiation that spores undergo initiates with the formation of an asymmetric septum near to one pole of the cell, forming a smaller compartment, the forespore, and a larger compartment, the mother cell. This process continues with the complex morphogenesis of the spore as governed by an intricate series of interactions between forespore and mother cell proteins across the inner and outer forespore membranes. Given that these interactions occur at a particular place in the cell, a critical question is how the proteins involved in these processes get properly targeted, and we discuss recent progress in identifying mechanisms responsible for this targeting.

The first published reports of sporulation were by Ferdinand Cohn in *Bacillus subtilis* and Robert Koch in *Bacillus anthracis* in 1877 in the *Zeitschrift für Pflanzenbiologie* (1, 2). In addition to being among the earliest published reports of bacteria, these articles provided the initial demonstration that bacteria have an internal cellular organization. Cohn and Koch described intracellular membrane-bound compartments of different sizes and positions within the cell. Despite these striking original observations, bacteria were considered for much of the 20th century to be “bags of enzymes” that lacked any discernible spatial organization (3). However, in the past two decades, numerous examples, including dedicated intracellular compartments such as magnetosomes, the presence of a defined orientation of the chromosome, and the polarity of the chemotactic apparatus have emphatically demonstrated that bacterial cells exhibit an intracellular organization that, while less complex than eukaryotic cells, is nonetheless a critical and significant part of their physiology.

Spores look very different from growing cells. This morphological differentiation initiates with an asymmetric division near to one pole of the cell, resulting in the formation of a smaller cell, the forespore, and a larger cell, the mother cell. The sporulation septum is similar, but not identical to the normal mid-cell division septum, and contains a thinner layer of peptidoglycan separating the two compartments. Numerous proteins are specifically localized to this septum, some of which are present on only one side of the septum.

In sporulation, the smaller of the two cells resulting from the asymmetric division becomes encased within the larger cell in a process termed “engulfment” (Fig. 1A, panels iii to v). Following completion of asymmetric septation, the mother cell membranes move and eventually entirely encircle the forespore. While there are superficial similarities to membrane movements that occur during phagocytosis in eukaryotic cells, the analogy may be of limited relevance, since in sporulating cells there is a layer of peptidoglycan that surrounds the forespore and separates the two compartments. During the later stages of spore development, the inner and outer proteinaceous layers of the spore are assembled, and the spore cortex, consisting of a thick layer of

Received: 9 September 2012, Accepted: 4 April 2013, Published: 14 February 2014

Editors: Patrick Eichenberger, New York University, New York, NY, and Adam Driks, Loyola University Medical Center, Maywood, IL


Correspondence: J. Dworkin, jonathan.dworkin@columbia.edu

© 2014 American Society for Microbiology. All rights reserved.
peptidoglycan contained between the inner and outer spore membranes, is synthesized.

In this review, we focus on the intracellular targeting of sporulation-specific proteins that mediate these processes that occur at specific locations in the cell. Although this localization has implications for function in many cases, we do not discuss in detail how these proteins work, and direct the interested reader to several reviews that provide a more general perspective (4–7). Finally, as a note of caution, most of the studies described here use fluorescent protein fusions to monitor localization. While in most cases these fusions complement the endogenous proteins, very recently it has been reported that the particular intracellular distribution observed for a number of proteins such as the MreB helix (8) or the ClpX and ClpP puncta (9) is likely an artifact of the fluorescent protein fusion. Whether this bias is seen in other protein localization studies is not yet known.

ASYMmetric SEPTATION

Like many other bacteria, B. subtilis divides symmetrically during growth. However, upon entry into sporulation, an asymmetric septum forms near one pole with roughly equal probability near either the “old” or the “new” pole (10). In addition to this difference in position, the peptidoglycan layer of the sporulation septum is noticeably thinner than vegetative septa. As with vegetative septa, the initial component of the sporulation septum is the polymeric, tubulin-like protein FtsZ. In fact, the first reported subcellular localization of a soluble protein, FtsZ, was at mid-cell (11). While the precise molecular nature of FtsZ polymer in vivo is not yet clear.
it appears to form a coil-like structure that is centered at mid-cell (13). Under certain circumstances, FtsZ rings are capable of generating a contractile force (14), and, while theoretical analyses suggest that this force may be sufficient to drive cytokinesis (15, 16), whether this is true in vivo is not known.

When cells enter sporulation, the FtsZ ring switches its location from mid-cell to the cell poles (17) (Fig. 1B, top). While this switch maintains cellular symmetry, it should be noted that structures such as FtsZ rings that appear to be symmetric are, at higher resolution, actually asymmetric since they are composed of protein subunits with intrinsic asymmetry (18). The switch is governed by the transcription factor Spo0A, since spo0A mutants fail to form polar FtsZ rings (17), and requires increases in the level of FtsZ and expression of the FtsZ interacting protein SpoIIE that are both dependent on Spo0A (19). The shift in FtsZ-ring localization occurs via a spiral intermediate along the long axis of the cell in a “slinky-like” movement (19), although what causes this process to overcome the MinCD-dependent inhibition of FtsZ-ring formation near the pole is not known. Recently, a new protein, RefZ, has been described that appears to mediate the transition of FtsZ rings into spirals (20). RefZ is a DNA-binding protein that recognizes specific chromosomal sequences suggesting that the chromosome itself plays a direct role in the FtsZ ring→spiral transition (20), but the underlying mechanism has not yet been worked out.

Sporulating cells rarely make two mature polar septa (5), although this event can be observed in so-called diphasic mutants that have a terminal asporogenous phenotype (21). This event is symmetry breaking since sporulating cells go from being symmetric to asymmetric with respect to septum position (22) (although the coccal bacterium Sporosarcina ureae undergoes symmetric division in sporulation [23]). An important clue to the mechanism underlying the transition is that the two polar septa are formed sequentially, not simultaneously. One interpretation of this observation is that cells are able to only construct a single septum at a time (24) perhaps from insufficient levels of factor(s) necessary for the simultaneous synthesis of two septa. Clearly, however, cells have sufficient levels of some key cytokinetic proteins (e.g., FtsZ) to form multiple structures. Whatever the mechanism underlying the sequential nature of septation, the cellular asymmetry is maintained by a checkpoint system whereby σ^F activation in the mother cell leads to the production of proteins (SpoIID, SpoIIM, and SpoIIP) that act together to inhibit formation of a second septum (24, 25).

A key consequence of asymmetric septation is that the septum forms over one of the two chromosomes. Thus, the origin-proximal one-third of that chromosome is located in the forespore, the smaller compartment, and genes at origin distal positions are located outside the forespore. Until the remainder of the chromosome is translocated, origin-distal genes are excluded from the forespore. This transient asymmetry has important implications for sporulation (26), since the differential activation of the compartment-specific transcription factors σ^F and σ^E is dependent on the chromosomal position of genes encoding the proteins, SpoIAB (27) and SpoIIR (28), respectively, which are necessary for their activation. The DNA-binding protein RacA, which is under control of Spo0A, is essential for the proper attachment of this chromosome origin to a cell pole, ensuring the establishment of this polarity (29, 30). RacA localizes to the cell poles through an interaction with the protein DivIVA (29), a peripheral membrane protein that preferentially localizes to negatively curved membranes (31).

**SpoIIE**

FtsZ serves as a nucleating factor for proteins that mediate other cytokinetic functions (32, 33). One sporulation-specific protein that interacts directly with FtsZ is the membrane PP2C-type phosphatase SpoIIE (34). The localization of SpoIIE, at least initially, to polar positions depends on FtsZ (35, 36) (Fig. 1C), but, as is seen with FtsZ, SpoIIE becomes unipolar in its localization pattern (35, 37). Although (as discussed below), the main function of SpoIIE is in the pathway leading to forespore-specific activation of σ^F, SpoIIE may be involved also in septum formation since ΔspoIIE mutants have an unusually thin septum (38).

A key aspect of sporulation is the compartment-specific activation of sigma factors in the mother cell and the forespore, which follow a crisscross pattern of regulation where the activation of a sigma factor in one compartment leads to the subsequent activation in the other compartment (39). SpoIIE plays a key role in the activation of the initial forespore transcription factor σ^F by dephosphorylating SpoIIA-P, which then relieves SpoIIAB-dependent inhibition of σ^F (40). Early models of σ^F activation hypothesized that SpoIIE was targeted to both faces of the asymmetric septum (41) and that the greater surface-to-volume ratio of the forespore in comparison with the mother cell was sufficient to account for preferential forespore activity. However, measurements of SpoIIE-green fluorescent protein (GFP) distribution in sporangia found that SpoIIE is enriched
in the forespore, indicating that the relative enrichment due to surface-to-volume ratio may not be sufficient (35). In addition, detailed analysis of SpoIIIE localization in single sporulating cells using PSICIC (Projected System of Internal Coordinates from Interpolated Contours) software indicates that SpoIIIE is preferentially distributed on the forespore face (42) (Fig. 1C). How this asymmetry arises is unclear, but, since SpoIIIE interacts with FtsZ (34), the intrinsic asymmetry of the FtsZ polymers may be responsible.

**SpoIIIE**

For sporulation to proceed, the distal two-thirds of one of the two replicated chromosomes must be pumped into the forespore. SpoIIIIE, a large, polytopic membrane protein belonging to the FtsK family of DNA transporters, is responsible for this translocation. Members of this family have diverse physiological roles including chromosome partitioning during vegetative growth and conjugative transfer of plasmid DNA. SpoIIIIE, like related proteins, is an ATPase and contains a DNA-binding domain. These proteins form hexameric aqueous channels that translocate one single double-stranded DNA molecule at a time (43). Recent evidence using superresolution microscopy indicates that DNA translocation is mediated by 45-nm complexes each composed of ~50 SpoIIIIE molecules that form a single pore (44).

SpoIIIIE is observed at the asymmetric septum and is enriched toward its center (45) (Fig. 2A, panels i and ii). SpoIIIIE fails to localize to the asymmetric division septum during sporulation when genetic perturbations result in the absence of DNA from the forespore, suggesting that it only assembles at the sporulation septum when DNA is trapped by the membrane (46). SpoIIIIE forms a focus only in the mother cell side of the septum in wild-type cells (47). Consistent with the role of the asymmetric distribution of specific chromosomal sequences in maintaining polarity of translocation for FtsK (48), alterations in chromosome architecture switched SpoIIIIE assembly to the forespore, and DNA translocation-defective SpoIIIIE proteins assembled in both cells. Thus, DNA determines the “macroscale” septal localization of SpoIIIIE, but it also determines the “microscale” partitioning of SpoIIIIE between the forespore and mother cell faces of the septal membrane.

SpoIIIIE is also observed following septation at sites of membrane fission between the mother cell and the forespore (Fig. 2A, panel iii) (49), and some spoIIIIE mutations result in engulfment defects. SpoIIIIE is observed at the leading edge of the migrating septal mem-

brane as engulfment progresses (50), suggesting a model for passive redistribution of SpoIIIIE to the poles as a consequence of this membrane movement. The basis for this movement is the subject of active study, although the synthesis (51) and degradation (52) of the peptidoglycan (as mediated by the SpoIID and SpoIIP proteins, see below) that lies between the mother cell and forespore membranes both appear to contribute. FisB is a recently identified protein that also localizes to the sites of membrane fission and is required for optimal fission (53). FisB interacts closely with cardiolipin (53), and these interactions may mediate its localization to the engulfing membrane, which has a high degree of negative curvature (54).

**SpoIIIGA/SpoIIR**

One of the first genes to be expressed in the forespore following σE activation is spoIIR, which encodes a secreted protein that stimulates conversion of pro-σE to σE only in the mother cell. SpoIIR is likely exported into the intermembrane space of the septum where it activates the membrane-associated pro-σE-processing enzyme SpoIIGA. A key question has been how σE is selectively activated in the mother cell. Pro-σE is distributed uniformly along all membrane surfaces and is not confined to the mother cell face of the septum (55). Thus, the asymmetric localization of pro-σE is not responsible for the differential activation. In addition, SpoIIR shows a septal pattern of localization (56) that is dependent on SpoIIGA (57), indicating that the localization of SpoIIGA is central. SpoIIGA is seen very early on in polar septum formation, suggesting that it interacts with a component of the division machinery (Fig. 2B). SpoIIGA localization is not dependent on SpoIIE (58), but the role of other proteins found at the polar septum is not known. SpoIIGA may become enriched in the mother cell through a diffusion-and-capture model, but the “anchor” protein has not been identified (59). It is possible that like SpoIIE, SpoIIGA becomes enriched on the mother cell membrane through an interaction with a protein polymer like FtsZ that is intrinsically asymmetric. Thus, restriction of SpoIIR action to the mother cell face of the septum likely originates in the asymmetric distribution of SpoIIGA.

**SpoIIB/SpoIID/SpoIIM/SpoIIP**

The peptidoglycan located in the intermembrane space between the outer forespore and inner forespore membranes is attached to the peptidoglycan that surrounds the combined mother cell and forespore. This peptidoglycan makes a rigid connection that must be severed for
FIGURE 2 Localization of septal proteins early in sporulation. A. The SpoIIIE DNA translocase (red) localizes to the asymmetric septum (i) because of the presence of DNA (blue) and mediates DNA pumping into the forespore (ii). Following completion of septation, SpoIIIE is found in the forespore membrane at the last point of contact with the mother cell (iii). B. The pro-$\sigma^E$ processing enzyme SpoIIGA (yellow) initially localizes to the sites of incipient septum formation (i) and then to the mother cell face of the asymmetric septum (ii). The SpoIIR signaling protein (green) is made in the forespore and crosses the forespore membrane where it presumably interacts with and activates SpoIIGA, although it is also seen in the forespore following completion of septation. C. SpoIIB (aqua) initially colocalizes with FtsZ (orange) during the process of Z-ring constriction and remains in the polar septum following completion of septation. The SpoIM (light blue), SpoIIP (dark blue), and SpoIID (purple) proteins proceed to localize to the now curved asymmetric septum.

engulfment to occur. Mutations in spoIID, spoIIM, or spoIIP result in the bulging of the forespore compartment into the mother cell and prevent engulfment from proceeding \(^{(60)}\). SpoIID is a peptidoglycan hydrolyase \(^{(61)}\) and SpoIIP is an autolysin \(^{(62)}\) that is both an amidase and endopeptidase that removes the stem peptides from the cell wall and cleaves their cross-links \(^{(52)}\). Together with SpoIIM, these proteins form a so-called DMP complex that hydrolyzes the peptidoglycan between the two membranes, thereby facilitating both interactions between mother cell and forespore proteins (e.g., SpoIIQ and SpoIIIAH \(^{(63, 64)}\); see below) as well as the movement of the mother cell membrane around the forespore \(^{(62, 65)}\). Localization of SpoIID depends on SpoIIP, which itself depends on SpoIIM \(^{(62)}\). The sporulation protein SpoIIB of unknown enzymatic activity also localizes to the septal membrane \(^{(66)}\), and SpoIIM depends on SpoIIB for its septal localization, leading to a hierarchical model of localization: SpoIIB \(\rightarrow\) SpoIIM \(\rightarrow\) SpoIIP \(\rightarrow\) SpoIID \(^{(Fig. 2C)}\) \(^{(65)}\). However, the DMP complex can localize in the absence of SpoIIB by apparently using the SpoIVFAB complex as a secondary mechanism \(^{(65)}\) (see below for discussion of SpoIVFAB). Finally, in addition to their role in engulfment, SpoIID, SpoIIM, and SpoIIP play a key role in maintaining cellular asymmetry by preventing the formation of a second polar septum and the resulting formation of a terminal disporic cell \(^{(24, 25)}\).

**SpoIIQ/SpoIIIAH**

When the septal peptidoglycan of sporulating cells is enzymatically removed by lysozyme, the cells engulf even in the absence of the DMP complex and instead require the forespore protein SpoIIQ and its mother cell partner SpoIIIAH \(^{(67)}\). SpoIIQ is an integral membrane protein under the control of \(\sigma^I\) that is essential for the synthesis and activation of the late forespore-specific transcription factor \(\sigma^G\) \(^{(68)}\). The extracellular portion of SpoIIQ contains a LytM domain found typically in endopeptidases. However, SpoIIQ is probably not an endopeptidase since its LytM domain lacks a key catalytic residue, it does not appear to interact directly with peptidoglycan \textit{in vitro} \(^{(69)}\), and two recent crystal structures indicate that it does not coordinate Zn\(^{2+}\) \(^{(70, 71)}\). Following asymmetric septation, a SpoIIQ fluorescent protein fusion is observed at the forespore membrane, and, when engulfment initiates, it moves around the forespore. Finally, near the completion of engulfment, SpoIIQ is seen as punctate membrane-associated fluorescence \(^{(56)}\) \(^{(Fig. 3A, purple)}\). SpoIIQ localization is dependent on the degradation of septal peptidoglycan by SpoIID and SpoIIP, and this localization is apparently stabilized by an interaction with its partner protein SpoIIIAH \(^{(63, 64)}\).

The polytopic membrane protein SpoIIIAH is expressed under control of \(\sigma^E\) in the mother cell. SpoIIIAH is part of the mother cell SpoIIA complex, composed of eight membrane proteins, that is necessary for activation of \(\sigma^G\) \(^{(72)}\). This complex forms a channel between the mother cell and the forespore \(^{(73, 74)}\) and may allow the movement of small molecules from the mother cell into the forespore \(^{(75)}\). SpoIIIAH is initially observed in all of the mother cell membranes, but, consistent with its role in intercompartmental signaling, SpoIIIAH becomes localized to the septum \(^{(Fig. 3A, yellow)}\). This enrichment is dependent on SpoIIQ \(^{(76, 77)}\) and is mediated by an interaction between the LytM domain of SpoIIQ and the extracellular YscJ domain of SpoIIAH \(^{(69, 75)}\) in the sporulation septum \(^{(Fig. 3B)}\). Recent crystal structures of the SpoIIQ-SpoIIIAH complex are consistent with these biochemical studies and demonstrate that SpoIIQ and SpoIIIAH both form ringlike structures \(^{(70, 71)}\).

This capture results in the assembly of SpoIIIAH with SpoIIQ into helical arcs and foci around the forespore \(^{(76)}\). Photobleaching experiments indicate that the SpoIIQ multimer does not freely diffuse, so SpoIIQ and SpoIIIAH complex could function as a ratchet to irreversibly drive engulfment \(^{(67)}\). Not much is known about how the other components of the SpoIIIA complex are targeted, but SpoIIIAH and SpoIIQ are necessary for the localization of a cyan fluorescent protein-SpoIIAG fusion protein to the mother cell-forespore interface \(^{(78)}\). The septal targeting of SpoIIIAH by SpoIIQ is an example of the mechanism of “diffusion and capture” \(^{(79)}\). In other examples of this mechanism (e.g., SpoIVFASpoIVFB, see below) it is often unclear how the landmark anchoring protein is initially targeted. However, in the case of SpoIIAH-SpoIIQ, one distinguishing feature of the incipient outer forespore membrane is that it is adjacent to the inner forespore membrane. Thus, specific gene expression \(^{(\sigma^E\text{-dependent} spoIIQ)}\) in one compartment leads to asymmetrical protein distribution in another compartment. This is another example of how the intrinsic asymmetry of the chromosome is converted to a spatial asymmetry \(^{(26)}\).

**SpoIVFA/SpoIVFB/BofA**

The final sigma factor to be activated is \(\sigma^K\) in the mother cell. \(\sigma^K\) is present as a pro-protein and the processing of pro-\(\sigma^K\) to \(\sigma^K\) is mediated by the membrane-embedded metalloprotease SpoIVFB, which itself is held inactive by two other membrane proteins SpoIVFA and BofA. Pro-\(\sigma^K\) and all three of these proteins are enriched at the

---

**Dworkin**

---
outer forespore membrane as demonstrated both by immunofluorescence (pro-σ^K [80] and SpoIVFA and SpoIVFB [81]) and by complementing GFP fusions (SpoIVFA [82], SpoIVB [81], and BofA [82]). σ^K processing depends on a forespore-produced protein SpoIVB that cleaves the inhibitory protein SpoIVFA thereby freeing the SpoIVFB to process pro-σ^K (83). The signal that SpoIVB responds to is unknown, but SpoIVB fails to accumulate when engulfment is perturbed, suggesting that SpoIVB levels act as a checkpoint for the progression of engulfment (84). Given the presence of this checkpoint, it is imperative for σ^K processing to occur on the outer forespore membrane and not on the mother cell membrane. Since localization of both BofA and SpoIVFB is dependent on SpoIVFA (82), understanding the localization of SpoIVFA is key to understanding the targeting of the entire complex.

While SpoIVFB is initially observed on all accessible mother cell membranes, it becomes selectively enriched at the outer forespore membrane via a diffusion-and-capture mechanism (Fig. 3C). Following insertion of SpoIVFB randomly into the mother cell membrane, it undergoes free diffusion. SpoIVFB is then captured in the forespore membrane since, when the forespore membrane is not contiguous with the mother cell membrane, targeting does not occur (79). The actual anchor responsible for the “capture” is not known, but a direct biochemical interaction between the extracellular domains of SpoIIIah and SpoIIQ and that of SpoIVFA has been demonstrated (77), and both proteins are necessary for proper SpoIVFA localization (65). While it is possible that the SpoIIQ-SpoIIIah complex is sufficient for SpoIVFA localization, the reported dependence of this localization on SpoID, SpoIM, and SpoIIp (77)
suggests that a complex, multiprotein network may serve to anchor SpoIVFA to the outer forespore membrane (Fig. 3D).

**SpoVM and SpoIVA**

The assembling coat is synthesized in the mother cell and is targeted to the outer forespore membrane by SpoIVA (85). SpoIVA binds and hydrolyzes ATP, allowing it to self-assemble into cable-like structures (86) that form a basement layer that serves as a platform for coat assembly. A forthcoming review by A. Driks and P. Eichenberger contains a detailed discussion of protein targeting to the different layers of the coat. SpoIVA localizes to the outer forespore membrane (Fig. 4A, blue), and proper targeting of SpoIVA depends on SpoVM, a 26-amino-acid amphipathic helical peptide that is expressed under σE control in the mother cell (87). A SpoVM-GFP fusion is targeted to the forespore via amino acid side chains on the hydrophobic face of the helix (88), suggesting that the SpoVM helix is oriented parallel to the membrane with the hydrophobic face buried in the lipid bilayer (89).

SpoVM-GFP produced after engulfment still was targeted to the forespore, even though this membrane was now topologically isolated (90), suggesting that the targeting was not occurring through a diffusion-and-capture mechanism. While SpoVM could be targeted by an interaction with an anchor protein present in the outer forespore membrane, its very small size poses a potential problem because of the apparent lack of a domain that might mediate this interaction. Alternatively, following engulfment, the outer forespore membrane has a high positive curvature, suggesting that SpoVM could be detecting this feature. To examine this possibility, purified SpoVM was incubated with phospholipid vesicles similar in size to the forespore (90). The binding of SpoVM was quantitatively related to the vesicle size (curvature) and was reduced by a mutation in the SpoVM amphipathic helix that also abolished localization of SpoVM to the outer forespore membrane in vitro (90). However, while in vitro the membrane curvature appears to be sufficient, the in vivo localization of SpoVM also depended on SpoIVA (89). The ability of a spoIVA suppressor mutation to allow the correct targeting of a mislocalized SpoVM mutant protein indicates that this interaction is direct. Thus, the localization of SpoVM and SpoIVA is interdependent and depends on the extreme positive curvature of the outer forespore membrane following engulfment. A key question for future work is to determine how SpoVM senses this curvature despite its small size and its inability to multimerize in vitro (90), characteristics that would be expected to preclude direct measurement of curvature.

Insight into this process underlying SpoVM targeting is key not just for understanding SpoIVA targeting, but also for other proteins. For example, the small CmpA protein localizes to the outer forespore membrane in a fashion dependent on SpoVM, and CmpA is involved in spore cortex assembly (91). In addition, the proper localization of the SpoVID coat protein depends on both SpoVM and SpoIVA, although the effects of ΔspoVM and ΔspoIVA mutations on SpoVID localization differ (85). Also, whereas SpoVM localization was unaffected in cells carrying a ΔspoVID mutation, SpoIVA locali-
zation was incomplete (85). This complicated set of interactions is critical for the process of spore encasement where the full shell of spore coat proteins is constructed around the entire circumference of the spore (92).

**SpoVD/SpoVE/SpoVB/YkvU**

As a consequence of engulfment, two distinct membranes separated by a thin layer of peptidoglycan surround the developing forespore. The initial layer, called the germ cell wall, is greatly thickened following completion of engulfment by the production of the peptidoglycan cortex (see also reference 100). This results from the action of genes expressed in the mother cell compartment under control of σ^K including spoVE that encodes a SEDS (shape, elongation, division, and sporulation) protein. ΔspoVE mutants fail to form a cortex and they accumulate cytoplasmic peptidoglycan precursors (93), suggesting a defect in peptidoglycan polymerization. Complementing SpoVE-GFP fusion proteins localize to the outer forespore membrane (Fig. 4B) and mutations in several conserved residues result in protein mislocalization (94). SpoVE targeting to this membrane is dependent on SpoIIQ, SpoIIIAH, and SpoIVFA (A. Fay and J. Dworkin, unpublished data), but it is not known whether this dependence is the result of a direct protein-protein interaction. SpoVE interacts directly both in vivo and in vitro with SpoVD, a penicillin-binding protein that is also required for spore cortex synthesis. SpoVD also localizes to the outer forespore membrane and this targeting is dependent on SpoVE (Fig. 4B and C) (95).

The SpoVB and YkvU proteins are sporulation-specific homologs of *Escherichia coli* MviN, a broadly conserved protein involved in an as yet not well-characterized fashion in peptidoglycan synthesis. SpoVB (96) and YkvU (97) proteins fused to GFP localize to the outer forespore membrane, consistent with the absence of cortex seen in ΔspoVB mutants (98), but nothing is known about this targeting.

**FUTURE DIRECTIONS**

The dramatic morphological asymmetry of sporulating *B. subtilis* has greatly facilitated the characterization of mechanisms responsible for targeting proteins to particular intracellular loci. An important question is: to what extent do these mechanisms operate in targeting proteins in other cells? Given the particularities of the morphological transformation of sporulation—specifically, the formation of a cell within a cell and the creation of a membrane-bound chromosome—some of the targeting mechanisms described above will not be generally relevant. However, since all cells localize proteins to active sites of septum formation, and positively curved membranes are seen at these positions, the mechanism that SpoVM uses to detect curvature might be generally applicable. One clear future direction is increased spatial resolution in light microscopy (e.g., references 44 and 99), which will facilitate progress on several critical issues. For example, is SpoIIIR on both membranes in the space between the forespore and the mother cell? Do the FtsZ rings early in sporulation exhibit differences in structure? With increasing evidence that proteins exist in cells as part of multiprotein complexes, increased spatial resolution will also facilitate understanding of the organization of these complexes. The study of these complexes in sporulation (e.g., BofA/SpoIVFA/SpoIVFB) will likely reveal general principles underlying the nature of analogous complexes in other microbiological (and, in fact, more general cellular) pathways.

**ACKNOWLEDGMENTS**

Work on sporulation in my laboratory is supported by the National Institutes of Health (R01GM081368).

**REFERENCES**


Dworkin


