SpoIIQ ANCHORS MEMBRANE PROTEINS ON BOTH SIDES OF THE SPOURATION SEPTUM IN BACILLUS SUBTILIS*
Nathalie Campo, Kathleen A. Marquis and David Z. Rudner
From the Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts, 02115
Running Title: SpoIIQ anchors SpoIIE in the engulfing septum
Address correspondence to: David Z. Rudner, Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Ave., Boston, MA 02115, Tel: 617 432-4455; Fax: 617 738-7664; E-Mail: rudner@hms.harvard.edu

During the process of spore formation in Bacillus subtilis, many membrane proteins localize to the polar septum where they participate in morphogenesis and signal transduction. The forespore membrane protein SpoIIQ plays a central role in anchoring several mother-cell membrane proteins in the septal membrane. Here, we report that SpoIIQ is also responsible for anchoring a membrane protein on the forespore side of the sporulation septum. Co-immunoprecipitation experiments reveal that SpoIIQ resides in a complex with the polytopic membrane protein SpoIIE. During the early stages of sporulation, SpoIIE participates in the switch from medial to polar division and co-localizes with FtsZ at the polar septum. We show that after cytokinesis, SpoIIE is released from the septum and transiently localizes to all membranes in the forespore compartment. Upon the initiation of engulfment, it specifically re-localizes to the septal membrane on the forespore side. Importantly, the re-localization of SpoIIE to the engulfing septum requires SpoIIQ. These results indicate that SpoIIQ is required to anchor membrane proteins on both sides of the division septum. Moreover, our data suggest that forespore membrane proteins can localize to the septal membrane by diffusion-and-capture as has been described for membrane proteins in the mother cell. Finally, our results raise the intriguing possibility that SpoIIE has an uncharacterized function at a late stage of sporulation.

Despite their small size and apparent simplicity, bacteria exhibit remarkably complex spatial organization, in which proteins localize to particular sites within the cell. Well-characterized examples include the cell division proteins, which assemble into a cytokinetic ring at mid-cell (1-3); the chemotaxis receptors, which localize to the flagellated cell pole (4); the actin-like cytoskeletal proteins that form spiral-like structures along the long-axis of the cell (5); and the secretion apparatus, which localizes at discrete sites in the cell membranes (6-8). Although the number of proteins that display specific patterns of localization continues to grow, our knowledge of the cellular landmarks that anchor them at particular sites remains poorly understood.

A powerful system in which to identify the anchors responsible for localizing proteins at particular sites is the process of sporulation in B. subtilis. Many proteins involved in spore formation localize to defined subcellular positions and this localization is critical for their activity. Upon the initiation of sporulation, the developing cell (called the sporangium) divides into two compartments of unequal size and dissimilar fates. The smaller compartment (the forespore) matures into a dormant spore, while the larger cell (the mother cell) nurtures the spore, packaging it in a protective coat. Through out the course of this developmental process, the two cells follow completely different programs of gene expression that are set in motion by the compartment-specific transcription factors $\sigma^F$ in the forespore and $\sigma^E$ in the mother cell.

The first landmark event in the process of sporulation is the formation of an asymmetric septum. The switch from medial to polar division is brought about by a change in the subcellular position of the cytokinetic FtsZ-ring from mid-cell to the cell poles (9). The membrane phosphatase SpoIIE, which is synthesized at the onset of sporulation, co-localize with FtsZ and plays an important role in re-positioning the FtsZ ring (10-13). After the septum is complete, SpoIIE has a second function: the activation of the forespore-
specific transcription factor, $\sigma^F$ (14-16). The localization of SpoIE at the polar septum plays a critical role in coupling developmental gene expression to the completion of this morphological event (17).

Shortly after polar division, the mother cell engulfs the forespore in a phagocytic-like process generating a cell-within-a-cell. As a result of engulfment, the forespore is surrounded by two membranes: its own referred to as the inner forespore membrane and one derived from the mother cell called the outer forespore membrane. Many proteins involved in spore morphogenesis and signal transduction specifically localize to the engulfing septal membranes (18-23). Mother cell membrane proteins achieve this localization by insertion into the cytoplasmic membranes followed by diffusion to and capture in the septum (24). However, in most cases it is not clear how these proteins are captured at this particular site. One example in which the anchoring mechanism is well understood is the mother-cell membrane protein SpoIIIAH. SpoIIIAH localizes to the engulfing septal membranes and this localization depends on a forespore membrane protein SpoIIQ (19,20). Importantly, the extracellular domains of SpoIIIAH and SpoIIQ interact in the space between the mother cell and forespore membranes (19,20). Thus, the zipper-like interaction between SpoIIQ and SpoIIIAH anchors SpoIIIAH in the engulfing septum. SpoIIQ itself localizes to the septal membrane on the forespore side but the mechanism by which it is anchored at this site is unknown (21). Interestingly, SpoIIIAH and SpoIIQ are also required for the proper localization of several other mother-cell membrane proteins (20,25). However, a direct link between these protein and SpoIIIAH or SpoIIQ has not been established.

In this report, we show that SpoIIQ is responsible for anchoring membrane proteins on the forespore side of the septal membrane. We demonstrate that SpoIIQ resides in a membrane complex with SpoIE. Re-examination of the subcellular localization of SpoIE revealed that after asymmetric division, SpoIE is released from the polar septum and transiently localizes to all membranes of the forespore compartment. Upon the initiation of engulfment, it specifically re-localizes to the septal membrane. We show that SpoIIQ is required to anchor SpoIE in the engulfing septal membrane. These results indicate that SpoIIQ plays a major role in organizing membrane proteins on both sides of the division septum. Moreover, they suggest that forespore membrane proteins can achieve proper localization by diffusion-and-capture. Finally, these data raise the possibility that SpoIE has a third function in sporulation during or after the morphological process of engulfment.

**EXPERIMENTAL PROCEDURES**

**General Methods-** All *B. subtilis* strains were derived from the prototrophic strain PY79 (26) and are listed in Table S1. All strains used for the immunoprecipitation experiments contain a *spoIVB* mutation to prevent degradation of proteins that have domains in the intermembrane space (K.A.M., N.C. and D.Z.R., unpublished observations). Sporulation was induced by resuspension at 37°C according to the method of Sterlini-Mandelstam (27).

**Anti-GFP antibody resin-** Affinity-purified anti-GFP antibodies (4 mg) (22) were batched absorbed to 1 ml protein A-sepharose (Amersham) for 1 hour at 4°C. The antibody resin was washed four times with phosphate buffered saline (PBS) and the antibody was covalently crosslinked to the protein A-sepharose by the addition of Disuccinimidyl Suberate (Pierce) to a final concentration of 5 mM. After 30 minutes the reaction was quenched by the addition of Tris pH 7.5 to a final concentration of 100 mM. The antibody resin was washed with 100 mM Glycine pH 2.5 to remove uncrosslinked antibody and then neutralized with 1X PBS.

**Preparation of crude membranes and detergent solubilization of membrane proteins-** 50 ml cultures were harvested at hour 2.5 after the initiation of sporulation and washed two times with 1X SMM (0.5 M Sucrose, 20 mM MgCl₂, 20 mM Maleic acid pH 6.5) at room temperature. Cells were resuspended in 1/10 volume 1X SMM and protoplasted with Lysozyme (0.5 mg/ml). Protoplasts were collected by centrifugation and flash frozen in N₂(l). Thawed protoplasts were disrupted by osmotic lysis with 3 ml hypotonic buffer (Buffer H) (20 mM Hepes pH 8, 200 mM NaCl, 1 mM DTT, with protease inhibitors: 1 mM PMSF, 0.5 µg/ml leupeptin, 0.7 µg/ml pepstatin). MgCl₂ and CaCl₂ were added to 1 mM and lysates

Downloaded from http://www.jbc.org/ by guest on March 25, 2020
were treated with DNAseI (10 µg/ml) (Worthington) and RNAseA (20 µg/ml) (USB) for 1 hour on ice. The membrane fraction was separated by centrifugation at 100,000X g for 1 hour at 4˚C. The supernatant was carefully removed and the membrane pellet was dispersed in 200 µl Buffer G (Buffer H with 10% Glycerol). Crude membranes were aliquoted and flash frozen in N2(l). 50-100 µl crude membranes were diluted 5-fold with Buffer S [Buffer H with 20% Glycerol and 100 µg/ml Lysozyme] and membrane proteins were solubilized by the addition of the nonionic detergent Digitonin (Sigma) to a final concentration of 0.5%. The mixture was rotated at 4˚C for 1 hour. Soluble and insoluble fractions were separated by centrifugation at 100,000X g for 1 hour at 4˚C.

Co-immunoprecipitation from detergent-solubilized membrane fractions- The soluble fraction from the Digitonin-treated membrane preparation (the load) was mixed with 20 µl affinity-purified anti-GFP antibody resin and rotated for 4 hours at 4˚C. The resin was pelleted at 5Krpm and the supernatant (the flow through) was removed. The resin was washed 4 times with 1 ml Buffer S + 0.5% Digitonin. Immunoprecipitated proteins were eluted by the addition of 50µl of sodium dodecyl sulfate (SDS) sample buffer [0.25 M Tris pH 6.8, 6% SDS, 10 mM EDTA, 20% Glycerol] and heated for 15 minutes at 50˚C. The resin was pelleted and the supernatant (the IP) was transferred to a fresh tube and 2-Mercaptoethanol was added to a final concentration of 10%. The load, flow through and immunoprecipitate were analyzed by immunoblot and SDS-PAGE followed by silver staining (28).

Immunoblot analysis- Samples were heated for 15 minutes at 50˚C prior to loading. Proteins were separated by SDS-PAGE on 12% polyacrylamide gels, electrophoresed onto PVDF membranes (PerkinElmer) and blocked in 5% nonfat milk in phosphate-buffered saline (PBS)-0.5% Tween-20. The blocked membrane was probed with anti-GFP (22), anti-SpoIIQ (29), anti-SpoIE (10), or anti-EzrA (30) antibodies. Primary antibodies were diluted 1:10,000 into 3% BSA in PBS-0.05% Tween-20. Primary antibodies were detected using horseradish peroxidase-conjugated goat, anti-rabbit (anti-GFP, anti-SpoIQ and anti-EzrA) or anti-rat (anti-SpoIE) immunoglobulin G (BioRad and Pierce) with the Western Lightning substrate (PerkinElmer).

Mass Spectrometry Analysis- Individual bands were excised from silver-stained gel and trypsinized. Extracted peptides were then separated on a nanoscale C18 reverse-phase HPLC capillary column, and were subjected to electrospray ionization followed by MS using an LCQ DECQ ion-trap mass spectrometer.

Fluorescence microscopy- Fluorescence microscopy was performed with an Olympus BX61 microscope as previously described (31). SpoIE-GFP (and SpoIE-YFP) was visualized on agarose pads as described previously (12). All other fluorescent proteins were visualized on glass slides with poly-L lysine-treated coverslips. Fluorescent signals were visualized with a phase contrast objective UplanF1 100x and captured with a monochrome CoolSnapHQ digital camera (Photometrics) using Metamorph software (Universal Imaging). The lipophylic membrane dye TMA-DPH (Molecular Probes) was used at a final concentration of 0.05 mM and exposure times were typically 200 ms. Images were analysed, adjusted and cropped using Metamorph software.

RESULTS

SpoIE reside in a membrane complex with SpoIIQ. The forespore membrane protein SpoIIQ interacts across the sporulation septum with the mother cell membrane protein SpoIIIAH. We and others have shown that these two proteins are required for the efficient localization of proteins involved in morphogenesis and cell-cell signaling (19,20). To identify other proteins that reside in a complex with SpoIIQ and SpoIIIAH, we immunopurified SpoIIQ from detergent-solubilized membrane fractions. For these experiments, we took advantage of a high-affinity anti-GFP antibody resin (32) and a strain (BTD665) harboring a functional CFP-SpoIIQ fusion (20). At hour 2.5 of sporulation, cells were harvested and disrupted by osmotic lysis. A crude membrane fraction was isolated and solubilized by the addition of the nonionic detergent digitonin. Immunoprecipitations were performed on the clarified detergent-solubilized fraction and the immunoprecipitates were analyzed by SDS-PAGE followed by silver staining (Fig. 1A and see
Experimental Procedures). As controls, immunoprecipitations were performed on detergent-solubilized lysates derived from a strain (BNC689) lacking a GFP fusion and a strain (BNC1384) harboring a GFP fusion to an unrelated *E. coli* membrane protein (MalF) (Fig. 1A) (21). The *malF-gfp* fusion was expressed from the *spoIIG* promoter (*P*<sub>*spoIIQ*). Accordingly, this heterologous membrane protein was synthesized at the same time and in the same cellular compartment as SpoIIQ.

Silver staining revealed that several other proteins co-purify with CFP-SpoIIQ (Fig. 1A). Importantly, no more than a few faint bands were detected in the immunoprecipitates from the lysate lacking a GFP fusion (Fig. 1A). Similarly, only a small number of proteins co-immunoprecipitated with the Mal-GFP fusion. We note that the heavy and light chains from the anti-GFP antibodies were barely detectable in these experiments due to the efficient crosslinking procedure we used to generate the antibody resin (see Experimental Procedures). To identify the proteins that were present in complexes with SpoIIQ, we cut out individual bands from the silver-stained gel, digested them with trypsin and analyzed the trypic fragments by Mass Spectrometry (MS). By this method, we identified several mother-cell-specific proteins and several proteins that are made during vegetative growth (N.C. and D.Z.R., unpublished).

In addition, we identified the sporulation membrane protein SpoIIIE. SpoIIIE is synthesized at the onset of sporulation prior to asymmetric division and is present in both the mother cell and forespore compartments (33-35). Most of the proteins that co-immunoprecipitated with SpoIIQ also co-purified with CFP-SpoIIIAH in support of the idea that many proteins reside in the zipper complex with SpoIIQ and SpoIIIAH (N.C. and D.Z.R., unpublished). Interestingly, SpoIIIE did not co-immunoprecipitate with SpoIIQ (Supplemental Fig. S1) suggesting that the complex that contains SpoIIQ and SpoIIIE is different from the zipper complex that contains SpoIIQ and SpoIIIAH.

To validate the MS results, we analyzed the immuno-affinity purifications by immunoblot (Fig. 1B). Anti-GFP antibodies revealed that the purifications were very efficient with greater than 80% of the GFP-fusion proteins immuno-depleted from the detergent-solubilized lysates (Fig. 1B compare Load (L) and Flow Through (FT)). Immunoblotting with anti-SpoIIIE antibodies confirmed that SpoIIIE efficiently co-immunoprecipitated with CFP-SpoIIQ but not with MalF-GFP or the untagged control (Fig. 1B). Moreover, an unrelated *B. subtilis* membrane protein (EzrA) was not detected in any of the immunoprecipitates (Fig. 1B). Finally, to determine whether the conditions used for immunoprecipitation maintained the reported interaction between SpoIIQ and SpoIIIAH (19,20), we probed the immuno-purifications with anti-SpoIIIAH antibodies. Under our conditions, SpoIIIAH remained complexed with CFP-SpoIIQ and was efficiently immuno-depleted (Supplemental Fig. S2).

To more rigorously assess whether SpoIIQ and SpoIIIE reside in a complex, we performed a reciprocal immunoprecipitation using a strain (BNC1203) harboring a functional SpoIIIE-GFP fusion (33). Analysis of individual bands in the silver-stained gel by MS confirmed the presence of SpoIIQ among the proteins co-purifying with SpoIIIE-GFP (Fig. 1A). Immunoblot analysis with anti-SpoIIIAH antibodies further supported this conclusion (Fig 1C). We note that a relatively small amount of SpoIIQ co-immunoprecipitated with SpoIIIE-GFP since very little SpoIIQ was immunodepleted from the lysate (Fig. 1C, compare Load (L) and Flow Through (FT)). Nonetheless, the amount of SpoIIQ present in this immunoprecipitate was significantly higher than in control immunoprecipitations (Fig. 1C). Finally, SpoIIIAH could not be detected by immunoblot in the SpoIIIE-GFP immunoprecipitate (data not shown). Altogether, these results indicate that SpoIIIE resides in a complex with the forespore membrane protein SpoIIQ. Moreover, our data suggests that the SpoIIQ-SpoIIIE complex is different from the zipper complex composed of SpoIIQ and SpoIIIAH.

**SpoIIIE has a dynamic localization pattern.** SpoIIIE is a polytopic membrane protein with ten transmembrane segments in the amino-terminal portion of the protein and a PP2C-like phosphatase domain in its carboxyl-terminus (36,37). SpoIIIE plays two distinct roles during the early stages of sporulation. First, at the onset of sporulation, SpoIIIE participates in the switch from medial to polar septation. Consistent with this activity, SpoIIIE-GFP has been shown to co-localize with...
the cell division protein FtsZ forming polar “E-rings” (10,12,33,38). Second, after asymmetric division, SpoIIE remains associated with the polar septum (33,34) and plays a critical role in the activation of the forespore-specific transcription factor σF (14-16).

Since SpoIIQ is synthesized in the forespore under the control of σF and the lysates for our immunoprecipitations were derived from cells at hour 2.5 of sporulation (well after the completion of both events that SpoIIE participates in), it was unexpected to find SpoIIE in a complex with SpoIIQ. With our interaction data in mind, we re-examined the localization pattern of SpoIIE-GFP. As described previously (10,33,34), prior to polar septation (hour 1.25), the fusion protein localized in bipolar “E-rings” at both potential division sites (Fig. 2). Surprisingly, immediately after asymmetric division but prior to the initiation of engulfment (flat polar septa), SpoIIE-GFP was not retained at the septum and instead was present throughout the forespore membranes (Fig. 2, hour 1.5). Importantly, this localization pattern was transient and as soon as engulfment initiated (slightly curved polar septa), SpoIIE-GFP re-localized to the septal membranes (Fig. 2, hour 1.75). SpoIIE-GFP remained present in the septal membranes throughout the engulfment process (Fig. 2, hour 2). Finally, when engulfment was nearly complete, weak SpoIIE-GFP foci could be observed in the forespore membranes (Fig. 2, hour 2). The presence of the SpoIIE-GFP fusion so late in sporulation was not due to stabilization of SpoIIE-GFP as has been observed for other GFP fusions (39). Native SpoIIE and SpoIIE-GFP were present at similar levels throughout the process of sporulation as assessed by immunoblot (Supplemental Fig. S3). Altogether, these results show that the localization of SpoIIE is more dynamic than previously appreciated. We suspect that the transient nature of the intermediate SpoIIE localization pattern likely explains why this dynamic behavior had not been observed previously.

Rubio and Pogliano have reported previously that all membrane proteins synthesized in the forespore (including heterologous ones like E. coli MalF) localize to the engulfing septal membrane (21). We therefore wondered whether the septal localization of SpoIIE-GFP during engulfment was specific or part of this default pathway for forespore membrane proteins. To investigate this, we compared the localization of SpoIIE-GFP with one of the membrane proteins (MalF-GFP) used in the original study by Rubio and Pogliano. The localization patterns of these two membrane proteins during sporulation were qualitatively different (Supplemental Fig. S4). In the case of SpoIIE-GFP, once engulfment had initiated, virtually all the protein was present in the septal membrane with almost no fluorescence detectable in the cytoplasmic membranes of the forespore (Fig. 2 and S4). By contrast, although there appeared to be some enrichment of MalF-GFP in the engulfing septal membrane in some of the cells, there was always fluorescent signal in the cytoplasmic membranes of the forespore (Fig. S4). These results suggest that the localization of SpoIIE to the engulfing septal membrane is specific and likely involves a protein or set of proteins that anchors it at this site.

The localization of SpoIIE to the engulfing septum requires mother cell gene expression. To determine whether the localization of SpoIIE to the engulfing septal membrane was dependent on forespore proteins under the control of σF and/or mother cell proteins under the control of σE, we visualized SpoIIE-GFP in strains lacking these transcription factors. In the absence of either sigma factor, sporulating cells fail to initiate engulfment and a second asymmetric division occurs at the distal pole, creating a sporangium with two forespores separated from the mother cell compartment by flat septa (40). Consistent with the idea that mother cell gene expression is required for the septal localization of SpoIIE, SpoIIE-GFP remained present in all forespore membranes in the σE mutant (Fig. 3; middle column). Importantly, at this same time point (hour 2), SpoIIE-GFP specifically localized to the engulfing septum in wild-type cells (Fig. 3; left column). Similar results were obtained in the σF mutant (data not shown). However, since σF is required for σE activity (40), this experiment cannot distinguish between a role for forespore proteins in anchoring SpoIIE or in activating mother-cell gene expression. From these experiments, we can conclude that the re-localization of SpoIIE to the septal membranes requires mother cell gene expression under the control of σF.
SpoIIQ is required for the septal localization of SpoIIE during engulfment. Our biochemical data indicates that SpoIIQ and SpoIIE reside in a membrane complex. Moreover, the localization pattern of SpoIIE during engulfment closely resembles the one previously described for SpoIIQ (21). Finally, both proteins require $\sigma^E$ for septal localization (21). These data raised the possibility that one of these proteins might anchor the other in the engulfing septal membranes. Since SpoIIE is essential for the activation of $\sigma^F$ and this transcription factor directs the synthesis of SpoIIQ, we were unable to test whether the localization of SpoIIQ requires SpoIIE. However, we were able to examine the localization of the SpoIIE-GFP fusion in a SpoIIQ mutant. In the absence of SpoIIQ, SpoIIE-GFP failed to re-localize to the septal membrane upon the initiation of engulfment (Fig. 3; right column). At this same time point (hour 2), SpoIIE-GFP specifically localized to the engulfing septum in wild-type cells (Fig. 3; left column). These results indicate that SpoIIQ anchors SpoIIE in the engulfing septal membranes. Moreover, they nicely explain why SpoIIQ requires $\sigma^E$ for proper localization (Fig. 3; middle column). It has been shown previously that the septal localization of SpoIIQ requires mother cell gene expression under the control of $\sigma^E$ (21). Thus, in the absence of $\sigma^E$, SpoIIQ is mislocalized, resulting in the mislocalization of SpoIIE.

SpoIIE and SpoIIQ co-localize in the engulfing septal membranes. To compare the dynamic localization of SpoIIE and SpoIIQ in the same cells, we analyzed a strain (BKM1553) that contained a YFP-fusion to SpoIIE (SpoIIE-YFP) and a CFP-fusion to SpoIIQ (CFP-SpoIIQ). Early during sporulation prior to asymmetric division, SpoIIE-YFP localized in polar E-rings as observed previously and CFP-SpoIIQ was undetectable (data not shown). Immediately after polar division, SpoIIE-YFP was visible throughout the forespore membranes (Fig. 4; white carets). At this time, $\sigma^E$ becomes active and the synthesis of SpoIIQ commences. Accordingly, CFP-SpoIIQ was undetectable at this stage (Fig. 4; white carets). Upon the initiation of engulfment, SpoIIE-YFP re-localized to the engulfing septal membrane. Consistent with the idea that SpoIIQ anchors SpoIIE at this site, CFP-SpoIIQ co-localized with SpoIIE-YFP (Fig. 4; yellow carets).

DISCUSSION

We have shown that after asymmetric division, the SpoIIE protein present at the polar septum is transiently released into the membranes of the forespore. Upon the activation of $\sigma^E$ in the forespore and the subsequent synthesis of SpoIIQ, SpoIIE re-localizes to the engulfing septal membranes where it is held in place by SpoIIQ. These results indicate that SpoIIQ is not only involved in anchoring proteins on the mother cell side of the membrane (Supplemental Fig. S2 and (20,25)) but also in anchoring proteins on the forespore side. Our data suggest that forespore membrane proteins can achieve proper localization by diffusion-and-capture as was reported for mother-cell membrane proteins (24). Furthermore, our observation that SpoIIE is released into the forespore membranes upon completion of polar division, coincident with $\sigma^E$ activation, raises the intriguing possibility that the release of SpoIIE from the polar septum is the trigger for sigma factor activation. Finally, the specific interaction between SpoIIE and a protein synthesized after SpoIIE has completed its two known functions leads us to hypothesize that this membrane phosphatase has a third function late in sporulation.

SpoIIE localizes to the engulfing septal membrane by diffusion-and-capture. We have shown that immediately after the completion of polar division, SpoIIE-GFP becomes uniformly distributed in the forespore membranes. Moreover, moments later, upon the activation of $\sigma^E$, it specifically localizes to the septal membrane in a manner that depends on SpoIIQ. We have previously shown that membrane proteins that reside in the engulfing septal membrane on the mother-cell side achieve this subcellular localization by insertion into the cytoplasmic membrane followed by diffusion to and capture in the septum (24). We hypothesize that SpoIIE becomes localized on the forespore side of the engulfing septum by a similar mechanism (Fig. 5).
In this scenario, once SpoIIQ is synthesized in the forespore, the SpoIIE protein that was released into the forespore membranes diffuses into the septal membranes where it is captured by SpoIIQ.

It is formally possible that the SpoIIE-GFP present in the engulfing septal membrane was derived from the mother cell and the SpoIIE-GFP present in the forespore was degraded upon its release from the septum. However, protoplast-treatment to separate the mother cell and forespore compartments revealed that at least half of the SpoIIE-GFP in the sporangium was in the forespore membranes (Supplemental Fig. S6 and (33,34)). This result strongly suggests that the SpoIIE-GFP released into the forespore was not degraded and therefore re-localized to the engulfing septal membrane by diffusion-and-capture.

The role of SpoIIE in cell-type specific activation of σ^F. The forespore transcription factor σ^F is made early during sporulation prior to asymmetric division, yet, its activation is restricted to the forespore compartment. The SpoIIE phosphatase (and its phosphatase activity) is directly involved in the establishment of this cell-type specificity but the mechanism by which it selectively and robustly triggers σ^F activation in the forespore is still unresolved. One of the models to explain forespore-specific activation of σ^F is that SpoIIE is equally distributed on both sides of the polar septum, which would result in a higher concentration of the membrane phosphatase in the forespore due to the smaller volume of this compartment (15,33). In this model, the increased concentration of SpoIIE is sufficient to tip the balance in favor of σ^F activation in the forespore. Alternatively, it has been proposed that SpoIIE is sequestered to the forespore face of the septum (16,34,41). Our observations that SpoIIE is released into the forespore membranes upon completion of asymmetric division appear to support this second model. We note that we cannot rule out the possibility that half of the septum-associated SpoIIE was released into the mother cell membranes and that we were unable to detect this protein because the signal was too diffuse or masked by the SpoIIE-GFP present at the distal “E-ring”. However, the SpoIIE-GFP signal at the division site was similar in total intensity to the signal in the forespore membranes after cytokinesis, suggesting that all (or most) of the SpoIIE at the septum ends up in the forespore compartment.

The observation that SpoIIE is released into the forespore membranes moments before σ^F becomes active raises the intriguing possibility that release of SpoIIE from the division machinery serves as an activating step. One possibility that we favor is that release of SpoIIE into the forespore membranes triggers high phosphatase activity. Since SpoIIE does not activate σ^F in strains depleted of the cell division protein FtsZ, this model would require that SpoIIE achieves a new (and more active) conformation through its association with the division machinery but that this high phosphatase activity would only be unleashed after release from the septum. In this scenario, the division machinery would serve as a chaperone for SpoIIE. In support of the idea that release from division machinery alters the conformation of SpoIIE, the ability to visualize SpoIIE-GFP in cytoplasmic membranes is only observed after release from the septum. For example, SpoIIE-GFP appears as a cytoplasmic haze in cells in which FtsZ is depleted (38). Moreover, in protoplast experiments, the SpoIIE-GFP in the mother cell, which is derived from the unused E-ring, appears cytoplasmic while SpoIIE-GFP in the forespore is membrane-associated (Fig. S6 and (33,34)). In addition, when SpoIIE-GFP is artificially synthesized during vegetative growth under conditions that promote division at the cell poles and mini-cell formation, SpoIIE-GFP only localizes to the cytoplasmic membranes of the minicells (S. Ben-Yehuda and D.Z.R., unpublished). Finally, we have found that the localization of SpoIIE-GFP to the cytoplasmic membranes of the forespore is only observed after the protein is released from the septum (Fig. 2). SpoIIE has ten transmembrane segments and it is therefore unlikely that it resides in the cytoplasm prior to division. Nonetheless, these cytological data are consistent with the idea that a new conformation of the protein is achieved through association with and release from the division septum. Although very speculative, we hypothesize that the release of SpoIIE into the forespore membranes upon completion of cytokinesis triggers high phosphatase activity resulting in compartment-specific activation of σ^F.
predivisional cell and in the mother cell at the distal “E-ring” would have low phosphatase activity and thus $\sigma^F$ would remain inactive.

**A third function for SpoIIE.** Our co-immunoprecipitation and microscopy experiments indicate that SpoIIE is retained in the engulfing septal membranes long after it has fulfilled its two known functions in sporulation (participation in the switch from medial to polar Z-ring formation and activation of $\sigma^F$). Moreover, the re-localization of SpoIIE to the engulfing septal membrane depends on SpoIIQ, a forespore protein made under the control of $\sigma^F$. The fact that SpoIIE is specifically recruited to the septum by a protein that is synthesized under the control of the transcription factor that SpoIIE itself activates, strongly suggests to us that this membrane phosphatase has another function at this late stage.

We hypothesize that SpoIIE is involved in a late step in the process of sporulation in the engulfing septal membranes. For example, SpoIIE might be necessary to anchor forespore and/or mother cell proteins in the engulfing septum. Alternatively, SpoIIE could play a direct role in the process of engulfment. It is also possible that SpoIIE participates in receiving a signal from the mother cell (or sensing the completion of engulfment) to activate $\sigma^G$ in the forespore. Finally, the interaction between SpoIIE and SpoIIQ could serve to modulate the phosphatase activity of SpoIIE. In this last model, interaction between SpoIIE and SpoIIQ might increase phosphatase activity to maintain $\sigma^F$ activity in the forespore. Conversely, once engulfment is complete or near complete, SpoIIQ could shut down phosphatase activity and turn off $\sigma^F$ to transition to late forespore gene expression under $\sigma^G$ control. Since SpoIIE is essential for the early stages of sporulation, any of these hypothetical late functions for SpoIIE would have been missed. The development of clever strategies for temporally-controlled inactivation of proteins after their early roles are complete will be needed to investigate additional functions for this and other sporulation proteins.

**REFERENCES**

1. Barak, I., and Wilkinson, A. J. (2007) *FEMS Microbiol Rev* 31(3), 311-326
2. Errington, J., Daniel, R. A., and Scheffers, D. J. (2003) *Microbiol Mol Biol Rev* 67(1), 52-65, table of contents
3. Vicente, M., Rico, A. I., Martinez-Arteaga, R., and Mingorance, J. (2006) *J Bacteriol* 188(1), 19-27
4. Kentner, D., and Sourjik, V. (2006) *Curr Opin Microbiol* 9(6), 619-624
5. Carballido-Lopez, R. (2006) *Microbiol Mol Biol Rev* 70(4), 888-909
6. Campo, N., Tjalsma, H., Buist, G., Stepiak, D., Meijer, M., Veenhuis, M., Westermann, M., Muller, J. P., Bron, S., Kok, J., Kuipers, O. P., and Jongbloed, J. D. (2004) *Mol Microbiol* 53(6), 1583-1599
7. Rosch, J., and Caparon, M. (2004) *Science* 304(5676), 1513-1515
8. Shiomi, D., Yoshimoto, M., Homma, M., and Kawagishi, I. (2006) *Mol Microbiol* 60(4), 894-906
9. Levin, P. A., and Losick, R. (1996) *Genes Dev* 10(4), 478-488
10. Arigoni, F., Pogliano, K., Webb, C. D., Stragier, P., and Losick, R. (1995) *Science* 270(5236), 637-640
11. Barak, I., Behari, J., Olmedo, G., Guzman, P., Brown, D. P., Castro, E., Walker, D., Westpheling, J., and Youngman, P. (1996) *Mol Microbiol* 19(5), 1047-1060
12. Ben-Yehuda, S., and Losick, R. (2002) *Cell* 109(2), 257-266
13. Khvorova, A., Zhang, L., Higgins, M. L., and Piggot, P. J. (1998) *J Bacteriol* 180(5), 1256-1260
14. Arigoni, F., Duncan, L., Alper, S., Losick, R., and Stragier, P. (1996) *Proc Natl Acad Sci U S A* **93**(8), 3238-3242
15. Duncan, L., Alper, S., Arigoni, F., Losick, R., and Stragier, P. (1995) *Science* **270**(5236), 641-644
16. Feucht, A., Magnin, T., Yudkin, M. D., and Errington, J. (1996) *Genes Dev* **10**(7), 794-803
17. Rudner, D. Z., and Losick, R. (2001) *Dev Cell* **1**(6), 733-742
18. Abanes-De Mello, A., Sun, Y. L., Aung, S., and Pogliano, K. (2002) *Genes Dev* **16**(24), 3253-3264
19. Blaylock, B., Jiang, X., Rubio, A., Moran, C. P., Jr., and Pogliano, K. (2004) *Genes Dev* **18**(23), 2916-2928
20. Doan, T., Marquis, K. A., and Rudner, D. Z. (2005) *Mol Microbiol* **55**(6), 1767-1781
21. Rubio, A., and Pogliano, K. (2004) *Embo J* **23**(7), 1636-1646
22. Rudner, D. Z., and Losick, R. (2002) *Genes Dev* **16**(8), 1007-1018
23. van Ooij, C., and Losick, R. (2003) *J Bacteriol* **185**(4), 1391-1398
24. Rudner, D. Z., Pan, Q., and Losick, R. M. (2002) *Proc Natl Acad Sci U S A* **99**(13), 8701-8706
25. Jiang, X., Rubio, A., Chiba, S., and Pogliano, K. (2005) *Mol Microbiol* **58**(1), 102-115
26. Youngman, P. J., Perkins, J. B., and Losick, R. (1983) *Proc Natl Acad Sci U S A* **80**(8), 2305-2309
27. Harwood, C. R., and Cutting, S. M. (1990) *Molecular Biological Methods for Bacillus*, John Willey & Sons Ltd, Chichester, United Kingdom
28. Rudner, A. D., Hall, B. E., Ellenberger, T., and Moazed, D. (2005) *Mol Cell Biol* **25**(11), 4514-4528
29. Doan, T., and Rudner, D. Z. (2007) *Mol Microbiol* **64**(2), 500-511
30. Haeusser, D. P., Schwartz, R. L., Smith, A. M., Oates, M. E., and Levin, P. A. (2004) *Mol Microbiol* **52**(3), 801-814
31. Campo, N., and Rudner, D. Z. (2007) *J Bacteriol* **189**(16), 6021-6027
32. Campo, N., and Rudner, D. Z. (2006) *Mol Cell* **23**(1), 25-35
33. King, N., Dreesen, O., Stragier, P., Pogliano, K., and Losick, R. (1999) *Genes Dev* **13**(9), 1156-1167
34. Wu, L. J., Feucht, A., and Errington, J. (1998) *Genes Dev* **12**(9), 1371-1380
35. York, K., Kenney, T. J., Satola, S., Moran, C. P., Jr., Poth, H., and Youngman, P. (1992) *J Bacteriol* **174**(8), 2648-2658
36. Adler, E., Donella-Deana, A., Arigoni, F., Pinna, L. A., and Stragier, P. (1997) *Mol Microbiol* **23**(1), 57-62
37. Arigoni, F., Guerout-Fleury, A. M., Barak, I., and Stragier, P. (1999) *Mol Microbiol* **31**(5), 1407-1415
38. Levin, P. A., Losick, R., Stragier, P., and Arigoni, F. (1997) *Mol Microbiol* **25**(5), 839-846
39. Resnekov, O., and Losick, R. (1998) *Proc Natl Acad Sci U S A* **95**(6), 3162-3167
40. Stragier, P., and Losick, R. (1996) *Annu Rev Genet* **30**, 297-241
41. Lewis, P. J., Wu, L. J., and Errington, J. (1998) *J Bacteriol* **180**(13), 3276-3284
42. Broder, D. H., and Pogliano, K. (2006) *Cell* **126**(5), 917-928
FOOTNOTES

*We thank members of the Rudner laboratory, Karen Carniol and Sigal Ben-Yehuda for valuable discussions, Petra Levin for anti-EzrA antisera, Kit Pogliano for strains, and Adam Rudner for advice on immuno-affinity purification and silver staining. We acknowledge Sigal Ben-Yehuda for the initial observations of SpoIIE-GFP dynamics. This work was supported in part by National Institute of Health Grant GM073831-01A1 and the Giovanni Armenise-Harvard Foundation. D.Z.R. is a Damon Runyon Scholar supported by the Damon Runyon Cancer Research Foundation (DRS 44-05).

FIGURE LEGENDS

Fig. 1. SpoIIE and SpoIIQ reside in a membrane complex. Immunoprecipitations using anti-GFP resin were performed on detergent-solubilized membrane fractions derived from *B. subtilis* cells at hour 2.5 of sporulation. Immunoprecipitates from a strain lacking GFP (BNC689), and strains containing a CFP-SpoIIQ fusion (BTD665), a SpoIIE-GFP fusion (BNC1203) and a MalF-GFP fusion (BNC1384) are shown. (A) Silver-stained gel of immunoprecipitates. Bands corresponding to the GFP fusion proteins are indicated (black circles). Bands corresponding to SpoIIE (IIE) and SpoIIQ (IIQ) are shown with red circles. The immunoprecipitations are from 7.5ml of sporulating cells. (B-C) Immunoblots from the immuno-affinity purifications. The detergent-solubilized membrane fraction prior to immunoprecipitation (L), the supernatants after immunoprecipitation (FT), and the immunoprecipitates (IP) were subjected to immunoblot analysis. Equivalent amounts of the load (L) and flow through (FT) were analyzed. (B) Immuno-purifications from the strain lacking GFP and strains containing CFP-SpoIIQ and MalF-GFP. Proteins were detected using anti-GFP (top panel), anti-SpoIIE (middle panel) and anti-EzrA (bottom panel) antibodies. (C) Immuno-purifications from the strain lacking GFP and strains containing SpoIIE-GFP and MalF-GFP. Proteins were detected using anti-GFP (top panel), anti-SpoIIQ (middle panel) and anti-EzrA (bottom panel) antibodies.

Fig. 2. SpoIIE has a dynamic localization pattern. Subcellular localization of SpoIIE-GFP in strain BDR1845 was analyzed by fluorescence microscopy at the indicated times (in hours) after the initiation of sporulation. The membranes from the same field were visualized using the fluorescent dye TMA-DPH. As indicated, the top row of images shows SpoIIE-GFP fluorescence. The 2nd row of images shows fluorescence from the membrane dye TMA-DPH. The 3rd row shows the overlay of the two. The yellow carets at hour 1.25 indicate the localization of SpoIIE-GFP in “E-rings” at potential division sites prior to septation. The yellow carets at hour 1.75 and hour 2 highlight the SpoIIE-GFP concentrated at the engulfing septal membranes. The white carets in all four images show the transient localization SpoIIE-GFP throughout the forespore membranes. The membrane staining indicates that these cells have flat septa and have therefore just completed polar division. Schematic diagrams depicting SpoIIE-GFP (green) localization are shown below the images. Fluorescent images of individual cells from the same fields are shown below the schematic diagrams. Scale bar 1 µm.

Fig. 3. SpoIIQ is required to anchor SpoIIE in the engulfing septal membranes. SpoIIE-GFP localization was analyzed in a wild-type strain (wt, BDR1845), a σE mutant (sigEΔ, BNC1264) and a spoIIQ mutant (IIQΔ, BNC1266) at hour 2 of sporulation. The membranes from the same fields were visualized using the fluorescent dye TMA-DPH. As indicated, the top row of images shows SpoIIE-GFP fluorescence. The 2nd row of images shows fluorescence from the membrane
The localization of SpoIIE-GFP at the engulfing forespore membranes in wild-type cells is indicated (yellow carets). In the absence of $\sigma^E$, the sporulating cells contain two flat septa, and SpoIIE-GFP is present throughout the forespore membranes (white carets). Similarly, in the absence of SpoIIQ, SpoIIE-GFP fails to relocalize to the engulfing septal membranes (white carets). Schematic diagrams depicting SpoIIE-GFP (green) localization in the different strains are shown below the images. Scale bar 1 $\mu$m.

**Fig. 4.** SpoIIE and SpoIIQ co-localize during the process of engulfment. SpoIIE-YFP (IIE-YFP) and CFP-SpoIIQ (CFP-IIQ) were visualized in strain BKM1553 at hour 1.5 of sporulation. The membranes from the same field were stained with the fluorescent dye TMA-DPH. As indicated, the first image in the top panel shows an overlay of fluorescence from the membrane dye (blue), IIE-YFP (false-colored green) and CFP-IIQ (false-colored red). All other images are as indicated. Immediately after polar division, SpoIIE-YFP localizes to all membranes in the forespore (white caret). At this stage CFP-SpoIIQ is undetectable. During the engulfment process IIE-YFP and CFP-IIQ co-localize in the septal membrane (yellow carets). Scale bar 1 $\mu$m.

**Fig. 5.** SpoIIE localizes to the engulfing septal membrane by diffusion-and-capture. Schematic diagram of SpoIIE. Prior to polar division, SpoIIE (green balls) co-localizes with FtsZ (not shown) in “E-rings”. Upon completion of cytokinesis, SpoIIE is released into the membranes of the forespore compartment. Moments later, $\sigma^F$ is activated in the forespore and directs the synthesis of SpoIIQ. SpoIIQ (Q) specifically localizes to the septal membranes by an unknown mechanism that depends on mother cell gene expression. SpoIIE present in the forespore diffuses into the septum where it is captured by SpoIIQ. The interaction between SpoIIE and SpoIIQ is depicted as direct but this need not be the case.

**Fig. S1.** SpoIIE resides in a complex with SpoIIQ but not with SpoIIIAH. Immunoprecipitations using anti-GFP resin were performed on detergent-solubilized membrane fractions derived from *B. subtilis* cells at hour 3.5 of sporulation. Immunoprecipitates from strains containing a CFP-SpoIIQ fusion (strain BTD665) and a CFP-SpoIIIAH fusion (strain BNC1195) are shown. Detergent-solubilized membrane fractions prior to immunoprecipitation (L), the supernatants after immunoprecipitation (FT), and the immunoprecipitates (IP) were subjected to immunoblot analysis using anti-GFP (upper panels) and anti-SpoIIE (lower panel) antibodies. Equivalent amounts of the load (L) and the flow through (FT) were analyzed. The anti-SpoIIQ immunoblot was from the same gel (and the same autoradiography film) but control lanes were removed for clarity.

**Fig. S2.** SpoIIQ and SpoIIIAH reside in a membrane complex. Immunoprecipitations using anti-GFP resin were performed on detergent-solubilized membrane fractions derived from *B. subtilis* cells at hour 3.5 of sporulation. Immunoprecipitates from a strain lacking GFP and one containing a CFP-SpoIIQ fusion (strain BTD665) are shown. Detergent-solubilized membrane fractions prior to immunoprecipitation (L), the supernatants after immunoprecipitation (FT), and the immunoprecipitates (IP) were subjected to immunoblot analysis using anti-GFP (upper panels); anti-SpoIIIAH (middle panels); and anti-EzrA (lower panels) antibodies. EzrA is an unrelated integral membrane protein and serves as a negative control. Equivalent amounts of the load (L) and the flow through (FT) were analyzed. The immunoblots were from the same gels (and the same autoradiography films) but control lanes were removed for clarity.
**Fig. S3.** SpoIIE persists at late times during sporulation. Wild-type (wt) (strain PY79), a spoIIE null mutant (SpoIEΔ) (BDR1736) and a strain containing a SpoIIE-GFP fusion (BDR1845) were induced to sporulate by resuspension. At indicated times (in hours) after the initiation of sporulation, samples were collected. Whole cell extracts were prepared and analyzed by immunoblot using anti-SpoIIE antibodies.

**Fig. S4.** The localization of MalF-GFP in the forespore is different from the septal localization of SpoIIE-GFP. Subcellular localization of MalF-GFP (AR4), SpoIIE-GFP (BDR1845) and CFP-SpoIIQ (BTD231) during sporulation. The membranes from the same field were visualized using the fluorescent dye TMA-DPH. As indicated, the top row of images shows fluorescence from the GFP fusions. The middle row of images shows fluorescence from the membrane dye TMA-DPH. The bottom row shows an overlay of the two. Carets indicate cells in which the process of engulfment is not complete. In these cells, MalF-GFP (white carrets) is present throughout the forespore membranes. By comparison, SpoIIE-GFP and CFP-SpoIIQ are restricted to the engulfing septal membranes (yellow carrets).

**Fig. S5** SpoIIE and SpoIIQ co-localize during the engulfment process. A strain containing SpoIIE-YFP (IIE-YFP) and CFP-SpoIIQ (CFP-IIQ) (BKM1553) was induced to sporulate by resuspension and analyzed by fluorescence microscopy at hour 2. The membranes from the same field were visualized using the fluorescent dye TMA-DPH. As indicated, the first image in the top panel shows an overlay of fluorescence from the membrane dye (blue), IIE-YFP (false-colored green) and CFP-IIQ (false-colored red). The second image in the top panel shows the overlay of IIE-YFP and CFP-IIQ. All other images are as indicated. During the engulfment process, IIE-YFP and CFP-IIQ both localize uniformly in the septal membranes (yellow carrets). Once engulfment is nearly complete, the two fusion proteins co-localize to discrete foci in the forespore membranes (white carrets).

**Fig. S6.** SpoIIE-GFP is present in the forespore during engulfment. SpoIIE-GFP was visualized in the forespore and mother cell compartments after protoplast treatment. A strain containing a SpoIIE-GFP fusion (BNC1395) was induced to sporulate by resuspension. At hour 2.5, once engulfment had begun, protoplasts were generated by treatment with lysozyme in the presence of hypertonic buffer (33,34) and SpoIIE-GFP was analyzed by fluorescence microscopy. For comparison, the membranes from the same field stained with the fluorescent dye TMA-DPH, and the corresponding phase contrast images are shown. SpoIIE-GFP is clearly visible in the forespore membranes after protoplast treatment. The SpoIIE-GFP signal in the mother cell is observed as a diffuse cytoplasmic haze (33,34). The cytological difference between SpoIIE-GFP localization in the mother cell and forespore is consistent with the idea that SpoIIE achieves a new conformation through its association with the division machinery (see Discussion). To prevent protoplast engulfment (42), BNC1395 also contained a mutation in spoIIIAH.
Figure 2

**SpoIE-GFP**

**membranes**

**overlay**

T1.25  T1.5  T1.75  T2

Campo et al.

Downloaded from http://www.jbc.org/ by guest on March 25, 2020
Figure S1: SpoIIE resides in a complex with SpoIIQ but not with SpoIIIAH. Immunoprecipitations using anti-GFP resin were performed on detergent-solubilized membrane fractions derived from B. subtilis cells at hour 3.5 of sporulation. Immunoprecipitates from strains containing a CFP-SpoIIQ fusion (strain BTD665) and a CFP-SpoIIIAH fusion (strain BNC1195) are shown. Detergent-solubilized membrane fractions prior to immunoprecipitation (L), the supernatants after immunoprecipitation (FT), and the immunoprecipitates (IP) were subjected to immunoblot analysis using anti-GFP (upper panels) and anti-SpoIIE (lower panel) antibodies. Equivalent amounts of the load (L) and the flow through (FT) were analyzed. The anti-SpoIIQ immunoblots were from the same gel (and the same autoradiography film) but control lanes were removed for clarity.
Figure S2. SpoIIQ and SpoIIIAH reside in a membrane complex. Immunoprecipitations using anti-GFP resin were performed on detergent-solubilized membrane fractions derived from *B. subtilis* cells at hour 3.5 of sporulation. Immunoprecipitates from a strain lacking GFP and one containing a CFP-SpoIIQ fusion (strain BTD665) are shown. Detergent-solubilized membrane fractions prior to immunoprecipitation (L), the supernatants after immunoprecipitation (FT), and the immunoprecipitates (IP) were subjected to immunoblot analysis using anti-GFP (upper panels); anti-SpoIIIAH (middle panels); and anti-EzrA (lower panels) antibodies. EzrA is an unrelated integral membrane protein and serves as a negative control. Equivalent amounts of the load (L) and the flow through (FT) were analyzed. The immunoblots were from the same gels (and the same autoradiography films) but control lanes were removed for clarity.
Figure S3 SpolIE persists at late times during sporulation. Wild-type (wt) (strain PY79), a spoIIE null mutant (SpoIIEΔ) (BDR1736) and a strain containing a SpoIIE-GFP fusion (BDR1845) were induced to sporulate by resuspension. At indicated times (in hours) after the initiation of sporulation, samples were collected. Whole cell extracts were prepared and analyzed by immunoblot using anti-SpoIIE antibodies.
Figure S4

The localization of MalF-GFP in the forespore is different from the septal localization of SpoIIE-GFP. Subcellular localization of MalF-GFP (AR4), SpoIIE-GFP (BDR1845) and CFP-SpoIIQ (BTD231) during sporulation. The membranes from the same field were visualized using the fluorescent dye TMA-DPH. As indicated, the top row of images shows fluorescence from the GFP fusions. The middle row of images shows fluorescence from the membrane dye TMA-DPH. The bottom row shows an overlay of the two. Carets indicate cells in which the process of engulfment is not complete. In these cells, MalF-GFP (white carrets) is present throughout the forespore membranes. By comparison, SpoIIE-GFP and CFP-SpoIIQ are restricted to the engulfing septal membranes (yellow carrets).
Figure S5 SpoIIE and SpoIIQ co-localize during the engulfment process. A strain containing SpoIIE-YFP (II-E-YFP) and CFP-SpoIIQ (CFP-IIQ) (BKM1553) was induced to sporulate by resuspension and analyzed by fluorescence microscopy at hour 2. The membranes from the same field were visualized using the fluorescent dye TMA-DPH. As indicated, the first image in the top panel shows an overlay of fluorescence from the membrane dye (blue), IIE-YFP (false-colored green) and CFP-IIQ (false-colored red). The second image in the top panel shows the overlay of IIE-YFP and CFP-IIQ. All other images are as indicated. During the engulfment process, II-E-YFP and CFP-IIQ both localize uniformly in the septal membranes (yellow carets). Once engulfment is nearly complete, the two fusion proteins co-localize to discrete foci in the forespore membranes (white carets).
Figure S6 SpoIIE-GFP is present in the forespore during engulfment. SpoIIE-GFP was visualized in the forespore and mother cell compartments after protoplast treatment. A strain containing a SpoIIE-GFP fusion (BNC1395) was induced to sporulate by resuspension. At hour 2.5, once engulfment had begun, protoplasts were generated by treatment with lysozyme in the presence of hypertonic buffer (33,34) and SpoIIE-GFP was analyzed by fluorescence microscopy. For comparison, the membranes from the same field stained with the fluorescent dye TMA-DPH, and the corresponding phase contrast images are shown. SpoIIE-GFP is clearly visible in the forespore membranes after protoplast treatment. The SpoIIE-GFP signal in the mother cell is observed as a diffuse cytoplasmic haze (33,34). The cytological difference between SpoIIE-GFP localization in the mother cell and forespore is consistent with the idea that SpoIIE achieves a new conformation through its association with the division machinery (see Discussion). To prevent protoplast engulfment (42), BNC1395 also contained a mutation in spoIIIAH.
| strain   | genotype                                                                 | reference                  |
|----------|---------------------------------------------------------------------------|----------------------------|
| BTD665   | spoIVB::erm, spoIIQ::spec, sacA::P_{spoIIQ^-cfp(Bs)-spoIIQ} (tet)         | This work                  |
| BDR1845  | spoIIE-gfp (kan)                                                          | King et al, 1999          |
| BNC689   | spoIVB::phleo                                                             | Campo and Rudner, 2006     |
| AR4      | amyE::P_{spoIIQ^-malF12-gfp} (cat)                                        | Rubio and Pogliano, 2004  |
| BNC1203  | spoIVB::phleo, spoIIE-gfp (kan)                                           | This work                  |
| BNC1264  | spoIVB::erm, spoIIE-gfp (kan)                                             | This work                  |
| BNC1266  | spoIIQ::spec, spoIIE-gfp (kan)                                            | This work                  |
| BNC1384  | spoIVB::spec, amyE::P_{spoIIQ^-malF12-gfp} (cat)                          | This work                  |
| BKM1553  | spoIIQ::cat, sacA::P_{spoIIQ^-cfp(Bs)-spoIIQ} (tet), spoIIE-yfp (spec)    | This work                  |
SpoIIQ anchors membrane proteins on both sides of the sporulation septum in *Bacillus subtilis*
Nathalie Campo, Kathleen A. Marquis and David Z. Rudner

*J. Biol. Chem.* published online December 11, 2007

Access the most updated version of this article at doi: [10.1074/jbc.M708024200](http://dx.doi.org/10.1074/jbc.M708024200)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

Supplemental material:
[http://www.jbc.org/content/suppl/2008/05/28/M708024200.DC1](http://www.jbc.org/content/suppl/2008/05/28/M708024200.DC1)