INTERCOMPARTMENTAL SIGNAL TRANSDUCTION DURING SPORULATION IN *BACILLUS SUBTILIS*

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Many of the chapters in this book describe cell-cell signaling among bacteria within their communities. Here we consider a much more intimate conversation that occurs between two siblings: the two cells that comprise the sporangium during spore formation in *Bacillus subtilis*. The conversation involves three separate signal transduction pathways that together coordinate the transcriptional programs of the two cells, called the mother cell and the forespore. These two cells follow different programs of developmental gene expression, in which several classes of coordinately regulated genes are sequentially activated. Cell-cell signaling ensures that the developmental programs in the mother cell and forespore are maintained in register with each other such that earlier events are initiated or in some cases completed before later events can begin. Our understanding of the molecular mechanisms underlying each of these three pathways is at a different stage of maturity. But it is already clear that each pathway has something different to teach us about how cells send and interpret signals. After a brief introduction to sporulation in *B. subtilis*, we will describe each pathway in turn, discuss the current state of understanding, and suggest testable hypotheses. Spore formation in *B. subtilis* has served as an important model for cell-cell signaling in bacteria. As you will see, the conversation between the forespore and mother cell provides insight into how and why cells communicate and highlights the diversity of ways in which organisms transduce information across their membranes.

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INTRODUCTION TO SPORULATION

B. subtilis enters the sporulation pathway in response to nutrient deprivation (15, 20, 45, 61). The sporulating cell proceeds through a series of well-defined morphological stages that culminate in the production of a dormant cell type known as the spore (or more properly, the endospore). The first landmark morphological event during sporulation is the formation of an asymmetrically positioned septum that divides the developing cell (or sporangium) into two daughter cells of unequal size: a forespore (the smaller cell) and a mother cell (Color Plate 1). Initially, the two cells lie side-by-side, but later in development the mother cell engulfs the forespore. Engulfment is a

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phagocytic-like process in which the forespore is pinched off within the mother cell, creating a cell within a cell (Color Plate 1). At this late stage, the mother cell packages the forespore in a protective proteinaceous coat while the forespore prepares for dormancy. Later in development, the mother cell lyses, liberating the fully mature spore.

A cascade of developmental transcription factors are activated in a stage- and cell-type-specific manner during this developmental process (37). Upon asymmetric division, the RNA polymerase sigma factor σ^F is activated in the forespore followed by σ^E in the mother cell. After engulfment is complete (or near complete), σ^G replaces σ^F in the forespore and, in turn, σ^K replaces σ^E in the mother cell (34). The stage- and compartment-specific activities of these four transcription factors were originally demonstrated using promoter fusions to *lacZ* and heroic immunoelectron microscopy (12, 38, 71). Today, the stage- and compartment-specific activities of these four sigma factors can be visualized with ease by fluorescence microscopy using strains in which sigma-dependent promoters direct the expression of the genes encoding the yellow and cyan fluorescent proteins (*yfp* and *cfp*) (Color Plate 1). For many years it was unclear whether the mother cell and forespore followed independent developmental programs like two separate free-running clocks. Elegant genetic analysis has revealed that, in fact, these two cells communicate with each other throughout the sporulation process. Signaling between the forespore and mother cell was first demonstrated in the early 1990s when it was discovered that gene expression in the mother cell under the control of σ^K was blocked by a mutation in the forespore transcription factor σ^G (7). The identification of mutants that restored mother cell gene expression in the absence of σ^G argued in favor of a bona fide signal transduction pathway. Finally, the discovery that these bypass mutations in a wild-type background uncouple mother cell and forespore gene expression and cause a defect in spore formation demonstrated that the conversation between mother and forespore plays a key role in coordinating the two developmental programs and that keeping these programs in register is critical for morphogenesis and successful sporulation.

It is now clear that there are three signal transduction pathways between the mother cell and forespore (constituting a true conversation) that ensure that gene expression in one compartment is linked to gene expression in the other throughout the sporulation process (Color Plate 1). Here we examine each signaling pathway separately and in chronological order: (i) σ^F in the forespore initiates a pathway that triggers activation of σ^E in the mother cell; (ii) σ^E in the mother cell is then responsible for the activation of σ^G in the forespore; and (iii) σ^G in the forespore sets in motion events that activate σ^K in the mother cell (Color Plate 1). These three pathways highlight the creative and diverse ways evolution has solved the challenge of transducing information across a lipid bilayer.

The Forespore Starts the Conversation by Sending a Signal to the Mother Cell

In response to starvation, two stationary-phase transcription factors, Spo0A and σ^H, are activated in the predivisional cell (45). Spo0A and σ^H direct the transcription of early-acting sporulation genes, including those involved in repositioning the division machinery to an asymmetric site and the synthesis of the first two compartment-specific transcription factors, σ^F and σ^E. Both σ^F and σ^E are synthesized prior to septation in the predivisional cell but are held inactive until the formation of the polar septum. Shortly after the septum is complete, σ^F becomes active in the forespore compartment. σ^F is the first cell type-specific transcription factor, and its activity confers forespore identity on the small cell and commits it to the sporulation program (13, 45). The activation of σ^F is thought to be directly coupled to the completion of septation through the action of a membrane phosphatase that plays a key role in shifting the division site from the mid-cell to the pole (20, 53). The exact mechanism by which septation triggers σ^F activation

remains unclear and a source of some controversy (5, 23, 24, 31, 66). Focus and scrutiny on this key morphological event in committing the cell to the sporulation pathway are well justified as it defines how one cell type (the predivisional cell) becomes two different cell types. We refer the interested reader to several recent reviews about this important control point in this developmental pathway (15, 20, 53).

The first signal transduction pathway between the forespore and mother cell has as input σ^F activity in the forespore and as output the activation of σ^E in the mother cell. σ^E is the first mother-cell-specific transcription factor in the large compartment and, as such, confers mother cell identity upon it. Activation of σ^E acts as a switch that sets in motion the mother cell developmental program and at the same time halts the program of the predivisional cell (45).

In the absence of σ^E, the large compartment continues to follow the program of the predivisional cell, and a second asymmetric division occurs at the distal pole, creating a sporangium with two forespore compartments (45). These disporic sporangia are terminally arrested in sporulation. Thus, σ^E-dependent gene expression is required to prevent septation at the unused polar site. In fact, σ^E activation appears to occur minutes after σ^F activation. If the activation of σ^E is delayed 10 to 15 min after septation, then the efficiency of sporulation is reduced, with a concomitant increase in disporic sporangia (30, 72). Indeed, simultaneously visualizing σ^F and σ^E activity using a σ^F-responsive promoter fusion to *cfp* and a σ^E-responsive promoter fusion to *yfp* in the same strain, it is hard to find a cell with CFP fluorescence (σ^F activity) that does not also have YFP fluorescence (σ^E activity) (Color Plate 1). On the other hand, if σ^E appears prematurely, that is, in the predivisional cell, then formation of the first polar septum is inhibited (14, 17). These findings indicate that the cell-cell signaling pathway serves as a timing device to ensure that σ^E is activated during a critical temporal window following the completion of the polar septum (14, 46).

σ^E is derived from an inactive proprotein precursor called pro-σ^E (33, 63) (Color Plate 2). This precursor has an amino-terminal extension of 27 amino acids that must be proteolytically removed in order for the transcription factor to associate efficiently with RNA polymerase and direct gene expression. The prodomain both holds σ^E inactive and localizes the proprotein to the membrane (Color Plate 2) (17, 21, 27). The pathway that begins with σ^F in the forespore and results in pro-σ^E processing in the mother cell is thought to be mediated by a secreted protein ligand and a putative membrane protease. The signaling molecule (SpoIIR, hereafter referred to as R) is encoded by a gene under the control of σ^F (22, 28, 36). R is translocated into the space between the forespore and mother cell membranes (51) where it activates SpoIIGA (hereafter referred to as GA), resulting in the conversion of pro-σ^E to its mature and active form (Color Plate 2). GA is a polytopic membrane protein with homology in its cytoplasmic domain to aspartyl proteases (60). This putative membrane-tethered protease localizes to the polar septum by an unknown mechanism, where it is perfectly positioned to receive an activating signal from the forespore (Color Plate 2) (16). The putative catalytic residues in the GA protease have not been shown to be critical for pro-σ^E processing nor has this cleavage reaction been reconstituted in vitro. However, cells engineered to artificially synthesize GA, pro-σ^E, and R during vegetative growth can process pro-σ^E to its mature and active form (60). This result indicates that GA and R are the only sporulation-specific proteins required to activate σ^E and suggests that GA is indeed the pro-σ^E processing enzyme.

To ensure that σ^E is activated in a timely fashion after polar division, both pro-σ^E and its putative processing enzyme GA are made predivisionally under the control of the Spo0A transcription factor (45). Thus, both proteins are present in the mother cell and forespore compartments. Since R can activate pro-σ^E processing in *cis* and in *trans* (60), why is σ^E activity restricted to the mother cell? The

answer appears to be that pro-σ^E is specifically degraded in the forespore compartment (Color Plate 2) (17). The protease (or proteases) responsible for clearing pro-σ^E has not yet been identified. In addition, there is a second mechanism that contributes to compartmentalized σ^E activity. After polar division, Spo0A acts preferentially in the mother cell, resulting in increased accumulation of pro-σ^E and GA in this compartment (17, 18).

The mechanism by which information is transduced across the lipid bilayer by R and GA is not yet known. We favor a model in which the cytosolic aspartyl protease domain of GA is active only as a dimer and the R signaling protein promotes dimerization by binding GA in the intermembrane space (Color Plate 2). Signal transduction by ligand-induced dimerization has been well characterized for receptor tyrosine kinases (55, 56) and seems like a compelling mechanism here. Alternatively, R binding to the extracellular face of GA could cause a conformational change in the protease that activates the catalytic center or allows the protease to bind its substrate. Dissection of the molecular mechanisms of R-mediated GA activation and GA-mediated pro-σ^E processing promises to reveal general principles of how information can be transduced across a lipid bilayer.

The Mother Cell Responds to the Forespore

The second signal transduction pathway, the activation of σ^G in the forespore under the control of σ^E in the mother cell, has been the most refractory to genetic and molecular dissection and therefore is the least well understood of the three signaling pathways described here. In part, this is because σ^G activity is regulated at multiple levels and appears to be coupled to the morphological process of engulfment (53, 61). The gene encoding σ^G is under the control of the forespore transcription factor σ^F. However, transcription of the σ^G gene is delayed by approximately 30 to 45 min relative to other genes in the σ^F regulon, until the time when engulfment is complete (or nearly complete) (44). It is not clear how σ^G gene transcription is regulated, but it appears to require σ^E-dependent gene expression (61). σ^G is also controlled posttranslationally. Premature expression of the σ^G gene (by fusing it to a strong σ^F-dependent promoter that is transcribed 30 to 45 min earlier) has no effect on the timing of σ^G activity (61).

σ^G only becomes active when engulfment is complete (or nearly complete), and it is thought that σ^G activation is linked to this morphological process (this linkage is referred to as morphological coupling) (53). In support of this idea, all mutants that block engulfment prevent σ^G activation but do not block transcription of the σ^G gene (35, 41, 44, 58). It remains unclear how the completion of engulfment is sensed and how this triggers σ^G activity. The surveillance of engulfment may be achieved by a putative complex of eight proteins (encoded by the *spoIIIA* operon) that are synthesized in the mother cell under σ^E control and localize to the mother cell membrane that surrounds the forespore (1, 9, 29). The *spoIIIA* locus (referred to as IIIA) is not required for the engulfment process but is essential for σ^G activation (29). Perhaps this putative membrane complex monitors the engulfment process and, once it is complete, transduces a signal to the forespore to activate σ^G (Color Plate 3). With this view of σ^G regulation, the "cell-cell" signaling pathway between the mother cell and forespore would be indirect and therefore considered nontraditional. The role of σ^E would be to promote transcription of the engulfment proteins (45) and the IIIA surveillance complex (Color Plate 3), and not to transcribe a signaling molecule like R in the first pathway. In this scenario, IIIA transduces an "engulfment signal" and cell-cell signaling is only a genetic formalism.

While this model is certainly reasonable, we hypothesize that σ^G activity requires both the completion of engulfment and a more traditional signal sent by the mother cell (Color Plate 3). In the parlance of systems biology, these two signals would constitute an "AND gate" and the regulation of σ^G would act as a coincidence sensor. In this model, a key function of the IIIA complex is to transduce the

mother cell signal (and could, in fact, encode the signal itself). Although no signaling protein under σ^E control has been identified in the activation of σ^G, it is likely that we have not yet found (or characterized) all the players in this pathway. In support of this idea, we still do not know what holds σ^G inactive prior to receiving a signal from the mother cell (see below). Importantly, there are no data to our knowledge that can distinguish between a role for the IIIA in monitoring engulfment and a role in a classical cell-cell signaling pathway.

What might be the engulfment signal? It has been proposed previously that the completion of engulfment is sensed as the separation of the forespore from the external environment (61). Once engulfment is complete, the forespore becomes a free protoplast within the mother cell and is isolated from the outside world. At this time, the intermembrane space between mother cell and forespore could change in its redox potential, electrochemical gradient, or metabolic state. Any of these changes could trigger σ^G activation. In this scenario, IIIA, which is thought to reside on the mother cell side of this double membrane (Color Plate 3), would probably have no role in surveillance or in transducing the engulfment signal.

Alternatively, it is possible that the activity of the IIIA complex in transducing a classical cell-cell signal to the forespore is sensitive to the engulfment status of the cell. For example, before the completion of engulfment (or if engulfment is perturbed) the IIIA proteins might be unable to fold or assemble into an active signaling complex. Thus, only when engulfment is complete would IIIA become competent to receive and transduce the mother cell signal. In this model, IIIA would serve both to transduce a classical σ^E-dependent signal from the mother cell and to sense engulfment. At first glance, the distinction between a classical signaling pathway and morphological coupling may seem artificial; however, we believe considering them separately has both mnemonic and predictive value.

It remains unclear whether additional proteins participate with IIIA in transducing the mother cell signal or engulfment status to the forespore. No other proteins have been identified except SpoIIIJ, which probably does not play a direct role in signal transduction (42). Proteins that function at earlier steps in sporulation could have a second role in σ^G signaling. These factors would likely not be identified in genetic screens because mutations would block the sporulation pathway at an earlier step. For example, any of the engulfment proteins could participate in both the mechanics of engulfment and in signal transduction. Currently, the best candidate of this type is the forespore protein SpoIIQ (referred to as IIQ). IIQ is required at a late stage for normal engulfment and for activation of σ^G (35, 62). Recently, it has been shown that IIQ localizes to the septal membrane on the forespore side (51) where it interacts directly with one of the membrane proteins in the IIIA complex (IIIAH) (Color Plate 3) (1, 9). In fact, IIQ is required to anchor IIIAH and presumably the rest of the putative IIIA complex in the mother cell membrane that surrounds the forespore (1, 9). Based on the physical interaction between IIIAH on the mother cell side of the septum and IIQ on the forespore side, it was proposed that IIQ might participate in triggering σ^G activation (1). This exciting and provocative hypothesis awaits further investigation.

Another outstanding question in the regulation of σ^G is what holds the transcription factor inactive prior to completion of engulfment. It was previously hypothesized that the SpoIIAB protein, which serves as an anti-σ^F factor, might also inhibit σ^G (29). However, more recent evidence suggests that SpoIIAB, which is indeed capable of holding σ^G inactive, does not perform this function in the forespore (57). Nonetheless, these data reinforce the possibility that the understanding of the activation of σ^G might require the identification and characterization of proteins required at earlier stages in sporulation, and spur the development of clever strategies for temporally controlled inactivation of proteins after their early roles are complete (18).

The Forespore Talks Back to the Mother

The third and final signal transduction pathway, the activation of σ^K in the mother cell under the control of σ^G in the forespore, is the most well understood of the three. The requirement for σ^G-dependent gene expression for the activation of σ^K was first recognized when it emerged that the *cotA* gene, responsible for the characteristic brown pigment of spores, was itself controlled by σ^K (7, 39). In σ^G mutants the spores failed to become pigmented, indicating that activation of σ^K in the mother cell required σ^G-dependent gene expression in the forespore. If σ^K activation is uncoupled from its dependence on σ^G, expression of σ^K-controlled genes commences approximately 30 min early, resulting in a reduction in sporulation efficiency (7). Thus, σ^K is synthesized in the mother cell before the activation of σ^G but remains inactive until the forespore signals that it is time for late mother cell gene expression.

σ^K, like σ^E, is derived from an inactive proprotein precursor called pro-σ^K (32) (Color Plate 4). This precursor has an amino-terminal extension of 20 amino acids with no detectable sequence similarity to the prodomain of σ^E. However, like pro-σ^E, the prodomain on σ^K serves as a covalently attached antisigma factor, which prevents interaction with core RNA polymerase (26, 69). Also, like the prodomain on σ^E, the amino-terminal prodomain of σ^K localizes the proprotein to the membrane (69). The polytopic membrane protein, SpoIVFB (hereafter referred to as B), is absolutely required for the proteolytic activation of pro-σ^K and is likely to be the processing enzyme (Color Plate 4) (8, 49). Expression of B and pro-σ^K in *Escherichia coli* is sufficient to trigger processing (70). B is a founding member of a family of membrane-embedded metalloproteases whose catalytic centers reside adjacent to or within the lipid bilayer (52, 67). The other founding member of this family of proteases is the Site-2 protease (47, 68), which is required for proteolytic activation of the sterol response element binding protein, a transcription factor required for the activation of genes involved in cholesterol metabolism and uptake (2). It is now clear that members of this family play key roles in signaling pathways in many bacteria and have been implicated in transducing a variety of signals (40). The B processing enzyme is regulated by two other integral membrane proteins, SpoIVFA (referred to as A) and BofA (7, 8, 50). In the absence of either of these proteins, B is capable of processing pro-σ^K without a forespore signal. All three proteins are synthesized in the mother cell under the control of σ^E and reside in a multimeric complex in the mother cell membrane that surrounds the forespore, perfectly positioned to receive a signal emanating from the forespore compartment (Color Plate 4) (48, 54). The A protein is required for the proper localization of B and BofA and is necessary for their interaction (Color Plate 4) (54). In turn, A is held in the forespore membrane through a network of interactions along and across the sporulation septum (9, 25). Indeed, A requires both IIIAH on the mother cell side and IIQ on the forespore side for its localization. Our current view of the regulation of pro-σ^K processing is that A anchors the complex in the mother cell membrane that surrounds the forespore and acts as a platform bringing BofA and B together, wherein BofA inhibits the processing activity of B until a signal has been received from the forespore (54).

The signaling protein SpoIVB (referred to as IVB) is produced in the forespore under σ^G control (6, 19) and is translocated across the membrane into the space between the forespore and mother cell membranes (3). IVB is a serine protease (64) and is capable of cleaving and releasing itself from the membrane (10). IVB then cleaves the extracellular domain of the regulatory protein A at multiple sites (3, 11). These cleavages are critical for efficient σ^K activation. A mutant A protein that cannot be cleaved by IVB delays pro-σ^K processing by over 2 h but does not completely block it (3). When IVB is unable to cleave A, a second signaling protease, CtpB, is able to compensate (3). CtpB, like IVB, is a serine protease with similar overall domain structure (3, 43). In its absence, pro-σ^K processing is delayed by ~30 min, suggesting that the

normal function of CtpB is to fine-tune the timing of σ^K activation. CtpB, like IVB, is capable of cleaving the extracellular domain of A (3). Importantly, an A mutant that cannot be cleaved by either of the two signaling proteases is blocked in pro-σ^K processing, and sporulation efficiency is significantly impaired (3). CtpB is synthesized in both the mother cell and the forespore compartments (43, 59, 65). However, synthesis in the forespore is both necessary and sufficient for CtpB activity (Color Plate 4) (4). Thus, there are two forespore signaling proteases that both target the same regulatory protein. We originally hypothesized that IVB cleaves and activates CtpB (3). CtpB is indeed a substrate for IVB both in vitro and in vivo; however, this cleavage does not appear to be critical for CtpB activity (4). Since IVB is absolutely required to trigger pro-σ^K processing but CtpB becomes essential for signaling only when IVB is present but cannot cleave A, these results suggest that IVB has yet another target (other than A and CtpB) in this signaling pathway.

It is clear that once the time is right, the forespore takes no chances in the activation of σ^K. The primary forespore signal IVB triggers pro-σ^K processing by cleaving A at one of two possible sites. If IVB is unable to cleave A, then CtpB compensates. It remains unknown whether these are fail-safe mechanisms for σ^K activation or whether the exact timing of σ^K activation is critical, and if so, why. Under laboratory conditions, subtle differences in the temporal activation of σ^K have very modest impacts on sporulation efficiency. Nonetheless, in the wild (and in the course of multiple cycles of sporulation and germination), small defects in the timing of late mother cell gene expression might have more pronounced consequences for the organism.

In summary, σ^G activity in the forespore sends a signal to the mother cell via a two-step proteolytic cleavage pathway that results in activation of σ^K. In this signaling pathway information is transduced across the lipid bilayer by the action of IVB and CtpB on one side of the membrane that triggers cleavage of pro-σ^K within or adjacent to the membrane by the membrane-embedded metalloprotease B. We hypothesize that cleavage of the regulatory protein A in the intermembrane space causes a conformational change in the B-A-BofA signaling complex that allows pro-σ^K access to the caged interior of the membrane metalloprotease B (3). We anticipate that further elucidation of the molecular mechanism of B activation and the consequences of the branched structure of the pathway will reveal general principles of how these membrane-embedded proteases are regulated and employed for signal transduction.

SUMMARY

We have described a conversation between two siblings during the developmental process of sporulation. These signal transduction pathways ensure that gene expression in the mother cell is coordinated with gene expression in the forespore and vice versa. As we have seen, if these development programs are uncoupled, sporulation efficiency is impaired. Both signaling pathways that emanate from the forespore result in the proteolytic activation of membrane-tethered transcription factors, yet the molecular mechanisms that govern information transduction are completely different in the two pathways. In the case of the signaling pathway emanating from the mother cell, it remains unclear whether this conforms to a traditional cell-cell signaling pathway or acts indirectly via a mechanism that couples gene expression to morphogenesis. We hypothesize that the signaling components in this pathway serve to monitor the completion of engulfment and to transduce a more classical signal from the mother cell. All three of these signaling pathways have served as powerful models for studying cell-cell signaling in bacteria. Their further elucidation will continue to provide insights into how information is transduced across a lipid bilayer.

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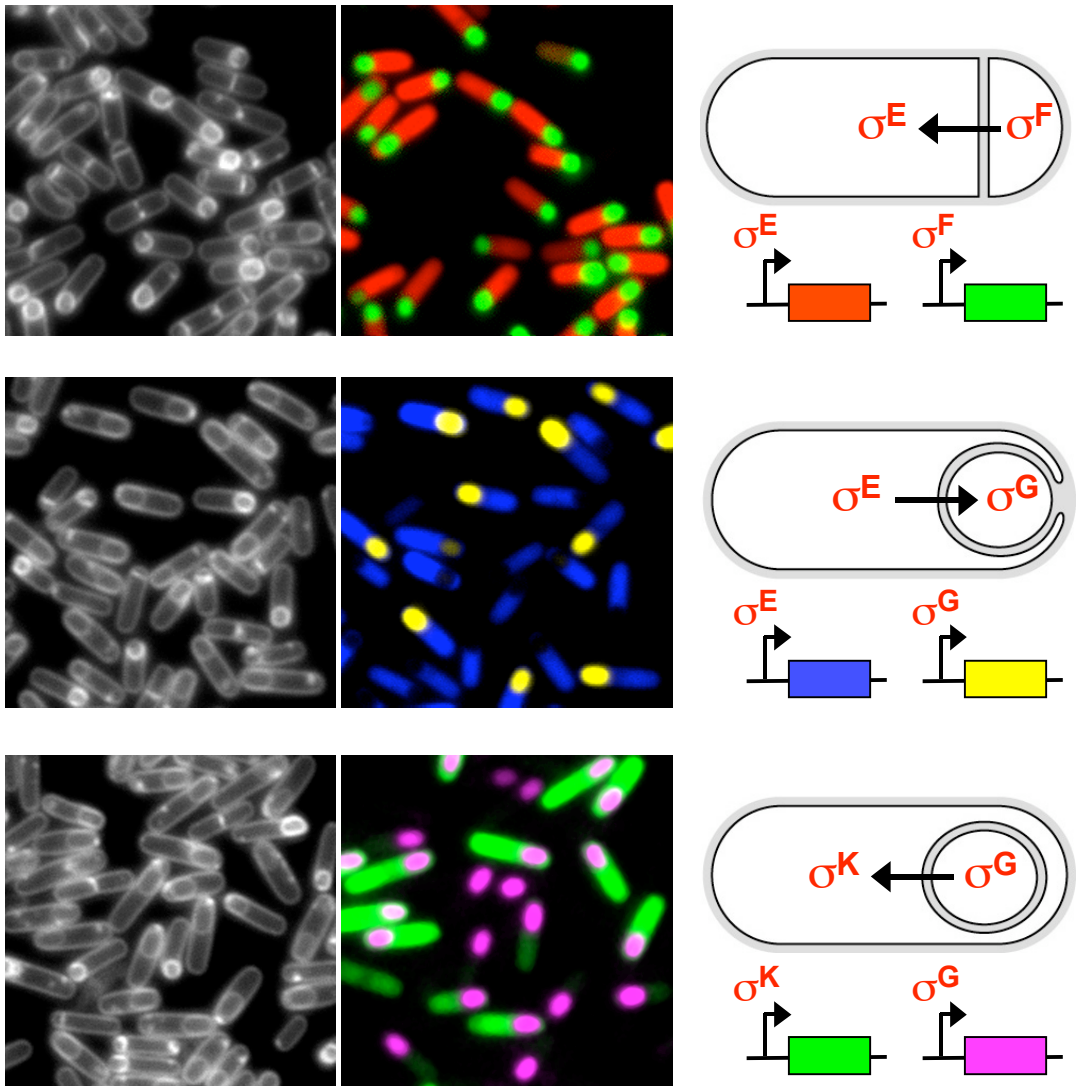
REFERENCES

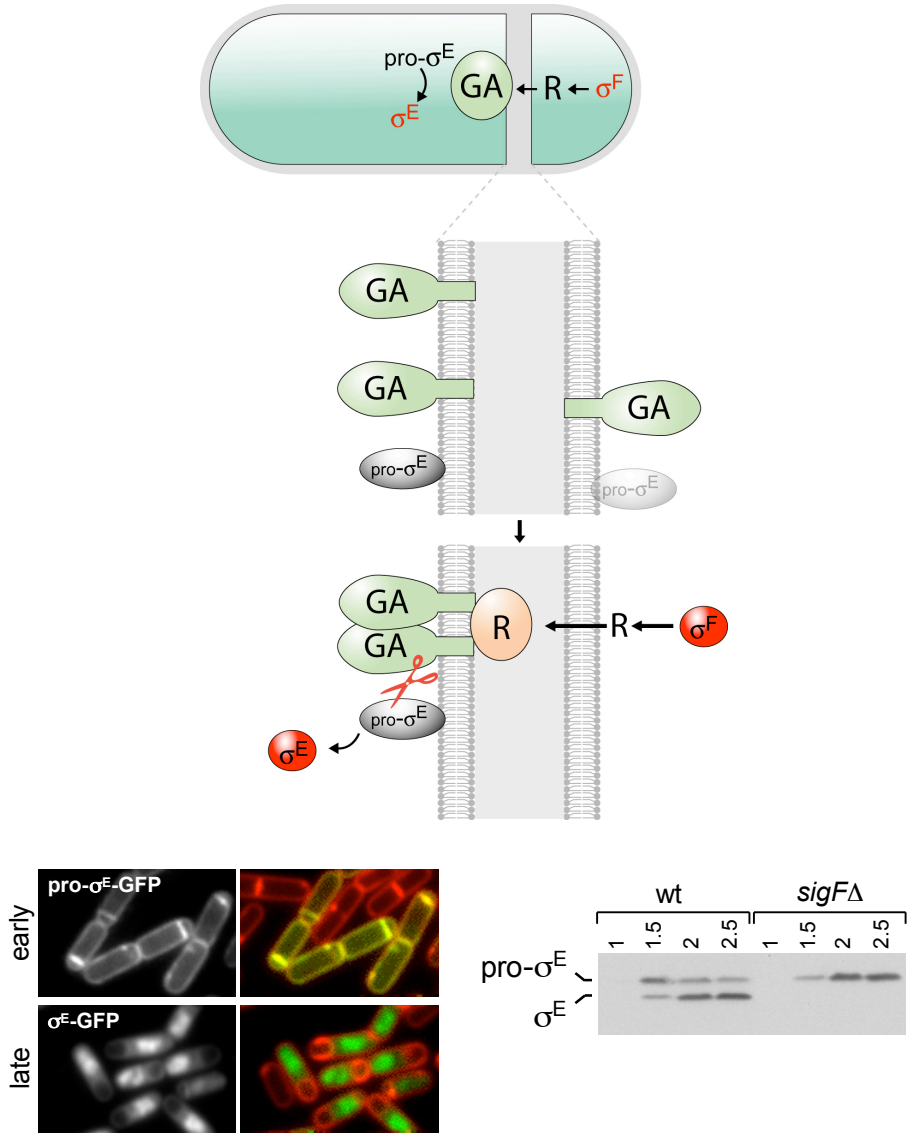
1. **Blaylock, B., X. Jiang, A. Rubio, C. P. Moran, Jr., and K. Pogliano.** 2004. Zipper-like interaction between proteins in adjacent daughter cells mediates protein localization. *Genes Dev.* **18:**2916–2928.
2. **Brown, M. S., and J. L. Goldstein.** 1997. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* **89:**331–340.
3. **Campo, N., and D. Z. Rudner.** 2006. A branched pathway governing the activation of a developmental transcription factor by regulated intramembrane proteolysis. *Mol. Cell* **23:**25–35.
4. **Campo, N., and D. Z. Rudner.** 2007. SpoIVB and CtpB are both forespore signals in the activation of the sporulation transcription factor sigmaK in *Bacillus subtilis*. *J. Bacteriol.* **189:**6021–6027.
5. **Clarkson, J., I. D. Campbell, and M. D. Yudkin.** 2004. Efficient regulation of sigmaF, the first sporulation-specific sigma factor in *B. subtilis*. *J. Mol. Biol.* **342:**1187–1195.
6. **Cutting, S., A. Driks, R. Schmidt, B. Kunkel, and R. Losick.** 1991. Forespore-specific transcription of a gene in the signal transduction pathway that governs Pro-sigma K processing in *Bacillus subtilis*. *Genes Dev.* **5:**456–466.
7. **Cutting, S., V. Oke, A. Driks, R. Losick, S. Lu, and L. Kroos.** 1990. A forespore checkpoint for mother cell gene expression during development in *B. subtilis*. *Cell* **62:**239–250.
8. **Cutting, S., S. Roels, and R. Losick.** 1991. Sporulation operon spoIVF and the characterization of mutations that uncouple mother-cell from forespore gene expression in *Bacillus subtilis*. *J. Mol. Biol.* **221:**1237–1256.
9. **Doan, T., K. A. Marquis, and D. Z. Rudner.** 2005. Subcellular localization of a sporulation membrane protein is achieved through a network of interactions along and across the septum. *Mol. Microbiol.* **55:**1767–1781.
10. **Doan, T., and D. Z. Rudner.** 2007. Perturbations to engulfment trigger a degradative response that prevents cell-cell signaling during sporulation in *Bacillus subtilis*. *Mol. Microbiol.* **64:**500–511.
11. **Dong, T. C., and S. M. Cutting.** 2003. SpoIVB-mediated cleavage of SpoIVFA could provide the intercellular signal to activate processing of Pro-sigmaK in *Bacillus subtilis*. *Mol. Microbiol.* **49:**1425–1434.
12. **Driks, A., and R. Losick.** 1991. Compartmentalized expression of a gene under the control of sporulation transcription factor sigma E in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. USA* **88:**9934–9938.
13. **Dworkin, J., and R. Losick.** 2005. Developmental commitment in a bacterium. *Cell* **121:**401–409.
14. **Eichenberger, P., P. Fawcett, and R. Losick.** 2001. A three-protein inihibitor of polar septation during sporulation in *Bacillus subtilis*. *Mol. Microbiol.* **42:**1147–1162.
15. **Errington, J.** 2003. Regulation of endospore formation in *Bacillus subtilis*. *Nat. Rev. Microbiol.* **1:**117–126.
16. **Fawcett, P., A. Melnikov, and P. Youngman.** 1998. The *Bacillus* SpoIIGA protein is targeted to sites of spore septum formation in a SpoIIE-independent manner. *Mol. Microbiol.* **28:**931–943.
17. **Fujita, M., and R. Losick.** 2002. An investigation into the compartmentalization of the sporulation transcription factor sigmaE in *Bacillus subtilis*. *Mol. Microbiol.* **43:**27–38.
18. **Fujita, M., and R. Losick.** 2003. The master regulator for entry into sporulation in *Bacillus subtilis* becomes a cell-specific transcription factor after asymmetric division. *Genes Dev.* **17:**1166–1174.
19. **Gomez, M., S. Cutting, and P. Stragier.** 1995. Transcription of spoIVB is the only role of sigma G that is essential for pro-sigma K processing during spore formation in *Bacillus subtilis*. *J. Bacteriol.* **177:**4825–4827.
20. **Hilbert, D. W., and P. J. Piggot.** 2004. Compartmentalization of gene expression during *Bacillus subtilis* spore formation. *Microbiol. Mol. Biol. Rev.* **68:**234–262.
21. **Hofmeister, A.** 1998. Activation of the proprotein transcription factor pro-sigmaE is associated with its progression through three patterns of subcellular localization during sporulation in *Bacillus subtilis*. *J. Bacteriol.* **180:**2426–2433.
22. **Hofmeister, A. E., A. Londono-Vallejo, E. Harry, P. Stragier, and R. Losick.** 1995. Extracellular signal protein triggering the proteolytic activation of a developmental transcription factor in *B. subtilis*. *Cell* **83:**219–226.
23. **Iber, D., J. Clarkson, M. D. Yudkin, and I. D. Campbell.** 2006. The mechanism of cell differentiation in *Bacillus subtilis*. *Nature* **441:**371–374.
24. **Igoshin, O. A., C. W. Price, and M. A. Savageau.** 2006. Signalling network with a bistable hysteretic switch controls developmental activation of the sigma transcription factor in *Bacillus subtilis*. *Mol. Microbiol.* **61:**165–184.
25. **Jiang, X., A. Rubio, S. Chiba, and K. Pogliano.** 2005. Engulfment-regulated proteolysis of SpoIIQ: evidence that dual checkpoints control sigma activity. *Mol. Microbiol.* **58:**102–115.

26. **Johnson, B. D., and A. J. Dombroski.** 1997. The role of the pro sequence of *Bacillus subtilis* sigmaK in controlling activity in transcription initiation. *J. Biol. Chem.* **272:**31029–31035.
27. **Ju, J., Luo, T., and W. G. Haldenwang.** 1997. *Bacillus subtilis* pro-sigmaE fusion protein localizes to the forespore septum and fails to be processed when synthesized in the forespore. *J. Bacteriol.* **179:**4888–4893.
28. **Karow, M. L., P. Glaser, and P. J. Piggot.** 1995. Identification of a gene, spoIIR, that links the activation of sigma E to the transcriptional activity of sigma F during sporulation in *Bacillus subtilis. Proc. Natl. Acad. Sci. USA* **92:**2012–2016.
29. **Kellner, E. M., A. Decatur, and C. P. Moran, Jr.** 1996. Two-stage regulation of an anti-sigma factor determines developmental fate during bacterial endospore formation. *Mol. Microbiol.* **21:**913–924.
30. **Khvorova, A., V. K. Chary, D. W. Hilbert, and P. J. Piggot.** 2000. The chromosomal location of the *Bacillus subtilis* sporulation gene spoIIR is important for its function. *J. Bacteriol.* **182:** 4425–4429.
31. **King, N., O. Dreesen, P. Stragier, K. Pogliano, and R. Losick.** 1999. Septation, dephosphorylation, and the activation of sigmaF during sporulation in *Bacillus subtilis. Genes Dev.* **13:**1156–1167.
32. **Kroos, L., B. Kunkel, and R. Losick.** 1989. Switch protein alters specificity of RNA polymerase containing a compartment-specific sigma factor. *Science* **243:**526–529.
33. **LaBell, T. L., J. E. Trempy, and W. G. Haldenwang.** 1987. Sporulation-specific sigma factor sigma 29 of *Bacillus subtilis* is synthesized from a precursor protein, P31. *Proc. Natl. Acad. Sci. USA* **84:**1784–1788.
34. **Li, Z., and P. J. Piggot.** 2001. Development of a two-part transcription probe to determine the completeness of temporal and spatial compartmentalization of gene expression during bacterial development. *Proc. Natl. Acad. Sci. USA* **98:**12538–12543.
35. **Londono-Vallejo, J. A., C. Frehel, and P. Stragier.** 1997. SpoIIQ, a forespore-expressed gene required for engulfment in *Bacillus subtilis. Mol. Microbiol.* **24:**29–39.
36. **Londono-Vallejo, J. A., and P. Stragier.** 1995. Cell-cell signaling pathway activating a developmental transcription factor in *Bacillus subtilis. Genes Dev.* **9:**503–508.
37. **Losick, R., and P. Stragier.** 1992. Crisscross regulation of cell-type-specific gene expression during development in *B. subtilis. Nature* **355:**601–604.
38. **Losick, R., P. Youngman, and P. J. Piggot.** 1986. Genetics of endospore formation in *Bacillus subtilis. Annu. Rev. Genet.* **20:**625–669.
39. **Lu, S., R. Halberg, and L. Kroos.** 1990. Processing of the mother-cell sigma factor, sigma K, may depend on events occurring in the forespore during *Bacillus subtilis* development. *Proc. Natl. Acad. Sci. USA* **87:**9722–9726.
40. **Makinoshima, H., and M. S. Glickman.** 2006. Site-2 proteases in prokaryotes: regulated intramembrane proteolysis expands to microbial pathogenesis. *Microbes Infect.* **8:**1882–1888.
41. **Margolis, P. S., A. Driks, and R. Losick.** 1993. Sporulation gene spoIIB from *Bacillus subtilis. J. Bacteriol.* **175:**528–540.
42. **Murakami, T., K. Haga, M. Takeuchi, and T. Sato.** 2002. Analysis of the *Bacillus subtilis* spoIIIJ gene and its paralogue gene, *yqjG. J. Bacteriol.* **184:**1998–2004.
43. **Pan, Q., R. Losick, and D. Z. Rudner.** 2003. A second PDZ-containing serine protease contributes to activation of the sporulation transcription factor sigmaK in *Bacillus subtilis. J. Bacteriol.* **185:**6051–6056.
44. **Partridge, S. R., and J. Errington.** 1993. The importance of morphological events and intercellular interactions in the regulation of prespore-specific gene expression during sporulation in *Bacillus subtilis. Mol. Microbiol.* **8:**945–955.
45. **Piggot, P. J., and R. Losick.** 2002. Sporulation genes and intercompartmental regulation, p. 483–517. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), Bacillus subtilis *and Its Closest Relatives: from Genes to Cells.* ASM Press, Washington, DC.
46. **Pogliano, J., N. Osborne, M. D. Sharp, A. Abanes-De Mello, A. Perez, Y. L. Sun, and K. Pogliano.** 1999. A vital stain for studying membrane dynamics in bacteria: a novel mechanism controlling septation during *Bacillus subtilis* sporulation. *Mol. Microbiol.* **31:**1149–1159.
47. **Rawson, R. B., N. G. Zelenski, D. Nijhawan, J. Ye, J. Sakai, M. T. Hasan, T. Y. Chang, M. S. Brown, and J. L. Goldstein.** 1997. Complementation cloning of S2P, a gene encoding a putative metalloprotease required for intramembrane cleavage of SREBPs. *Mol. Cell* **1:**47–57.
48. **Resnekov, O., S. Alper, and R. Losick.** 1996. Subcellular localization of proteins governing the proteolytic activation of a developmental transcription factor in *Bacillus subtilis. Genes Cells* **1:**529–542.
49. **Resnekov, O., and R. Losick.** 1998. Negative regulation of the proteolytic activation of a developmental transcription factor in *Bacillus subtilis. Proc. Natl. Acad. Sci. USA* **95:**3162–3167.
50. **Ricca, E., S. Cutting, and R. Losick.** 1992. Characterization of bofA, a gene involved in intercompartmental regulation of pro-sigma K processing during sporulation in *Bacillus subtilis. J. Bacteriol.* **174:**3177–3184.

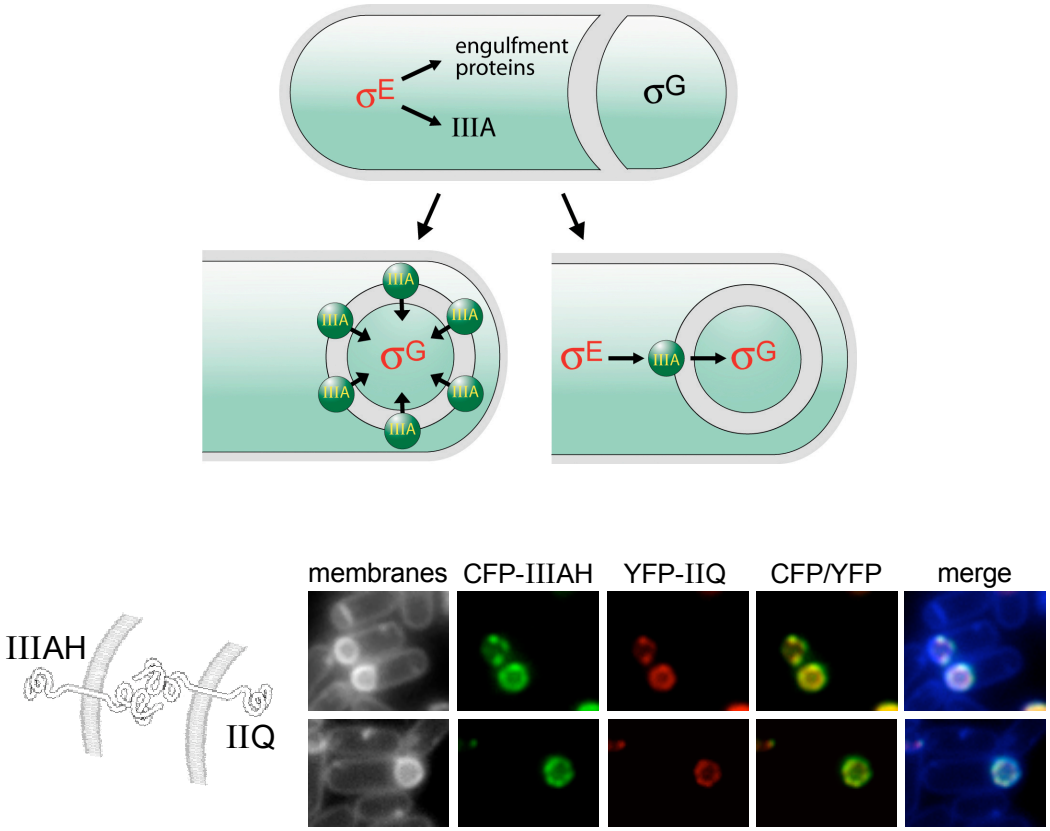
51. **Rubio, A., and K. Pogliano.** 2004. Septal localization of forespore membrane proteins during engulfment in *Bacillus subtilis*. *EMBO J.* **23:**1636–1646.
52. **Rudner, D. Z., P. Fawcett, and R. Losick.** 1999. A family of membrane-embedded metalloproteases involved in regulated proteolysis of membrane-associated transcription factors. *Proc. Natl. Acad. Sci. USA* **96:**14765–14770.
53. **Rudner, D. Z., and R. Losick.** 2001. Morphological coupling in development: lessons from prokaryotes. *Dev. Cell* **1:**733–742.
54. **Rudner, D. Z., and R. Losick.** 2002. A sporulation membrane protein tethers the prosigmaK processing enzyme to its inhibitor and dictates its subcellular localization. *Genes Dev.* **16:**1007–1018.
55. **Schlessinger, J.** 2000. Cell signaling by receptor tyrosine kinases. *Cell* **103:**211–225.
56. **Schlessinger, J.** 2002. Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. *Cell* **110:**669–672.
57. **Serrano, M., A. Neves, C. M. Soares, C. P. Moran, Jr., and A. O. Henriques.** 2004. Role of the anti-sigma factor SpoIIAB in regulation of sigmaG during *Bacillus subtilis* sporulation. *J. Bacteriol.* **186:**4000–4013.
58. **Smith, K., M. E. Bayer, and P. Youngman.** 1993. Physical and functional characterization of the *Bacillus subtilis* spoIIM gene. *J. Bacteriol.* **175:**3607–3617.
59. **Steil, L., M. Serrano, A. O. Henriques, and U. Volker.** 2005. Genome-wide analysis of temporally regulated and compartment-specific gene expression in sporulating cells of *Bacillus subtilis*. *Microbiology* **151:**399–420.
60. **Stragier, P., C. Bonamy, and C. Karmazyn-Campelli.** 1988. Processing of a sporulation sigma factor in *Bacillus subtilis*: how morphological structure could control gene expression. *Cell* **52:**697–704.
61. **Stragier, P., and R. Losick.** 1996. Molecular genetics of sporulation in *Bacillus subtilis*. *Annu. Rev. Genet.* **30:**297–241.
62. **Sun, Y. L., M. D. Sharp, and K. Pogliano.** 2000. A dispensable role for forespore-specific gene expression in engulfment of the forespore during sporulation of *Bacillus subtilis*. *J. Bacteriol.* **182:**2919–2927.
63. **Trempy, J. E., J. Morrison-Plummer, and W. G. Haldenwang.** 1985. Synthesis of sigma 29, an RNA polymerase specificity determinant, is a developmentally regulated event in *Bacillus subtilis*. *J. Bacteriol.* **161:**340–346.
64. **Wakeley, P. R., R. Dorazi, N. T. Hoa, J. R. Bowyer, and S. M. Cutting.** 2000. Proteolysis of SpolVB is a critical determinant in signalling of pro-sigmaK processing in *Bacillus subtilis*. *Mol. Microbiol.* **36:**1336–1348.
65. **Wang, S. T., B. Setlow, E. M. Conlon, J. L. Lyon, D. Imamura, T. Sato, P. Setlow, R. Losick, and P. Eichenberger.** 2006. The forespore line of gene expression in *Bacillus subtilis*. *J. Mol. Biol.* **358:**16–37.
66. **Wu, L. J., A. Feucht, and J. Errington.** 1998. Prespore-specific gene expression in *Bacillus subtilis* is driven by sequestration of SpoIIE phosphatase to the prespore side of the asymmetric septum. *Genes Dev.* **12:**1371–1380.
67. **Yu, Y. T., and L. Kroos.** 2000. Evidence that SpoIVFB is a novel type of membrane metalloprotease governing intercompartmental communication during *Bacillus subtilis* sporulation. *J. Bacteriol.* **182:**3305–3309.
68. **Zelenski, N. G., R. B. Rawson, M. S. Brown, and J. L. Goldstein.** 1999. Membrane topology of S2P, a protein required for intramembranous cleavage of sterol regulatory element-binding proteins. *J. Biol. Chem.* **274:**21973–21980.
69. **Zhang, B., A. Hofmeister, and L. Kroos.** 1998. The prosequence of pro-sigmaK promotes membrane association and inhibits RNA polymerase core binding. *J. Bacteriol.* **180:**2434–2441.
70. **Zhou, R., and L. Kroos.** 2004. BofA protein inhibits intramembrane proteolysis of pro-sigmaK in an intercompartmental signaling pathway during *Bacillus subtilis* sporulation. *Proc. Natl. Acad. Sci. USA* **101:**6385–6390.
71. **Zuber, P., and R. Losick.** 1983. Use of a *lacZ* fusion to study the role of the *spoO* genes of *Bacillus subtilis* in developmental regulation. *Cell* **35:**275–283.
72. **Zupancic, M. L., H. Tran, and A. E. Hofmeister.** 2001. Chromosomal organization governs the timing of cell type-specific gene expression required for spore formation in *Bacillus subtilis*. *Mol. Microbiol.* **39:**1471–1481.



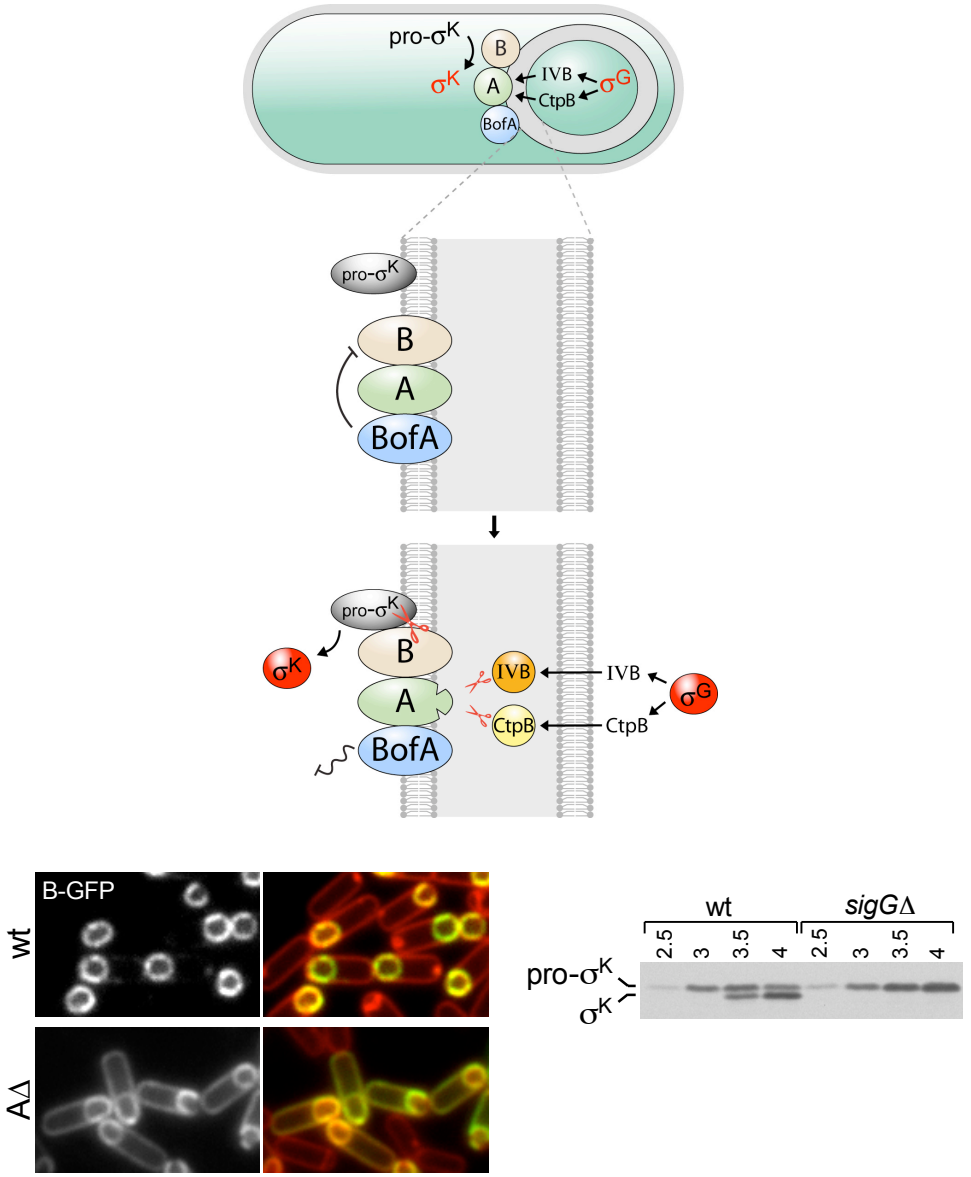
COLOR PLATE 1 (chapter 1) Stage- and compartment-specific gene expression during sporulation. Sporulating cells harboring fluorescent fusions to forespore- and mother-cell-specific promoters were visualized by fluorescence microscopy. In the top panels, the cells (from hour 2 of sporulation) contained σ^F- and σ^E-responsive promoters fused to *cfp* (false-colored green) and *yfp* (false-colored red), respectively. Note that all sporangia that have σ^F-dependent expression of *yfp* also have σ^E-dependent expression of *cfp*. In the middle panels, the cells (from hour 3 of sporulation) contained σ^E- and σ^G-responsive promoters fused to *cfp* (blue) and *yfp* (yellow), respectively. Note that some sporangia that have σ^E-dependent expression of *cfp* do not yet have detectable σ^G-dependent expression of *yfp*. In the bottom panel, the cells (from hour 4 of sporulation) contained σ^G- and σ^K-responsive promoters fused to *cfp* (false-colored purple) and *yfp* (false-colored green), respectively. Note that some sporangia that have σ^G-dependent expression of *cfp* do not yet have σ^K-dependent expression of *yfp*. Schematic diagrams to the right show the three signal transduction pathways that comprise the conversation between the mother cell and forespore. The membrane dye (TMA-DPH) stains the double membrane that surrounds the forespore more intensely prior to the completion of engulfment.



COLOR PLATE 2 (chapter 1) Activation of σ^E in the mother cell is controlled by a signal transduction pathway emanating from the forespore under the control of σ^F. Schematic diagram of the signal transduction pathway. Proteolytic activation of pro-σ^E in the mother cell requires the putative membrane-tethered aspartyl protease GA. GA localizes to the septal membrane and is inactive in its default state. The signaling protein R is produced in the forespore compartment under the control of σ^E and is secreted into the space between the forespore and mother cell membranes, where it activates the GA processing enzyme. Both pro-σ^E and GA are synthesized in the predivisional cell and are therefore present in both the mother cell and forespore compartments. Pro-σ^E is degraded by an unknown mechanism in the forespore. It is hypothesized that the signaling protein R activates GA by promoting its dimerization. Fluorescence micrographs of wild-type cells harboring a functional pro-σ^E-green fluorescent protein (GFP) fusion. Early during sporulation pro-σ^E localizes to all the membranes of the sporangia. Cell-cell signaling results in proteolytic processing and release of σ^E-GFP into the mother cell cytosol, where it localizes to the nucleoid and directs gene transcription. Pro-σ^E processing requires forespore gene expression under the control of σ^F. Pro-σ^E and σ^E were visualized by immunoblot analysis of whole cell lysates from wild-type and σ^F mutant (*sigF*Δ) cells in a sporulation time course. Time (in hours) is indicated.



COLOR PLATE 3 (chapter 1) Two models for the activation of σ^G in the forespore by σ^E in the mother cell. σ^E in the mother cell directs the synthesis of several engulfment proteins as well as the *IIIA* locus. In the first model, the IIIA proteins monitor the engulfment process. Upon completion of engulfment, they transduce a signal that triggers σ^G activation. In the second model, the IIIA complex is involved in transducing an unknown mother cell signal. In this second model, the requirement for engulfment must also be satisfied to activate σ^G, and this need not require IIIA. The engulfment protein IIQ localizes to the septal membrane on the forespore side and anchors IIIAH in the septal membrane on the mother cell side. The interaction between these two proteins has been established biochemically and cytologically. Fluorescence micrographs of a sporulating cell harboring *cfp-IIIAH* and *yfp-IIQ* fusions.



COLOR PLATE 4 (chapter 1) The activation of σ^K in the mother cell is controlled by a signal transduction pathway emanating from the forespore under the control of σ^G. Schematic diagram of the signaling pathway. Proteolytic activation of pro-σ^K in the mother cell requires the putative membrane-embedded metalloprotease B. B resides in a multimeric membrane complex with A and BofA. A anchors the complex in the mother cell membranes that surround the forespore and serves as a platform to bring B and BofA into close proximity, wherein BofA holds B inactive. Two signals from the forespore under the control of σ^G trigger pro-σ^K processing. Both signaling proteins (IVB and CtpB) are serine proteases, and both target the regulatory protein A. Cleavage of A triggers activation of the B metalloprotease and pro-σ^K processing. It is hypothesized that cleavage of A results in a conformational change in the complex that allows pro-σ^K access to the caged interior of the membrane-embedded protease. The putative pro-σ^K processing enzyme B localizes to the engulfing septal membranes and is anchored there by the regulatory protein A. Wild-type and A mutant cells harboring a B-GFP fusion were visualized during sporulation by fluorescence microscopy. Pro-σ^K processing requires forespore gene expression under the control of σ^G. Pro-σ^K and σ^K were visualized by immunoblot analysis of whole cell lysates from wild-type and σ^G mutant (*sigGΔ*) cells in a sporulation time course. Time (in hours) is indicated.