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Bacillus subtilis sporulation and stationary phase gene expression

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Abstract. *Bacillus subtilis* cells entering stationary phase due to nutrient deprivation have a number of options. Complex interconnected regulatory circuits govern differential gene expression patterns that channel the cell along the path it has sensed is most advantageous for survival in the environment. The actual choice depends upon the activity of an elaborate signal transduction network (the phosphorelay) that ultimately affects the activity of two key transcription factors, Spo0A and AbrB. Should the cell commit to sporulation, a temporally and spatially controlled cascade of RNA polymerase sigma factors leads to the development and release of an endospore from within the terminally differentiated, apoptotic mother cell.

Key words. Spo0A; AbrB; gene regulation; cellular differentiation; transcription factors.

Introduction

Upon depletion of nutrients, bacterial cells enter into a semiquiescent state known as stationary phase. In order to survive, the cells redirect their metabolism and physiology to cope with the hostile conditions. Some species, notably those in the genera *Bacillus* and *Clostridium*, have the ability to undergo a cellular differentiation process leading to the formation of a dormant spore. The *Bacillus* spore is metabolically inactive, highly resistant to various environmental assaults and serves to protect the organism's genome until growth-favoring conditions return [see articles by Popham and Watabe in this issue]. Sensing mechanisms incorporated in the spores can then be activated which ultimately lead to germination and resumption of active vegetative growth [see article by A. Moir in this issue]. Complex regulatory circuits govern the alterations in gene expression that occur upon entry into stationary phase. In *Bacillus subtilis*, the best-characterized Gram-positive spore-forming bacterium, over 100 genes are dedicated to, or required for, the sporulation process. But sporulation is not the only developmental option open to *B. subtilis* cells. A multitude of other genes, themselves not directly necessary for spore formation (at least not under laboratory conditions), undergo changes in expression that are required for general adaptive and survival-enhancing properties precipitated by entry into stationary phase. Additionally, regulatory mechanisms serving to integrate available metabolic, environmental and physiological information must be in place in order to determine which developmental option is most suitable in the given conditions. Once the choice is made, expression of genes specific for alternate paths must be shut off, and changes must be made that either enhance or are necessary for commitment to the chosen path.

In this review, we will attempt to provide an overview of the genetics and regulatory circuits of stationary phase and sporulation in *B. subtilis*. Our focus will be primarily upon events occurring during entry into stationary phase (termed the transition state) and during the early stages of the sporulation process. More detailed discussions and in-depth treatments of topics, only briefly touched upon here, can be found in several recent reviews [1–10] and in the other articles of this multi-author review series.

The transition state: decisions, decisions

Facing imminent entry into stationary phase due to sensing that the environment can no longer support replicative

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growth, the cell's first priorities are to express functions needed to scavenge alternative nutrients and to effectively compete with other species for the scarce resources [10]. During the transition state, *B. subtilis* expresses various extracellular proteases and other degradative enzymes, transport functions and numerous alternate pathways to seek out (chemotaxis) and maximize utilization of nutrients. A variety of antibiotics and antimicrobials are pumped out of the cell in an effort to outcompete other nearby microbial species. Under appropriate conditions, there is a finite probability that a cell will enter a distinct physiological state and express functions allowing it to become competent for uptake of exogenous DNA [2]. Sporulation is usually considered as a last resort to be embarked upon only when all other attempts to grow, compete and survive have been exhausted. But the timing of the decision to commit to spore formation is extremely critical. Once committed, there is no turning back: if the cell fails to produce a complete spore (about 6–8 h at 37 °C in laboratory cultures), the ability to propagate its genetic information is irretrievably lost. Since sporulation is an energy-intensive process, the cell must make the decision to sporulate based upon having sensed that enough scarce resources will still be available for completion of the process. In addition to sensing the quantity and nature of available nutrients, *B. subtilis* cells also utilize a type of quorum-sensing mechanism [11] to monitor the local population density of their species. Using information gathered from the combination of these sensing mechanisms is probably a strategy that has evolved as an attempt to maximize the survival potential of as many individuals as possible in the local population (all likely to be clones).

The transition state is a crossroads in the life cycle of the cell when information is gathered and processed, and when functions are expressed that will allow entry into whichever metabolic state or developmental path is ultimately chosen, but before any final commitment is made. In the laboratory the transition state is quite transitory, but in the soil habitat of *B. subtilis*, where nutrients are not usually abundant, the predominant growing state of the cell is probably more akin to the transition state than to rapid logarithmic growth in rich media. Not surprisingly, a complex network of regulatory mechanisms direct gene expression during the transition state and early stationary phase. Various two-component signal transduction systems sense environmental signals and alter gene expression accordingly [6, 12, 13]. In response to extracytoplasmic stimuli, alternate RNA polymerase sigma factors drive expression of adaptive and survival functions. Small peptide-signaling pheromones are excreted and imported by the cells (see below). Numerous other regulatory proteins are involved in either activating, repressing or modulating transcriptional events. A variety of critical global regulators provide links between various physiological and developmental options. One important group of these regulators have been termed transition-state regulators. The primary function of this group of proteins is to prevent the inappropriate expression (during replicative growth) of functions that are only needed during stationary phase [14]. Some also serve to modulate the expression of genes during slow, but active, replicative growth. The most important members of this class of regulatory proteins are AbrB, ScoC (a.k.a. Hpr), SinR, CodY and Abh. All are DNA-binding global regulators of numerous genes, and there are many overlaps between their individual regulons. AbrB appears to be a lynchpin interconnecting most, if not all, of these various regulatory networks.

The AbrB protein

Over 40 different genes are subject to regulation due to direct AbrB binding to their promoters or regulatory regions [10, 15, 16; M. A. Strauch, unpublished]. Many other genes are indirectly controlled by AbrB since it is a regulator of other regulatory proteins, including ScoC, Abh, SinR and SigH. Additional genes possibly controlled by AbrB (either directly or indirectly) have been identified by transcriptional profiling [17] and await further investigation. AbrB-controlled genes function in a wide variety of metabolic and physiological processes, including production of extracellular degradative enzymes, nitrogen utilization and amino acid metabolism, motility, synthesis of antibiotics and their resistant determinants, development of competence, transport systems, oxidative stress response, phosphate metabolism, cell surface components and sporulation. Knockout mutations in *abrB* do not seem to significantly affect the cells ability to sporulate under many common laboratory conditions, but in certain situations the loss of AbrB can be seen to alter the onset of the sporulation process [M. A. Strauch, unpublished]. At the other end of the spectrum, overexpression of AbrB can repress sporulation [18]. Thus, although not usually considered essential for either viability, growth or sporulation in the laboratory, AbrB probably is an essential function for fitness, adaptation and survival in the wild.

Mutations in the *abrB* gene were first isolated as second-site revertants, relieving many of the pleiotropic phenotypes exhibited by cells possessing mutations in genes which prevented the initiation of sporulation (*spo0* genes). Among the phenotypes reverted were those associated with production of, and resistance to, various antibiotics (hence the mnemonic *abr* = antibiotic resistance). AbrB had been shown to repress synthesis of the tyrocidin synthetase I gene from *B. brevis* [19] and functions responsible for resistance to polymixin B from *B. polymixa* [20]. But until recently the antibiotic genes targeted for AbrB regulation in *B. subtilis* were largely un-

known. In the past 2 years it has been found that AbrB regulates genes responsible for production of the antilisterial subtilosin [21] and a novel antimicrobial called TasA [22]. Additionally, there is evidence [M. A. Strauch and J. Helmann, unpublished] that AbrB regulates expression of *sigW*, which encodes an RNA polymerase sigma factor responsible for transcription of antibiotic genes and resistance determinants [23]. Undoubtedly, AbrB proteins (and their homologues) play critical roles in the production of antibiotics and toxins in other *Bacillus* and *Clostridium* species. In fact, it has recently been observed that AbrB regulates expression of the *pag* gene of *B. anthracis* [24].

The structure of the AbrB DNA-binding surface has recently been elucidated [25] and contains a unique motif termed the looped-hinge helix fold. Multimerization between identical monomeric subunits (10,500 Da) is absolutely required to form the active surface and is believed to be a key factor in the ability of AbrB to differentially regulate various target genes (see below). Dimerization positions two identical short α-helical segments, one from each subunit, in a slightly offset, antiparallel linear alignment at the base of a saddle-like cleft. The DNA target fits into the cleft with the protein contacting one face of the DNA helix. Examination of over 40 chromosomal sites of AbrB binding, and over 80 high-affinity binding sites selected using in vitro methods, failed to derive a consensus base sequence that could adequately explain AbrB site selection and recognition [15, 16, 26]. It has been hypothesized that AbrB recognizes a three-dimensional DNA architecture that is shared by a finite subset of base sequences [26, 27]. It appears that the major factor accounting for AbrB's flexible sequence recognition is the dynamic flexibilty of the looped-hinge helix motif of the protein. The flexible hinges allow spatial repositioning of the α-helical segments to conform to the target DNA such that optimal contacts may be made.

DNA binding by AbrB affects gene expression in three different ways [10, 14, 28]. For some genes, AbrB appears to be the sole negative regulator (repressor): expression of these genes is constitutive during all phases of growth in *abrB* mutants. But the most common form of AbrB negative control is what has been termed a preventer role: AbrB acts as one factor in a series of redundant regulatory networks that ensure no one regulator has complete control over genes that must remain silent during active growth. Genetic studies have also implicated AbrB as an activator of some genes. However, there is no evidence that AbrB directly activates RNA Polymerase. Rather, it appears that the activation is the result of AbrB interference with the expression or DNA binding site availability of other negative regulators (i.e. two negatives = a positive).

What mechanism effectively maintains AbrB-mediated regulation during logarithmic growth but is rapidly counteracted as the cells enter stationary phase? During active growth transcription of the *abrB* gene is autoregulated [29]. This maintains the intracellular concentration of AbrB at a narrow threshold range of regulatory effectiveness. In response to nutrient deprivation and the onset of the transition state, a regulatory cascade is activated which increases the intracellular concentration of the phosphorylated form of a critical regulatory protein, Spo0A (see below). Phosphorylated Spo0A is a potent repressor of *abrB* transcription, acting independently of AbrB concentration [30]. The concentration of AbrB drops below its threshold of effectiveness, and the AbrB-dependant regulatory effects are lifted.

There is evidence that AbrB can bind both as a dimer and as a tetramer to some targets while only able to bind to others as a tetramer [25]. It is possible that future investigations will uncover instances where only a dimeric form can bind. Since changes in the intracellular concentration of AbrB would be expected to affect the dimer:tetramer ratio, it has been hypothesized that this may be a mechanism to achieve differential regulatory effects at different subsets of AbrB-controlled promoters [25], possibly in response to different Spo0A ~P levels. A striking example of differential effects of intracellular AbrB levels upon gene expression is seen in the case of competence development [18]. AbrB impinges upon the competence pathway at multiple points, with both negative and positive effects. Competence development is maximal at a very narrow range of AbrB levels: small deviations either above or below this level significantly decrease the ability of the cell to become competent [18]. (A more detailed discussion of the competence pathway can be found in a recent review [2].) The combination of multimer state-dependent binding and an ability to flexibly conform to specific subsets of base sequences makes AbrB ideally suited to its role as a global regulator.

Other transition-state regulators and interconnected regulatory circuits

Overproduction of either ScoC or SinR inhibits *B. subtilis* sporulation, among other effects [31, 32]. The ScoC protein (a.k.a. Hpr, not to be confused with the *ptsH* gene product – HPr – of bacterial PEP-sugar phosphotransferase systems) is a DNA-binding regulator of protease production, oxidative stress responses, permease complexes and other physiological processes [14, 33]. ScoC is a member of the MarR family of transcriptional regulators [34]. Expression of *scoC* is regulated by Spo0A, AbrB [31] and possibly by autoregulation and at least one nonprotein effector molecule [M. A. Strauch, unpublished]. The negative effect on sporulation when ScoC is overproduced is due, at least in part, to ScoC repression of oligopeptide permease systems responsible for trans-

port of small peptide pheromones into the cell [35] and ScoC repression of *sinI* expression (see below).

The gene encoding SinR is the second in a dicistronic operon which also contains *sinI*. SinR is a DNA-binding global regulator of degradative enzymes synthesis, motility, competence and sporulation [33, 36]. The targets of its negative effects on sporulation are the *spoIIA*, *spoIIG* and *spoIIE* operons (see below). During vegetative growth, repressive levels of SinR are maintained due to transcription of *sinR* by an internal promoter in the *sinIR* operon [37]. At the onset of stationary phase, transcription of a promoter (P1) upstream of the *sinI* cistron leads to accumulation of SinI protein. AbrB and ScoC are negative regulators of *sinIR* P1 during growth, whereas Spo0A in its phosphorylated state activates transcription during early stationary phase [T. Leighton, I. Smith and M. A. Strauch, unpublished]. SinI interacts with SinR to sequester the latter's activity, thus relieving SinR repressive effects [38].

CodY is a global regulator exerting nutritional repression in response to the availability of certain mixtures of amino acids. During active growth in rich media, CodY represses motility, competence, dipeptide permeases and genes required for utilization of alternate carbon and nitrogen sources such as acetate and histidine [39]. Furthermore, negation of CodY repressive effects are required for the full induction of the Krebs cycle that is required for sporulation [40; A. L. Sonenshein, personal communication]. Interestingly, recent work indicates that GTP binds to CodY as a corepressor [A. L. Sonenshein, personal communication].

The amino-terminal regions of the SpoVT and Abh proteins share a high degree of amino acid sequence identity to the DNA-binding domain of AbrB. SpoVT is a regulator of numerous forespore specific genes [41] that are expressed at later times during sporulation (see below) and so is not a true transition-state regulator by the strict definition. The Abh regulon has not yet been elucidated, but transcription of *abh* requires either of the extracytoplasmic function sigma factors σ^X and σ^W [42], and is subject to AbrB repression [M. A. Strauch, unpublished].

Of the approximately 35 two-component signal transduction systems present in *B. subtilis* [43], two in particular deserve mention in this section. The DegS/DegU pair regulates expression of various proteases, enzymes degrading a variety of carbohydrates, surfactin and polyketide biosynthesis functions, and the regulatory proteins DegQ, DegR and ComK [6]. ComK is required for expression of late competence genes, some SOS functions and general stress factors, and an anti-sigma factor directed toward the sigma factor (σ^D) needed to transcribe genes necessary for motility [2]. (ComK expression is also controlled by the AbrB, SinR and CodY transition-state regulators.) The ComP/ComA two-component system is required for inactivation of functions leading to degradation of ComK activity and for the expression of phosphatases and peptide pheromones affecting various signal transduction pathways (see below). A number of genes in other metabolic pathways are suspected to be regulated by ComA [4]. It is still not entirely clear how all the different metabolic and environmental data that the cell gathers as it approaches stationary phase are then relayed and integrated through these complex regulatory circuits in order to funnel the cell to the most advantageous survival response. What does seem certain, however, is that conditions that activate sporulation inhibit competence, and vice versa. Furthermore, all available consistent evidence points to the ultimate factor in the response choice being the intracellular level of the phosphorylated form of Spo0A, which is the subject of the following section.

Initiating spore formation: The phosphorelay and Spo0A

The initiation of sporulation is controlled by a complex assemblage of protein kinases, phosphorylatable proteins and phosphatases known as the phosphorelay (fig. 1). The multi-component nature of the phosphorelay allows for input and integration of multiple, discrete metabolic and environmental signals and thus affords numerous points for checks and balances. The crucial output of this signal transduction system is phosphorylation of the Spo0A transcription factor. Spo0A ~P plays two crucial roles in the cell's adaptive responses to nutrient limitation. The first role, accomplished at relatively low intracellular concentrations, is to repress transcription of *abrB*. As mentioned above, this leads to lifting AbrB-dependent regulation of transition-state-associated gene expression. If the Spo0A ~P concentration then reaches a higher critical level, it is capable of activating expression of genes required for the entry and commitment to sporulation (see below).

Four different histidine kinases (KinA, B, C and D) have been shown capable of initiating the phosphorylation cascade, and another (KinE) is suspected of having the capability [44]. The nature of the particular signals that each kinase responds to is largely unknown. The target of each of the kinases activity is the Spo0F protein, which serves as a type of secondary messenger. The phosphoryl group from Spo0F ~P is then transferred to the Spo0B protein and from thence to the Spo0A protein. The most critical of the kinases necessary for sporulation (at least under laboratory conditions) is KinA, followed in importance by KinB. The roles of KinC and KinD seem to be generation of a level of Spo0A ~P that regulates the onset of transition state gene expression (via repression of *abrB*), but which is insufficient to activate sporulation-specific gene expression. Interestingly, both KinC and KinD (but not KinA or KinB) may be capable of directly phospho-

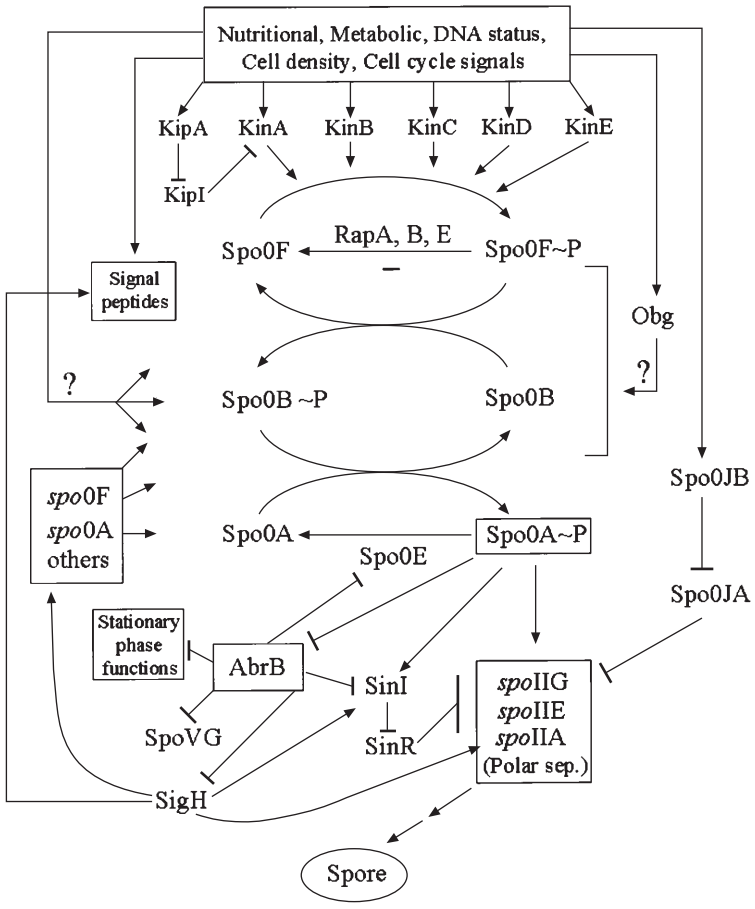

Figure 1. The Phosphorelay. The main interactions that occur within and associated with the phosphorelay are illustrated; for further explanation, see text. Arrows indicate activation, and barred lines indicate repression. Phosphorylated forms of proteins are indicated by ~P. Unknown regulatory mechanisms are indicated with a question mark.

rylating Spo0A under certain circumstances and thus bypass the Spo0F-Spo0B intermediaries [44].

Signals regulating the activity of the kinases may be relayed through other proteins as well as directly acting upon the kinases themselves. KinA activity is regulated by the KipA and KipI proteins (fig. 1). Expression of the *kipA* and *kipI* genes is subject to control by carbon and nitrogen sources [45]. Although KinA is a soluble protein in the cytoplasm and not membrane bound, its activity may be affected by the presence of certain types of fatty acids [46]. KinB is located in the cell membrane, and its activity is dependent upon another integral membrane protein (KbaA) and a lipoprotein (KapB) attached to the outer side of the membrane [47, 48]. There is some evidence that a signal related to one-carbon-unit metabolism (perhaps involving a methylation event) is an effector of the KinB-KbaA-KapB complex [49]. Although some of the kinase genes have been shown to be subject to transcriptional regulation, the significance of differential expression patterns is not entirely clear and does not seem to correlate with the times when the kinases are maximally active [44].

Presumably, the use of Spo0F and Spo0B proteins as intermediaries between the kinases and Spo0A is to provide additional modules upon which to impose controls regulating the phosphate flux through the pathway. At least three separate protein phosphatases (RapA, B and E) are capable of dephosphorylating Spo0F ~P [50]. The activities of the phosphatases themselves are regulated by small peptides that result from the proteolytic processing of larger precursors (Phr) that occurs during an import-export cycle. At least some of these peptides can be thought of as pheromones and quorum-sensing molecules providing information on local cell density and perhaps other environmental cues. A total of 11 members of the Rap family of phosphatases are present in *B. subtilis*. Seven of these are cotranscribed with Phr peptide precursors. Besides RapA, RapB and RapE, the only other phosphatase of this class to have its target tentatively identified is RapC, which is believed to dephosphorylate the ComA ~P competence regulator (see above). It is unclear whether each type of Phr signal peptide can be excreted into the medium as a free entity or whether some types remain attached, and are imported back, only to the

cell from whence they came. In at least one case (PhrA), an argument has been made that the temporal sequence of export, processing and import may provide a proscribed and finite time frame for information processing and decision making. Space limitations preclude a more detailed description of these fascinating regulators; luckily, two excellent, recent reviews cover this field of investigation [4, 7].

Although nutrient limitation is usually the precipitating event leading to sporulation initiation, the cell monitors the status of several metabolic and physiological conditions and adjusts the activity of the phosphorelay accordingly. Among the factors considered are cell density (sensed via the small peptide pheromones), status of DNA replication and DNA damage, and functionality of the Krebs TCA cycle. In many cases neither the exact signaling entity nor its impingement point on the phosphorelay is known [1, 8]. One metabolic change long known to be associated with the onset of sporulation is a drop in the intercellular GTP and GDP pools. However, the 'cause-and-effect' functional relationship between regulation of sporulation initiation and GTP/GDP pools is still not clear. One candidate for providing a link is Obg, an essential GTP-binding protein having homology to eukaryotic Ras proteins and whose gene is cotranscribed with *spo0B* [51]. In addition to (or perhaps instead of) a possible role in sporulation initiation, Obg is necessary for general stress responses activated by σ^B, an effect that may be mediated through ribosome components or functions [52].

If all available information channeled through the phosphorelay indicates that sporulation may be the preferred survival option, and as Spo0A ~P levels continue to increase, positive feedback loops begin to kick in [53, 54] that increase expression of the *spo0A* and *spo0F* operons. These loops involve both direct activation of transcription by Spo0A ~P and an indirect effect of Spo0A ~P upon the expression of the *spo0H* gene. The *spo0H* (*sigH*) gene encodes an RNA polymerase sigma factor (σ^H) that recognizes alternate promoters located upstream of *spo0A* and *spo0F*. The *phrC* and *phrE* genes encoding inhibitors of phosphatases acting on Spo0F ~P (see above) are also subject to σ^H control [4]. Transcription of *sigH* is negatively regulated by AbrB [16, 55] and thus Spo0A ~P repression of *abrB* (see above) leads to increased σ^H levels. The activity of σ^H may also be subject to posttranscriptional control by unknown mechanisms [56].

Like *sigH*, transcription of *spo0E* is subject to AbrB repression and thus also rises in response to Spo0A ~P repression of *abrB* [27, 57]. Spo0E is a phosphatase whose specific substrate is Spo0A ~P [58]. Why a phosphatase acting on Spo0A ~P should increase in concentration coincidentally with increasing levels of Spo0A ~P is still a conundrum. Perhaps an as yet undiscovered mechanism modulates Spo0E activity. Or perhaps it is a 'race' or timing mechanism to see whether Spo0A ~P accumulation (due to strong persistent signals activating the phosphorelay) can overwhelm the action of Spo0E.

Spo0A ~P is absolutely required for activation of the *spoIIA*, *spoIIG* and *spoIIE* operons, as well as unidentified targets necessary for proper asymmetrical septation (the function of the products encoded by these operons will be described in subsequent sections). Phosphorylation of Spo0A increases its affinity for binding DNA sites in the regulatory regions of its targets [30, 59]. Direct activation of transcription involves an interaction with RNA polymerase which appears to facilitate a step subsequent to closed-complex formation [60, 61]. Spo0A also has two important indirect means that it uses to increase expression of these targets. Both are the result of Spo0A ~P repression of *abrB*: increasing σ^H levels (*spoIIA* is σ^H-dependent) and decreasing SinR activity/repression at *spoIIA*, *spoIIG*, *spoIIE* (see above and fig. 1).

There is one final checkpoint that can short-circuit Spo0A ~P-dependent activation at these promoters, even subsequent to DNA binding. This checkpoint apparently provides a link ensuring that compartmentalization of gene expression is tied to proper partitioning of a chromosome into the developing forespore (see next section). The Spo0JA protein can specifically dissociate Spo0A ~P-RNAP initiation complexes [62]. Spo0JA activity is regulated by an interaction with the Spo0JB protein, the latter being a component of a mitotic-like partitioning complex responsible for proper chromosome segregation into the forespore compartment [63].

Commitment to sporulation: asymmetric septation and compartmentalization

Stage II of sporulation is defined by the formation of an asymmetric septum that divides the cell (now termed a sporangium) into unequally sized compartments: the forespore and the mother cell. It is at this time that commitment to sporulation occurs. Prior to this the cell was still able to revert to active growth or pursue an alternate stationary phase state had the appropriate environmental signals been received.

Polar septation begins before chromosome segregation is completed, temporarily trapping only a small portion (~30%) of a chromosome into the forespore compartment [64]. After a short period (~15 min) the remainder of the chromosome is pumped across the septum into the forespore via the DNA translocase activity of the SpoIIIE protein [63, 65]. The small portion of DNA that initially enters the forespore normally comprises the origin of replication and surrounding genes, including *spoIIIE*. The origin-proximal position of *spoIIIE* appears to be important for the function of its encoded protein [66]. Proper partitioning and positioning of the chromosome is

also dependent upon Spo0JB, as mentioned above. It has been reasoned that since the forespore temporarily has a reduced genetic complement, this brief genetic imbalance between the forespore and the mother cell plays an important role in establishing compartment specific gene expression [67].

The change from medial to polar septation depends upon genes expressed under Spo0A ~P and σ^H control. Initially, FtsZ rings form at both poles of the cell, an event that is Spo0A ~P dependent [68]. One of these is chosen (seemingly at random) to be the actual site of septation. One or more σ^H-dependant functions are then required for formation of the septum at this locale [68]. The non-chosen Z-ring persists at the opposite end of the sporangium (in what is the mother cell) until σ^E activity (see below) causes it to be dismantled [5]. Another Spo0A ~P-dependent gene, *spoIIE*, is required for efficient polar septation. SpoIIE is a phosphatase that has been observed to associate with FtsZ rings [69] but its actual mechanistic role in septation is not known.

Although the septum physically separates the sporangium into separate compartments, each destined for a different fate and subject to distinct patterns of gene expression, communication between compartments is not lost. In fact, for proper spore formation to occur, the left hand must know what the right hand is doing, as we shall see in the following section.

Compartment-specific gene expression: a cascade of sigma factors

Prior to completion of the polar septum, two RNA polymerase sigma factors (σ^F and σ^E) are synthesized in inactive forms and thus are present in both compartments of the sporangium. However, only in the forespore will σ^F become active and only in the mother cell will σ^E become active. Early forespore-specific gene expression directed by σ^F is necessary not only for the appearance of the late forespore-specific σ^G, but also for the activation of σ^E in the mother cell. Likewise, early-mother-cell-specific expression due to σ^E leads not only to the appearance of the late-mother-cell-specific σ^K, but is also required for activation of σ^G in the forespore, the latter then being necessary for activation of σ^K back in the mother cell (see below and fig. 2). This temporal cascade of sigma factors, subject to 'criss-cross' regulation [70] between cellular compartments, provides an excellent experimental system with which to study molecular communication mechanisms responsible for coordinate regulation of cell-type-specific gene expression occuring during a cellular differentiation process.

σ^F (*spoIIAC*) is the first sporulation-specific sigma factor activity to appear. It is upon σ^F that the entire cascade hinges. The regulator proteins SpoIIAA and SpoIIAB are synthesized along with σ^F prior to division of the sporangium, and σ^F is maintained in an inactive state by association with the SpoIIAB anti-sigma factor [71]. Once polar septation has taken place, the association between σ^F and SpoIIAB in the forespore is broken. However, the σ^F-SpoIIAB association is maintained in the mother cell after septation, thus ensuring that active σ^F is present only in the forespore. SpoIIAB acts in three ways to ensure σ^F inactivity in the mother cell: as an anti-sigma factor; as a kinase that phosphorylates SpoIIAA, rendering the latter inactive [72]; and by forming a persistent complex with dephosphorylated SpoIIAA, in an ADP-dependent reaction [73].

In the forespore, σ^F becomes active due to SpoIIAA counteracting the SpoIIAB effects. SpoIIAA in its active form is able to displace SpoIIAB from its complex with σ^F, thus forming a SpoIIAA:SpoIIAB complex and liberating free σ^F. The concentration of active SpoIIAA is dependent upon the kinase activity of SpoIIAB and the phosphatase activity of SpoIIE. Phosphorylated SpoIIAA cannot interact with SpoIIAB. Since SpoIIE is also synthesized in the predivisional cell, its phosphatase activity must be stalled until after septation and then only appear in the forespore. The mechanisms that delay SpoIIE activity and ensure it is only active in the forespore have been the subject of much speculation but have yet to be conclusively identified (see [5] for discussion). Two critical factors appear to be its localization to the septal membrane and the proper functioning of the SpoIIIE DNA translocase [74, 75].

σ^F directs transcription of *spoIIIG* and *spoIIR,* among others. The *spoIIIG* (*sigG*) gene encodes σ^G, which is responsible for late forespore gene expression (see below and fig. 2). SpoIIR is required for the appearance of σ^E activity in the mother cell. It appears to be secreted from the forespore into the cavity between the two membranes of the polar septum, where it activates the SpoIIGA protease [76]. SpoIIGA cleaves an inactive precursor protein, pro-σ^E, to release active σ^E. The exact mechanisms used to restrict pro-σ^E processing only to the mother cell are not entirely clear [66, 77] and may include a dependence upon de novo synthesis of certain fatty acids [78]. But it does seem clear that restriction of σ^E activity only to the mother cell depends upon the activity of the SpoIIIE DNA translocase [66] and does not require σ^F activity in the forespore.

In the forespore, activation of σ^G is dependent upon σ^E-directed expression (in the mother cell) of a complex of membrane proteins encoded by the *spoIIIA* operon. These proteins appear to change their membrane association as a function of the forespore engulfment process (stage III). Prior to engulfment they are associated with the mother cell membrane, but they become part of the forespore's outer membrane after it is engulfed [9]. The means by which these proteins release σ^G from inhibition have not

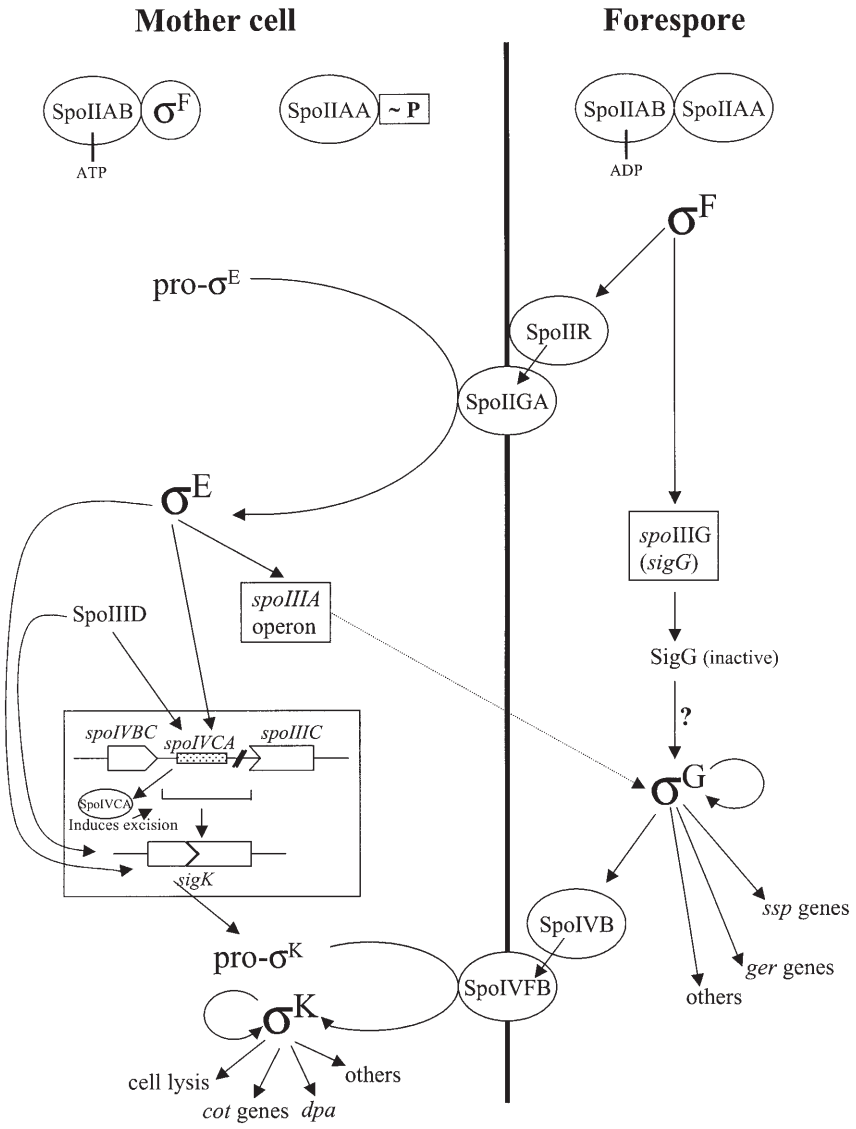

Figure 2. The mechanisms involved in the regulation of sigma factors in the compartmentalized sporulating cell. The symbol σ and appropriate letter indicates the sigma factors, and precursor proteins of these sigma factors are indicated as pro-σ and the appropriate letter. The status of sigma F (σ^F) in each compartment post septation is indicated at the top of the figure; for further explanation, see text. A vertical line represents the asymmetric septum, which divides the mother cell and the forespore. Arrows indicate activation. Circular arrows indicate autoregulation. Those proteins associated with the membrane are illustrated as embedded in the septum. Unknown regulatory mechanisms are indicated with a question mark.

yet been identified. It appears that the SpoIIAB antisigma factor inhibits σ^G activity [79], but how this is accomplished is quite mysterious, given the previous sequestering of SpoIIAB by SpoIIAA in the forespore (see above).

The final sigma factor to make an appearance during sporulation is σ^K, whose activity is restricted to the mother cell. An intact contiguous copy of the *sigK* gene is not normally present in a number of *B. subtilis* strains. In these strains, the *sigK* reading frame is interrupted by a 48-kbp element called *skin* [80]. Within the *skin* element is encoded the SpoIVCA site-specific recombinase protein that excises the *skin* sequences to generate an uninterrupted *sigK* gene. Transcription of *spoIVCA* and subsequent transcription of the intact *sigK* gene is directed by σ^E and a regulatory protein known as SpoIIID [81]. As is the case for *sigE* (*spoIIGB*), translation of *sigK* mRNA produces an inactive precursor protein, pro-σ^K. Processing of pro-σ^K requires a protease, SpoIVFB, located in the mother cell membrane. At least two proteins located in the mother cell (SpoIVFA and BofA) negatively regulate the activity of SpoIVFB [82, 83]. SpoIVB protein produced in the forespore (σ^G dependent) generates an unknown signal that reaches the SpoIVFB complex and overcomes the inhibitory effects of SpoIVFA and BofA [84, 85]. Recently, an additional regulator, BofC, has

been postulated to interact with SpoIVB in the forespore to ensure that SpoIVB activity (and thus, ultimately, pro-σ^K processing) is not inappropriately expressed at a premature time [85]. How BofC actually accomplishes this inhibition is not clear, nor is it clear how the inhibitory effects are reversed in response to temporal and spatial signals.

Once activated in their respective compartments, both σ^K and σ^G direct further transcription of their own genes in positive autoregulatory loops. These late-stage sporulation-specific transcription factors are then responsible for the expression of the gene products necessary for the final steps in assembly, maturation and release of the completed spore.

Concluding remarks

As a general rule, it is rare for a research or review article not to have near its end a paraphrasing of the sentiment 'while much has been learned, much remains to be learned'. This is usually accompanied by an optimistic statement concerning future progress in the field. This article will not provide an exception to the rule.

A final note: the literature reviewed for this submission covers the period up to early December 2000. We apologize to the many authors whose work could not be cited due to space limitations or whose recent work may have been over looked during our literature search.

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