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Cell Death Pathway that Monitors Spore Morphogenesis

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SUMMARY

The use of quality control mechanisms to stall developmental pathways or completely remove defective cells from a population is a widespread strategy to ensure the integrity of morphogenetic programs. Endospore formation (sporulation) is a well conserved microbial developmental strategy in the Firmicutes phylum wherein a progenitor cell that faces starvation differentiates to form a dormant spore. Despite the conservation of this strategy, it has been unclear what selective pressure maintains the fitness of this developmental program, composed of hundreds of unique genes, during multiple rounds of vegetative growth when sporulation is not required. Recently, a quality control pathway was discovered in *Bacillus subtilis* which monitors the assembly of the spore envelope and specifically eliminates, through cell lysis, sporulating cells that assemble the envelope incorrectly. Here, we review the use of checkpoints that govern the entry into sporulation in *B. subtilis* and discuss how the use of regulated cell death pathways during bacterial development may help maintain the fidelity of the sporulation program in the species.

Keywords

apoptosis; programmed cell death; SpoIVA; SpoVM; nuclear envelope; lamins; gametogenesis

Sporulation in Gram-positive bacteria

Sporulation (see Glossary) in Gram-positive bacteria is a developmental program in which a progenitor cell differentiates to form a metabolically dormant cell type termed an “endospore” (usually simply referred to as a “spore”) as a response to unfavorable growth conditions. This process has been extensively studied in the model organism *Bacillus subtilis*, where insights into diverse phenomena, such as differential gene expression, intracellular signaling, cellular decision making, asymmetric cell division, developmental checkpoints, subcellular protein localization, chromosome segregation, and membrane dynamics have been extracted (1–4). Spore formation is also an important component of the lifestyles of several pathogenic bacteria such as *Bacillus anthracis*, *Bacillus cereus*, *Clostridium difficile*, and *Clostridium botulinum* (5, 6). Entry into sporulation may be

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induced by a number of stress factors. For example, a number of species within the human intestinal microbiota, such as *Clostridium* and *Metabacteria polyspora*, are strict anaerobes and sporulate to facilitate survival and transmission outside of the intestine (5, 7). In *B. subtilis*, sporulation is triggered by nutrient deprivation.

In favorable growth conditions, *B. subtilis* divides by binary fission to produce two genetically identical and morphologically similar daughter cells that ultimately exhibit similar cell fates (production of more bacterial cells). However, upon nutrient depletion, *B. subtilis* initiates the sporulation pathway by first dividing asymmetrically to produce two genetically identical, but morphologically dissimilar progeny: a larger mother cell and a smaller forespore that will each undergo different fates (Fig. 1). Next, the mother cell swallows the forespore (a step termed “engulfment”), whereupon the now double membrane-encased forespore slowly matures into a spore inside the mother cell cytosol. During this maturation, the mother cell deposits an architecturally complex protein shell (the **coat**), composed of ~80 proteins atop the forespore surface (8). Subsequent to initiating coat assembly, a second shell, the **cortex**, made of specialized peptidoglycan, is constructed between the double membrane envelope encasing the forespore (9). Eventually, the forespore core is dehydrated, the mother cell lyses, and the mature spore is released into the environment. The genetic material inside the dormant spore is protected from environmental insults such as high heat, desiccation, radiation, and caustic chemicals. This resistance is mediated in part by the two concentric coat and cortex shells surrounding the core (10). The resumption of favorable growth conditions triggers the dormant spore to germinate, after which it rapidly initiates normal growth and cell division (11).

Sporulation in *B. subtilis* is a complex process that requires a large energy investment during a time when resources are scarce. The payoff for this investment is that the genetic material for the species may be carefully preserved until favorable growth conditions resume. However, despite the importance of the ~600 genes that are preferentially expressed during sporulation (12–14), these genes are not under any obvious selective pressure for maintenance during prolonged vegetative growth in favorable conditions (15). As such, long-term vegetative growth could result in the accumulation of mutations in sporulation genes that allow entry into sporulation, but result in the production of defective, albeit partially functional, spores. It is conceivable that permitting the gradual accumulation of such mutations could eventually compromise the integrity of sporulation such that the program may even gradually fail to be maintained in the species. Here, we review several early checkpoints that govern the entry of cells into the sporulation pathway that aim to monitor the quality of the genetic material. We also discuss a recently described late-acting mechanism that actively eliminates faulty spores to prevent such strains from taking over a population and corrupting the integrity of the sporulation program.

To sleep, perchance to dream: checkpoints that govern entry to sporulation

After elaboration of the polar septum, *B. subtilis* commits to complete the sporulation program, even if nutrients suddenly become available after this point. This commitment step is not simply linked to polar septum formation, but absolutely requires activation of σ^E in the mother cell, which occurs after activation of σ^F in the forespore (16, 17). Specifically,

activation of σ^E requires a cascade of feed-forward loops that robustly drives σ^E activation to ensure commitment. Achieving robust σ^E activation requires that at least one principal kinase that signals the onset of sporulation be activated beyond a threshold level (17).

Most sporulation checkpoints identified to date act soon after the initiation of sporulation, before the cell irreversibly commits to the pathway. These checkpoints monitor chromosome copy number and genomic integrity before permitting the cell to enter the sporulation program. Entry into sporulation only occurs at a specific time during the cell cycle, when the cell harbors exactly two replicated chromosomes but has not initiated a further round of DNA replication. This temporal regulation ensures that the mother cell and forespore each receive exactly one copy of the genetic material. A genetic selection strategy identified a previously unannotated gene, termed *sda* that participates in this process (18). Non-functional variants of Sda permitted mutant *B. subtilis* strains to sporulate that were otherwise restricted from entry into sporulation due to DNA replication defects. In contrast, overexpression of wild type *sda* inhibited entry into sporulation by preventing activation of a master regulator of sporulation (**Spo0A**). Later studies (19–21) showed that Sda binds directly to a critical histidine kinase that governs the activity of Spo0A, thereby resulting in decreased levels of phosphorylated Spo0A. Taken together, a model emerged in which Sda would prevent entry into sporulation if initiation of chromosome replication was impaired by DNA damage. However, it remained unclear how, in undamaged cells, sporulation would initiate only after the generation of precisely two copies of the chromosome. Veening *et al.* suggested that this phenomenon, too, was mediated by Sda (22). Phosphorylated Spo0A levels were shown to fluctuate throughout the cell cycle, where levels were lowest at the time of replication initiation. Interestingly, Sda levels also fluctuated during the cell cycle, but Sda levels were maximal at the time of replication. This stabilization of Sda was mediated by the active form of the DNA replication initiator protein, thereby connecting DNA replication to entry into sporulation.

Whereas Sda levels drop upon entry into sporulation, SirA, a highly conserved gene among endospore-forming species of *Bacillus* that has been implicated in the control of proper chromosomal ploidy, is expressed at the onset of sporulation (23). Consistent with such a role, deletion of *sirA* resulted in cells harboring more than two chromosomal origins, and *sirA* overexpression produced anucleated cells. In vegetative cells, induced expression of *sirA* disrupted DnaA localization to the replication initiation site, *oriC*, indicating that *sirA* abrogates chromosome replication by preventing DnaA binding to *oriC* (24). Interestingly, SirA did not itself localize to *oriC*, but instead co-localized with DnaA at the mid-cell replisome, suggesting that SirA prevents DnaA binding to *oriC* by tethering DnaA to the replisome and preventing rebinding at *oriC* (25). Taken together, SirA is instrumental in directly preventing additional chromosomal replication by inhibiting initiation, as well as ensuring that the replicated chromosome is properly positioned for successful chromosome segregation into the forespore.

Another early sporulation checkpoint involves monitoring the integrity of the genetic material prior to differentiating into a spore. This function is mediated by **DisA**, a sporulation-specific checkpoint protein that delays the initiation of sporulation in response to chromosomal damage (26). Deletion of *disA* greatly reduced the sporulation efficiency of

cells compared to wild type cells after exposure to mutagenic compounds that induced double strand breaks, but showed no effect on the sporulation of cells that were not similarly exposed, suggesting that DisA is active only in response to DNA damage. At the onset of sporulation, DisA scans along the chromosome as an octameric complex (27) and stalls upon sensing a lesion in the DNA. Within the DisA complex, Witte et al. identified three distinct domains, one of which possessed diadenylate-cyclase activity, which was later revealed to be required for DisA focus formation on the chromosome (28). c-di-AMP synthesis was repressed when DisA was stalled on DNA, thereby correlating low c-di-AMP levels in the cell to the presence of DNA damage (27, 28). A current model predicts that c-di-AMP acts as a secondary messenger to signal whether the cell should proceed with sporulation (28). While c-di-AMP targets in *B. subtilis* are currently unknown, low c-di-AMP levels in *Listeria monocytogenes* have been shown trigger solute uptake in response to osmotic stress (29), suggesting that low c-di-AMP levels in *B. subtilis* may facilitate additional nutrient uptake in an effort to delay entry into sporulation. Alternatively, c-di-GMP has been previously shown to bind to and activate proteins in *E. coli* (30) suggesting that the presence of high levels of c-di-AMP may similarly trigger entry into sporulation. Interestingly, DisA has also been implicated in scanning for DNA damage during *B. subtilis* spore germination and outgrowth, presumably to restore any oxidative damage that accrued while in the spore state (31).

Taking one for the team: rationale for destroying improperly forming spores

In the previously discussed checkpoints, each cell verifies that it meets a standard (proper ploidy or absence of gross DNA damage), and halts or slows the entry into sporulation until the problems are rectified. However, it has been unclear if mechanisms exist to rectify mistakes once a cell irreversibly commits to sporulate. In the absence of such mechanisms, two outcomes are possible: (i) the defective spore would be unable to withstand harsh environmental conditions and would be subsequently destroyed, thereby preventing the propagation of an unfavorable trait, or (ii) the spore would be marginally functional enough to survive, especially in the absence of a strong environmental selective pressure, after which the cell could germinate, multiply, and pass on faulty sporulation genes.

Possessing a checkpoint mechanism to monitor the progression of forespore maturation in individual cells would be advantageous for the successful maintenance of the sporulation program in the entire population. Without such a quality control mechanism, should a cell acquire a mutation that permits sporulation, but at the expense of overall spore quality, it is conceivable that the sporulation-defective strain could ultimately overtake the population. For instance, consider a starting population of 10^9 cells, of which half are wild type and the other half lack sporulation checkpoint pathways but are otherwise wild type. Under normal conditions, the population growth of the bacteria can be modeled at early time points with the Malthusian equation:

$$x(t) = x_0 e^{rt} \quad (1)$$

where x_0 is the initial population, r is the growth rate (*B. subtilis* has a doubling time of approximately 30 minutes), and t is the number of hours in log phase (under optimal conditions, *B. subtilis* grows exponentially for approximately 5 hours). We assume that 75% of cells enter the sporulation pathway (32) and those that do not sporulate die of starvation. We also impose on this model that the produced spores are not subject to additional stringent environmental selection such as excessive temperature fluctuations or exposure to harsh chemicals, likely similar to routine physiological circumstances where lack of nutrients is the major environmental pressure. We next suppose that 99% of wild type cells successfully complete the sporulation program (the remaining 1% are eliminated from the population by quality control mechanisms for making a defective spore). In contrast, we suppose that 100% of mutants that lack checkpoints successfully form spores. Consistent with this supposition, several studies (18, 33) have indeed reported that some sporulation-defective mutants can be rescued by removing checkpoints, demonstrating that mutations in sporulation genes per se may not result in a reduction in sporulation efficiency; rather, the inability to sporulate is due to checkpoint mechanisms that prevent progression along the sporulation pathway.

We next impose that both populations develop mutations within sporulation genes that would normally result in detection and elimination by the checkpoint mechanisms at the rate of 3 in 1000 cells (34). As a result, sporulation-defective cells are removed from the population, while checkpoint-less cells harboring mutations in sporulation genes remain and ultimately result in the creation of marginally functional spores that can survive nutrient deprivation, but may be defective under harsher environmental conditions. Vegetative growth, sporulation, germination, and regrowth of three sub-populations of cells (WT, checkpoint-less cells, and checkpoint-less cells that acquire mutations in sporulation genes) can be modeled using the following series of equations (*veg*: vegetative cells; *spo*: sporulating cells; *WT*: wild type cells; *check*: cells lacking checkpoints; *mutant*: sporulation mutant; *C*: constant):

$$x_{veg,WT} = (x_{initial,WT}) e^{rt} - C_{mutation} (x_{initial,WT}) e^{rt} \quad (2)$$

$$x_{veg,check} = (x_{initial,check}) e^{rt} - C_{mutation} (x_{initial,check}) e^{rt} \quad (3)$$

$$x_{veg,check,mutant} = (x_{initial,check,mutant}) e^{rt} + C_{mutation} (x_{initial,check}) e^{rt} \quad (4)$$

$$x_{spo,WT} = 0.75 (x_{veg,WT}) \quad (5)$$

$$x_{spo,check}=0.75(x_{veg,check}) \quad (6)$$

$$x_{spo,check,mutant}=0.75(x_{veg,check,mutant}) \quad (7)$$

$$x_{initial,WT}=0.99x_{spo,WT} \quad (8)$$

$$x_{initial,check}=1.00x_{spo,check} \quad (9)$$

$$x_{initial,check,mutant}=1.00x_{spo,check,mutant} \quad (10)$$

$$P_{WT} = \frac{x_{initial,WT}}{x_{initial,WT} + x_{initial,check} + x_{initial,check,mutant}} \times 100 \quad (11)$$

$$P_{check} = \frac{x_{initial,check}}{x_{initial,WT} + x_{initial,check} + x_{initial,check,mutant}} \times 100 \quad (12)$$

$$P_{check,mutant} = \frac{x_{initial,check,mutant}}{x_{initial,WT} + x_{initial,check} + x_{initial,check,mutant}} \times 100 \quad (13)$$

For simplicity, this equation set assumes that 100% of the released spores will successfully germinate and begin vegetative growth; therefore, each subsequent cycle has an initial starting number of cells equivalent to the number of completed spores from the cycle before. This model defines a cycle as 5 hours of exponential growth followed by synchronized induction of sporulation and a sufficient time of nutrient deprivation such that any non-sporulated cells would die. The results of the simulation in Fig. 2 shows that wild type cells (blue) were rapidly outcompeted by checkpoint-less cells (red), which took over the population after 103 cycles. However, the fraction of the third subpopulation, checkpoint-less cells that had accumulated sporulation defects (green), steadily rose, such that the fraction of checkpoint-less cells (which were otherwise wild type) began to decline after briefly reaching a peak at cycle 103. By cycle 232, checkpoint-less mutant cells made up the majority of the population and, by 900–1000 cycles, eventually took over the population. Thus, under environmental conditions that only impose a lack of nutrients, checkpoint-less

mutants that produce inferior spores could thrive and out-compete cells that actively prevent or eliminate such mutations from accumulating. It is then conceivable that the further accumulation of mutations may even ultimately result in the failure to retain the sporulation program. A quality control mechanism capable of eliminating incorrectly sporulating cells on an individual basis would therefore be one mechanism by which the integrity of the sporulation program could be protected and maintained within the population.

Destroying the wall brick by brick: regulated proteolysis drives regulated cell death

One such quality control pathway to eliminate cells that incorrectly assembled the spore coat was recently discovered. In contrast to the early-acting sporulation checkpoint pathways described above, this pathway monitors the assembly of the spore envelope, which occurs late in the sporulation program. In *B. subtilis*, the basement layer of the spore coat contains **SpoVM**, a 26 aa protein (35) that preferentially localizes to the positively curved membrane surrounding the forespore to mark it as the site for coat assembly (36–38); and **SpoIVA**, an unusual ATPase derived from a family of GTPases which harnesses the energy from ATP hydrolysis to drive its irreversible polymerization into a static platform atop which the rest of the spore coat assembles (39–42). Mutations in *spoVM* or *spoIVA*, but not the ~80 other coat proteins (43–45), result not only in defective coat formation but also failure to initiate cortex assembly, suggesting that the assemblies of the coat and cortex are somehow coordinated by SpoVM and SpoIVA (46, 47).

To study this phenomenon, Ebmeier *et al.* first isolated an allele of *spoVM* that permitted initiation of coat assembly, but abrogated cortex assembly (48). Strains harboring this allele largely failed to produce heat-resistant spores, but a genetic selection revealed a heat-resistant extragenic loss-of-function suppressor mutation in a small unannotated open reading frame encoding a 37 aa protein that was termed **CmpA**. The *cmpA* gene is controlled by the same mother cell-specific sporulation sigma factor (σ^E) that drives expression of *spoVM* and *spoIVA*, and also by a slightly later-acting transcription factor (SigIID) which likely ensures a slightly delayed expression of *cmpA* relative to *spoVM* and *spoIVA* (49). The *cmpA* gene is widely conserved in spore-forming species of the *Bacillales* order, but not found in *Clostridiales*. Whereas deletion of *cmpA* restored sporulation in several sporulation-defective strains, overproduction of CmpA in an otherwise wild type strain resulted in lysis of sporulating cells (50), and therefore a sporulation defect, indicating that CmpA plays an inhibitory role during sporulation. CmpA-GFP could be detected surrounding the forespores of wild type cells only at an early time point during sporulation; at a later time point at which cells had successfully elaborated phase-bright forespores (coincident with dehydration of the forespore core), CmpA-GFP could no longer be detected. However, in mutant cells that harbored a coat assembly defect, CmpA-GFP persisted around phase-bright forespores, suggesting that CmpA is selectively maintained in cells with a sporulation defect and eventually eliminated in successfully sporulating cells (48).

To elucidate the function of CmpA, Tan *et al.* employed a genetic strategy to select for additional suppressor mutants that would correct the sporulation defect in a strain harboring the same *spoVM* allele that yielded the suppressor in *cmpA* (50). This yielded two mutations in *spoIVA*, suggesting that CmpA participates in a pathway with the two principal components of the spore coat basement layer (SpoVM and SpoIVA). Next, Tan *et al.* isolated suppressor mutants that corrected the lysis and sporulation defect in a CmpA-overproducing strain. This yielded three mutations in the *clpX* gene, which encodes the ATPase subunit of the ClpXP protease (51, 52), and one mutation in *clpP*, which encodes the proteolytic subunit of ClpXP. All three *clpX* mutations resulted in amino acid substitutions which reduced the function of the N- domain of ClpX, which had been implicated in substrate binding (53), suggesting that these mutants were no longer able to recognize and degrade a particular substrate. The *clpP* mutation resulted in an amino acid substitution near the region that interacts with ClpX, likely reducing the interaction between ClpX and ClpP (54). Taken together, a model was proposed in which CmpA could be a ClpXP adaptor protein that delivers SpoIVA for degradation specifically in cells that harbor a coat assembly defect (Fig. 3, Key Figure).

Consistent with this model, co-purification experiments demonstrated that SpoIVA, ClpX, and CmpA could exist in a complex in a CmpA-dependent manner. In contrast, N-domain variants of ClpX that suppressed the CmpA overproduction defect failed to interact with CmpA (and, by extension, SpoIVA). Similarly, SpoIVA variants that suppressed the *spoVM* defect failed to co-purify with CmpA and ClpX. Examination of SpoIVA stability in vivo demonstrated that, in wild type cells, SpoIVA was largely stable over time. However, in cells harboring a coat assembly defect, SpoIVA was rapidly degraded in a CmpA-dependent manner. The suppressor mutants were therefore able to overcome CmpA-mediated degradation of SpoIVA by either acquiring a mutation in *spoIVA* that rendered SpoIVA largely resistant to degradation or by acquiring mutations in *clpX* that produced a faulty N-domain in ClpX that failed to recognize the CmpA adaptor.

What mediates the degradation of SpoIVA specifically in cells that exhibit a coat assembly defect? SpoIVA, CmpA, and ClpXP are all present at the same time in the mother cell after asymmetric division, but SpoIVA degradation does not occur until after the cells have reached a phase-bright forespore state approximately 2 hours later. Indeed, co-production of ClpX, CmpA, and SpoIVA in vegetative cells did not result in SpoIVA degradation, suggesting that this pathway requires an unidentified factor or factors that mediate SpoIVA degradation. As the degradation is delayed until the cells reach phase-brightness, it is likely that the missing factor is under the control of the final sigma factor that is activated during sporulation, since deletion of this sigma factor suppressed the degradation of GFP-SpoIVA in mutant cells. It is likely that identification of this factor or factors will reveal the mechanistic basis for how the assembly of the coat is monitored and how the cell may make the decision to either finish the sporulation program or destabilize the spore coat and lyse.

Taken together, the CmpA pathway appears to participate in a spore quality control pathway that recognizes the occurrence of a coat assembly defect and prevents the progression of the defective spore to completion by degrading SpoIVA by ClpXP, thereby destabilizing the spore coat and cortex, leading to cell lysis and removal of the defective cell from the

population. We propose that this prevents the propagation of defective sporulation genes and ensures the maintenance of the sporulation pathway in a population.

Concluding remarks

Apoptosis, the controlled death of particular cells that occurs normally during development to benefit the organism as a whole, is a hallmark of multicellular organisms (55). Indeed, the lysis of the mother cell at the end of sporulation represents an example of programmed cell death in a simple multicellular organism (the sporangium, consisting of just two cells: the mother cell and forespore). The existence of such altruistic examples of regulated cell death in independently growing bacteria and single-celled eukaryotes, though, has been controversial (56–58), especially since manifestations of this phenomenon are usually reported as a stress response of cells to an environmental insult rather than as a normal part of the cell cycle (59–61). A recent example of programmed cell death identified in the unicellular yeast *Saccharomyces cerevisiae*, though, suggested that during gametogenesis (also referred to as sporulation) aborted meiotic products that naturally arise are actively destroyed in a fashion that exhibits several key features of eukaryotic apoptosis, including genome fragmentation, in an effort to preserve the integrity of the germline (62). This suggested that, as opposed to a regulated cell death event that may occur only in the face of selective pressure, a unicellular organism may employ a version of programmed cell death during the normal course of a developmental program, in a fashion that is conceptually similar to the discarding of unfit sporangia during *B. subtilis* sporulation by the CmpA pathway. Another phenomenon with similarities to the CmpA pathway occurs in plant cells of *Arabidopsis*, which specifically mark damaged chloroplasts (a vestige of bacteria within plant cells) for destruction (63). In this scenario, chloroplasts that overproduce reactive oxygen species are selectively targeted by ubiquitination for degradation. Similar to the spore monitoring pathway in *Bacillus*, regulated proteolysis leads to destabilization of an organelle to preserve the stability of the population of cells as a whole.

On a more molecular level, the CmpA pathway may reveal mechanisms by which the proper assembly of micron-sized supramolecular structures may be monitored by nanometer-sized molecules during development. For example, in mammalian cells, nuclear lamins are static intermediate filaments that maintain the structural integrity of the nuclear envelope (analogous to the static polymers formed by SpoIVA during sporulation that protect the integrity of the developing forespore). Interestingly, incorrect assembly of nuclear lamins is a trigger that induces apoptosis (64), suggesting that a mechanism must also exist in eukaryotes that monitors the morphogenesis of static structural features. Ultimately, a combination of genetic and biochemical approaches may reveal the full repertoire of factors in the CmpA pathway and may reveal how cells monitor the assembly of large structures and mount a cellular response in the face of incorrectly assembled structures (see Outstanding Questions).

Outstanding Questions Box

- What other factors are involved in the CmpA pathway? How is the improper assembly of the basement layer recognized at the molecular level? How is the CmpA pathway turned off in properly sporulating cells?
- In *B. subtilis*, cortex assembly initiates only after initiation of coat morphogenesis. How are the assemblies of both spatially separated structures linked? How does degradation of SpoIVA lead to spore instability and lysis?
- *Clostridiales* do not harbor a CmpA ortholog. Do they harbor a similar spore quality control system that monitors spore envelope assembly?

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Glossary

ClpX	ATPase subunit of the ClpXP protease that unfolds substrates and delivers it for proteolysis by ClpP. Some substrates require a specific adaptor protein to bind to and be recognized by ClpX
ClpP	Proteolytic subunit of the ClpXP protease that is widely conserved in bacteria and mitochondria and plays a major role in the degradation of misfolded proteins
CmpA	Cortex morphogenic protein A is a small protein (37 amino acids) that is an adaptor that binds to improperly polymerizing SpoIVA and delivers it for degradation by the ClpXP protease
Cortex	The cortex is a peptidoglycan shell built between the two membranes of the forespore, underneath the proteinaceous coat. This layer expands and contracts in response to temperature fluctuations to control the water content of the interior core. This integrity of this shell is linked to heat resistance of spores
Coat	The outermost surface of the <i>B. subtilis</i> spore. The coat is an architecturally complex protein shell that surrounds the mature spore and is composed of approximately 80 proteins. The coat acts as a shield to protect the spore core from degradative enzymes and toxic molecules
DisA	DNA integrity scanning protein A acts as part of a DNA-damage checkpoint just after the initiation of sporulation.

DisA forms octameric foci that rapidly scans the chromosome and pauses at double-stranded breaks, thereby temporarily stalling sporulation until the DNA is repaired

Sda Sporulation inhibitor, or suppressor of DnaA, inhibits the phosphorylation of Spo0A, response to DNA replication defects. Its function is to ensure that the cell does not begin to asymmetrically divide until there are precisely two replicated chromosomes

SirA Sporulation inhibitor of replication A, inhibits DnaA binding to *oriC*, thereby preventing chromosomal replication during active sporulation and maintaining the proper ploidy for both the mother cell and forespore

Sporulation In this article, refers to “endospore formation”, an alternative life cycle found exclusively in the Firmicutes phylum, wherein a cell undergoes a massive physiological change to differentiate into a metabolically dormant cell type (an endospore, or simple a “spore”). In *B. subtilis* sporulation is triggered by nutrient deprivation. Upon the return of favorable growth conditions, the spore germinates and grows normally and divides by binary fission

Spo0A Stage 0 sporulation protein A is a transcription factor that serves as a master regulator that drives the expression of early sporulation genes. As such, it is a major target for regulatory proteins that mediate entry into the sporulation program

SpoIVA Stage IV sporulation protein A is the major structural protein in the basement layer of the spore coat. It anchored on the forespore surface by SpoVM and self-polymerizes in an ATP hydrolysis-dependent manner to form a platform around the forespore, atop which the coat assembles

SpoVM Stage V sporulation protein M is a small protein (26 amino acids) that marks the surface of the developing forespore during engulfment as the site for coat assembly. It recognizes and binds preferentially to the positively curved (convex) membranes, such as that surrounding the forespore

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TRENDS BOX

- Sporulation is a bacterial developmental pathway that is maintained in Firmicutes despite the lack of obvious selective pressure during vegetative growth.
- The CmpA pathway is a quality control mechanism that acts late in the sporulation pathway in *Bacillus subtilis* to monitor the proper assembly of the spore envelope.
- Improperly formed spores are actively removed from a population of sporulating bacteria by the CmpA pathway to ensure the integrity and maintenance sporulation in the species.

Figure 1. Vegetative growth and sporulation in *B. subtilis*

Left (green): During the vegetative life cycle, cells divide symmetrically, resulting in two genetically identical and morphologically similar daughter cells. Right (blue): When faced with nutrient starvation, *B. subtilis* sporulates to produce a dormant spore. To this end, sporulating cells first divide asymmetrically to create two genetically identical but morphologically different cells; the large mother cell (MC) and the smaller forespore (FS). The forespore is then engulfed by the mother cell to create a double-membrane bound organelle-like structure. Next, the proteinaceous spore coat (red) is constructed atop the forespore, after which the peptidoglycan cortex (dashed gray) is built between the double membranes. Once the spore is fully matured, the mother cell lyses and releases the completed spore into the environment. Membranes: yellow; peptidoglycan cell wall: solid gray.

Figure 2. Mutant cells without a checkpoint mechanism accumulate mutations and eventually take over the population

The system was modeled in Python Spyder (v. 2.7) using equations 2–13 as described in the text. The simulation was initiated with 10^9 cells, half of which were wild type (blue) and the other half consisting of otherwise wild type cells that did not harbor checkpoints to monitor the progress of sporulation (red). Both cell types accumulated mutations in sporulation genes at a rate of .003. A third population, checkpoint-less cells that accumulated mutations in sporulation genes (green) arose from the checkpoint-less population (red) and, in the absence of additional environmental insults, ultimately took over the population.

Figure 3. CmpA mediates a quality control mechanism that monitors proper spore coat assembly

Top: SpoVM (red), SpoIVA (green), and CmpA (purple) localize to the surface of the forespore (orange double membranes). Left: in a single cell shown in the population, SpoIVA misassembles the basement layer of the coat (due, for example, to a mutation in *spoIVA*). Middle: in the cell harboring a mis-assembled coat, CmpA, the ClpXP adaptor protein, is stabilized and leads to the ClpXP-mediated degradation of SpoIVA. Right, in a cell in which SpoIVA properly assembles and polymerizes, CmpA is degraded. Bottom: Cells that correctly assembled the spore envelope complete the sporulation program to produce dormant spores, whereas cells in which SpoIVA was degraded are removed from the population by lysis. Adapted from (50).