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Spore germination

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The germination of dormant spores of *Bacillus* species is the first crucial step in the return of spores to vegetative growth, and is induced by nutrients and a variety of non-nutrient agents. Nutrient germinants bind to receptors in the spore's inner membrane and this interaction triggers the release of the spore core's huge depot of dipicolinic acid and cations, and replacement of these components by water. These latter events trigger the hydrolysis of the spore's peptidoglycan cortex by either of two redundant enzymes in *B. subtilis*, and completion of cortex hydrolysis and subsequent germ cell wall expansion allows full spore core hydration and resumption of spore metabolism and macromolecular synthesis.

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Abbreviations

AGFK	mixture of asparagine, glucose, fructose and K^+
DPA	dipicolinic acid
MPa	megaPascals

Introduction

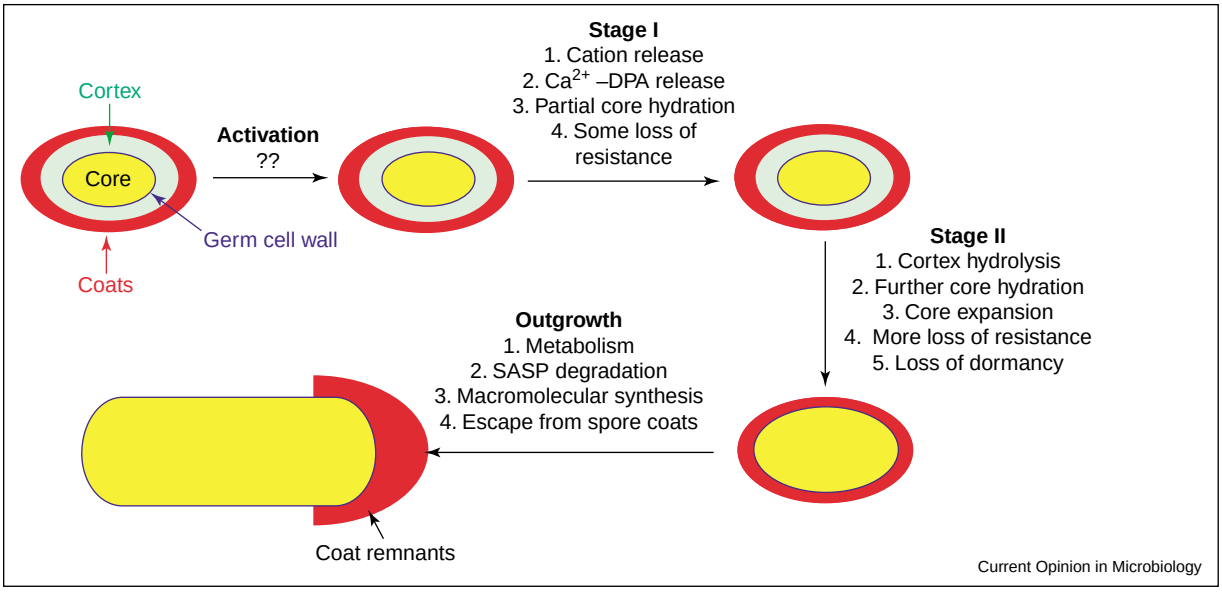
Spores of *Bacillus* species are formed in sporulation, a process triggered by starvation. These spores are metabolically dormant and resistant to heat, radiation, desiccation, pH extremes and toxic chemicals [1]. The dormant spore also monitors its environment, and when conditions are again favorable for growth, the spore germinates and goes through outgrowth, ultimately being converted back into a growing cell [2]. In addition to its intrinsic interest, spore germination has attracted applied interest, as it is through germination that spores ultimately cause food spoilage and foodborne disease. Similarly, it is via germination in lung macrophage that spores of *Bacillus anthracis* cause pulmonary anthrax [3].

Spores in nature germinate probably only in response to nutrients, termed germinants. These germinants are generally single amino acids, sugars or purine nucleosides, but there are also combinations of nutrients that trigger spore germination, one being a mixture of asparagine, glucose, fructose and K^+ (AGFK) that triggers *B. subtilis* spore germination [2]. Within seconds of mixing spores and germinants, the spore becomes committed to germinate, and germination will proceed even after removal of the germinant. However, the mechanism of commitment is not known. Subsequent steps in germination, in the order they take place [2] (Figure 1), are: first, release of spore H^+, monovalent cations and Zn^{2+}, probably from the spore core (release of H^+ elevates the core pH from ~6.5 to 7.7, a change essential for spore metabolism once spore core hydration levels are high enough for enzyme action [4]); second, release of the spore core's large depot (~10% of spore dry wt) of pyridine-2, 6-dicarboxylic acid (dipicolinic acid [DPA]) and its associated divalent cations, predominantly Ca^{2+}; third, replacement of DPA by water, resulting in an increase in core hydration and causing some decrease in spore wet-heat resistance (although this initial increase in core hydration is not sufficient for protein mobility or enzyme action in the spore core [5,6]); fourth, hydrolysis of the spore's peptidoglycan spore cortex; and fifth, swelling of the spore core through further water uptake and expansion of the germ cell wall [5]. Only after this further increase in core hydration does protein mobility in the core return, thus allowing enzyme action [5,6]. These events take place without detectable energy metabolism, and comprise the process of germination [2]. This process is divided into two stages (Figure 1), stage I comprises the first three steps of the process, and stage II comprises the fourth and fifth steps. The two stages can be separated experimentally by either chemical treatments or mutations [2,5,7]. The initiation of enzyme action in the spore core after completion of stage II allows initiation of spore metabolism, followed by the macromolecular synthesis that converts the germinated spore into a growing cell [2]. This period is termed spore outgrowth and will not be considered in this review.

In addition to nutrients, spores are germinated by other agents, including lysozyme, salts, high pressures, Ca^{2+}–DPA and cationic surfactants such as dodecylamine [8]. Although these 'non-nutrient' agents are probably not physiological, they use several of the components of the nutrient germination pathway. In addition, it is possible that Ca^{2+}–DPA released from one spore may stimulate the germination of other neighboring spores.

In this review, I will concentrate on recent work on spore germination. This work has mainly been carried out with *B. subtilis*, and results are with this organism, unless noted otherwise. Recent work has focused on three major

Figure 1



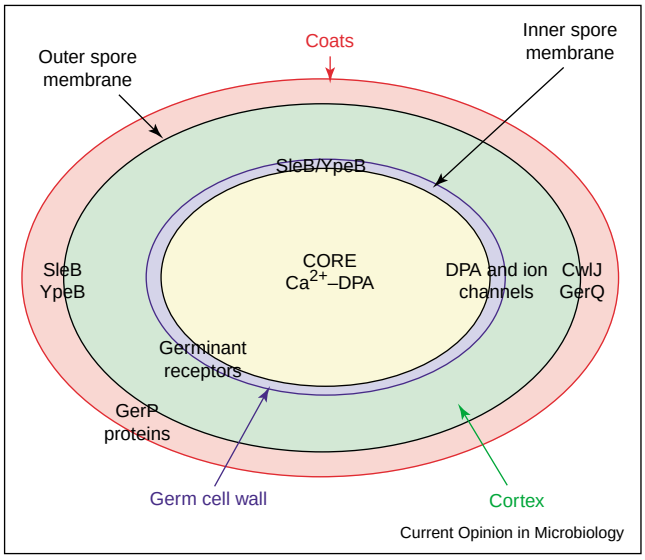
Events in spore germination. The process of spore activation can potentiate and thus synchronize the germination of spores of some species. A common activation treatment is a sub-lethal heat shock, but the mechanism of spore activation is not well understood [2], hence the question marks. Spore germination is divided into two stages, as cortex hydrolysis is not required for stage I. SASP degradation denotes the hydrolysis of the large depot of small, acid-soluble spore proteins (SASP) that make up 10–20% of the protein in the spore core. One type of SASP, the α/β-type, saturates spore DNA and prevents many types of DNA damage [1]. Whereas metabolism and SASP degradation (which require enzyme action in the spore core) are shown as taking place only after stage II is complete, these events may begin partway through stage II when the core water content has risen sufficiently for enzyme action. Also shown in this figure is that the spore's germ cell wall must expand significantly to complete stage II of germination. This figure is adapted from Figure 1 in reference [2], and does not show the spore's inner and outer membranes (see Figure 2). The events in stage I may take only seconds for an individual spore, although there may be a lag of several minutes after addition of a germinant before these events begin, and spore cortex degradation may take several minutes for an individual spore. However, because of significant variation between individual spores, particularly in the times for the initiation of the first events in stage I after addition of a germinant, these events may take many minutes for a spore population.

components of the germination apparatus (Figure 2): the receptors that respond to nutrient germinants; the channels that allow ion and DPA movement out of the spore; and the lytic enzymes that degrade the spore cortex. There has also been progress in understanding germination by non-nutrient agents, and all of this knowledge has provided new insight into how the various components of the germination apparatus interact and function together.

Germinant receptors

The action of nutrient germinants is initiated by binding to receptors located in the spore's inner membrane (Figure 2). In *B. subtilis* these receptors are encoded by the homologous tricistronic *gerA*, *gerB* and *gerK* operons (termed *gerA* operon homologs) expressed in the forespore late in sporulation [2,9]. Similar GerA-type proteins are present in spores of other *Bacillus* and *Clostridium* species [2,9]. In *B. subtilis* spores, the GerA receptor recognizes L-alanine, whereas the GerB and GerK receptors are required together for germination with AGFK. *B. subtilis* also has two additional *gerA* operon homologs of unknown function [10]. The evidence that the *gerA* operon homologs encode the spore's germinant receptors includes the isolation of mutations in *B. subtilis* that allow spores to germinate in D-alanine [2,9,11]. These mutations are in the *gerBA* or *gerBB* cistrons (termed *gerB** mutations), and analysis of strains carrying both the *gerB** and *gerB* alleles provided strong evidence that GerBA and GerBB physically interact, as has been suggested for the proteins encoded by other *gerA* operon homologs. The proteins encoded by these operons are probably membrane proteins, and at least GerAA, GerAC and GerBA are in the spore's inner membrane (Figure 2) [12,13]. The proteins encoded by the *B* cistrons of the *gerA* operon homologs also show limited sequence similarity to the superfamily of amino acid/polyamine/organocation transporters [14]. Taking all of these data into account, the simplest model is that each germinant receptor is composed of the three proteins encoded by each *gerA* operon homolog, with this protein complex being located in the spore's inner membrane. However, the topology of these proteins in the membrane, the stoichiometry of proteins in the receptor, and the possible physical interaction between different germinant receptors and with additional proteins is not clear (see below).

Figure 2



Spore structure and locations of components of the spore germination apparatus. The sizes of the various spore layers are not drawn to scale, and the outermost spore layer, the exosporium, is not shown, as it has no known role in germination. Note that the outer spore membrane may not be a good permeability barrier in dormant spores, unlike the inner spore membrane [2]. The location of the spore's nutrient germinant receptors is shown in the inner spore membrane [12,13]. The cortex-lytic enzyme CwlJ is shown in the spore coat layer adjacent to the spore cortex on which it acts during germination, even though the precise location of CwlJ in the coats is not known. CwlJ is shown adjacent to GerQ (originally YwdL), also a coat protein and needed for the presence of CwlJ in spore coats [31•,35,36•]. The cortex-lytic enzyme SleB and the YpeB protein needed for the appearance of SleB in spores are shown in both the inner spore membrane and at the cortex/coat boundary, although the location of these proteins in the spore's outer layers has not been precisely determined [31•]. Whereas CwlJ is shown to be adjacent to GerQ, and SleB adjacent to YpeB, there is no direct evidence that these protein pairs physically interact, although GerQ is essential for CwlJ's presence in the coats and YpeB is needed for the presence of SleB [31•,36•]. Note that SleB and YpeB are also shown to be adjacent to each other in the spore's inner membrane. The GerP proteins, at least some of which may be important in allowing nutrients to access the germinant receptors, are shown in the spore coats. The presence of DPA and ion channels in the inner spore membrane is also noted, although the components of these channels have not yet been definitively identified.

B. subtilis spores lacking all germinant receptors do not germinate with nutrients [10]. However, these mutant spores exhibit a slow constant rate of spontaneous germination, the mechanism of which is not understood. These 'receptorless' spores do, however, germinate normally with lysozyme, Ca^{2+}–DPA and dodecylamine [10,15].

Major unknown aspects of the germinant receptors are the proteins these receptors interact with, including other germinant receptors. This is of particular interest, as some germinants, for example AGFK in *B. subtilis*, require participation of two different germinant receptors (GerB and GerK) [2,9]. This is also the case for some germinants of *B. cereus* and *B. anthracis* spores [16–19]. However, at least the GerB* receptor can function alone to cause germination in D-alanine [11,20•]. Overexpression of the GerA or GerB* receptors 20- to 200-fold increased rates of spore germination considerably with L-alanine or D-alanine, respectively, but the increases were not commensurate with the level of receptor expression [20•]. Overexpression of the GerB or GerK receptor or both receptors also did not increase the rate of spore germination with AGFK, and overexpression of either the GerA or GerK receptors significantly inhibited spore germination with D-alanine and the GerB* receptor [20•]. These findings indicate that germinant receptors can interact directly either with each other and/or with additional components needed for spore germination, with likely candidates for the latter being proteins involved in DPA and cation movement. Although the identities of proteins that interact with the germinant receptors are not known, at least one other type of bacterial membrane receptor physically interacts with other receptors, forming complexes that function 'collaboratively' [21,22].

In addition to the *gerA* operon homologs, there are several other genes whose products play roles in *B. subtilis* spore germination [2]. These include: *gerF*, whose product adds diacylglycerol to membrane proteins, probably including the proteins encoded by the *B* cistrons of the *gerA* operon homologs; *gerC*, which encodes an enzyme of menaquinone biosynthesis; and *gerD*, whose product is needed in some unknown fashion for nutrient germination.

Ion/DPA channels

The release of cations (the question of parallel anion release is not resolved), followed by release of DPA and associated divalent cations early in spore germination suggests that one or more channels for these ions must be opened in the inner spore membrane upon binding of a germinant to its receptor. Similarly, during sporulation, there must be a mechanism for the uptake of DPA into the forespore from its site of synthesis in the mother cell compartment. DPA uptake into the forespore probably requires energy, given that extremely high concentrations of DPA are accumulated, but there is no obvious need for energy in the release of DPA and cations in germination. Unfortunately, the proteins involved in these ion movements are not known. Recent work [23] confirmed an earlier suggestion [24] that proteins encoded by the *spoVA* operon are involved in DPA uptake in sporulation. However, it is not clear if these same proteins are involved in DPA efflux during spore germination.

A Na^+/H^+-K^+ antiporter termed GerN has been identified in *B. cereus* as a possible participant in cation movement during spore germination [25,26]. The *gerN* gene is a homolog of a *B. megaterium* gene termed *grmA* mutation of which blocks spore germination in nutrients [27]. Mutation of *B. cereus gerN* does not affect growth or sporulation

but significantly reduces spore germination with inosine, although less so with L-alanine and not at all with Ca^{2+}–DPA. However, mutation of possible *B. subtilis gerN* homologs has no effect on spore germination [25]. Consequently, the role of GerN in spore germination is not clear, and perhaps this protein functions in spore formation and not in spore germination.

Additional proteins that may play some role in movement of small molecules during spore germination are those encoded by the *gerP* locus [28]. This hexacistronic operon is transcribed in the mother cell compartment at approximately the time of DPA synthesis. In *B. cereus* and *B. subtilis*, mutations in several *gerP* cistrons give spores that are defective in nutrient germination. This defect is suppressed by removal of spore coats, and in *B. subtilis* by a mutation that disrupts spore coat assembly. It has been suggested that GerP proteins are required for some aspect of spore coat structure that is crucial in allowing nutrients to access the germinant receptors. However, the specific role of the GerP proteins in this process is unclear.

Another small molecule that exhibits significant movement in spore germination, but in the opposite direction of ions and DPA, is water. The *B. subtilis* spore core volume increases 2- to 2.5-fold through stage II of germination, and does so by water uptake [2]. However, the mechanism for this water uptake is not known, and *B. subtilis* has no homologs of the aquaporins found in other organisms, save for GlpF, the facilitator for glycerol uptake. As yet, the role of GlpF in spore germination has not been tested.

Cortex-lytic enzymes

In *B. subtilis* two enzymes, CwlJ and SleB, play redundant roles in the degradation of the spore's peptidoglycan cortex during germination [2,5,29,30,31•,32,33]. Germination of *cwlJ* and *sleB* spores is relatively normal, and even *cwlJ sleB* spores go through stage I of germination relatively normally. However, the double mutant spores cannot degrade their cortex and do not progress beyond stage I of germination. CwlJ and SleB require muramic-δ-lactam in peptidoglycan for their action, with SleB being a lytic transglycosylase while the specificity of CwlJ is unknown [31•,33]. The muramic-δ-lactam requirement for peptidoglycan cleavage by CwlJ or SleB ensures that the spore's germ cell wall (Figure 2), which lacks this modification, is not degraded during germination and becomes the cell wall of the outgrowing spore [9,30]. Note that the germ cell wall must expand considerably to encompass the increased volume of the stage II germinated spore core (Figures 1,2). This expansion can be blocked by some treatments of spores [34], but its mechanism is not understood.

CwlJ and SleB are synthesized only in sporulation, CwlJ in the mother cell and SleB in the forespore; neither is synthesized in zymogen form, although SleB is synthesized with a signal peptide that is rapidly removed [2,31•]. CwlJ is located in the spore coat fraction and is readily removed by decoating procedures [31•,35]. CwlJ is also absent from *cotE* spores, which have a severe coat defect. SleB is found in the spore integuments (coats, outer membrane and cortex) [31•]. Since SleB has a potential peptidoglycan-binding domain, this protein may be located in the cortex or the coat/cortex boundary (Figure 2) [31•]. However, a significant amount of SleB is also associated with the inner spore membrane [31•].

Both CwlJ and SleB require specific proteins for assembly and/or stability in spores [31•,36•]. For SleB the protein required is YpeB [31•,32]. The *ypeB* gene is co-transcribed with *sleB*, and YpeB is in the same location in spores as SleB (Figure 2). Similarly, in most *Bacillus* species, *cwlJ* is co-transcribed with *gerQ* (originally called *ywdL*). Although this is not the case in *B. subtilis*, even in this organism, *gerQ* and *cwlJ* are transcribed in the mother cell at the same time in sporulation [36•]. GerQ is essential for the presence of CwlJ in spores and GerQ is, like CwlJ, a coat protein (Figure 2). The reason for the absence of CwlJ in *gerQ* spores is not clear, but *gerQ* spores have no gross coat defects and GerQ is not needed for *cwlJ* transcription.

Because both SleB and CwlJ are synthesized in a mature form, there must be a mechanism (or mechanisms) keeping these enzymes in an inactive state in dormant spores. For SleB, this mechanism is not clear, although it may be that SleB only works on a cortex in which the peptidoglycan has a level of stress much higher than that in dormant spores [37,38]. However, the inactivity of CwlJ in dormant spores and its activation after stage I of germination is probably because CwlJ requires Ca^{2+}–DPA for its action (Figures 2,3 and see below) [39••].

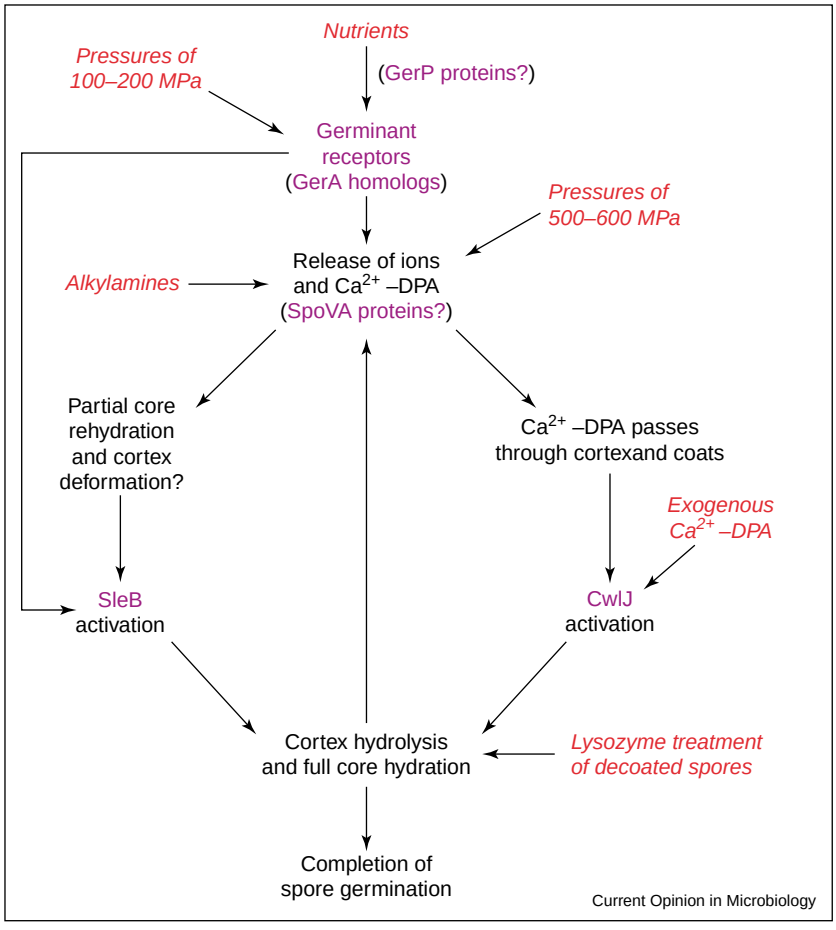
Whereas CwlJ and SleB are the only enzymes needed for cortex degradation during *B. subtilis* spore germination, other enzymes may be involved in this process, yet not be essential [31•,40]. One such enzyme is the *yaaH* gene product that encodes a cortex-lytic enzyme (termed SleL in *B. cereus*) [31•,40]. The situation in spores of *Clostridium perfringens* may be somewhat different from that in *Bacillus* spores, as *C. perfringens* spores have a cortex-lytic enzyme (termed SleC) that is activated by proteolysis in the first minutes of spore germination [29,41,42].

Non-nutrient germination

In addition to nutrients, spores are germinated by a variety of non-nutrients [8], including lysozyme, Ca^{2+}–DPA, cationic surfactants, high pressures and salts. These various non-nutrients can bypass individual components of the nutrient germination pathway (Figure 3).

Because lysozyme can degrade the cortex of most spores, this is a potential pathway for spore germination, although lysozyme will also degrade the spore's germ cell wall. In

Figure 3



Model for nutrient and non-nutrient spore germination in *B. subtilis*. Nutrients activate the germinant receptors and this causes the release of ions including Ca^{2+}–DPA from the spore core, which in turn triggers CwlJ action as the released Ca^{2+}–DPA flows past this enzyme. SleB action may be triggered by germinant receptor activation upon nutrient binding, and/or by changes in the stress upon the spore cortex due to the core hydration in stage I of germination. Perhaps binding of nutrients to germinant receptors activates SleB in the spore's inner membrane, whereas cortical stress activates both this SleB as well as the SleB located in the coat fraction. SleB and/or CwlJ then catalyze the cortex hydrolysis that is needed for completion of stage II of germination upon germ cell wall expansion. High pressures either activate the germinant receptors (100–200 MPa) or Ca^{2+}–DPA release (500–600 MPa), whereas alkylamines activate Ca^{2+}–DPA release either directly or indirectly by effects at the spore's inner membrane. External Ca^{2+}–DPA activates CwlJ, which then causes cortex hydrolysis, and this process might be amplified by consequent release of endogenous Ca^{2+}–DPA. Lysozyme treatment also causes cortex lysis and this in turn somehow causes Ca^{2+}–DPA release.

order for spores to be germinated by lysozyme, the spore coats must first be removed. With this pretreatment, spores are readily germinated by lysozyme, undergo DPA release and, if the lysozyme treatment is in a hypertonic medium, give rise to colonies.

Exogenous Ca^{2+}–DPA also is a good spore germinant. Spores lacking all germinant receptors germinate normally with Ca^{2+}–DPA, as do *sleB* spores [10]. However, *cwlJ* spores do not germinate with Ca^{2+}–DPA, and spores that lack SleB as well as DPA due to lack of DPA synthase also do not germinate with nutrients, although they do germinate with Ca^{2+}–DPA [39••]. These findings strongly suggest that germination by exogenous Ca^{2+}–DPA is via some direct or indirect activation of CwlJ.

Spore germination by cationic surfactants like dodecylamine, was recognized over 40 years ago [43]. Complete spore germination induced by dodecylamine requires either CwlJ or SleB, but does not require the spore's germinant receptors [15]. Because dodecylamine causes rapid Ca^{2+}–DPA release from spores that cannot degrade their cortex, yet causes no release of other small molecules from these spores, this agent may open the spore's channels for Ca^{2+}–DPA.

Spores of many species can be germinated at very high pressures (100–600 megaPascals [MPa]) [2,8]. At lower pressures (100–200 MPa), germination is caused by the activation of the germinant receptors [44]. However, at higher pressures (500–600 MPa), spores that lack nutrient

receptors trigger germination rapidly, suggesting that these pressures somehow open the spore's Ca^{2+}–DPA channels [45].

Finally, spores of some species, for example *B. megaterium*, only require salts such as KBr for germination. However, essentially nothing is known about the mechanism of germination induced by salt.

Conclusions and future directions

Significant progress has been made recently in understanding the process of spore germination (Figure 3). The function of the germinant receptors in recognition of nutrient germinants has been established, and the localization of the receptors to the spore's inner membrane is now consistent with the relative lack of effect of removal of the spore's outer layers on nutrient germination [2]. However, the proteins these receptors interact and 'talk' with are not known and thus the picture of receptor function is not yet clear. Also not clear are how nutrient binding to a receptor results in the transduction of a signal to other components of the germination apparatus and how different receptors interact to trigger spore germination with some germinants.

Another unknown in spore germination is the mechanism through which DPA and cations are lost from spores. This mechanism probably involves channels in the spore's inner membrane that are closed in dormant spores and opened upon germinant-receptor binding. One formal possibility is that the germinant receptors are themselves ion channels, but this seems unlikely, as spores lacking all germinant receptors germinate normally with the non-nutrient germinants Ca^{2+}–DPA, dodecylamine or lysozyme [10,15,36•,39••]. However, perhaps the opening of some cation channels is not needed for germination with non-nutrients. Unfortunately, with the exception of the SpoVA proteins, there are no good candidates for spore-specific constituents of DPA and cation channels in the spore's inner membrane. An added complication in understanding the function of molecules that are embedded in the spore's inner membrane is that the great majority of lipid molecules in this membrane are immobile (Cowan AE, Melly E, Koppel DE *et al.*, unpublished data). This lipid immobility is consistent with the extremely slow passage of small molecules such as water and methylamine through the inner spore membrane [2,46], and will undoubtedly affect membrane protein function, but in ways that are not clear.

A later part of the signal transduction pathway in spore germination is the activation of lytic enzymes that degrade the spore cortex, allowing progression through stage II of germination. The mechanism of activation of SleB by stage I events is not known, although SleB activation has been suggested to be caused by nutrient binding to germinant receptors and/or changes in the stress on the cortical peptidoglycan [2,31•,39••]. However, the mechanism of CwlJ activation is clearer, as this protein requires exogenous or endogenous Ca^{2+}–DPA for its activation [36•,39••]. Since Ca^{2+}–DPA release is a hallmark of stage I of germination, the released Ca^{2+}–DPA then activates CwlJ, resulting in progression to stage II. Whereas the mechanism of the activation of CwlJ by Ca^{2+}–DPA is not clear (it may be direct or indirect), the outline of the signal transduction pathway leading to completion of spore germination through CwlJ action is clear. Unfortunately, CwlJ activity has not been demonstrated *in vitro*. Clearly, much remains to be learned about the process of spore germination.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Setlow P: **Resistance of bacterial spores**. In *Bacterial Stress Responses*. Edited by Storz G, Hengge-Aronis R. Washington, DC: American Society for Microbiology; 2000: 217-230.

2. Paidhungat M, Setlow P: **Spore germination and outgrowth**. In *Bacillus subtilis and its Relatives: From Genes to Cells*. Edited by Hoch JA, Losick R, Sonenshein AL. Washington, DC: American Society for Microbiology; 2002: 537-548.

3. Guidi-Rontani C, Weber-Levy M, Labruyere E, Mock M: **Germination of *Bacillus anthracis* spores within alveolar macrophage**. *Mol Microbiol* 1999, **31**:9-17.

4. Jedrzejas MJ, Setlow P: **Comparison of the binuclear metalloenzymes diphosphoglycerate-independent phosphoglycerate mutase and alkaline phosphatase: their mechanism of catalysis by a phosphoserine intermediate**. *Chem Rev* 2001, **101**:607-618.

5. Setlow B, Melly E, Setlow P: **Properties of spores of *Bacillus subtilis* blocked at an intermediate stage of spore germination**. *J Bacteriol* 2001, **183**:4894-4899.

6. Cowan AE, Koppel DE, Setlow B, Setlow P: **A cytoplasmic protein is immobile in the cytoplasm of dormant spores of *Bacillus subtilis*: implications for spore dormancy**. *Proc Natl Acad Sci USA* 2003, **100**:4209-4214.

7. Setlow B, Loshon CA, Genest PC, Cowan AE, Setlow C, Setlow P: **Mechanisms of killing of spores of *Bacillus subtilis* by acid, alkali and ethanol**. *J Appl Microbiol* 2002, **92**:362-375.

8. Gould GW: **Germination**. In *The Bacterial Spore*. Edited by Gould GW, Hurst A. New York: Academic Press; 1969: 397-444.

9. Moir A, Corfe BM, Behravan J: **Spore germination**. *Cell Mol Life Sci* 2002, **59**:403-409.

10. Paidhungat M, Setlow P: **Role of Ger proteins in nutrient and non-nutrient triggering of spore germination in *Bacillus subtilis***. *J Bacteriol* 2000, **182**:2513-2519.

11. Paidhungat M, Setlow P: **Isolation and characterization of mutations in *Bacillus subtilis* that allow spore germination in the novel germinant D-alanine**. *J Bacteriol* 1999, **181**:3341-3350.

12. Hudson KD, Corfe BM, Kemp EH, Feavers IM, Coote PJ, Moir A: **Localization of GerAA and GerAC germination proteins in the *Bacillus subtilis* spore**. *J Bacteriol* 2001, **183**:4317-4322.

13. Paidhungat M, Setlow P: **Localization of a germinant receptor protein (GerBA) to the inner membrane of *Bacillus subtilis* spores**. *J Bacteriol* 2001, **183**:3982-3990.

14. Jack DL, Paulsen IT, Saier MH Jr: **The amino acid/polyamine/organocation (APC) superfamily of transporters specific for amino acids, polyamines and organocations**. *Microbiology* 2000, **146**:1797-1814.

15. Setlow B, Cowan AE, Setlow P: **Germination of spores of *Bacillus subtilis* with dodecylamine**. *J Appl Microbiol* 2003, **95**:637-648.

16. Ireland JAW, Hanna PC: **Macrophage-enhanced germination of *Bacillus anthracis* endospores requires *gerS***. *Infect Immun* 2002, **70**:5870-5872.

17. Weiner MA, Read TD, Hanna PC: **Identification and characterization of the *gerH* operon of *Bacillus anthracis* endospores: a differential role for purine nucleosides in germination**. *J Bacteriol* 2003, **185**:1462-1464.

18. Ireland JAW, Hanna PC: **Amino acid- and purine ribonucleoside-induced germination of *Bacillus anthracis* ΔSterne endospores: *gerS* mediates responses to aromatic ring structures**. *J Bacteriol* 2002, **184**:1296-1303.

19. Barlass PJ, Houston CW, Clements MO, Moir A: **Germination of *Bacillus cereus* spores in response to L-alanine and to inosine: the roles of *gerL* and *gerQ* operons**. *Microbiology* 2002, **148**:2089-2095.

20. • Cabrera-Martinez R-M, Tovar-Rojo F, Vepachedu VR, Setlow P: **Effects of overexpression of nutrient receptors on germination of spores of *Bacillus subtilis***. *J Bacteriol* 2003, **185**:2457-2464.

Overexpression, by 20–200 times, of the GerA, GerB, GerB* or GerK receptors in spores is shown to significantly increase spore germination rates when only a single receptor is needed (GerA or GerB*), but not when two receptors are required, as in germination on AGFK. However, the findings that even when only a single receptor is needed, the increases in spore germination rates are well below the levels of receptor overexpression, and that overexpression of some receptors decreases rates of germination with germinants that require other receptors, together suggest that different germinant receptors interact physically and/or compete for some limiting component that is essential for spore germination.

21. Ames P, Studdert CA, Reiser RH, Parkinson JS: **Collaborative signalling by mixed chemoreceptor teams in *Escherichia coli***. *Proc Natl Acad Sci USA* 2002, **99**:7060-7065.

22. Gestwicki JE, Riessling LL: **Inter-receptor communication through arrays of bacterial chemoreceptors**. *Nature* 2002, **415**:81-84.

23. Tovar-Rojo F, Chander M, Setlow B, Setlow P: **The products of the *spoVA* operon are involved in dipicolinic acid uptake into developing spores of *Bacillus subtilis***. *J Bacteriol* 2002, **184**:584-587.

24. Errington J: ***Bacillus subtilis* sporulation: regulation of gene expression and control of morphogenesis**. *Microbiol Rev* 1993, **57**:1-33.

25. Thackray PH, Behravan J, Southworth TW, Moir A: **GerN, an antiporter homologue important in germination of *Bacillus cereus* endospores**. *J Bacteriol* 2001, **183**:476-482.

26. Southworth TW, Guffanti AA, Moir A, Krulwich T: **GerN, an endospore germination protein of *Bacillus cereus*, is an Na^+/H^+-K^+ antiporter**. *J Bacteriol* 2001, **183**:5896-5903.

27. Tani K, Watanabe T, Matsuda H, Nasu M, Kondo M: **Cloning and sequencing of the spore germination gene of *Bacillus megaterium* ATCC 12872: similarities to the NaH- antiporter gene of *Enterococcus hirae***. *Microbiol Immunol* 1996, **40**:99-105.

28. Behravan J, Chirakkal H, Masson A, Moir A: **Mutations in the *gerP* locus of *Bacillus subtilis* and *Bacillus cereus* affect access of germinants to their targets in spores**. *J Bacteriol* 2000, **182**:1987-1994.

29. Makino S, Moriyama R: **Hydrolysis of cortex peptidoglycan during bacterial spore germination**. *Med Sci Monit* 2002, **8**:RA119-RA127.

30. Popham D: **Specialized peptidoglycan of the bacterial endospore: the inner wall of the lockbox**. *Cell Mol Life Sci* 2002, **59**:426-433.

31. • Chirakkal H, O'Rourke M, Atrih A, Foster SJ, Moir A: **Analysis of spore cortex lytic enzymes and related proteins in *Bacillus subtilis* endospore germination**. *Microbiology* 2002, **148**:2383-2392.

The location of SleB in both the integument (coats/outer membrane/cortex) and inner membrane is shown, as is the presence of YpeB in these same locations. CwlJ is also shown to be in the integument, and neither CwlJ nor SleB are found to be subject to proteolysis upon spore germination. The roles of CwlJ and SleB and several other proteins in cortex hydrolysis are also presented.

32. Boland FM, Atrih A, Chirakkal H, Foster SJ, Moir A: **Complete spore-cortex hydrolysis during germination of *Bacillus subtilis* 168 requires SleB and YpeB**. *Microbiology* 2000, **146**:57-64.

33. Atrih A, Foster SJ: ***In vivo* roles of germination specific lytic enzymes of *Bacillus subtilis* 168**. *Microbiology* 2001, **147**:2925-2932.

34. Melly E, Cowan AE, Setlow P: **Studies on the mechanism of killing of *Bacillus subtilis* spores by hydrogen peroxide**. *J Appl Microbiol* 2002, **93**:316-325.

35. Bagyan I, Setlow P: **Localization of the cortex lytic enzyme CwlJ in spores of *Bacillus subtilis***. *J Bacteriol* 2002, **184**:1219-1224.

36. • Ragkousi K, Eichenberger P, van Ooij C, Setlow P: **Identification of a new gene essential for germination of *Bacillus subtilis* spores with Ca^{2+}–dipicolinate**. *J Bacteriol* 2003, **185**:2315-2329.

A screen for mutants that are defective in spore germination with Ca^{2+}–DPA identifies mutations in either *cwlJ* or *gerQ* (initially called *ywdL*). GerQ is shown to be a spore coat protein that is essential for the assembly or stability of CwlJ in spore coats.

37. Foster SJ, Johnstone K: **Purification and properties of a germination-specific cortex-lytic enzyme from spores of *Bacillus megaterium* KM**. *Biochem J* 1987, **242**:573-579.

38. Makino S, Ito N, Inoue T, Miyata S, Moriyama R: **A spore-lytic enzyme released from *Bacillus cereus* spores during germination**. *Microbiology* 1994, **140**:1403-1410.

39. •• Paidhungat M, Ragkousi K, Setlow P: **Genetic requirements for induction of germination of spores of *Bacillus subtilis* by Ca^{2+}–dipicolinate**. *J Bacteriol* 2001, **183**:4886-4893.

CwlJ but not SleB is shown to be essential for spore germination with Ca^{2+}–DPA supplied from either exogenous sources or from the spore core. This leads to a model for the mechanism of activation of cortex hydrolysis during spore germination.

40. Chen Y, Fukuoka S, Makino S: **A novel peptidoglycan hydrolase of *Bacillus cereus*: biochemical characterization and nucleotide sequence of the corresponding gene, *sleL***. *J Bacteriol* 2000, **182**:1499-1506.

41. Shimamoto S, Moriyama R, Sugimoto K, Miyata S, Makino S: **Partial characterization of an enzyme fraction with protease activity which converts the spore peptidoglycan hydrolase (SleC) precursor to an active enzyme during germination of *Clostridium perfringens* S40 spores and analysis of a gene cluster involved in the activity**. *J Bacteriol* 2001, **183**:3742-3751.

42. Okamura S, Urakami K, Kimata M, Aoshima T, Shimamoto S, Moriyama R, Makino S: **The N-terminal prepeptide is required for the production of spore cortex-lytic enzyme from its inactive precursor during germination of *Clostridium perfringens* S40 spores**. *Mol Microbiol* 2000, **37**:821-827.

43. Rode LJ, Foster JW: **Germination of bacterial spores with alkyl primary amines**. *J Bacteriol* 1961, **81**:768-779.

44. Wuytack EY, Soons J, Poschet F, Michiels CW: **Comparative study of pressure- and nutrient-induced germination of *Bacillus subtilis* spores**. *Appl Environ Microbiol* 2000, **66**:257-261.

45. Paidhungat M, Setlow B, Daniels WB, Hoover D, Papafragkou E, Setlow P: **Mechanisms of induction of germination of *Bacillus subtilis* spores by high pressure**. *Appl Environ Microbiol* 2002, **68**:3172-3175.

46. Westphal A, Price PB, Leighton TJ, Wheeler KE: **Kinetics of size changes of individual *Bacillus thuringiensis* spores in response to changes in relative humidity**. *Proc Natl Acad Sci USA* 2003, **100**:3461-3466.