The Spore Coat

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ABSTRACT Spores of Clostridiales and Bacillales are encased in a complex series of concentric shells that provide protection, facilitate germination, and mediate interactions with the environment. Analysis of diverse spore-forming species by thin-section transmission electron microscopy reveals that the number and morphology of these encasing shells vary greatly. In some species, they appear to be composed of a small number of discrete layers. In other species, they can comprise multiple, morphologically complex layers. In addition, spore surfaces can possess elaborate appendages. For all their variability, there is a consistent architecture to the layers encasing the spore. A hallmark of all Clostridiales and Bacillales spores is the cortex, a layer made of peptidoglycan. In close association with the cortex, all species examined possess, at a minimum, a series of proteinaceous layers, called the coat. In some species, including Bacillus subtilis, only the coat is present. In other species, including Bacillus anthracis, an additional layer, called the exosporium (Fig. 1; see also references 8 and 9). In B. subtilis, where the coat has been most deeply studied, three layers are visible by TEM (Fig. 1). A fourth layer, the basement layer, is not distinguishable by TEM, but corresponds to the innermost layer atop which the rest of the coat assembles and contains several of the coat proteins required to initiate coat assembly (i.e., SpoIVA, SpoVM, and SpoVID, see below and reference 7). Located on top of the basement layer, the inner coat is characterized by a series of fine, lightly staining lamellae. This morphology is superficially reminiscent of a cross section through the myelin sheath of a neuronal axon (while being entirely distinct biochemically). Not all species possess a coat layer with this appearance, but many do, including Clostridium difficile (10–12). Surrounding the B. subtilis inner coat is a coarsely layered, darkly staining layer known as the outer coat. Finally, the outermost layer of the B. subtilis coat is called the crust (13). The crust was detected only recently, because visualizing it requires applying the stain ruthenium red. While not typically used for imaging bacterial resolution, and in the decades since (1–7). The coat is readily distinguished from the cortex (see reference 178) because of its higher electron density. In a large subset of species, the spore also possesses an additional layer surrounding the coat, called the exosporium (Fig. 1; see also references 8 and 9).

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spores, ruthenium red was also shown to significantly enhance features in the Bacillus anthracis exosporium (see below) \( (14) \). It is notable that so many years after the first detailed analyses of coat structure, there is still the opportunity for new discoveries using classical methodologies.

In contrast to the morphologically complex, multilayered B. subtilis coat, the B. anthracis coat possesses a single compact layer that only occasionally resolves into two layers \( (4, 15) \) (to be explicit, here we are not considering the exosporium, which is described in detail in reference \( 8 \)). Because the inner layer stains more lightly than the outer one, these layers are also referred to as inner and outer coats \( (16) \). Other species have different coat layer arrangements \( (17) \), usually with one to three readily discerned layers \( (Fig. \, 1) \). Regardless of the number, all the layers usually appear to be in close contact in any given spore, suggesting that they are connected by chemical bonds (although TEM images occasionally show B. subtilis spores in which the inner and outer layers have partially separated, suggesting that between-layer bonds are weaker than within-layer bonds).

Taken together, these observations raise an important question: what are the benefits to spores of such extreme diversity in coat structure? It is intuitive to propose that this diversity is driven by adaptation to varying environments. While this is very likely, it does not explain the counterintuitive observation that, in many specific environments such as a single soil sample, a large number of highly diverse spore-forming species can be found (see also reference \( 179 \)). Perhaps, this is due to further levels of diversity in a conventional soil sample. Alternatively (and not mutually exclusively), even in a single niche, there are adaptive benefits to a diverse community of sporeformers. This possibility, in turn, provides a motivation to better understand how the coat facilitates survival in nature. Indeed, a more sophisticated understanding of how spores interact with and survive the challenges of their niches might reveal the functions of species-specific features of the coat and, therefore, their adaptive benefits.

In most species, including B. subtilis, B. anthracis, and C. difficile, the coat appears to possess folds around its entire circumference \( (Fig. \, 1) \). The folds serve a remarkable purpose; by unfolding, they accommodate expansion in the volume of the spore core that occurs when the relative humidity changes \( (18, 19) \). It is possible that the ability to respond to changes in interior volume that take place over short timescales is important in natural environments. The dynamic properties of the coat likely have additional important and still poorly appreciated functions during dormancy and germination, as is discussed later.

**COAT PROTEIN COMPOSITION**

In B. subtilis, the coat is composed of at least 80 different proteins \( (Table \, 1) \) \( (20–22) \). Coat protein composition has not been characterized in as much detail in other species (even though, in the past decade, several novel coat proteins have been identified in B. anthracis and C. difficile \( (23–27) \)). It is likely, however, that most sporeformers also assemble dozens of protein species in their coat. Comparative genomic analyses indicate that coat proteins are the most species specific of all the sporulation proteins, suggesting that coat composition is highly influenced by the ecosystem in which a species resides. This is not unexpected considering that coat composition may influence the surface properties that will mediate spore dispersal in the environment or attachment to specific hosts or surfaces.

Homologs of B. subtilis coat proteins are found primarily in other Bacillaceae, more rarely in Clostridia, and almost never in non-spore-forming species \( (28) \) (see

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**FIGURE 1** Thin-section TEM analysis of spores from diverse species. Spores were prepared as described in McKenney et al. \( (13) \). Images in the top row were fixed using ruthenium red. Other images were conventionally fixed. Images are not to scale; each image was sized to facilitate comparison. Two images of Bacillus amyloliquefaciens are shown (one showing a section along the long axis, the other showing a section along the short axis) to point out the thick caps of coat at the poles. The difference in thickness between the two caps is a consistent feature of this species. Two images of Brevibacillus laterosporus are also shown to emphasize the variation in morphology of the distinctive structure (indicated with a brown bracket) associated with the coat. The mother cell envelope, which is still present in these two spores, is indicated with a green bracket. The image of Clostridium difficile is taken from Semenyuk et al. \( (142) \). The crust (Cr), outer coat (OC), and inner coat (IC) are indicated in the image of Bacillus subtilis in the upper left. The coat and, where it is present, the exosporium are indicated with blue and red brackets, respectively. The image of Bacillus megaterium is courtesy of Dr. Joel Bozue at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID).
### TABLE 1 B. subtilis coat proteins (strain 168)

<table>
<thead>
<tr>
<th>Name</th>
<th>Paralogs</th>
<th>Operon</th>
<th>Regulators (transcription)</th>
<th>Function</th>
<th>Domains</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Morphogenetic coat proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CotE (BSU17030)</td>
<td>cotE</td>
<td></td>
<td>(\sigma^E/\sigma^K) GerE((-))</td>
<td>Assembly of the outer coat (major)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CotH (BSU36060)</td>
<td>cotH</td>
<td></td>
<td>(\sigma^E/\sigma^K) GerE((-)) SpolIID(\text{+})</td>
<td>Assembly of the outer coat (minor)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CotO (YjoX) (BSU11730)</td>
<td>cotO</td>
<td></td>
<td>(\sigma^E)</td>
<td>Assembly of the outer coat (minor)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CotX (BSU11760)</td>
<td>cotX</td>
<td></td>
<td>(\sigma^E/\sigma^K) GerE(\text{+}) GerR(\text{+})</td>
<td>Assembly of the crust</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CotY (BSU11750)</td>
<td>CotZ</td>
<td>cotYZ</td>
<td>(\sigma^E/\sigma^K) GerE(\text{+}) GerR(\text{+})</td>
<td>Assembly of the crust</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CotZ (BSU11740)</td>
<td>CotY</td>
<td>cotYZ</td>
<td>(\sigma^E/\sigma^K) GerE(\text{+}) GerR(\text{+})</td>
<td>Assembly of the crust</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SafA (YrbA) (BSU27840)</td>
<td>safA-coxA</td>
<td></td>
<td>(\sigma^E)</td>
<td>Assembly of the inner coat</td>
<td>LysM</td>
<td>150, 151</td>
</tr>
<tr>
<td>SpoIVA (BSU22800)</td>
<td>spoIVA</td>
<td></td>
<td>(\sigma^E) SpolIID(\text{(-)})</td>
<td>Spore cortex formation, coat assembly and anchoring</td>
<td>ATPase (ATP-dependent irreversible polymerization)</td>
<td>94, 95, 100, 102</td>
</tr>
<tr>
<td>SpoVM (BSU15810)</td>
<td>spoVM</td>
<td></td>
<td>(\sigma^E)</td>
<td>Spore cortex formation, coat assembly, spore encasement</td>
<td>Atypical amphipathic a-helix (NMR structure: 2MVH and 2MVJ)</td>
<td>97–101, 149</td>
</tr>
<tr>
<td>SpoVID (BSU28110)</td>
<td>spoVID-yxSE</td>
<td></td>
<td>(\sigma^E)</td>
<td>Spore encasement</td>
<td>LysM</td>
<td>93, 96, 104, 107, 120</td>
</tr>
<tr>
<td><strong>B. Other coat proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>B.1. Basement layer (SpoIVA-dependent, SafA-independent, CotE-independent)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CotJA (BSU06890)</td>
<td>cotJ-yesJ</td>
<td>cotJ</td>
<td>(\sigma^E)</td>
<td>May provide protection against oxidative stress</td>
<td>Mn catalase (ferritin-like domain)</td>
<td>150, 151</td>
</tr>
<tr>
<td>CotJB (BSU06900)</td>
<td>cotJ-yesJ</td>
<td>cotJ</td>
<td>(\sigma^E)</td>
<td>Adaptor for ClpXP protease, promotes degradation of SpoIVA if coat is misassembled</td>
<td>Phospholipase (GDSL lipolytic enzyme family)</td>
<td>59, 60</td>
</tr>
<tr>
<td>CotJC (BSU06910)</td>
<td>YdbD, YjoC</td>
<td>cotJ-yesJ</td>
<td>(\sigma^E)</td>
<td>Spore lipolytic enzyme</td>
<td>Halocid dehydrogenase superfamily</td>
<td>126</td>
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<tr>
<td>CmpA</td>
<td>cmpA</td>
<td></td>
<td>(\sigma^E) SpolIID(\text{+})</td>
<td></td>
<td>ATP binding</td>
<td>152</td>
</tr>
<tr>
<td>LipC (YcsK) (BSU04110)</td>
<td>lipC</td>
<td></td>
<td>(\sigma^K) GerE(\text{+}) SpolIID(\text{(-)})</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>YhaX (BSU09830)</td>
<td>yhaX-hemZ</td>
<td>yhelD</td>
<td>(\sigma^E) SpolIID(\text{(-)})</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>YheD (BSU09770)</td>
<td>yheCD</td>
<td>yhelD</td>
<td>(\sigma^E) SpolIID(\text{+})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YjzB (BSU11320)</td>
<td>yjzB</td>
<td></td>
<td>(\sigma^K) GerE(\text{+})</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>YppG (BSU22250)</td>
<td>yppG</td>
<td></td>
<td>(\sigma^E/\sigma^K) GerE(\text{+})</td>
<td></td>
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<tr>
<td><strong>B.2. Inner layer (SpoIVA- and SafA-dependent)</strong></td>
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<tr>
<td>CotD (BSU22200)</td>
<td>cotD</td>
<td></td>
<td>(\sigma^K) SpolIID(\text{(-)}) GerE(\text{+}) GerR(\text{+})</td>
<td></td>
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<tr>
<td>CotF (BSU40530)</td>
<td>cotF</td>
<td></td>
<td>(\sigma^E/\sigma^K) SpolIID(\text{+}) GerE(\text{(-)})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CotP (YdfT) (BSU05550)</td>
<td>ydgBA-cotP</td>
<td></td>
<td>(\sigma^E) GerE(\text{(-)})</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**References:**
- 13, 109, 109, 119, 145, 146, 94, 95, 100, 102, 103, 107, 147, 148, 97–101, 149, 150, 151, 152, 153.
### CotT (BSU12090)
- **cotT**
- $\sigma^E / \sigma^K$ SpoIIID(+) GerE(-)
- Spore cortex lytic enzyme
- Cell wall hydrolase

### CwlJ (YcbQ) (BSU02600)
- **cwlJ**
- $\sigma^E / \sigma^K$ SpoIIID(+)
- CwlJ inhibitor.
- Not to be confused with the germinant receptor GerQ in *B. cereus*

### GerQ (YwdL) (BSU37920)
- **gerQ**
- $\sigma^E$
- CwlJ inhibitor.
- Not to be confused with the germinant receptor GerQ in *B. cereus*

### OxdD (YoaN) (BSU18670)
- **oxdD**
- $\sigma^E$
- May provide protection against toxic compounds
- Oxalate decarboxylase (Cupin superfamily)

### Tgl (BSU31270)
- **tgi**
- $\sigma^E$
- Introduction of cross-links in the coat (GerQ)
- Transglutaminase (X-ray diffraction structure: 4P8I and 4PAS)

### YaaH (SleL) (BSU00160)
- **yaaH**
- $\sigma^E$
- SpoIIID(-)
- N-Acetylglucosaminidase
- LysM (2x), glycoside-hydrolase family 18 (X-ray diffraction structure: 4S3J)

### YeeK (BSU06850)
- **yeeK**
- $\sigma^E$
- May provide protection against oxidative stress
- Mn catalase (ferritin-like domain)

### YhjR (BSU10610)
- **yhjRQ**
- $\sigma^E$
- Ferritin-like domain

### YisY (BSU10900)
- **yisY**
- $\sigma^E$
- $\alpha / \beta$ hydrolase superfamily

### YjqC (BSU12490)
- **yjqC**
- $\sigma^E$
- May provide protection against oxidative stress
- Mn catalase (ferritin-like domain)

### YmaG (BSU17310)
- **ymaG**
- $\sigma^E$
- GerE(+)
- Ferritin-like domain

### YsnD (BSU28320)
- **ysnD**
- $\sigma^E$
- $\alpha / \beta$ hydrolase superfamily

### YmaG (BSU17310)
- **ymaG**
- $\sigma^E$
- GerE(+)
- Ferritin-like domain

### YsnD (BSU28320)
- **ysnD**
- $\sigma^E$
- $\alpha / \beta$ hydrolase superfamily

### YxqC (BSU12490)
- **yjqC**
- $\sigma^E$
- May provide protection against oxidative stress
- Mn catalase (ferritin-like domain)

### YutH (BSU32270)
- **yutH**
- $\sigma^E$
- SpoIIID(-)
- BSK (bacterial spore kinase)
- Kinase-like domain

### YzbC (BSU31730)
- **yzbC**
- $\sigma^E$
- SpoIIID(-)
- BSK (bacterial spore kinase)
- Kinase-like domain

### YxeE (BSU39580)
- **yxeE**
- $\sigma^E$
- GerE(+)
- $\alpha / \beta$ hydrolase superfamily

### YxbR (BSU40630)
- **yxbR**
- $\sigma^E$
- $\alpha / \beta$ hydrolase superfamily

### CotA (BSU06300)
- **cotA**
- $\sigma^E$
- Spore pigmentation
- Multicopper oxidase (laccase); (X-ray diffraction structure: 2X87, 2BHF)

### CotB (BSU36050)
- **cotB**
- $\sigma^E$
- SpoIIID(+)
- GerE(+)
- Multicopper oxidase (laccase); (X-ray diffraction structure: 2X87, 2BHF)

### CotC (BSU17700)
- **cotC**
- $\sigma^E$
- SpoIIID(-)
- GerE(+)
- Multicopper oxidase (laccase); (X-ray diffraction structure: 2X87, 2BHF)

### CotG (BSU36070)
- **cotG**
- $\sigma^E$
- GerE(+)
- $\alpha / \beta$ hydrolase superfamily

### CotM (BSU17970)
- **cotM**
- $\sigma^E$
- GerE(+)
- $\alpha / \beta$ hydrolase superfamily

### CotQ (YvdP) (BSU34520)
- **cotQ**
- $\sigma^E$
- GerE(+)
- FAD-linked oxidoreductase

### CotS (BSU30900)
- **cotS**
- $\sigma^E$
- SpoIIID(-)
- BSK (bacterial spore kinase)
- Kinase-like domain

### CotT (BSU12090)
- **cotT**
- $\sigma^E$
- SpoIIID(+)
- GerE(-)
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- Cell wall hydrolase

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- **yhjRQ**
- $\sigma^E$
- Ferritin-like domain

### YisY (BSU10900)
- **yisY**
- $\sigma^E$
- $\alpha / \beta$ hydrolase superfamily

### YjqC (BSU12490)
- **yjqC**
- $\sigma^E$
- May provide protection against oxidative stress
- Mn catalase (ferritin-like domain)

### YmaG (BSU17310)
- **ymaG**
- $\sigma^E$
- GerE(+)
- Ferritin-like domain

### YsnD (BSU28320)
- **ysnD**
- $\sigma^E$
- $\alpha / \beta$ hydrolase superfamily

### YxqC (BSU12490)
- **yjqC**
- $\sigma^E$
- May provide protection against oxidative stress
- Mn catalase (ferritin-like domain)

### YutH (BSU32270)
- **yutH**
- $\sigma^E$
- SpoIIID(-)
- BSK (bacterial spore kinase)
- Kinase-like domain

### YzbC (BSU31730)
- **yzbC**
- $\sigma^E$
- SpoIIID(-)
- BSK (bacterial spore kinase)
- Kinase-like domain

### YxeE (BSU39580)
- **yxeE**
- $\sigma^E$
- GerE(+)
- $\alpha / \beta$ hydrolase superfamily

### YxbR (BSU40630)
- **yxbR**
- $\sigma^E$
- $\alpha / \beta$ hydrolase superfamily

### CotA (BSU06300)
- **cotA**
- $\sigma^E$
- Spore pigmentation
- Multicopper oxidase (laccase); (X-ray diffraction structure: 2X87, 2BHF)

### CotB (BSU36050)
- **cotB**
- $\sigma^E$
- SpoIIID(+)
- GerE(+)
- Multicopper oxidase (laccase); (X-ray diffraction structure: 2X87, 2BHF)

### CotC (BSU17700)
- **cotC**
- $\sigma^E$
- SpoIIID(-)
- GerE(+)
- Multicopper oxidase (laccase); (X-ray diffraction structure: 2X87, 2BHF)

### CotG (BSU36070)
- **cotG**
- $\sigma^E$
- GerE(+)
- $\alpha / \beta$ hydrolase superfamily

### CotM (BSU17970)
- **cotM**
- $\sigma^E$
- GerE(+)
- $\alpha / \beta$ hydrolase superfamily

### CotQ (YvdP) (BSU34520)
- **cotQ**
- $\sigma^E$
- GerE(+)
- FAD-linked oxidoreductase

### CotS (BSU30900)
- **cotS**
- $\sigma^E$
- SpoIIID(-)
- BSK (bacterial spore kinase)
- Kinase-like domain

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(continued)
<table>
<thead>
<tr>
<th>Name</th>
<th>Paralogs</th>
<th>Operon</th>
<th>Regulators (transcription)</th>
<th>Function</th>
<th>Domains</th>
<th>References</th>
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<tbody>
<tr>
<td>CotU (YnzH)</td>
<td>CotC</td>
<td>cotU</td>
<td>σ^E</td>
<td>Not to be confused with the GerT antiporter in <em>B. cereus</em></td>
<td>Hsp20 family</td>
<td>23, 162, 168</td>
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<td>GerT (YozR)</td>
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<td></td>
<td>σ^E</td>
<td></td>
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<td>169</td>
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<td>SpsB (BSU37900)</td>
<td>sps</td>
<td>YfnH</td>
<td>σ^E, σ^K</td>
<td>Spore polysaccharide synthesis (rhamnose synthesis)</td>
<td></td>
<td>71, 71–73</td>
</tr>
<tr>
<td>SpsI (BSU37840)</td>
<td>sps</td>
<td>YtdA</td>
<td>σ^E, σ^K</td>
<td></td>
<td></td>
<td>71, 71–73</td>
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<tr>
<td>YknT (Cse1S)</td>
<td>yknT</td>
<td></td>
<td>σ^E</td>
<td>SpolliID(+)</td>
<td></td>
<td>13, 21, 170</td>
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<tr>
<td>YibD (BSU14970)</td>
<td>yibDE</td>
<td></td>
<td>σ^E, GerE(+)</td>
<td></td>
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<td>13, 21</td>
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<tr>
<td>YncD (AlrB)</td>
<td>yncD</td>
<td>ykvP-ykvQ-ykzR-ytdA</td>
<td>σ^E, GerE(+)</td>
<td>Conversion of L-Ala to D-Ala</td>
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<td>13, 21, 72</td>
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<tr>
<td>YtdA (BSU30850)</td>
<td>sps</td>
<td>YfnH</td>
<td>σ^E</td>
<td>Spore polysaccharide synthesis (putative)</td>
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<td>72</td>
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<tr>
<td>YtxO (BSU30890)</td>
<td>cotSA-cotS-ytxO</td>
<td></td>
<td>σ^E, GerE(+)</td>
<td>Glucose-1-phosphate uridylytransferase (X-ray diffraction structure: 1KNV)</td>
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<td>13, 21</td>
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**B.4. Crust (SpoIVA-, CotE-, and CotX/Y/Z-dependent)**

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<tr>
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<th>Paralogs</th>
<th>Operon</th>
<th>Regulators (transcription)</th>
<th>Function</th>
<th>Domains</th>
<th>References</th>
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<tr>
<td>CgeA (BSU19780)</td>
<td></td>
<td>cgeAB</td>
<td>σ^E, GerE(+)</td>
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<td>55, 111, 171</td>
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<tr>
<td>CgeB (BSU19790)</td>
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<td>cgeAB</td>
<td>σ^E, GerE(+)</td>
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<td>CotV (BSU11780)</td>
<td></td>
<td>cotVW</td>
<td>σ^E, σ^K, GerE(+)</td>
<td></td>
<td></td>
<td>111–113, 116, 144</td>
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<tr>
<td>CotW (BSU11770)</td>
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<td>cotVW</td>
<td>σ^E, σ^K, GerE(+)</td>
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<td>111–113, 116, 144</td>
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**B.5. Layer-specific localization not determined**

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<th>Regulators (transcription)</th>
<th>Function</th>
<th>Domains</th>
<th>References</th>
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<tr>
<td>Cotl (YtaA)</td>
<td>CotS</td>
<td>cotl</td>
<td>σ^E, GerE(+)</td>
<td>BSK (bacterial spore kinase)</td>
<td>Kinase-like domain</td>
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<td>CotR (YvdO)</td>
<td>cotR</td>
<td></td>
<td>σ^E, σ^K, GerE(+)</td>
<td>Spore lipolytic enzyme</td>
<td>Phospholipase (papatin family)</td>
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<td>CotSA (YtxN)</td>
<td>cotSA-cotS-ytxO</td>
<td></td>
<td>σ^E, GerE(+)</td>
<td>Transfer of glycosyl groups</td>
<td>Glycosyltransferase 1 family</td>
<td>172</td>
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<tr>
<td>GerPA (YisH)</td>
<td>GerPF</td>
<td>gerP</td>
<td>σ^E, GerE(+)</td>
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<td>61, 62</td>
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<tr>
<td>GerPB (YisG)</td>
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<td>σ^E, GerE(+)</td>
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<td>GerPC (YisF)</td>
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<td>61, 62</td>
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<tr>
<td>GerPD (YisE) (BU10690)</td>
<td>gerP</td>
<td>σ^E</td>
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<td>GerPE (YisD) (BU10680)</td>
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<td>σ^K</td>
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<td>GerPF (YisC) (BU10670)</td>
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<td>σ^E</td>
<td>Nucleotide-sugar-dependent glycosyltransferase</td>
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<td>SodA (BU25020)</td>
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<td>Not under sporulation control</td>
<td>Superoxide dismutase (X-ray diffraction structure: 2RCV)</td>
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<td>SscA (YhzE) (BU09958)</td>
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<td>YabG (BU00430)</td>
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<td>YdhD (BU05710)</td>
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<td>YhbB (BU08920)</td>
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<td>Amidase</td>
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<tr>
<td>YheC (BU09780)</td>
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<td>ATP binding</td>
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<td>YkvP (BU13780)</td>
<td>ykvP-ykvQ-</td>
<td>σ^K</td>
<td>LysM</td>
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<tr>
<td>YkvQ (BU13790)</td>
<td>ykvP-ykvQ-</td>
<td>σ^E</td>
<td>Glycosylase</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>YwrJ (BU36040)</td>
<td>cotB-ywrJ</td>
<td>σ^E/σ^K</td>
<td>Glycosylase</td>
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</table>

*Transcriptional regulation is based on information compiled in references 21 and 72.

Numbers associated with protein structures are from the Protein Data Bank.
also reference 29). Similarly, many of the coat proteins identified in C. difficile (Table 2) are not conserved in B. subtilis or other Bacillaceae (11, 24–27). The coat morphogenetic proteins, SpoIVA and SpoVM (see below), however, are found in most sporeformers (28). Two proteins that are not limited to spore-forming species are CotA, a multicopper oxidase (30–32), and Tgl, a transglutaminase (33, 34). It is intriguing that so few coat proteins have discernible homologs in the protein databases. This suggests that most coat proteins have functions distinct from other proteins. But it also raises the possibility that assembly of the coat, like that of the flagellum or the pilus, is governed by a distinctive mechanism that makes the unique mechanical properties and functions of the coat (and perhaps additional as-yet-undiscovered structures) possible. If this view is correct, then the study of coat assembly could reveal fundamentally novel mechanisms of macromolecular assembly.

**FUNCTIONS OF THE COAT: PROTECTION, CONTROL OF GERMINATION, AND INTERACTIONS WITH ENVIRONMENT**

The coat protects against a wide range of assaults, including digestion by predatory microbes and challenges by various chemicals (35–40). The mechanisms of these resistance properties remain obscure (see also reference 41), but it seems reasonable to assume that, at least to a significant degree, the coat acts as a barrier, passively excluding degradative enzymes and toxic molecules. However, the coat is not impermeable, because germinants pass through it en route to the germinant receptors in the inner membrane (see reference 180). Since the coat is porous, protection against small toxic molecules is unlikely to be due to a simple barrier function. It is possible that coat porosity increases its effective surface area (see reference 181). If the coat surface can also detoxify reactive molecules (42, 43), then the rather nonspecific protection that the coat confers against these molecules could be explained. On the other hand, more specific resistance properties, for example, against oxidative compounds, could be provided by the catalases and other enzymes that the coat possesses. Notably, catalases are found in the coat of species as distantly related as B. subtilis and C. difficile (Tables 1 and 2).

Although it is well documented that the coat protects the spore, it has been challenging to determine the role of individual coat proteins in any specific protective function (Table 1). In most cases, inactivating any coat protein gene has no measurable effect on spore resistance properties. For instance, as mentioned above, the B. subtilis spore coat contains the multicopper oxidase CotA, which is responsible for the characteristic pigmentation of spore-forming colonies (31, 32, 44, 45). Even though cotA mutant spores have altered surface topography (46), they do not seem to be severely impaired in their resistance properties. Not all coat protein gene mutations are phenotypically silent; these exceptions are in the morphogenetic coat protein genes, controlling assembly of the other coat proteins (see below). Therefore, while it can be inferred that protection is compromised when certain large subsets of coat proteins are absent (cases that always result in a major structural defect), it is unclear whether the coat’s protective role depends on specific biochemical or structural properties of the individual coat proteins or requires that the coat reach a certain thickness and degree of architectural complexity.

Studies of spores lacking one or another coat layer (achieved, in initial studies, by chemical treatment [4, 47] and, later, by genetic methods [48]) revealed another function for the coat: modulating germination. CwlJ is a B. subtilis coat protein with a relatively well-understood role in germination (49–51). It is a cell wall hydrolase that, along with SleB, degrades the cortex peptidoglycan during stage II of germination (see reference 180). During dormancy, CwlJ is stored in the inner layer of the B. subtilis spore coat, where it is held inactive, most likely by forming a complex with the coat protein GerQ (not to be confused with the germination receptor GerQ in the so-called B. cereus group of species that includes B. anthracis) (52). Interestingly, GerQ is cross-linked by the transglutaminase Tgl; however, tgl mutant spores do not exhibit any readily detected defect in known coat functions, whereas gerQ mutant spores show germination defects (53, 54). Another cell wall hydrolase called YaaH in B. subtilis (Sle in B. anthracis; see reference 178) is present in the inner coat (13, 55) and contributes to peptidoglycan hydrolysis upon germination (56). Importantly, C. difficile spores also contain a spore-cortex lytic enzyme, SleC (CD630_05510) (57, 58). In addition to cell wall hydrolases, the B. subtilis spore coat contains two phospholipases (LipC and CotB) and at least one of them (LipC) is required for efficient germination. It has been hypothesized that LipC could play a role in the degradation of the spore outer membrane (59, 60).

The GerPA-GerPF group of coat proteins has also been implicated in germination, but their roles remain obscure (61). The gerP operon consists of six genes expressed at a late stage in sporulation. Initial studies showed that gerP mutant spores have a defect in receptor-mediated germination, and that this defect is overcome
when a major portion of the coat is removed. These experiments are consistent with the view that GerP proteins reside in the coat and facilitate the passage of germinants. A more recent study confirms these observations (62). In particular, it strengthens the view that GerP proteins somehow mediate the flow of germinants through the coat. How this could occur remains unclear, but the gerP mutation reveals an intrinsic impermeability of the coat to small molecule nutrient germinants. It is possible that GerP proteins form specialized structures within the coat that act as channels for these molecules. Alternatively, GerP proteins could be distributed throughout the coat, globally altering its chemical properties such that germinant molecules pass through easily en route to the germinant receptors in the inner membrane.

An intriguing further possible role for the coat in germination is suggested by the presence of alanine racemases. Because alanine racemases can convert the germinant l-alanine to d-alanine, which does not stimulate germination in species studied so far, alanine racemases can reduce or inhibit germination when l-alanine is present. Such a role has been demonstrated in B. anthracis for an exosporium-associated alanine racemase (63). However, so far, no corresponding phenotype has been observed for a mutation in the B. subtilis coat-associated alanine racemase, YncD (64). Because there is more than one alanine racemase in B. subtilis, redundancy is a possible confounding factor. Interestingly, C. difficile also expresses a gene encoding an alanine racemase during sporulation (65, 66), even though amino acids are not sufficient to trigger germination in that species.

All spores can germinate, but the responses to specific germinants are species specific (180). For instance, in C. difficile, where germination must occur in the gastrointestinal tract of the host, bile is a key germinant (67–69). Furthermore, it is interesting to note that proteins involved in germination are located both in the spore interior, a structure that does not otherwise vary dramatically among species, and in the coat, which varies considerably. As already discussed, we suggest that coat variation is adaptive in survival in diverse environments. It is possible that variation in the coat also facilitates adaptive evolution of germination. In particular, it is appealing to speculate that variation in the coat provides the opportunity for optimizing the response to germinants.

There are reasons to speculate that the coat has roles beyond protection and germination. First, in some species, TEM reveals coat structures with no known functions. These include, for example, the spikelike structures that decorate the Bacillus clausii coat (17) (Fig. 1). Second, in species lacking the exosporium, the coat is the outermost structure and, therefore, mediates interactions with surfaces in the environment (70). Understanding these functions in any species will require better characterization of the ecologically relevant stresses that spores encounter. Therefore, future research in coat function may be driven, to a large degree, by advances in microbial ecology.

Recent work has revealed that the B. subtilis coat contains enzymes involved in polysaccharide synthesis (71, 72). In particular, two glucose-1P nucleotidyltransferases, Spsl and YtdA, are produced in the mother cell late in sporulation and localize to the outer layer of the spore coat. The sps (spore polysaccharide synthesis) operon, which includes spsl, is partially conserved in B. anthracis. In both species, it is required for production of rhamnose (73), a prominent carbohydrate of the spore surface (74). Furthermore, a specific sequence has been identified by phage display as required for docking of SpsC to the forespore surface (75), while the crystal structure of SpsA, a nucleotide-diphospho-sugar transferase, has been reported (76). It has also been shown that spore surface properties are influenced by the presence of spore polysaccharides. For instance, disruption of spsM or spsI renders B. subtilis spores more hydrophobic, thus affecting spore dispersal properties (71, 72, 77). We consider spore surface properties in more detail below.

Another group of coat proteins that includes CotI (YtaA), CotS, YutH, and YsxE has been investigated recently in B. subtilis (78). These proteins are collectively referred to as bacterial spore kinases (BSKs), because they share a structural motif also found in eukaryotic kinases. Importantly, homologous proteins are present in other spore-forming species. It has been suggested that the majority of these BSKs are catalytically inactive because they lack some key amino acids in the active site; however, they may be able to interact with specific substrates. Interestingly, in both B. subtilis and B. anthracis, cotI and cotS are adjacent to the ytdA locus (which is likely involved in spore polysaccharide synthesis, see above). The simultaneous presence of BSKs and genes involved in polysaccharide synthesis or glycosyl transfer is also observed in some Clostridia species (78).
**TABLE 2** *C. difficile* coat proteins (strain 630)

<table>
<thead>
<tr>
<th>Name</th>
<th>Homologous coat proteins in <em>B. subtilis</em></th>
<th>Operon</th>
<th>Regulators (transcription)</th>
<th>Function</th>
<th>Domains</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>Alr2 (CD630_34630)</td>
<td>YncD</td>
<td>alr2</td>
<td>σ^E</td>
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<td>Alanine racemase</td>
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<td>BclA1 (CD630_03320)</td>
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<td>BclA2 (CD630_32300)</td>
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<td>CD630_02130</td>
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<td>YisY</td>
<td>cd2864</td>
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<td>σ^K</td>
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<td>CotA (CD630_16110)</td>
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<td>cotE</td>
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<td>Peroxiredoxin (glycoside hydrolase family 18)</td>
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<td>σK</td>
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<td>SipL</td>
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<td>σE</td>
<td>Spore encasement (functional homolog of <em>B. subtilis</em> SpoVID), interacts with SpoIVA</td>
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<td>SleC</td>
<td>No</td>
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<td>σE</td>
<td>Spore cortex lytic enzyme</td>
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<td>SodA</td>
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<td>SpoIVA</td>
<td>No</td>
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<td>σK</td>
<td>Spore coat assembly, dispensable for cortex formation</td>
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*Transcriptional regulation is based on information from references 65 and 66.*
formation, considering that as much as 1 to 2% of the *B. subtilis* genome encodes coat proteins and at least 15% of all sporulation proteins are coat proteins. Moreover, because several hours are necessary for the synthesis, assembly, and maturation of the coat, it is also one of the most extended multiprotein assembly processes in bacteria.

Morphologically distinct stages of sporulation in *B. subtilis* (Fig. 2; see also Fig. 1 from reference 79) were identified in the 1960s by TEM analysis, and a large number of mutants blocked at these stages were isolated, and their mutations were mapped to specific loci in early studies (80, 81). About 2 h into sporulation, sporulating cells divide asymmetrically to produce a small forespore and a larger mother cell. Shortly after asymmetric division, two cell-specific lines of gene expression are established: one in the forespore, initiated under the control of the alternative sigma factor, σ^E^, and the other in the mother cell, under the control of another alternative sigma factor, σ^K. About half of the known coat proteins are produced from genes that belong to the σ^E^ regulon (21); thus, synthesis of the first group of coat proteins commences shortly after asymmetric division, specifically in the mother cell. The next developmental stage corresponds to the engulfment of the forespore by the mother cell in a process analogous to phagocytosis. Once engulfment is complete, the forespore is physically separated from the mother cell cytoplasm by two membranes (the inner and outer forespore membranes). This morphological transition is accompanied by a transcriptional switch in both the forespore (where σ^G^ takes over from σ^K) and the mother cell (where σ^K replaces σ^E^).

Hence, synthesis of the second group of coat protein genes is controlled by σ^K, also exclusively in the mother cell. Additional transcription factors contribute to the regulation of expression of coat genes: SpoIID in the σ^K regulon and GerE in the σ^K regulon with minor contributions of GerR (82–89). The ultimate stage of sporulation corresponds to the lysis of the mother cell and the release of the spore in the environment. Nevertheless, coat maturation appears to continue even after mother cell lysis, in particular, because cross-linking of coat proteins may be ongoing for several days (22, 53, 90, 91).

This intricate transcriptional regulation has consequences for the production and subcellular localization dynamics of coat proteins in the mother cell (92). With the use of epifluorescence microscopy to analyze fusions of coat proteins to fluorescent proteins, six classes of coat proteins were defined based on localization kinetics (21) (Fig. 3). Most coat proteins localize to the nascent coat in two steps: initially, to the mother cell proximal (MCP) pole of the forespore (the only pole accessible before completion of engulfment) and, subsequently, to the mother cell distal (MCD) pole. The first three classes are composed of σ^K-dependent proteins, whose synthesis begins before completion of engulfment, whereas the last three correspond to σ^K-dependent proteins (i.e., produced after engulfment, when σ^K becomes active). The second factor taken into account in this classification is the stage when coat proteins complete spore encasement (in other words, the amount of time necessary to transition from a single polar cap to a complete shell surrounding the forespore [93]). Class I proteins complete encasement shortly after engulfment, as soon as the MCD pole becomes available, class II proteins when spores become visible by phase-contrast microscopy (during the main stage of cortex synthesis), and class III proteins when phase dark spores turn bright. The degree of spore brightness is correlated with the degree of dehydration of the spore core (i.e., late in sporulation, Ca^{2+}-dipicolinic acid produced in the mother cell is imported in the forespore to replace water, see references 41 and 178 and Fig. 2). The three classes of σ^K-dependent coat proteins are distinguished based on the same morphological transitions: class IV proteins complete encasement as soon as they are produced, class V when spores are still dark, and class VI when spores become phase bright.

In addition to transcriptional regulation, coat assembly is controlled by coat morphogenetic proteins. Deposition on and around the outer forespore membrane is dependent on the coat morphogenetic protein SpoIVA, which functions as a coat anchor (94, 95). Sporulating cells harboring a mutation in spoIVA complete engulfment, but fail to synthesize the cortex. In addition, swirls of coat material are readily detected by TEM in the mother cell cytoplasm. Interestingly, these coat fragments retain the multilayer aspect of the mature coat, suggesting that to a significant degree, coat assembly does not depend on contact with the forespore. A related phenotype (i.e., presence of coat swirls in the cytoplasm) is observed in a spoVID mutant (96), albeit with an important difference, the presence of a cortex, implying that, unlike SpoIVA, SpoVID is not involved in cortex formation. In addition to SpoIVA and SpoVID, the initiation of coat assembly is further controlled by the 26-amino-acid peptide, SpoVM, which is also necessary for cortex production (97). The structure of SpoVM has recently been solved by nuclear magnetic resonance (NMR) and corresponds to an atypical amphipathic helix. This geometry is consistent with a model...
FIGURE 2 Model of spore coat assembly during *B. subtilis* sporulation. In the left column, we list the stages of sporulation as they appear by TEM, phase-contrast microscopy, or fluorescence microscopy in the presence of a membrane stain. The center column contains diagrams of spore coat morphogenesis. Layers of the spore coat are color coded (cyan = basement layer; yellow = inner coat; blue = outer coat; maroon = crust). In the right column, we list the stages of spore coat assembly. DPA, dipicolinic acid. Modified from McKenney et al. (21). See text for details.
where SpoVM would insert deeply into membranes where it could sense variations in acyl chain packing resulting from membrane curvature (98).

It has been suggested that SpoVM is able to sense positive membrane curvature and therefore preferentially localizes to the convex outer membrane of the forespore, as opposed to the plasma membrane of the mother cell, which is negatively curved around the periphery (99). This hypothesis is well supported by in vitro experiments demonstrating preferential localization of SpoVM-green fluorescent protein (GFP) to membrane vesicles of similar size to the B. subtilis forespore and to 2-μm silica beads coated with lipid bilayers (100). In sporulating cells, however, spoIVA is necessary for preferential localization of SpoVM-GFP to the outer forespore membrane (93, 101). Although it is yet unclear
if there is a hierarchy in the recruitment of SpoVM and SpoIVA to the outer forespore membrane, both proteins are essential for proper initiation of coat assembly.

The biochemical characterization of SpoIVA has revealed that it is an ATPase and further demonstrated that ATP hydrolysis is necessary for irreversible polymerization of SpoIVA into cablelike structures (102, 103). Addition of SpoVM and SpoIVA to beads coated with membranes is sufficient to reconstitute the basement layer of the coat in vitro (100). In sporulating cells, however, SpoIVA-GFP will not complete spore encasement in the absence of spoVID (93). The view that SpoIVA assembly requires SpoVID is further supported by biochemical data implying that SpoIVA and SpoVID physically interact (104). Similarly, SpoIVA was shown to interact directly with SpoVM (103). Therefore, the assembly of the innermost layer of the coat (the basement layer) requires the combined contributions of three proteins: SpoIVA, SpoVM, and SpoVID. All three are dependent on σE for synthesis and finish spore encasement as soon as engulfment is completed (i.e., they are class I proteins) (21).

Coat assembly and cortex synthesis are largely independent events as they occur in different compartments of the sporulating cell (the intermembrane space for cortex formation versus the mother cell for coat assembly) and involve a different set of sporulation proteins (cell wall synthesis enzymes versus structural proteins). Nevertheless, as mentioned above, SpoVM and SpoIVA are necessary for both processes and provide a link between the two. In fact, a quality control mechanism has recently been characterized, suggesting that sporulating cells with defective coats are eliminated because they are unable to complete cortex synthesis (105, 106). SpoIVA resides at the core of this mechanism and is targeted for proteolysis in response to coat misassembly. The presence of spore coat defects causes the stabilization of CmpA, a small sporulation protein produced under the control of σE and SpoIIID (thus, shortly after the synthesis of SpoIVA, SpoVM, and SpoVID). CmpA is an adaptor protein that binds to SpoIVA and delivers it to the ClpXP protease for degradation. After elimination of SpoIVA, cortex formation is inhibited and the sporulating cell eventually lyses. Conversely, in the absence of envelope defects, CmpA is rapidly eliminated by ClpXP-mediated proteolysis.

Assembly of coat layers beyond the basement layer is dependent on additional morphogenetic proteins (107). Importantly, the formation of each layer can be associated with the presence of a specific protein: inner layer assembly is dependent on SafA (108, 109), while CotE controls morphogenesis of the outer layer (110). On top of the outer coat, a group of three proteins, CotX, CotY, and CotZ, is necessary for crust formation (13, 111). CotY and CotZ are cysteine-rich proteins that were shown to interact directly (112, 113) and may be subjected to cross-linking by formation of disulfide bonds. Purified CotY even displayed an ability to self-assemble, forming lattices reminiscent of the structure seen in the exosporium of B. cereus group species (114–116). Based on the mapping of genetic dependencies for more than 40 coat protein-GFP fusions, an extended network of interactions between coat proteins has been characterized (13, 117). Coat protein-GFP fusions dependent on safA for localization were assigned to the inner coat. Conversely, if localization required cotE, these proteins were thought to reside in the outer layer. An approximately equal number of coat proteins were assigned to the inner and outer layers, implying that the protein composition of both layers is of similar complexity. In all cases tested, genetic inferences were supported by image analysis at subpixel resolution (13, 55). A subset of cotE-dependent proteins was shown to also be dependent on the cotX cotYZ gene cluster and therefore categorized as crust proteins (13, 111). In addition, atomic force microscopy (AFM) was used to compare the morphology of wild-type spores and various spore coat mutants (including spoVID, safA, and cotE mutants [118]). Remarkably, the AFM data were consistent with the coat assembly model inferred from TEM and fluorescence microscopy studies.

Each morphogenetic protein is connected to many individual coat proteins and therefore represents a hub of the coat interaction network (13). Because recruitment of both SafA and CotE is dependent on SpoIVA, whereas recruitment of CotX, CotY, and CotZ is dependent on CotE, the organization of the coat interaction network is highly hierarchical. While SpoIVA is at the top of the hierarchy for recruitment of coat proteins, SpoVM and SpoVID are at the top of the spore encasement hierarchy. Moreover, it has been shown that encasement by CotE-GFP and SafA-GFP is dependent on spoVM and spoVID, and that SpoVID interacts directly with SafA and CotE (93, 104, 119, 120). Therefore, a model integrating the formation of multiple coat layers and the dynamics of spore encasement has been proposed (7, 21, 107). In this model (Fig. 2), a scaffold organized in multiple layers is initially assembled on one pole of the forespore. Assembly of this multilayered polar cap is dependent on morphogenetic proteins, which all localize to this structure early during coat assembly (i.e., during engulfment of the forespore by the...
mother cell). Subsequently, the coat encases the spore in multiple coordinated waves. The coordination of the successive waves is dependent on transcriptional regulation. Importantly, additional coat proteins can be added to this scaffold even after completion of encasement. This property implies that the coat remains permeable to proteins of a certain size until a late stage in sporulation. For instance, the coat protein CotD is among the last to be produced (class VI), but eventually localizes to the inner coat and will have to cross the nascent outer coat layer to reach its final destination (21).

Morphogenetic proteins in addition to those described above have been documented in _B. subtilis_ including, in particular, CotH and CotO (121–123). By TEM, single mutant spores of cotH and cotO have roughly similar phenotypes, characterized primarily by a disorganized outer coat structure (123). Furthermore, both proteins interact with CotE, since CotH forms a complex with CotE (124, 125), while localization of Cot-O-GFP (YjbX-GFP) to the coat is dependent on cotE (126). Nevertheless, structural differences were noted between the cotH and cotO mutants by AFM (118, 123). CotB and CotG are two outer coat proteins that have been shown to be dependent on CotH (and possibly CotO) for assembly (127). Interestingly, the cotH gene is surrounded by cotB and cotG on either side, and the 5′ UTR of the cotH mRNA overlaps with the divergently transcribed cotG mRNA (128). Unexpectedly, a recent study has suggested that a cotE mutant could be rescued, at least in part, by the overexpression of cotH (129).

Notably, several proteins are proteolyzed in the process of coat assembly. This was noted soon after the first coat proteins were isolated and characterized (130). It is likely that proteases, including the coat protein YabG (and possibly ClpXP, which can degrade SpoIVA), are responsible for at least some proteolytic activity in the coat and/or during coat maturation (106, 131–133). In addition to proteolysis, another important factor in spore coat maturation is protein cross-linking, which, as mentioned above, may occur even after release of the spore from the mother cell (22, 53, 90, 91).

**PHYSICAL AND CHEMICAL PROPERTIES**

The chemical properties of the spore surface have a major impact on the interactions that spores may establish with cells and surfaces in the environment. Therefore, it is reasonable to assume that these properties play important roles in adaptation to diverse environments. For example, the ability of pathogenic spores to adhere to host tissue, and the transport of spores through soil in the environment will be determined, to a large degree, by the chemical properties of the spore surface. These properties are still incompletely understood, in part, because of the relatively few studies that have been conducted and the complexity of this type of analysis. In the case of _B. subtilis_, for example, the spore surface has been reported to have hydrophilic or hydrophobic characteristics, depending on the study (70, 77, 134). The differences among these studies are intriguing and underscore the need for a better understanding of which analytic methods are most useful for spore surface analysis. It is important to point out that this discussion does not address exosporium surface chemistry, which is governed by distinct proteins (70, 135–137). As alluded to above, characterizing spore surface chemical properties is important to understanding aspects of coat function in nature. Spore surface properties are also a major concern in several industries including food preparation (138), where industrial machinery can be fouled by bacteria, including spores, that adhere to equipment surfaces and interfere with the flow of materials through the equipment.

Until recently, the mechanical properties of the spore surface have received little if any study. This is somewhat surprising given that a key feature of the coat, its capacity to fold and unfold, has been evident from observations in the literature extending over decades (18, 19, 46, 139, 140). The coat’s mechanical properties should be of interest to the microbiologist, as an example of a striking biological adaptation with potentially important implications for cell function, and to the biochemist, for what it might teach us about the molecular basis of flexibility at the nanoscale. In this regard, it is striking to note that electron microscopic data clearly indicate that the layers of the coat are not distorted at a fold or in its vicinity, strongly suggesting that the coat does not experience an irreversible mechanical failure when a fold appears, as might be expected from a simple model of coat structure. The molecular basis of this resiliency remains unknown.

Intriguingly, the folds are irregular; they are not uniformly spaced around the circumference of any given spore, and their sharpness and number vary from spore to spore in a population. These observations suggest that the number and heights of the folds are not dictated by specifically positioned molecular cues within the spore. More likely, the folds are an emergent property resulting from more global spore properties, and the variations in the folds’ heights are the result of a variation among
spores. A model at least partially explaining fold morphology and variation argues that a key event in the formation of coat folds is the decrease in spore core volume that occurs after cortex maturation (18). This event induces stress energy in the coat that will resolve by the separation of the coat from the forespore surface at several locations, dictated largely by the spore geometry, the coat stiffness (the Young’s modulus), and the forces binding the coat to the forespore. These separation or delamination events are irreversible (because the cross-linked cortex prevents the core from expanding sufficiently for complete unfolding) and define the locations of the folds for the remainder of each spore’s existence. Folds will change in height after spore release, allowing for the further increase in cell volume that occurs after cortex maturation (19). This insight, in turn, raises the possibility of using spores to store and then releasing this energy to do practical mechanical work (141).

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