OUTLOOK

Spore germination: Two ion channels are better than one

Patrick Eichenberger

Department of Biology, New York University, New York, New York 10003, USA

Germination is the process by which spores emerge from dormancy. Although spores can remain dormant for decades, the study of germination is an active field of research. In this issue of *Genes & Development*, Gao and colleagues (pp. XXX–XXX) address a perplexing question: How can a dormant spore initiate germination in response to environmental cues? Three distinct complexes are involved: GerA, a germinant-gated ion channel; 5AF/FigP, a second ion channel required for amplification; and SpoVA, a channel for dipicolinic acid (DPA). DPA release is followed by rehydration of the spore core, thus allowing the resumption of metabolic activity.

Bacterial endospores (spores) are extraordinarily resilient dormant cells, displaying resistance to high temperatures, desiccation, UV radiation, and many bactericidal chemicals (Setlow and Christie 2023). These resistance properties depend on the correct assembly of the spore structure, which consists of a partially dehydrated core that contains the bacterial chromosome bound to saturation by small acid-soluble spore proteins (SASPs). The core itself is surrounded by a highly impermeable inner membrane with low lipid mobility, concentric protective structures (including a cortex made of modified peptidoglycan), and a multilayered proteinaceous coat. The outermost layer of the spore is called the crust in *Bacillus subtilis* and the exosporium in *Bacillus anthracis*. Both contain glycoproteins that serve as anchors for polysaccharides (Nakaya et al. 2023). A key feature of the core is the presence of a large pool of dipicolinic acid (DPA) chelated with divalent cations (primarily Ca^{2+}) that can represent as much as 25% of the dry weight of the spore. The presence of Ca-DPA is essential for the maintenance of dormancy. Therefore, one of the first steps in germination, the process by which spores emerge from dormancy, is the release of Ca-DPA, allowing its replacement by water in the core (Christie and Setlow 2020). Once a certain threshold of rehydration has been reached, metabolic activity can resume, and the protective structures will either be shed (the coat) or degraded by dedicated enzymes (cell wall hydrolases for the cortex, and proteases for the SASPs).

Spores are ubiquitous, but their presence is not always welcome. For instance, strict procedures are followed in hospitals to avoid nosocomial infections by *Clostridioides difficile* spores (Serrano et al. 2024). Even nonpathogenic spore formers such as *B. subtilis* can cause food spoilage (Wen et al. 2022). Because germinated spores lose most of their resistance properties, an understanding of how germination proceeds at the molecular level will likely have clinical and economic impacts. The triggers for germination are species-specific. Specificity is controlled by receptors that recognize small molecules called germinants, which are often amino acids, like L-alanine, or monosaccharides, like glucose. However, once the germinant is bound to its receptor, it is assumed that the mechanism of germination is largely conserved.

The best-characterized germinant receptor in *B. subtilis* is the GerA complex, which responds to the presence of L-alanine (Fig. 1A). It is composed of two polytopic membrane proteins (GerAA and GerAB) and a lipoprotein (GerAC). In spores, germinant receptors are clustered in a structure called the germinosome (Christie and Setlow 2020). Modeling data using AlphaFold2-Multimer (Evans et al. 2022) suggested that both GerAA and GerAC can form pentamers that produce channel-like structures. These observations implied that the GerA complex could be a ligand-gated ion channel. The binding of L-alanine to GerAB would open the channel and cause the release of monovalent cations (H^+, K^+, and Na^+), increasing the pH of the core (Fig. 1B). The electrochemical activity directed by the GerA complex has been demonstrated in fluorescence microscopy experiments by expressing the complex in vegetative cells and using a potentiometric dye (Gao et al. 2023).

The second important contributor to the so-called stage 1 of germination is the SpoVA complex, which serves as the DPA channel (Gao et al. 2022). During sporulation, an asymmetric cell division generates two compartments.

[*Keywords*: germination; sporulation; dormancy; ion channel; GerA; signal transduction]
Corresponding author: pe19@nyu.edu
Article published online ahead of print. Article and publication date are online at http://www.genesdev.org/cgi/doi/10.1101/gad.351554.124.

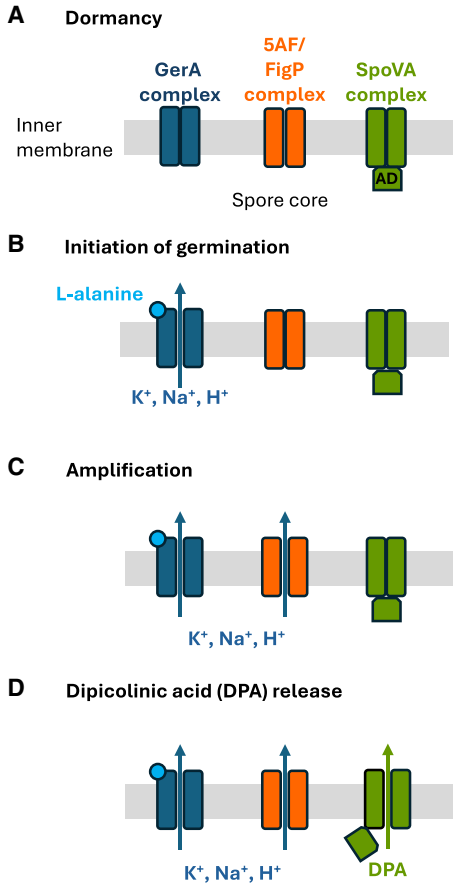

Figure 1. Spore germination in *B. subtilis*. (*A*) Three protein complexes are present in dormant spores: GerA, 5AF/FigP, and SpoVA. (*B*) In the presence of L-alanine, a germinant, the GerA complex is activated, and the resulting opening of the ion channel releases monovalent cations. (*C*) The export of monovalent cations is amplified by the opening of the 5AF/FigP complex. (*D*) The release of monovalent cations causes the opening of the SpoVA complex (the SpoVAD plug is dislodged), and the large pool of DPA in the spore core is expelled. DPA is replaced by water in the core, and metabolism can resume.

The smaller of the two is defined as the forespore, whereas the larger is called the mother cell, which will lyse once the forespore has matured into a spore. DPA is synthesized in the mother cell by the dipicolinate synthase SpoVF. Two channels are then used for importing DPA into the forespore: SpoVV in the outer forespore membrane, and the SpoVA complex in the inner forespore membrane (Ramírez-Guadiana et al. 2017). In contrast, only the SpoVA complex is necessary for DPA export, because the outer spore membrane (if it even still exists) no longer constitutes a permeability barrier in mature spores. However, a key question remained: How is the information translocated from the GerA complex to the SpoVA complex? Since it does not seem that the two complexes interact directly, the simplest explanation is that the release of monovalent cations somehow triggers the opening of the SpoVA complex (Gao et al. 2023). The structural components of the DPA channel are encoded in the *spoVA* locus. Intriguingly, only SpoVAC, SpoVAEb, and SpoVAD are required for DPA export (Fig. 1D). Even more surprisingly, SpoVAF (5AF) is homologous to GerAA and is predicted to form a pentamer resembling the structure found in the germinant-gated ion channel.

The characterization of 5AF and its partner, YqhR (FigP), is the focus of the current study by Gao et al. (2024). The two proteins are shown to form another ion channel, which is not involved in germinant recognition, as it is unresponsive to L-alanine in the absence of the GerA complex. In the absence of the 5AF/FigP complex, however, spores were delayed in ion release. Thus, this second ion channel is required to amplify the response following germinant binding to the GerA complex (Fig. 1C). FigP was identified in a transposon-screening approach (along with four other proteins: YktB, YmfD, YmfJ, and YtpA, whose roles remain to be established). A loss of function of *figP* suppresses the phenotype of the A360V substitution in *5AF*. This allele is characterized by a high frequency of premature germination and is thought to stabilize the open channel conformation. Importantly, in fluorescence microscopy experiments, the 5AF/FigP complex colocalizes with the GerA complex. Nevertheless, clustering of the receptors does not appear to be essential for 5AF activation, leaving open the question of how the 5AF/FigP complex is activated in response to germinant binding. One possibility is that, like the DPA channel, it is responsive to ion release from the GerA complex. Another complication is that some spore formers do not have the 5AF/FigP complex, so it is not viewed as essential for germination. It becomes critical, however, when germinant concentrations are low. Thus, having two distinct ion channels is clearly better than relying on just the GerA complex for efficient spore germination.

The *gerA* and *spoVA* loci had been linked to spore germination for more than three decades, and some structural information was already available (Christie and Setlow 2020); however, the main clue that led to the new model was the ability to use AlphaFold2-Multimer to predict the structure of the complexes (Evans et al. 2022), leading to the realization that they could be channels. Will it soon be possible to confirm these predictions with structural data, perhaps with cryo-electron microscopy? The second important innovation in Gao et al. (2024) was the use of transposon sequencing (Tn-seq) to identify FigP as 5AF's partner. We already know from transcriptomics approaches that there are hundreds of sporulation genes whose roles remain elusive (Arrieta-Ortiz et al. 2015). Tn-seq is bound to become a prominent tool in the discovery of these hidden functions.

References

Arrieta-Ortiz ML, Hafemeister C, Bate AR, Chu T, Greenfield A, Shuster B, Barry SN, Gallitto M, Liu B, Kacmarczyk T, et al.

2015. An experimentally supported model of the *Bacillus subtilis* global transcriptional regulatory network. *Mol Syst Biol* **11:** 839. doi:10.15252/msb.20156236

Christie G, Setlow P. 2020. *Bacillus* spore germination: knowns, unknowns and what we need to learn. *Cell Signal* **74:** 109729. doi:10.1016/j.cellsig.2020.109729

Evans R, O'Neill M, Pritzel A, Antropova N, Senior A, Green T, Žídek A, Bates R, Blackwell S, Yim J, et al. 2022. Protein complex prediction with AlphaFold-Multimer. bioRxiv. doi:10.1101/2021.10.04.463034

Gao Y, Barajas-Ornelas RDC, Amon JD, Ramírez-Guadiana FH, Alon A, Brock KP, Marks DS, Kruse AC, Rudner DZ. 2022. The SpoVA membrane complex is required for dipicolinic acid import during sporulation and export during germination. *Genes Dev* **36:** 634–646. doi:10.1101/gad.349488.122

Gao Y, Amon JD, Artzi L, Ramírez-Guadiana FH, Brock KP, Cofsky JC, Marks DS, Kruse AC, Rudner DZ. 2023. Bacterial spore germination receptors are nutrient-gated ion channels. *Science* **380:** 387–391. doi:10.1126/science.adg9829

Gao Y, Amon JD, Brogan AP, Artzi L, Ramirez-Guadiana FH, Cofsky JC, Kruse AC, Rudner DZ. 2024. SpoVAF and FigP assemble into oligomeric ion channels that enhance spore germination. *Genes Dev* (this issue). doi:10.1101/gad.351353.123

Nakaya Y, Uchiike M, Hattori M, Moriyama M, Abe K, Kim E, Eichenberger P, Imamura D, Sato T. 2023. Identification of CgeA as a glycoprotein that anchors polysaccharides to the spore surface in *Bacillus subtilis*. *Mol Microbiol* **120:** 384–396. doi:10.1111/mmi.15126

Ramírez-Guadiana FH, Meeske AJ, Rodrigues CDA, Barajas-Ornelas RDC, Kruse AC, Rudner DZ. 2017. A two-step transport pathway allows the mother cell to nurture the developing spore in *Bacillus subtilis*. *PLOS Genet* **13:** e1007015. doi:10.1371/journal.pgen.1007015

Serrano M, Diogo Martins D, Henriques AO. 2024. *Clostridioides difficile* sporulation. *Adv Exp Med Biol* **1435:** 273–314. doi:10.1007/978-3-031-42108-2_13

Setlow P, Christie G. 2023. New thoughts on an old topic: secrets of bacterial spore resistance slowly being revealed. *Microbiol Mol Biol Rev* **87:** e0008022. doi:10.1128/mmbr.00080-22

Wen J, Smelt JPPM, Vischer NOE, de Vos AL, Setlow P, Brul S. 2022. Heat activation and inactivation of bacterial spores: is there an overlap? *Appl Environ Microbiol* **88:** e0232421. doi:10.1128/aem.02324-21

Spore germination: Two ion channels are better than one

Patrick Eichenberger

Genes Dev. published online February 5, 2024
Access the most recent version at doi:10.1101/gad.351554.124