BACTERIAL PHYSIOLOGY

New alarm call for spores

Dormant endospores can survive tough conditions over prolonged periods of time, but what cues reactivate them from their torpor? Nutrients are a well-characterized signal for germination of spores but now muropeptides released by growing bacteria have been identified as a general signal for germination, according to a paper published in *Cell*.

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Reasoning that the growth of bacteria could serve as a marker of an environment that is conducive to growth, the authors set out to probe whether growing bacteria produced signals that could induce spore germination. Filtered bacterial culture supernatants from *Bacillus subtilis* and *Escherichia coli*, but not *Staphylococcus aureus*, induced spore germination. Intriguingly, only growing cells produced the spore-germination signal. During growth, enzymes lyse the mature peptidoglycan sac that maintains bacterial cell shape to allow insertion of new peptidoglycan monomers, releasing muropeptides in the process. Gram-negative bacteria can recycle muropeptides, whereas Gram-positive bacteria simply release them into the environment. Because *B. subtilis* supernatants were more efficient germinants than *E. coli* supernatants, the authors proposed that liberated peptidoglycan fragments might induce germination.

Tiny amounts of purified and digested *B. subtilis* peptidoglycan were sufficient to induce spore germination, and high-performance liquid chromatography was used to pinpoint disaccharide tripeptide as the smallest molecule that could induce germination. Purification of peptidoglycan from a selection of Gram-positive and Gram-negative bacteria confirmed that only peptidoglycan with a meso-diaminopimelic acid residue in the third position of the stem peptide induced germination, which explained why *S. aureus* supernatant (L-Lys in the third position of the stem peptide) failed to induce germination.

The ~55 amino-acid PASTA (penicillin and Ser or Thr kinase-associated) domain has previously been proposed to bind peptidoglycan. Many (if not all) Gram-positive bacteria and all bacterial spore formers have at least one Ser or Thr membrane kinase with multiple PASTA repeats in their extracellular domains, and in *B. subtilis* this protein is $PrkC_{Bs}$. Spores of a mutant lacking $PrkC_{Bs}$ failed to respond to muropeptides or culture supernatants, but could germinate in response to nutrients. Fractionation of a tagged protein proved that $PrkC_{Bs}$ is localized to the spore inner membrane, and is therefore ideally positioned to bind small peptidoglycan fragments that can penetrate the spore coat. To test if PrkC discriminates amongst muropeptide signals, the authors replaced the *B. subtilis* $PrkC_{Bs}$ with the *S. aureus* $PrkC_{Sa}$ homologue. *S. aureus* peptidoglycan has L-Lys in the third position of the stem peptide. Spores of the *B. subtilis* mutant that expressed $PrkC_{Sa}$ responded to both meso-diaminopimelic acid and L-Lys-containing peptidoglycan. Finally, the ability of a kinase activator (bryostatin) and repressor (staurosporine) to regulate germination provided clear evidence that the kinase activity of PrkC is essential for peptidoglycan signalling.

Innate immune responses depend on the recognition of specific bacteria-derived molecules such as peptidoglycan. Research into how spores of the humble soil bacterium *B. subtilis* germinate has unexpectedly revealed a new interspecies signal (peptidoglycan fragments) that might have common functions in awakening dormant bacteria.

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ORIGINAL RESEARCH PAPER Shah, I. M., Laaberki, M-H., Popham, D. L. & Dworkin, J. A eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments. *Cell* **135**, 486–496 (2008)