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The Use of Cryotomography to Study the Complex Morphological Remodeling of Membranes in Bacteria

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Electron cryotomography provides 3-dimensional volumes of biological samples at macromolecular resolution [1]. As such, it is delivering long-awaited insights into the structures and cytoskeletal filaments, the cell envelope, flagellar motor, chemoreceptor arrays, and other ultrastructures of bacteria. Since the sample is flash-frozen prior to imaging, the technique is usually not considered as a viable approach to studying dynamic cellular processes. Here we present our data on bacterial endospore formation – a complex morphological process involving extensive membrane remodeling. We show that by having prior knowledge of the different stages of the process, we can selectively trap intermediates and reconstitute the whole process to high resolution using cryotomography.

Endospore formation begins with DNA replication, chromosome segregation and packing, asymmetric positioning of the Z-ring, and septation (reviewed in [2]). This yields a mother cell and a daughter cell, or “prespore”, that are separated by a double-membraned septum. After septum formation the mother cell engulfs the prespore in a process morphologically similar to phagocytosis. Inside the mother cell the forespore matures, adding several layers of cortex, a protein coat and in some species an exosporium. Finally, when the mother cell lyses, the mature spore is released. When favorable conditions return, the spores germinate and new progeny emerge via outgrowth. For decades, the model organism for studying both sporulation and the "Gram-positive" cell type has been the bacterium *Bacillus subtilis* (Figure 1A) [3]. It is a member of the phylum *Firmicutes* and is surrounded by a single membrane and a thick layer of peptidoglycan. *Acetonema longum* is also a member the *Firmicutes* and forms endospores, however, the bacteria are surprisingly "Gram-negative": two membranes and a thin layer of peptidoglycan envelop them (Figure 1B)[4].

Mechanistic clues about how endospore formation came from comparing images of sporulating *B. subtilis* (monoderm) and *A. longum* (diderm) cells (Figure 2) [3-4]. Images of vegetative, sporulating and germinating cells revealed that both monoderm (*B. subtilis*) and diderm (*A. longum*) bacteria produced spores that were surrounded by two membranes. Furthermore, in both cases the two membranes originated from the inner/cytoplasmic membrane of the mother cell. Some time between mid to late spore development and germination, *B. subtilis* loses its outer spore membrane to become a monoderm, "Gram-positive" vegetative cell, whereas *A. longum* retains both spore membranes and, amazingly, the outer spore membrane emerges as an OM. Therefore, endospore formation offers a novel hypothesis for how the bacterial OM could have evolved: a primordial monoderm cell may have first developed the ability to form endospores, and then this process could have given rise to diderm vegetative cells[5].

In addition to cryotomography providing a high resolution imaging of a complex morphological process such as endospore formation, it has further sparked a novel hypothesis about the last common ancestor of all bacteria. We hypothesize that starting with a presumably monoderm primordial cell, the process of endospore formation evolved early. Further evolution of a monoderm bacterium able to sporulate could

then have given rise to a species such as the modern *A. longum* that retained its outer spore membrane. Such a diderm, sporulating bacterium was likely the last common ancestor of all endospore-forming and diderm bacteria (Figure 2)[5].

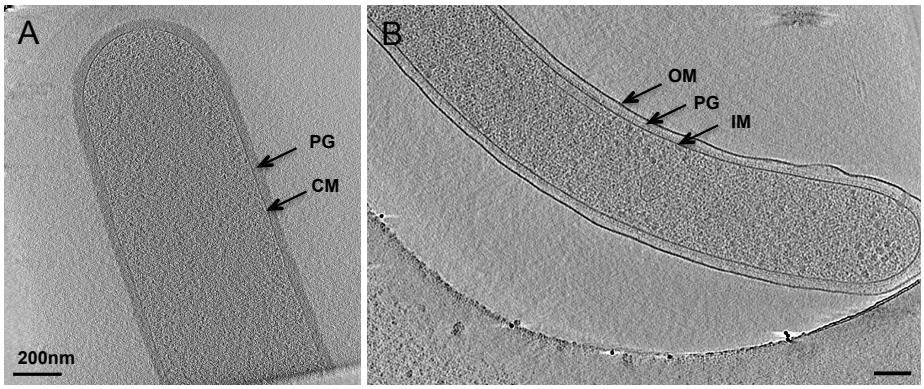

Figure 1. Tomographic slices showing the cell envelope architecture of A) Gram-positive *B. subtilis* cell and B) Gram-negative *A. longum* cell. CM: cytoplasmic membrane, PG: peptidoglycan, IM: inner membrane, OM: outer membrane.

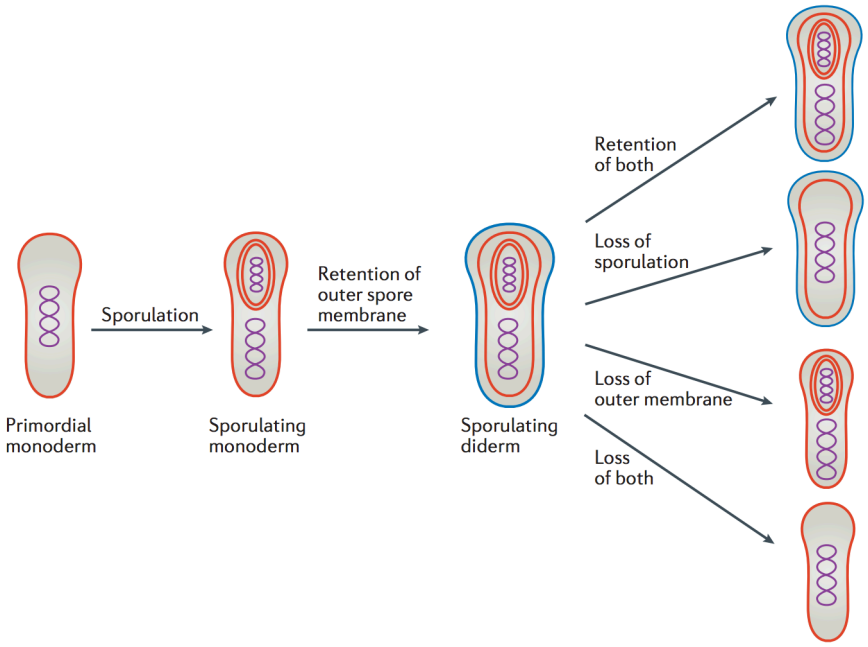

Figure 2. Model for how the outer membrane arose as a byproduct of sporulation and how losses then led to the diversity of modern bacterial cell plans.

References:

[1] Jensen, G. J., & Briegel, A. (2007). How electron cryotomography is opening a new window onto prokaryotic ultrastructure. *Curr Opin Struct Biol, 17*(2), 260-267.

[2] Margolin, W. (2002). Bacterial sporulation: FtsZ rings do the twist. *Curr Biol, 12*(11), R391-392

[3] Tocheva, E. I., Lopez-Garrido, J., Hughes, H. V., Fredlund, J., Kuru, E., Vannieuwenhze, M. S., Brun Y. V., Pogliano K., Jensen, G. J. (2013). Peptidoglycan transformations during *Bacillus subtilis* sporulation. *Mol Microbiol, 88*(4), 673-686.

[4] Tocheva, E. I., Matson, E. G., Morris, D. M., Moussavi, F., Leadbetter, J. R., & Jensen, G. J. (2011). Peptidoglycan remodeling and conversion of an inner membrane into an outer membrane during sporulation. *Cell, 146*(5), 799-812.

[5] Tocheva, E. I., Ortega, D. R., & Jensen, G. J. (2016). Sporulation, bacterial cell envelopes and the origin of life. *Nat Rev Micro, 14*, 535-542.

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