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Ultrastructural analyses of dormant and germinating bacterial endospores

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The soil bacterium *Bacillus subtilis*, like other *Bacillus* and *Clostridium* species, forms spores to survive unfavorable environmental conditions. Spores are robust and can resist heat, radiation, desiccation, pH extremes and toxic chemicals [1]. The presence of nutrients or other chemicals, so called germinants, lead to the activation of a spore and to the transformation into a metabolically active bacterium, which is called germination. During the germination process significant structural and molecular changes occur, like release of molecules from the spore core, rehydration of the core and degradation of the cortex and coats [1]. Although basic ultrastructural data about the germination are available from Santo *et al*. [2], analysis by modern microscopy techniques and correlation with molecular events are still lacking. Thus, we have started a project to get more detailed information about the structural and topochemical aspects of the dormant spore and their germination by using current methods for ultrastructural research.

To study the germination of *Bacillus subtilis* spores we firstly established a germination assay that uses the activation of spores by tryptone soy broth which leads to a rapid and homogenous activation of the entire spore population. Activation of spores was measured over time by phase-contrast light microscopy as a change of the spore´s refraction revealing the time course of activation and germination. To analyze the morphological changes by transmission electron microscopy (TEM) activated spores were fixed at different time points during germination using high pressure freezing. The fixed spores were freeze-substituted, embedded in epon and cut into ultrathin sections. In parallel, samples were chemically fixed and analyzed by light microscopy and by conventional scanning electron microscopy (SEM).

The analysis by TEM revealed six different morphological stages (Fig. 1 A-F) during the germination process. The increase in volume of the spore core (Fig. 1 B) and the distinct rupture within the coats (Fig. 1 D) are obvious hallmarks of the germinating spore. The change of the spore´s refraction in phase-contrast light microscopy correlates with the change from stage one (Fig. 1 A) to stage three (Fig. 1 C), with stage two as an intermediate stage (Fig. 1 B). Furthermore we found particular morphological structures in some stages, for example vesicle-like formations of the inner spore membrane at the poles of the spores. With SEM we could determine five different morphological stages which we correlated with results from TEM and light microscopy. The dormant *Bacillus subtilis* spore possesses a complex structure with a core, a cortex and a multilayered coat. In addition to these well-known structures, we could find features, like crystalline regions within the core and membrane-like loops localized underneath the inner spore membrane. Analysis by cryo-electron microscopy of vitreous sections (CEMOVIS, [3]) proved the presence of these structures in samples that were structurally preserved close to native state. Ongoing investigations are focused on the analysis of the particular structures by using methods like electron tomography and immunocytochemistry. We have also started to analyze the germination process of other species like *Bacillus thuringiensis*, *B. anthracis*, *B. cereus* and *Clostridium difficile* to find out about the similarities and differences in the transformation process from a spore to a vivid bacterium.

1. P. Seltow, Curr. Opin. Microbiol. 6 (2003), p. 550
2. L. Y. Santo and R. H. Doi, J. Bacteriol. 120 (1974), p. 475
3. H. Han, J. Huebinger, M. Grabenbauer, J. Struct. Biol. 178 (2012), p. 84

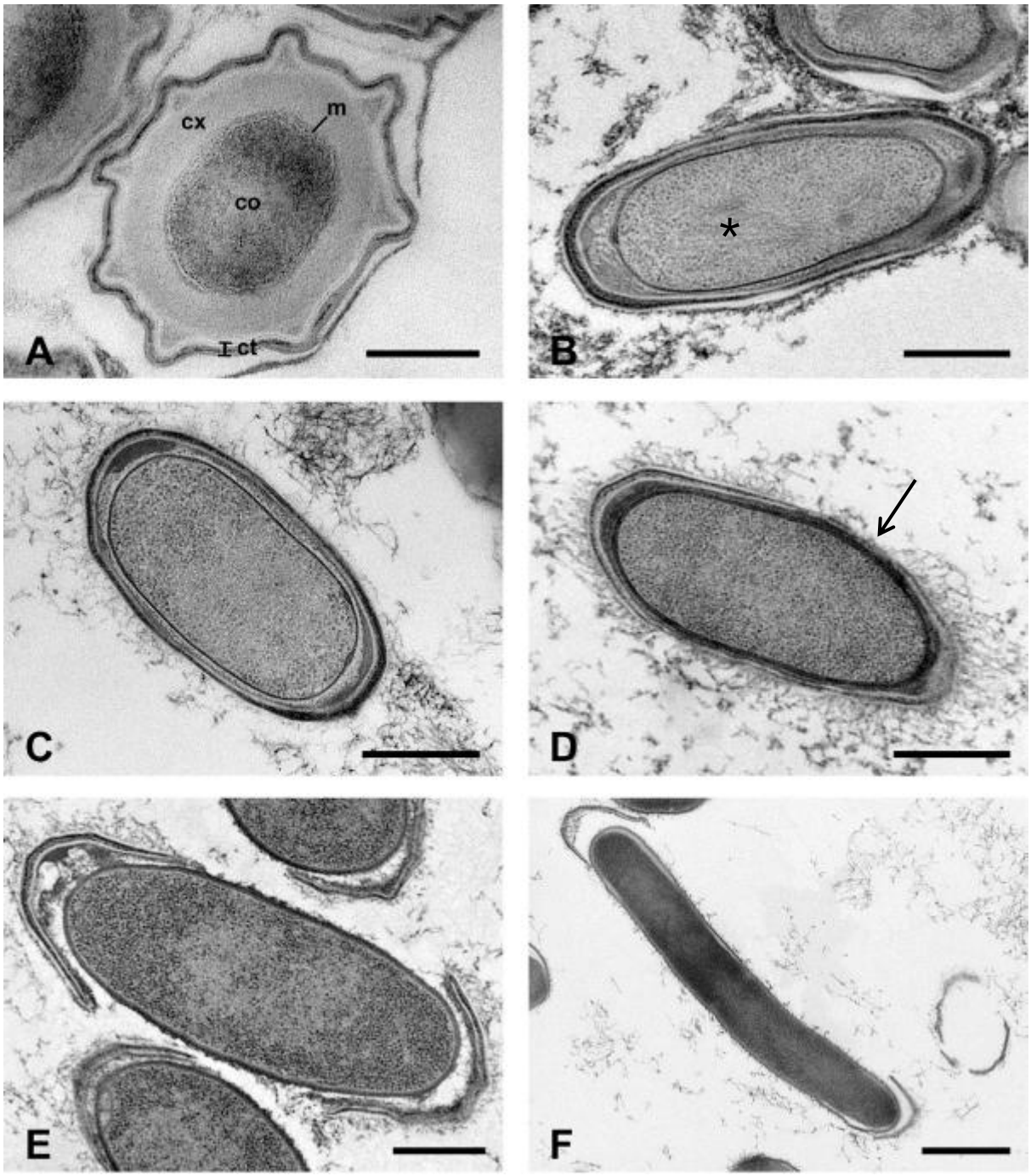

Figure 1. Transmission electron microscopy of ultrathin sections of germinating *Bacillus subtilis* ATCC 6633 spores after high pressure freezing. Six morphological stages could be distinguished. **A** Stage one reveals the typical spore structure with core (co), inner spore membrane (m), cortex (cx) and coats (ct). **B** The spores from stage two show an increased core and beginning cortex degradation. The core exhibit filament-like structures (*). **C** In stage three the cortex is almost degraded and the core has further expanded. **D** The rupture in the coats (arrow) is visible in stage four. **E** The outgrowing spore that has stripped the coat characterizes stage five. **F** Stage six represents the vegetative bacteria, which immediately starts cell division. (Bars: A = 200 nm; B - E = 500 nm; F = 1 µm)