

Bacterial Spore Germination

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Abstract

Some species of the orders Bacillales and Clostridiales can generate spores in sporulation when environments are not conducive to growth. Formed spores are metabolically dormant and can survive for years under harsh conditions, but return to life within minutes in the process of germination when triggered by chemical agents. Some new mechanisms have been identified during spore germination, including dipicolinic acid (DPA) leakage and spore germination memory, but the precise molecular details of the signal transduction process remain to be identified. The review focused on the mechanism of DPA leakage and spore germination memory and hypothesis for the future investigation.

Keywords: Spores; Germination; DPA leakage; Germination memory

Overview of Germination

Many members of the Firmicutes phylum, in particular Bacilli and Clostridia, can form spores under adverse conditions for growth, such as starvation. These spores are metabolically dormant and are extremely resistant to a variety of environmental stresses. Although spores can keep dormant for years, they can restore vegetative growth in several minutes when conditions become favorable [1,2]. Several major events occur in a defined order during spore germination [3,4]. Initially, spore sensor environment, and some spores commitment to germinate, even if the germinant is removed or displaced from its cognate germinant receptor (GR). This commitment step is followed by release of monovalent cations, as well as the spore core's large pool (ca. 25% of core dry weight) of pyridine-2,6-dicarboxylic acid (dipicolinic acid [DPA]) along with divalent cations, predominantly Ca²⁺, that are chelated with DPA (CaDPA). CaDPA release triggers spores' peptidoglycan (PG) cortex

degradation by cortex-lytic enzymes (CLEs). Subsequently, cell wall PG expands, spore core swells and water is up-taken, and core protein and inner spore membrane lipid mobility was restored. When the core water content rises to ca. 80% of wet weight, equal to that in the growing cell, metabolism in the core begins, followed by macromolecular synthesis, ultimately converting the germinated spore into a growing cell in the process of outgrowth. Consequently, spore resistance loses [5].

DPA Leakage and Commitment

When spores sense nutrient or non-nutrient germinant, some spores initiate slow CaDPA leakage [6]. D-alanine block experiments indicated that all spores that initiate slow CaDPA leakage are committed to germinate. Other work has confirmed that the time at which individual spores of Bacillus species commit to germinate is at or very near the time at which the permeability of the

spores' IM increases. The increase of permeability of the spores' IM leads the monovalent cations Na⁺, K⁺, and H⁺ rapid release and CaDPA is also rapidly released by heat treatment such as that at 70°C that have no effects on dormant spores [7,8]. Furthermore, the change of channel protein SpoVA number affects the DPA leakage speed [6]. All the results indicated the equal of CaDPA leakage beginning and commitment occurrence. However, there are a number of unanswered questions and uncertainties as follows. (i) What is the change that triggers a spore to actually initiate slow CaDPA leakage and commit to germinate; (ii) Under high temperatures, the SpoVA channel is much easily to partially activate and initiate CaDPA release, but the molecular mechanism for this phenomenon is far from known; (iii) It is unknown that the values of slow CaDPA leakage differ so much in germination triggered by nutrients, dodecylamine, and CaDPA. It is speculated that all CaDPA release is via partial activation of SpoVA channels. Therefore, more information is needed on the composition, structure, and regulation of the function of SpoVA channels for CaDPA in order to completely understand the early events in the germination of bacterial spores.

Spore Germination Memory and Signal Transduction

A recent finding about spore germination is that spores display memory of germinant stimuli such that spores given a 1st germinant pulse exhibit greater germination after a 2nd pulse soon after the 1st one, but this memory decays with the increase of interval period between two pulses [9]. This memory of a 1st germinant pulse can be transferred between nutrient and non-nutrient germinants, and has been observed in germination with *Bacillus* and *C. difficile* spores. Spore memory can significantly enhance sensitivity to low nutrient germinant concentrations. Furthermore, it is speculated that memory is mostly likely generated and stored in the CaDPA release channel, perhaps by a protein(s)' conformational change [3]. However, the detailed signal transduction pathway is worthwhile to be further investigated.

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