The Ser/Thr protein kinase PrkC imprints phenotypic memory in *Bacillus anthracis* spores by phosphorylating the glycolytic enzyme enolase

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Bacillus anthracis **is the causative agent of anthrax in humans, bovine, and other animals.** ***B. anthracis*** **pathogenesis requires differentiation of dormant spores into vegetative cells. The spores inherit cellular components as phenotypic memory from the parent cell, and this memory plays a critical role in facilitating the spores' revival. Because metabolism initiates at the beginning of spore germination, here we metabolically reprogrammed** ***B. anthracis*** **cells to understand the role of glycolytic enzymes in this process. We show that increased expression of enolase (Eno) in the sporulating mother cell decreases germination efficiency. Eno is phosphorylated by the conserved Ser/Thr protein kinase PrkC which decreases the catalytic activity of Eno. We found that phosphorylation also regulates Eno expression and localization, thereby controlling the overall spore germination process. Using MS analysis, we identified the sites of phosphorylation in Eno, and substitution(s) of selected phosphorylation sites helped establish the functional correlation between phosphorylation and Eno activity. We propose that PrkC-mediated regulation of Eno may help sporulating** ***B. anthracis*** **cells in adapting to nutrient deprivation. In summary, to the best of our knowledge, our study provides the first evidence that in sporulating** ***B. anthracis*****, PrkC imprints phenotypic memory that facilitates the germination process.**

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This article contains Figs. S1–S7 and Table S1.
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Deciphering the role of metabolic enzymes that orchestrate morphogenic transition states in bacteria is a fundamental question. *Bacillus anthracis* is the causative agent of anthrax in humans, bovine, and other animals (1, 2). It is known to survive hostile environments by forming spores that remain quiescent and retain minimum metabolic activity (3, 4). As a pathogen, the success of *B. anthracis* depends on the spore's ability to develop into a growing vegetative cell. Under favorable conditions, spore metabolism is triggered to support energy needs and to develop into a fully functional cell (5). The metabolic checkpoints and energy reserves in the spore provide different stimuli at an early growth stage and ensure the completion of the developmental program. Therefore, the transformation of a dormant spore into a vegetative cell is a key step in the pathogenic cycle of *B. anthracis*. However, the molecular events leading to successful spore dormancy and later to germination remain to be fully elucidated.

During the process of germination, some spores disintegrate their protective structure and grow into vegetative cells (5). Sporulating cells carry a substantial set of macromolecules (including several proteins) through their journey from progenitor cell to spore (6). The role of these proteins remains largely unknown. This carryover of cellular components is termed "phenotypic memory" (7). The efficiency of spore germination has been shown to be determined by this phenotypic memory inherited from the parent cell (7, 8). In a recent study, the role of one such protein, alanine dehydrogenase, which controls alanine-induced outgrowth in cellular memory, was described (7). Because the protein cargo remains constant in the spore, these proteins decide the fate of the reviving spore depending on environmental conditions and sensory signaling molecules (9). There have been considerable efforts toward understanding the spore germination process and involvement of signaling mechanisms (10–12).

The link between metabolism and the spore revival process is not explored well. At the onset of germination, metabolism resumes without requiring new macromolecular synthesis (13). Reports in *Bacillus subtilis* have highlighted the essentiality and

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