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# Overview: development in bacteria: spore formation in *Bacillus subtilis*

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**Abstract.** Like eukaryotes, bacteria possess complex developmental programs that drive environmental adaptation and morphological differentiation. In some species, these morphological changes are quite elaborate and result in major changes in cell appearance, including the formation of ornate appendages. The ease with which some bacteria can be manipulated makes them highly attractive model systems for developmental analysis. In this set of reviews, we tackle the best studied of these systems, spore formation in *Bacillus subtilis*. Construction of a spore initiates in response to starvation, takes each cell about 8 h and is directed by a tightly controlled genetic program. First, the cell creates an internal protoplast with its own copy of the chromosome. Over the next several hours, development continues as proteins synthesized within the protoplast as well as in the surrounding cell cytoplasm coalesce into the various complex structures that comprise the spore. The resulting cell is metabolically dormant and as close to indestructible as any cell found on earth. Nonetheless, the spore retains the ability to revive almost immediately when nutrient returns to the environment. Here, we review the genetic control of spore formation, the structure and assembly of several major spore components, the process of germination, and the environmental and disease implications of spores. As these reviews document, spore formation in *B. subtilis* has been among the most productive systems for understanding both the broad themes and the molecular basis of development. Not only does this system continue to add to our understanding of these questions, but it provides a particularly powerful means to address the cell biological dimension of development.

**Key words.** Development; spore; *Bacillus subtilis*; sporulation; bacteria; assembly; adaptation.

The capacity to generate a variety of morphologically distinct cell types permits complex multicellular organisms to arise from a single fertilized cell. This marvelous ability is not restricted to higher organisms; bacteria also possess developmental programs. As in higher organisms, development in bacteria can be as subtle as the alteration of expression of just a few genes or as profound as a major reorganization of the cellular architecture. Several well-studied examples of bacteria which radically alter cellular morphology during development include the members of the genera *Streptomyces* [1] and *Anabena* [2], and the bacterium *Caulobacter crescentus* [3].

The greatest degree of morphological complexity is found among the Myxobacteria [4, 5]. These predatory organisms can live as single cells when their preferred food, other bacteria, is abundant. When nutrient is limiting, however, about $10^5$ cells coalesce into a dense swarm, guided by chemotactic signals [6]. After the appropriate number of bacteria have congregated, they begin to form a discrete multicellular structure called a fruiting body. In some species, this structure resembles a mound. In others, it is quite dramatic in appearance, possessing a series of elegant protruding stalks [5, 7]. This developmental process results in the production of terminally differentiated cells that can be dispersed and repopulate new niches. For some species, these specialized cells reside in the interior of the mound; in other species, they are at the tips of the stalks. From extensive work in many labs, the molecular events that direct these elaborate events are coming to light.

These and other bacterial systems are exciting models for development because of the ease and rapidity with which they can manipulated. It should come as no surprise that many of the fundamental questions in development originally posed in eukaryotic systems, such as establishment of cell polarity, and temporal and spatial control of gene

expression, are relevant to bacteria as well [8, 9]. As a result, studies of bacterial development have and will continue to inform our understanding of this process in all organisms.

The reviews here address one of the best-understood examples of bacterial development: the formation of endospores by *Bacillus subtilis*. Although we will consider only *B. subtilis* and a few of its close relatives in detail, many Gram-positive bacteria, including members of the Bacilli and Clostridia, produce endospores according to a program similar to that of *B. subtilis* [10–12]. When confronted by nutrient depletion, these normally rod-shaped organisms produce an oval, dormant cell called a spore, in a process called sporulation. The spore is entirely distinct from the vegetative cell, possessing several molecules and cellular structures seen nowhere else in nature [13–16]. These unique components contribute to the spore's most striking characteristics: it is metabolically inactive, highly resilient to environmental assault and stable for extreme periods of time. In spite of its inert state, the spore can sense the reappearance of even minute amounts of nutrient in the environment, and respond by converting back to a vegetatively growing cell [17]. This process, called germination, is reviewed here by Moir. Sporulation is not simply a way to survive the occasional stress. Rather, as reviewed by Nicholson, spore forming organisms take advantage of their special capabilities to populate a variety of inhospitable environments including the soil and the ocean [11, 18]. As discussed by Aronson, some spore formers have learned to use sporulation as a mode of pathogenesis.

The study of spore-forming bacteria dates back to some of the earliest studies on transmissible disease. 124 years ago, Robert Koch described the morphological events during sporulation in *Bacillus anthracis*, the causative agent of anthrax [19]. In the same issue of *Beiträge zur Biologie der Pflanzen*, Ferdinand Cohn reported sporulation in *Bacillus subtilis* [20]. Using the light microscope, these early investigators made the extraordinary observation that the initial stages of sporulation involve the formation of an internal compartment, called the forespore, within the rod shaped bacterium. After several hours the surrounding cell, called the mother cell, lyses, exposing the internal cell, now a dormant spore, to the outside world. Modern studies of sporulation have beautifully elaborated this initial view and we can now draw a relatively detailed picture of the molecular events underlying these morphological transitions.

We now know that sporulation begins well before the first morphological changes detected by light microscopy [21]. Cells must sense and measure a complex variety of parameters, of which nutrient levels are only one, before they can commit to sporulation. The mechanisms used by the cell to make this decision are described in the review by Strauch. After the cell commits to sporulation (referred to as stage 0, fig. 1A), it builds a septum (marking stage II, fig. 1B). Unlike the septum that appears during vegetative cell division, this one is positioned off to one side, generating a smaller forespore compartment and a larger mother cell compartment. Soon afterward, an endocytosis-like event converts the forespore into a protoplast, possessing two membranes (during stage III, fig. 1C). Popham reviews the synthesis and function of a unique organelle, composed of two peptidoglycan structures called the germ cell wall and cortex, that is deposited between these membranes (during stage IV) and which is essential to spore dormancy. The next structure to be built is a thick protein shell that encases the forespore, called the coat (at stage V, fig. 1D). Watabe discusses the complex assembly events that guide deposition of the many coat proteins. Taken together, these reviews present a comprehensive picture of the molecular control of development during sporulation and the ecological consequences of this specialized life style.

A word is in order about the nomenclature used to refer to sporulation and germination genes. Generally, these genes are named according to the morphological stage at which mutations in them halt spore development. For example, mutations in *spoII* genes arrest development at stage II and do not allow progression to stage III. Genes whose products are required for the initiation of sporulation and the proper formation of the asymmetrically positioned septum are denoted as *spo0*. Different operons are distinguished by the addition of a single capital letter (e.g. *spoIIA*, *spoIIB* and so on). Different cistrons within a given operon are denoted by additional letters (e.g. *spoIIAA*, *spoIIAB* and so on). Protein products of these genes are denoted using standard rules (SpoIIAA, SpoIIAB and so on). However, not all genes and proteins involved in sporulation are identified with this nomenclature. Many genes have been named (or renamed) based

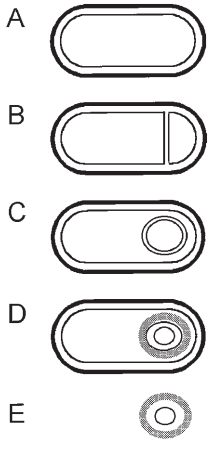


Figure 1. Stages of sporulation. (*A*) Stage 0/I, (*B*) stage II, (*C*) stage III, (*D*) stage IV, (*E*) released spore. The hatched line around the spore in panels *D* and *E* is the coat.

upon the biochemical function of their protein products: for example, *spo0H* has been shown to encode a sigma factor of RNA polymerase and has been renamed sigH ($\sigma^H$).


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