16 Prokaryotic Life Cycles

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Introduction

Prokaryotic development generates a cell with a different purpose from the parent particularly (1) dormancy, (2) nutrient acquisition, or (3) dispersal. Some species produce specialized cells that simultaneously perform two of these functions, such as baeocytes and zoospores, which function in both dormancy and dispersal. Some organisms have complex life cycles that produce several different types of specialized cells, such as *Anabaena* and *Rhodomicrobium*, and are best viewed with a more holistic bend that focuses on the entire life cycle. This chapter compares the structure, function, and, where known, the mechanism of differentiation of the three classes of specialized cell types. This approach has the advantage of illustrating and emphasizing similarities and differences in origin, means, and mechanics.

Resting cells are usually generated in response to nutrient limitation and provide a metabolically quiescent state that permits survival during famine or drought. The *Bacillus* endospore is the most carefully studied resting cell. However, dormant cells are formed by many mechanisms, each with a unique evolutionary origin, attesting at once to the efficacy of this survival approach (Table 16.1). An interesting twist to endospore formation is found in the closely related genera *Metabacterium*, which forms two or more internal endospores, and *Epulopiscium*, which produces two live offspring internally. In *Metabacterium*, binary fission is no longer the sole means of reproduction. Instead, endospore formation has become hardwired into the cell division cycle and may be the primary means of proliferation (Angert and Losick 1998). Endospore formation was modified further in *Epulopiscium* to produce live offspring internally (Angert et al. 1996). With a few modifications to the endospore developmental program, the purpose of the life cycle seems to have shifted from dormancy to reproduction.

Some cyanobacteria produce specialized cells for fixing nitrogen. All living cells require organic nitrogen, but only a few bacteria can reduce atmospheric nitrogen (N_2) to ammonia (NH_3), an ATP-dependent process that utilizes the enzyme nitrogenase. Nitrogenase is oxygen labile. While some bacteria deal with the O_2 sensitivity of nitrogenase by growing in anaerobic or microaerophilic environments, the problem is compounded in organisms where oxygenic photosynthesis is the primary means of growth. There are a wide variety of symbioses involving nitrogen-fixing bacteria, for example, the *Rhizobium*-legume plant symbiosis. In this symbiosis, the legume produces an O_2 binding protein known as leghemoglobin to protect nitrogenase. The most well studied example of a single species producing a specialized cell type for nitrogen fixation is the *Anabaena* heterocyst. Nitrogen-fixing cyanobacteria have devised two strategies to deal with nitrogenase sensitivity to O_2. Some species use a circadian clock to separate the two processes temporally. Other filamentous species, like *Anabaena*, use a heterocyst to fix nitrogen thus separating the two processes spatially. In *Anabaena*, the fixed nitrogen is shuttled from the heterocysts into the photosynthetic cells.

Nongrowing, motile cells are differentiated in some species as a mechanism for dispersal (Table 16.1). The most well studied example is the swarmer cell of *Caulobacter crescentus*, which is produced by a sessile stalked cell as an obligatory part of the cell division cycle. Dispersal mechanisms are found in many different phyla suggesting their utility in long-term survival of the species. Among them are zoospores that are both dormant and flagellated. Zoospores are produced by certain Actinobacteria like *Kineococcus* and the baeocytes of *Pleurocapsa*. The hormogonia of *Nostoc* are multicellular filaments that move by gliding on surfaces and serve as the infective units during symbiosis with certain plants.

Prokaryotic development is strictly asexual unlike eukaryotic development, which generates progeny with genotypes that differ from either parent. Prokaryotic development is often a direct or indirect response to nutrient limitation particularly carbon, nitrogen, and/or phosphorus. In *Caulobacter crescentus*, growth in oligotrophic (nutrient-limited) environments led to a life cycle whereby a sessile parent produces motile progeny that disperse to reduce competition with the parent. Here, the life cycle is hardwired and not directly induced by nutritional cues, but long-term growth in oligotrophic environments may have shaped the developmental program to reduce competition with the stalked parent.

This chapter is meant to serve as an introduction to the fascinating diversity of life cycles among prokaryotes. Precious

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Table 16.1
Types of prokaryotic differentiated cells

Function	Cell	Representative genus	Phylum
Dormancy	Aerial spore	*Streptomyces*	Actinobacteria
	Akinete	*Anabaena*	Cyanobacteria
	Baeocyte	*Pleurocapsa*	Cyanobacteria
	Cyst	*Azotobacter*	γ-Proteobacteria
	Cyst	*Rhodospirillum*	α-Proteobacteria
	Cyst (myxospore)	*Myxococcus*	δ-Proteobacteria
	Elementary body	*Chlamydia*	Chlamydia
	Endospore	*Bacillus*	Firmicutes
	Endospore	*Metabacterium*	Firmicutes
	Exospore	*Methylosinus*	α-Proteobacteria
	Exospore	*Rhodomicrobium*	α-Proteobacteria
	Zoospore	*Kineococcus*	Actinobacteria
Nutrient acquisition	Heterocyst	*Anabaena*	Cyanobacteria
Dispersal	Attack phase cells	*Bdellovibrio*	δ-Proteobacteria
	Baeocyte	*Pleurocapsa*	Cyanobacteria
	Hormogonium	*Nostoc*	Cyanobacteria
	Swarmer cell	*Caulobacter*	α-Proteobacteria
	Swarmer cell	*Rhodomicrobium*	α-Proteobacteria
	Swarmer cell	*V. parahaemolyticus*	γ-Proteobacteria
	Zoospore	*Kineococcus*	Actinobacteria

little is known about the details of most prokaryotic developmental cycles. Nevertheless insights into certain genetic and biochemical strategies are evident from those few organisms that have been studied.

Differentiation Leading to Dormancy

Resting cells are generated in response to nutritional stress and can maintain dormancy for long periods of time until conditions favor growth. Various types of resting cells include aerial spores, akinetes, baeocytes, cysts, elementary bodies, endospores, exospores, and zoospores, which are formed by different physical processes (◉ *Table 16.1*). Nevertheless, they have some structural and functional similarities: (1) dormancy is achieved by dehydrating the cytoplasm, (2) additional layers around the cell enhance resistance to environmental stresses, and (3) storage molecules are produced to aid in germination.

Aerial Spores. Streptomyces, one of the genera comprising the Actinobacteria, is distinguished by hyphal growth. Following spore germination, vegetative hyphae are produced that form a thick network on the substrate surface. Cell division is rare in the vegetative hyphae and not essential for growth or viability (McCormick 2009). Next, aerial hyphae are extended vertically. The multinucleate aerial hyphae coil and then synchronously septate many times to produce a chain of spores (◉ *Fig. 16.1*). The production of spores on aerial hyphae gives the colonies a powdery appearance. Many genes that mediate development have been identified. The *bld* (bald) mutants cannot form aerial hyphae, and the *whi* (white) mutants cannot form the white aerial spores.

The *bld*-signaling cascade acts as a checkpoint between growth of vegetative hyphae and production of aerial hyphae (Willey et al. 2006). The *bld*-signaling cascade includes 5 extracellular oligopeptides and 'A'-factor, a γ-butyrolactone, whose role appears to be activation of the *ram* (rapid aerial mycelium) and chaplin operons. The *ramCSAB* genes synthesize and secrete surface-active peptide SapB, product of the *ramS* gene. SapB is initially synthesized as a 42 kDa protein then processed and modified to form a double cyclic structure similar to lantibiotics (◉ *Fig. 16.2*). The *bld* genes also stimulate production of the surface-active chaplin proteins that bind to the cell walls of aerial hyphae. The chaplins and SapB initiate escape of the aerial hyphae from the colony surface by reducing surface tension.

The *whi* genes mediate spore differentiation of the multinucleate aerial hyphae. WhiH activates expression of the cell division gene *ftsZ* to initiate septation of the aerial hyphae. This is a critical and specific developmental step since FtsZ is not essential for hyphal growth due to the lack of cross-walls. The spores are metabolically quiescent resting cells and are resistant to desiccation and to slightly elevated temperatures (i.e., 55 °C).

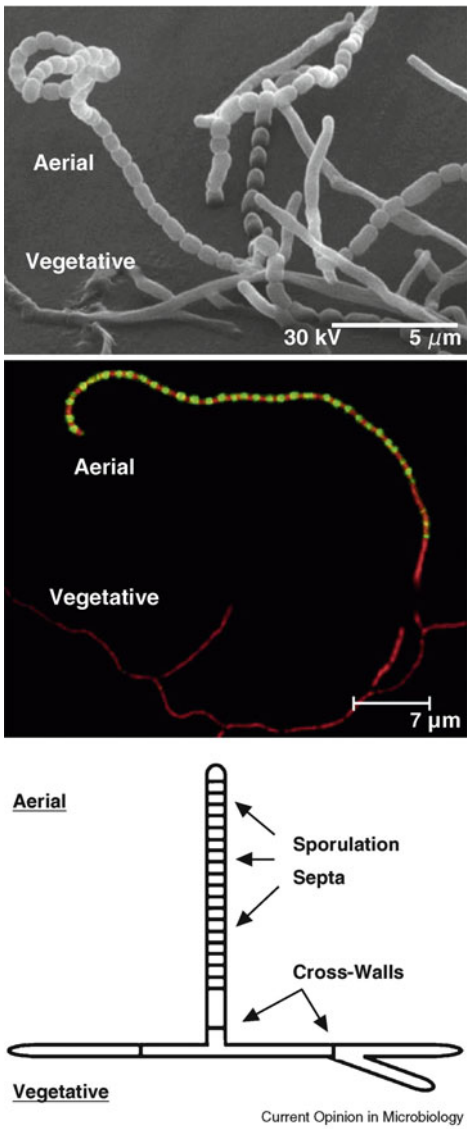


Fig. 16.1
Microscopic images of *S. coelicolor* sporulation and synchronous developmentally regulated cytokinesis. (*Top*) A scanning electron micrograph illustrates the culmination of development, the production of a string of spores from a long apical cell of an aerial hypha (aerial). Branching syncytial vegetative hyphae are located near the bottom (vegetative). (*Middle*) A laser confocal scanning micrograph of a wild-type strain expressing FtsZ–EGFP (*green*) is shown. Nucleic acid is stained with propidium iodide (*red*). Evenly spaced FtsZ rings are spatially restricted to the differentiating aerial hypha (aerial). Branching syncytial vegetative hyphae (vegetative) are devoid of FtsZ rings, and the genomic material is not segregated into well-defined nucleoids. (*Bottom*) Diagram shows the locations of widely spaced vegetative cross-walls and evenly spaced sporulation septa (From McCormick 2009)

Akinetes. Resting cells produced by some cyanobacteria, akinetes are formed from a vegetative cell that has become enlarged by cell wall thickening. *Anabaena* akinetes often form next to heterocysts (Fig. 16.3). *Nostoc punctiforme* and *Anabaena* strain CA convert to akinetes at the end of exponential growth suggesting that the conversion is caused by nutritional shift down. A DNA microarray consisting of 6,893 *N. punctiforme* genes suggested that akinete formation is accompanied by downregulation of genes involved in primary and secondary metabolism (Campbell et al. 2007). Akinetes often possess storage materials such as glycogen, a polymer of glucose, and cyanophycin, a polymer of arginine and aspartate. *Anabaena cylindrica* (PCC 6309) akinetes exhibit resistant to desiccation and subfreezing temperatures but sensitivity to ultraviolet light and temperatures above 60 °C (Olsson-Francis et al. 2009).

When the akinetes germinate, the spore coat usually ruptures, and the germline begins to grow and divide. When this happens to a chain of akinetes, an unusual pattern forms (Fig. 16.4). If germination takes place in the absence of fixed nitrogen, heterocysts appear at approximately every seventh cell. The process of heterocyst production is discussed in section "Nutrient Acquisition."

Cysts. A single vegetative cell rounds up and accumulates a thick coat to produce a cyst. Cyst formation has been observed in many bacterial groups including the myxobacteria, *Azospirillum, Azotobacter*, and the purple photosynthetic bacterium *Rhodospirillum*. Technically speaking, the akinete is a cyst, though for historical reasons, it is given a separate name. Given the phylogenetic diversity of organisms that undergo encystment and the diversity of cyst wall structures, cysts are likely to have several evolutionary origins.

Azotobacter vinelandii is an aerobic soil bacterium that fixes nitrogen while simultaneously protecting nitrogenase from oxygen damage (Fig. 16.5). When carbon is limiting, *Azotobacter* forms cysts that are resistant to desiccation (Setubal et al. 2009). The cells shed their flagella, cease nitrogen fixation, gradually become round, and finally become optically refractile. The cyst is surrounded by a multilayered outer coat called the exine, consisting of lipoprotein, polysaccharide, and phenolic lipids (Fig. 16.6). Alginate, a polysaccharide composed of 1-4-linked beta-D-mannuronic acid and alpha-L-guluronic acid, protects nitrogenase from oxygen damage but is also a major component of the cyst coat. Germination, which occurs when the cysts are placed in the presence of a carbon source such as glucose, immediately induces respiration, macromolecular synthesis, and the conversion of the cyst to the vegetative cell. Alginate can be degraded by alginate lyases, which hydrolyze the polysaccharide using a beta-elimination reaction. While *Azotobacter vinelandii* strains produce several different alginate lyases, mutants lacking alginate lyase AlyA3 germinate poorly compared to wild-type cells suggesting that this enzyme is responsible for degrading the exine (Gimmestad et al. 2009).

In *Rhodospirillum centenum*, development of a cyst involves accumulation of polyhydroxybutyrate (PHB) storage granules, loss of flagella, and change in cell shape. An exine protective outer coat typically surrounds four to eight cells and provides resistance to desiccation. One of the three chemotaxis-like signal transduction cascades initiates cyst development. Another

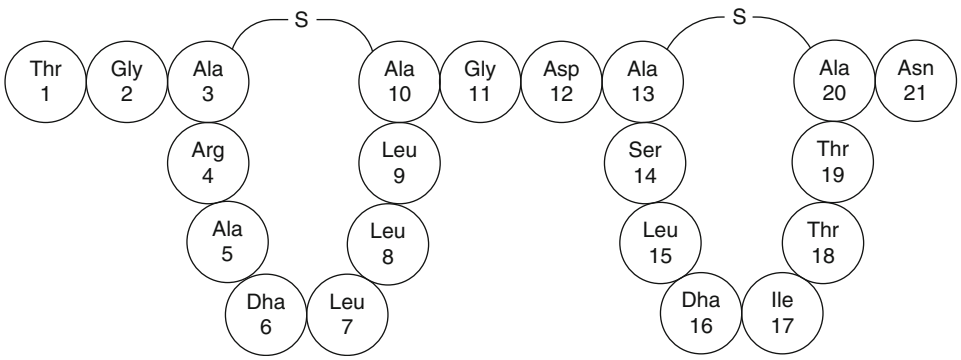


Fig. 16.2
SapB primary sequence. SapB maturation is believed to occur in three stages. First, serine residues are dehydrated giving rise to 2,3 didehydroalanine (Dha) residues. Next, nucleophilic attack by the cysteine sulfhydryl residues at positions 31 and 41 (10 and 20 in the mature protein) on the β-carbon of Dha at positions 24 and 34 (3 and 13 in the mature protein) gives rise to intramolecular cross-links, each of which consists of two alanine residues connected by a thioether linkage. Finally, the leader is removed to generate mature SapB (Adapted from Willey et al. 2006)

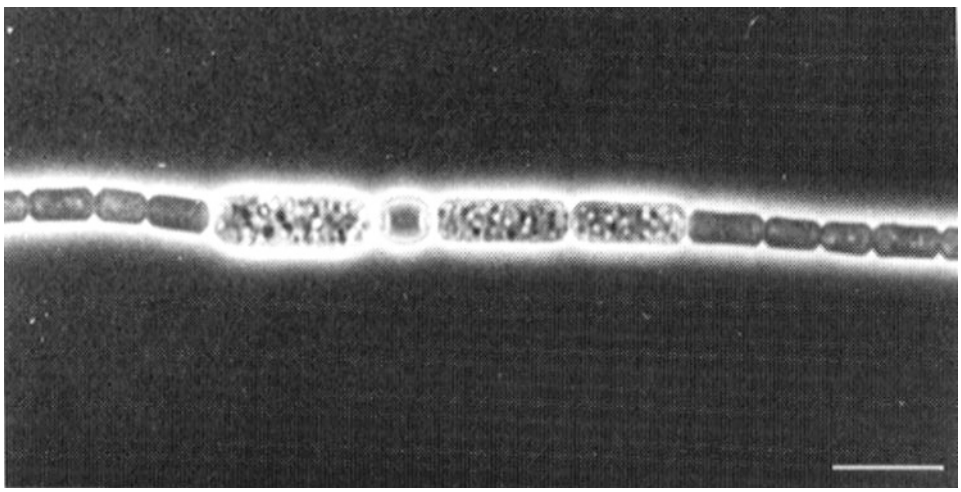


Fig. 16.3
Phase contrast photomicrograph of part of a filament of *Anabaena cylindrica*, showing a heterocyst with akinetes on either side and vegetative cells to the far *left* and *right* (From Nichols and Adams 1982)

controls chemotactic and phototactic responses, and the third controls flagellation (Berleman and Bauer 2005).

The myxobacteria also produce cysts known as myxospores as part of a more complicated developmental cycle involving formation of a multicellular fruiting body. Fruiting body development will be discussed in section ❯ "Fruiting Body Development in *Myxococcus xanthus*."

Elementary Bodies. Chlamydia sp. is an obligate intracellular parasite that causes several prevalent human diseases including trachoma, the leading cause of human blindness, and a sexually transmitted disease. *Chlamydia* alternates between extracellular and intracellular states using the elementary body (EB) and the reticulate body (RB), respectively (Abdelrahman and Belland 2005). The EB is metabolically inactive, about 300 nm in diameter, resistant to desiccation, and highly infectious. The DNA inside EBs is compacted by histone-like proteins HctA and HctB. Although EBs lack peptidoglycan, proteins in the outer membrane are extensively cross-linked by disulfide bonds. EBs also produce a type III secretion system that injects toxins into host cells.

The life cycle of *Chlamydia* begins when an EB binds to an epithelial cell and delivers the actin-recruiting protein TARP into the host with the type III secretion system. Actin filaments in the vicinity of the EB induce phagocytosis that engulfs the EB, internalizing it and surrounding it with a membrane to form a phagosome. The EB begins to enlarge and differentiate into an RB. Differentiation requires disruption of DNA-histone interactions by the small metabolite 2-C-methylerythritol 2, 4-cyclodiphosphate and the *euo* gene product, which may be a histone-specific protease. Chromosome unpacking leads to

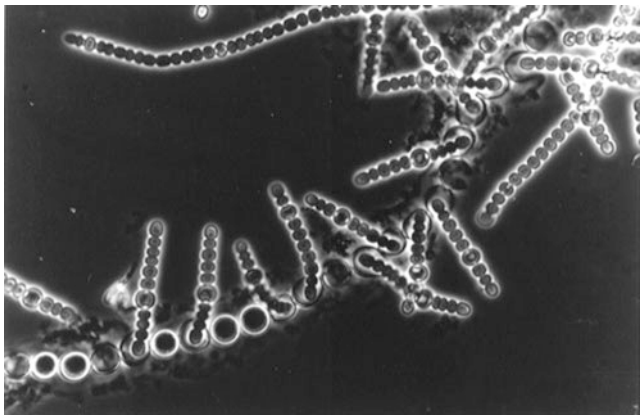


Fig. 16.4
Phase contrast photomicrograph of a filament of germinated akinetes of *Anabaena*. Germlings emerge from the akinete then produce chains of cells, each containing a heterocyst (From Nichols and Adams 1982)

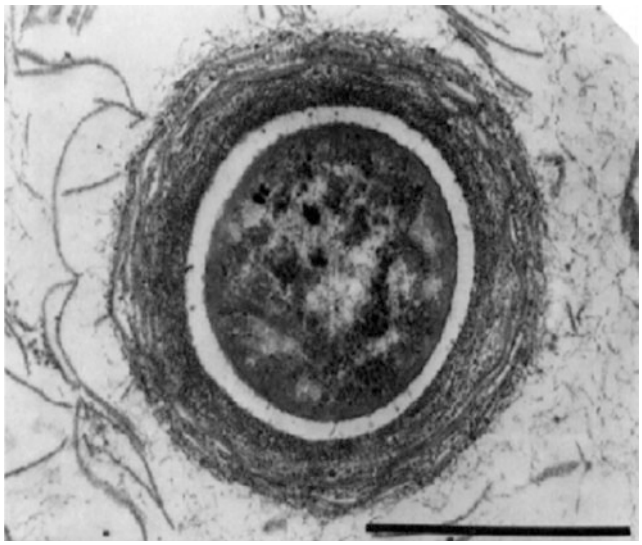


Fig. 16.6
Thin section of a mature cyst of *Azotobacter vinelandii*. Bar = 1 μm (From Hitchins and Sadoff 1970)

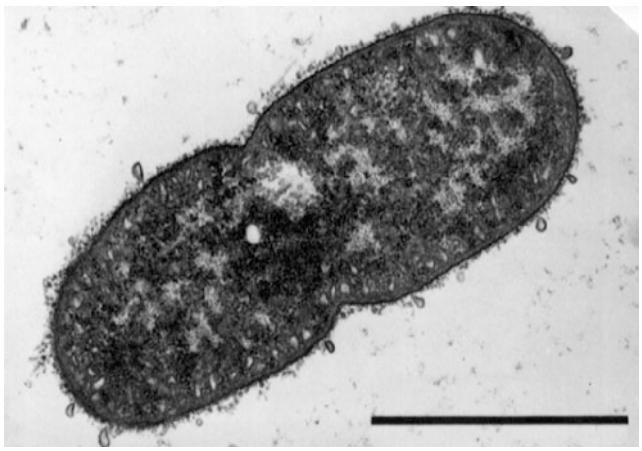


Fig. 16.5
Thin section of a dividing vegetative cell of *Azotobacter vinelandii*. Bar = 1 μm (From Hitchins and Sadoff 1970)

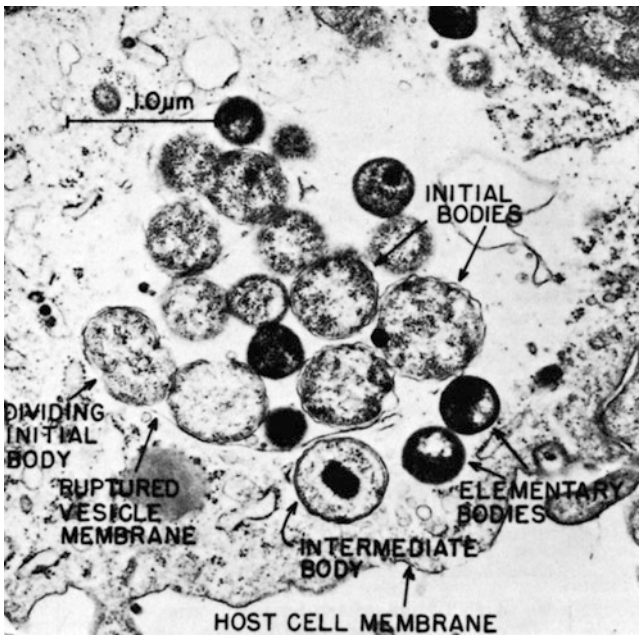


Fig. 16.7
Thin section of an animal cell infected with *Chlamydia psittaci*. The "initial bodies" are the reticulate bodies (From Cutlip 1970)

transcription of genes involved in nutrient assimilation including ABC transporters, oligopeptide permeases, and ATP transporters. Within 10–15 h, the RB synthesizes ribosomes, reorganizes the DNA, acquires a different cell wall, and begins to divide by binary fission. After a period of rapid growth and division, the RBs begin to differentiate into EBs. The genes expressed during this stage include those involved in making the cross-linked outer membrane complex and those involved in condensing the chromosome. The EBs eventually occupy the entire host cytoplasm. EBs are released by two pathways, one involving extrusion through the host membrane and the other involving lysis of the host cell. A phagosome filled with both types of cells and some intermediary forms is shown in *Fig. 16.7*.

Endospores. Endospores are produced inside a mother cell and released when the mother cell lyses. In addition to *Bacillus*, a variety of other Firmicute genera also form endospores. The morphological events leading to endospore formation in other Firmicutes seem relatively similar except for the fact that some genera like *Metabacterium* produce multiple endospores.

Endospores are the most durable cells known. Endospore-forming bacteria have been isolated from bees trapped in

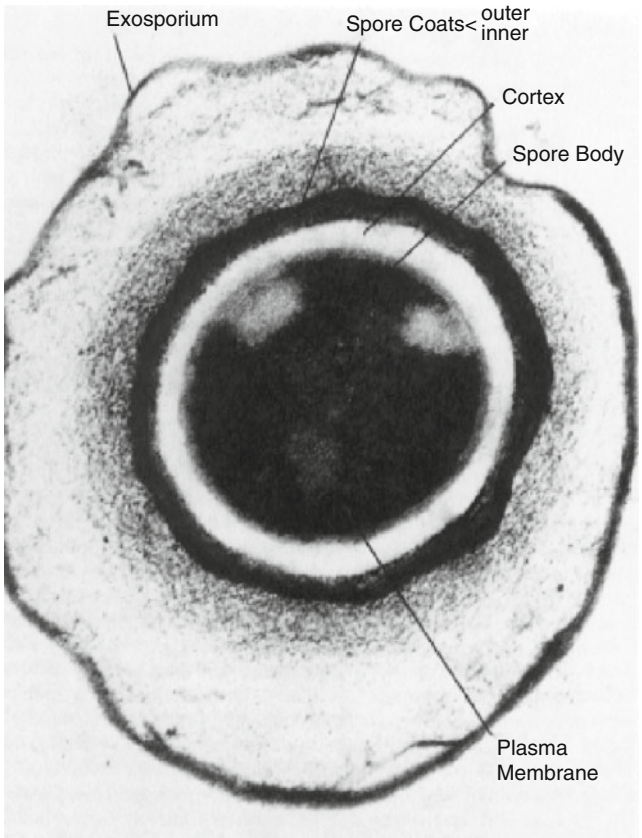


Fig. 16.8
Thin section of a *Bacillus sphaericus* endospore (Courtesy of Dr. S. Holt)

25–40-million-year-old amber (Cano and Borucki 1995) and from 250-million-year-old salt crystals (Vreeland et al. 2000). The durability of the endospore is due in part to the many thick layers surrounding it, which surpass those of any other type of spore. Starting from the outside and proceeding inward, the spore layers include the exosporium, inner and outer coats, outer membrane, cortex, germ cell wall, inner membrane, and central core (*Fig. 16.8*). The unique structure of the endospore is due to the unique manner of its synthesis. The outermost layers including the exosporium, coats, outer membrane, and cortex are contributed by the mother cell using a genetic program different from that of the spore interior. The mechanism of endospore formation is known in extraordinary detail for *Bacillus subtilis* and will be discussed in section "Endospore Formation in *Bacillus subtilis*."

The composition of the endospore layers and the contribution of each layer to the resistance properties of the endospore are known in some detail (*Fig. 16.8*). Beginning with the outermost layer and working inward, the exosporium is a loose-fitting structure made of protein found on endospores of some but not all species. The inner and outer spore coats contain about 70 different proteins in *B. subtilis*. The coat confers resistance to some chemicals and to external lytic enzymes but has little or no role in resistance to heat and radiation. The precise function of the outer membrane is unclear because its removal has no affect on spore resistance. The cortex is composed of peptidoglycan that is similar to vegetative peptidoglycan but with a few spore-specific modifications (Popham 2002). The cortex is essential for dormancy and dehydration. The germ cell wall is also composed of peptidoglycan, probably identical to vegetative cell peptidoglycan, and becomes the cell wall during germination. The inner membrane composition is similar to that of the plasma membrane of growing cells. However, the inner membrane is compressed and the lipid molecules largely immobile until germination. The core contains most spore enzymes and the molecular machinery for germination and growth.

Endospores exhibit extraordinary resistance to heat, desiccation, and radiation (Setlow 2006). Resistance to wet heat is due to dehydration of the spore core. While water comprises 75–80% of the wet weight of the vegetative cell cytoplasm, it comprises only 27–55% of the spore core wet weight. The core also contains dipicolinic acid at concentrations well above solubility, which further contributes to the dehydrated state of the core. Dehydration restricts macromolecular movement and reduces modification of macromolecules by soluble toxic agents. Saturation of the spore DNA with α/β-type small, acid-soluble spore proteins (SASP) alters DNA structure and increases its resistance properties.

The mechanism of chemical resistance varies with the chemical. There are many mechanisms including interaction with one of the spore coat proteins, the impermeability of the spore membrane that restricts access to the spore core, protection of the spore DNA by α/β-type SASP, and DNA repair systems that become active upon germination. The mechanism of spore resistance to γ-radiation is not understood but does not involve α/β-type SASP. Protection to ultraviolet (UV) irradiation is due, in part, to binding of α/β-type SASP to the DNA, the presence of dipicolinic acid, and the use of DNA repair pathways during germination.

Very little is known about germination with any spore type; perhaps the most is known about endospore germination (Moir 2006). The endospore is an exquisite biosensor that germinates in response to specific germinants. The types of germinants vary with the species and include specific amino acids, sugars, or nucleosides. Mutants with defects in germination have been examined genetically and biochemically. A composite of results from several different species have enabled construction of a rough draft of the germination process.

The first step in germination involves passage of the germinant through the outer coat and cortex before coming in contact with the germinant receptors. The *gerP* mutants seem to have a defect in germinant passage through the outer layers and germinate poorly unless the coat is first removed (Moir 2006). The germination receptors appear to be contained within the *gerA* and *gerB* operons which are widespread among *Bacillus* species. Though the Ger proteins are somewhat variable between species, probably due to differences in germinants, they tend to be integral membrane proteins and lipoproteins that reside in the inner membrane. Point mutations in these proteins result in strains that require higher concentrations of germinant for

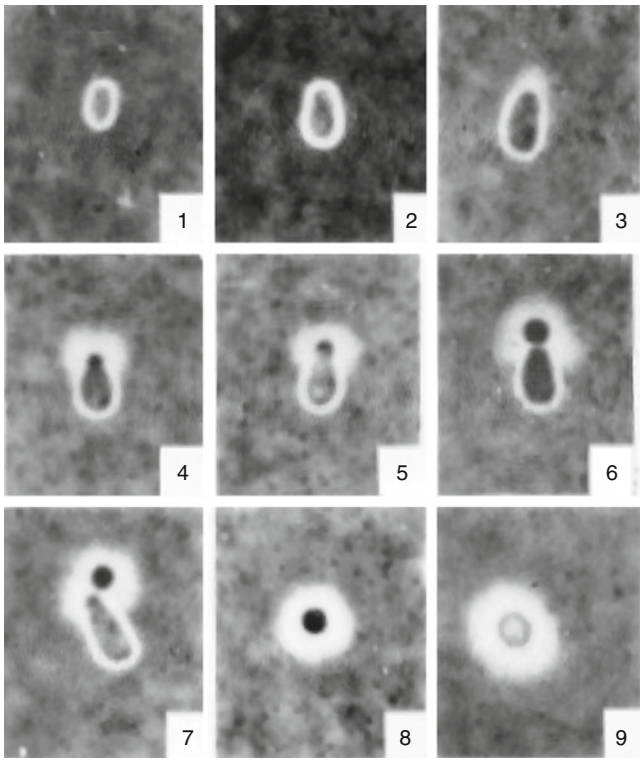


Fig. 16.9
Phase contrast micrographs of exospore formation by *M. trichosporium*. The capsulated, rod-shaped, vegetative cell becomes pear-shaped, and the tapered end buds off the cell that is eventually released as the exospore (From Whittenbury et al. 1970)

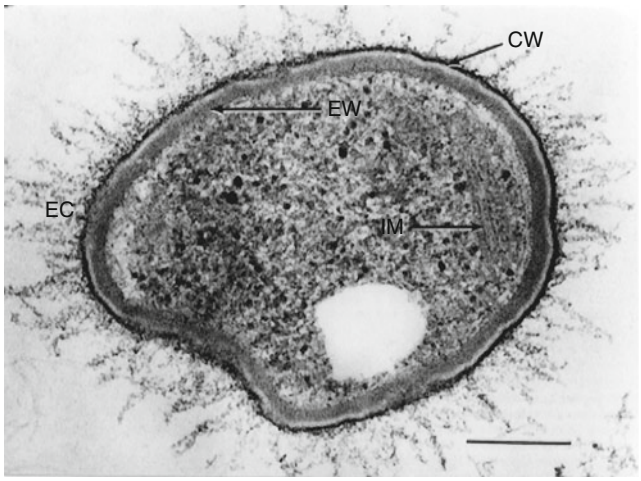


Fig. 16.10
Thin section of *Methylosinus trichosporium* exospore. Bar = 0.2 μm. *CW* cell wall, *EC* exospore capsule, *EW* exospore wall, and *IM* intracytoplasmic membranes (From Reed et al. 1980)

germination. None of these proteins resemble transport proteins, and there appears to be little or no transport of the germinant. Rather, these proteins appear to be receptors that work in a manner that is, as yet, unknown to initiate germination.

Many types of ion fluxes are observed early in germination. H^+, K^+, Na^+, Ca^{+2}, and dipicolinic acid are released from the spore core. The release of Ca-dipicolinic acid is significant since it comprises 10% of the spore dry weight. The SpoVA proteins, implicated in dipicolinic acid uptake during spore formation, may also mediate dipicolinic acid release during germination. The Na^+/H^+K^+ antiporter protein GerN seems to play a role in inosine-stimulated germination in *B. cereus*. It is possible that this germinant receptor moves ions as part of the mechanism initiating germination.

One of the major steps in germination is degradation of the spore cortex with lytic enzymes CwlJ and SleB which are embedded in the outer layers of the spore. SleB is a muramidase and is somehow activated during germination.

Exospores. Exospores are produced by a process resembling budding in several α-proteobacteria, including the methylotroph *Methylosinus trichosporium* and the phototroph *Rhodomicrobium vannielii*. When *M. trichosporium* cells reach stationary phase, some of the cells elongate, become tapered, and bud off rounded bodies that gradually become optically refractile (*Fig. 16.9*). Exospores are resistant to desiccation and to elevated temperatures of at least 78 °C but, in general, are not as durable as endospores. Electron micrographs of thin sections through exospores demonstrate a much simpler and thinner spore coat than the endospore (*Fig. 16.10*). Germination occurs slowly when the cells are placed under conditions conducive to growth and resembles budding (*Fig. 16.11*).

Rhodomicrobium vannielii is a phototrophic, budding bacterium that produces both exospores and motile swarmer cells. A batch culture of *R. vannielii* may simultaneously contain flagellated rods, ovoid cells linked together by branched, mycelial connections, and angular exospores. Swarmer cells can be separated from the budding mycelial forms (*Fig. 16.12*) by passing the culture through glass wool. Thus, homogeneous populations of each cell type can be obtained and the sequence of developmental events connecting them determined (Whittenbury and Dow 1977). *Figure 16.13* illustrates spore formation and *Fig. 16.14* diagrams events following germination. The swarmer cells will be discussed in section "Dispersal Strategies."

Endospore Formation in *Bacillus subtilis*

The events leading to endospore formation are customarily divided into seven stages diagrammatically represented in *Fig. 16.15*. The definition of these stages is based on morphological changes discerned through transmission electron microscopy and mutants that are blocked in a particular stage. The developmental program is driven in large part by stage and compartment-specific sigma factors, also shown in *Fig. 16.15*. A recent review describing the transcription network in detail may be found in de Hoon et al. and is summarized in *Fig. 16.16* (de Hoon et al. 2010).

Endospore formation begins only after a round of DNA replication has been completed to ensure that two chromosomes

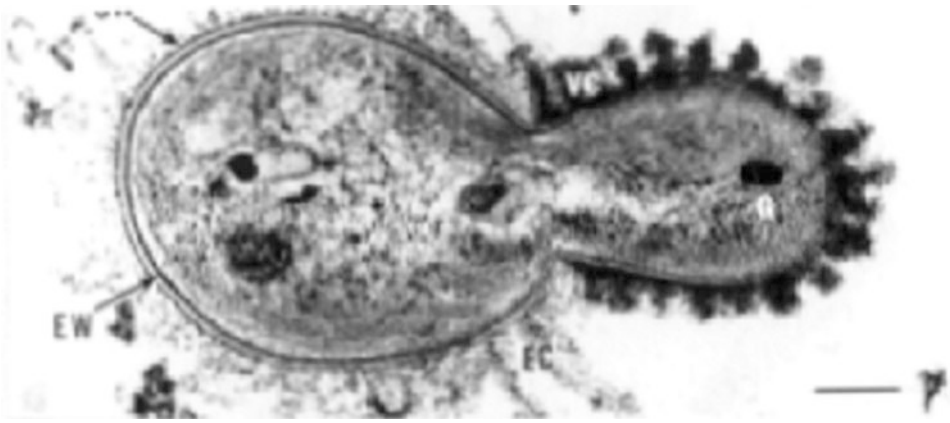


Fig. 16.11
Thin section of a germinating exospore of *Methylosinus trichosporium*. Bar = 0.2 μm (From Reed et al. 1980)

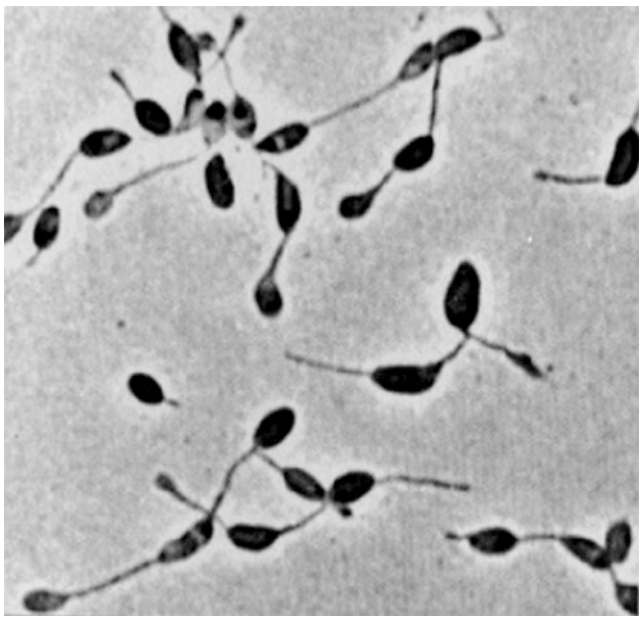


Fig. 16.12
Electron micrograph of growing *Rhodomicrobium* cells. Ovoid cells are linked together by mycelial connections (Courtesy of Dr. P. Hirsch.)

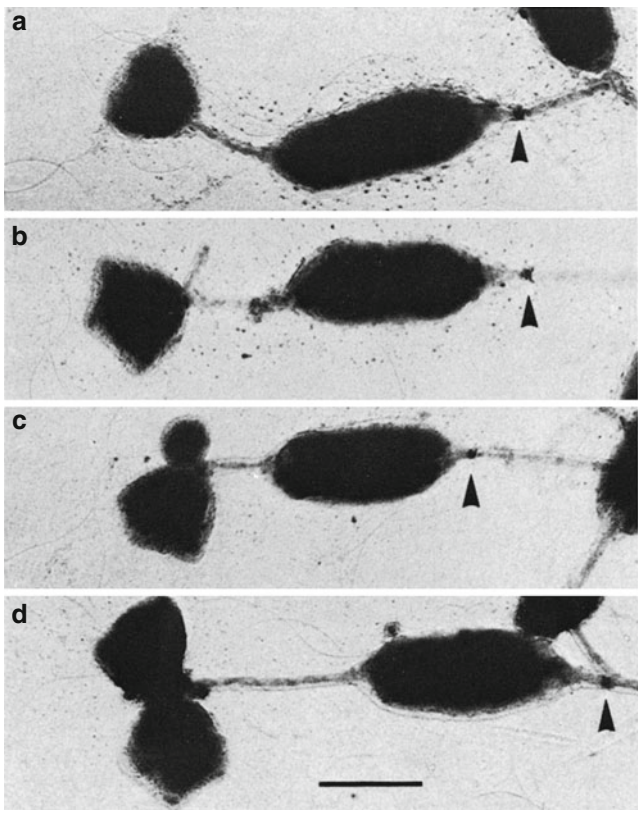


Fig. 16.13
Electron micrographs of *Rhodomicrobium* cells forming exospores. (a) First exospore formed. (b) Filament for second exospore is extended. (c) Beginning of second exospore. (d) Completion of second exospore. *Arrowheads* indicate filament plug separating the mother cell from the rest of the vegetative cells. Bar = 1 μm (From Whittenbury and Dow 1977)

are available in the predivisional cell. The chromosomes are anchored with their origin of replication at a pole (*Fig. 16.15*, red lines). Endospore formation commences with asymmetric cell division. The smaller compartment, the forespore, eventually becomes the endospore. The larger compartment becomes the mother cell. Shortly after asymmetric cell division, different programs of gene expression are established in each compartment and coordinated through intercompartmental signaling to maintain spatial and temporal checkpoints for endospore formation. The forespore is engulfed by the mother cell by a process analogous to phagocytosis resulting in the deposition of another membrane around the forespore. The cortex is synthesized between the two forespore membranes. At least 70 different coat proteins are synthesized by the mother cell and deposited in layers on top of the cortex. Finally, the mother cell lyses to release the dormant endospore.

Endospore formation is regulated by a cascade of compartment-specific sigma factors. The master regulator of

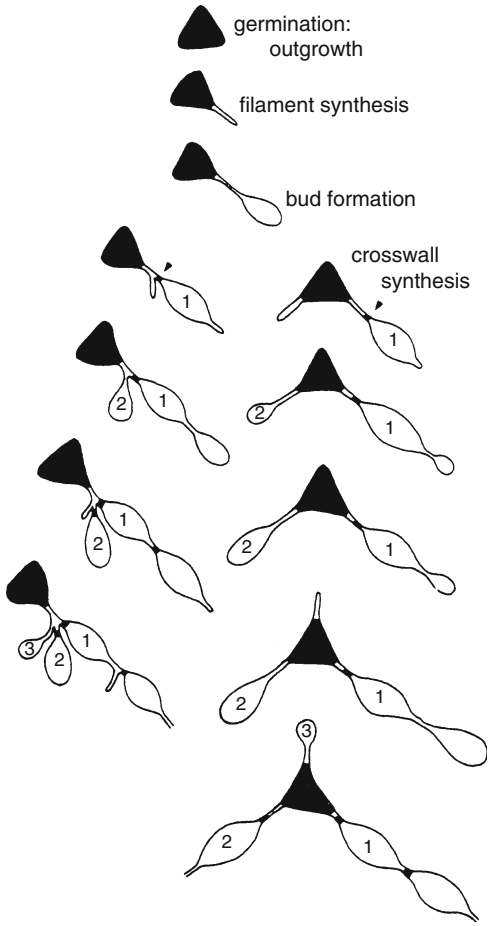


Fig. 16.14
Diagrammatic representation of exospore germination in *Rhodomicrobium*. The *left* and *right* sides show two different exospores (From Whittenbury and Dow 1977)

sporulation, SpoOA∼P, is not a sigma factor but a response regulator whose concentration and phosphorylation state determine the initiation of sporulation. Phosphorylation of SpoOA alters the transcription of more than 500 genes, about 120 directly. SpoOA∼P is an indirect activator of *sigH* to produce σ^H, the first sigma factor in this cascade. σ^H directs transcription of *sigF* to produce the major sigma factor in the forespore following asymmetric cell division, σ^F. σ^F directs transcription of about 50 genes including σ^G. σ^G, the final forespore-specific transcription factor, regulates about 100 genes. In the mother cell, σ^E is produced by a σ^A-specific promoter that is activated by SpoOA∼P. σ^E directs transcription of about 270 genes. σ^E directs expression of σ^K, the last sigma factor in the mother cell.

The forespore and mother cell lines of expression are connected by intercompartmental signaling to ensure coordinated regulation. These points of coordination are known as developmental checkpoints. Three such checkpoints have been identified. The first checkpoint occurs in the predivisional cell which accumulates both the forespore sigma factor σ^F and the mother cell sigma factor σ^E, as both are induced by SpoOA∼P (◉ *Fig. 16.16*). However, these sigma factors are held in an inactive state until asymmetric division is completed. σ^F is sequestered by two molecules of the anti-sigma factor SpoIIAB, while σ^E is produced with a 27-amino acid leader sequence that renders it inactive (pro-σ^E). The anti-anti-sigma factor SpoIIAA binds to the SpoIIAB$_2$-σ^F complex to stimulate σ^F release. SpoIIAB is a kinase that phosphorylates SpoIIAA. To effect σ^F release, SpoIIAA needs to be in an unphosphorylated state. After completion of asymmetric cell division, the septum-associated phosphatase SpoIIE dephosphorylates SpoIIAA, facilitating release of σ^F and initiating the forespore-specific genetic program. In the other compartment, the processing of pro-σ^E is mediated by the membrane-associated protease SpoIIGA. SpoIIGA is stimulated by contact with SpoIIR, produced by the forespore and one of the first proteins expressed in response to σ^F activation. Timing is critical. A delay in SpoIIR production is catastrophic and results in forespores at both poles and a mother cell devoid of DNA.

The second checkpoint involves the activation of σ^G in the forespore by a mechanism that remains unknown. SpoIIIAH in the mother cell interacts with SpoIIQ in the forespore to form a channel. One thought is the channel imports a regulatory factor that activates σ^G. Another idea is that the channel functions as a feeding tube to shuttle small metabolites into the forespore.

The third checkpoint involves the activation of the mother cell sigma factor pro-σ^K by the SpoIVFB protease. Proteolysis of pro-σ^K is stimulated by SpoIVB, a forespore-specific protein produced by σ^G.

Endospore formation involves five feed-forward motifs, each consisting of a sigma factor and a downstream transcription factor: σ^F, RsfA; σ^G, SpoVT; σ^E, GerR; σ^E, SpoIIID; and σ^K, GerE (◉ *Fig. 16.16*). In each of these motifs, a sigma factor induces production of a transcription factor and, along with the transcription factor, jointly regulates expression of downstream sporulation genes. The regulatory modules of each sigma factor are conserved among 14 different genera of endospore-forming bacteria (de Hoon et al. 2010). The feed-forward motifs are less conserved than the sigma factors but more conserved than the downstream sporulation genes they regulate. Overall, the level of conservation is remarkable, given more than a billion years of evolution. These results stand in marked contrast to the developmental cycle of the myxobacteria which shows little conservation over a comparable period of evolution (see below).

Fruiting Body Development in *Myxococcus xanthus*

Myxobacteria are δ-proteobacteria that move on surfaces without the use of flagella. During the vegetative phase of their life cycle, they grow as carnivores or scavengers swarming across surfaces in search of food (◉ *Fig. 16.17*). When faced with starvation, they use their motility to form large, spore-filled fruiting bodies. The spores germinate in the presence of nutrients to resume the life cycle. Multicellularity maximizes the ability of a myxobacterial community to prey on other bacteria

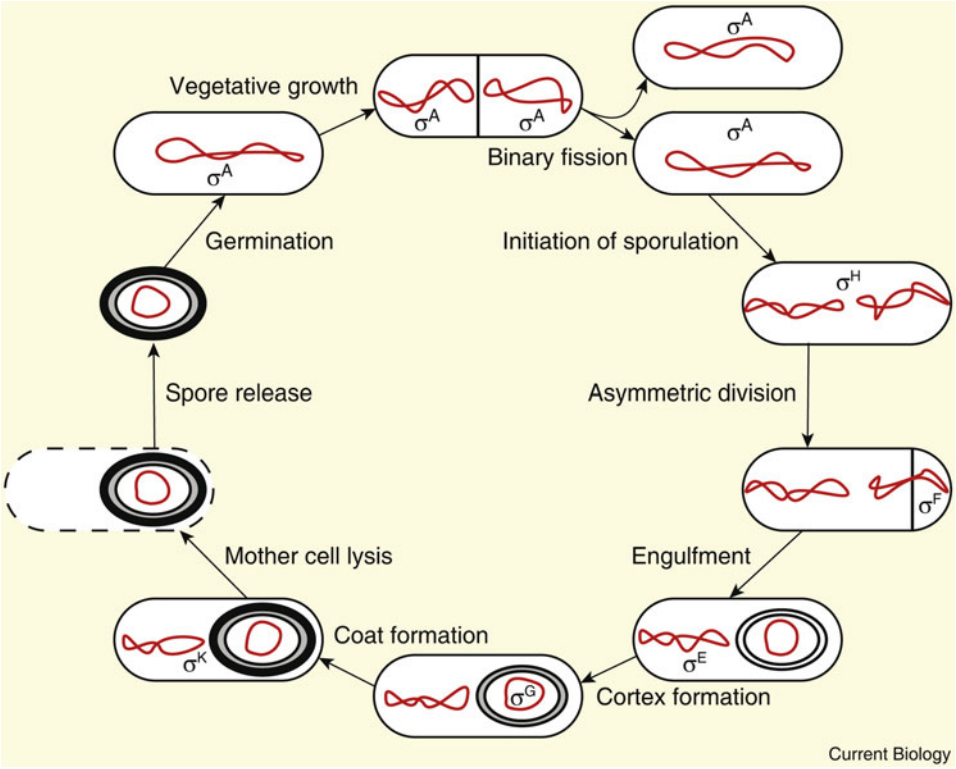


Fig. 16.15

Morphological stages of the *B. subtilis* life cycle. The temporal and compartment-specific activity of each sporulation sigma (σ) factor is indicated. During vegetative growth, cells divide by binary fission to generate two identical daughter cells. Sporulation is initiated in response to starvation. In the predivisional sporulating cell, the chromosomes (*red*) are oriented with their origin-proximal region anchored at the cell poles. During asymmetric division, two membrane-bounded compartments are generated: a small forespore and a large mother cell. After asymmetric division, the remainder of the forespore chromosome (i.e., the origin-distal region) is pulled into the forespore by translocation. Engulfment of the forespore by the mother cell results in the release of the forespore as a free protoplast in the mother cell. The cortex (composed of modified peptidoglycan, *gray*) is synthesized between the two membranes surrounding the forespore. The coat (*black*) is a complex structure made of at least 70 distinct proteins that assemble around the forespore surface. Following mother cell lysis, the mature spore is released into the environment. *B. subtilis* cells can remain in a dormant spore state for an extended period of time, but spores will germinate in response to the presence of particular small molecules that act as germinants and resume vegetative growth (From de Hoon et al. 2010)

and hydrolyze macromolecular substrates such as cell walls, proteins, lipids, polysaccharides, and nucleic acids. Fruiting body formation ensures that a population of cells optimized for feeding will emerge following germination. A recent book edited by D. C. Whitworth (2008) describes many aspects of the life cycle of this fascinating group of bacteria.

Fruiting bodies are 10–1,000 μm in size (*Fig. 16.18*). The fruiting bodies generated by *Myxococcus* species are spherical. The *Chondromyces* and *Stigmatella* species form numerous sporangia-containing spores. The sporangia are held by stalks that are branched in certain *Chondromyces* species or consist of tubules in *Stigmatella aurantiaca*. The fruiting bodies contain thousands of myxospores that are metabolically quiescent and resistant to heat and UV radiation. *M. xanthus* myxospores are protected by a thick electron-dense coat composed of carbohydrates and proteins.

Fruiting body development of *M. xanthus* is induced by amino acid limitation using the stringent response (*Fig. 16.19*). Inhibition of protein synthesis stimulates pppGpp and ppGpp synthesis by RelA. ppGpp activates genes involved in the production of the extracellular A-signal, consisting of a mixture of amino acids (Trp, Pro, Phe, Tyr, Leu, and Ile). A-signal is sensed by histidine protein kinase SasS, which activates the response regulator SasR. SasR along with σ^{54} activates a set of developmental genes. During the period of A-signaling between 0 h and 8 h, no specific changes in cell morphology or multicellular arrangement can be discerned (*Fig. 16.19*). The second phase is accompanied by accumulation of intracellular lipid droplets and a sudden burst of motility. Swarms of cells come together and form numerous small aggregates three or four layers thicker than the cell mat. These move across the surface of the biofilm sometimes disappearing into

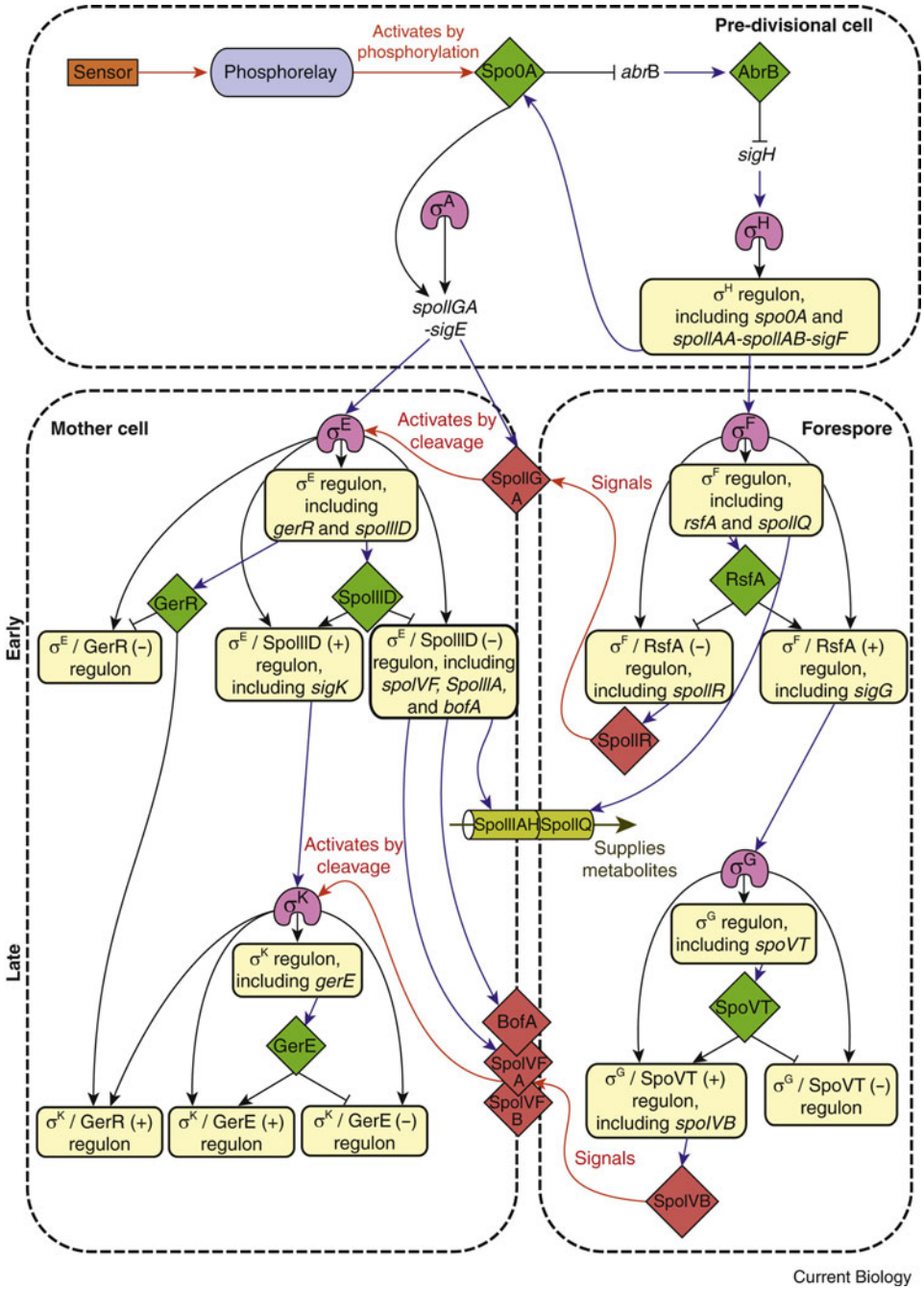


Fig. 16.16

Modular architecture of the sporulation regulatory network in *B. subtilis*. The temporal progression of sporulation is from top to bottom. Each cellular compartment (predivisional cell, forespore, and mother cell) is surrounded by *dotted lines*. Sigma factors are shown in *pink*, transcription factors in *green*, and regulons in *yellow*. Signaling proteins are shown in *red* and the feeding tube in *olive*. Transcriptional regulation is indicated by *black arrows*, gene expression (protein synthesis) by *blue arrows*, and signaling pathways by *red arrows*. Coherent feed-forward loops are indicated by a *plus sign* and incoherent feed-forward loops by a *minus sign* (From de Hoon et al. 2010)

the cell mat and other times fusing with neighboring aggregates. With time, most of the small aggregates disappear leaving only large aggregates. The formation and stability of the large aggregates require the C- and E-extracellular signals along with the Dif chemosensory system.

The large aggregates grow taller by adding tiers consisting of a single layer of cells to the uppermost layer. The cells emerge through a hole on the top tier, and the layer expands to fill the entire surface. This structure eventually becomes encapsulated with polysaccharide. Within the

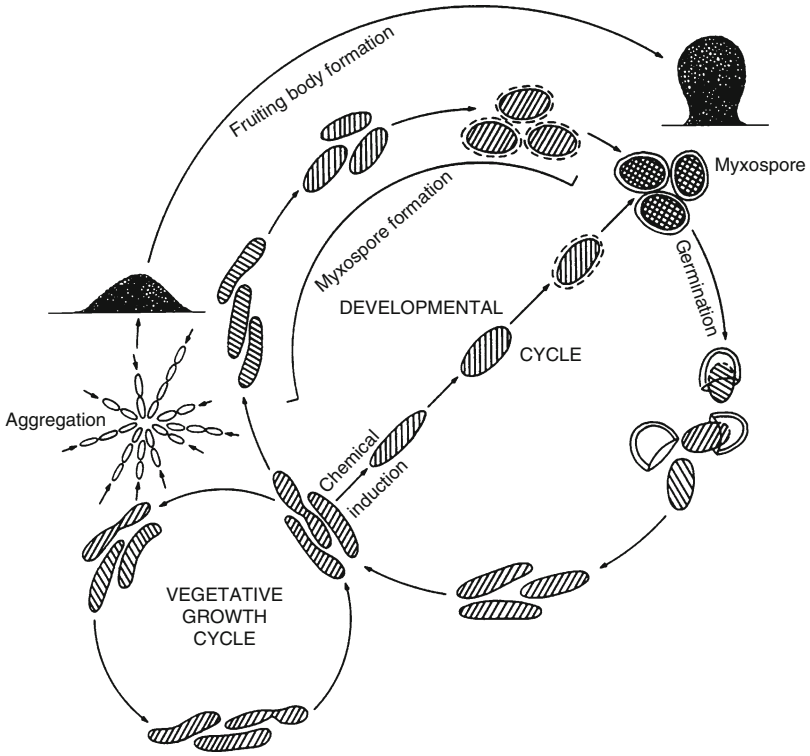


Fig. 16.17
Diagram of the life cycle of *Myxococcus xanthus*. The fruiting body is not drawn to scale, but it is a few hundredths of a mm in diameter, in contrast to the vegetative cells, which are about 5–7 μM (From Dworkin 1985)

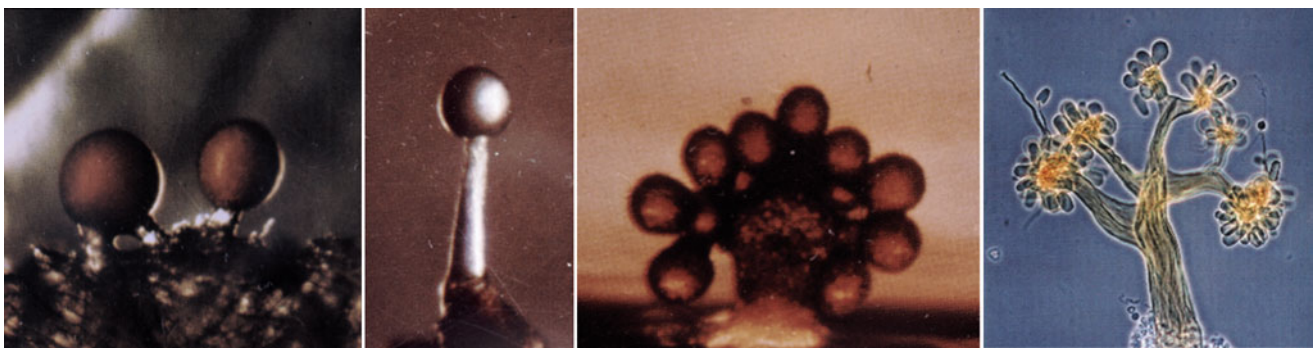


Fig. 16.18
Fruiting bodies. *Myxococcus fulvus*, *M. stipitatis*, *Stigmatella aurantiaca*, *Chondromyces crocatus* (Individual pictures courtesy of Hans Reichenbach)

aggregates, the majority of the vegetative cells undergo programmed cell death. The remaining cells differentiate into myxospores.

The C-signal, E-signal, and Dif chemosensory system govern several stages of myxobacterial development. The E-signal is a lipid containing a branched chain fatty acid that plays a role in fruiting body morphogenesis, programmed cell death, and sporulation. The C-signal is required for the formation of fruiting bodies as well as spores. C-signal transmission leads to transcription of several developmental genes through the phosphorylation of the response regulator FruA. Although FruA is synthesized soon after starvation is sensed, FruA is activated in response to the C-signal. In addition to its role as a transcriptional activator, FruA activates FrzCD, a methyl-accepting chemotaxis protein (MCP). FrzCD relays the signal to FrzE, a response regulator, which controls cell reversals. FrzE∼P also inhibits FrzCD methylation by a negative feedback loop. The Frz-phosphorylated intermediates oscillate forming the "frizillator." FruA∼P levels, initially low because of little C-signaling, set a rhythm to the reversal of gliding direction every eight minutes. When cells collide, the frizillator

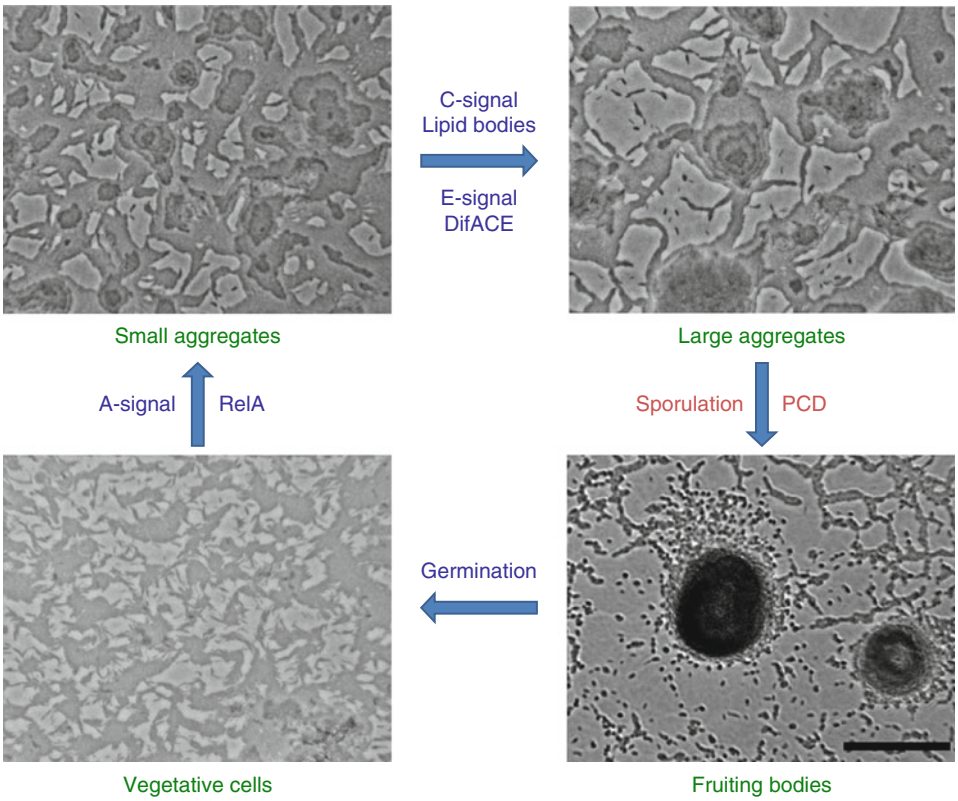


Fig. 16.19

Stages of fruiting body morphogenesis in *Myxococcus xanthus*. Vegetative cells (*lower left*) form a thick biofilm. Cells at the top of the biofilm are arranged in layers visualized as different shades of gray. Fruiting body morphogenesis begins with nutrient limitation detected via a RelA-dependent stringent response. A-signaling is used to determine that a sufficient cell density is available for development to proceed. Morphogenesis begins with vigorous cell movement leading to the formation of many small aggregates raised several layers above the surface of the cell mat (*upper left*). These small aggregates move rapidly, sometimes fusing with other aggregates and sometimes receding into the cell mat. With time, large, spatially stable aggregates form and are extended vertically by adding successive layers to the top of the fruiting body (*upper right*). Inside the aggregates, cells undergo programmed cell death (PCD) and sporulation leading to the production of mature fruiting bodies (*lower right*). Fruiting bodies maintain dormancy until nutrients initiate germination. Bar is 0.1 mm (Modified from data found in Curtis et al. 2007)

becomes synchronized due to increased C-signaling and cells rarely reverse direction leading to streams of cells that move toward aggregation foci. Within the aggregation centers, C-signaling is maximized by cell contact leading to activation of *devRST* operon. *devRST* expression is regulated spatially ensuring sporulation only within fruiting bodies.

The Dif chemosensory system also controls fruiting body morphogenesis and sporulation. DifA, a methyl-accepting chemotaxis protein (MCP); DifC, a CheW-like coupler; and DifE, a CheA-like histidine kinase form a ternary signaling complex. *difACE* mutants form small aggregates that are several layers thick but fail to mature beyond that stage. DifACE are essential for extracellular matrix (ECM) production, S-motility, and chemotaxis to several lipids including one containing a rare fatty acid, 16:1ω5c (a fatty acid of 16 carbons with one point of unsaturation at position 11 from the carboxyl group). S-motility is not the critical output because cells can make fruiting bodies using only A-motility. The DifACE pathway has two sensory inputs mediated by PilA and FibA leading to different outputs for ECM production and lipid chemotaxis, respectively. Either input mediates development, but loss of both inputs eliminates fruiting body morphogenesis.

Whereas the basic program for endospore formation is conserved in all endospore-forming bacteria, there is little conservation of developmental genes among the myxobacteria (Huntley et al. 2011). A set of 95 *M. xanthus* developmental genes was examined for representation in the genomes of other fruiting body forming bacteria. Genes for entire signal transduction pathways important for fruiting body formation in *M. xanthus* are conserved in the closely related species *S. aurantiaca*, whereas only a minority of these genes are conserved in the more distantly related species *S. cellulosum* and *H. ochraceum*. These comparative analyses suggest that the genetic programs for fruiting body formation in *M. xanthus* and *S. aurantiaca* are highly similar in spite of major differences in fruiting body structure and significantly different from the genetic program directing fruiting body formation in *S. cellulosum* and *H. ochraceum*.

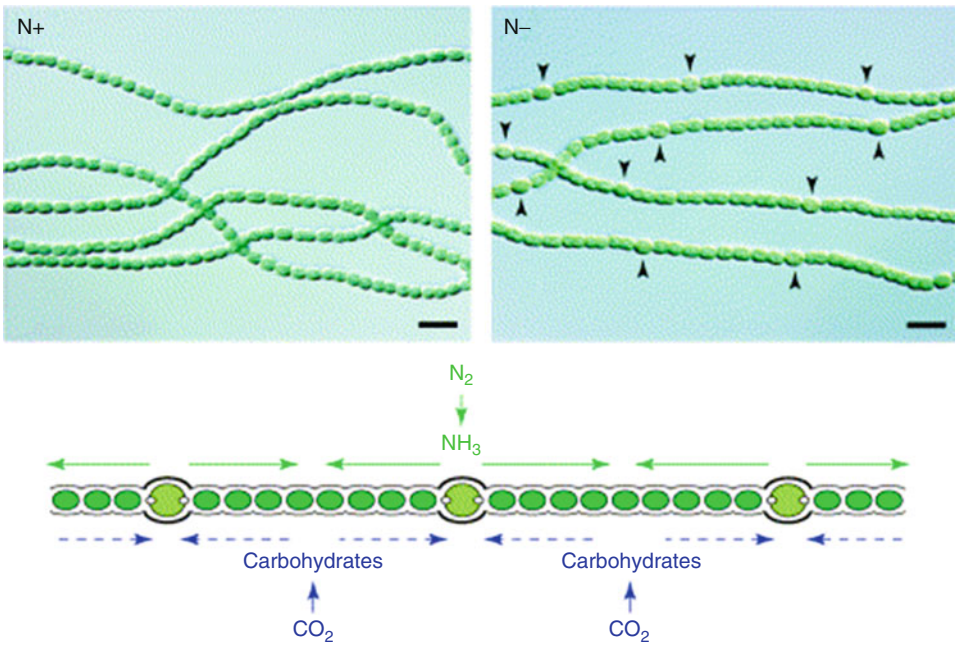


Fig. 16.20
The filamentous cyanobacterium *Anabaena* PCC 7120 grown with (N+) or without (N−) combined nitrogen. Heterocysts are indicated by *arrowheads*. Heterocysts supply fixed nitrogen as glutamine and other amino acids to the neighboring vegetative cells, which supply heterocysts with fixed carbon produced by photosynthesis. Filaments are composed of individual cells, each with its own plasma membrane and cell wall but enclosed by a common outer membrane. The diagram is not drawn to scale (From Golden 2003 #3877)

Nutrient Acquisition

The most prominent examples of specialized cells for nutrient acquisition involve nitrogen fixation in which atmospheric nitrogen (N_2) is converted to ammonia (NH_3). Oxygenic photosynthesis and nitrogen fixation are incompatible processes as nitrogenase is exquisitely sensitive to oxygen. *Anabaena* solves this problem by using a heterocyst to fix nitrogen. The reader is directed to a recent review on heterocysts (Kumar et al. 2010). Heterocysts are larger than vegetative cells due to a thick cell envelope containing glycolipid and polysaccharide that protects nitrogenase from oxygen. Oxygenic photosystem II is dismantled during heterocyst differentiation. Cyanophycin granules are found at poles adjacent to vegetative cells. Vegetative cells supply heterocysts with a source of carbon, possibly sucrose, and glutamate. Heterocysts convert the glutamate to glutamine and other amino acids that are shuttled to the vegetative cells.

Heterocyst formation in *Anabaena* is suppressed in nitrogen-rich media (*Fig. 16.20*). The photosynthetic vegetative cells grow in long filaments and each cell undergoes binary fission to increase the length of the filament. In the absence of nitrate, heterocysts appear at regular intervals. The heterocysts are green in *Fig. 16.21* due to the presence of the *patS-gfp* reporter gene, which produces the green fluorescent protein. The heterocysts supply the vegetative cells with organic nitrogen that diffuses from cell to cell along the chain. Heterocysts are incapable of cell division, and with intervening vegetative cell division, the

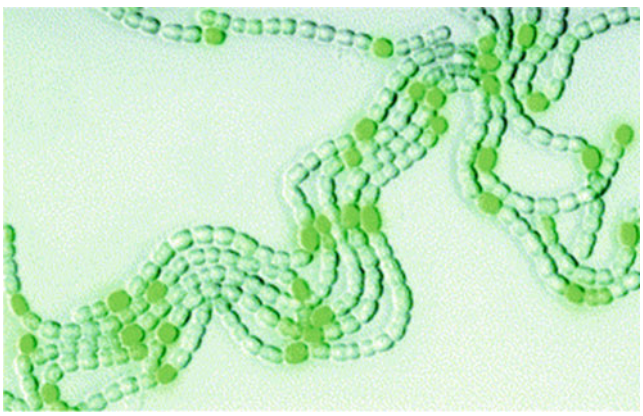


Fig. 16.21
Filaments of *Anabaena* PCC 7120 carrying a *patS-gfp* reporter 27 h after nitrogen step-down. The image is an overlay of a grayscale bright-field micrograph and the corresponding GFP fluorescence micrograph in *green*. The *bright green* and somewhat larger cells are heterocysts (From Golden 2003 #3877)

spacing between the heterocysts increases until vegetative cells some distance from the heterocyst are faced with nitrogen limitation. These nitrogen-stressed vegetative cells differentiate into heterocysts to maintain the proper spacing and the flow of nitrogen. In a *patS* mutant, heterocyst production is excessive relative to organic nitrogen availability. A few heterocysts are found in nitrogen-rich medium, and in nitrogen-limiting medium, the

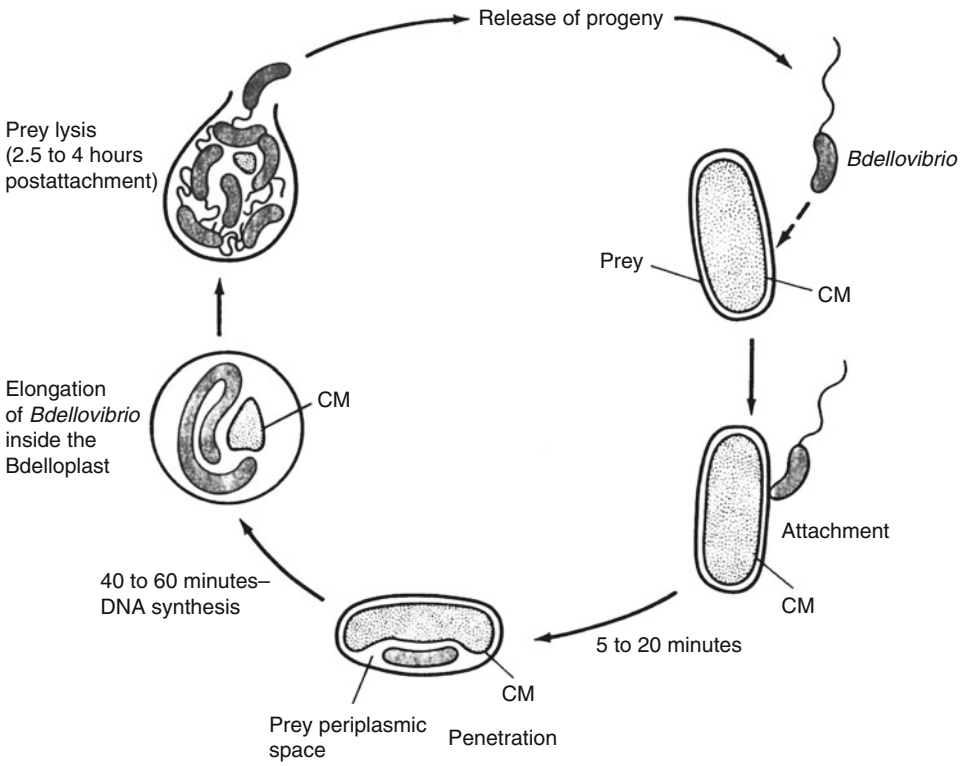
spacing between heterocysts is dramatically decreased and irregular. PatS is an intercellular peptide signal that suppresses heterocyst formation. The prevailing model is that PatS, produced in the heterocyst, diffuses from cell to cell along with organic nitrogen (glutamine and other amino acids) as concentration gradients that lessen with distance from the heterocyst. At a point where both PatS and organic nitrogen diminish below a certain threshold, a new heterocyst is induced.

Heterocyst differentiation begins when a vegetative cell senses nitrogen limitation. The intracellular signal for nitrogen imitation is 2-oxoglutarate, an intermediate in the Krebs cycle. In cyanobacteria, the Krebs cycle is incomplete due to an absence of 2-oxoglutarate dehydrogenase, so the primary function of 2-oxoglutarate is anabolic including serving as the carbon skeleton for ammonium assimilation. NtcA, a transcriptional regulator, responds to excess 2-oxoglutarate by activating many genes involved in carbon and nitrogen metabolism including, indirectly, *hetR*, whose protein product is the master regulator of heterocyst differentiation. HetR is an autoactivator that indirectly induces production of nitrogenase and many of the changes associated with heterocyst differentiation. HetR autoactivation is subject to several levels of control, among them regulation by PatS. The heterocyst inhibitory peptide PatS interferes with HetR DNA-binding activity to diminish the level and activity of HetR.

Dispersal Strategies

While nonmotile cells can be passively dispersed by wind and rain, some organisms produce specialized motile cells to force the issue. Production of dispersal cells is achieved by upregulating genes encoding a motility motor. As observed in *C. crescentus, Vibrio parahaemolyticus*, and *Bdellovibrio bacteriovorus*, this involves generation of flagella. Dispersal cells can also use other types of motility. Baeocytes produced by *Pleurocapsa* cyanobacteria and hormogonia, short motile filaments that are released from filamentous cyanobacteria, move by gliding on solid surfaces without flagella (◉ *Table 16.1*).

Attack Phase Cells. Bdellovibrio bacteriovorus, a member of the δ-proteobacteria, is an obligate intracellular parasite of other gram-negative bacteria (Rendulic et al. 2004; Sockett 2009). *B. bacteriovorus* cells alternate between a motile infectious attack phase and a nonmotile reproductive phase (◉ *Fig. 16.22*). They



◘ Fig. 16.22

Diagrammatic representation of the life cycle of *B. bacteriovorus*. In the predatory phase, *Bdellovibrio* attack phase cells are free-swimming while seeking prey. After collision with a prey cell, *B. bacteriovorus* penetrates the outer membrane and peptidoglycan layer of a gram-negative bacterium. *Bdellovibrio* loses its motility and resides in the periplasm of the prey bacterium. In the growth phase, the host converts into a spherical bdelloplast, while *Bdellovibrio* elongates into a filamentous cell that consumes the prey's nutrients. When the nutrients are exhausted from the prey cell, the filament partitions and the progeny develop into small, highly motile flagellated predator cells. The remainder of the prey cell lyses to release the progeny (From Brock and Madigan 1988 with permission of Prentice-Hall, Inc., Englewood Cliffs, NJ)

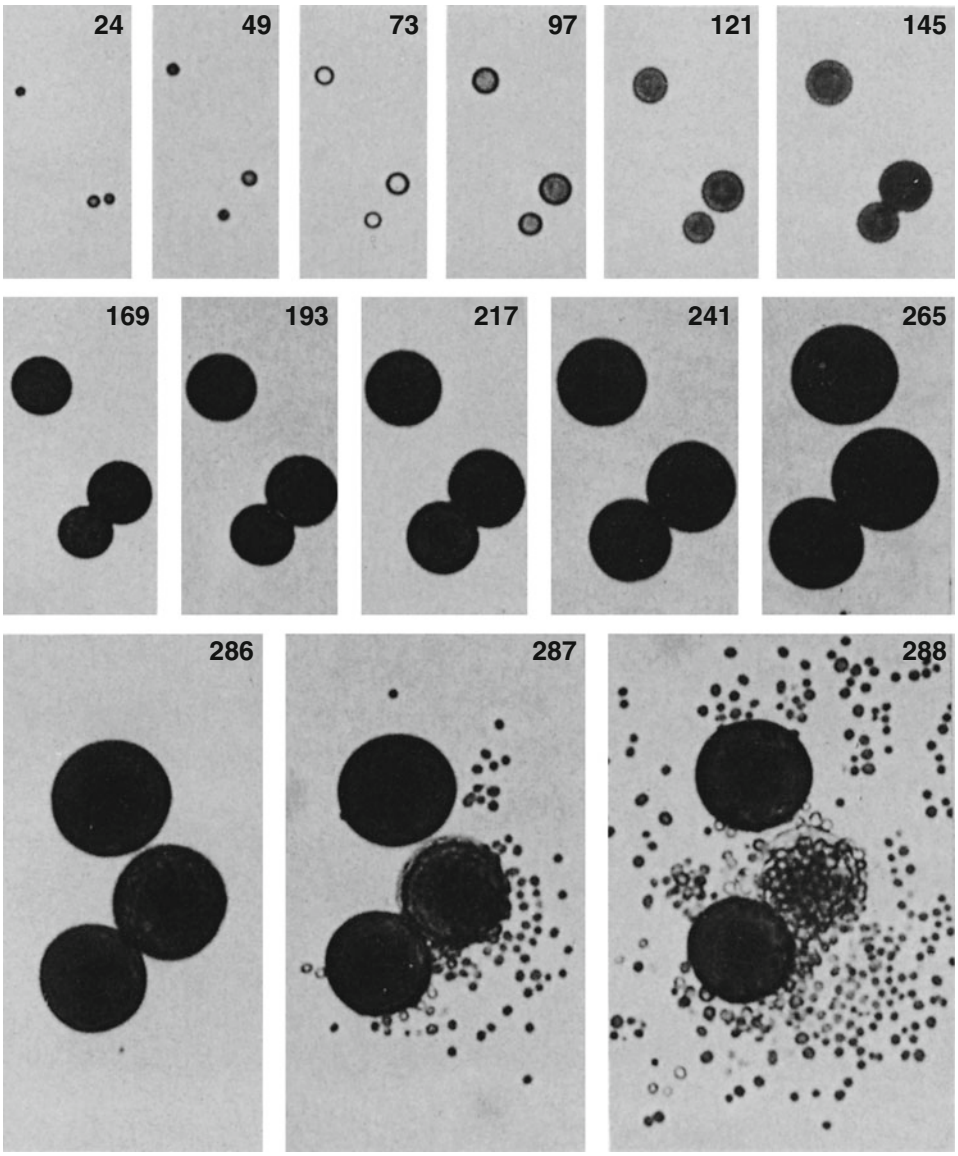


Fig. 16.23
Phase contrast photomicrographs illustrating the development of *Dermocarpa*. The *number* on each photo indicates the elapsed time in hours since the initial observation (From Waterbury and Stanier 1978)

invade the host cell by forming a hole in the outer membrane where they reside in the periplasm (the region between the inner and outer membrane) feeding on biopolymers with hydrolytic enzymes. Greater than 200 genes encoding lytic enzymes have been found in the genome. Within three hours of invasion, the host cell is full of attack phase cells that are released by lysing the host.

Attack phase cells are motile using a single polar flagellum. Whether they actively seek prey cells or happen upon them by chance remains unknown. *B. bacteriovorus* probably attaches to prey cells using type IV pili since inactivation of the pilin gene eliminates predation (Evans et al. 2007). A small opening in the prey cell outer membrane and peptidoglycan layer is made then resealed once *Bdellovibrio* is inside the host cell. Growth and DNA replication occur without cell division to form a multinucleate filament. *B. bacteriovorus* transforms the shape of the prey cell to a spherical, osmotically stable bdelloplast where the *B. bacteriovorus* transports nutrients from the host cytosol. *B. bacteriovorus* can synthesize only 11 amino acids and is dependent on host cell amino acids for protein synthesis. The filamentous *B. bacteriovorus* cell undergoes multiple septation events to generate many progeny. The progeny develop flagella then dissolve the outer membrane and peptidoglycan layer of the bdelloplast to emerge as mature attack phase cells.

Baeocytes. The pleurocapsalean cyanobacteria reproduce by multiple fission to produce many motile baeocytes (*Fig. 16.23*). The baeocyte becomes covered by a thick, fibrous sheath and increases in size as much as 1,000-fold in some

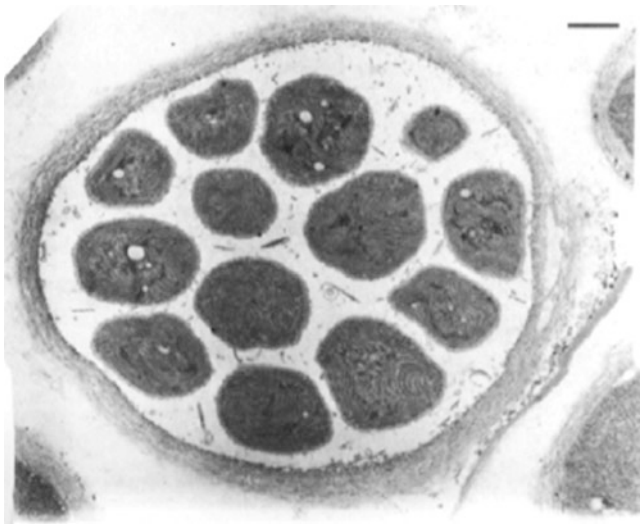


Fig. 16.24
Thin section of a *Dermocarpa* cell. The cell has undergone multiple fission events and is filled with baeocytes, each of which is surrounded by layers of peptidoglycan and outer membrane. Bar, 1 μm (From Waterbury and Stanier 1978)

species such as *Dermocarpa*. When the maximum size has been reached, the cell undergoes multiple fissions within the fibrous sheath (Fig. 16.24). The parental cell then ruptures, releasing numerous small baeocytes. The baeocytes are phototactic and motile by gliding until the cells become covered by the fibrous sheath. At this point, they tend to attach to a solid surface. Unfortunately, little work is done with this intriguing system.

Hormogonia. The most carefully examined dispersal cell using gliding motility is the hormogonium, produced by several filamentous cyanobacteria (Meeks and Elhai 2002). Hormogonia are short filaments composed of nongrowing cells that are motile on surfaces by a mechanism that remains unknown. Cyanobacteria form symbiotic associations with a wide range of eukaryotic hosts including plants, fungi, sponges, and protists. The most carefully studied cyanobacterial symbioses are those with plants in which the cyanobacteria infect the roots, stems, leaves, and, in the case of the liverworts and hornworts, the thallus. The symbionts are usually *Nostoc* spp, that gain entry to the host by means of hormogonia where they resume filamentous growth and develop enhanced N_2 fixation, with much of the fixed nitrogen being destined for the plant.

The differentiation of hormogonia results from a round of synchronous cell division that decreases cell size followed by fragmentation of the filament at the heterocyst–vegetative cell junctions to release short filaments lacking heterocysts. Gene expression during hormogonia differentiation in *Nostoc punctiforme* was examined with DNA microarrays (Campbell et al. 2007). The number of genes expressed in hormogonia producing cells is nearly fivefold higher than those expressed in akinete-forming or nitrogen-fixing cultures. This result is startling because hormogonia are nongrowing and unable to fix nitrogen. The upregulation of 944 genes (out of 2,935 total) suggests that hormogonia are metabolically active. Of the upregulated genes, 85 are involved in signal transduction, 18 are involved in chemotaxis, and 20 are involved in transcriptional regulation arguing that hormogonia are highly tuned to the environment. This result may reflect the fact that the plant exerts strong influence over the differentiation and colonization processes (Meeks and Elhai 2002).

Swarmer Cells. The term "swarmer cell" is ambiguous in that it can refer to several different types of cells. Three examples will be offered. The first uses *V. parahaemolyticus* as the model organism but is represented by a wide variety of Proteobacteria that use swimming cells to move in liquid and swarmer cells to move on solid surfaces. The other two examples, *Rhodomicrobium* and *Caulobacter*, are more similar to each other than to *Vibrio*.

V. parahaemolyticus exists as a swimmer (vegetative) cell in liquid environments or as a swarmer cell on solid surfaces, allowing it to colonize a variety of niches (Fig. 16.25). Swimmer cells are small, uninucleate, and have a single polar flagellum. Swarmer cells are 20–30 times larger than swimming cells and possess a large number of lateral flagella that differ from the polar flagellum. The polar flagellum contains four flagellin subunits, FlaA, B, C, and D, while the lateral flagella contain a single flagellin protein LafA. The polar flagellum is protected by a membranous sheath, which is absent in the lateral flagella. Although the energy used for both types of motility is the electrochemical gradient, the coupling ions are different. The polar flagellum utilizes Na^+ ions, while the lateral flagella use H^+.

Swarmer cell differentiation is induced by growth on solid surfaces or viscous environments because resistance to polar flagellum rotation signals differentiation. Swarmer cells are also differentiated in response to iron limitation. Cell division is inhibited but not cell growth, leading to polynucleated, elongated cells. On solid surfaces, swarming occurs for several hours and then ceases as rapid cell division produces swimmer cells by a process known as consolidation. Concentric rings or terraces are formed on agar plates due to repeated cycles of swarming and consolidation.

Rhodomicrobium vannielii is a phototrophic, budding bacterium that produces both exospores and motile swarmer cells in addition to nonmotile vegetative cells. The polarly flagellated swarmer cell is, like its counterpart in *Caulobacter*, a nongrowing cell whose function is to maximize dispersal. Swarmer cell formation in *R. vannielli* is induced by conditions of low light intensity and high levels of CO_2. At higher light intensities, the swarmer cells shed their flagella and undergo morphogenetic conversion to the reproductive budding phase.

The strategy of alternating swarmer and stalked cells in *Caulobacter* (described in section "*Caulobacter*") is similar but different in a fundamental way. A *Caulobacter* stalked cell cannot give rise to another stalked cell but only to a swarmer cell whose production is an obligate output of the cell division cycle. The sessile, budding cell of *R. vannielii*, on the other hand, has the option of either producing a swarmer cell, or continuing to produce cells connected by hyphae. *R. vannielli* can also generate a resistant, resting exospore as described in section

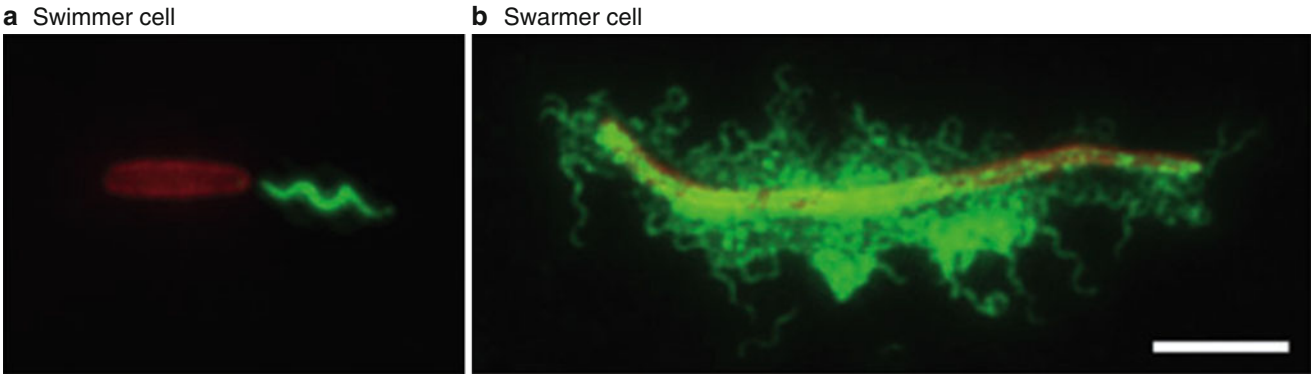


Fig. 16.25
Swimmer and swarmer cells of *V. parahaemolyticus*. LM5674 (wild-type) swimmer cell grown in liquid (a) and swarmer cell grown on a surface (b) are profoundly different. Cells were fixed and examined by immunofluorescence microscopy. Both panels are of the same magnification and the bar indicates 5 μm. Cells were stained with membrane dye FM 4–64 (*colored red*) and anti-polar flagellin antiserum (a, *colored green*) or anti-lateral flagellin antiserum (b, *colored green*) (From Gode-Potratz et al. 2011)

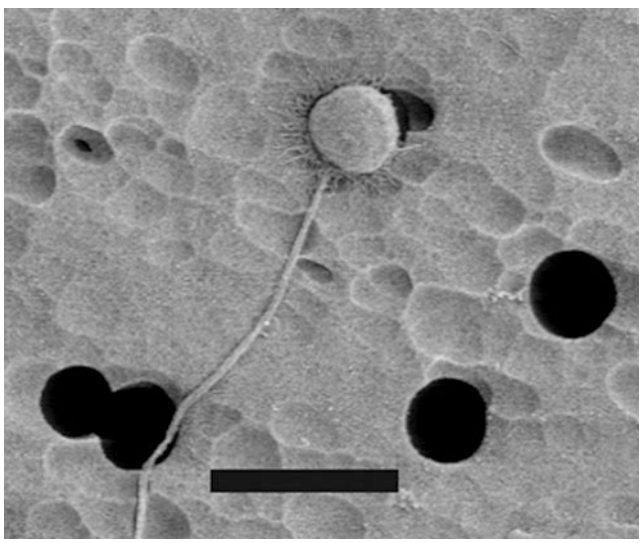


Fig. 16.26
Scanning electron micrograph of a *Kineococcus radiotolerans* SRS30216^T zoospore exhibiting a single flagellum. Bar, 2 μm (From Phillips et al. 2002)

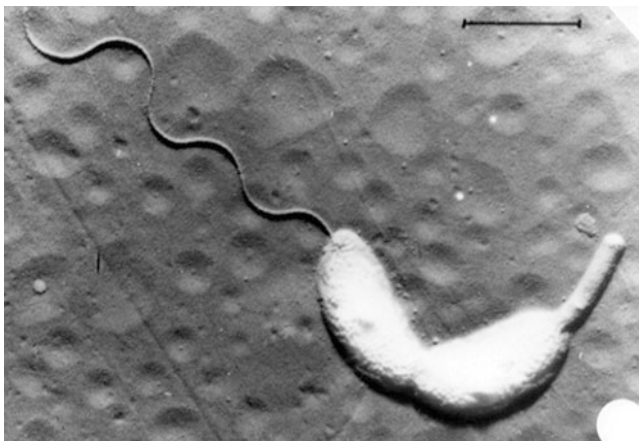


Fig. 16.27
Electron micrograph of a shadowed cell of *Caulobacter crescentus*. The stalked mother cell is in the process of dividing, leading to the formation of a flagellated swarmer cell. Bar, 1 μm (From Poindexter 1964)

"Differentiation Leading to Dormancy". In addition to this remarkable panoply of developmental options, *R. vannielli* can grow either anaerobically as a phototroph or aerobically as a chemotroph, so it is an extremely versatile organism.

Zoospores. The production of motile zoospores is widespread but patchy among members of the Actinobacteria (*Fig. 16.26*). *Kineosporia* zoospores are produced at the tips of substrate hyphae and in clusters on sporangioles, though there is a great deal of variation in the manner in which zoospores are produced in other genera. A question of interest is how the zoospores maintain dormancy and at the same time exhibit rapid movement and tactic behavior. *Kineosporia* SR11 zoospores move at remarkable speeds of up to 160 μm/s and can move up chemical gradients of a variety of inorganic compounds (Radajewski and Duxbury 2001). The *Kineococcus* genome contains complete pathways for flagellar biogenesis and chemotaxis arguing that zoospore dispersal is designed to locate new niches for growth (Bagwell et al. 2008).

Caulobacter

During the *C. crescentus* cell cycle, a stalked parent always produces flagellated progeny (*Fig. 16.27*). A comprehensive review of this dimorphic life cycle is found in Curtis and Brun (2010) and can be divided into cyclic and noncyclic phases

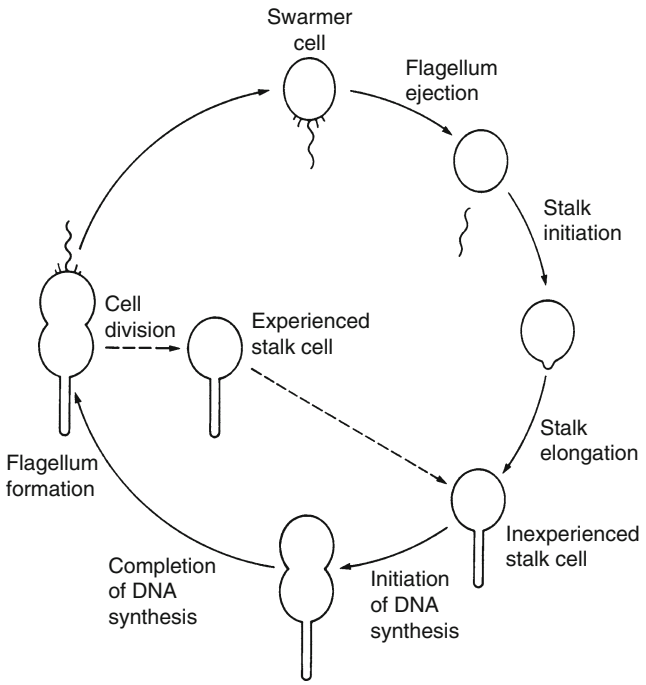


Fig. 16.28

Diagram of the life cycle of *Caulobacter crescentus*. The cyclic developmental program begins with a stalked cell with an adhesive holdfast at the tip of the stalk. The stalked cell enters S phase, a cell state where it is competent for DNA replication. As the cell grows and replicates its DNA, it becomes a predivisional cell. During this time, the cell becomes incompetent for DNA replication, entering the G2 phase. In the late predivisional stage, a flagellum is formed at the swarmer cell pole. After compartmentalization, flagellar rotation is activated and pili are produced. Cell separation leads to two different cell types. One cell is a stalked cell, which reenters the cyclic developmental program and S phase, completing the circle. The other cell is a swarmer cell. The swarmer cell cannot replicate its chromosome yet is distinct from the predivisional cell and therefore is in a separate phase, referred to as G1. The holdfast is formed predominantly during the swarmer cell stage. Later, the swarmer cell differentiates into a stalked cell. This differentiation comprises the noncyclic developmental program. (From Dworkin 1985)

(*Fig. 16.28*). The cyclic phase begins with a stalked cell using the terminal holdfast to attach to a substrate. The stalked cell initiates chromosome replication, and by analogy with the eukaryotic cell cycle, this is referred to as the S phase. At the conclusion of DNA replication and growth, the cell emerges from S phase into the predivisional (G2) phase. In late G2, a flagellum forms at the opposite cell pole from the stalk and begins rotating (*Fig. 16.27*). Pili are produced on the same pole. Following cell division, the flagellated swarmer cell swims away. The stalked cell repeats the cycle, each time producing a swarmer cell. The cyclic portion of the life cycle is controlled by three master regulators, CtrA, GcrA, and DnaA, to move the cell through the cell cycle in a variety of fascinating ways. The complete regulatory circuit involves approximately 550 cell cycle–dependent genes.

After swimming for some time, the swarmer cell grows a holdfast at the same pole as the flagellum, sheds the flagellum, and eventually grows a stalk to become a new stalked cell. At this point, it enters the cyclic developmental phase. CtrA is a response regulator that regulates chromosome replication initiation. Phosphorylated CtrA (CtrA∼P) interacts with DNA in the origin of replication (*oriC*) to block initiation of replication locking the swarmer cell into the G1 phase. The swarmer to stalked cell transition utilizes the unusual response regulator PleD to produce a second messenger, cyclic diguanylic acid (c-di-GMP). PleD activity is needed to degrade the flagellar anchor protein FliF, which coincides with flagellum ejection. Less is known about the mechanisms behind pilus loss, holdfast induction, and stalk formation. Stalk formation has been difficult because there seem to be two pathways for making a stalk as there are no stalkless mutants. The final step in the transition from swarmer cell to stalked cell involves becoming replication competent. This event also begins the cyclic portion of the life cycle for all stalked cells.

The mechanism by which the stalked cell produces the swarm cell is known with stunning clarity and includes temporal induction of cell cycle events and spatial control of protein localization into the stalked cell compartment and the swarmer cell compartment. CtrA is degraded by ClpXP, an ATP-dependent protease releasing the major block to chromosome replication. Degradation of CtrA is accompanied by DnaA synthesis. DnaA binds to *oriC* to initiate DNA replication. Free DnaA, which is present only during the start of each cell cycle, also acts as a transcriptional activator of GcrA expression to activate genes required for DNA replication. CtrA also represses transcription GcrA, and proteolysis of CtrA enables transcription of GcrA. DnaA also acts as a transcriptional activator of cell division initiator FtsZ, which localizes at the site of cell division.

Cell polarity governs correct localization of the flagellum and stalk in the predivisional cell. Cell polarity is achieved with the regulators of polarity, the DivJ and PleC histidine kinases, and the DivK response regulator. The cyclic developmental cycle begins with DivJ and DivK∼P localized to the stalked pole. DivL and PleC are delocalized in the inner membrane. DivK phosphorylation leads to inactivation of CtrA, which, along with DnaA, leads to *gcrA* transcription. DnaA and GcrA produce PodJ, which localizes to the nascent swarmer pole and serves as a localization factor for PleC. Now, DivJ and PleC are located at opposite poles and mark the stalked pole and the pole that will develop the flagellum, respectively. DivK is phosphorylated by DivJ at the stalked pole and localizes there. Some DivK∼P also diffuses to the swarmer pole but is dephosphorylated by PleC causing it to diffuse back across the cell where it becomes rephosphorylated by DivJ at the stalked pole. DivK phosphorylation/dephosphorylation cycling continues as the cell cycle progresses. After cytokinesis, DivJ and PleC enzymatic activities are separated from each other. As a consequence, DivJ activity leads to DivK phosphorylation in the stalked cell, which then localizes at the stalked pole. In the swarmer cell compartment, PleC

activity leads to DivK∼P dephosphorylation and delocalization. The phosphorylation state of DivK affects activation/inactivation of CtrA in each cell compartment. DivK∼P favors CtrA inactivation encouraging further rounds of cell division. In the swarmer cell, DivK favors CtrA activation blocking chromosome replication initiation and reproduction.

Conclusion

The startling diversity of prokaryotic developmental cycles attests at once to the ability of specialized cells to enhance survival of the species in ever-changing environments. Differentiation of multiple cell types achieves a division of labor that maximizes the resources of these simple but cunning creatures. One may justify an interest in prokaryotic life cycles solely on the ground that locked within them is a vast array of undiscovered secrets stemming from the delicate interactions between an organism and its environment. The spatial and temporal cues and their exquisitely balanced sensory pathways manifested in the systems described in this chapter are but the beginning of a journey of discovery that will enlighten and entertain for decades to come.

Acknowledgment

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