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# Protein Localization and Cell Fate in Bacteria

Lucy Shapiro and Richard Losick

A major breakthrough in understanding the bacterial cell is the discovery that the cell is highly organized at the level of protein localization. Proteins are positioned at particular sites in bacteria, including the cell pole, the incipient division plane, and the septum. Differential protein localization can control DNA replication, chromosome segregation, and cytokinesis and is responsible for generating daughter cells with different fates upon cell division. Recent discoveries have revealed that progression through the cell cycle and communication between cellular compartments are mediated by two-component signal transduction systems and signaling pathways involving transcription factor activation by proteolytic processing. Asymmetric cell division in *Caulobacter crescentus* and sporulation in *Bacillus subtilis* are used as paradigms for the control of the cell cycle and cellular morphogenesis in bacterial cells.

Temporal and spatial constraints restrict how a bacterium gleans information from its genome. These constraints dictate the ordered expression of cell cycle events, the three-dimensional organization of the cell, and the biogenesis of subcellular organelles and altered cellular forms. Each of these general classes of cellular functions is programmed by a genetic network that includes a pathway of regulatory events. The combination of bacterial genetics and new ways of using cytological methods to examine bacterial cell organization has not only led to the identification of the components of these regulatory pathways, but has placed the pathway outcomes at specific sites in the cell. Analysis of two bacteria separated by large evolutionary distance, the Gram-negative, aquatic bacterium *Caulobacter crescentus* and the Gram-positive, soil bacterium *Bacillus subtilis*, has elucidated common mechanisms that serve as paradigms for prokaryotic cell cycle control and cellular morphogenesis.

The use of immunogold electron microscopy and fluorescence microscopy to study the subcellular organization of bacterial cells has revealed a surprising extent of protein compartmentalization and localization. In some instances, localized proteins are not essential for viability, as is the case for the flagellum and the chemosensory apparatus (*1*), actin-recruiting virulence proteins in certain pathogenic bacteria (*2*), and certain morphogenetic and regulatory proteins involved in the process of sporulation (*3*–*5*). In other cases, such as the proteins involved in DNA replication, chromosome segregation, or cell division, the site of protein deposition is an integral part of the regulatory machinery that mediates these essential functions. A critical question now facing the prokaryotic community is how proteins are deployed in a targeted three-dimensional array in the absence of known cytoskeletal components. Notwithstanding free diffusion in the microenvironment of the bacterial cell, how are proteins directed to specific sites in the cell, and how are they kept there? What controls the rearrangement of proteins during morphogenesis? As we will show, *C. crescentus* and *B. subtilis* offer numerous and striking examples of subcellular localization of proteins. These, and examples from other bacteria, have changed our view of the bacterial cell and demonstrated that it is important not only to understand what a protein does but where and when it acts.

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## Asymmetric Cell Division

Differentiation in *C. crescentus* and *B. subtilis* results from a modified process of cell division (*6*, *7*). Unlike the familiar process of binary fission in which a dividing cell generates identical progeny, *C. crescentus* and sporulating *B. subtilis* undergo cell division asymmetrically, each generating dissimilar progeny with distinct fates (Fig. 1). Asymmetric division in *C. crescentus* is an obligatory feature of its cell cycle. Thus, a dividing cell generates a motile, swarmer cell, which is propelled by a rotary flagellum, and a nonmotile, stalked cell. The stalked cell replicates its chromosome and undergoes differentiation into a predivisional cell with a nascent swarmer cell on one side of the division plane and the stalked cell on the other. Meanwhile, after an interval in which DNA replication is repressed, the swarmer sheds its flagellum and undergoes metamorphosis into a stalked cell, which repeats the cycle.

Asymmetric division in sporulating *B. subtilis*, in contrast, is an environmentally induced adaptation to conditions of nutritional stress. *Bacillus subtilis* normally propagates by binary fission. However, when nutrients become limiting, it undergoes a switch from symmetric to asymmetric division by the formation of a polar septum. The polar septum partitions the developing cell into a small, forespore cell and a large, mother cell. Initially, the forespore and the mother cell lie side by side in the sporangium. Later, the forespore becomes wholly engulfed by the mother cell in a phagocytic-like process to create a cell within a cell. The forespore ultimately becomes a spore and gives rise to future progeny, whereas the mother cell is discarded by lysis when maturation of the spore is complete.

## Polarity and Protein Localization

In *C. crescentus*, the cell cycle perpetuates asymmetry that is present before cell division. The predivisional cell is itself asym-

metric, having a nascent flagellum and chemoreceptors at one pole and a stalk at the other (Fig. 2) (*8*, *9*). Positioning of the flagellum and of the chemoreceptors appears to be mediated by separate targeting mechanisms. In the case of flagellum biogenesis, the first structure assembled at the cell pole is the MS ring, an integral membrane component of the flagellar motor. The MS ring is composed of FliF protein subunits, which are synthesized relatively early in the cell cycle, before formation of the "swarmer" and "stalked" cell compartments (*9*). Ring protein synthesized early in the cell cycle is recovered only in the daughter swarmer cell, providing evidence that the MS ring is targeted to the pole that will bear the flagellum. It is not known how this targeting occurs, but FliF could recognize a receptor or other landmark left at the future site of flagellum biogenesis at the time of the previous cell division.

The positioning of the chemoreceptors at the cell pole opposite the stalk is independent of flagellar positioning. Interestingly, clustering of chemoreceptors at the cell pole is also observed in *Escherichia coli*, although in this case the receptors are found at both ends of the cell (Fig. 2) (*1*). The *E. coli* chemoreceptor Tsr, a transmembrane protein, is held in a complex with the cytoplasmic histidine kinase CheA by way of the adapter protein CheW (*10*). In the absence of CheA and CheW, the Tsr chemoreceptor loses its polar position and appears at random sites around the cell (*1*). Similarly, in the absence of Tsr, CheA and CheW are randomly distributed throughout the cytoplasm. Therefore, complex formation between Tsr, CheA, and CheW is essential for polar localization of the chemosensory apparatus, with CheW playing a pivotal role in the positioning of the ternary complex. Newly synthesized chemoreceptors might be inserted in the membrane only at the cell pole, but retention at the pole requires the formation of a ternary complex. Such a higher order complex also forms when the MS ring protein is inserted at the cell pole in C. *crescentus*, creating a platform for the assembly of other flagellar proteins.

During sporulation in *B. subtilis*, polarity is created as a consequence of asymmetric division and does not appear to exist before septum formation (*7*). The polarity created by asymmetric division is reinforced by differential gene expression (below) and by the targeting of proteins to the septum and to other sites within the forespore and the mother cell. For example, a membrane-bound phosphatase (SpoIIE) involved in the establishment of cell type (below) initially localizes to potential division sites near both poles of the sporangium (*3*). Later, it becomes concentrated at the polar septum, disappearing from the distal pole of the sporangium (Fig. 2). Meanwhile, morphogenetic proteins (SpoIVA and CotE) involved in the assembly of the spore coat and two regulatory proteins (SpoIVFA and SpoIVFB) involved in the activation of a transcription factor late in the sporulation process localize around the mother cell membrane that surrounds the forespore at the postengulfment stage of sporulation (*4*, *5*). Yet other sporulation proteins (SASPs) drive the forespore chromosome, but not that of the mother cell, into a striking doughnut-like structure (Fig. 2) (*5*). Some of this localization is due to cell-specific gene expression. SpoIVA, SpoIVFA, SpoIVFB, and CotE are produced in the mother cell, whereas SASPs are produced in the forespore (*7*). But how are we to explain the targeting of proteins to specific locations within a compartment? How do some proteins recognize the septal membranes but not the rest of the cytoplasmic membrane? How do other proteins adhere to the mother cell membrane that surrounds the engulfed forespore but not other membrane surfaces in the mother cell? Al-

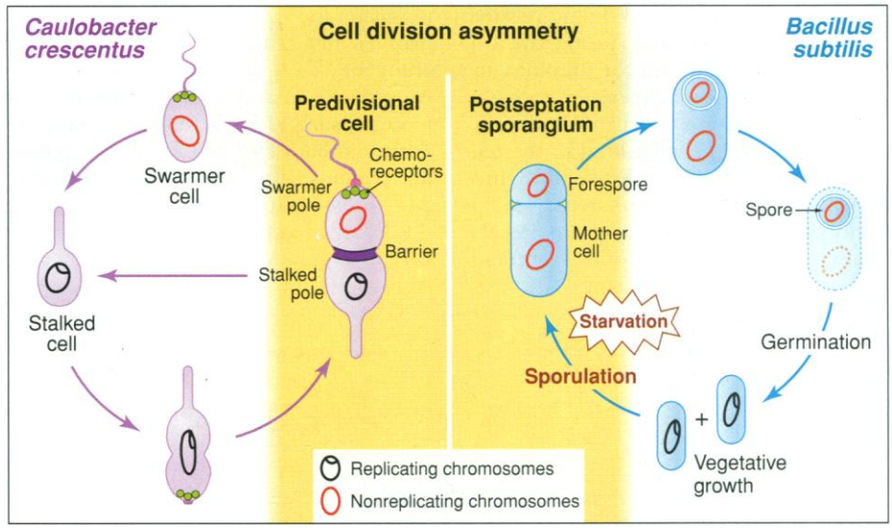


**Fig. 1.** Cell division asymmetry characterizes both the *C. crescentus* predivisional cell (left) and the *B. subtilis* postseptation sporangium (right). The black theta structures represent chromosomes capable of initiating DNA replication, and the red circles represent nonreplicating chromosomes. The green dots at the *C. crescentus* swarmer pole represent the chemoreceptors and the purple bar at midcell represents the barrier formed between the two cellular components. In *B. subtilis*, asymmetric division is followed by engulfment of the forespore by the mother cell. When maturation of the spore is complete, the mother cell and the mother cell chromosome disintegrate, as indicated by dashed lines.

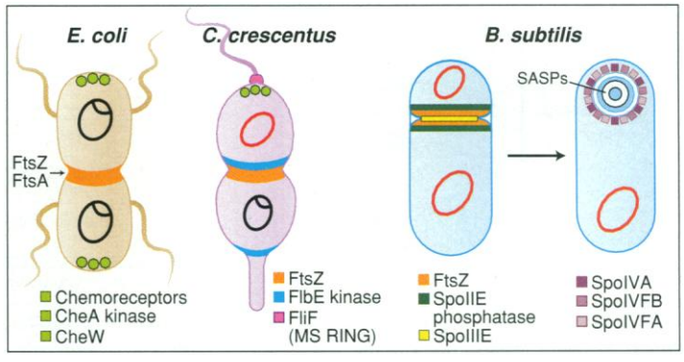


**Fig. 2.** Protein localization in *E. coli*, *C. crescentus*, and sporulating *B. subtilis*. The flagella in *E. coli* are peritrichous, and the single flagellum in *C. crescentus* is at one pole of the cell. The tubulin-like protein FtsZ assembles at incipient sites of cell division and then disassembles when the septum is completely formed (*13*). In *B. subtilis*, FtsZ and the SpoIIE phosphatase initially localize to potential division sites near both poles (*3*, *17*). After asymmetric division, FtsZ disappears from both poles, but SpoIIE persists at the site at which the septum has formed. For simplicity, FtsZ and SpoIIE are only shown at the site at which the polar septum is being formed. After engulfment, the forespore chromosome is packaged into a doughnut-like ring (white) by the SASPs (thereby obscuring the chromosome in red) and the SpoIVA, SpoIVFA, and SpoIVFB proteins adhere to the outside surface of the mother cell membrane that surrounds the forespore.

though little is known about these processes, the differentiation of membrane domains is likely to contribute to protein targeting in the bacterial cell.

## Chromosome Segregation and Cell Division

Cell division in bacteria involves alternating and coordinated cycles of DNA replication and cytokinesis. Because bacteria lack a conspicuous mitotic apparatus, the nature of the prokaryotic machinery for segregating newly duplicated DNA and ensuring that each daughter cell receives a chromosome has eluded description. Nevertheless, it has recently become possible to visualize the position during the cell cycle of specific sites on the chromosome (*11*). These and other experiments involving the visualization of "chromosome partition" proteins (*12*) that bind to the replication origin region indicate that chromosome segregation is mediated by an apparatus that is responsible for moving the origin region of newly formed chromosomes to opposite poles of the cell.

After chromosome segregation, a cytokinetic ring is formed at the midcell position by polymerization of the FtsZ protein (Fig. 2) (*13*). FtsZ is a tubulin-like protein that localizes to the plane of division (*14*). The discovery of localized FtsZ demonstrated that bacteria are not only capable of localizing cytoplasmic proteins, but that there are also proteins in the bacterial cell that function in a manner analogous to cytoskeletal components of eukaryotic cells. As the division process proceeds, rings of FtsZ can be detected at the leading edge of the newly forming septum. Cytokinesis is believed to be effected by constriction of these rings in a guanosine 5′-triphosphate–dependent manner. Just as the Tsr chemoreceptor recruits the CheA and CheW proteins to the cell pole, FtsZ may recruit other division proteins, such as FtsA, to the septum (*15*). Although both FtsZ and FtsA are cytoplasmic proteins, other integral-membrane proteins are likely to be involved in generating the localized apparatus that drives cytokinesis. As a consequence of the growth of the septum at the leading edge of the contracting cytokinetic ring, the cell is divided into two daughter cells, each containing a chromosome.

Sporulation in *B. subtilis* involves a switch in the site of cell division and a surprising modification of the mechanism of DNA segregation. At the start of sporulation, the sporangium contains two complete chromosomes, each attached near an opposite pole by way of a site at or near the origin of DNA replication (*11*, *16*). Chromosome attachment is not, however, followed by chromosome segregation, nor by the formation of a medially situated FtsZ ring. Instead, two rings of FtsZ assemble at potential cell division sites near opposite ends of the cell while the two chromosomes remain spread across the length of the cell (*17*). The switch from the assembly of a medially positioned FtsZ ring to the formation of two polar rings occurs under the control of the master regulator for entry into sporulation, the transcription factor Spo0A (below), and is a pivotal event in the transition from growth to sporulation (*17*). In a subsequent regulatory event, a septum is formed at one of the sites, thereby creating the forespore and mother cell compartments (Fig. 2). Because septum formation precedes chromosome segregation, the forespore initially contains only the origin-proximal region of the chromosome (*16*). The remainder of the chromosome is subsequently translocated into the forespore by an inferred DNA translocase (SpoIIIE), which is located in the polar septum (*16*).

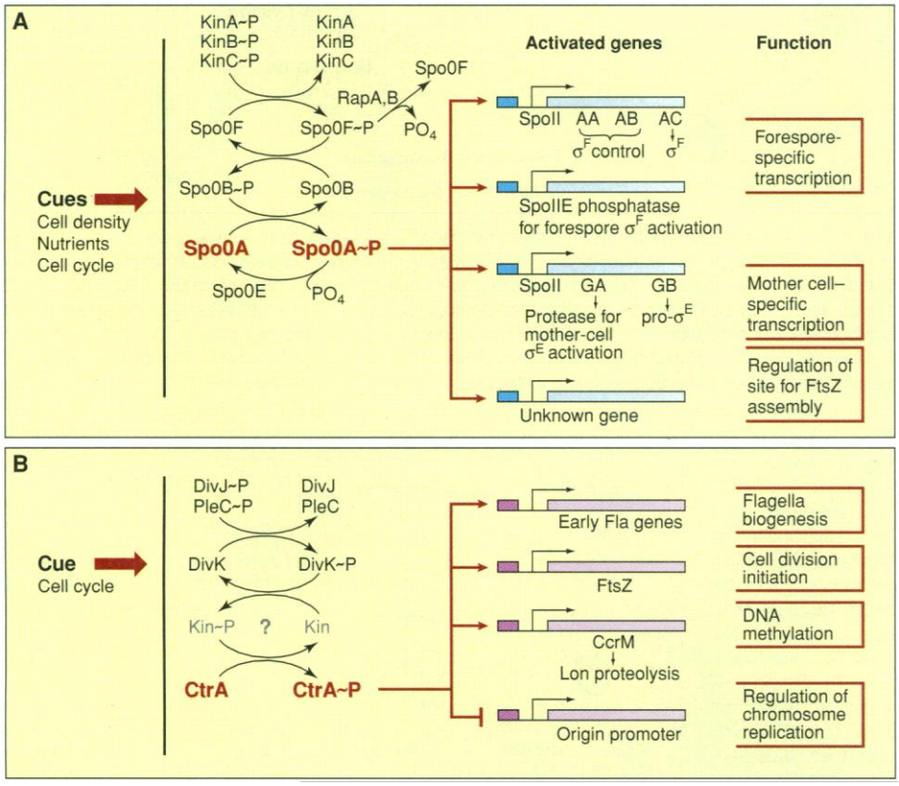


**Fig. 3.** (**A**) The phosphorelay that activates the transcriptional regulator Spo0A in *B. subtilis* sporulation. Activated Spo0A~P turns on the genes that govern forespore- and mother cell–specific transcription factors and an unknown gene or genes that are required for switching the site of assembly of the tubulin-like protein FtsZ to the cell pole. (**B**) The hypothetical phosphorelay (*24*) that activates the cell cycle transcriptional regulator CtrA in *C. crescentus*. Activated CtrA~P turns on the early flagellar genes, regulates the gene encoding FtsZ protein required for cell division initiation, and activates the gene encoding the essential DNA methyltransferase CcrM, while repressing a promoter in the origin of replication only in the swarmer cell, thereby allowing replication to proceed in the stalked cell. The function of the genes activated by Spo0A~P and CtrA~P is indicated in the boxes at the right.

## Master Regulators Govern the *C. crescentus* Cell Cycle and *B. subtilis* Entry into Sporulation

The coordination of a multigene pathway that directs complex cellular behavior is a universal problem faced by all organisms. The regulatory networks that govern the *C. crescentus* cell cycle and *B. subtilis* sporulation initiation exhibit conserved circuit logic that is based on two-component signal transduction systems (*18*, *19*). Two-component systems are composed of an integral membrane or cytoplasmic sensor kinase that catalyzes the transfer of a phosphate from adenosine 5′-triphosphate to an internal histidine residue in response to extracellular or intracellular signals, and a cognate cytoplasmic response regulator that catalyzes the transfer of the phosphate from the sensor kinase to an aspartate residue in its own receiver domain. Many response regulators have a DNA-binding domain that mediates transcriptional regulation. Some two-component systems are components of a multistep phosphorelay with alternating histi-

dine-aspartate phosphotransfers (*20*).

In C. *crescentus*, cell cycle progression appears to be cued by internal signals, whereas B. *subtilis* sporulation initiation is cued by the integration of external cell density signals and by internal nutritional and cell cycle signals. In each case, a signal transduction pathway culminates in the phosphorylation of a response regulator that controls the transcription of a wide spectrum of genes, whose expression is directly involved in determining cell fate (Fig. 3). Three histidine kinases are involved in governing the initiation of sporulation in *B. subtilis* (*21*) (Fig. 3A). All three kinases donate phosphate to the single-domain response regulator, Spo0F, to create Spo0F~P. Phosphate from Spo0F~P is then transferred to Spo0B, a phosphoprotein phosphotransferase, which completes the chain by, in turn, relaying the phosphate to the master transcriptional regulator Spo0A (*22*).

An important concept to emerge from studies of the phosphorelay in *B. subtilis* is that phosphate flow through the relay is regulated as much by specific phosphatases, which drain phosphates from the relay, as by kinases, which feed phosphates into the relay (*21*, *23*). Three such phosphatases are known, two of which dephosphorylate Spo0F~P (RapA and RapB) and one which acts on Spo0A~P (Spo0E) (Fig. 3A). Thus, phosphorylation and dephosphorylation integrate a variety of signals and thereby set the cellular concentration of the active form of the master regulator, Spo0A~P.

The comparable response regulator in C. *crescentus* is CtrA, which in its phosphorylated form regulates the transcription of multiple cell cycle genes (Fig. 3B). Only some possible members of a CtrA phosphorelay have been identified, but it is attractive to suppose that they constitute a *B. subtilis*–like pathway (*24*). If so, it will be interesting to see whether cell cycle signals in C. *crescentus* are modulated by the action of phosphatases as well as kinases.

How do Spo0A and CtrA play a pivotal role in differential cell fate? Once a sufficient supply of Spo0A~P builds up in the cell (*25*), the transcription of the *spoIIA* and *spoIIG* operons and the *spoIIE* gene is induced (*26*); these transcription units encode the sigma factors, $\sigma^F$ and $\sigma^E$, and proteins required for their differential activation in the forespore and the mother cell (Fig. 3A) (*7*). In addition, Spo0A~P induces a still unknown gene or genes that are required for the switch from symmetric to asymmetric cell division (*17*). Thus, integration of external signals through the phosphorelay culminates in an increase in the cellular concentration of Spo0A~P and thereby triggers a chain of events that converts the predivisional cell into a sporangium with two cellular compartments that follow dissimilar pathways of differentiation.

CtrA~P similarly plays a central role in the C. *crescentus* cell cycle by governing the ordered activation of many cell cycle events (*27*) (Fig. 3B). These include (i) initiation of the flagellar transcriptional cascade; (ii) transcription of the gene for an essential DNA methyltransferase, CcrM, that converts hemi-methylated DNA to the fully methylated DNA after chromosome duplication (*28*); (iii) transcription of the cell division gene *ftsZ* (*29*); and (iv) repression of DNA replication in the swarmer compartment of the predivisional cell (*27*, *30*).

Two of these regulatory events, transcription of flagellar genes and repression of DNA replication, generate progeny cells with different fates. After the initiation of DNA replication in the C. *crescentus* stalked cell, CtrA~P accumulates to threshold levels in the early predivisional cell. This initiates the induction of the early flagellar genes. Among the flagellar genes under the control of CtrA~P are those encoding the proteins in the type III transport system that mediate the polar assembly of the flagellum, the *fliF* gene encoding the protein component of the MS ring, and the gene for the FlbD response regulator that is used for the differential activation of the late flagellar genes in the swarmer compartment of the predivisional cell (Fig. 4B).

Once chromosome replication is completed and the two chromosomes become fully methylated by the CtrA~P-induced DNA methyltransferase, the swarmer and stalked compartments are separated by the formation of a barrier of undefined composition. CtrA~P, which is present in the swarmer compartment, prevents the initiation of DNA replication by binding to and repressing a promoter within the origin of replication that appears to be essential for replisome formation (*27*, *31*). The chromosome in the stalked compartment is free to form the replisome (*31*). Thus, it appears that differential protein localization or function serves to effect the unique cell fate of the two progeny cells. The progeny swarmer cell differentiates into a stalked cell later in the cell cycle, becomes competent to initiate DNA replication, and then builds up sufficient quantities of CtrA to again turn on the series of cell cycle events that culminates in an asymmetric predivisional cell.

## Connecting Cell Fate to Asymmetric Distribution of Proteins

Asymmetric cell divisions that yield different progeny cells are a hallmark of sporulation in *B. subtilis* and the C. *crescentus* cell cycle (Fig. 1). How does asymmetric division in these organisms induce differential gene expression in the progeny cells after cytokinesis? Strikingly, in both organisms events at the division plane help dictate the dissimilar fates of the progeny (Fig. 4).

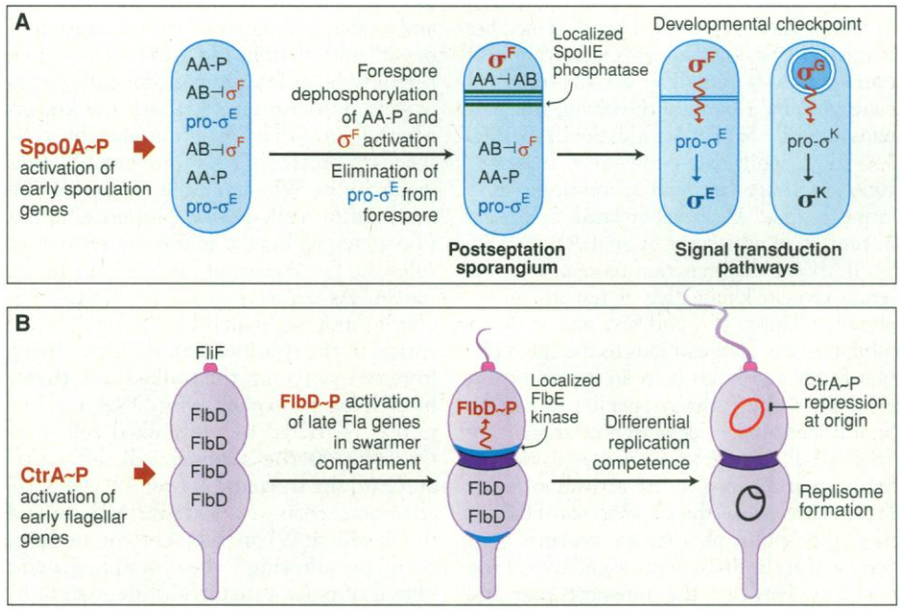


**Fig. 4.** Determination of cell fate by protein localization during sporulation in *B. subtilis* (**A**) and during the cell cycle in *C. crescentus* (**B**). Spo0A~P in (A) is shown inducing the synthesis of SpoIIAA (AA), SpoIIAB (AB), $\sigma^F$, and pro-$\sigma^E$. CtrA~P in (B) is shown inducing the synthesis of the MS ring protein FliF and the response regulator FlbD, although several other early flagellar genes are also under the control of CtrA~P.

The genes that encode the external components of the flagellum, such as the hook and the filament subunits, are transcribed in the swarmer compartment of the predivisional cell shortly after the formation of a barrier between the two compartments (*32*). Differential flagellar gene expression is governed by FlbD (*33*, *34*), a member of the two-component family of response regulators. FlbD is activated by a phosphate derived from the histidine kinase FlbE (*35*). FlbD~P both induces the transcription of the late flagellar genes and represses the transcription of the early flagellar genes whose products are no longer needed for flagellar biogenesis in the swarmer compartment (*33*).

This compartment-specific transcription is attributed to selective phosphorylation of FlbD in the swarmer compartment (*34*) (Fig. 4B). An illuminating finding is that the FlbE kinase is not distributed uniformly throughout the predivisional cell (*35*). Rather, it is localized to the pole of the stalked compartment and to the division plane at the midcell position (Fig. 4B). When the predivisional cell is partitioned into two compartments, FlbE at the midcell position is somehow selectively captured by the swarmer compartment, where the kinase activates the FlbD transcription factor. FlbE kinase molecules at the pole of the stalked cell are apparently inactive. Thus, events at the division plane ensure that the phosphodonor for FlbD, and hence FlbD-directed transcription, occurs in one cellular compartment.

In *B. subtilis*, differential gene expression in the forespore is governed by $\sigma^F$, a member of the RNA polymerase sigma factor family of transcription factors. The activity of $\sigma^F$ is controlled by a pathway involving the proteins SpoIIE, SpoIIAA, and SpoIIAB (Fig. 3A) (*36*). SpoIIE is a phosphatase that activates SpoIIAA by dephosphorylation at a serine residue. Dephosphorylated SpoIIAA, in turn, is an antagonist of SpoIIAB. Finally, SpoIIAB is a dual function protein: It is the serine protein kinase that is responsible for phosphorylation of SpoIIAA, and it is an inhibitor of $\sigma^F$, which binds to the transcription factor and holds it in an inactive complex. Thus, the pathway operates in part by SpoIIE-mediated dephosphorylation of SpoIIAA-P, which leads to inactivation of SpoIIAB and hence to the activation of $\sigma^F$. Provocatively, like the *C. crescentus* FlbE kinase, the SpoIIE phosphatase becomes concentrated at the division plane and thus at the boundary between the forespore and the mother cell (*3*). Evidence (*37*) indicates that preferential dephosphorylation of SpoIIAA-P in the forespore is responsible for restricting $\sigma^F$-directed transcription to the small chamber of the sporangium (Fig. 4A). How could this occur? If SpoIIE molecules are present or active only on the forespore side of the septum, then dephosphorylation of SpoIIAA-P would be restricted to one cell. Even if SpoIIE molecules are displayed equally on both sides of the septum, however, the asymmetric placement of the septum would bring about a higher ratio of SpoIIE phosphatase molecules to the SpoIIAB kinase molecules in the small, forespore chamber than in the large, mother cell chamber and thus contribute to preferential dephosphorylation of SpoIIAA-P in the forespore. Whatever the precise mechanisms that limit dephosphorylation of SpoIIAA-P to the forespore, and phosphorylation of FlbD to the swarmer cell, it is noteworthy that in two unrelated microorganisms cell fate is established by proteins that localize to the site of cell division.

The mother cell counterpart to $\sigma^F$ is $\sigma^E$. The $\sigma^E$ factor is produced in the predivisional sporangium as an inactive proprotein (pro-$\sigma^E$) that does not become active until after polar septation when $\sigma^E$-directed transcription is confined to the mother cell (*38*). The $\sigma^E$ factor is subject to two regulatory mechanisms. One mechanism, which is considered below, operates at the level of the conversion of pro-$\sigma^E$ to mature $\sigma^E$ and is responsible for delaying the time of $\sigma^E$ activation until after polar division (*39*). The other mechanism seems to be responsible for restricting the activity of $\sigma^E$ to the mother cell. Immunofluorescence microscopy experiments show that shortly after polar division pro-$\sigma^E$ and $\sigma^E$ are present in the mother cell but absent in the forespore, and evidence indicates that elimination of pro-$\sigma^E$ and $\sigma^E$ from the forespore could be responsible, at least in part, for cell-specific gene transcription (*40*). It is not known whether pro-$\sigma^E$/$\sigma^E$ is eliminated by cell-specific proteolysis or by some other mechanism. Whatever the nature of the mechanism, cell-specific protein elimination plays strikingly analogous regulatory roles in *C. crescentus* as well as in *B. subtilis*. As we have seen, the master regulator CtrA~P, which blocks DNA replication in the swarmer cell, is either absent from or inactive in the stalked cell, thereby helping to explain why DNA replication is restricted to the stalked cell. Furthermore, as the swarmer cell differentiates into the stalked cell (Fig. 1), the polar chemoreceptors (*8*) and the MS ring of the flagellum (*9*) are subject to proteolysis, thereby allowing the morphogenetic switch of polar structures while maintaining asymmetry throughout the cell cycle. Thus, differential protein distribution in *B. subtilis* and *C. crescentus* helps govern the dissimilar fates of the progeny of asymmetric division.

## Developmental Checkpoints That Link Gene Expression to Morphogenesis

Progression through the *C. crescentus* cell cycle and the sporulation pathway of *B. subtilis* involves, as we have seen, the temporally ordered and spatially controlled expression of several sets of genes, whose products drive morphogenesis. However, the reverse is also true: Gene expression at several critical junctures is dependent on certain landmark events in morphogenesis. Checkpoints exist that ensure that a particular stage of morphogenesis has been reached before the next round of gene expression can ensue. These developmental checkpoints are timing devices that keep the program of gene expression in check with the course of morphogenesis. An example of such a coupling device is offered by flagellum biogenesis. The expression of flagellar genes is governed by a regulatory hierarchy of three sets of genes (*6*). At the top of the hierarchy are genes that encode components of the basal body, the membrane-anchored motor that drives rotation of the flagellum. The expression of these genes is required for the expression of the next gene set, which includes additional basal body genes, and those that specify components of the hook, which links the motor to the flagellar filament. Finally, expression of this second set of genes is required to turn on flagellin genes, which encode components of the filament.

Remarkably, mutations in any one of a large number of morphogenetic genes prevents transcription of downstream genes in the hierarchy. This implies that gene transcription is somehow coupled to events in the morphogenesis of the flagellum. How does this coupling operate? The nature of the coupling mechanisms has not yet been elucidated in *C. crescentus*, but in the enteric bacteria *Salmonella typhimurium* and *Escherichia coli* this process is now understood (*41*). The transcription of flagellin genes is directed by a dedicated RNA polymerase sigma factor whose activity is negatively regulated by an inhibitory protein called FlgM. Assembly of the flagellum is mediated by a specialized export system that allows flagellar components to pass out of the cell through a channel in the basal body where they polymerize outside the cell into the elongating flagellum. Early in morphogenesis, the activity of the sigma factor is held in check by complex formation with FlgM. When, and only when, the hook and basal body are completed, the export channel allows FlgM to pass out of the cell, resulting in a free and active cytoplasmic sigma factor. Thus, the activation of a transcription factor is explicitly tied to the completion of the assembly of

a substructure in morphogenesis.

Gene expression in *B. subtilis* is similarly tied to morphogenesis at several points during the course of sporulation. First, in an example we have already considered, the activation of $\sigma^F$ requires both asymmetric division (*42*) and a protein phosphatase that localizes to the polar septum (*3*). Thus, the pathway governing $\sigma^F$ activity not only teaches us about cell-specific gene expression but also provides an explicit example of how gene expression is linked to a key event in morphogenesis. Polar septation additionally governs the activation of the mother-cell transcription factor $\sigma^E$ by a mechanism involving the proteolytic processing of pro-$\sigma^E$ (Fig. 4A). Pro-$\sigma^E$ is synthesized in the predivisional sporangium, but its conversion to mature $\sigma^E$ is delayed until the septum is formed (*38*). Proteolytic processing is mediated by an intercellular signal transduction pathway in which a membrane-bound receptor-protease in the mother cell is activated by a signaling protein produced in, and secreted from, the forespore under the control of $\sigma^F$ (*39*). Because activation of $\sigma^F$ requires asymmetric division, the conversion of pro-$\sigma^E$ to $\sigma^E$ is therefore also tied to septum formation through the dependence of processing on the product of a $\sigma^F$-controlled gene. In this way, pro-$\sigma^E$ processing is a timing device that indirectly couples the appearance of $\sigma^E$ to the formation of the sporulation septum.

Later in development, $\sigma^E$ is replaced in the mother cell by the late-acting transcription factor $\sigma^K$ which, like $\sigma^E$, is derived from an inactive proprotein (*43*). As in the case of pro-$\sigma^E$, the conversion of pro-$\sigma^K$ to $\sigma^K$ is controlled by a signal transduction pathway that couples proteolytic processing in the mother cell to a protein signal generated in and secreted from the forespore (Fig. 4A) (*43*). In this case, production of the signaling protein (under the control of the late-acting, forespore transcription factor $\sigma^G$) and its transmission require that the engulfment has been completed and the forespore is completely enclosed within the mother cell cytoplasm. Transmission of the signal is mediated by proteins (SpoIVFA and SpoIVFB) that are located in the mother cell membrane that surrounds the forespore and sit at the boundary between the forespore and mother cell compartments (Fig. 2).

The asymmetric localization of cell fate–determining proteins before cell division that culminates in specifying the fate in progeny cells is a fundamental mechanism of cell differentiation and development in eukaryotic organisms. The asymmetric inheritance of proteins such as Ash1P in *Saccharomyces cerevisiae* (*44*), NUMB and PROSPERO in *Drosophila melanogaster* (*45*), and Notch1 in the generation of the mammalian cortex (*46*) ensures that the progeny cells have different programs of gene expression. We have described here two evolutionarily distant bacteria that rely on asymmetric protein localization to control the fate of progeny cells. Clearly, the individual mechanisms that target proteins to specific cellular sites will differ between prokaryotes and eukaryotes, but the fundamental process has been conserved. In addition to protein localization, temporally and spatially controlled phosphorylation of regulatory proteins, and regulated proteolysis are emerging as central themes in the morphogenesis of all organisms.

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# Exploitation of Mammalian Host Cell Functions by Bacterial Pathogens

B. Brett Finlay and Pascale Cossart

Interest in bacterial pathogenesis has recently increased because of antibiotic resistance, the emergence of new pathogens and the resurgence of old ones, and the lack of effective therapeutics. The molecular and cellular mechanisms of microbial pathogenesis are currently being defined, with precise knowledge of both the common strategies used by multiple pathogenic bacteria and the unique tactics evolved by individual species to help establish infection. What is emerging is a new appreciation of how bacterial pathogens interact with host cells. Many host cell functions, including signal transduction pathways, cytoskeletal rearrangements, and vacuolar trafficking, are exploited, and these are the focus of this review. A bonus of this work is that bacterial virulence factors are providing new tools to study various aspects of mammalian cell functions, in addition to mechanisms of bacterial disease. Together these developments may lead to new therapeutic strategies.

Despite the extensive use of antibiotics and vaccination programs, infectious diseases, particularly microbial diseases, continue to be a leading cause of morbidity and mortality worldwide. Recent outbreaks and epidemiologic studies predict that their incidence will increase while the world's population continues to grow. The emergence of previously undescribed pathogens has been a feature of the end of this century. Increased global travel has contributed to the dissemination of pathogens previously confined to specific regions. In addition, it is now clear that bacterial pathogens cause diseases previously thought not to be infectious, such as the gastro-duodenal ulcers caused by *Helicobacter pylori*. And old diseases, such as tuberculosis, have returned with a vengeance, particularly in immunocompromised patients, accompanied by the emergence of antibiotic-resistant strains. No new class of antibiotic has been discovered in the past three decades, and derivatives of current antibiotics soon encounter resistance. New anti-infective agents are thus desperately needed to counter diseases previously treated by conventional antibiotics. Development of these reagents, however, requires a better understanding of how bacteria can cause disease.

Knowledge in the field of microbial pathogenesis—the study of the molecular basis of microbial diseases—has increased dramatically in recent years (Table 1) with contributions from several different directions. Research on pathogens such as *Salmonella*, *Shigella*, *Yersinia*, and *Listeria* species that are relatively easy to genetically manipulate has led the way, but new techniques have been developed that allow most bacterial pathogens to be studied at the molecular and cellular levels. Many pathogens share common mechanisms of interaction with the host, but each species has also evolved a repertoire of unique approaches to exploit host processes (*1*). The study of the molecular interactions between bacterial factors and cellular components or signaling pathways in vitro has been called cellular microbiology (*2*). Recent advances in identifying and detecting virulence factors in vivo rather than in culture have also helped open up the field of microbial pathogenesis, with the use of approaches such as sensitive imaging systems to follow light production or green fluorescent protein expression (*3*). More importantly, research with genetic techniques (*4*) to identify genes induced when the bacteria are inside an animal but not in culture, or to identify genes are essential for virulence in an animal, indicates that additional relevant virulence factors will be identified in the near future. Another source of knowledge has come from progress in cell biology. This progress includes new information on cell physiology; the development of in vitro systems; the ongoing development of fluorescence, confocal, video, and electron microscopy; and the development of new techniques such as the ability to generate and express transdominant negative forms of various cytoskeleton proteins or signaling molecules and the ability to change the intracellular composition by microinjection. In turn, bacteria have provided cell biologists with valuable tools to dissect cellular processes, such as cytoskeleton rearrangements and signaling pathways.

This article highlights some of the recent findings concerning the cellular and molecular interactions that occur between bacterial pathogens and their host cells. It is organized according to the successive interactions that occur at different stages during the infectious process, including microbial adherence to host cells, pathogen uptake into mammalian cells, bacterial survival and replication inside mammalian cells, and cell intoxication and death caused by bacterial products.

## Adhesion to Mammalian Cells

Bacterial adherence to host cells or surfaces is often an essential first stage in disease because it localizes pathogens to appropri-

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