Morphological Coupling in Development: Lessons from Prokaryotes

Review

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At certain junctures in development, gene transcription is coupled to the completion of landmark morphological events. We refer to this dependence on morphogenesis for gene expression as "morphological coupling." Three examples of morphological coupling in prokaryotes are reviewed in which the activation of a transcription factor is tied to the assembly of a critically important structure in development.

The translation of genetic information into form during development is guided by a complex interplay between genes and their protein products. Morphogenesis in systems ranging in complexity from bacteria to the mammalian embryo is characterized by the expression of sets of genes in highly ordered temporal sequences. At the same time, but less widely recognized, is the opposite circumstance, in which the activation of gene expression is tied to key events in morphogenesis. Coupling transcription at critical junctures to developmental landmarks helps to ensure that gene activation is kept in register with the course of cellular differentiation. We refer to this reverse linkage of gene expression to morphogenesis, the subject of this review, as "morphological coupling."

It is instructive to compare morphological coupling in development to checkpoints that link the ordered events in the cell cycle (Hartwell and Weinert, 1989). As is the case in the construction of morphological structures, the order of events in the cell cycle is ensured by dependent relationships in which the initiation of late events requires the completion of early events. If the link between these two ordered events is informational (regulatory in nature), then there must, by definition, be a control (surveillance) mechanism to monitor the completion of the first event and trigger the second (Hartwell and Weinert, 1989; Nasmyth, 1996; Tobey, 1975). Hartwell and Weinert (1989) called these control mechanisms checkpoints. In this context, the control mechanisms that couple gene expression to the completion of a morphological event can be viewed as morphological (or developmental) checkpoints (Losick and Shapiro, 1993).

An illustrative example of a cell cycle checkpoint that links a landmark morphological event with the activation of a later, downstream event is the spindle position checkpoint in the budding yeast *Saccharomyces cerevisiae* (Figure 1; Hoyt, 2000). Microtubules emanating from the spindle pole body (SPB) during mitosis transport the dividing nucleus into the neck between the mother cell and bud whereupon the cell exits mitosis, completing nuclear division and cytokinesis. The dividing cell ensures that both mother and daughter cells

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receive a nucleus by coupling exit from mitosis to the physical movement of the nucleus into the neck. The control mechanism that links these events centers on a GTP binding protein, Tem1p, that associates with the SPB destined for the bud (Figure 1). It is presumed that in its active (GTP-bound) state, Tem1p can trigger exit from mitosis. However, it is maintained in its inactive (GDP-bound) state by a GTPase-activating enzyme complex. Importantly, the putative guanine nucleotide exchange factor for Tem1p localizes to the bud during mitosis. Movement of the dividing nucleus into the neck brings the G protein into close proximity with its exchange factor, resulting in its activation. Thus, the initiation of a downstream event, exit from mitosis, is coupled to a landmark morphological event, movement of the nucleus into the neck.

Here we consider three examples of morphological coupling in bacteria in which a downstream event, transcription of a particular set of developmental genes, is coupled to a landmark event in morphogenesis. First, we review a classic example of morphological coupling, the assembly of the bacterial flagellum in which the activation of a transcription factor is coupled to the completion of an intermediate structure in flagellum assembly. We then turn in more depth to two emerging examples of morphological coupling that operate during the process of sporulation. In one, a developmental regulatory protein is activated in response to the formation of a morphological structure known as the polar septum, and in the other, the activity of a regulatory protein is coupled to a phagocytic-like process called engulfment. All three examples center on transcription factors that are members of a prokaryotic family of gene control proteins called alternative (or dedicated) sigma factors. Alternative sigma factors are transcriptional activators that bind to the (core) RNA polymerase in place of the primary or housekeeping sigma factor, conferring on the enzyme the capacity to recognize and transcribe from a particular class of promoters. In the examples that follow, morphological coupling is mediated via three such alternative sigma factors called σ^{28}, σ^F, and σ^G.

Coupling Gene Transcription to Hook-Basal Body Formation during Flagellum Assembly

Morphogenetic events during assembly of the locomotor organelle, the flagellum in *E. coli* and *S. typhimurium*, closely follow sequential changes in gene expression (Chilcott and Hughes, 2000). *Early* genes in flagellar assembly, which are activated by environmental cues, encode transcription factors that activate the *middle* genes. The *middle* genes primarily encode structural components of an intermediate structure in flagellar assembly known as the hook-basal body (the hook is the torque generator and the basal body is the energy transducer; Figure 2). An alternative sigma factor, σ^{28}, which is encoded by one of the *middle* genes, in turn activates *late* gene expression. The *late* genes encode the force generators of the flagellum as well as the structural

- ● Temp1p-GDP (inactive)
- ● Temp1p-GTP (active)
- ● Bub2p/Bfa1p (GAP)
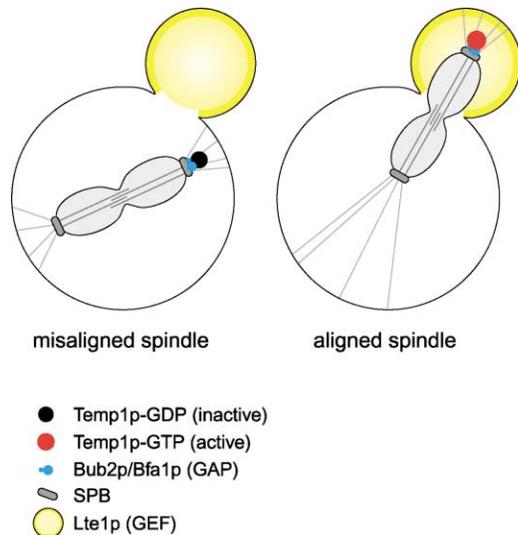- ▱ SPB
- ◯ Lte1p (GEF)

Figure 1. Mitosis Is Coupled to Migration of the Dividing Nucleus into the Mother Bud Neck

A model of the spindle position checkpoint in budding yeast (adapted from Hoyt, 2000). Microtubules (gray) emanating from the nuclear envelope-embedded spindle pole body (SPB) transport the dividing nucleus into the neck. The small GTP binding protein, Tem1p, localizes to the SPB (with preference for the pole destined to enter the bud; Bardin et al., 2000; Pereira et al., 2000). It is presumed that GTP-bound Tem1p can trigger exit from mitosis (Schmidt et al., 1997). However, Tem1p is maintained in its inactive GDP-bound state (black circle) by its association at the SPB with the two-component GTPase-activating enzyme (GAP) composed of Bub2p and Bfa1p (blue circles; Pereira et al., 2000). Importantly, the putative guanine nucleotide exchange factor (GEF) for Tem1p, Lte1p, becomes localized in the bud during mitosis (concentrated at the cortex; yellow haze; Bardin et al., 2000; Pereira et al., 2000). Migration of the dividing nucleus into the neck brings GDP-bound Tem1p in close proximity to its nucleotide exchange factor, resulting in exchange for GTP. Active, GTP-bound Tem1p (red circles) then triggers exit from mitosis.

proteins of the helical flagellar filament. Recent high-resolution analysis of the order of flagellum gene expression based on transcriptional profiling (Laub et al., 2000) and the use of GFP fusions (Kalir et al., 2001) reveals that the timing of gene expression is fine tuned in a manner that closely resembles the sequence of the assembly.

An early clue to the idea that flagellum gene expression might also be coupled to morphogenesis was the observation that mutations in any of more than 30 genes required for the assembly of the hook-basal body prevented the transcription of the *late* temporal class of flagellum genes, which are under the control of σ^{28} (Chilcott and Hughes, 2000). This suggested the hook-basal body itself was necessary for the activation of *late* gene transcription. The control mechanism that links the two events involves a negative regulator of σ^{28} called FlgM. FlgM is an anti-sigma factor that binds tightly to the transcription factor, holding it in an inactive state. Normally, FlgM holds σ^{28} inactive until the assembly of the hook-basal body is complete (Figure 2). When this stage of flagellum morphogenesis is finished, inhibition of σ^{28} by FlgM is relieved and *late* gene expression ensues.
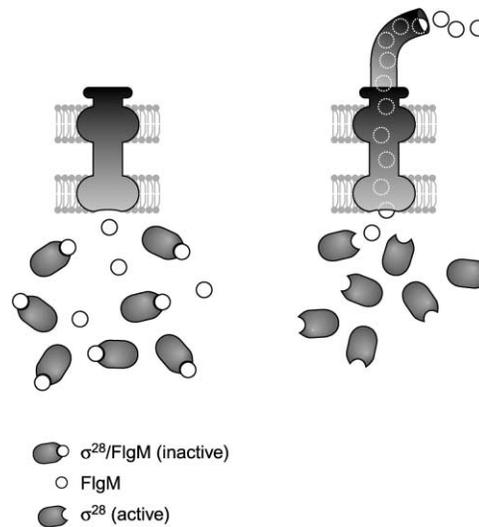
How does FlgM link completion of the intermediate

- ◖◗ σ^{28}/FlgM (inactive)
- ◯ FlgM
- ◖ σ^{28} (active)

Figure 2. Activation of σ^{28} Is Coupled to Completion of the Hook-Basal Body

σ^{28} is held inactive by the anti-sigma factor FlgM during the assembly of the hook-basal body. Upon completion, the tube-like structure pumps FlgM out of the cell, relieving the inhibition imposed on σ^{28}. σ^{28} activates *late* gene transcription, including the genes encoding the flagellar filament (not shown).

structure to *late* gene expression? The flagellum is a hollow tube-like structure. Construction of the flagellum (both the hook-basal body and the flagellar filament) occurs at the distal tip by secretion of proteins through the conduit where they are added onto the growing structure (Chilcott and Hughes, 2000). The FlgM anti-sigma factor is also secreted through the tube-like structure but is not incorporated into the growing flagellum (Hughes et al., 1993). Instead, it is excreted out of the cell (Figure 2). Completion of the intermediate hook-basal body triggers secretion of FlgM. The resulting drop in intracellular pools of FlgM relieves the inhibition imposed on σ^{28}, resulting in activation of *late* gene transcription.

Thus, flagellar assembly is an ordered pathway of events with dependent relationships in which the initiation of late events (σ^{28}-dependent *late* gene transcription) is dependent upon an earlier morphological event (the completion of the hook-basal body). The link between these events is informational, as FlgM mutants uncouple *late* gene transcription from completion of the hook-basal body (Chilcott and Hughes, 2000). With this view of morphogenesis, the anti-sigma factor FlgM could be considered a component of the "hook-basal body morphological checkpoint." FlgM is part of the surveillance mechanism that ensures completion of an earlier event before the initiation of a later event. If the morphological checkpoint is not satisfied (the intermediate hook-basal body is not completed), inhibition of σ^{28} by FlgM is not relieved.

Morphological Coupling during Sporulation in *Bacillus subtilis*

A more complex developmental system in which traditional and molecular genetic evidence has pointed to
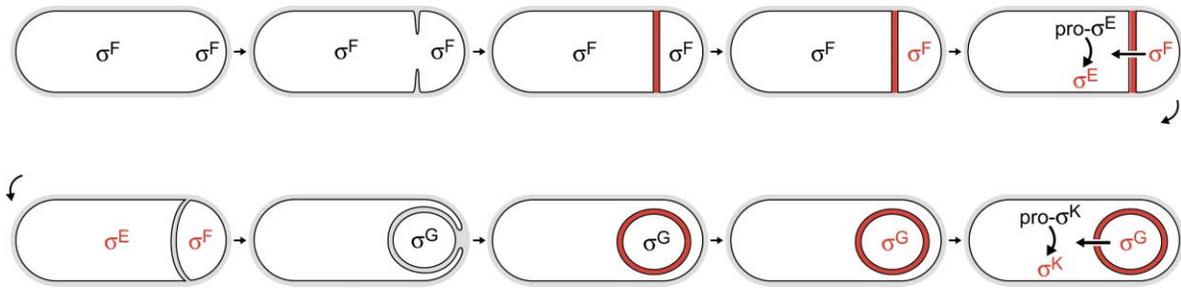
Figure 3. The Forespore Sigma Factors σ^F and σ^G Are Coupled to Landmark Morphological Events during Sporulation

Schematic representation of the process of sporulation with landmark morphological events and activated transcription factors highlighted in red. σ^F is synthesized in the predivisional cell but is held inactive (black) prior to asymmetric division. Completion of the sporulation septum triggers activation of σ^F in the forespore. σ^G is synthesized during engulfment or shortly after its completion but requires engulfment itself (or its completion) for activation. Gene expression in the mother cell is tied to these morphological events through intercompartmental signal transduction pathways emanating from the forespore.

the existence of regulatory linkages between morphogenesis and gene expression is spore formation in the bacterium *Bacillus subtilis*. For many years it had been appreciated that the process of sporulation is an ordered pathway of events with dependent relationships (Piggot and Coote, 1976). With the introduction of methods for creating *lacZ* fusions to individual developmental genes, it became clear that mutations in large numbers of genes blocked the expression of other genes (Losick et al., 1986; Zuber and Losick, 1983). These observations suggested that morphogenesis must progress to critical stages in order for genes that act later in development to become activated. A similar conclusion was reached from molecular genetic studies of the gene that is responsible for the characteristic brown pigment of spores. Sporulation mutants were classically identified by their failure to produce this brown pigment. When it emerged that the appearance of pigment is due to the transcriptional activation of a single gene called *cotA* (or *pig*), it became clear that transcription late in development is dependent upon the completion of morphological events that had occurred earlier (Sandman et al., 1988). Thus, some kind of coupling must exist between developmental gene expression and morphogenesis itself. Presumably, this morphological coupling involves control mechanisms that monitor the completion of landmark events in morphogenesis and trigger gene activation.

Sporulation and Its Control
B. subtilis enters the sporulation pathway in response to conditions of nutrient limitation (Piggot and Losick, 2002). The sporulating cell proceeds through a series of well-defined morphological stages that culminate after 7 or 8 hr in the production of a dormant cell type known as the spore (or more properly, the endospore). A hallmark of sporulation is the formation of an asymmetrically positioned septum that divides the developing cell (or sporangium) into two daughter cells of unequal size: a forespore (the smaller cell) and a mother cell (Figures 3 and 4). Initially, the two cells lie side by side, but later in development the mother cell engulfs the forespore. Engulfment is a phagocytic-like process in which the forespore is pinched off within the mother cell, creating a cell within a cell (Figures 3 and 4). At this late stage,

the mother cell packages the forespore in a protective proteinacious coat while the forespore prepares for dormancy. Later in development the mother cell lyses, liberating the fully mature spore. Throughout this morphological process the forespore and the mother cell follow different programs of gene expression (Figure 4). Yet the two programs are linked to each other by signal transduction pathways that ensure that gene expression in one compartment is kept in register with gene expression in the other (Figure 3; Losick and Stragier, 1992).

As is the case with the flagellar assembly described above, sporulation is largely driven by the sequential activation of a series of developmental transcription factors. Two factors (Spo0A and σ^H) are activated in the predivisional cell in response to environmental cues upon the initiation of sporulation (Piggot and Losick, 2002). These regulatory proteins direct the transcription of early acting sporulation genes, including those involved in repositioning the division machinery to an asymmetric site and the synthesis of the first two compartment-specific transcription factors σ^F and σ^E. Both σ^F and σ^E are synthesized prior to septation in the predivisional cell but are held inactive until the formation of the polar septum. Shortly after the septum is complete, σ^F becomes active in the forespore compartment (Figure 3). An intercompartmental signal transduction pathway emanating from the forespore under the control of σ^F then causes the appearance of σ^E in the mother cell. This signal transduction pathway operates at the level of the processing of an inactive proprotein precursor called pro-σ^E (Figure 3). σ^F and σ^E, in turn, direct the transcription of compartment-specific genes (Figure 4), including those involved in engulfment of the forespore as well the genes for a second set of alternative transcription factors, σ^G (in the forespore) and σ^K (in the mother cell).

σ^G is activated during or just after the process of engulfment when it replaces σ^F (Figure 3; Li and Piggot, 2001; Piggot and Losick, 2002). σ^K, like σ^E, is synthesized as an inactive precursor known as pro-σ^K. Pro-σ^K is proteolytically processed to its mature and active form as a result of a signal transduction pathway emanating from the forespore under the control of σ^G (Figure 3). Just as σ^G replaces σ^F in the postengulfment forespore, σ^K replaces σ^E in the mother cell (Li and Piggot, 2001).
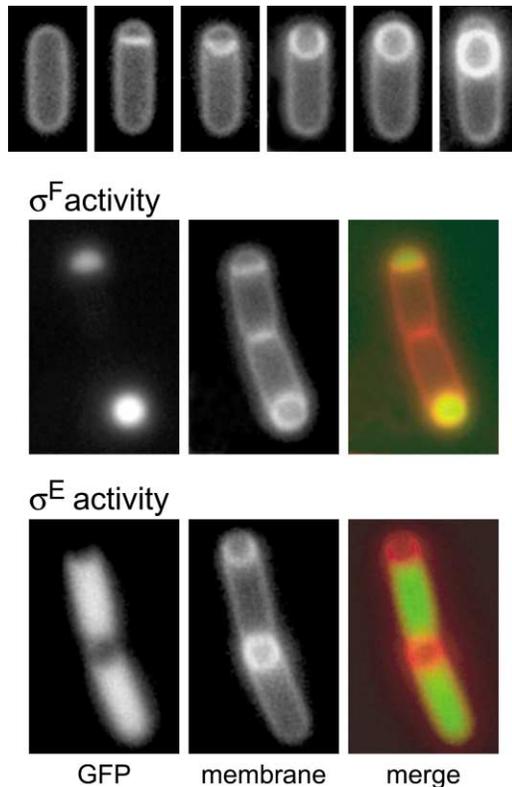
Figure 4. Landmark Morphological Events in Sporulation and the Resulting Compartment-Specific Transcriptional Activation

Sporulating cells of *B. subitilis* stained with the membrane dye TMA-DPH. The sequence at the top shows (from left to right) a cell at the predivisional stage progressing through cells at the stages of polar division and engulfment. The asymmetric septum and membranes engulfing the forespore stain more intensely because they are composed of two layers of membrane. Compartment-specific gene transcription was visualized using σ^F- and σ^E-dependent promoters fused to the gene encoding green fluorescent protein (GFP). The membrane stain was false-colored red in the merged image. The middle images show two cells in which σ^F-dependent synthesis of GFP is confined to the forespore compartments. The bottom images show two cells in which σ^E-dependent synthesis of GFP is confined to the mother cell compartments.

σ^G in the forespore and σ^K in the mother cell are responsible for the expression of late-acting sporulation genes, including those responsible for condensing the forespore nucleoid and those responsible for packaging the forespore in a proteinacious coat (Piggot and Losick, 2002).

In summary, the forespore and mother cell lines of gene expression can be described as two parallel pathways involving the sequential action of σ^F and σ^G in the small chamber of the sporangium and σ^E and σ^K in the large chamber. At the same time, intercompartmental signal transduction pathways explicitly tie the activation of σ^E and σ^K in the mother cell to the action of σ^F and σ^G in the forespore, respectively (Losick and Stragier, 1992).

Activation of σ^F Is Coupled to Formation of the Polar Septum

Once it was appreciated that σ^F activity was restricted to the forespore compartment, it was hypothesized that its activation was tied to completion of the septum (Margolis, 1993). Support for this idea comes from the observation that mutations in all the cell division genes that prevent polar septation also block activation of σ^F (Beall and Lutkenhaus, 1991; Levin and Losick, 1994). A particularly clear example of this dependent relationship is the depletion of the cell division protein FtsL (using an inducible promoter fusion to the *ftsL* gene; Daniel et al., 1998). The absence of inducer during sporulation blocks polar division and σ^F activation. However, if *ftsL* transcription is induced in these sporulating cells stalled prior to asymmetric cell division, polar septation is completed and σ^F activation ensues. Thus, σ^F, which is present in the predivisional cell, is held inactive until the polar septum is formed. Upon completion of this morphological event σ^F inhibition is relieved, triggering compartment-specific gene expression.

What is the control mechanism that links completion of septation to σ^F activation? Three proteins have been identified that regulate the activity of σ^F: SpoIIAB, SpoIIAA, and SpoIIE (hereafter referred to as AB, AA, and E, respectively; Figure 5A; Piggot and Losick, 2002). Like σ^F, all three proteins are synthesized in the predivisional cell. AB is an anti-sigma factor, which, like FlgM, binds tightly to σ^F, holding it in an inactive state (Duncan and Losick, 1993). AA is a phosphoprotein which, in its dephosphorylated state, can activate σ^F by disrupting the interaction between AB and σ^F (AA has been referred to as an anti-anti-sigma factor; Alper et al., 1994; Diederich et al., 1994). The AB anti-sigma factor is also a serine kinase that can inactivate AA by phosphorylation (Min et al., 1993). Finally, E is a polytopic membrane phosphatase, which is capable of dephosphorylating and thereby activating AA, enabling it to disrupt the AB-σ^F complex (Figure 5A; Arigoni et al., 1996; Duncan et al., 1995). Thus, a competition is set up between phosphorylation (and inactivation) of AA by AB and dephosphorylation (and activation) of AA by E. Upon completion of asymmetric cell division, dephosphorylated AA relieves the inhibition imposed on σ^F by disrupting its interaction with AB.

How σ^F activation is coupled to septation is not completely understood, but two possible control mechanisms have been proposed. Protein localization studies of the membrane phosphatase E provided the first hint at a surveillance mechanism (Arigoni et al., 1995). Prior to septation, E colocalizes with the division machinery in a bipolar pattern at both potential division sites (Figure 5B; Arigoni et al., 1995; King et al., 1999; Wu et al., 1998). This localization requires the cell division protein FtsZ, a tubulin-like molecule that forms polymeric rings at the nascent division sites (Levin et al., 1997). E preferentially accumulates at the polar site that is destined to become the division plane (Figure 5B; King et al., 1999). During septation or shortly after its completion, E invades the septal membranes. The localization of E, a positive regulator of σ^F activation, to the morphological structure being monitored is suggestive of a component of a surveillance mechanism (Arigoni et al., 1995). If E is present in equal amounts on both faces of the septal membranes, the concentration of E in the smaller forespore compartment is expected to be about 5-fold higher than in the mother cell (Arigoni et al., 1995; King et al., 1999). Such
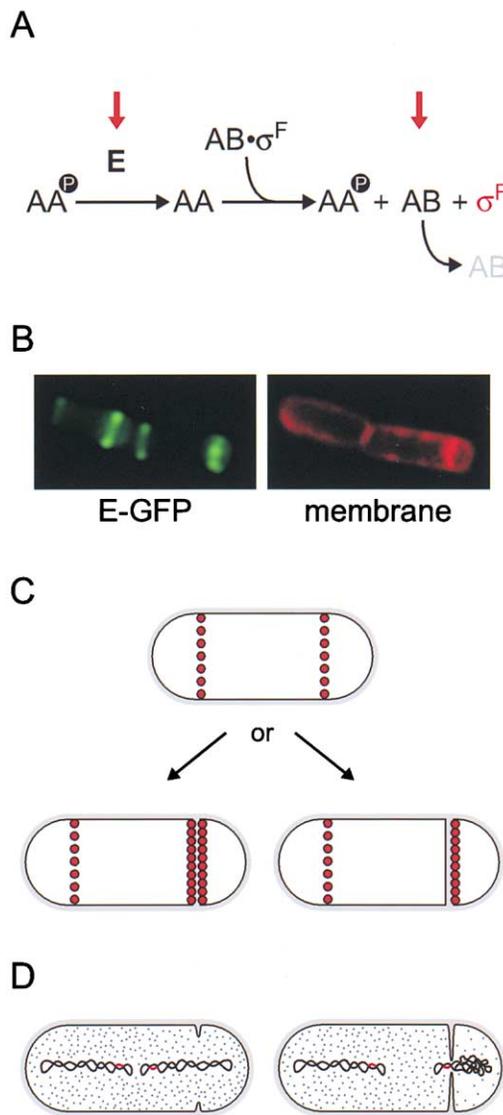
an increase of E phosphatase activity could tip the balance in favor of dephosphorylated AA, resulting in release of σ^F from inhibition by AB (Figure 5C). Alternatively, it has been proposed that the E phosphatase is only inserted into the septal membrane on the forespore face of the division septum (Duncan et al., 1995; Wu et al., 1998). This localization pattern would even more dramatically shift the balance in favor of dephosphorylated AA, resulting in the activation of σ^F (Figure 5C). In either scenario, E is predicted to play a central role in coupling gene expression to cell division.

Further characterization of E has raised an intriguing model for how it might directly monitor septation. Analysis of E activity prior to septation revealed that some dephosphorylated AA is present in the predivisional cell, yet σ^F remains inactive until division is complete (Feucht et al., 1999; King et al., 1999). In fact, mutations in genes involved in cell division that block septation accumulate significant amounts of dephosphorylated AA without activating σ^F. These observations on their face would suggest that E plays a constitutive (and nonregulatory) role in σ^F activation, simply balancing the kinase activity of AB. However, mutations in E have been identified that dephosphorylate AA as efficiently as wild-type but do *not* activate σ^F after septation is complete (King et al., 1999). Evidently, E itself is involved in making AA competent for disrupting the AB-σ^F complex. It has been hypothesized that the activity of E changes upon insertion into the septal membrane and that the mutations in E that have phosphatase activity but fail to activate σ^F may reveal a sensing domain that monitors this event (King et al., 1999). In particular, dephosphorylated AA might be inefficiently released from E prior to insertion into the septum. Upon insertion, the conformation of E (dependent on an intact sensor domain) changes, which, in turn, permits more efficient product release. Free AA is then competent to disrupt the AB-σ^F complex, triggering activation of σ^F (King et al., 1999). While there is no direct evidence for such a model, resolving the paradoxical behavior of the E phosphatase will help get at the heart of the molecular basis for morphological coupling in this system.

That the localization of E to the septum is probably not the only control mechanism that links septation with σ^F activation emerged from the observation that an E mutant that lacked its transmembrane segments and localized throughout the cytoplasm is capable of supporting σ^F activation and sporulation, albeit at a reduced level (Arigoni et al., 1999). In this soluble E mutant there is a delay in the activation of σ^F, and in many cells, σ^F becomes active in both compartments (or prior to septation), indicating that proper localization of E plays an important role in efficient morphological coupling (Arigoni et al., 1999). However, if the septal localization of E were the only control mechanism linking morphogenesis to gene expression, the defect in sporulation

Figure 5. The Control Mechanisms that Couple σ^F Activation to Septation

(A) A simplified summary of the regulation of σ^F activity. Dephosphorylation of the anti-anti-sigma factor, AA-P by the E phosphatase generates the active form of AA. Dephosphorylated AA in turn activates σ^F (red) by inducing the release of σ^F from the anti-sigma factor AB. Free AB is susceptible to proteolysis (gray). Red arrows indicate potential control mechanisms that couple σ^F activation to septation.
(B) The localization pattern of the E phosphatase fused to green fluorescent protein (E-GFP). Prior to septation, the protein localizes in rings at the potential division sites with preference for the site destined to become the septum (left cell). After asymmetric division, the protein colocalizes with the septal membranes (right cell). (Adjacent cells in the same field were removed from these images for clarity.)
(C) Schematic representation of the localization of the E phosphatase, the first potential control mechanism that couples σ^F activation to septation. The E phosphatase (red circles) is present on one or two faces of the septal membranes after polar division.
(D) Schematic representation of transient genetic asymmetry and its consequences for the AB protein, the second potential control mechanism. Prior to asymmetric division, the AB protein (gray circles) is free to diffuse throughout the cytoplasm. Upon septation, the origin-proximal third of the forespore chromosome is trapped in the forespore compartment. The AB gene (red line) resides in a terminal region and is initially excluded from the forespore. After septation, a DNA translocase (not shown) pumps the remaining DNA into the forespore. During the period of transient genetic asymmetry the AB protein lost to proteolysis cannot be replenished.

would be expected to be much more severe. This observation suggested that a second control mechanism exists for coupling σ^F activation to completion of asymmetric division.

An important contribution to a second control mechanism comes from the unique manner in which the chromosomes are segregated during sporulation. Unlike DNA segregation during vegetative growth, during sporulation, segregation does not occur prior to cytokinesis. During asymmetric division, the origin-proximal third of the chromosome becomes trapped in the forespore compartment while the rest remains in the mother cell (Figure 5D; Wu and Errington, 1994; Wu et al., 1995). After septation is complete, a DNA translocase pumps the remaining two thirds of the chromosome into the forespore compartment (Bath et al., 2000). Thus, after septation but before DNA translocation is complete, there is a period of transient genetic asymmetry when only part of the genome is present in the forespore compartment. Genetic experiments designed to assess the importance of this transient state led Frandsen et al. (1999) to propose a chromosome position model coupling septation to activation of σ^F. An additional clue came from the observation that the anti-sigma factor AB is susceptible to proteolysis (Figure 5A; Pan et al., 2001). Importantly, the gene encoding the AB anti-sigma factor is located in the terminal proximal region of the genome and is therefore excluded from the forespore for some period of time after septation. It was therefore hypothesized that the loss of AB protein to proteolysis in the forespore compartment after septation but prior to translocation of the AB gene participates in tipping the balance toward σ^F activation (Figure 5D; Dworkin and Losick, 2001). This control mechanism monitors a consequence of completion of polar division: the loss of diffusion between the two compartments resulting in the inability to replenish the AB lost to degradation in the forespore.

In support of this model, movement of the AB gene from its terminal position to several origin-proximal sites severely impairs sporulation when combined with the soluble E mutant (Dworkin and Losick, 2001). Importantly, movement of the gene to other terminal positions has no effect in this mutant background. These results indicate that transient genetic asymmetry of the AB gene and the instability of the AB protein could serve as a second control mechanism linking σ^F activation to completion of the septum. Finally, it is likely that only some of the AB protein is lost to degradation in the forespore prior to σ^F activation (a point we will come back to later). The time between asymmetric cell division and establishment of σ^F activation has been estimated to be 10–15 min (Partridge and Errington, 1993; Pogliano et al., 1999), and the half-life of the AB protein in the absence of its partner proteins AA and σ^F is ~25 min (Pan et al., 2001). Thus, if this control mechanism plays a role in morphological coupling, it would suggest that a small drop in AB levels in the forespore is sufficient to trigger σ^F activation (perhaps through a self-reinforcing cycle).

Evidently, the activation of σ^F is linked to septum formation through at least two pathways. One pathway involves the association of the E phosphatase with the septum itself and the other involves the transient exclusion of the gene for the AB anti-sigma factor from the forespore prior to chromosome translocation.

The Appearance of σ^E Is Indirectly Coupled to Septum Formation

The appearance of σ^E, the earliest acting transcription factor in the mother cell line of gene expression, is also critically linked to septation. In the absence of σ^E, a second asymmetric division occurs at the distal pole, creating a sporangium with two forespore compartments (Piggot and Losick, 2002). These disporic sporangia are terminally arrested in sporulation. Thus, σ^E-dependent gene expression is required to prevent septation at the unused polar site (Lewis et al., 1994). In fact, if σ^E appears prematurely, that is, in the predivisional cell, then formation of the first polar septum is inhibited (Eichenberger et al., 2001; Fujita and Losick, 2001). On the other hand, if the appearance of σ^E is delayed 10–15 min after septation, then the efficiency of sporulation is reduced with a concomitant increase in disporic sporangia (Khvorova et al., 2000; Zupancic et al., 2001). These findings indicate that σ^E must appear during a critical window of time following the completion of the polar septum (Eichenberger et al., 2001; Pogliano et al., 1999).

How is the appearance of σ^E tied to the completion of the polar septum? The answer is that the activation of σ^E is governed by an intercompartmental signal transduction pathway that depends on σ^F (Piggot and Losick, 2002). As indicated above, σ^E is derived from an inactive proprotein precursor called pro-σ^E (LaBell et al., 1987; Trempy et al., 1985). This precursor has an amino-terminal extension which must be proteolytically removed in order for the transcription factor to associate efficiently with RNA polymerase and direct gene expression. The prodomain both holds σ^E inactive and localizes the proprotein to the membrane (Fujita and Losick, 2001; Hofmeister, 1998; Ju et al., 1997). The conversion of pro-σ^E to mature σ^E is mediated by a putative processing enzyme, SpoIIGA (hereafter referred to as GA; Stragier et al., 1988). GA is synthesized in the predivisional cell, but it is incapable of processing pro-σ^E in its default state. GA is a polytopic membrane protein with homology in its cytoplasmic domain to aspartyl proteases. The putative membrane protease localizes to septum, where it is perfectly placed to receive an activating signal from the forespore compartment (Figure 6; Fawcett et al., 1998). The signaling molecule (SpoIIR, hereafter referred to as R) is encoded by a gene under the control of σ^F (Hofmeister et al., 1995; Karow et al., 1995; Londono-Vallejo and Stragier, 1995). The activation of σ^F in the forespore as a result of morphological coupling to septation results in the synthesis of the R signaling protein. R is a secreted protein, and it is predicted to be secreted into the space between the forespore and mother cell membranes where it activates GA, resulting in proteolytic processing of σ^E (Figure 6). Thus, σ^E activation is tied to polar septation as suggested by Stragier et al. (1988), but in an indirect manner that depends on the compartment-specific activation of σ^F.
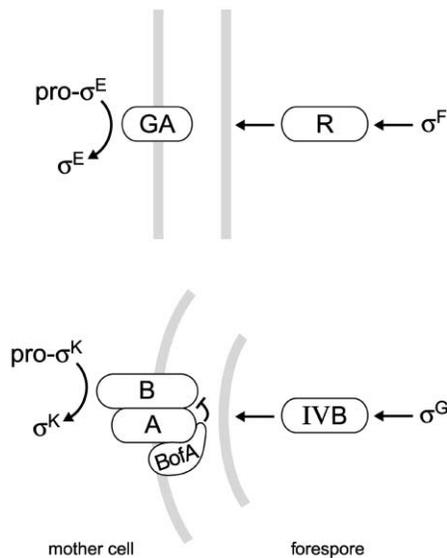
Figure 6. Parallel Intercompartmental Signal Transduction Pathways Link Activation of σ^E and σ^K in the Mother Cell to σ^F and σ^G in the Forespore

Proteolytic activation of pro-σ^E in the mother cell requires the putative aspartyl protease GA. GA localizes to the septal membrane and is inactive in its default state. The signaling protein R is produced in the forespore compartment under the control of σ^F and is probably secreted into the space between the forespore and mother cell membranes where it activates the GA processing enzyme. Proteolytic activation of pro-σ^K in the mother cell requires the putative membrane-embedded metalloprotease, B. B is present in a complex with its two regulators, A and BofA. The A protein anchors the complex in the mother cell membrane that surrounds the forespore and acts as a platform bringing BofA and B together, whereby BofA inhibits B processing activity. The forespore signaling molecule, IVB, activates B (perhaps by cleaving one of its regulators). IVB is made in the forespore compartment under control of σ^G.

Activation of σ^G Is Coupled to Engulfment

The first hint that the activation of the second forespore-specific sigma factor σ^G was coupled to engulfment of the forespore by the mother cell was suggested by mutants that prevent engulfment. As was the case with the activation of σ^F and its dependence on the cell division proteins, all the known mutants that prevent engulfment also impair efficient activation of σ^G (Cutting et al., 1989; Frandsen and Stragier, 1995; Margolis et al., 1993; Sandman et al., 1988; Smith et al., 1993). Evidently, some aspect of engulfment is monitored by the forespore, and upon its completion, σ^G is activated. In fact, σ^G-controlled gene expression is regulated at two levels: expression of the σ^G gene and activation of the σ^G protein (Stragier and Losick, 1996). The gene encoding σ^G is under the control of σ^F. However, unlike the other genes in the σ^F regulon, transcription of the σ^G gene is delayed by approximately 1 hr until the time when engulfment is complete (or nearly complete; Partridge and Errington, 1993). It is not clear how this delay in gene transcription is achieved, but it appears to require a signal from the mother cell under the control of σ^E. The second level of control of σ^G is in its activation, and this control appears to be coupled to the engulfment process itself. Importantly, all the mutants that block engulfment and prevent

efficient activation of σ^G do not affect transcription of the σ^G gene (Margolis et al., 1993; Partridge and Errington, 1993; Smith et al., 1993). In fact, premature expression of the σ^G gene (by fusing it to a strong σ^F-dependent promoter that is transcribed 1 hr earlier) has no effect on the timing of σ^G activity (Stragier and Losick, 1996). Thus, it is likely that σ^G is held inactive prior to the completion of engulfment. Upon completion of this landmark morphological event the inhibition imposed on σ^G is relieved, and late forespore-specific gene expression ensues.

Much less is known about how σ^G is held inactive prior to engulfment. It is, however, intriguing that a putative complex of eight membrane proteins (encoded by the *spoIIIA* operon) that appear to localize to the mother cell membrane that surrounds the forespore (C. van Ooij and R.L., personal communication) is required for efficient activation of σ^G (Kellner et al., 1996). Interestingly, *spoIIIA* mutants engulf efficiently but fail to activate σ^G, suggesting they may play a role in monitoring the process of engulfment. The anti-sigma factor AB may also be part of the control mechanism. σ^G, like σ^F, binds to AB. Moreover, a σ^G mutant that is impaired in its interaction with AB partially bypasses the requirement for *spoIIIA* (Kellner et al., 1996). A critical test (that has yet to be reported) in support of the idea that AB is part of the surveillance mechanism is whether the σ^G mutant also bypasses the block to engulfment. It also remains unclear whether AA and E play a role in the activation of σ^G and how σ^F can escape from AB while σ^G is held inactive. It has been difficult to address these questions because of the requirement for these proteins in the regulation of σ^F. Clever strategies that circumvent these difficulties will be needed if the mechanistic basis for the coupling of σ^G activation to engulfment is to be elucidated.

The Appearance of σ^K Is Indirectly Coupled to Engulfment

The activation of σ^K, the second mother cell-specific sigma factor, is tied to engulfment through its dependence on σ^G. The requirement for σ^G-dependent gene expression for the activation of σ^K was first recognized when it emerged that the *cotA* gene, responsible for the characteristic brown pigment of spores, was itself controlled by σ^K (Cutting et al., 1990; Lu et al., 1990). In σ^G mutants the spores failed to become pigmented, indicating that activation of σ^K in the mother cell required σ^G-dependent gene expression in the forespore. If σ^K activation is uncoupled from its dependence on σ^G, expression of σ^K-controlled genes commences approximately 30 min early, resulting in a reduction in sporulation efficiency (Cutting et al., 1990). Thus, σ^K is synthesized in the mother cell prior to the activation of σ^G in the forespore, but remains inactive until the completion of engulfment triggers σ^G activation. Coupling σ^K activation to σ^G ensures that events in one compartment are coordinated with events in the other (Losick and Stragier, 1992).

The activation of σ^K in the mother cell is governed by an intercompartmental signal transduction pathway that emanates from the forespore under the control of σ^G (Cutting et al., 1990). As described above, σ^K, like σ^E,

is derived from an inactive proprotein precursor called pro-σ^K (Kroos et al., 1989). This precursor has an amino-terminal extension with no detectable sequence similarity to the prodomain of σ^E. However, like pro-σ^E, the prodomain on σ^K serves as a covalently attached anti-sigma factor, which prevents interaction with core RNA polymerase (Zhang et al., 1998). Also, like the prodomain on σ^E, the amino-terminal prodomain of σ^K localizes the proprotein to the membrane (Zhang et al., 1998). The polytopic membrane protein SpoIVFB (hereafter referred to as B) is absolutely required for the proteolytic processing of pro-σ^K and is likely to be the processing enzyme (Figure 6; Cutting et al., 1991; Resnekov and Losick, 1998). B is a founding member of a family of putative membrane-embedded metalloproteases whose catalytic centers reside adjacent to or within the membrane (Rudner et al., 1999; Yu and Kroos, 2000). The other founding member of this family is the Site-2 protease (Rawson et al., 1997; Zelenski et al., 1999), which is required for proteolytic activation of the sterol response element binding protein, a transcription factor required for the activation of genes involved in cholesterol metabolism and uptake (Brown and Goldstein, 1997). The B processing enzyme is regulated by two other integral membrane proteins, SpoIVFA (referred to as A) and BofA (Cutting et al., 1990, 1991; Ricca et al., 1992). In the absence of either of these proteins, B is capable of processing pro-σ^K independently of a forespore signal. All three proteins exist in a complex in the mother cell membrane that surrounds the forespore, perfectly positioned to receive a signal emanating from the forespore compartment (Figure 6; Resnekov et al., 1996). The A protein is required for the proper localization of B and BofA, and is likely to be necessary for their interaction (D.Z.R. and R.L., unpublished observations). Our current view of the regulation of pro-σ^K processing is that A anchors the complex in the mother cell membrane that surrounds the forespore and acts as a platform bringing BofA and B together, whereby BofA inhibits B processing activity until a signal has been received from the forespore.

SpoIVB (hereafter referred to as IVB) is the only forespore protein produced under σ^G control that is required for activation of σ^K and is likely to be the signaling protein (Figure 6; Cutting et al., 1991; Gomez et al., 1995). IVB is probably secreted into the space between the forespore and mother cell membranes where it relieves the inhibition imposed on B by BofA. Interestingly, IVB itself appears to be a serine protease (Wakeley et al., 2000), suggesting that IVB activates B by cleaving one of its regulators. Thus, the proteolytic activation of σ^K in the mother cell is indirectly tied to engulfment through an intercompartmental signal transduction pathway under the control of σ^G.

Summary

The interplay between gene expression and morphogenesis during development is a two-way street. Not only is morphogenesis driven by the orderly expression of genes, but gene expression is sometimes explicitly coupled to landmark events in morphogenesis, in a manner analogous to the checkpoints that operate during the cell cycle. Two bacterial systems in which morphogenesis is both driven by and drives gene expression are flagellum biosynthesis and spore formation, the subjects of this review. As we have seen, the coupling of gene transcription to landmark events in morphogenesis centers on the activation of a transcription factor in response to the completion of a critical event in morphogenesis. In the case of flagellum biosynthesis, the key event is the assembly of the hook-basal body, whereas in sporulation, the key events are the formation of the sporulation septum and the engulfment of the forespore by the mother cell.

The nature of the mechanism that couples gene activation to morphogenesis in flagellum biosynthesis is relatively well understood (hook-basal body-mediated export of an anti-σ^{28} factor), but the mechanisms that link gene transcription to morphogenesis during sporulation have not been fully elucidated. In the case of σ^F, at least two pathways seem to be involved in tying the activation of the transcription factor to septation: one involves a regulatory protein phosphatase that is part of the septum and the other the delayed translocation of the gene for an unstable anti-σ^F factor across the septum into the forespore. In the case of σ^G, the nature of the connection between transcriptional activation and the phagocytic-like process of engulfment is largely unknown.

While the direct targets of the surveillance mechanisms that monitor septation and engulfment are the forespore transcription factors σ^F and σ^G, gene expression in the mother cell is additionally but indirectly linked to these landmark morphological events through two intercompartmental signal transduction pathways. Both pathways operate in an analogous manner: a proprotein precursor to the mother cell transcription factor (pro-σ^E, on the one hand, and pro-σ^K, on the other) is converted to the mature and active form of the transcription factor by a dedicated processing enzyme whose activity is dependent upon a forespore-produced signaling protein. Yet remarkably, the nature of the processing enzymes, the logic of how their activities are regulated, and the sequences of the signaling proteins bear little or no resemblance to each other. Evolution has evidently seized on a good idea, piggy backing on existing surveillance mechanisms, and did so more than once.

The logic of tying gene expression to morphogenesis seems compelling. Morphological coupling helps to keep programs of gene expression in register with the course of morphogenesis. A developmental program might not operate in lock step with morphogenesis if it were entirely governed by a free-running clock. Explicit links to morphogenesis help to ensure that transcription factors are activated at just the right time and just the right place. In some cases, as in the example of the sporulation transcription factor σ^E, it matters that a transcription factor is activated in a narrow window of time set by a landmark event in morphogenesis. In light of these considerations and the parallel to the existence of surveillance mechanisms in the cell cycle, it seems safe to anticipate that explicit (informational) links between landmark events in morphogenesis and transcription factors will emerge as a common feature of development in organisms of many kinds.

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