Checkpoints That Couple Gene Expression to Morphogenesis

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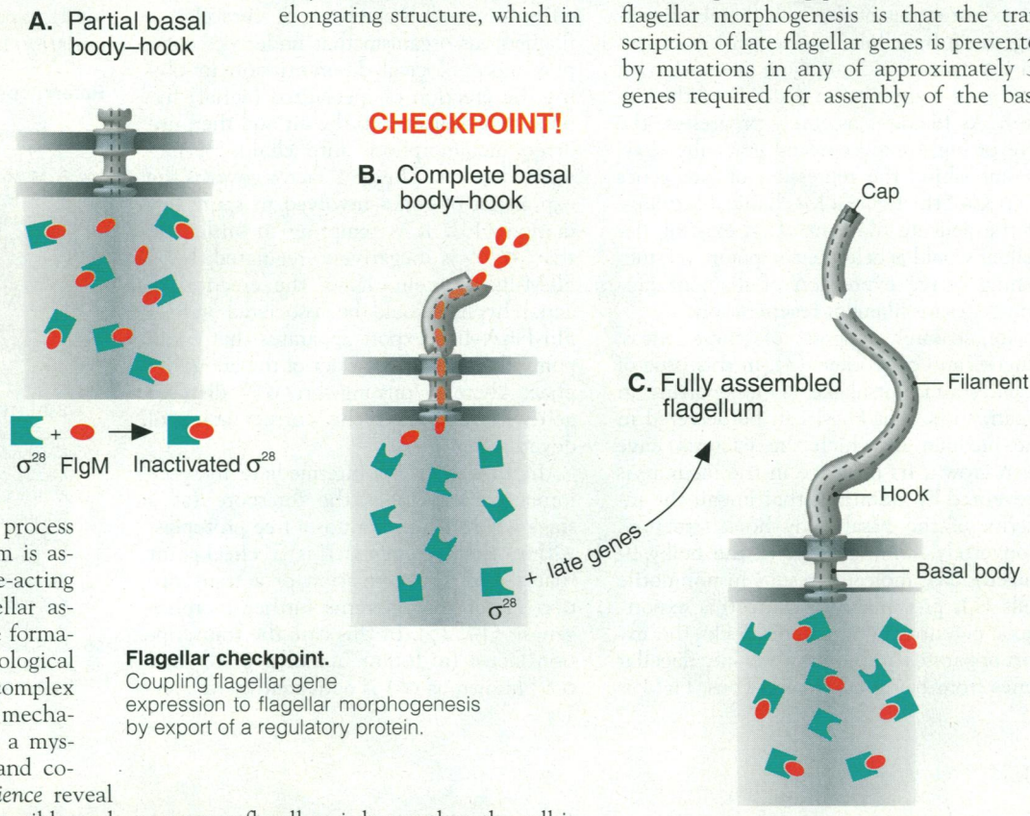
A fundamental tenet of developmental biology is that cell differentiation and morphogenesis are governed in part by the expression of sets of genes in temporally ordered sequences. Systems ranging in complexity from spore formation in bacteria to chorion assembly in insects are orchestrated by well-defined programs of gene expression that are played out over the course of several hours. It is possible, however, that the reverse is also true. That is, the activation of certain genes could depend upon, and indeed be coupled to, the attainment of certain landmark events in morphogenesis. Developmental checkpoints of this kind could keep the program of gene expression in register with the course of morphogenesis. One can imagine, at least in principle, how the ordered appearance of structural proteins could dictate the sequential events of morphogenesis. But how could a morphogenetic event regulate the expression of genes?

One of the earliest and most striking examples of morphogenetic coupling is the process by which the bacterial flagellum is assembled. The expression of late-acting genes in the pathway for flagellar assembly is tightly coupled to the formation of an intermediate morphological structure, the hook–basal body complex (*1*, *2*). Until now, however, the mechanism for this control remained a mystery. The findings of Hughes and coworkers (*3*) in this issue of *Science* reveal the answer, which is at once sensible and surprising.

The flagellum is a complex organelle consisting of a basal body (a rotary motor), which is contained within the cell envelope, and two structures, a hook (a flexible coupling) and a helical filament (a propeller), which are external to the cell (*2*). The motor drives the filament either clockwise or counterclockwise, depending on the output of the cell's chemosensory system (*4*). The power is provided by protons that pass through the motor down a transmembrane electrochemical gradient. The hook and the filament are assembled from protein subunits that are exported by a specialized apparatus, presumably associated with the basal body. The subunits are believed to move across the cell envelope through a hollow channel in the axis of the basal body (*2*). The subunits are added, remarkably, at the distal end of the elongating structure, which in a mature flagellum is longer than the cell itself. Distinguishing characteristics of the flagellum export apparatus are that the proteins it translocates (unlike most exported proteins) lack a cleavable signal sequence and that it discriminates against nonflagllar proteins. Two putative components of the export apparatus are FliI, a protein that resembles the catalytic subunit of the F_0F_1 adenosine triphosphatases, and FlhA, an integral membrane protein (*5*). A fascinating recent insight into the flagellum export apparatus is the discovery that FliI and FlhA appear to be members of a superfamily of specialized export systems that includes those that export the virulence proteins of certain bacterial pathogens (*5*).



Flagellar checkpoint. Coupling flagellar gene expression to flagellar morphogenesis by export of a regulatory protein.

Genes involved in the assembly and functioning of the flagellum are regulated in an ordered sequence of three classes (*1*). The early genes control the expression of the intermediate genes, which are responsible for the production of the basal body (including the motor switch and the export apparatus) and the hook. Next, the late genes determine the assembly of the filament as well as the production of the components of the machinery for chemotactic behavior. Late genes are transcribed under the control of a regulatory protein known as σ^{28} (*6*, *7*), a member of a family of prokaryotic transcription initiation factors that work by binding to RNA polymerase and directing it to the specialized promoters of genes under its control.

A long-standing paradox in the field of flagellar morphogenesis is that the transcription of late flagellar genes is prevented by mutations in any of approximately 30 genes required for assembly of the basal body and hook (*1*, *2*, *8*). It is difficult to imagine that all 30 gene products could be directly involved in controlling the activity of σ^{28}. Rather the action of σ^{28} must somehow depend on the presence of an intermediate structure in flagellum assembly.

The action of σ^{28} appears to be linked to flagellum assembly through an additional regulatory protein known as FlgM (*8*, *9*), an anti-σ factor that binds σ^{28} and holds it in an inactive state (*10*). Mutants lacking FlgM express late flagellar genes constitutively, regardless of the integrity of any of the 30 or so genes needed for basal body

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and hook formation. Hughes and co-workers (3) hypothesized that the basal body–hook structure, which is responsible for the export of filament subunits, might also be able to excrete FlgM into the surrounding medium. According to this hypothesis, the fully assembled basal body–hook structure functions as a protein export apparatus that governs σ^{28} activity by lowering the intracellular concentration of FlgM. Thus, the completion of the basal body–hook complex is a checkpoint in flagellar morphogenesis (see figure). Mutations in any gene required for the assembly of the basal body–hook structure would block FlgM export and thus would prevent expression of late genes by allowing FlgM to accumulate intracellularly and sequester σ^{28} (part A of the figure). During normal morphogenesis in wild-type cells, once the basal body–hook structure is completed, FlgM would be exported out of the cell, thereby lowering the intracellular concentration of this anti-σ factor and allowing activation of late gene transcription (part B of the figure). As filament assembly progresses, the rate of FlgM export would gradually slow, reestablishing the repression of late genes (part C of the figure). Mechanical breakage of the delicate filament of an existing flagellum would accelerate the pump, reestablishing active expression of filament protein genes for filament regeneration.

In striking support of these ideas, Hughes and co-workers (3), in this issue of *Science*, and Kutsukake (*11*), reporting in Japan, show that FlgM can be detected in the medium in which the bacteria have been grown. Its presence in the medium is prevented by mutations that impair the integrity of the basal body–hook structure. Conversely, joining FlgM to the bulky β-galactosidase molecule results in nonmotile cells (3), presumably because this export-incompetent fusion protein blocks the export apparatus and so prevents late flagellar genes from being expressed. Thus, FlgM is the first example of a transcription factor whose presence is regulated by being exported out of the cell, a mechanism that neatly explains the mystery of how gene expression can be coupled to the assembly of a transmembrane structure! An interesting close relative of FlgM exists in the virulence protein export system of *Yersinia enterocolitica* (*9*, *12*), suggesting that here too control of the activity of an anti-σ factor may be achieved by its export from the cells.

There are as yet few systems in which the steps in cellular morphogenesis are described in the molecular detail that is known for the bacterial flagellum. However, it could be argued that both the production of aerial hyphae in *Streptomyces coelicolor* and spore formation in *Bacillus subtilis* use the paradigm of morphological checkpoints in gene expression. A close homolog of σ^{28}, σ^{whiG}, is found in the decidedly nonmotile bacterium *S. coelicolor*, a filamentous organism that undergoes complex morphological differentiation involving the erection of specialized (aerial) hyphae that project into the air and then undergo metamorphosis into chains of pigmented spores. The σ^{whiG} factor governs the expression of genes involved in spore formation (*13*). It is tempting to anticipate that σ^{whiG} is negatively regulated by a FlgM-like protein. If so, the erection of aerial hyphae could be associated with a FliI-FlhA–like export apparatus that could pump the anti-σ factor out of the aerial hyphae, thereby turning on σ^{whiG}-directed gene expression at the correct stage of development.

In *B. subtilis*, an intermediate in spore formation known as the forespore (at a stage when it is present as a free protoplast within the sporangium) is a checkpoint that signals the activation of a transcription factor that governs further morphogenesis (*14*, *15*). In this case the transcription factor (a distant homolog of σ^{28} and σ^{whiG} known as σ^{K}) is activated by its conversion from an inactive proprotein rather than by its export from the cell.

The morphogenesis checkpoint hypothesis could be applicable in a wider context. Linking the activity (or presence) of regulatory proteins to morphogenetic cues might have consequences other than altering the balance of specific transcription factors within the cell. In instances in which groups of differentiating cells must communicate with one another to build multicellular structures, cell assemblages might influence the activities of regulatory proteins in ways that govern subsequent morphogenesis. In fact, intimate cell packing in *Myxococcus xanthus* is a checkpoint that triggers the expression of genes for subsequent morphogenesis (*15*, *16*). The concept of coupling gene expression to morphogenesis is applicable to many events in development and could provide a framework for future experiments on the control of differentiation in a wide range of organisms.

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