The Bacterial Flagellum: From Genetic Network to Complex Architecture

Minireview

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A rotating propeller at the cell surface, driven by a transmembrane proton gradient, provides many bacteria with the ability to move and thus respond to environmental signals. To acquire this powerful capability, the bacterial cell is faced with the challenge of building a tiny rotary engine at the base of the propeller. Although the motor is anchored in the cytoplasmic membrane, a significant portion of the entire mechanism extends into the cytoplasm and, at the other end, out into the environment (Figure 1). At least 20 individual proteins are used as parts for this complex structure and another 30 are used for its construction, function, and maintenance (Macnab, 1992).

To carry out the feat of coordinating the ordered expression of about 50 genes, delivering the protein products of these genes to the construction site, and moving the correct parts to the upper floors while adhering to the design specifications with a high degree of accuracy, the cell requires impressive organizational skills. The construction scheme must deal with fundamental questions in structural and developmental biology. How does the cell measure the length of a component made up of polymerized subunits? When the appropriate length is reached, how does the cell turn off the assembly of one part of the structure and switch on the assembly of the next part? Are there checkpoint mechanisms that determine whether one flagellum component has been completed and that it is okay to start construction of the next component? How is this information conveyed to the expression of the flagellar genes? Because the assembly of the flagellum proceeds in large measure by the passage of structural proteins through a central channel to its distal tip (Iino, 1969; Emerson et al., 1970), what is the export mechanism and how does it choose the proteins that are allowed entry into the pipeline? This minireview addresses some of the mechanisms used by the cell to solve these problems in subcellular morphogenesis, many of which provide unique insight into the versatility and ingenuity of the bacterial cell, and some of which provide new paradigms for cellular morphogenesis.

Both the design of the flagellum and some of the newly discovered regulatory tricks used to organize its construction have been conserved among diverse and evolutionarily distant bacteria. What's more, some of the regulatory mechanisms used for flagellar construction appear also to be used for phage assembly and, surprisingly, for the selective transport of virulence factors from the bacterial cell to their animal or plant hosts (Russel, 1994; Rosqvist et al., 1994).

Flagellar Structure

The bacterial flagellum has three subassemblies: a transmembrane motor (basal body), a propeller (filament), and a universal joint (hook) that permits articulation between the motor and the filament (Figure 1). The basal body is composed of a compound ring at the inner (cytoplasmic) membrane, a shaft (rod) that extends from the inner membrane ring, through the cell wall, to the outer membrane, and two periplasmic rings that are threaded on the rod. A cytoplasmic hollow cylinder (Francis et al., 1994; Khan et al., 1992), part of the C ring switch complex, sits below the inner membrane ring and is composed of the proteins that respond to chemotactic signals, communicating the direction of rotation to the motor, as well as participating in the rotation mechanism itself. It is believed that a complex of motor proteins, which surrounds the M ring in the plane of the membrane (Figure 2), is the stator and that the MS ring–shaft assembly (and possibly the C ring) is the rotating part of the motor. Another complex associated with the inner membrane ring (and possibly with the switch complex) includes export proteins that are involved in the transport of axial flagellar proteins from their site of synthesis inside the cell through a central channel to the point of assembly. This directed transport obviates the need of flagellar proteins to traverse the membranes hydrophobic milieu, while conferring selectivity on the export process. The axial proteins, which include those composing the rod, the hook, the hook–filament junction, the filament, and the filament cap, are exported without the aid of cleavable signal sequences by this novel export apparatus (Macnab, 1992).

Construction of the Basal Body and Hook Subassemblies

Flagellar assembly is initiated by the insertion of the MS ring into the inner membrane (Figure 2). Although the MS ring appears in electron micrographs as two individual rings, it is actually a continuous structure assembled as a multimer of a single polypeptide (Ueno et al., 1992, 1994). Electron microscopic analysis of basal bodies composed of monomers with deleted regions revealed that, in fact, three structural domains (the two rings and portion of the proximal rod) result from the circular aggregation of identical monomers, each of which has amino acid sequence
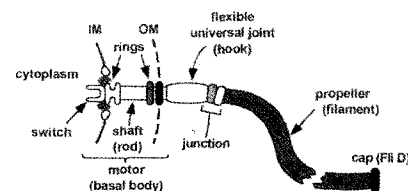

Figure 1. The Bacterial Flagellum

IM, inner membrane; OM, outer membrane. The cell wall is not shown. The component parts are not drawn to scale.
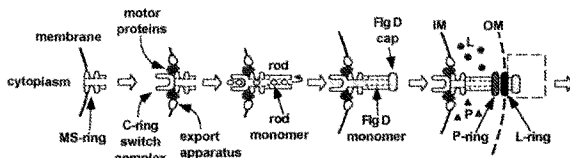
Figure 2. The Pathway of Flagellar Basal Body Assembly

IM, inner membrane; OM, outer membrane. The cell wall, which lies in the plane of the L ring, is omitted from the diagram.

domains that correspond to the three visible structures. The center of this remarkable complex forms a channel that is "physiologically" closed (Ueno et al., 1992) to all but axial flagellar proteins, most likely owing to the flagellar-specific export apparatus functioning as a gatekeeper.

The order of assembly of the switch complex, motor proteins, and export apparatus is not known. However, the formation of the export apparatus must be coincident with or soon after MS ring assembly if it controls the gate and mediates assembly of the next flagellar component, the rod. Rod monomers are the first to be allowed to pass through the transmembrane channel and are believed to be added distally to the nascent structure, which then elongates to become the completed rod (an interesting but unanswered question is how the cell knows that rod assembly has been completed). The next monomer to be sent down the pipeline is a scaffolding protein, FlgD (Ohnishi et al., 1994). The FlgD cap does not remain in the fully assembled flagellum, but does play a crucial role in the addition of hook monomers and the completion of the basal body–hook structure (see Figure 3). While the FlgD protein is "capping" the newly completed rod, the periplasmic subunits for the P and L rings (Figure 2) are assembled around the rod adjacent to the cell wall (not illustrated in Figure 2) and the outer membrane, respectively. In contrast with the axial flagellar proteins, the P and L ring monomers have cleavable signal sequences, and their export to the periplasm is assumed to be SecA mediated, though this has never been directly shown.

Coordination of Flagellar Gene Expression and Assembly

In the enteric bacteria Escherichia coli and Salmonella typhimurium and in Caulobacter crescentus, it is clear that the full constellation of flagellar proteins are not being churned out simultaneously. Rather, the expression of the flagellar genes is highly ordered and responsive to the state of flagellar assembly (Macnab, 1992; Hughes et al., 1993; Kutsukake, 1994; Brun et al., 1994).

Flagellar genes are grouped into several classes based on their epistatic relationships. For example, in the enterics, expression of genes in class II (encoding basal body and hook subassemblies) is dependent on the prior expression of genes in class I (encoding transcriptional activators; Liu and Matsumura, 1994), and expression of genes in class III (encoding the filament subassembly and various motility and chemotaxis functions) is dependent on the prior expression of both class I and class II genes (Macnab, 1992). In C. crescentus, the expression of the class II basal body genes are required for the expression
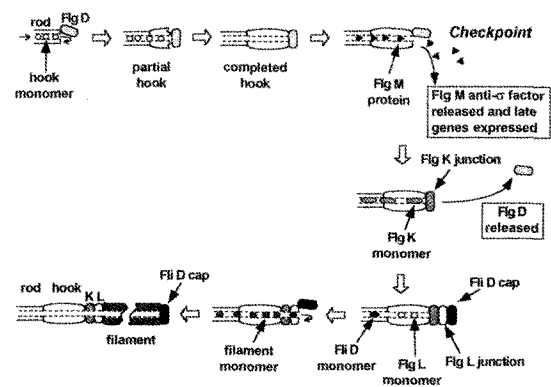

Figure 3. The Pathway of Flagellar Hook and Filament Assembly

Shown beginning with the region boxed in the last part of Figure 2. The filament is composed of flagellin monomers.

of the class III late-basal body and hook genes, which are required for the expression of the class IV flagellin genes (Brun et al., 1994). Although details of the flagellar hierarchy in the enteric bacteria and Caulobacter differ, the general organization and regulatory mechanisms have been conserved. In both cases, the order of expression of the flagellar genes reflects the order of assembly of their gene products. Furthermore, in each case, an alternative σ factor is encoded by a class II gene that is then used to confer specificity to the transcription of genes lower in the hierarchy.

A puzzling observation in this hierarchical arrangement of gene expression is that a mutation in any one of a large number of genes in class II prevents the expression of the genes in class III. It is hard to imagine a mechanism whereby every gene product in a given class acts both as a structural protein and as a regulatory protein. Recently, however, evidence has been presented that helps explain these epistatic relationships and provides a unique paradigm for a feedback mechanism that couples the state of morphogenesis to the regulation of subsequent gene expression (Hughes et al., 1993; Kutsukake, 1994; Losick and Shapiro, 1993). In the enterics, it has been shown that one of the class II genes encodes an alternative σ factor that is specific for the expression of class III genes. Meanwhile, an anti-σ factor (FlgM in Figure 3) is synthesized that holds this σ factor in an inactive state (Ohnishi et al., 1992). Once the basal body (Figure 2) and the hook (Figure 3) structures have been completed, then—and only then—does the channel become competent to export the FlgM anti-σ factor out of the cell (Figure 3), thereby "freeing" the σ factor to get on with the job of transcribing the class III genes encoding the junction and filament proteins. Thus, the bacterial cell uses a checkpoint mechanism that allows the transcriptional machinery to be directly responsive to the physical state of the partially assembled structure.

Inherent in this mechanism is the need for a gatekeeper at the cytoplasmic end of the channel to control passage of the FlgM anti-σ factor. At least seven flagellar genes are believed to encode proteins that mediate a localized

export machine. One of these proteins, FlhB, performs a remarkable dual function (Kutsukake et al., 1994). Its hydrophobic N-terminal portion is needed for the polarized export of rod and hook monomers. Later, FlhB also facilitates the export of the filament proteins. While the rod and hook are being assembled, the C-terminal portion of FlhB functions as a gatekeeper, preventing the release of FlgM from the cell. When the hook is completed, FlgM can be released from the cell, thereby allowing expression of the late-flagellar genes. This checkpoint mechanism thereby accomplishes two goals: it prevents flagellins from competing for entry into the channel, and it prevents the energy-intensive synthesis of filament proteins that cannot be used.

Construction of the Filament Subassembly

The transition from the completed basal body–hook subassembly requires not only the release of the anti-σ factor, but the release of the FlgD scaffold protein that was used for hook assembly (Figure 3). The newly synthesized junction proteins FlgK and FlgL are then sent through the channel to take up residence at what will be the junction of the hook and the filament. Before starting the assembly of the filament, however, a filament cap, FliD, is positioned at the "growing tip" of the structure. This epiglottis-like cap allows the flagellin monomers coming down the pipeline to be assembled into the elongating filament rather than being released into the medium. Although both the rod and the hook are under strict length control, the filament is not and it can grow to many times the length of the cell.

A General Paradigm for Selective Export?

A connection between virulence and motility has long been observed in many pathogens, some of which require the expression of flagellar genes for virulence (Eaton et al., 1992), and others in which motility must be suppressed for virulence (Akerley et al., 1995 [this issue of Cell]). In a remarkable convergence of disparate challenges faced by the bacterial cell—how to get virulence factors directly into a mammalian or plant host and how to construct a flagellum that is essentially situated outside the cell—a common solution has emerged. All seven proteins currently implicated in the selective export of axial flagellar proteins have homologs in proteins that mediate the export of virulence factors in a wide range of bacterial pathogens. Two examples are the causative agent of bubonic plague, Yersinia pestis, and the plant pathogen Erwinia carotovora (Forsberg et al., 1994). These common export proteins include an ATPase homolog that might facilitate energy transfer for protein translocation (Macnab, 1992) and the FlhB gatekeeper that controls transport of specific flagellar proteins (Kutsukake et al., 1994). The concept that a specific transport apparatus might mediate selective export is supported by the observation that the transfer of a Yersinia cytotoxin into its mammalian host requires tight binding between the two cells (Rosqvist et al., 1994). It has been proposed that the Yersinia cell has a mechanism that "senses" the completion of a channel between the bacterial surface and the host cell, which results in the derepression of virulence factor expression and the ensuing direct transfer of the newly synthesized factor into the host cell (Rosqvist et al., 1994). This is a clear parallel to the check-point mechanism in flagellar biogenesis that coordinates gene expression with selective transfer through the export channel. Indeed, it may be that the coupling between the completion of the intercell channel and the expression of virulence factors will prove to be mediated by the release of anti-σ factor homologs. As this continuing story unfolds, a new paradigm for communication between the bacterial cell and its outside world is being established that links mophogenesis, gene expression, and selective protein export.

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