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Review

Prokaryotic motility structures

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Prokaryotes use a wide variety of structures to facilitate motility. The majority of research to date has focused on swimming motility and the molecular architecture of the bacterial flagellum. While intriguing questions remain, especially concerning the specialized export system involved in flagellum assembly, for the most part the structural components and their location within the flagellum and function are now known. The same cannot be said of the other apparati including archaeal flagella, type IV pili, the junctional pore, ratchet structure and the contractile cytoskeleton used by a variety of organisms for motility. In these cases, many of the structural components have yet to be identified and the mechanism of action that results in motility is often still poorly understood. Research on the bacterial flagellum has greatly aided our understanding of not only motility but also protein secretion and genetic regulation systems. Continued study and understanding of all prokaryotic motility structures will provide a wealth of knowledge that is sure to extend beyond the bounds of prokaryotic movement.

Overview

Motility is widespread throughout the prokaryotes, yet no one structure confers motility to all organisms in all circumstances. Of the motility structures, the bacterial flagellum has received the most attention from researchers. There exist a number of variations on the classical flagellum, such as different lateral and polar flagella on the same cell, and the periplasmic flagella of spirochaetes. Motility is also conferred by flagella in the domain Archaea, yet these structures bear little similarity to their bacterial counterparts. Rather, archaeal flagella demonstrate similarity to another bacterial motility apparatus, type IV pili. Additional structures involved in bacterial motility include the junctional pore complex and the ratchet structure involved in gliding motility, and the unique contractile cytoskeleton of *Spiroplasma*.

Bacterial flagella

Without a doubt the most common and best studied of all prokaryotic motility structures is the bacterial flagellum (Aldridge & Hughes, 2002; Macnab, 1999). Composed of over 20 protein species with approximately another 30 proteins required for regulation and assembly, it is one of the most complex of all prokaryotic organelles (Fig. 1). Well understood in its own right as a motility structure, it has become a model system for type III secretion systems in general (Aldridge & Hughes, 2002).

The bacterial flagellum is a rotary structure driven from a motor at the base, with the filament acting as a propeller. The flagellum consists of three major substructures: the filament, the hook and the basal body. The filament is typically about 20 nm in diameter and usually consists of thousands of copies of a single protein called flagellin. Less commonly the filament is composed of several different flagellins. At the tip of the flagellum is the capping protein HAP2. Connecting the filament to the basal body is the hook region, composed of a single protein. The junction of the hook and filament requires the presence of a small number of two hook-associated proteins called HAP1 and HAP3. The basal structure consists of a rod, a series of rings, the Mot proteins, the switch complex and the flagellum-specific export apparatus. The rings anchor the flagellum to the cytoplasmic membrane (MS ring), the peptidoglycan (P ring) and the outer membrane (L ring). Gram-positive bacteria have flagella that lack the P and L rings. The switch proteins (FliG, FliM and FliN) allow the flagellum to switch rotation thus changing the direction of swimming in response to attractants or repellents in the environment, which cells detect with a complex chemotaxis system. The result of environmental sensing is the direct contact of a phosphorylated CheY protein with the FliM switch protein (Bourret *et al.*, 2002). MotA and MotB proteins form a channel through which the protons that power the rotation of the flagellum flow. They form the stator, or nonrotating portion, of the structure with MotB apparently attached to the peptidoglycan layer. The rotor extends into the cytoplasm forming the C ring and consisting of several proteins including the three switch proteins.

As a structure the flagellum assembles from the base, with the MS ring being the first partial structure visible by electron microscopy (Aizawa, 1996). Other basal anchoring proteins are added along with the hook portion before the

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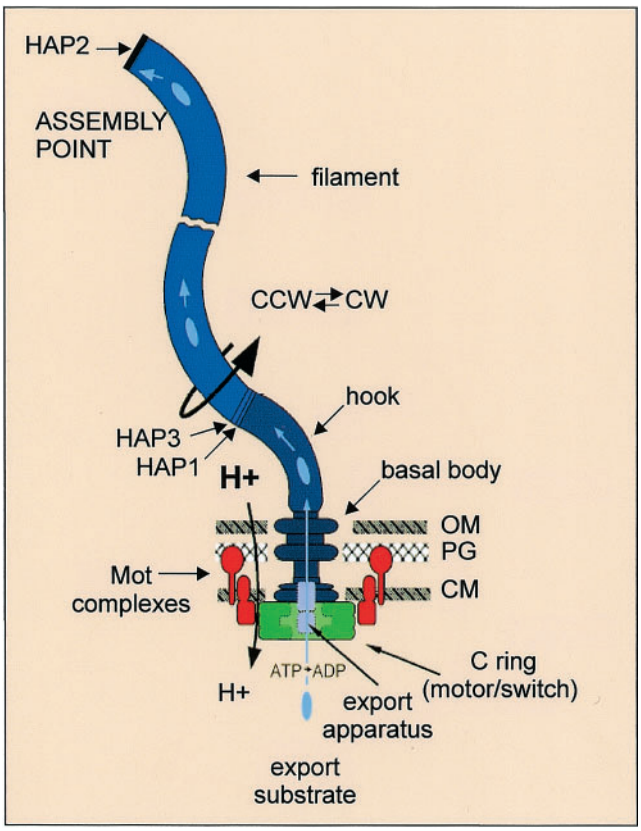

Fig. 1. The bacterial flagellum. The motor consists of stators or Mot complexes (red) and a rotor or C ring (green), which functions as the switch. The flagellin subunits cross the plane of the cytoplasmic membrane in an ATP-dependent manner and are delivered into a central channel in the basal body–hook filament structure where they eventually reach their assembly point at the distal end of the structure. Diameter of the filament is approximately 20 nm. From Macnab (1999). Reprinted with permission of the American Society for Microbiology.

filament is made. Unlike the filament portion of the flagellum structure, the hook region has a defined length (55 nm, approximately 120 subunits of FlgE). A recently presented model explains how the length is regulated (Makishima *et al.*, 2001). Hook length is controlled by the C ring (cytoplasmic ring, located directly beneath the basal body) composed of the switch proteins but also known to be involved in flagella assembly. In the model, FlgE subunits fill the C ring (which acts in the capacity of a measuring cup) and are then exported and assembled en masse. The length of the hook is determined by the capacity of the C ring and its binding sites for the hook subunits.

The growth of the filament is unusual since the individual flagellin monomers that make up the filament are added, not at the base closest to the cell surface, but at the distal tip furthest from the cell. The capping protein is needed to allow the new flagellin monomers to assemble and not to diffuse into the surrounding environment. Recent data have indicated the likely mechanism for addition of new flagellin subunits at the end of the filament under the cap protein (Yonekura *et al.*, 2000). Electron cryomicroscopy revealed the cap (a pentamer of HAP2) to be a plate-like structure with five legs protruding downwards interacting with the filament. There is a gap under each leg with one gap larger than the others and estimated to be large enough to accommodate one flagellin subunit, allowing its folding before incorporation into the filament. As each subunit is added, the cap rotates or walks along the end of the helical filament, with a complete rotation of the cap occurring for every 55 flagellin subunits added. The model has been likened to the unscrewing of a lid from a jar except that the lid in this case, formed by HAP2, never comes off (Hughes & Aldridge, 2001). This remarkable assembly mechanism is possible because the entire flagellar structure from the basal body through the hook and the filament is hollow, allowing for passage of new subunits.

Insight into how changes in the arrangement of the flagellin subunits assembled in the filament lead to a switch in bacterial motility, from swimming to tumbling, has been obtained from flagellin crystals (Samatey *et al.*, 2001). Every flagellar filament is composed of 11 protofilaments, each composed of thousands of flagellin molecules, one on top of the other. Each of the 11 protofilaments can be in a left-handed (L) or a right-handed (R) state, with all the subunits within that protofilament being in the same state (L or R). The L state has a slightly longer (by 0·8 Å) intersubunit distance. In a normal case, a filament will contain a mix of both L and R protofilaments. In *Salmonella*, there are 9 L and 2 R type protofilaments, resulting in an overall left-handed helix while the cells are swimming. Conversion of just two of the L form protofilaments to the R state is enough to change the filament to a right-handed coil, resulting in cell tumbling.

Samatey *et al.* (2001) managed to crystallize a truncated version of flagellin which is missing both N-terminal and C-terminal amino acids involved in filament formation, and determined its structure at 2 Å resolution. The crystals were however only of the R state. Using computer modelling, they stretched the R state in the hope of simulating the L state. Initially the stretching resulted in no major changes in the structure but then over a short step, a beta hairpin shifted into a new position allowing for the 0·8 Å expansion to the L state. This point at the intersubunit interface in domain D1 of flagellin may be the key to switching.

A complex type III export system is located likely in the patch of membrane inside the MS ring (Macnab, 1999). Interestingly this means that all the substrates transported by this mechanism, such as the hook, hook-associated proteins, rod proteins and flagellin are made without cleaved leader sequences, usually a prerequisite for export of proteins out of the cell. Much progress has been made recently concerning the composition and interactions of the components of the specialized export system used to assemble bacterial flagella (Zhu *et al.*, 2002). Until recently,

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there were thought to be six membrane components and three cytoplasmic components of the system, as well as some specific chaperones. The membrane components (FlhA, FlhB, FliO, FliP, FliQ and FliR) are believed to be found in the region of the cytoplasmic membrane located within the confines of the MS ring: some have been shown to interact physically with the MS ring. Of the soluble components, FliJ is a general chaperone for both rod/hook and filament type substrates while FliI and FliH are an ATPase essential for substrate translocation and its specific inhibitor, respectively. More recently, it has been reported that both FliI and FliH localize to the cytoplasmic membrane, even in the absence of potential docking components (Auvrey *et al.*, 2002), meaning that the only cytoplasmic components of the export system may be the chaperones.

The interaction between FliI and FliH has been extensively studied (Minamino *et al.*, 2001): a heterotrimeric complex of two FliH and one FliI has much less ATPase activity than FliI alone. While FliI has significant sequence similarity to the beta-catalytic subunit of the proton-translocating F_0F_1 ATPase, it also possesses a flagellum-specific N-terminal extension which is involved in its interaction with the C-terminal approximately 100 aa of FliH (Gonzalez-Pedrajo *et al.*, 2002). Presumably, the role of FliH is to inhibit the ATPase activity of FliI until it can be productively used for exporting flagellar substrates (Minamino *et al.*, 2002). The FliI–FliH complex also interacts with FliJ as well as at least some members of the membrane-embedded components of the export system, specifically the C-terminal (and predicted cytoplasmic) domains of both FlhA and FlhB.

A key problem to be determined is how and when the different substrates are selected for secretion (Aldridge & Hughes, 2001). This appears to involve a number of potential factors ranging from specific chaperones to mRNA signals and specific secretion signals at the N terminus of substrates. Among the unsolved mysteries of the assembly process are how the many different substrates used in the structure of the flagellum are recognized and assembled in the proper order, and the mechanism of switching of the substrates as one part of the structure is completed and another must be started. The C-terminal domain of FlhB, an integral membrane protein, binds rod/hook substrates more strongly than it does filament type substrates and is involved in the substrate switching process of the entire system (Minamino & Macnab, 2000).

Polar vs lateral flagella

One of the unusual variations on bacterial flagellation is the presence in certain organisms of both a polar and a lateral flagellation system. In *Escherichia coli*, *Proteus* sp., *Salmonella typhimurium* and *Serratia marcescens*, it has been demonstrated that the flagella of swimming and swarming cells are the same, although their numbers are different (Harshey & Matsuyama, 1994). Other organisms assemble two different organelles. Best studied in the *Vibrionaceae*, the polar system is constitutively expressed in swimming and surface-grown cells while the lateral flagellation system is only expressed under certain conditions in viscous environments, upon surface contact or if the rotation of the polar flagella are specifically inhibited under laboratory conditions (Kirov *et al.*, 2002). The common feature of the above is the restriction of the polar flagella, either physically or genetically. There has been direct evidence that the polar flagellum acts as a mechanosensor (Kawagishi *et al.*, 1996), and it is proposed to work possibly by sensing swimming speed or rotation rate. Polar and lateral flagella are encoded by different sets of genes, including separate ones for the motor and switch proteins of the two flagellar types; no single mutation that abolishes both polar and lateral flagella formation has been isolated (McCarter & Silverman, 1990).

An intriguing aspect of the polar/lateral flagellation systems in various *Vibrio* species is that the polar flagellum is sheathed (possibly an extension of the cell membrane) and driven by a sodium ion gradient, while the lateral flagella are unsheathed (and hence thinner; Fig. 2) and driven by a proton gradient (Atsumi *et al.*, 1992; Kawagishi *et al.*, 1995). The mechanism of sheath formation is unknown, and whether the polar flagellum rotates within the sheath or whether the two rotate as a unit remains to be unveiled. Because both systems can be expressed at once, it has been suggested that this would be an excellent model system for

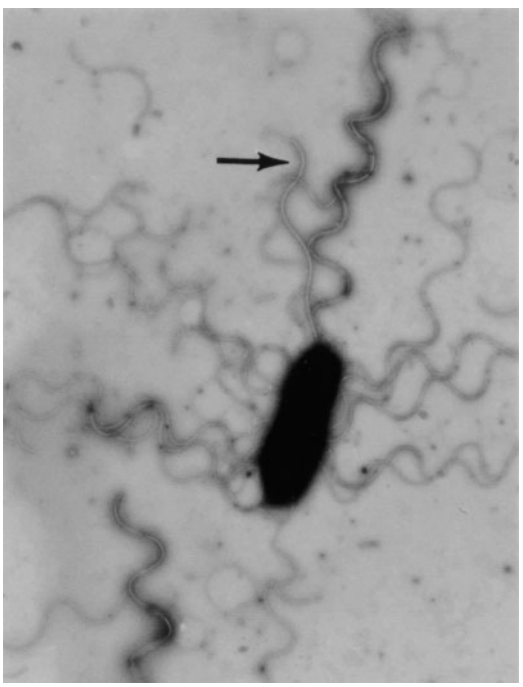

Fig. 2. Electron micrograph of *V. parahaemolyticus* harvested from a plate and negatively stained with 0·5 % phosphotungstic acid. Both polar and lateral flagella are observed with an arrow indicating the polar flagellum. Cell diameter is 0·5 μm. Photograph courtesy of Linda McCarter, University of Iowa.

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dissecting the signals involved in type III secretion since the polar and lateral systems presumably possess different signals to prevent misguided incorporation of the wrong components (McCarter, 2001).

Unusual aspects of the *Vibrio* system include the presence of six flagellins in the polar flagellum system, all of which are incorporated into the structure and none of which singularly are essential. Their spatial distribution within the filament is unknown. The presence of the sheath adds other peculiarities: the sheath appears to compensate for the loss of the capping protein since *Vibrio parahaemolyticus* HAP2 mutants appear to possess a normal polar flagellum, while in enteric species loss of HAP2 leads to secretion of flagellin into the medium without polymerization into a filament. The lateral flagellation system has only one flagellin but it has four *mot* genes (*motA*, *motB*, *motX* and *motY*) instead of the typical two found in H^+ driven flagellar motors (McCarter, 2001).

Periplasmic flagella of spirochaetes

Perhaps the most unusual case of bacterial flagellation is that of the spirochaetes. Here flagella are located in the periplasm between the outer membrane sheath and cell cylinder, subterminally attached to one end of the cell cylinder (Fig. 3). The number of periplasmic flagella and whether the flagella overlap at the centre of the cell varies among species (Li *et al.*, 2000a). The flagella function by rotating within the periplasmic space. Unlike some other bacteria in which flagellation depends on environmental changes, the spirochaete periplasmic flagella are expressed throughout the cell's life-cycle and are believed to have vital skeletal and motility functions (Li *et al.*, 2000b; Motaleb *et al.*, 2000). Due to their continuous presence, the complex regulatory controls observed for motility gene expression in many bacteria seem to be absent in at least certain spirochaetes.

Spirochaete periplasmic flagella are compositionally some of the most complex yet described. They are comprised of FlaA sheath proteins and usually multiple (two to four) FlaB core proteins. The FlaA proteins are made with a leader peptide and are likely secreted via a sec-dependent pathway into the periplasm before assembly onto the flagellar filament. FlaA proteins bear no sequence similarity to the FlaB proteins which make up the filament proper. The FlaB proteins have N- and C-terminal sequence similarity to other bacterial flagellins and are not processed at their N terminus. They are presumed to be secreted through the hollow basal body–hook structure via the type III mechanism found for other bacterial flagellins.

The spirochaete flagellar motion is driven by the proton-motive force (PMF) and the cellular movement depends on asymmetrical rotation of the two ends of the cell (Li *et al.*, 2000a). In other words, when the periplasmic flagella located at either end of the spirochaete are rotating in the same direction the cells do not swim. One of the interesting aspects to be determined for spirochaete motility is how the cell controls the rotation of the flagella at the opposite ends of the cell so that both structures rotate in opposite directions. Since some unique motility genes are believed to exist in spirochaetes it has been speculated that some of these might be present to address this problem (Li *et al.*, 2000a).

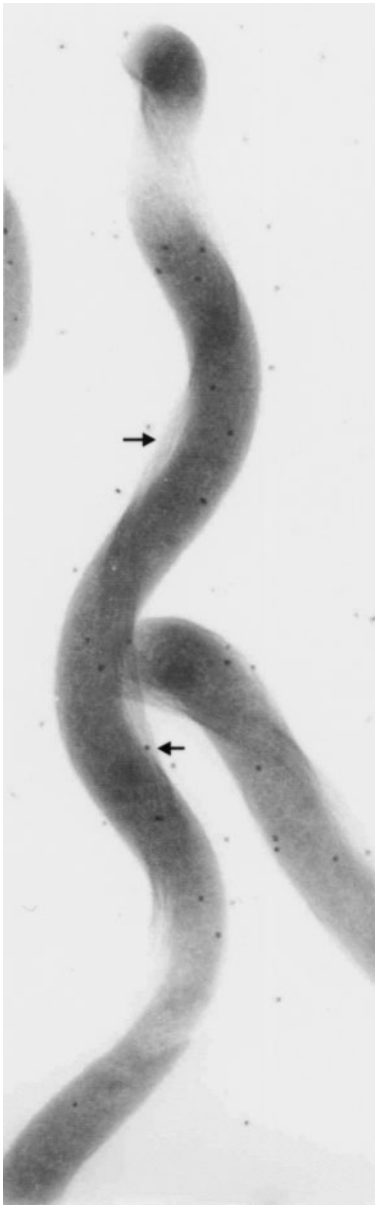

Fig. 3. High-voltage electron micrograph of *Borrelia burgdorferi* illustrating the bundle of periplasmic flagella running lengthwise along the organism (indicated by arrows). Cell diameter is 0·33 μm. Photograph courtesy of K. Buttle, Biological Microscopy and Image Reconstruction Resource, NIH Biotechnological Resource, Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York, and N. W. Charon, West Virginia University.

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Archaeal flagella

The other major subdivision of prokaryotes is the domain Archaea. Members are motile via a structure that appears to be fundamentally distinct from the bacterial counterpart in composition and, likely, assembly (Thomas *et al.*, 2001). The archaeal flagellum is a rotary structure, driven by a proton gradient, and it is thinner than typical bacterial flagella. While a hook structure is evident, convincing demonstration of a basal body with rings has been lacking. This may be due to a different anchoring mechanism being present in archaea, perhaps because of cell wall differences compared to bacteria or because the structure is more fragile than its bacterial counterpart. Fundamental differences between archaeal and bacterial flagella have become obvious from analysis of complete genome sequences of many flagellated archaea. Genes encoding bacterial proteins involved in structure or assembly of flagella have not been reported in archaeal genomes. If archaeal flagella have an anchoring structure it appears to be composed of proteins that are archaea-specific. Even the archaeal flagellins, which compose the major portion of the flagellar filament, lack sequence similarity to bacterial flagellins. In several ways they appear more similar to type IV pilins which themselves form a structure involved in other forms of motility, such as twitching motility (see below). There is sequence similarity between type IV pilins and archaeal flagellins over the first 50 aa, which are extremely hydrophobic. In addition, both type IV pilins and archaeal flagellins are made as preproteins with short positively charged leader peptides. These proteins are processed by specific leader peptidases, distinct from the leader peptidase I equivalent. Mutations in the leader peptidase in either system prevent the assembly of a detectable structure. In the case of the archaeal flagellum this strongly suggests that the assembly mechanism is distinct from the bacterial one involving a type III secretion mechanism with flagellins that lack leader peptides.

In archaea, only one major gene cluster involving up to 12 genes has been reported to be involved with flagellation. Mutations in a number of these genes result in nonflagellated cells. Recently the gene encoding a preflagellin peptidase has been reported (Bardy & Jarrell, 2002). In *Methanococcus jannaschii* it is part of the large gene cluster involved in flagellation but in other *Methanococcus* species it is located quite removed from the flagellin gene cluster. All flagellated archaeal species have three conserved genes, termed *flaHIJ*, located near the genes for the flagellins. Interestingly, FlaI is a homologue of TadA, an ATPase involved in type IV pilus production in *Actinobacillus*, while FlaJ is similar to TadB, a multitopic membrane protein needed in the same system (Planet *et al.*, 2001).

In archaea, there are always multiple (2–6) flagellin genes present (*Sulfolobus solfataricus* appears to be an exception). Thus far the only components of the archaeal flagellum identified are the flagellins themselves, where it appears that the multiple flagellins are all present as structural components of the assembled flagellum. Recent work indicated that the hook protein might in fact be a minor flagellin, FlaB3 in the case of *Methanococcus voltae* (Bardy *et al.*, 2002) (Fig. 4). The flagellins are often, perhaps even universally, posttranslationally modified, usually by glycosylation although only in the case of halobacteria have the associated carbohydrate moieties been determined. Flagellin glycosylation may be necessary for proper flagellar assembly.

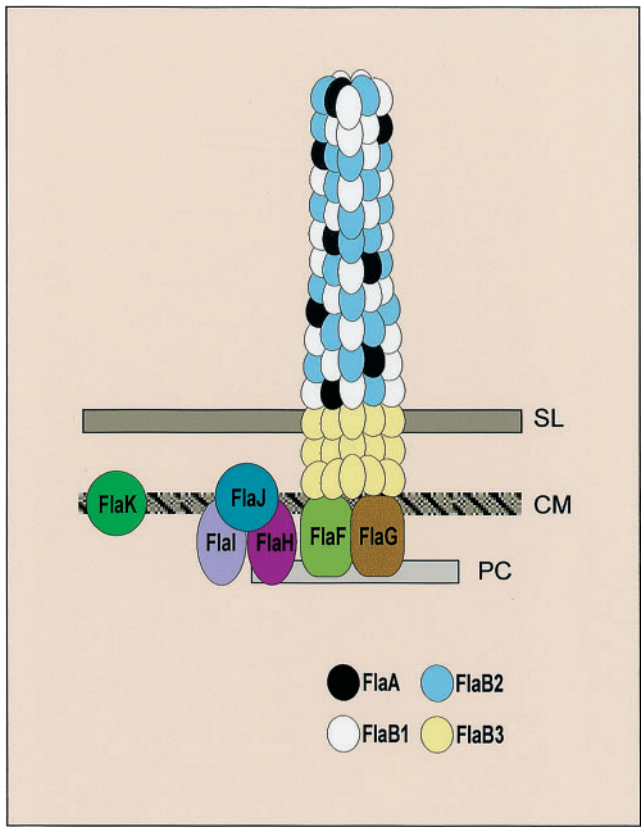

Fig. 4. Schematic speculative representation of the archaeal flagellum. Flagellin subunits are synthesized as preproteins, with the leader peptide cleaved by the preflagellin peptidase FlaK prior to the incorporation of the flagellins into the filament. Flagellin FlaB3 localizes proximal to the cell surface and may form the hook section of the flagellar filament. FlaI is a putative NTP-binding protein. The identification and function of other components of the archaeal flagellum remains unknown. Diameter of the filament is approximately 12 nm. SL, S layer; CM, cytoplasmic membrane; PC, polar cap.

Polarity of growth of archaeal flagella has not yet been determined. The similarities to the type IV pilus system, especially the pilin-like short leader peptides present on the archaeal preflagellins, suggest that archaea may be building flagella in a novel way. New subunits may be added to the base, similar to the way pili are assembled. Recent support for this theory comes from a study on the structure of *Halobacterium salinarum* flagella which found no evidence for a central channel (Cohen-Krausz & Trachtenberg, 2002), eliminating the bacterial flagellum mode of assembly.

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One fascinating feature of the archaeal flagellation system is that despite its distinctness compared to its bacterial counterpart, it presumably interacts with a very bacterial-like chemotaxis system. In *Halobacterium*, where the chemotaxis system is best studied amongst the archaea (Rudolph & Oesterhelt, 1996), homologues to most bacterial chemotaxis proteins have been found. This includes CheY, a protein that in bacteria binds in its phosphorylated form to the switch protein FliM to alter the rotation of the flagellum. As yet, no FliM homologue has yet been reported for any archaeon.

Type IV pili

Type IV pili (Fig. 5) are responsible for various types of flagellar-independent motility, such as twitching motility in *Pseudomonas aeruginosa* (Whitchurch *et al.*, 1991) and social gliding motility found in *Myxococcus xanthus* (Spormann, 1999). Twitching motility is described as a method of surface translocation, characterized by short, intermittent jerks, which requires a minimum number of cells to be present (Wall & Kaiser, 1999). It can be detected on plates by monitoring colony expansion. The region of twitching motility appears as a thin halo surrounding the original colony, which consists of a thin layer of cells (Semmler *et al.*, 1999). The length of the pilus fibre varies, up to several micrometres in length. The outer diameter of the pilus fibre is 6 nm, and unlike the bacterial flagellum, there is no channel in the centre of the fibre (Wall & Kaiser, 1999), precluding a flagella-like growth pattern of monomers assembling at the pilus tip after passing through the internal structure. Analysis of the crystal structure of *Neisseria gonorrhoeae* MS11 pilin revealed that the N terminus of the pilin forms α-helices, which compose the core of the pilus fibre. The outside of the pilus fibre is composed of β-sheets packed against the core (Forest & Tainer, 1997) and an extended C-terminal tail. The C-terminal tail is not an integral part of the pilus fibre, as it can withstand the addition of epitopes without affecting the assembly of the fibre (Mattick, 2002). The pilus fibre is composed of five pilin monomers per helical turn, with a rise of approximately 41 Å per turn (Mattick, 2002).

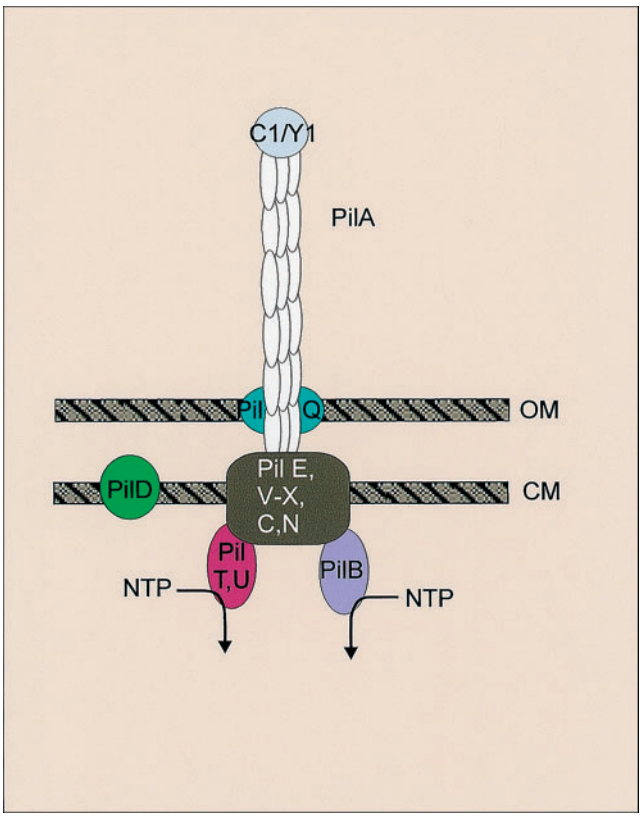

Fig. 5. Schematic speculative representation of type IV pili. The major pilin (PilA) as well as pilin-like proteins (PilE and PilV–X) are synthesized as preproteins with the leader peptide cleaved by a prepilin peptidase, PilD. PilD is also responsible for methylation of the PilA N-terminal amino acid. The secretin PilQ is required for the type IV pilus to cross the outer membrane (OM). NTP binding proteins (PilT/U and PilB) are required to provide energy for retraction and assembly. Diameter of the filament is approximately 6 nm. CM, cytoplasmic membrane. Modified from Thanassi & Hultgren (2000) and Mattick (2002).

Although there are variations in the number of proteins involved in the creation of type IV pili and the resulting twitching motility, the best studied system in *P. aeruginosa* involves at least 35 genes (McBride, 2001). There are three NTP binding proteins required for assembly and function. Mutations in two of these (PilU and PilT) result in hyperpiliated, non-motile cells. It is thought that these proteins may provide energy for retraction. Mutations in the third NTP binding protein (PilB) results in nonpiliated cells, indicating that PilB provides energy essential for assembly. The pilus fibre is composed of a single pilin protein, PilA. PilA is initially made as a prepilin with a short (6 aa) leader peptide cleaved by PilD, a bifunctional enzyme responsible not only for the N-terminal processing of the prepilin but also for methylation of the N-terminal amino acid of the mature protein (Alm & Mattick, 1997), although N-methylation is not essential for assembly of the pilins into the pilus. There are additional prepilin-like proteins, PilE, PilV, FimT and FimU, all of which are processed by PilD. While the functions of these proteins are unknown, mutations in PilE, PilV and FimU result in non-piliated cells, while a FimT mutant resembles wild-type (Alm & Mattick, 1997). The large protein PilY1 has C-terminal homology to the *Neisseria* tip adhesin PilC, and the *pilY1* gene is located immediately upstream of *pilE* (Mattick, 2002).

PilQ is required to allow type IV pili to cross the outer membrane. This protein is a member of the secretin superfamily, whose members form highly stable complexes of 12–14 subunits, with central channels that range from 5 to 10 nm in diameter (Thanassi & Hultgren, 2000). This is in agreement with the outer diameter of type IV pili. Most of the genes implicated in type IV pilus assembly do not have well defined roles as yet: a significant gap in our knowledge

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of assembly and structure of this important organelle. Recently, detailed mutagenic and localization studies of the type IV pili (bundle-forming pili) of enteropathogenic *E. coli* (EPEC) has led to the prediction of interaction of many of the proteins of the *bfp* operon to form an assembly complex for the elaboration of the pili (Ramer *et al.*, 2002).

Pilus retraction has recently been shown directly in *P. aeruginosa* and other organisms. In *P. aeruginosa*, visualization of type IV pili was done through the labelling of non-flagellated cells with an amino-specific dye (Skerker & Berg, 2001). The type IV pili were evident as filaments extending from the cell body, and their extension and retraction was clearly visible (Fig. 6). Movement of cells was only visible during the retraction of the pili; the cells appeared to be pulled by the pili. Movement of the cell bodies was not visible during the extension of the pili; it was speculated that type IV pili are probably too flexible to push a cell.

Included in type IV pili systems are homologues to the chemotaxis proteins for flagellar-based motility. A mutation in any of the corresponding genes results in non-piliated cells, suggesting that these Che-like proteins are involved in both the regulation of pilus biogenesis and twitching motility (Wall & Kaiser, 1999). As is the case with archaeal flagella, the mechanism of how a chemotaxis system similar to the one that interacts with bacterial flagella also interacts with type IV pili is undetermined (Shi & Sun, 2002). It is likely that the CheY homologue interacts with either PilT or PilU, as these proteins are required to supply energy for retraction (Wall & Kaiser, 1999).

M. xanthus moves by gliding motility, which occurs at an interface (solid–liquid, solid–air or solid–solid) and is characterized by a smooth motion (Wall & Kaiser, 1999). There are two gliding systems in *M. xanthus* – social gliding (S motility) and adventurous gliding (A motility). Social gliding is dependent on type IV pili (Wall & Kaiser, 1999) while adventurous gliding is driven by slime extrusion (see below). Individual cells cannot move by social motility when completely isolated, yet are able to move when located 1–2 μm from their neighbour, indicating dependence on cell-to-cell interactions (Wall & Kaiser, 1999). Most of the genes involved in type IV pili in *M. xanthus* are homologous to ones already described in *P. aeruginosa* including the major pilin and NTPases involved in pilus formation and retraction, although three additional genes thought to form a ABC transporter are also observed. Deletions in any of these three genes abolish gliding motility. The pilus-mediated motility in *M. xanthus* may be more complicated than twitching motility as other unique features are evident, such as the Tgl protein (Rodriguez-Soto & Kaiser, 1997). By a process called stimulation, strains that are tgl^{+}, even ones that lack pili, have the ability to allow tgl^{-} strains to assemble pili and engage in social gliding. Presumably this involves transfer of the Tgl protein between the two strains. Unusual features of Tgl include six copies of the 34 aa repeat (tetratrico peptide repeat motif, TPR) and its likely lipid modification. Its necessary role in pilus formation remains unclear.

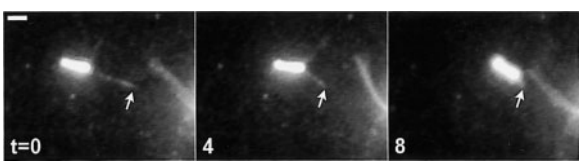

Fig. 6. Retraction of type IV pili leads to cell movement. Type IV pili are visualized on non-flagellated *P. aeruginosa* cells labelled with an amino-specific fluorescent dye Cy3. Arrow indicates point of pilus attachment to quartz slide. The sequence of photographs represents stills from a time-lapse video. Elapsed time (t) in s. (Bar, 2 μm.) The video may be observed at http://www.rowland.org/bacteria/jsmovies.html From Skerker & Berg (2001). Copyright (2001) National Academy of Sciences, USA.

The junctional pore complex

Gliding motility has been defined as 'a smooth translocation of cells over a surface by an active process that requires the expenditure of energy' (McBride, 2001). Many different mechanisms, however, seem to be responsible for this type of motility, depending on the organism. In cyanobacteria and myxobacteria, it has recently been determined that the likely mechanism involves the extrusion of copious amounts of slime through a unique nozzle-like structure called a junctional pore complex that provides the thrust for locomotion (Wolgemuth *et al.*, 2002; Hoiczyk & Baumeister, 1998) (Fig. 7). In cyanobacteria, these pores are 14–16 nm in diameter and form rows that encircle both sides of the cross wall. The pore structure likely forms part of a larger complex that spans the cell wall. Non-motile mutants lacked the pore complex. Examination of slime secretion indicated an origin near the site of the pore complex and a rate comparable to the speed of these gliding bacteria (about 3 μm s^{-1}). Forward motion is obtained by slime secretion from a row of pore complexes on one side of the cross wall while reversals in movement are explained by slime secretion from the other side of the cross wall.

In *M. xanthus*, nozzle-like structures, similar though somewhat smaller than those seen in cyanobacteria, are found at both poles of the cells and this is where slime is secreted to drive adventurous motility (Wolgemuth *et al.*, 2002). It has been proposed in this case that the slime enters the nozzle by an unknown mechanism near the cytoplasmic membrane, where it is hydrated. The resulting expansion of the slime forces it out of the nozzle causing a propulsive thrust. The model predicts that mutants that are defective in adventurous motility should include ones that are defective in the nozzle structure, although these have yet to be reported.

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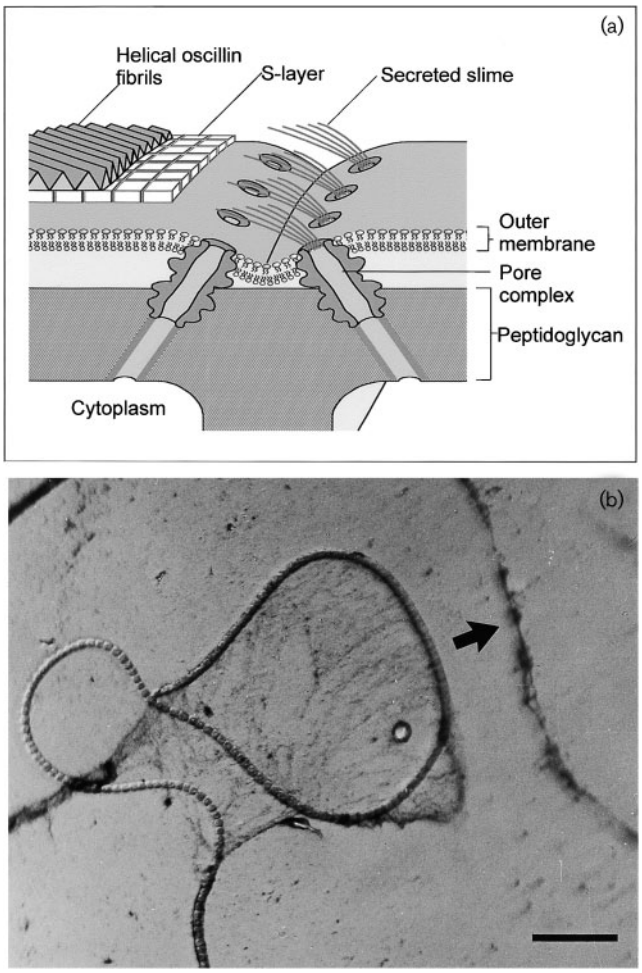

Fig. 7. Junctional pore complex. (a) Schematic diagram of the junctional pore complex in cyanobacteria. The pore organelle spans the entire cell wall complex and possesses all the features expected for a structure that is involved in the rapid and efficient secretion of extracellular carbohydrates. Polysaccharide secreted through the junctional pores is proposed to propel the multicellular filament. (b) Slime release in the cyanobacterium *Anabaena variabilis* studied using India ink as a stain for the secreted mucilage. If secretion of mucilage provides the force for movement of the organism then it is expected that the slime must be secreted perpendicularly to the long axis of the filament. This is what is observed in the figure. Slime is produced only at the concave flank of the filament, which pushes the filament aside. This is seen at the upper bent part of the filament where the India ink stained slime bands point in the opposite direction of movement (which is indicated by the large arrow). Bar, 100 μm. Reprinted from Current Biology, Vol. 8, E. Hoiczyk & W. Baumeister, The junctional pore complex, a prokaryotic secretion organelle, is the molecular motor underlying gliding motility in cyanobacteria, pages 1161–1168, Copyright 1998 with permission of Elsevier Science.

Ratchet structure

Members of the *Cytophaga–Flavobacterium* group of bacteria appear to glide by a yet different mechanism (McBride, 2001). Here it seems motility at a rate of 2–10 μm s^{-1} is the result of the movement of cell surface components. This has been readily demonstrated through the use of latex beads which can be observed to move along the surface of cells in complex paths. One proposed model for gliding in *Flavobacterium johnsoniae* and related organisms is specific motility proteins anchored in the cytoplasmic and outer membrane. Movement of the cytoplasmic proteins may be driven by the PMF and their interaction with the outer-membrane proteins in a ratcheting mechanism may propel the cells forward (Fig. 8). The outer-membrane proteins may be anchored to the peptidoglycan, forming tracks. Several genes have been implicated in gliding motility in *F. johnsoniae* including three (*gldA*, *gldF* and *gldG*) whose products may form an ATP transporter required for movement (Hunnicutt *et al.*, 2002). The exact function of any of these proteins in gliding is unknown. Another possibility is that gliding in this organism is due to slime extrusion and subsequent uptake with the *gld* gene products forming the transporter for import or export.

Contractile cytoskeleton

Spiroplasma melliferum is one of the smallest free-living organisms on earth with a genome size about half that of *E. coli*. Surprisingly, this bacterium is motile, though nonflagellated, and it lacks any genes analogous to ones

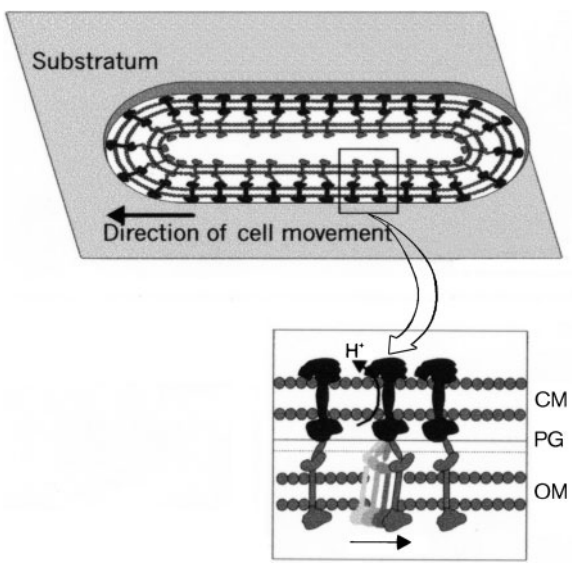

Fig. 8. Schematic speculative representation of the ratchet structure involved in gliding motility in *Flavobacterium* and related organisms. Proteins in the cytoplasmic membrane (CM) are proposed to harvest the PMF and propel the outer membrane (OM) proteins along tracks that are anchored to the peptidoglycan (PG). Temporary attachment of the OM proteins to the substratum results in cell movement. From McBride (2001). With permission, from the Annual Review of Microbiology, Volume 55 ©2001 by Annual Reviews www.annualreviews.org

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involved in flagellation as well as known gliding genes. This organism lacks a cell wall but has a membrane-bound internal cytoskeleton, composed primarily of a unique 59 kDa protein, which is thought to act as a linear motor, in contrast to the rotary motor of the flagellum (Trachtenberg, 1998) (Fig. 9). The cytoskeleton is attached to the cytoplasmic membrane, possibly through one or more of the approximately seven proteins that co-purify with it. The cytoskeleton is involved in motility due to its linear contraction and its close interaction with the cytoplasmic membrane (Trachtenberg & Gilad, 2001). The cytoskeleton exists as a seven fibril ribbon that extends the length of the cell. A conformational switch in the monomer leads to length changes: because of the strong interconnectiveness of the cytoskeleton subunits, changes in any part of the fibril are transmitted to neighbours and ultimately to the attached membrane. Though poorly studied at present, this motility structure represents a truly novel approach to motility using what appears to be a much smaller complement of genes than that required for flagellation. Identification of the roles of the cytoskeleton co-purifying proteins will be a major advance in the elucidation of this motility structure.

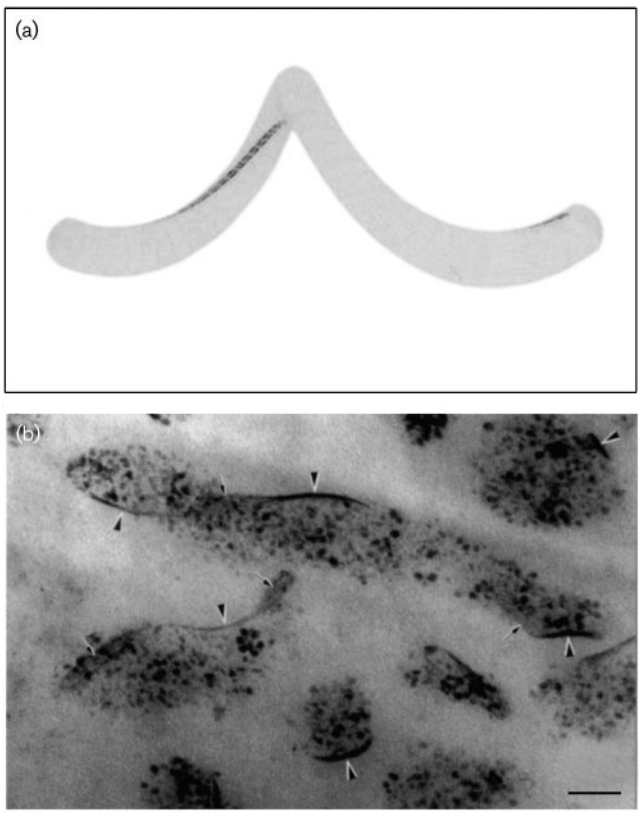

Fig. 9. (a) A model of the *Spiroplasma* cell (only one helical repeat long) with the cytoskeleton ribbon following the shortest helical path. (b) Contractile cytoskeleton of *Spiroplasma melliferum* BC3. The specimen was slam-frozen and freeze-substituted in acetone/uranyl acetate. The membrane and cytoplasm are extracted under these conditions and only the cytoskeleton and ribosomes are well preserved. The helical path of the flat cytoskeletal ribbon is clearly visible in cross-sections (arrowheads) and tangential sections (arrows). Bar, 1000 Å. Both parts reprinted from Journal of Structural Biology, Vol. 124, S. Trachtenberg, Mollicutes – wall-less bacteria with internal skeletons, pages 244–256, Copyright 1998, with permission of Elsevier Science.

In summary

While motility is commonplace among the prokaryotes, it is important to note the variety of structures responsible for motility. These structures vary depending not only on the organism in question, but also on the particular environment. Study of the bacterial flagellum has provided insights into many aspects of prokaryotic cellular activities including genetics and regulation, physiology, environmental sensing, protein secretion and assembly of complex structures. Continued study of all prokaryotic motility structures will provide knowledge that is likely to reach far beyond the topic of motility.

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