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Pulling Together with Type IV Pili

Eric Nudleman Dale Kaiser

Stanford University, Departments of Biochemistry and of Developmental Biology, Stanford, Calif., USA

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Social motility · Twitching motility · Biofilms · PilT · PilQ · Secretins · Fibrils

Abstract
Type IV pili are an efficient and versatile device for bacterial surface motility. They are widespread among the β-, γ-, and δ-proteobacteria and the cyanobacteria. Within that diversity, there is a core of conserved proteins that includes the pilin (PilA), the motors PilB and PilT, and various components of pilus biogenesis and assembly, PilC, PilD, PilM, PilN, PilO, PilP, and PilQ. Progress has been made in understanding the motor and the secretory functions. PilT is a motor protein that catalyzes pilus retraction; PilB may play a similar role in pilus extension. Type IV pili are multifunctional complexes that can act as bacterial virulence factors because pilus-based motility is used to spread pathogens over the surface of a tissue, or to build multicellular structures such as biofilms and fruiting bodies.

Where Are Type IV Pili Found?

Henrichsen [1972] was the first to observe the association between surface-dependent motility and type IV pili (tfp). Henrichsen collected and studied the movement of bacteria capable of spreading over surfaces, typically the surface of moist agar. He observed that some of the organisms had flagella and could swim in suspension, but could also swarm over the surface. Other bacteria, lacking flagella, were nevertheless capable of rapid swarm-spreading by motility that was described as 'gliding' or 'twitching'. These organisms formed spreading zones at the colony edge that frequently were one, or at most a few layers of cells – so thin as to be barely visible to the naked eye. Henrichsen also observed the correlation between twitching among *Moraxella* strains and *Acinetobacter* strains and the presence of polar pili, or fimbriae recognized by electron microscopy [Henrichsen et al., 1972; Henrichsen and Blom, 1975]. *Pseudomonas aeruginosa* was shown to have tfp by Bradley [1973, 1974], Darzins [1994] and Mattick et al. [1996]. MacRae and McCurdy [1976] discovered a correlation between pili and gliding motility among myxobacteria in the 1970s. These and later investigations have enlarged Henrichsen's list of strains possessing tfp to those shown in figure 1. The list is based on a

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Dale Kaiser
Department of Biochemistry and Developmental Biology
Stanford University School of Medicine
Stanford, CA 94305 (USA)
Tel. +1 650 723 6165, Fax +1 650 725 7739, E-Mail kaiser@cmgm.stanford.edu

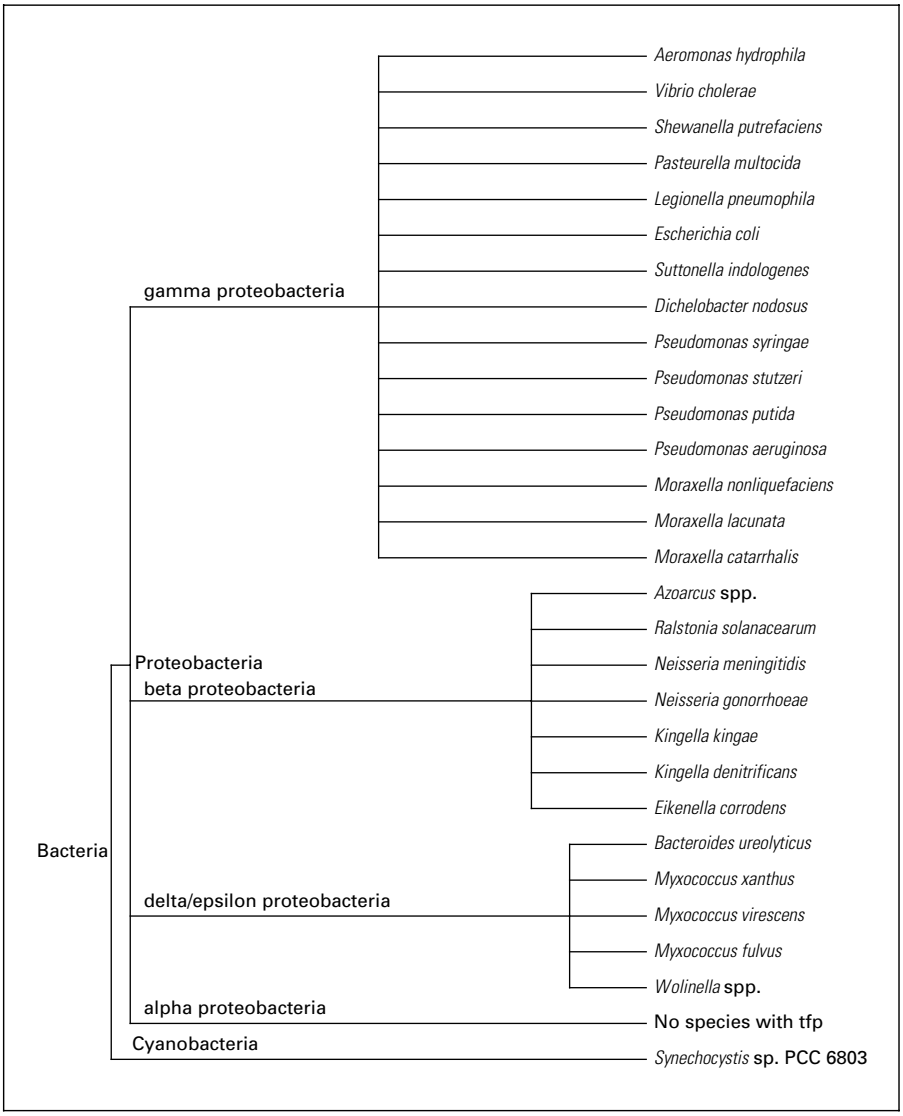

Fig. 1. Phylogeny of tfp. Organisms that have the conserved core of tfp genes are shown classified by their taxonomic lineage. The tree is simplified to show the subdivisions of the Proteobacteria that are common to these organisms, but no further branches. The tree was generated using the NCBI Taxonomic classifications and the TreeView Software [Page, 1996].

twitching motility phenotype, the presence of polar pili, or the presence of genes encoding tfp in the sequenced genome, as suggested by Mattick [2002].

A wide distribution that extends across the β-, γ-, and δ-proteobacteria and the cyanobacteria might be taken to imply that the common ancestor of these groups had tfp (fig. 1). If so, tfp would have been expected among the α-proteobacteria, but they have *cpa* pili instead [Skerker and Shapiro, 2000]. Rather than postulating common ancestory, the high degree of structural similarity across organisms may instead indicate that groups of tfp genes have spread across these diverse genera by lateral gene transfer. Lateral transfer might also account for the finding that groups of tfp genes are often clustered, or reside on plasmids [Giron et al., 1991; Gophna et al., 2003; Stone et al., 1996; Wall and Kaiser, 1999]. Significantly, many of the piliated strains are important pathogens of animals and plants. Type IV pili endow bacteria with social motility, and cooperating cells might have an advantage in overcoming the barriers to infection erected by a potential host, or be able to move into better environmental conditions.

Fluid Mechanics and Tiny Organisms

The movement of tiny organisms requires different physical mechanisms from the swimming of large organisms. For instance, a fish can swim by imparting rearward momentum to the water around it [Vogel, 1994]. But bac-

teria are too small to use momentum, rather they must deal with viscosity, drag, and the reversibility of fluid flow at low Reynolds numbers [Purcell, 1977]. Pulling the narrow, streamlined front end of a cylindrical bacterium forward is a good way to translate force into movement across a surface that is covered with a viscous fluid.

Retraction

David Bradley obtained the first evidence that tfp were engines of movement. He observed that the *P. aeruginosa* bacteriophage PP7 attached initially to the distal end of a pilus, and that the average pilus length was significantly reduced after incubation with phage [Bradley, 1972]. From these and related data, Bradley hypothesized that the pili retract, pulling the phage to the cell surface. Bradley also reported that both the nonpiliated and the hyperpiliated mutants lacked twitching motility, and he concluded that retractile pili were the mechanical basis for twitching motility [Bradley, 1980]. Mattick [2002] extensively reviews Bradley's historic experiments.

Direct evidence that tfp forcefully retract has been reported recently. Using an optical trap on *Neisseria gonorrhoeae* tfp, Merz et al. [2000] measured the velocity, timing, and force of retraction. Single diplococci were immobilized on a bead attached to a coverslip. The tfp were pulled by manipulating a bead coated with anti-pilin monoclonal antibody that was held in a laser trap. When the cell retracted tfp that were bound to the bead, the bead was pulled from the laser trap with a force greater than 80 pN. These tfp did not retract continuously; spates of retraction were separated by intervals of 1–20 s. A retraction velocity of 1.17 ± 0.49 μm s^{-1} was recorded, which was independent of the length of a given pilus. This value correlates well with the rate at which cells crawled on coverslips and also pulled out of the laser trap towards microcolonies (~ 1 μm s^{-1}), suggesting that the measured force of retraction is responsible for the cell movement observed.

An objection was raised to the measurements of Merz et al. [2000] in that several tfp from the same cell may have been attached to a single bead. If more than one pilus had retracted, the force would not apply to a single tfp. To address this uncertainty, Maier et al. [2002] used an inducible promoter to express low levels of pilin in order that 80% of the cells would have no tfp, and the rest would either have a single pilus or several well-separated tfp. Force measurements under these new conditions agreed with the previously reported values; the stall forces averaged 110 ± 30 pN. The initial velocity of pilus retraction was also confirmed as 1.2 ± 0.2 μm s^{-1}.

The diameter of a single tfp being much less than the wavelength of visible light, they are very difficult to see, except by electron microscopy. However, cells labeled with a fluorescent dye that binds amino groups rendered those tfp in live *P. aeruginosa* cells visible with total internal reflection microscopy [Skerker and Berg, 2001]. As had been observed in *N. gonorrhoea* a tfp of *P. aeruginosa* would retract, pause, then retract further. When the distal tip of the pilus had adhered to the substratum, the cell body was pulled forward by the retraction. The speed of pilus retraction (~ 0.5 μm s^{-1}) agreed with the velocity of cell movement (0.31 ± 0.21 μm s^{-1}). Following retraction, some tfp re-extended, now with labeled pilin, suggesting that fluorescently labeled monomers released by retraction had been stored in the membrane, then reassembled into a new pilus. Extension was not associated with cell movement, suggesting that, although tfp can pull, they cannot push.

Observations consistent with tfp retraction have been made in *Myxococcus xanthus*. Piliated cells were seen to adhere end-on to a polystyrene surface, then to 'jiggle', which was taken to indicate pilus retraction [Sun et al., 2000]. Wild-type cells from which the tfp had been sheared, and mutants that lacked tfp were unable to attach. A *pilT* mutant adhered, but failed to jiggle. Cells that had jiggled were then occasionally observed to lie flat on the surface and to glide short distances [Sun et al., 2000].

The tfp Apparatus

Among the tfp proteins common to *M. xanthus*, *P. aeruginosa*, *N. gonorrhoeae*, and *Synechocystis* PCC6803, the most highly conserved are PilA, PilB, PilC, PilD, PilM, PilN, PilO, PilP, PilQ, and PilT, using the gene designation terminology of *M. xanthus* and *P. aeruginosa* (table 1). Protein localization is also conserved. In *M. xanthus*, this entire gene set is in a single cluster [Wall and Kaiser, 1999].

PilA, the Pilin

Type IV pilins, which are encoded by the *pilA* gene, share a conserved amino terminal region of about 60 amino acids [reviewed in Strom and Lory, 1993; Wu and Kaiser, 1995]. All pilins are synthesized as prepilins, and the prepilins are processed by PilD, a peptidase. Satisfying the substrate specificity of PilD would partly account

Table 1. Core tfp genes

Protein	Function	Cellular localization	Reference
PilA	Pilin: monomer of the tfp filament	Inner membrane and pilus fiber	see text
PilB	Pilus extension	Periplasm/inner membrane	see text
PilT	Pilus retraction	Cytoplasm/inner membrane	see text
PilC	Unknown	Inner membrane	Nunn et al. [1990], Wu et al. [1997]
PilD	PilA leader peptidase	Inner membrane	see text
PilM	ATPase, unknown	Inner membrane	Martin et al. [1995]
PilN	Unknown	Periplasm	Martin et al. [1995]
PilO	Unknown	Periplasm	Martin et al. [1995]
PilP	PilQ stability in *N. gonorrhoeae*	Anchored in outer membrane	Drake et al. [1997], Martin et al. [1995]
PilQ	Secretin	Outer membrane	see text

for the conservation of the pilin sequence around the cleavage site. A second constraint on the amino acid sequence of the pilin amino terminus is that it must form an α-helix that is capable of staggered coiling with several copies of itself.

Structure of the tfp Fiber

The structure of a pilus fiber is important for its mechanical strength and flexibility, for pilus assembly, and for pilus retraction. A tfp is a fibrous repeating polymer made of many thousands of copies of the processed pilin, encoded by *pilA*. The fiber is a layered structure of α-helices surrounded by β-strands illustrated in figure 2. The N-terminal amino acids of adjacent monomers (after processing of the prepilin), shown in blue in figure 2A, form an α-helical coiled coil [Parge et al., 1995]. The parallel, staggered α-helices coil around and make hydrophobic bonds with one another. In *N. gonorrhoeae,* this solid inner layer of the fiber is then covered with the C-terminal regions of adjacent monomers that form a scaffold of β-strands; individual β-strands are shown as green ribbons in figure 2A. A 2.6-angström resolution X-ray crystal structure of *N. gonorrhoeae* pilin dimers was the first to be obtained, and the dimer interactions were used to infer the structure of the fiber [Parge et al., 1995]. The fiber model was confirmed with anti-peptide antibodies that distinguished between regions of pilin that were buried and regions that were exposed in the assembled fiber [Forest et al., 1996; Forest and Tainer, 1997]. The fiber of *N. gonorrhoeae* was predicted to have 5 pilin monomers per helical turn, a rise of about 4 nm per monomer, and an outer diameter of about 6 nm. A space-filling model of the assembled structure is shown in figure 2B. Helix parameters and helix diameter of *N. gonorrhoeae* also agree with fiber and crystal diffraction of *P. aeruginosa* PAK tfp [Craig et al., 2003; Folkhard et al., 1981; Hazes et al., 2000].

Toxin-coregulated pili of *Vibrio cholerae* (TCP), and the bundle forming pili of enteropathogenic *Escherichia coli* (EHEC) are both type IVb. They have a different structure that defines a subtype different from the type IVa pili of *N. gonorrhoeae* and *P. aeruginosa*. Nevertheless, TCP and EHEC tfp share with the other tfp an assembly pathway, a core gene set, a pilin C-terminal disulfide bond, and sequence similarity in the N-terminal 60 residues of pilin, but have little or no other similarity in the C-terminal domains of pilin [Giron, 1997; Strom and Lory, 1993]. X-ray crystallography and cryo-electron microscopy of TCP show 5 β-strands (one more than the type IVa pilin) and a new overall protein fold. Nevertheless, as shown by comparing figure 2C with 2A and 2B, TCP have a structural scaffold that consists of α-helices and β-sheets [Craig et al., 2003]. The difference from the type IVa pili lies in the folded arrangement of the β-sheets.

Perhaps the hydrophobic bonding and the flexibility of α-helices in all tfp allow them to bend, twist, or bundle with other pili. In all tfp, the β-sheets of one pilin monomer interact with the β-strands of the next, as shown in figure 2C, to give mechanical strength to the fiber. Despite its narrow diameter of 6 nm, the fiber can withstand tension stresses of more than 100 pN. The absence of a channel in the center of that narrow fiber implies that the pilus cannot be assembled from the tip like a flagellum, but rather must be assembled from its base. Also, the base is inferred to be the locus of disassembly, because of the location of PilT in the inner membrane (table 1).

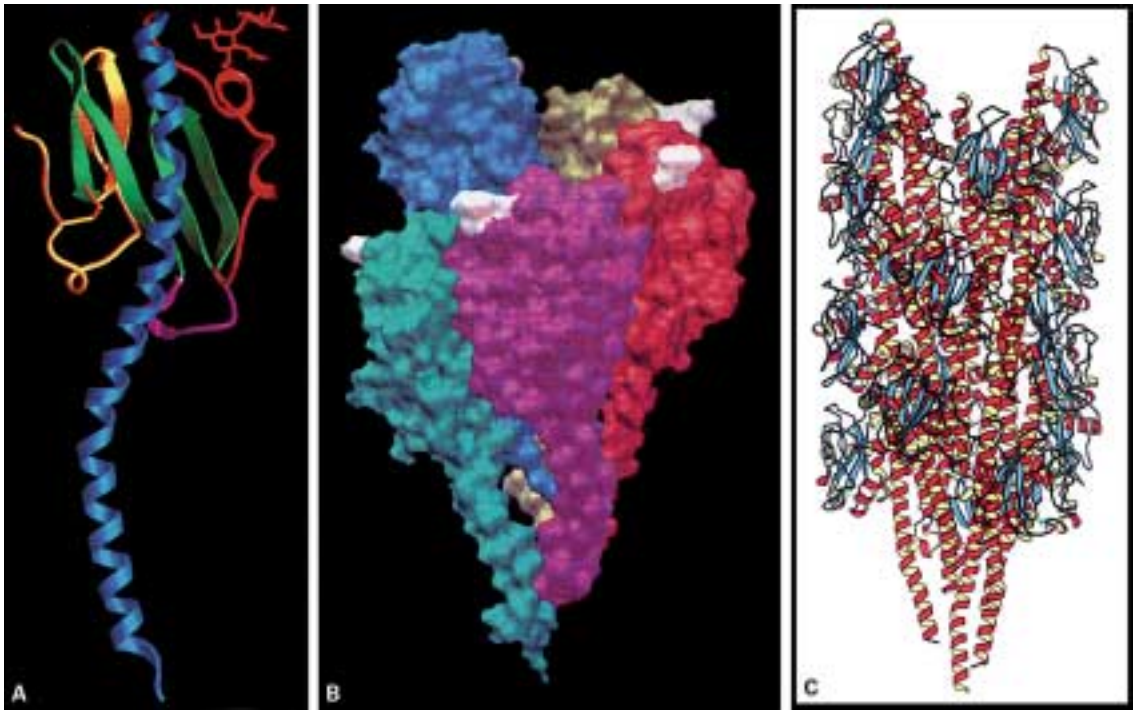

Fig. 2. Structure of pilin in the tfp fiber. **A** The secondary structure of the pilin monomer from *N. gonorrhoeae*, type IVa. Reprinted from Parge et al. [1995], with permission. The highly conserved N-terminus (blue) is part of the coiled-coil core that is tucked inside the pilus fiber. Also shown are the four major β-strands (green), the conserved disulphide region (yellow), and the sugar loop (red) of each monomer. **B** Space-filling model of five, helically staggered monomers, as they are arranged in the pilus fiber. Each monomer has the structure shown in **A**. [From Parge et al., 1995]. Successive monomers are shown in green, purple, red, yellow, and blue. While the N-terminal α-helices of the 5 monomers of one helical turn wrap around each other, the surface of the fiber is made up entirely of residues from the carboxyl domains of the monomers. **C** Structure-based model of TCP filament from *V. cholerae,* typeIVb [Craig et al., 2003]. The model illustrates the packing of the N-terminal α-helices (red/orange) that form the core. The model also shows the interactions between the β-sheets of adjacent monomers in the filament (blue). The image was generated from PDB code 1OR9 using VRML 2.0 software [Suhnel, 1996].

PilD

PilD is a leader peptidase that recognizes an N-terminal pre-sequence of PilA, which is distinct from the sequences recognized by signal peptidases I and II [Nunn and Lory, 1991]. PilD is identical with XcpA, the leader peptidase used in the main terminal branch of the type II general secretory pathway [reviewed by Pugsley et al., 1997]. PilD proteolytically removes the leader sequence from several other proteins of the general secretory pathway, which for that reason are called pseudopilins. PilD is a bifunctional enzyme that also methylates the newly created N-terminal amino acid of the pilin or pseudopilin. In vitro and mutational studies in *P. aeruginosa* have shown that the two activities of this integral membrane protein are at two adjacent active sites in the enzyme protein [Pepe and Lory, 1998].

PilT and PilB, Complementary Motor Proteins

The hyperpiliated mutants that Bradley found to be phage resistant and to lack twitching motility were found to have internal deletions in the *pilT* gene [Whitchurch et al., 1990a]. Mutations in the *pilT* homologues of *P. aeruginosa*, *M. xanthus* and *Synechocystis* PCC6803 were also found to be hyperpiliated, nonmotile, and unable to retract their tfp [Bhaya et al., 2000; Skerker and Berg, 2001; Sun et al., 2000; Whitchurch et al., 1990b; Wu et al., 1997]. The *pilT* gene is the most highly conserved of those required for tfp [Wall and Kaiser, 1999].

PilT has an NTP-binding cassette, or 'Walker box', that is necessary for retraction [Herdendorf et al., 2002; Wu et al., 1997]. PilT in *M. xanthus* has significant sequence homology to PilB (32% identity, 50% similarity). Mutations in *pilB* destroy the cells' capacity to assemble tfp. The two proteins appear to have opposing roles:

PilT is required for pilus retraction, PilB for pilus extension. *Synechocystis* PCC6803 has 2 *pilT* genes *(pilT1, pilT2)* and a *pilB* gene. Mutants in *pilT1* are hyperpiliated; interestingly, mutants in *pilT2* exhibit negative phototaxis (wildtype cells are positively phototactic) [Bhaya et al., 2000].

The PilT protein is a (distant) member of the AAA family of motor ATPases that generally form hexameric complexes, and that mediate the unidirectional disassembly of macromolecular complexes [Vale, 2000]. PilT purified from the gliding thermophile *Aquifex aeolicus* has an ATPase activity of 15.7 U/mg of protein in the presence of Mg^{2+}. The protein formed stable oligomers of 5–6 subunits [Herdendorf et al., 2002]. Purified PilT from *Synechocystis* PCC6803 had a similar specific ATPase activity of 18.4 U/mg [Okamoto and Ohmori, 2002]. By regulating the level of the PilT protein in cells, Maier et al. have obtained evidence that the PilT motor undergoes multiple cycles of ATP hydrolysis as it advances along the pilus filament [Maier et al., 2002]. Reducing the level of PilT in cells reduced the frequency of retraction events, but the length of the pauses between spurts of retraction was similar in cells with a low level of PilT as in those with normal levels of PilT. Neither the stall force nor the force-velocity dependence was affected by reducing the level of PilT. This suggests that once a (hexameric) complex has formed at the base of a pilus, it can catalyze retraction at the maximum rate.

The PilT sequence has no evident transmembrane domains [Herdendorf et al., 2002; Wu et al., 1997], and the protein is thought to localize to the cytoplasmic face of the inner membrane where it would be in a position to encircle the base of the pilus fiber. G. Oster has suggested that the PilT oligomer might deliver its power stroke by a mechanism similar to that of the F_1-ATPase [reported in Kaiser, 2000]. Similarity of catalytic sites and a hexameric structure align the F_1-ATPase with a predicted PilT/PilB motor. The PilT oligomer would break the protein-protein interaction between monomers in the fiber in an ATP-dependent manner, and dissolve the pilus into a monomer pool in the membrane, from which they could be recycled [Herdendorf et al., 2002; Merz et al., 2000; Skerker and Berg, 2001]. Retraction is rapid: an estimated 1,500 pilin monomers are disassembled per second [Merz et al., 2000].

PilQ, a Secretin

Tfp cross the outer membrane through a large oligomeric channel made of a single protein [Bitter et al., 1998; Collins et al., 2001; Drake and Koomey, 1995; Liu et al., 2001; Martin et al., 1995; Schmidt et al., 2001; Wall et al., 1999; Yoshihara et al., 2001]. The PilQ of *Neisseria* spp., *Pseudomonas* spp., *M. xanthus* and *Synechocystis* PCC6803 are members of the large secretin family, proteins that form multimeric pores in the outer membranes of gram-negative bacteria [Genin and Boucher, 1994]. Secretins facilitate the passage of folded proteins, filamentous phage particles, DNA, and other macromolecules across the outer membrane [Dubnau, 1999; Genin and Boucher, 1994; Linderoth et al., 1997]. PilQ is essential for tfp biogenesis [Drake and Koomey, 1995; Liu et al., 2001; Wall et al., 1999; Yoshihara et al., 2001].

Secretins were first identified as proteins required for secretion that form highly stable complexes, resistant to boiling in detergent [Chen et al., 1996; Hardie et al., 1996a; Kazmierczak et al., 1994; Newhall et al., 1980]. Electron microscopy of purified PilQ multimers from *P. aeruginosa, N. meningitidis,* and *E. coli* (EPEC) have revealed ring-shaped structures with 12-fold symmetry [Bitter et al., 1998; Collins et al., 2001; Schmidt et al., 2001]. By electron microscopy, the PilQ pore has an internal diameter of 5–7 nm, which matches the 6-nm diameter of the pilus fiber.

Secretins are most highly conserved at their C-termini. This region, embedded in the outer membrane, is predicted to form a β-barrel composed of β-strands from adjacent monomers in the complex [Brok et al., 1999; Daefler et al., 1997; Guilvout et al., 1999; Wall et al., 1999]. The C-terminal domain also interacts with specific cognate lipoproteins that are necessary for secretin multimerization [Daefler et al., 1997; Daefler and Russel, 1998].

Secretins form electrochemically gated channels, and purified protein embedded in planar lipid bilayers form voltage-gated, ion-conducting channels [Brok et al., 1999; Nouwen et al., 1999]. As the applied membrane potential is ramped up, the secretin channel conductance increases nonlinearly, suggesting that the conformation of the channel proteins can change with the voltage applied. A recent 2.5-nm resolution structure of the PilQ complex of *N. meningitidis* revealed a funnel-shaped structure that constricts to a closed point that presumably lies in the periplasm [Collins et al., 2003]. Thus, the channel conductance may reflect the conformational flexibility of the closed tip of the funnel. Structural comparisons of the open and closed conformations might give insight into the gating mechanism, and how the pilus might trigger the channel to open.

Small lipoproteins are often required to aid assembly and localization of the secretin to the outer membrane

[Crago and Koronakis, 1998; Daefler et al., 1997; Daefler and Russel, 1998; Hardie et al., 1996a, b; Koster et al., 1997; Nouwen et al., 1999; Shevchik and Condemine, 1998]. In *N. gonorrhoeae,* mutations in the *pilP* lipoprotein reduce assembly of PilQ multimers [Drake et al., 1997]. In *E. coli* (EPEC), the secretin (BfpB) is itself a lipoprotein [Ramer et al., 1996]. In this case, the assembly of BfpB into a multimer requires the small (14 kD) protein, BfpG [Schmidt et al., 2001]. In *M. xanthus,* the Tgl lipoprotein is required for assembly of the PilQ secretin [Nudleman et al., unpubl.].

Binding Targets of tfp

Tfp mediated motility usually involves their contact with another cell. Although tfp have been reported to attach to inert surfaces, those reports offer little information about targets. Attachment of tfp to cells has been shown to lead to retraction. However, cells separated by more than a pilus length (several micrometers) rarely move [Kaiser, 1979; Merz et al., 2000; Semmler et al., 1999]. As a result, the formation of *Neisseria* microcolonies depends on functional tfp [Merz et al., 2000]. Cells with mutations in *pilT* can tether to other cells, or to inert surfaces, but they are not able to form microcolonies in *N. gonorrhoeae* or wide rafts of cells in *M. xanthus* [Merz et al., 2000; Skerker and Berg, 2001; Sun et al., 2000; Whitchurch et al., 1990b; Wu et al., 1997].

The surfaces of human epithelia are usually covered with harmless biofilms that may offer a measure of protection. Harmful biofilms are associated with persistent multicellular infections. Tfp-dependent cell-to-cell adhesions are required for biofilm formation in many bacteria pathogenic for humans [Costerton et al., 1999]. Such biofilms are found on surfaces of the middle ear, urinary tract, bone, and heart valves; biofilms also grow on the abiotic surfaces of implanted medical devices. Investigating the role of tfp in biofilm formation, O'Toole and Kolter [1998] found several different pil$^-$ mutants of *P. aeruginosa* in a screen for biofilm defects. Compared to the wild type, these pil$^-$ mutants attached poorly to the polyvinylchloride plastic surface. However, by 8 h, the mutant cells had scattered about on the plastic, but were unable to move or to aggregate, while the wild-type did both [O'Toole and Kolter, 1998]. Heydorn et al. [2002] compared the behavior of biofilms in a flow chamber formed by wild type with a pil$^-$ mutant. Their pil$^-$ mutants formed discrete, dense microcolonies after 98 h, whereas the wild-type cells formed a confluent, featureless flat sheet. Chiang and Burrows reported that under static (i.e., no flow) conditions, *pilT* mutants formed more dense biofilms after 24 h than wild type, while the pil$^-$ *(pilA)* mutants formed less dense biofilms [Chiang and Burrows, 2003]. Under flow conditions, the *pilA* mutants were unable to adhere, while the *pilT* mutant adhered and formed a dense mat. However, the *pilT* biofilm mushroom structures were less dense than the wild type.

Twitching motility is thus implicated in forming the dense mushroom-shaped structure of mature *P. aeruginosa* biofilms, particularly in the development of their mushroom caps [Klausen et al., 2003]. Tfp-based motility of *M. xanthus* is important for building its multicellular fruiting body that has a species-specific shape [Kaiser, 2003]. Induced to sporulate by starvation, fruiting body cells of *M. xanthus* must continue to move within the structure in order to signal each other and to reach the signaling threshold adequate for sporulation [Kim and Kaiser, 1990; Sager and Kaiser, 1993a, b]. In sum, tfp-based movement helps to give shape to multicellular structures in biofilms and in fruiting bodies.

Adhesion by tfp is often critical for pathogenesis. That tfp bind to tissues to initiate an infection is indicated by the reduced adhesion of bacteria that have lost their tfp [Chi et al., 1991; Collyn et al., 2002; Farinha et al., 1994; Rothbard et al., 1985; Ruehl et al., 1993; Strom and Lory, 1993; Zhang et al., 2000]. In addition to adhesion, several observations suggest that tfp are cytotoxic by virtue of retraction. *E. coli* (EPEC) cells with mutations in *bfpF* (the *pilT* homolog) are avirulent, even though they have tfp and adhere to tissue monolayers [Bieber et al., 1998]. Similarly, *pilT* mutants of *P. aeruginosa* and *N. meningitidis* lack cytotoxicity [Comolli et al., 1999; Pujol et al., 1999]. Retraction might also help the bacteria spread over the surface of a host cell, as proposed by Henrichsen [1983]. Spreading would also facilitate the establishment of a biofilm on the surface of a tissue [Costerton et al., 1999].

Retraction of tfp may bring about an intimate contact between a bacterial pathogen and its host cell, which might suffice to allow type II or type III secretion of toxins into the host. Type II secretion is reviewed by Sandkvist [2001]; type III by Cornelis and Van Gijsegem [2000]. It has been reported by Kirn et al. [2003] that a soluble toxin requires the TCP of *V. cholerae* to be translocated across the bacterial outer membrane. However, it has not been demonstrated that retraction is required.

tfp prefer to adhere by their tip when they bind the surface of quartz [Skerker and Berg, 2001]. The distal tip of the pilus filament is unique. Each pilin monomer at the

tip exposes a region of its surface that otherwise is part of the monomer-monomer interface [Forest and Tainer, 1997]. Carbohydrates have been identified as the cellular targets of pilus adhesion. In *P. aeruginosa,* the C-terminal disulfide bonded region of 12–17 residues of the monomer is exposed at the tip [Hazes et al., 2000] and is required for binding to the carbohydrate moiety of the glycosphingolipids asialo-GM1 and asialo-GM2 on epithelial cells [Lee et al., 1994; Sheth et al., 1994]. It has been reported that the PilC protein in *N. gonorrhoeae*, and its PilY1 homolog in *P. aeruginosa* are required for binding to host cell tissues [Scheuerpflug et al., 1999; Wolfgang et al., 2000]. PilC and PilY1 are found associated with the pilus filament and within the cell membrane. These proteins are required for tfp biogenesis, but pili can be restored to mutants defective in either gene by a mutation in *pilT* [Wolfgang et al., 1998]. Even though piliation is restored, the suppressed mutants cannot bind host tissues. Wolfgang et al. suggest that PilC may be required to cap or to stabilize the tfp filament. In their absence, the pilus would be retracted.

Myxobacterial tfp Prefer to Bind Fibrils

The fibrils of *M. xanthus* make up a linked network of amorphous strands, about 30 nm in diameter, that are often seen to join neighboring cells into a cluster [Behmlander and Dworkin, 1994a; Dworkin, 1999]. Fibrils consist of almost equal amounts of protein and of polysaccharide that contains galactose, glucosamine, glucose, rhamnose, and xylose. Several different fibrillar proteins can be distinguished from each other by their antigens as well as by their electrophoretic mobility [Behmlander and Dworkin, 1994b].

Most of the mutants of *M. xanthus* that lack social motility, lack tfp; social motility depends on tfp [Kaiser, 1979]. However, three groups of social motility mutants, the *pilT* mutants, the *dsp* (dispersed growth) mutants, and certain lipopolysaccharide-defective mutants retain tfp. Unlike the *pilT* mutants, which grow in clumps, the *dsp* mutants are less cohesive and grow dispersed in liquid culture [D. Morandi, unpubl. results]. Arnold and Shimkets [1988a, b] and Shimkets [1986] discovered that the *dsp* mutants lack fibrils, although dsp appears not to encode constituents of the fibrils. A group of chemosensory mutants, called *dif*, also lack fibrils [Yang et al., 2000].

Bowden and Kaplan [1998] have reported that mutants defective in lipopolysaccharide (LPS) O-antigen biosynthesis lack social motility, yet have tfp. In fact, the LPS mutants are hyperpiliated, like *pilT* mutants. In a separate study, it had been shown that *M. xanthus dif* mutants that lack fibrils also have more tfp than wild-type cells because their tfp do not retract. It was shown that the addition of crude fibril material brought the *dif* mutants down to normal levels of tfp. However, a *pilT* mutant was not brought down by addition of isolated fibrils or by a fibril donor strain [Li et al., 2003]. Using the same assay, Li et al. [2003] showed that protease-treated fibrils, or chitin, a β-1,4-linked polymer of N-acetyl glucosamine brought the *dif* mutants down to normal levels of tfp. Unlike the *dsp* and *dif* mutants, the LPS mutants have fibrils and can even donate fibrils to a *dif* mutant [Li et al., 2003]. They also have the pilus-based cohesivity of wild-type cells. Taking these data together, the observation that LPS is necessary for S-motility suggests that the O-antigen mutants are deficient in pilus retraction. Because chains of the O-antigen completely cover the cell surface, a retracting pilus sliding through this covering would be expected to interact repeatedly with the O-antigen chains, and the absence of O-antigen might therefore disable retraction.

Since social motility is not observed among cells that are more than a pilus length apart [Kaiser and Crosby, 1983], the observations together imply that a pilus extends ahead of an *M. xanthus* cell, adheres to fibrils on other cells, then retracts, pulling the leading end of the piliated cell forward toward the other cells. In this series of actions, *M. xanthus* offers a model for social gliding and twitching motility.

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