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MINIREVIEW

Type IV Pilus-Dependent Motility and Its Possible Role in Bacterial Pathogenesis

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Type IV pili (TFP) are very unique appendages on the bacterial surface. They are not only required for microbial adherence but also involved in bacterial movement, such as social gliding motility in *Myxococcus xanthus* and twitching motility in *Pseudomonas* and *Neisseria* species (33). How bacterial pili are involved in cellular movement has long been a mystery. Recent studies have revealed that TFP are motility apparatuses that extend and retract TFP filaments at the cellular pole (20, 29, 31). Furthermore, in *Myxococcus* and *Synechocystis* spp., the TFP action was found to be controlled by a chemotaxis-like system (3, 31). These findings provide insights at the molecular level into the involvement of TFP in cellular motility. They also suggest possible roles of TFP in directed cell movement, biofilm formation, guided tissue invasion, and other pathogenesis-related events.

TFP

Pili are fibrous organelles that are expressed on the surface of gram-negative bacteria (for a review, see reference 16). Historically, different families have been assigned on the basis of the specificity of host-receptor recognition and the seroreactivity of antibodies against pilin proteins. More recently, pili have been grouped on the basis of the deduced amino acid sequence of pilin genes and their assembly mechanisms. Although they differ in morphology and structure, most pili are adhesins involved in mediating bacterial interactions with the environment or other cells. For example, the type I and type P pili are assembled by the chaperone-usher pathway, expressed on the surface of uropathogenic strains of *Escherichia coli* and mediate bacterial attachment to host cells through specific carbohydrate binding proteins (25). TFP are conserved in their major pilin amino acid sequences. They are assembled through the general secretion pathway and are expressed in a divergent collection of gram-negative bacteria, including *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Moraxella bovis*, *Eikenella corrodens*, *Vibrio cholerae*, the cyanobacterium *Synechocystis* sp., enteropathogenic *E. coli* (EPEC), and enterotoxigenic *E. coli* (16, 30). TFP are 6- to 7-nm thick, rod-like fibers of variable length and relative flexibility and are usually polarly located.

Biochemical and genetic studies of TFP, mainly in *P. aeruginosa* and *N. gonorrhoeae,* have identified important components of TFP biogenesis. These components are homologous to those of the general (type II) secretion pathway, including pilin, prepilin peptidase, soluble proteins with or without essential nucleotide-binding motifs, inner membrane proteins, and outer membrane secretins that form oligomeric doughnut-shaped structures (for reviews on TFP biogenesis, see references 1, 13, 30, and 32, and for a review on the general secretion pathway, see reference 24). TFP are assembled primarily from one subunit, the pilin protein. The pilins are highly conserved at the N terminus, which is highly hydrophobic. They are synthesized in a precursor form from which the short, basic, amino-terminal signal peptide is cleaved by a prepilin peptidase. The peptidases are bifunctional in that they also methylate the first amino acid (Phe or Met) of the mature pilin. Based on accumulated genetic and biochemical evidence, the current model of TFP biogenesis is as follows (13). Prior to polymerization into pilus fibers, the pilin molecules are anchored to the inner membrane via the conserved hydrophobic region in the N-terminal domain. The pilins are then polymerized, possibly driven by energy-prone self-assembly in the presence of biogenesis machinery proteins, such as PilB and PilC in *P. aeruginosa* and PilF in *N. gonorrhoeae*. The polymerized pilus fibers thrust through an outer membrane pore consisting of oligomeric PilQ, which is homologous with the secretins of the general secretion pathway (5, 36). Many other genes, such as the *pilMNOPQ* operon in *P. aeruginosa*, also participate in TFP biogenesis (1). PilT, which is a cytoplasmic protein with a nucleotide-binding domain, is not required for pilus biogenesis, although in some cases it appears antagonistic to other *pil* mutants (35, 36). Nevertheless, PilT has a very important role in TFP function (see below).

TFP-ASSOCIATED CELLULAR FUNCTIONS AND TFP-DEPENDENT CELLULAR MOTILITY

The cellular functions of TFP have been extensively studied in various bacteria. Many studies have demonstrated that TFP proteins function as adhesins and mediate bacterial interactions with the environment and/or host cells (19, 23, 30). Thus, TFP are considered virulence factors in several important human and animal pathogens including *P. aeruginosa*, *N. gonorrhoeae*, *N. meningitidis*, *M. bovis*, *E. corrodens*, *V. cholerae*, EPEC, and enterotoxigenic *E. coli*. In nonpathogenic bacteria such as *M. xanthus*, TFP also act as adhesins required for bacterial agglutination (38).

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Besides serving as adhesins, TFP are also involved in the secretion and uptake of some macromolecules. For example, TFP of *P. aeruginosa* are essential components for toxin secretion (18). In *N. gonorrhoeae*, TFP are involved in transforming DNA into bacterial cells (34). These cellular functions may be related to the fact that the membrane portion of TFP may function as a general (type II) secretion pathway.

The most unique (and interesting) cellular function of TFP is their involvement in the bacterial movements on solid surfaces (33). It is now well documented that many bacteria can move on solid surfaces without the aid of flagella (7, 12, 22). Social gliding motility in *M. xanthus* and twitching motility in *P. aeruginosa* and *N. gonorrhoeae* are the best examples of these flagellum-independent movements. Interestingly, genetic and behavioral studies show that both the social gliding motility of *Myxococcus* and twitching motility of *Pseudomonas* and *Neisseria* are absolutely dependent on TFP (14, 15, 37). Some recent studies have shown that other bacteria (e.g., EPEC and *Synechocystis* sp.) engage in TFP-dependent cellular motility on solid surfaces (2, 4).

TFP ARE RETRACTABLE MOTILITY APPARATUSES FOR SURFACE MOVEMENT

How TFP are involved in cellular motility has long been a mystery. In 1970, Bradley found that pilus-specific phage particles which initially attach to pili later appear at the cell surface (6). Based on this observation, he proposed that TFP may generate movement through pilus retraction. Recent studies in three model bacteria, *M. xanthus*, *N. gonorrhoeae*, and *P. aeruginosa*, have provided more-direct evidence that TFP indeed retract to generate gliding or twitching motility (20, 29, 31). Sun et al. (31) developed a tethering assay for *M. xanthus* cells in a highly viscous medium (1% methylcellulose). Using this assay, they observed that some *M. xanthus* cells could be tethered perpendicular to a glass or polystyrene surface in a TFP-dependent manner. These tethered cells displayed a jiggling motion as the cell bodies were drawn closer to the tethering surface. Further analyses of the behavior of these tethered cells led to a theory that *M. xanthus* cells extrude their pili, which attach to a solid surface and retract, bringing the tethered cell bodies closer to the surface. The study also showed that *pilT* mutant cells, which are piliated but defective in gliding and twitching motility, could be tethered to a solid surface (38) but fail to retract closer to the tethering surface, suggesting that PilT is required for generating the retraction force.

Merz et al. (20) used laser tweezers to show that *N. gonorrhoeae* pili retract. In one of their experiments, individual *N. gonorrhoeae* cells were immobilized on latex beads via antibodies specific for the bacterium. Smaller beads were used to tether the TFP fibers through a monoclonal antibody specific for a pilus surface-exposed epitope. The smaller beads, held in optical tweezers, were repeatedly pulled toward the immobilized cells. *pilT* mutant cells, in contrast, were unable to generate such retractile forces.

Skerker and Berg (29) recently labeled the TFP of *P. aeruginosa* with an amino-specific Cy3 fluorescent dye and visualized them on a quartz slide using a total internal reflection microscope. They were able to directly observe the extension and retraction of TFP of *P. aeruginosa.* Frequently, the distal tip of a pilus was adsorbed to the substratum and the pilus was pulled taut. Occasionally, the cell body detached from the surface and was pulled forward by means of pilus retraction.

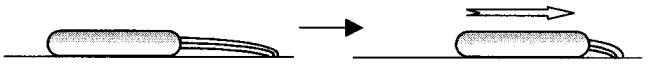

FIG. 1. Model of TFP-dependent motility. Based on recent studies (20, 29, 31), bacteria such as *M. xanthus* and *P. aeruginosa* extend TFP filaments at one pole of the cell, attach them at the distal tips, and retract to generate motility.

Through these studies, it is clear that TFP are motility apparatuses that extend pilus filaments, attach at their distal tips, and retract (Fig. 1). These findings greatly expand our knowledge about this mode of bacterial motility. Whereas flagellum-dependent motility works well in aquatic environments, TFP-dependent motility works well on solid surfaces. It would be interesting to discover how bacteria have evolved two very different motility mechanisms that are adapted for very different environments.

TFP-DEPENDENT MOTILITY IN *M. XANTHUS* AND *SYNECHOCYSTIS* SP. IS GUIDED BY A CHEMOTAXIS-LIKE SYSTEM

Previous studies have clearly shown that TFP-dependent motility is not a random movement. For example, the cyanobacterium *Synechocystis* sp. PCC6803 directs its TFP-dependent motility to perform phototaxis (2, 3). *Pseudomonas aeruginosa* requires its TFP-dependent motility to build up a sophisticated biofilm structure (21). The best example for directed TFP-dependent motility is the involvement of social gliding motility in the fruiting body formation of *M. xanthus*.

In response to starvation, hundreds of thousands of *M. xanthus* cells aggregate to form a multicellular structure called the "fruiting body," which creates a unique environment that enables the starving cells to support each other. In this cellular process, starved cells not only perform TFP-dependent social gliding motility but also respond to environmental signals and cell density to modulate their reversal frequency and direct themselves to move toward aggregation centers (17, 27, 28, 37). Detailed genetic and behavioral analyses have identified a chemotaxis-like system (called the *frz* system in *M. xanthus*) that is involved in controlling TFP-dependent motility (26, 31). The *frz* system contains homologs of the genes encoding the methyl-accepting chemotaxis protein (MCP), *cheA*, *cheY*, *cheW*, *cheB* and *cheR*, and regulates cellular reversal (28). Further studies have revealed some insights at the molecular level into this control process (31). It appears that TFP in *M. xanthus* are usually located at one pole of the cell (17) but can switch from one pole to another (31). The reversal of gliding direction is associated with switching active TFP from one pole of the cell to another and is controlled by the *frz* system (31) (Fig. 2). Given the fact that the *frz* system contains MCP, which can sense various environmental signals, it is reasonable to assume that chemotaxis-guided TFP motility in *M. xanthus* can be achieved in the following way. (i) The *frz* system recognizes various chemical signals in the environment and relays the signals to the sites of pilus extrusion. (ii) The extruded pili coordinate cell movements in the direction of the chemical

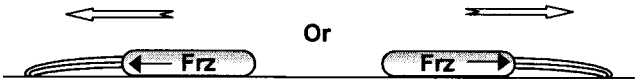

FIG. 2. Model of chemotaxis-guided TFP-dependent motility. Based on the study by Sun et al. (31), Frz chemotaxis homologs direct cell movements by controlling the sites of pilus extension.

gradient as a consequence of pilus retraction. (iii) Switching the sites of pilus extrusion from one pole of the cell to the other results in cellular reversals in *M. xanthus* motility. (iv) The pilus switching frequency between the two poles of a cell is controlled by the *frz* chemosensory system.

Recent studies showed that chemotaxis-like genes are also required for regulating TFP-dependent motility to perform phototaxis in *Synechocystis* sp. PCC6803 (3, 8). Therefore, it is likely that many other TFP-dependent motility systems may also be controlled by similar chemotaxis-like systems (10). The chemotaxis system has been well characterized for its role in directing flagellum-dependent motility. In the flagellum-chemotaxis system, it is known that the chemotaxis signal is relayed through a motor switching complex containing FliM-FliN-FliG (11). These homologs have not been found in gliding bacteria as yet. It would be interesting to elucidate the components that relay the chemotaxis signal to the TFP motility system and to find out how similar chemotaxis systems have developed to interact with two totally different motility systems. It is also worthwhile to point out that the genome sequence of *N. gonorrhoeae* suggests that such *che* homologs may not be present. It is unclear whether the TFP-dependent motility in *N. gonorrhoeae* is being controlled by a chemotaxis-like system.

TFP-DEPENDENT MOTILITY AND ITS ROLE IN PATHOGENESIS

The recent advances in understanding TFP at the molecular level and their physiological functions will help us to further understand the role of TFP in bacterial pathogenesis. It has recently been shown that TFP are an essential component for bacterial biofilm formation (such as in *P. aeruginosa*) (21). Without TFP, bacterial cells are still able to attach to solid surfaces but fail to build up multicell layers of the biofilm structure (21). Now, considering that TFP are motility apparatuses that can pull cells forward or upward and that the action of TFP can be directed by a chemotaxis-like system, it becomes relatively easy to understand how TFP may be involved in forming sophisticated biofilm structures. Furthermore, chemotaxis-guided, TFP-dependent motility may enable the bacteria to seek and locate appropriate target cells or find a weak area within tissue for effective penetration and invasion. This review has focused on the motility of TFP. However, TFP retraction and its involvement in pathogenesis may be beyond the twitching-gliding motility. For example, TFP is required for DNA uptake, phage infection, and adherence pattern formation and has some interaction with the type III secretion system (6, 9, 19, 25). These aspects, together with TFP-dependent motility, go beyond the traditional sense of pilus, inspire new ideas, and open new fields for future exciting studies of pili.

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