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Review

How Bacteria Use Type IV Pili Machinery on Surfaces

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The bacterial type IV pilus (T4P) is a versatile molecular machine with a broad range of functions. Recent advances revealed that the molecular components and the biophysical properties of the machine are well conserved among phylogenetically distant bacterial species. However, its functions are diverse, and include adhesion, motility, and horizontal gene transfer. This review focusses on the role of T4P in surface motility and bacterial interactions. Different species have evolved distinct mechanisms for intracellular coordination of multiple pili and of pili with other motility machines, ranging from physical coordination to biochemical clocks. Coordinated behavior between multiple bacteria on a surface is achieved by active manipulation of surfaces and modulation of pilus–pilus interactions. An emerging picture is that the T4P actively senses and responds to environmental conditions.

Biophysical Properties of Type IV Pili Are Adapted for Life at the Surface

Most bacteria in the biosphere live on surfaces. To efficiently colonize surfaces they must control attachment, surface-associated motility, and interactions between bacteria. A crucial extracellular organelle used for these purposes by bacteria is the T4P. The T4P is an organelle with polymeric organization that extends several micrometers from the cell body. Its physical properties make the pilus well suited for many roles important to surface colonization. The length of T4P is dynamic. They elongate by polymerization and retract by depolymerization. When the pilus adheres to an object during retraction, remarkably high force is applied to this object [1,2] (Figure 1A). This force can be translated into bacterial movement [3] and trigger downstream signaling in host cells [4,5]. Moreover, the physicochemical properties of the pilus surface are crucial for the organelle's adhesive strength on various surfaces.

Recent structural and molecular biological advances converge into a consistent picture of the structure and molecular mechanism of T4P assembly. While the proteins essential for pilus biogenesis are well conserved throughout bacteria and archaea [6], the molecular mechanisms and phenotypes involved in surface interaction and surface motility are diverse. In this review we aim to draw connections between current knowledge of molecular biology and biophysics of type IV pili. In particular, we focus on T4P behavior on different surfaces, and concentrate on results from recent advances in biophysical measurement techniques and strategies.

Composition and Structure of Type IV Pili

A consistent picture of the structure of the T4P machine is emerging. It is built of four subcomplexes that span the bacterial cell envelope [7–10]. It is important to note that homologues of the genes involved in T4P biogenesis have different notations for different species. Here, we adopt the notations for *Neisseria gonorrhoeae* and *Pseudomonas aeruginosa*. For *Myxococcus xanthus* an outside-in assembly pathway has been demonstrated [11]. Considering the homology of the T4P elements of other bacterial species, this pathway is likely to be general. The outer membrane secretin complex formed by PilQ is most likely anchored to the

Trends

The proteins forming the type IV pilus (T4P) machine are conserved throughout two of the three domains of life. Single-molecule analyses revealed that the T4P fiber can withstand and generate force on the order of 100 piconewtons and adjust to high tension by elongation.

Coordination of multiple type IV pili, and with other machines driving motility, has evolved differently across species. The mechanisms range from being consistent with purely mechanical coordination to molecular clocks that control the localization of the motors.

Physicochemical surface properties govern T4P-driven twitching motility. Recent experiments reveal that bacteria actively modify the surface properties by secretion of extracellular polymers, most likely for controlling group behavior.

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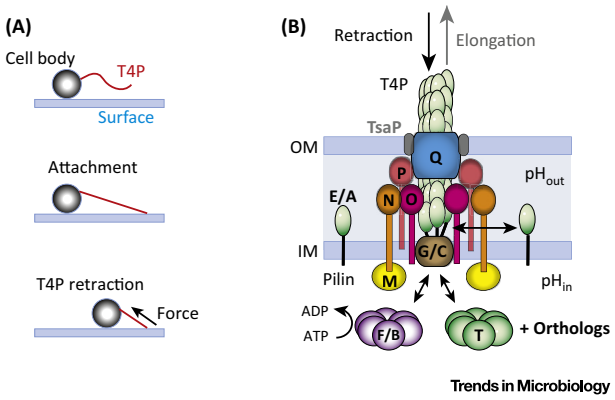


Figure 1. The Type IV Pilus (T4P) Machine Mediates Twitching Motility. (A) By cycles of T4P polymerization, surface adhesion, and retraction, the T4P pulls the cell body along surfaces. (B) Current molecular model of the T4P machine. *Neisseria gonorrhoeae*/*Pseudomonas aeruginosa* nomenclature for the Pil proteins is used (with only the distinctive letter shown in the figure). The T4P complex spans the bacterial cell envelope. The pilus fiber extends from the outer membrane. Its length dynamically changes through polymerization and depolymerization powered by the cytoplasmic ATPases PilF/B and PilT and the proton gradient. Abbreviations: IM, inner membrane; OM, outer membrane.

Glossary

EPS: EPS has been used to denote extracellular polysaccharides or exopolysaccharides, but has recently also been used to denote extracellular polymeric substances, since the biofilm matrix is known to include proteins and nucleic acids as well as polysaccharides. However, for the purpose of this review, we use EPS to denote exopolysaccharides.

peptidoglycan layer through TsaP [12]. PilQ recruits the PilNOP alignment subcomplex which resides within the periplasm and is connected to the inner membrane (Figure 1B). The PilNOP complex recruits the cytoplasmic protein PilM and the integral membrane protein PilG/C, also referred to as the platform protein [11]. The two cytoplasmic ATPases, PilF/B and PilT, power polymerization and depolymerization of the pilus polymer, respectively. They localize independently of other proteins of the T4P complex. Various proteins fine-tune the function and dynamics of the T4P, including minor pilins, PilT orthologs, and the PilC/Y1 proteins. An important open question is how the individual components of the T4P machine act together to drive pilus polymerization (see Outstanding Questions). Two mechanisms have been proposed. The piston model [13,14] predicts that the chemomechanical coupling works through ATP-driven conformational changes of PilF/B that push pilins through PilNO. The rotational mechanism has been proposed on the basis of conformational changes of PilG/C pili used by the type II secretion system of *Klebsiella oxytoca* [15]. In this model, the idea is that the elongation ATPase turns the platform protein, allowing pilins to be spooled into the nascent pilus. For detailed reviews about the components of the T4P system please refer to [7,16,17].

Mechanical Aspects of the Single T4P: Force Generation and Elasticity

Retraction of the T4P generates high mechanical force onto a load attached to the pilus [1]. In the case that the pilus is attached to a surface, the force generated by pilus retraction is used for bacterial movement [3] (Figure 1A). Various techniques have been developed for characterizing force generation by the T4P (Box 1) [18–20]. The force generated by a single T4P exceeds 100 pN, making the T4P one of the strongest molecular machines characterized so far [2]. High force generation is conserved in *N. gonorrhoeae* (gonococcus) and *M. xanthus* [21–23]. Multiple pili form bundles and cooperate to generate forces in the range of 1 nN [24].

The speed of T4P retraction is fine-tuned by environmental conditions and by PilT orthologs [25]. As expected for any molecular motor, the speed of retracting pili decreases as a function of the applied force (Box 1, Figure IF). Interestingly, at high forces the T4P switches between a high-speed mode and a low-speed mode [26,27]. This indicates that the T4P system can assume distinct states of T4P retraction. The two different modes are controlled by the concentration of oxygen and by its modulation of proton motive force in *N. gonorrhoeae* [27,28]. This finding implies that the proton motive force is a direct source of energy for gonococcal T4P retraction. Further fine-tuning of the T4P retraction speed occurs at the level of production of the PilT orthologs PilT2 and PilU [29]. So far it is unclear whether different PilT orthologs can form heterohexamers.

Box 1. Methods for Characterizing Single-Molecule Mechanics of the Type IV Pilus (T4P)

Type IV pili are remarkably powerful molecular machines. Various biophysical techniques have been adapted for characterizing kinetics and force generation by single pili and by assemblies of pili.

Laser tweezers (laser traps, optical tweezers) have proven useful for measuring the velocity of single pilus retraction and the force generated [18] (Figure IA,B). Laser light is focused by a microscope objective into a diffraction limited spot. A dielectric particle (such as a glass bead or a bacterium) experiences force, pulling the particle into the trap center. Displacements down to the subnanometer range (10^{-10} m) and forces in the range of piconewtons (10^{-12} N) can be measured under optimal conditions [107]. Figure IF shows the velocity–force relationship measured with laser tweezers.

Microscopic pillars can be deflected by pilus retraction [19] (Figure IC). If their stiffness is known, then the deflection of the pillar measured by microscopy can be converted into a measure of the force. This method is less sensitive at low forces and displacements but allows measurement of forces in the nanonewton range (10^{-9} N) at higher throughput.

Atomic force microscopes (AFMs) are particularly useful for characterizing the elastic properties of pili [20] (Figure ID). A bacterium is bound to a cantilever with known stiffness. When a pilus binds to the surface and the surface is displaced (downward) then force is applied on the pilus. In this way, the extension of the pilus as a function of the applied force can be determined.

Finally, microfluidic devices allow application of defined shear flows generating force on the cell body [108] (Figure IE).

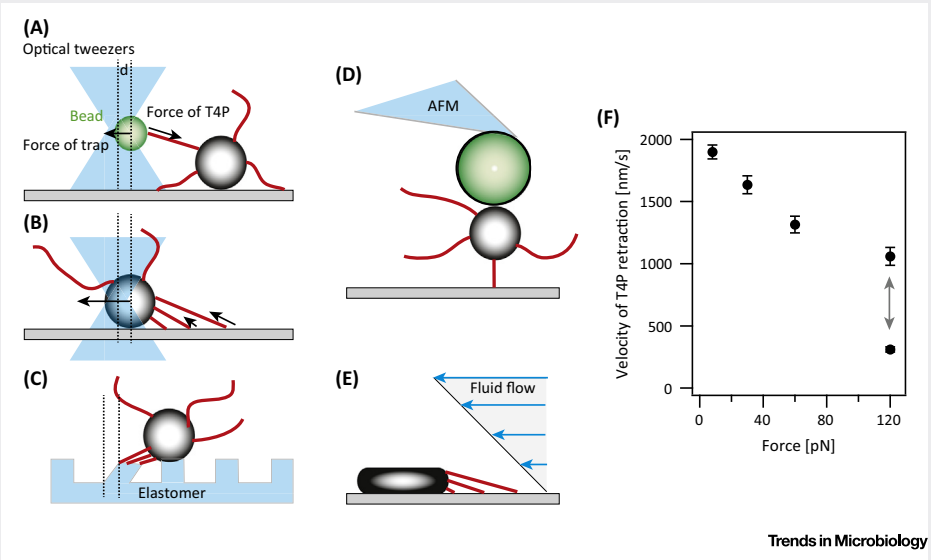


Figure I. Methods for Characterizing Mechanical Properties of the Type IV Pilus (T4P) System. (A) Laser tweezers measure force-dependent kinetics and maximum force of single pilus retraction and elongation. (B) Laser tweezers can trap single cells for determining the force applied by the entire cell onto the surface. (C) Elastomeric micropillars for characterizing force generation by an entire cell. (D) Atomic force microscopy (AFM) was used for characterizing the elastic properties of the T4P. (E) Application of surface shear stress using defined fluid flow. (F) Velocity of single pilus retraction measured with laser tweezers decreases as a function of applied force. At high force, the speed switches between different velocity modes (adapted from [36]).

Various nanomanipulation experiments provide evidence that the pilus fiber can withstand several hundreds of piconewtons of force [2,24,30]. In particular, atomic force microscopy (AFM) showed that individual fibers of *P. aeruginosa* are stable up to F = 250 pN [30]. Pilus elongation in response to external stress would decrease the probability that the pilus breaks. Elongation can be achieved either by pilus polymerization or by transitioning to a stretched conformational state. Currently, there is evidence for both mechanisms. For *N. gonorrhoeae*, stretching by the method of molecular combing causes the pilus fiber to extend three-fold [31]. Force plateaus obtained by AFM for *P. aeruginosa* are consistent with the transition of the pilus to a stretched conformational state [30]. Stretching was shown to reveal hidden

epitopes that are buried within the pilus fibers in the relaxed state [31]. Steered molecular dynamics simulations reproduced exposure of the relevant sequence of amino acids upon force application [32]. This simulation predicts that hydrophobic contacts between pilins within the core of the filament subunits are strong enough to maintain stability under tension. By contrast, there is evidence for force-induced T4P polymerization [26,33]. Using laser tweezers, the probability for pilus elongation was found to depend on the concentration of the PilT retraction ATPases. With decreasing PilT concentration, the T4P showed a high probability to elongate even at F <100 pN. Considering the switching times between retraction and elongation, their dynamics most likely reflects binding and unbinding of PilT to the T4P complex.

Motility Phenotypes and Intracellular Coordination of T4P

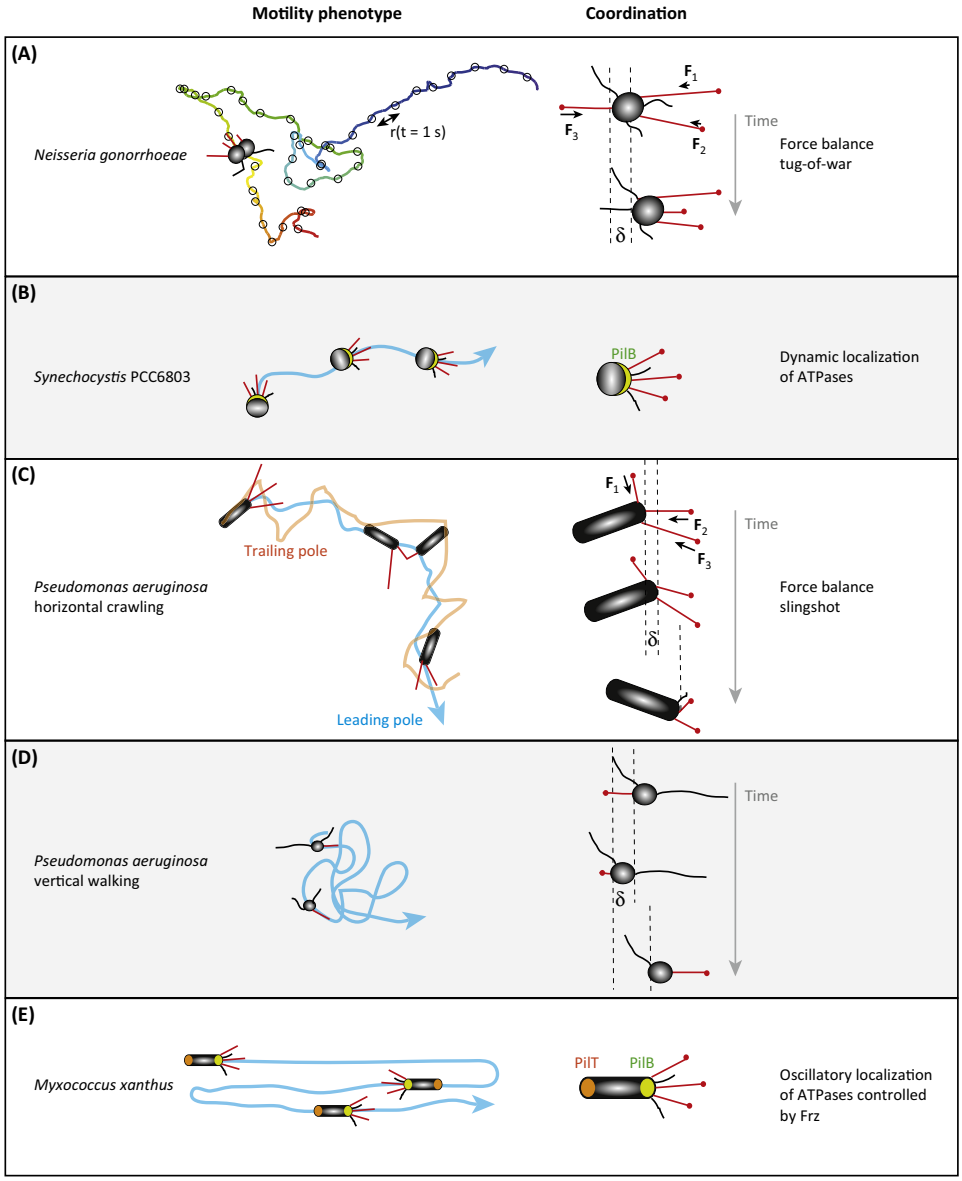
Type IV pili mediate surface motility in bacterial species that live in different habitats and are phylogenetically distinct. While the set of proteins required for motility and the molecular mechanism of force generation are well conserved [6], different bacterial species use distinct mechanisms for coordinating multiple pili.

Arguably the simplest mechanism of coordination between type IV pili is employed by *Neisseria* species. These round cocci are peritrichously piliated, that is, they generate their pili randomly around the cell contour [34,35] (Figure 2A). When attached to a surface, they perform a random walk with directional persistence at short time scale ($\alpha > 1$), indicating a specific type of coordination between pili (Box 2). A combined theory–experiment approach showed that coordination can be explained by a mechanical tug-of-war model with memory [36]. Memory is most likely introduced by T4P bundling and by the fact that the T4P complex is stable even after the pilus is fully retracted. Stability of the T4P complex has been demonstrated for *Thermus thermophilus* [8]. In agreement with theoretical predictions, the directional persistence increases with pilus density <10 pili per cell [34]. The speed of motility (~1.5 μm/s) is only slightly lower than single T4P retraction and shows oxygen-dependent speed-switching, suggesting that few pili are responsible for movement and that surface friction is not high [27]. At a very high density of ≈30 pili per cell, trapping occurs frequently because the forces are more likely to be balanced [37]. Interestingly, the symmetry of the cell body is broken in most gonococci because they form diplococci. The asymmetric shape orients the bacteria with their major axis perpendicular to the direction of movement [37].

Box 2. Random Walks and the Molecular Tug-of-war

Random walks describe the trajectory of a particle that steps in any direction in space with equal probability per unit time. For example, a bacterium in liquid without any means of active locomotion, will move due to random collisions with its surrounding molecules. These random movements are called diffusion or Brownian motion. The random walk can be described by the probability of finding a diffusing particle at a certain distance r after time t from its original position at time $t = 0$. The mean displacement will be zero since movement is equally likely in all directions. The mean squared displacement (MSD) increases linearly with time, $\left\langle r(t)^2 \right\rangle = aDt$, where a depends on the dimension, and D is the diffusion constant. Please refer to Philips *et al.* for further reading [109].

If pili are generated at random positions around a spherical cell and pull one after another, then the directional movement would be 'straight' at a time scale shorter than a single pilus retraction. At longer time scales, active pilus retraction would effectively increase the diffusion constant of the random walk. However, when these pili pull simultaneously in different directions, then the forces add up vectorially. The force acting on the pilus with the least neighboring pili will experience the highest force. This pilus most likely detaches from the surface. As a consequence, the initially random asymmetry of pilus bonds around the bacterium is amplified; the more pili bind in close vicinity, the lower the force per pilus and the lower the probability of detachment. This tug-of-war mechanism causes directional persistence of movement and has initially been formulated for describing vesicle transport by motors moving in opposite directions along the eukaryotic cytoskeleton [110]. Directional persistence is generally reflected in an altered relationship between the MSD and time, namely a power law $\left\langle r^2 \right\rangle = aDt^{\alpha}$. A coefficient $\alpha > 1$ for time scales longer than a single T4P retraction is indicative of pilus coordination.



Trends in Microbiology

Figure 2. Motility Phenotypes and Cellular Coordination of Type IV Pili. Left column: paths of bacteria moving along surfaces. (A) Experimental trace of *Neisseria gonorrhoeae* with the bacterium drawn to scale. The rainbow colors encode time with a total time of 42 s. The open symbols denote the location of the bacterium with $\Delta t = 1$ s. The bacterium moves the distance *r(t)* within time *t*. This distance is used for calculating the mean squared displacement (Box 2). When the trace appears straight over the distance of multiple pilus lengths, then the random walk is persistent at low time scales. (B) Schematic trace of *Synechocystis* PCC6803. The arrow indicates the direction of movement. (C) Schematic trace of crawling of *Pseudomonas aeruginosa*. The blue line depicts the locations of the leading pole, and the orange line depicts the locations of the lagging pole as a function of time. (D) Schematic walking trace of *P. aeruginosa*. (E) Schematic trace of *Myxococcus xanthus* oscillatory back-and-forth movements at the surface. Right column: schemes for mechanisms of coordination between pili that are consistent with current experimental and theoretical data. The force vectors (F) indicate the forces generated by individual T4P retractions. The symbol δ depicts the displacement of the bacterial cell body between different snapshots.

The cyanobacterium *Synechocystis* sp. PCC 6803 is another coccoid bacterium that employs T4P for surface movement (Figure 2B). Individual cells display 'random walk'-like trajectories with directional persistence at short time scales [38]. Recently, the intracellular distribution of the elongation ATPase PilB1 (PilF/B in *N. gonorrhoeae/P. aeruginosa* notation) has been characterized [39]. It forms 'crescents' adjacent to the cytoplasmic membrane that dynamically relocalize at a time scale of minutes. The direction of motility strongly correlates with the localization of PilB1. Interestingly, the crescents are more prevalent under low light conditions that favor phototactic motility. However, it is currently unclear how dynamics and localization of the T4P proteins couple to phototaxis as the latter involves complex group interactions [40].

In contrast to *N. gonorrhoeae* and *Synechocystis*, *P. aeruginosa* is rod-shaped and generates T4P at the cell poles. The T4P-driven surface motility of *P. aeruginosa* can switch between two different surface motility mechanisms [41–43] (Figure 2C,D): 'crawling,' by which the bacterium moves lengthwise parallel to the surface with high directional persistence, and upright 'walking' perpendicular to the surface, by which the bacterium moves with low directional persistence. While walking motility is nearly a random walk with a mean squared displacement (MSD) behavior characteristic of diffusion and can explore 2-D surface randomly, crawling motility is superdiffusive, with an MSD slope of $\propto \sim 1.4$, and can cover distances efficiently. Taken together, crawling and walking are the 2-D analog of *Escherichia coli* runs and tumbles in 3-D, although the physical and biological origins are different. Type IV pili play significant roles in facilitating transitions between vertical and horizontal cell orientations, which is important for a number of processes, such as progressive surface sensing and the change from 'reversible' to 'irreversible' surface attachment, and cell detachment, as can be seen in the launch sequence of *P. aeruginosa* from surfaces.

The multidirectional 'tug of war' model for T4P-driven movement described in Box 2 can generate a rich diversity of motility phenomena. When observed at high time resolution, T4P-driven horizontal crawling on surfaces (which microbiologists refer to as 'twitching' motility due to the seemingly random movements) has the characteristics of a sling-shot. This type of movement alternates between two kinds of actions: a slow (0.3 μm/s) translation of near-constant velocity and variable duration (0.3–10 s), and a fast translation (1 μm/s) of short, monodisperse duration (~100 ms) [44] almost always accompanied by a rotation of the bacterium (Figure 2C). It was proposed that these movements are due to multiple T4P pulling and to the rupture of a single T4P under the collective tension of all the other T4Ps, respectively. The existence of fast movements that are ~20× faster than the velocities from T4P pulling may enable cells to move efficiently on surfaces covered by exopolysaccharide (**EPS**: see Glossary) and other macromolecules, by reducing the local viscosity via shear thinning.

The rod-like *M. xanthus* tightly controls the location of its T4P through a molecular clock that triggers the process of switching the location of the T4P between both poles (Figure 2E). Pili assemble at the leading pole and upon directional reversal of movement, the pili switch the pole [45]. Polar localization of T4P proteins is ensured by the GTPase SofG [46,47]. Cellular reversals are induced by the Frz chemosensory system. The GTPase MglA in combination with its cognate activating protein MglB and the response regulator RomR stimulate motility by setting up the correct localization of the dynamically localized proteins [48,49]. In particular, the ATPases PilF/B and PilT accumulate at the leading and lagging poles, respectively, and relocate during reversals [50]. The Frz system also synchronized the T4P motors with the second motility system employed by *M. xanthus* as described in the following chapter.

Coordination with Other Motors

Bacteria often employ multiple motility-associated motors simultaneously. *M. xanthus* employs gliding motility (aka adventurous motility, A-motility) in addition to twitching motility. The motors

that drive gliding motility are related to the bacterial flagella homologs [51,52]. As these motors walk along a helical track around the cell along the cytoplasmic membrane [53], they exert propulsive force on the cell body. Both twitching motility and gliding motility are coordinated by the Frz system. In particular, recent studies indicate that gradients of the GTPase MglA are important in this respect. The AglR parts of the gliding motors have been shown to reverse their direction of movement frequently [54]. The reversal frequency is positively correlated with the gradient formed by MglA that also regulates the localization of PilT and PilF/B. This generates a bias of motor movement towards the lagging pole and propels the cell forward.

P. aeruginosa generates type IV pili and a single polar flagellum. The coordination between swimming and swarming motility driven by flagella and twitching motility driven by the T4P is currently fuzzy. Type IV pili can be located at one pole, or both poles, or they can be absent [55]. Concomitantly the localization of the ATPases seems to depend on the experimental conditions, including the presence of a surface [55,56]. In some studies, it appears that the localization of pili and flagella can be uncorrelated in terms of the pole they assemble at [55]. While the T4P localization depends on the cytoskeletal protein MreB, flagella localization is independent of MreB but dependent on the signal recognition particle – the GTPase FlhF, suggesting that the two structures localize by separate mechanisms. However, the Poc proteins affect both flagella localization and pilus production and localization, indicating a coupling mechanism [57]. Although the molecular mechanisms of intracellular coordination between T4P and flagella are not well understood, intercellular coupling affects the efficiency of swarming motility. Generation of T4P strongly reduced the swarming speed on agar plates [58]. It was shown that pilus–pilus interaction reduced nematic ordering of the rod-like cells at the swarm edges. Computer simulations predicted that pilus–pilus interactions increase the probability that cells move in clusters. When piliated and nonpiliated cells were co-cultured, nonpiliated cells dominated the swarm edge [58].

Vibrio cholerae constitutes another example of synergistic use of T4P with other motors. Cells of *V. cholerae* engage a diversity of surfaces with their T4P, including surfaces of phytoplankton, zooplankton, and crustaceans [59,60], as well as nonnutritive, abiotic surfaces, using a wealth of T4P types [mannose-sensitive hemagglutinin (MSHA) pili, virulence-associated toxin co-regulated pili (TCP), and chitin-regulated pili (ChiRP)]. *V. cholerae* can also skim over a surface using flagellum-driven swimming. Recent work has shown that different interactions between MSHA pili and the surface can create two types of motility [61]: (i) 'Orbiting motility' consists of near-circular trajectories that allow cells to naturally loiter over surface regions that interact more strongly with MSHA pili. (ii) 'Roaming motility' consists of low-curvature trajectories that allow cells to 'pass over' surface regions with weak interactions with MSHA pili. In this context, T4P interactions effectively facilitate mechanical scanning of a surface before *V. cholerae* attachment and subsequent biofilm development [61]. Whereas specific appendages are associated with specific motility modes, it is clear from these examples that the coordination of T4P with other motors can lead to a broad range of motility possibilities, which are currently being studied in detail.

The interplay between different adhesins is even more complex for *Caulobacter crescentus*. *C. crescentus* generates flagella, type IV pili, and a stalk. The interplay between pili and flagellum rotation stimulates the transition between reversible attachment and polysaccharide-dependent irreversible attachment [62]. Type IV pili mediate reversible attachment of *C. crescentus* to the surface. In addition, it was proposed that they mechanically arrest flagella rotation, triggering production of an adhesive polysaccharide holdfast for the stalk which renders attachment irreversible [62]. Furthermore, type IV pili are involved in deciding whether daughter cells adhere to the surface succeeding cell division. Importantly, the curved shape of *C. crescentus* increases the probability that the daughter cell attaches to the surfaces using the T4P [63]. This finding

suggests that T4P retraction may be important for surface attachment rather than for twitching motility in this species.

Bacterial Conditioning of Surfaces: Interactions between the T4P and Extracellular Polymers

Given that bacteria can colonize a diverse range of surfaces, it is not surprising that they can also modify surfaces via secretion of macromolecules. One of the defining characteristics of bacterial biofilms is the EPS matrix. The mature extracellular matrix of *P. aeruginosa* is composed mostly of EPS, proteins, and DNA [64–66]. Due to their interactions with the T4P, some of these macromolecular substances play interesting roles during early stages of biofilm formation. For example, the exopolysaccharide Psl from *P. aeruginosa* (PAO1) can function as a 'molecular glue' mediating early surface attachment [67–71]. Recent work has indicated that Psl can have a social function, through its interactions with the T4P [41]. The large-scale cell migration patterns that lead to microcolony formation, the first social step in biofilm organization, can be traced to T4P–EPS interactions. By using cell-tracking algorithms to extract the full motility history of every cell [41], and combining that information with fluorescent lectin stains of Psl, it was shown that the T4P-driven surface motility of *P. aeruginosa* is guided by a web of collectively secreted Psl. Since *P. aeruginosa* can secrete Psl and can also associate with Psl, the more Psl is deposited on a given patch of surface, the more likely a bacterium will visit that patch, and the more likely Psl will be secreted there again. Through this cycle of positive reinforcement, T4P–EPS interactions allow a bacterial community to self-organize like an economic system, via a 'rich-get-richer' amplification of Psl accumulation (Figure 3) that results in a measurable power law [72] (Box 3) in the surface visit distribution. In this organization, a small number of cells are extremely enriched in communally produced Psl. (It is interesting to note that both Psl-producing cells and non-Psl-producing cells have enhanced probabilities of adhering to surface areas with high concentrations of Psl, and thereby participate in microcolony formation.) In fact, since high local Psl levels are found to

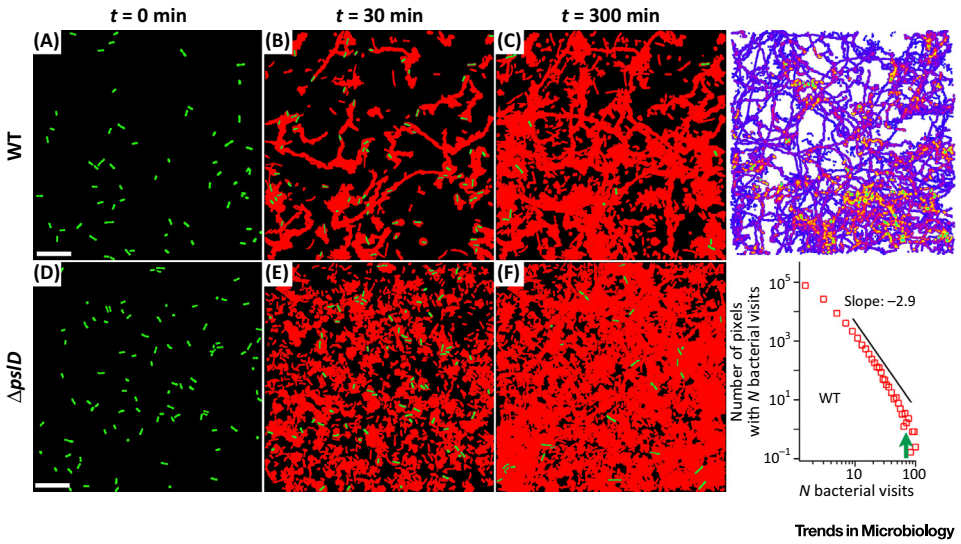


Figure 3. Type IV Pilus (T4P)–EPS Interactions and Microcolony Formation. (Left) History tracking shows that wild-type (WT) *Pseudomonas aeruginosa* PAO1 bacterial trajectories tend to cover a small fraction of the surface, whereas an Δ*pslD* mutant will cover the surface completely and efficiently. Three time points are shown, t = 0 min (A,D), 30 min (B,E), and 300 min (C,F). The top row is for WT, and bottom row is for Δ*pslD*. Red and black colors indicate 'used' surface areas (i.e., areas that have been previously covered by bacterial trajectories) and 'fresh' surface areas, respectively. Bacteria in the current frame are shown in green. Scale bars are 10 μm. (Right) Visit frequency distribution of WT *P. aeruginosa* (15 h of growth), and associated power law distribution of visit frequencies. This shows that the more often a given surface area is used by bacteria, the more likely it will be used again. This phenomenon leads to a 'rich get richer' amplification that results in microcolony formation [72].

Box 3. Power Laws

A power law is a functional relation of the form $f(x) = a\,x^n$. $f(x)$ varies as the nth power of x. A signature of power law relations is a straight line on a log-log plot of $f(x)$ vs x. This is a consequence of one of the well-known characteristics of power laws: scale invariance. Multiplying the argument x by a constant c will only produce a rescaling of the power law relation itself (multiplication by a constant factor of c^n). A broad range of self-organized phenomena, both natural and human-derived, follow power laws over orders of magnitude. This includes distributions of earthquake sizes and distributions of income. In the former, the Gutenberg–Richter law is the relationship between earthquake magnitude and frequency. In the latter, the Pareto distribution (or the '80–20' rule) tells us that the net worth of Americans is distributed according to a power law with an exponent of 2, or that 20% of the population have 80% of the wealth, with the top 4% (top 20% of the top 20%) having 64% (80% of 80%) of the wealth due to scale invariance. In the case of bacteria, power laws govern how often *Pseudomonas aeruginosa* visits each patch of surface, with a small fraction of the surface getting the lion's share of visits due to EPS secretion [72]. This effect facilitates the formation of microcolonies. Power laws also govern the distribution of step sizes as *Myxococcus xanthus* pulls itself along an EPS-covered surface using the T4P [81].

increase the probability of daughter cells remaining sessile after cell division, these Psl-rich cells become the founding population for initial microcolony development [72]. We expect the above effects to be amplified further since Psl can function as a signal to stimulate diguanylate cyclases, which produce cdiGMP that leads to the production of more EPS [73]. Recent work has shown that other complementary effects such as mechanical confinement may also play important roles in biofilm development [74]. At the leading edge of expanding *P. aeruginosa* biofilms on soft and semisolid media, bacteria can create furrows that can mechanically guide migration of cells. Throughout the network of furrows, it was found that extracellular DNA (eDNA), which can interact with T4P, and facilitates cell alignment [74].

Although EPS-T4P interactions commonly govern bacterial motility, subtle differences in the details of these interactions can lead to drastic phenotypic changes. *M. xanthus* is capable of organized social behavior [75]. For example, *M. xanthus* can form fruiting bodies composed of 10^4–10^6 cells to ensure community survival under starvation conditions [76]. This behavior [77] relies on the coordinated motility of large numbers of cells via social ('S')-motility, which is mediated by T4P [78]. Recent evidence suggests that the T4P can sense EPS secreted by other cells [79,80], which enables *M. xanthus* to coordinate movement along EPS tracks.

Recent work has dissected *M. xanthus* S-motility, using high-speed cell tracking, and found that these cell trajectories consist of aperiodic stick–slip movements, driven by T4P-generated force acting against EPS-derived friction [81]. At timescales shorter than the motility cycle (the time required for typical T4P pulls), the movements of *M. xanthus* cells are consistent with the dynamics of earthquakes [82] and a variety of other nonbiological systems [83,84]. These *M. xanthus* movements are characterized by discrete 'avalanches' which exhibit a statistical hierarchy governed by a power law distribution. Measured power law exponents for the *M. xanthus* system are consistent with mean field theoretical models of friction and other so-called 'crackling noise' systems [85,86]; this indicates that the frictional interaction between the surface and the bacterium, mediated by EPS, is being probed [87]. Interestingly, a quantitative analysis indicates that *M. xanthus* S-motility trajectories are in the 'chaotic stick–slip' regime, which is usually observed for advanced lubricants with highly branched molecular architectures. This suggests the interesting hypothesis that EPS of *M. xanthus* can promote surface adhesion and also function as a lubricant, which may alleviate the force generation requirements for multicell S-motility.

Physicochemical Properties, Surface Topography, and Fluid Flow Govern Twitching Motility

To better understand how external factors, including surface property and fluid flow, influence surface motility, biophysical experiments have been designed for rational control of these

parameters. Micropatterning and microfluidics have been used for analyzing single-cell behavior, cell adhesion, and biofilm formation [88–92]. Here, we focus on T4P-mediated interactions with surfaces.

Type IV pili adhere to almost all known abiotic and biotic surfaces, suggesting that the adhesion forces are unspecific. The rate of pilus detachment from surfaces correlates with the phenotypes of twitching motility. For example, the gonococcal T4P binds more strongly to polystyrene than to glass. BSA (bovine serum albumin) reduces the interaction strength [34,36]. In agreement with these observations, gonococci are trapped most frequently in the nonmotile state when moving on polystyrene surfaces followed by glass and BSA-coated glass surfaces [36,37]. Application of seminal plasma again increases the fraction of motile gonococci, reminiscent of BSA [93]. Using various bottom-up approaches, the effects of the physicochemical properties of the surface on twitching motility have been examined systematically. *Neisseria* species use their T4P to move on their human host cells [22,94]. Thus it is conceivable that the membrane properties influence their motility. The fluidity of lipid membranes can be controlled through lipid composition and

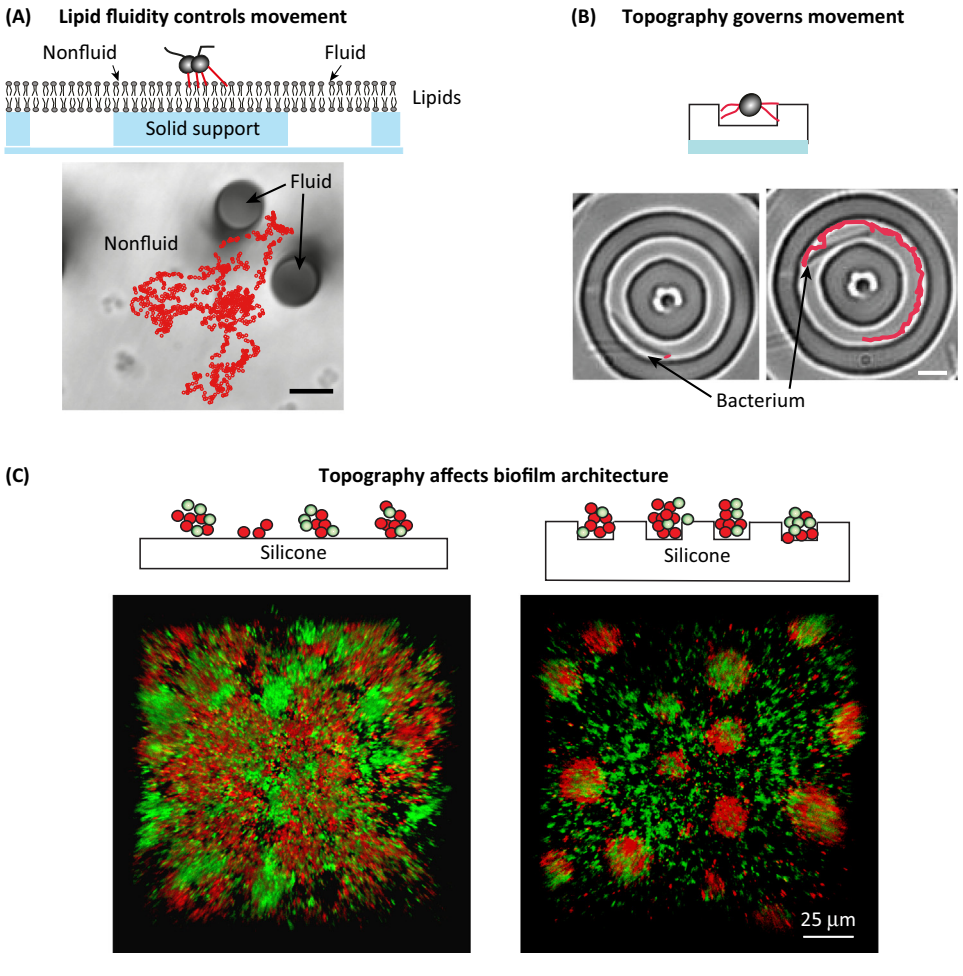


Figure 4. Designed Surfaces Control Twitching Motility and Biofilm Architecture. (A) Path of a single gonococcus moving on a lipid bilayer with varying fluidity. Red dots: trace of a single bacterium. Scale bar: 5 µm (adapted from [95]). (B) *Myxococcus xanthus* moving within circular grooves). Red line: trace of a single bacterium. Δt = 2 min between left and right image. Scale bar: 5 µm (adapted from [97]). (C) Gonococcal biofilm grown for 2 days on flat (left) and microstructured silicone surface (right). Red and green indicate gonococci generating mCherry and GFP, respectively (adapted from [98]).

solid support. The speed and persistence of gonococcal surface motility negatively correlate with lipid fluidity [95] (Figure 4A). On microstructured surfaces with alternating fluidity, bacteria avoid fluid areas. These findings suggest that pili require solid surfaces for translating their forces into movement. The effect of surface softness was recently examined for *P. aeruginosa* on surfaces coated with polymers that transition from a soft brush-like conformation to a stiffer collapsed conformation as a function of temperature [96]. Here, again, directional persistence was higher on stiffer surfaces. In nature, the adhesive surface encountered by bacteria is usually not flat. The effect of surface topography has been studied with micromolded silicone. *N. gonorrhoeae* and *M. xanthus* are guided by barriers as low as one micrometer [97] (Figure 4B). Interestingly, the surface topography also affects the architecture of gonococcal biofilms [98] (Figure 4C). The density is considerably lower and the surface roughness is higher when biofilms grow on microscopic grooves, reducing the biomass.

Shear stress on surface-bound bacteria affects both adhesion and motility. The residence time of *P. aeruginosa* increases with the rate of shear stress applied by fluid flow [99]. Deletion of flagella increases the residence time [99] in agreement with the finding that flagella facilitate detachment from the surface [43]. Interestingly, *P. aeruginosa* uses its T4P for moving upstream [100]. Since pili are generated at the pole, the bacterium can pivot around the point of attachment. Fluid flow tends to align the rod-shaped bacterium such that the bacterium moves upstream. Upstream movement of individual cells is characterized by counter-advection and lateral diffusion [101]. Recently, branched microfluidic devices (reminiscent of plant or animal vascular structures) have been used for studying the competitive advantage of twitching motility in the presence of fluid flow [101]. Under these conditions, *P. aeruginosa* moved upstream into previously noncolonized branches, segregating from species that have a higher growth rate under static conditions.

Concluding Remarks

To our current knowledge, the biophysical characteristics of a single T4P are well conserved between species. Individual type IV pili are remarkably strong molecular motors that generate and withstand 100 piconewtons of force. There is evidence that pilus elongation is involved in supporting high tension. The coordination of the dynamics of multiple pili relates to cell shape and to its necessity to coordinate with other motors. *P. aeruginosa* and *M. xanthus* condition their surfaces for governing group behavior, and twitching motility is affected by surface stiffness and topography.

It is tempting to speculate that the biophysical properties of T4P respond to the environmental conditions, and recent experiments support this hypothesis. During biofilm formation, oxygen can become limiting, establishing oxygen gradients [102]. Interestingly, the gonococcal T4P adjusts to oxygen levels within seconds by changing pilus–pilus bond-breakage forces governing assembly and disassembly of microcolonies [103]. Furthermore, structure predictions suggest that pilus–pilus interactions also control menigococcal microcolony formation during infection of endothelial cells [104]. Microcolonies formed during early infections disassemble as a consequence of enhanced post-translational modification of the pilin subunits promoting colonization of the host cells. Finally, there is evidence that the T4P is used for detecting surface attachment which, in turn, upregulates T4P production [105,106]. Thus, a picture emerges in which the T4P is used as an extracellular tool for adjusting the biophysical interactions between the bacterial cell and the adhesive surface.

Outstanding Questions

How do the components of the type IV pilus (T4P) system act together for T4P assembly and disassembly?

Are cooperative mechanisms involved in the typically large forces generated by T4P retraction and how does chemomechanical coupling work?

How do expression and localization dynamics of T4P components couple to twitching motility phenotypes and group behavior?

How do T4P density, localization, and force generation respond to physical cues like surface stiffness?

How do T4P–T4P and T4P–surface interaction forces evolve during biofilm formation?

Acknowledgments

We gratefully thank the Maier and Wong laboratories for helpful discussions. This work was supported by the Human Frontiers in Science Program (RGP0061/2013 to G.W. and B.M.), by the Deutsche Forschungsgemeinschaft (MA3898 to B.M.), and the National Science Foundation (DMR1411329 to G.W.).

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