Current Biology, Vol. 12, 000–000, April, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S0960-9822(02)000-X

Bacterial Surface Motility: Slime Trails, Grappling Hooks & Nozzles

Review

Alexey J. Merz[1] and Katrina T. Forest[2]

It has been known for decades that bacteria locomote over surfaces, but the mechanisms that power motility have been unclear. Recent experiments have begun to explain two modes of surface motility. Twitching or social gliding motility is powered by the retraction of type IV pili. Adventurous gliding motility is powered by the rearward secretion of carbohydrate slime. In both cases, cell movement depends on the translocation of enormous volumes of macromolecules through outer membrane pore complexes. In this review, we describe molecular models for surface motility and discuss how these models can inform studies of macromolecule secretion across bacterial membranes.

Introduction

When we think of bacterial motility, we usually think of cells swimming and tumbling through fluid media, propelled by rotary flagella. Over the last thirty years, studies of flagellar motility have yielded insights into molecular motor function, signal transduction and type III bacterial protein secretion. But bacterial life is not limited to the aqueous phase, and bacterial motility is not limited to swimming: many bacteria crawl, glide or twitch their way over solid substrates [1–5]. Bacterial surface locomotion is involved in many aspects of microbiology including morphogenesis, biofilm formation and microbe–host interactions. Studies of surface motility engines can also improve our understanding of protein export and of proteinaceous channels that conduct macromolecules.

We describe recent work on two modes by which Gram-negative bacteria move over surfaces. Adventurous gliding results from compressive forces generated by the hydration, expansion and rearward extrusion of polyelectrolyte slime. Twitching or 'social gliding' motility is due to tensile forces generated through the attachment and retraction of type IV pilus fibers. Many questions remain about the molecular machines that power twitching and social gliding, but they share at least one common feature: both rely on the flux of large volumes of macromolecules through proteinaceous pores in the bacterial outer membrane.

[1]Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755-3844, USA. alexey.merz@dartmouth.edu
[2]Department of Bacteriology, University of Wisconsin, Madison, WI 53706, USA. forest@bact.wisc.edu

Slime Trails

Several mechanisms have been proposed to account for gliding motility including treadmill-like motors on the cell surface [1,3,6] and secretion of surfactants that draw the cell forward [3,4]. Recent experiments support another idea: that the gliding of filamentous bacteria — linked chains of dozens to hundreds of cells — is powered by compressive forces arising from the rearward secretion of slime, a polyelectrolyte gel composed of complex carbohydrates [3].

Hoiczyk and Baumeister have presented three lines of evidence consistent with the slime secretion model. First, the cell surfaces of several filamentous bacteria are covered with crystalline surface layers (S-layers) of protein [7]. S-layers serve as scaffolds which in turn support arrays of proteinaceous ridges and grooves. In *Phormidium uncinatum* the ridge arrays are formed by the Ca^{2+}-binding protein oscillin, which is required for motility [8]. The surface striations are hypothesized to form channels which direct the flow of secreted slime. On filamentous species that rotate as they glide, the surface striations form helices around the bacterial filament, and the helical chirality matches the direction of filament rotation [9,10]. On species that glide but do not rotate, helical striations are absent.

Second, electron microscopy reveals in many gliding bacteria the presence of junctional pore complexes — cylindrical structures that span the inner and outer membranes [10,11]. Junctional pores are proposed to act as 'nozzles' through which slime is secreted [11]. The nozzles are positioned in circumferential bands adjacent to the junctions between cells in a filament and are tilted forward or back, again suggestive of a thrust-vectoring function. Electron microscopic studies of pore-enriched cell envelope fractions show that the outer membrane component of the isolated complex is a hollow tube with 7 nm openings at its ends, and a larger diameter at its midpoint. In some cases the complex is attached to a needle-like structure presumed to span the periplasm [11].

In the third line of evidence for the secretion model, light microscopic observation of slime secretion from living cells (Figure 1) shows that streams of slime do indeed emerge from the junctional locations where nozzles are found, at speeds similar to the gliding speed [11]. Like the filamentous gliders, the rod-shaped bacterium *Myxococcus xanthus* also secretes slime. New experiments published recently in *Current Biology* [12] show that streams of slime are secreted from *Myxococcus* cell poles, and at these sites electron microscopy again reveals the presence of pore-like structures that might serve as nozzles. Thus data from diverse organisms show that slime secretion occurs in a polarized fashion consistent with the

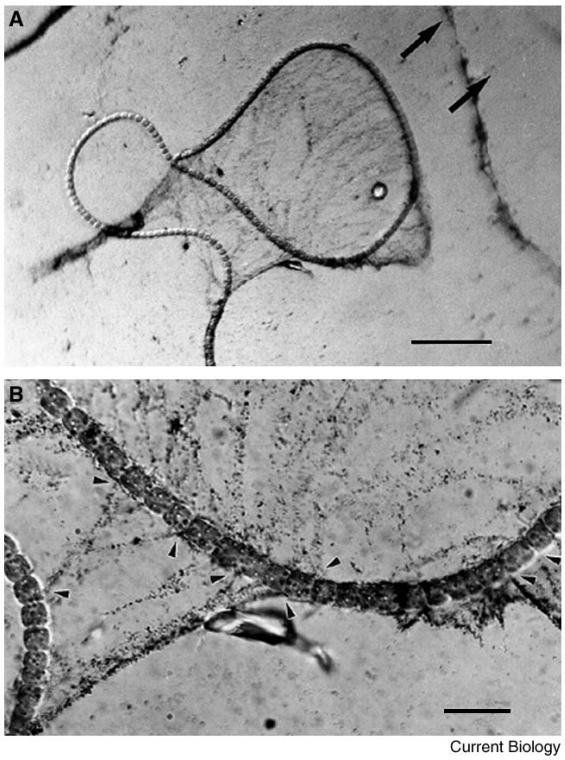

Figure 1. Slime secretion from cell–cell junctions in a filament of *Anabena variabilis*.

A stream of carbon particles was used to dissociate from the bacteria, and label, the slime streams emanating from junctional sites. Arrows in (A) show the direction of filament movement, which is opposite to the direction of slime secretion. Arrowheads in (B) show examples of slime streams originating at junctions. From [11].

observed directions and speeds of gliding, and show that secretion occurs at membrane sites coincident with nozzle-like pore structures.

To determine whether slime secretion could generate enough force for motility, Oster's group has developed a mathematical model [12] in which slime (a generic polyelectrolyte gel) is introduced into the nozzle cavity in a relatively dehydrated, and thus compact, state. Hydration of the slime causes it to swell, exerting force as it is expelled at the nozzle opening. Slime exiting the nozzle pushes against the substrate and against already-secreted slime; crosslinks between carbohydrate chains allow the gel to resist shear loads. In other words, slime behaves as a supramolecular spring [13], storing potential energy that is converted into kinetic energy as the slime swells in the nozzle chamber. Several analogous systems have been subjected to intense scrutiny, including DNA condensation and the compaction of actin gels [13,14]. Under a variety of conditions, slime hydration would provide enough force to move cells forward at observed speeds. The model does not specify how unswelled slime is introduced into the nozzle cavity, but Wolgemuth *et al.* [12] make a strong argument that hydration is the dominant force-generating mechanism.

Many questions remain regarding slime-secretion motility. Are the force–velocity relationships predicted by the hydration model observed experimentally? What is the chemical composition of the slime secreted by *Myxococcus*, and are its physical characteristics consistent with those assumed in the modeling? Which proteins are used to construct the nozzle, and which are needed to synthesize slime? Do defined mutations in the genes encoding these proteins abolish gliding? Where does slime polymerize, and how is it introduced into the nozzle? How are production of slime protomer, polymerization and secretion regulated, and are these systems linked to chemotactic regulatory circuits? It is tempting to imagine that slime secretion is a spatially directed form of capsule or exopolysaccharide secretion. In some such systems it has been proposed that carbohydrate polymerization and secretion are coupled processes occurring at co-located sites [15,16]. Do the slime-motility and capsule-biosynthetic systems share strong homology? Did slime-based motility evolve more than once?

Grappling Hooks

Twitching motility and social gliding motility require type IV pili — 6 nm thick protein filaments that extend up to 5 μm from the cell surface [2,4,5]. In rod-shaped bacteria, type IV pili are found at the cell poles. A correlation between the presence of pili, susceptibility to pilus-binding bacteriophages and cell motility was established in pioneering studies by Bradley [17–19], who proposed that pilus retraction could account for both cell movement and phage infection.

In Bradley's experiments, anti-pilus antibodies inhibited motility and infection by pilus-binding phage, and increased the number of pili per cell. Bradley also identified *Pseudomonas aeruginosa* mutants that were phage-resistant and immobile. A subset of these mutants had superabundant pili that could bind phage but apparently could not move the phage to the cell body [17–19]. Similar lines of evidence suggested that conjugal pili and the pili of *Caulobacter crescentus* could retract [20–22], but through the 1980s and 1990s only a handful of studies examined pilus retraction. Interest in pilus retraction as a motility mechanism was reignited by the discovery that PilT, a presumed ATPase, is required for twitching motility but not pilus assembly [23], and subsequent findings that PilT-like proteins or pilus-dependent motility are implicated in virulence, biofilm formation, morphogenesis, DNA uptake and protein export [5,24–30].

Type IV pilus retraction has now been directly observed in three systems. Using a laser tweezers trap [31], we found [32] that pili on *Neisseria gonorrhoeae* cells can form tethers between cells, or between cells and inert objects such as latex beads, and that these tethers forcefully retract (Figure 2 A–C). Retraction requires PilT and is abolished by a point mutation in the PilT ATPase domain. Quantitative experiments

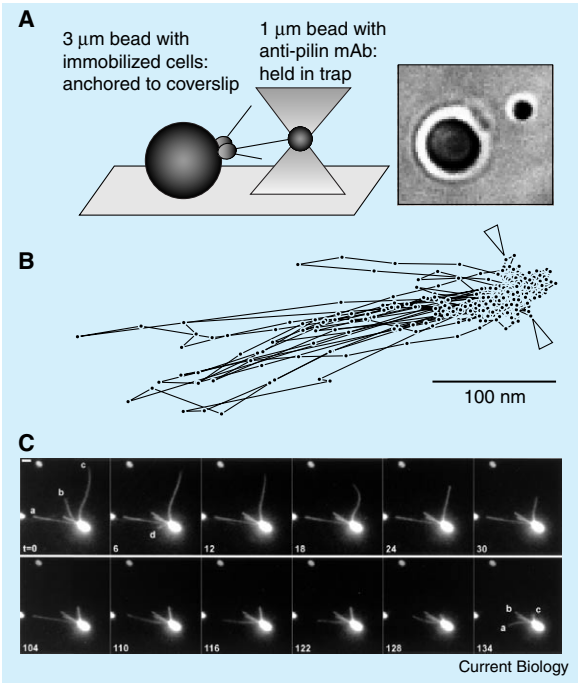

Figure 2. Type IV pilus retraction and extension.

(A,B) Laser tweezers analysis of pilus retraction, from [32]. (A) Experimental geometry. Bacterial cells (diplococci) are immobilized on 3 μm diameter beads that are anchored to the coverslip. A 1 μm anti-pilin-derivitized bead is suspended in the laser tweezers trap. When pili attach to the 1 μm bead and retract, the 1 μm bead is pulled out of the laser tweezers. The cartoon shows a side view; the micrograph shows the assay in progress from above, with scale indicated by the beads. (B) Direction of displacements. Trace from a recording of an immobilized cell that shows the locations of the 1 μm bead in the plane parallel to the coverslip surface. Eight retraction events are recorded in this trace. The center of the laser trap is indicated by arrowheads. The immobilized cell is in the same position relative to the laser trap as in the micrograph in (A). (C) Retraction and extension of fluorescently labelled pili, from [33]. Elapsed time is shown in seconds. Scale bar = 2 μm.

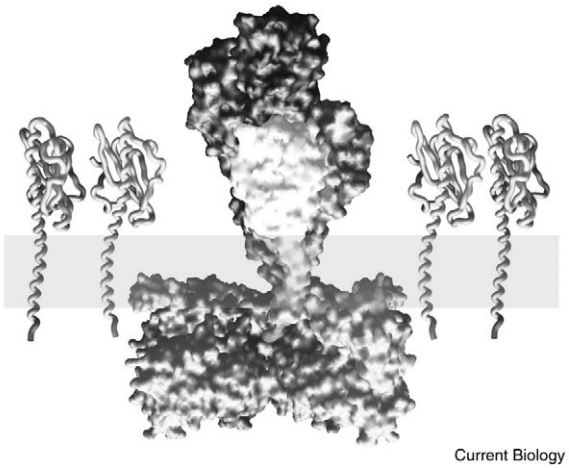

Figure 3. Simplified view of Type IV pilus assembly and retraction.

Monomeric pilin subunits (blue Cα tubes based on *N. gonnorhoeae* pilin structure; PDB code 2pil.pdb) form a pool in the inner membrane (grey, semitransparent bar). The pilus fiber is represented as molecular surfaces of five monomers, ranging from white to dark blue with the membrane-proximal subunit shaded according to electrostatic potential: grey represents neutral, red negative and blue positive potential. Assembly or retraction requires a cytoplasmic secretion/retraction GspE homolog associated with the inner membrane (represented here as an orange molecular surface of three monomers from the hexameric ring structure of the *Helicobacter pylori* type IV secretion ATPase ; PDB code 1g6o). The front of the ATPase ring is not shown, and other components of the secretion machinery including the secretin pore complex and pseudopilins are not depicted.

show that retraction occurs at average speeds of 1.2 μm s^{-1} and can generate tensile forces exceeding 80 pN per cell (a cell can have several pili) [32]. Skerker and Berg [33] covalently labeled the pili of *P. aeruginosa* cells with a fluorescent dye and were able to observe individual pili using evanescent wave microscopy (Figure 2D) [34]. They saw pili extend as well as retract, at speeds of ~0.5 μm s^{-1}. (The *Pseudomonas* experiments were done at room temperature, but most of the *Neisseria* work was done at 35°C. When *Neisseria* was observed at room temperature, pilus retraction speeds were the same as in *Pseudomonas*; B. Maier, A.J.M., M. So and M. Sheetz, unpublished data.)

Pili bend and flex due to Brownian motion. In some cases an extended pilus attached by its tip to the coverslip surface and was then pulled taut; from the lack of lateral deflection in such pili, a lower limit of 10 pN was set on the retraction force [33]. In another set of experiments, Shi and coworkers [35] used video microscopy to show the shortening of pilus tethers between *M. xanthus* cells and the substrate.

Significant forces are generated during pilus assembly as well as retraction. Extension forces are large enough to cause membrane protrusions and possibly membrane puncture [36]. These observations are consistent with forces larger than 5 pN — perhaps much larger [37,38]. Thus, explanations of pilus dynamics will need to account for high speeds and significant forces during assembly and retraction. Nevertheless, the available data suggest that pili are too flexible to mediate extension-based motility [32,33], and pilus retraction therefore appears to provide the main force for cell movement.

How Do Pili Work?

The pilus is a polymeric helical filament composed of pilin subunits [39,40]. In contrast to the subunits of other polymeric fibers, such as actin and tubulin, monomeric pilin is insoluble in water. Instead, the unassembled pilin subunit's highly conserved and very hydrophobic amino terminal domain is thought to span the inner membrane [41,42]. During assembly, the amino terminal domain moves from the membrane into a bundled coil of α-helices at the fiber's core [39,40].

The amino terminal domain forms approximately 2,600 Å^2 of the total 4,500 Å^2 intersubunit contact area (Figure 3; K.T.F., unpublished data). The assembled fiber translocates across the outer membrane through a bushing-like pore assembly, described in greater detail below. Pilus retraction is hypothesized to involve depolymerization from the fiber base, with free subunits entering a pool in the cytoplasmic membrane (Figure 3). In other words, the retracting filament is thought to melt into the membrane.

The polymerization and depolymerization of many macromolecular complexes, including actin and tubulin filaments, are dramatically influenced by increases in aqueous solvent entropy which occur as previously hydrated protein surfaces are buried in intersubunit contacts [43]. But if the pool of unassembled pilin monomers resides within the inner membrane and not in the aqueous phase, pilus polymerization and disassembly might exert comparatively modest effects on aqueous solvent entropy. Pilus polymerization is therefore likely to be dominated by energetic constraints different from those that control actin and tubulin polymerization. Nevertheless, actin, tubulin and type IV pili all assemble and disassemble *in vivo* at ~1000 subunits s^{-1} [13,32,33,43,44].

Several low-abundance proteins, including some with amino terminal homology to pilin ('pseudopilins') are necessary for fiber assembly and other pilus-related functions [29,30]. Pseudopilins are also required for type II protein export, a process that shares many similarities with type IV pilus assembly [29,30]. The biochemical functions of the minor subunits are for the most part uncharacterized. They might form fiber nucleation assemblies, like the eukaryotic Arp2/3 and γ-tubulin complexes that initiate actin and tubulin assembly *in vivo*, or they might form capping assemblies that terminate and stabilize already-formed fibers. Pseudopilins also might serve as mechanical anchors, or they might sequester or chaperone free subunits within the cytoplasmic membrane.

Pilus assembly and retraction are thought to be energized by a pair of homologous proteins. PilF (in *Neisseria*; the nomenclature varies in other systems) is required for pilus assembly. PilT is dispensible for assembly but is required for retraction [5,23]. These presumed ATPases both belong to the GspE family of hexameric ATPases, a subset of the AAA family of mechanoenzymes and chaperones [45]. GspE proteins are essential not only for pilus assembly but also for type II and IV secretion. They fractionate as cytoplasmic or peripheral inner membrane proteins, and in at least some cases have been shown to have weak ATPase activity, which may be stimulated by phospholipid [46–49].

GspE family members are believed to act at the cytoplasmic face of the plasma membrane, because the periplasm, an oxidizing environment, contains little or no ATP. Nevertheless, cytoplasmic traffic ATPases in at least three systems — SecA/YEG preprotein translocase, type I export (ABC) systems, and type IV export systems (distinct from type IV pili) — are thought to enter the cytoplasmic membrane. These enzymes are at least transiently exposed to the periplasmic compartment during their catalytic cycles [50–53]. Studies of type II and IV secretion have begun to map GspE interactions with the other secretion proteins [29], and the three-dimensional structure of a GspE homolog was recently determined [54]. Despite these advances, the mechanisms by which GspE family members promote protein export, pilus assembly and pilus retraction are unknown.

How Do Pili Do Work?

In Brownian ratchet models, type IV pili melt spontaneously into membranes — but not into the aqueous phase — and the retraction force is a consequence of energy stored in the filament during an energy-consuming polymerization reaction. Consistent with this idea, isolated type IV pilus filaments are efficiently dissociated by gentle detergents that do not disassemble actin or tubulin filaments or the extremely stable type I pili [55,56]. Furthermore, retraction of the conjugal F pilus, which like the type IV pilus is assembled from membrane-embedded monomers, is promoted by high temperature or energy depletion [20,21]. PilT could serve a regulatory function, such as catalyzing the removal of a stabilizing terminal cap from the pilus base and thereby triggering pilus retraction by a Brownian ratchet mechanism. [13,37,57,58]. Underscoring the possibility that PilT carries out regulatory functions, elegant genetic experiments with *Synechocystis* showed that a PilT homolog (PilT2) controls the direction of pilus-dependent phototaxis [59,60].

Pilus retraction might not be spontaneous. In alternative models, assembly is energetically favorable, or assembly and retraction are energetically equivalent. In these cases the fiber would not store useful energy, and PilT would be expected to participate more directly in the work of retraction. In the facilitated ratchet scenario, PilT acts as an ATP-dependant chaperone, and peels pilin subunits off the fiber base one at a time. As subunits are removed, new hydrophobic patches are exposed at the fiber base, causing the base to sink into the membrane's hydrophobic interior. In this model, PilT catalyzes disassembly, and disassembly causes retraction.

In power stroke models, suggested by Oster [2], PilT walks up the filament, driving the filament into the membrane and thereby causing subunits to melt off of the fiber's base. In these models, PilT catalyzes retraction, and retraction causes disassembly. Here, PilT might step up the fiber in a linear fashion, or it might wind its way up the helix screw, one subunit at a time. Because the linear step model implies larger steps and lower force per step, multiple motors may need to act on a single fiber to generate the large forces observed, as in myosin thick filaments [61].

Detailed biochemical and biophysical characterization of pilus dynamics will limit the range of possible models. Retraction is processive and retraction displacements ranging from 0.1 to over 5 μm — corresponding to 100 to over 4000 subunits — are routinely observed [32,33]. Although we analyzed hundreds of retraction events, in no case did a retracting fiber reverse direction and begin re-extending. Instead,

retraction events were either complete or terminated with release — possibly breakage — of the retracting fiber [32]. These observations suggest that the switch from assembly to retraction might be essentially irreversible, and that the equilibrium constant for retraction is large.

Large equilibrium constants and small steps are consistent with high forces [37,57,58]. Experiments with pili attached to *Pseudomonas* and *Neisseria* cells show that the stall force is in the 10–80 pN range [32,33]. This is substantially higher than forces generated by kinesin motors, which take 8 nm steps, and is similar to forces associated with spontaneous microtubule polymerization, which occurs in 0.61 nm steps [13,37,61]. Pilus retraction, if it occurs through dissociation of single subunits, has a step size of 0.80 nm [39]. If 600–1500 pilin subunits s^{-1} are removed one at a time, the spatial and temporal resolution required to observe single steps experimentally (one 0.8 nm step ms^{-1}) will approach the limits of available optical technology [61], but the larger, less frequent steps implied by the linear step model would be easier to observe.

Pilus depolymerization into the cytoplasmic membrane is presumed to occur, but has not been demonstrated experimentally. The energy requirements for pilus retraction need to be defined: genetic data suggest that ATPase activity is required but this has not been directly demonstrated. Similarly, a requirement for proton-motive force is possible but untested. It would be useful to know whether PilT touches the periplasm during its catalytic cycle. Experiments that constrain models for retraction will also constrain models for assembly. For example, assembly and retraction do mechanical work. If retraction can occur spontaneously, assembly will require the input of energy, and *vice versa*.

Many components, and presumably mechanisms, are conserved among type II protein secretion and type IV pilus biogenesis systems. Pilus assembly and type II secretion require pseudopilins. It has been hypothesized that the pseudopilins of type II systems polymerize into a short 'pseudopilus' — a reciprocating plunger that extends to push substrates through secretin channels and then retracts [29]. Consistent with this idea, experiments from Pugsley's group [62] show that under certain experimental conditions a type II export system can indeed produce long, pilus-like filaments. However, while type IV pilus systems often encode two or more GspE-type proteins — at least one for assembly and one for retraction — most type II secretion systems appear to encode only a single GspE homolog [63]. This raises the question of how reciprocating action could occur. But as the above models make clear, ATPase action is formally required during only one step of the cycle: it is needed during extension if retraction is spontaneous, or during retraction if extension is spontaneous. Additional ATPase activities may be present for regulation, in cases where additional mechanical force is required, or to impart directionality if extension and retraction are energetically balanced.

Nozzles: Dribble or Torrent?

The pilus fiber traverses the outer membrane through a bushing formed by a multimeric secretin complex. Secretins are a family of conserved outer membrane channel proteins that assemble into extremely stable transmembrane complexes containing 12–14 subunits. Secretin complexes are required not only in pilus biogenesis but in type II, III and IV secretion and in the export of filamentous bacteriophages [29,30,64]. In electron microscopic studies, the secretin complexes looks strikingly similar to the nozzle complex involved in gliding motility, with inside diameters of 7–8 nm, similar to the diameters of exported enzymes, bacteriophages and type IV pili [65–69]. Moreover, purified secretin complexes form gated large-conductance channels when incorporated into planar lipid membranes [65,67,70].

Some *in vivo* evidence supports the idea that protein filaments pass though secretin channels. First, Koomey's group [36] has shown that if pilus retraction is prevented by mutation of *pilT*, depletion of the secretin pore from *N. gonorrhoeae* cells is lethal. Assembled pilus fibers accumulate in the periplasm. Some of these fibers push out or even punch through and rupture the outer membrane. Thus pilus assembly occurs before the fiber translocates across the outer membrane, and translocation is secretin-dependent. Second, recent experiments [71] show that active filamentous phage extrusion decreases the outer membrane permeability of cells expressing a secretin mutant that otherwise confers high permeability. These experiments suggest that translocating phage can physically plug the secretin channel. Taken together, the data strongly suggest that observations of pilus extension and retraction are observations of protein translocation through secretin pores in the outer membrane.

Measurements of the speed of pilus extension and retraction, along with structural data, allow us to estimate the molecular flux through a secretin pore. The extension or retraction of a pilus at the observed speeds entails the translocation of 18,000–45,000 nm^3 s^{-1}, or 12–40 MDa protein s^{-1} across a single site — probably one secretin complex — in the outer membrane [32,33,39]. Remarkably, the calculations of Oster *et al.* [12] suggest that a very similar volume of polyelectrolyte slime is ejected from a nozzle during adventurous gliding motility. In the case of the relatively slow-moving *M. xanthus*, the volume of material ejected per pore is predicted to be ~3000 nm^3 s^{-1}, while in the faster filamentous species, the volume could be up 300,000 nm^3 s^{-1}. The mass of carbohydrate ejected in these cases would be 0.25–25 MDa s^{-1} $nozzle^{-1}$.

These numbers can be compared to the movement of other large polymers through protein channels. The mammalian nuclear pore is among the largest of all transmembrane channels, with the ability to accomodate substrates 9 to 26 nm in diameter. Recent experiments with intact and permeablized cells show maximum translocation rates of ~1000 substrate molecules or up to 100 MDa $pore^{-1}$ s^{-1} [72,73]. To take another example, DNA is considered to move through

the replisome at relatively high speed — around 1000 nt s^{-1} or 300 nm s^{-1} (linear) or ~0.65 MDa s^{-1} [74]. The replisome includes the toroidal helicase and sliding clamp assemblies with inside diameters of ~3 nm. Even accounting for the smaller cross-sectional area of the replisome complexes, the flux through secretin pores and nozzles can be at least an order of magnitude larger.

What limits translocation rates? It is possible that these processes are constrained by friction or specific binding between substrates and export channels. However, pilus extension and retraction rates are similar to the rates of actin and tubulin polymerization and depolymerization *in vivo* (~1000 monomers s^{-1}), and these processes are not constrained by a pore. Diffusion is similarly unlikely to limit the translocation rate, as transport through the nuclear pore does not seem to be limited by the pore's intrinsic capacity [72,73].

Synthesis of the translocated macromolecules may however be limiting. The energy requirements for slime synthesis during gliding are formidable [12]. And the flux of pilin protein moving through just one secretin pore during retraction, 12–40 MDa s^{-1}, is comparable to the entire cell's net synthetic capacity. A 'typical' *E. coli* cell contains 10^8 MDa of protein [75]. Doubling of this cell mass every 40 minutes requires a net protein synthesis of 40 MDa s^{-1} $cell^{-1}$. Thus, if a significant proportion of the pilus subunits released by retraction is degraded, and not recycled into new fibers, pilus-based motility is an expensive proposition indeed! The maximal rates of secretion through single channels appear to be enormous, but taken together these observations imply that in these cases macromolecule secretion rates are limited by underlying processes such as macromolecule synthesis, rather than by the intrinsic flux capacity of the translocation channels.

Acknowledgements

We thank N. Bose, K. Collins, R. Brennan, T. Economou, H. Higgs, M. Koomey, G. O'Toole, M. Sheetz, J. Skerker, R. Taylor, and T. Yahr for critical discussion of the ideas in this review. We apologize to colleagues whose work was not cited due to space constraints. A.J.M. is supported by Fellowship DRG–1598 of the Cancer Research Fund of the Damon Runyon-Walter Winchell Foundation. K.T.F. is supported by the NIH (GM59721) and the W.M. Keck Foundation.

References

1. Hoiczyk, E. (2000). Gliding motility in cyanobacterial: observations and possible explanations. Arch. Microbiol. *174*, 11–17.
2. Kaiser, D. (2000). Bacterial motility: how do pili pull? Curr. Biol. *10*, R777–780.
3. McBride, M.J. (2001). Bacterial Gliding Motility: Multiple Mechanisms for Cell Movement over Surfaces. Annu. Rev. Microbiol. *55*, 49–75.
4. Spormann, A.M. (1999). Gliding motility in bacteria: insights from studies of Myxococcus xanthus. Microbiol. Mol. Biol. Rev. *63*, 621–641.
5. Wall, D. and Kaiser, D. (1999). Type IV pili and cell motility. Mol. Microbiol. *32*, 1–10.
6. Lapidus, I.R. and Berg, H.C. (1982). Gliding motility of Cytophaga sp. strain U67. J. Bacteriol. *151*, 384–398.
7. Sara, M. and Sleytr, U.B. (2000). S-Layer proteins. J. Bacteriol. *182*, 859–868.
8. Hoiczyk, E. and Baumeister, W. (1997). Oscillin, an extracellular, Ca2+-binding glycoprotein essential for the gliding motility of cyanobacteria. Mol. Microbiol. *26*, 699–708.
9. Hoiczyk, E. (1998). Structural and biochemical analysis of the sheath of Phormidium uncinatum. J. Bacteriol. *180*, 3923–3932.
10. Hoiczyk, E. and Baumeister, W. (1995). Envelope structure of four gliding filamentous cyanobacteria. J. Bacteriol. *177*, 2387–2395.
11. Hoiczyk, E. and Baumeister, W. (1998). The junctional pore complex, a prokaryotic secretion organelle, is the molecular motor underlying gliding motility in cyanobacteria. Curr. Biol. *8*, 1161–1168.
12. Wolgemuth, C., Hoiczyk, E., Kaiser, D. and G. Oster. (2001). How *Myxobacteria* glide. Curr. Biol. *12*, 369–377.
13. Mahadevan, L. and Matsudaira, P. (2000). Motility powered by supramolecular springs and ratchets. Science *288*, 95–100.
14. Bloomfield, V.A. (1997). DNA condensation by multivalent cations. Biopolymers *44*, 269–282.
15. Bliss, J.M. and Silver, R.P. (1996). Coating the surface: a model for expression of capsular polysialic acid in *Escherichia coli* K1. Mol. Microbiol. *21*, 221–231.
16. Whitfield, C. and Roberts, I.S. (1999). Structure, assembly and regulation of expression of capsules in *Escherichia coli*. Mol. Microbiol. *31*, 1307–1319.
17. Bradley, D.E. (1972). Shortening of Pseudomonas aeruginosa pili after RNA-phage adsorption. J. Gen. Microbiol. *72*, 303–319.
18. Bradley, D.E. (1974). The adsorption of Pseudomonas aeruginosa pilus-dependent bacteriophages to a host mutant with nonretractile pili. Virology *58*, 149–163.
19. Bradley, D.E. (1980). A function of Pseudomonas aeruginosa PAO polar pili: twitching motility. Can. J. Microbiol. *26*, 146–154.
20. Novotny, C.P. and Fives-Taylor, P. (1974). Retraction of F pili. J. Bacteriol. *117*, 1306–1311.
21. Novotny, C.P. and Fives-Taylor, P. (1978). Effects of high temperature on *Escherichia coli* F pili. J. Bacteriol. *133*, 459–464.
22. Lagenaur, C. and Agabian, N. (1977). Caulobacter crescentus pili: structure and stage-specific expression. J. Bacteriol. *131*, 340–346.
23. Whitchurch, C.B., Hobbs, M., Livingston, S.P., Krishnapillai, V. and Mattick, J.S. (1991). Characterisation of a Pseudomonas aeruginosa twitching motility gene and evidence for a specialised protein export system widespread in eubacteria. Gene *101*, 33–44.
24. Wolfgang, M., Lauer, P., Park, H.S., Brossay, L., Hebert, J. and Koomey, M. (1998). PilT mutations lead to simultaneous defects in competence for natural transformation and twitching motility in piliated Neisseria gonorrhoeae. Mol. Microbiol. *29*, 321–330.
25. Merz, A.J. and So, M. (2000). Interactions of pathogenic neisseriae with epithelial cell membranes. Annu. Rev. Cell Dev. Biol. *16*, 423–457.
26. Comolli, J.C., Hauser, A.R., Waite, L., Whitchurch, C.B., Mattick, J.S. and Engel, J.N. (1999). Pseudomonas aeruginosa gene products PilT and PilU are required for cytotoxicity *in vitro* and virulence in a mouse model of acute pneumonia. Infect. Immun. *67*, 3625–3630.
27. Bieber, D., Ramer, S.W., Wu, C.Y., Murray, W.J., Tobe, T., Fernandez, R. and Schoolnik, G.K. (1998). Type IV pili, transient bacterial aggregates and virulence of enteropathogenic *Escherichia coli*. Science *280*, 2114–2118.
28. O'Toole, G.A. and Kolter, R. (1998). Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development. Mol. Microbiol. *30*, 295–304.
29. Sandkvist, M. (2001). Biology of type II secretion. Mol. Microbiol. *40*, 271–283.
30. Lory, S. (1998). Secretion of proteins and assembly of bacterial surface organelles: shared pathways of extracellular protein targeting. Curr. Opin. Microbiol. *1*, 27–35.
31. Sheetz, M.P. Laser tweezers in cell biology. (1998). (Academic Press).
32. Merz, A.J., So, M. and Sheetz, M.P. (2000). Pilus retraction powers bacterial twitching motility. Nature *407*, 98–102.
33. Skerker, J.M. and Berg, H.C. (2001). Direct observation of extension and retraction of type IV pili. Proc. Natl. Acad. Sci. U.S.A. *98*, 6901–6904.
34. Steyer, J.A. and Almers, W. (2001). A real-time view of life within 100 nm of the plasma membrane. Nat. Rev. Mol. Cell Biol. *2*, 268–275.
35. Sun, H., Zusman, D.R. and Shi, W. (2000). Type IV pilus of Myxococcus xanthus is a motility apparatus controlled by the frz chemosensory system. Curr. Biol. *10*, 1143–1146.
36. Wolfgang, M., van Putten, J.P., Hayes, S.F., Dorward, D. and Koomey, M. (2000). Components and dynamics of fiber formation define a ubiquitous biogenesis pathway for bacterial pili. EMBO J. *19*, 6408–6418.
37. Peskin, C.S., Odell, G.M. and Oster, G.F. (1993). Cellular motions and thermal fluctuations: the Brownian ratchet. Biophys. J. *65*, 316–324.

38. Dai, J. and Sheetz, M.P. (1999). Membrane tether formation from blebbing cells. Biophys. J. *77*, 3363–3370.
39. Parge, H.E., Forest, K.T., Hickey, M.J., Christensen, D.A., Getzoff, E.D. and Tainer, J.A. (1995). Structure of the fibre-forming protein pilin at 2.6 A resolution. Nature *378*, 32–38.
40. Forest, K.T. and Tainer, J.A. (1997). Type-4 pilus-structure: outside to inside and top to bottom—a minireview. Gene *192*, 165–169.
41. Watts, T.H., Worobec, E.A. and Paranchych, W. (1982). Identification of pilin pools in the membranes of Pseudomonas aeruginosa. J. Bacteriol. *152*, 687–691.
42. Dupuy, B., Taha, M.K., Pugsley, A.P. and Marchal, C. (1991). Neisseria gonorrhoeae prepilin export studied in *Escherichia coli*. J. Bacteriol. *173*, 7589–7598.
43. Inoue, S. and Salmon, E.D. (1995). Force generation by microtubule assembly/disassembly in mitosis and related movements. Mol. Biol. Cell *6*, 1619–1640.
44. Khan, S. and Sheetz, M.P. (1997). Force effects on biochemical kinetics. Annu. Rev. Biochem. *66*, 785–805.
45. Vale, R.D. (2000). AAA proteins. Lords Of the ring. J. Cell Biol. *150*, F13–20.
46. Christie, P.J., Ward, J.E., Jr., Gordon, M.P. and Nester, E.W. (1989). A gene required for transfer of T-DNA to plants encodes an ATPase with autophosphorylating activity. Proc. Natl. Acad. Sci. U.S.A. *86*, 9677–9681.
47. Krause, S., Pansegrau, W., Lurz, R., de la Cruz, F. and Lanka, E. (2000). Enzymology of type IV macromolecule secretion systems: the conjugative transfer regions of plasmids RP4 and R388 and the cag pathogenicity island of Helicobacter pylori encode structurally and functionally related nucleoside triphosphate hydrolases. J. Bacteriol. *182*, 2761–2770.
48. Sakai, D., Horiuchi, T. and Komano, T. (2001). Atpase activity and multimer formation of Pilq protein are required for thin pilus biogenesis in plasmid R64. J. Biol. Chem. *276*, 17968–17975.
49. Bhattacharjee, M.K., Kachlany, S.C., Fine, D.H. and Figurski, D.H. (2001). Nonspecific adherence and fibril biogenesis by Actinobacillus actinomycetemcomitans: TadA protein is an ATPase. J. Bacteriol. *183*, 5927–5936.
50. Kim, Y.J., Rajapandi, T. and Oliver, D. (1994). SecA protein is exposed to the periplasmic surface of the E. coli inner membrane in its active state. Cell *78*, 845–853.
51. Baichwal, V., Liu, D. and Ames, G.F. (1993). The ATP-binding component of a prokaryotic traffic ATPase is exposed to the periplasmic (external) surface. Proc. Natl. Acad. Sci. U.S.A. *90*, 620–624.
52. Economou, A. and Wickner, W. (1994). SecA promotes preprotein translocation by undergoing ATP-driven cycles of membrane insertion and deinsertion. Cell *78*, 835–843.
53. Dang, T.A. and Christie, P.J. (1997). The VirB4 ATPase of Agrobacterium tumefaciens is a cytoplasmic membrane protein exposed at the periplasmic surface. J. Bacteriol. *179*, 453–462.
54. Yeo, H.J., Savvides, S.N., Herr, A.B., Lanka, E. and Waksman, G. (2000). Crystal structure of the hexameric traffic ATPase of the Helicobacter pylori type IV secretion system. Mol. Cell *6*, 1461–1472.
55. Watts, T.H., Kay, C.M. and Paranchych, W. (1982). Dissociation and characterization of pilin isolated from Pseudomonas aeruginosa strains PAK and PAO. Can. J. Biochem. *60*, 867–872.
56. Sauer, F.G., Futterer, K., Pinkner, J.S., Dodson, K.W., Hultgren, S.J. and Waksman, G. (1999). Structural basis of chaperone function and pilus biogenesis. Science *285*, 1058–1061.
57. Hill, T.L. and Kirschner, M.W. (1982). Subunit treadmilling of microtubules or actin in the presence of cellular barriers: possible conversion of chemical free energy into mechanical work. Proc. Natl. Acad. Sci. U.S.A. *79*, 490–494.
58. Koshland, D.E., Mitchison, T.J. and Kirschner, M.W. (1988). Polewards chromosome movement driven by microtubule depolymerization *in vitro.* Nature *331*, 499–504.
59. Bhaya, D., Takahashi, A. and Grossman, A.R. (2001). Light regulation of type IV pilus-dependent motility by chemosensor- like elements in Synechocystis PCC6803. Proc. Natl. Acad. Sci. U.S.A. *98*, 7540–7545.
60. Bhaya, D., Bianco, N.R., Bryant, D. and Grossman, A. (2000). Type IV pilus biogenesis and motility in the cyanobacterium Synechocystis sp. PCC6803. Mol. Microbiol. *37*, 941–951.
61. Mehta, A.D., Rief, M., Spudich, J.A., Smith, D.A. and Simmons, R.M. (1999). Single-molecule biomechanics with optical methods. Science *283*, 1689–1695.
62. Sauvonnet, N., Vignon, G., Pugsley, A.P. and Gounon, P. (2000). Pilus formation and protein secretion by the same machinery in *Escherichia coli*. EMBO J. *19*, 2221–2228.
63. Sandkvist, M. (2001). Type II secretion and pathogenesis. Infect. Immun. *69*, 3523–3535.
64. Russel, M. (1994). Phage assembly: a paradigm for bacterial virulence factor export? Science *265*, 612–614.
65. Nouwen, N., Ranson, N., Saibil, H., Wolpensinger, B., Engel, A., Ghazi, A. and Pugsley, A.P. (1999). Secretin PulD: association with pilot PulS, structure and ion- conducting channel formation. Proc. Natl. Acad. Sci. U.S.A. *96*, 8173–8177.
66. Nouwen, N., Stahlberg, H., Pugsley, A.P. and Engel, A. (2000). Domain structure of secretin PulD revealed by limited proteolysis and electron microscopy. EMBO J. *19*, 2229–2236.
67. Brok, R., Van Gelder, P., Winterhalter, M., Ziese, U., Koster, A.J., de Cock, H., Koster, M., Tommassen, J. and Bitter, W. (1999). The C-terminal domain of the Pseudomonas secretin XcpQ forms oligomeric rings with pore activity. J. Mol. Biol. *294*, 1169–1179.
68. Linderoth, N.A., Simon, M.N. and Russel, M. (1997). The filamentous phage pIV multimer visualized by scanning transmission electron microscopy. Science *278*, 1635–1638.
69. Collins, R.F., Davidsen, L., Derrick, J.P., Ford, R.C. and Tonjum, T. (2001). Analysis of the PilQ secretin from Neisseria meningitidis by transmission electron microscopy reveals a dodecameric quaternary structure. J. Bacteriol. *183*, 3825–3832.
70. Marciano, D.K., Russel, M. and Simon, S.M. (1999). An aqueous channel for filamentous phage export. Science *284*, 1516–1519.
71. Marciano, D.K., Russel, M. and Simon, S.M. (2001). Assembling filamentous phage occlude pIV channels. Proc. Natl. Acad. Sci. U.S.A. *98*, 9359–9364.
72. Smith, A.E., Slepchenko, B.M., Schaff, J.C., Loew, L.M. and Macara, I.G. (2002). Systems analysis of Ran transport. Science *295*, 488–491.
73. Ribbeck, K. and Gorlich, D. (2001). Kinetic analysis of translocation through nuclear pore complexes. EMBO J. *20*, 1320–1330.
74. Baker, T.A. and Bell, S.P. (1998). Polymerases and the replisome: machines within machines. Cell *92*, 295–305.
75. Neidhardt, F.C. and Umbarger: Chapter 3: Chemical composition of *Escherichia coli*. In *Escherichia coli* and Salmonella: Cellular and Molecular Biology. (1996). Neidhardt, F.C. ed. (American Society for Microbiology Press).