Bacterial motility: **Secretory secrets of gliding bacteria**

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Many bacteria glide over surfaces without the aid of flagella. Gliding is still somewhat mysterious, but recent studies show that it involves specialized secretory systems that assemble membrane-associated filaments, and the recognition of extracellular components that trigger movement via transmembrane transducers.

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Motile bacteria either swim, by using flagella, or glide over surfaces by mechanisms that remain a mystery. Bacteria that glide can move towards or away from a variety of stimuli, including chemicals and light. Some gliders also display the fascinating behavior known as elasticotaxis: for example, *Myxococcus xanthus* will move toward a glass bead that is located hundreds of cell lengths away on the surface of an agar plate. How do bacteria move in the absence of flagellar propellers?

The answer to this question is not known, but recent advances have provided insights into some of the molecules and processes that are involved. Myxobacteria and pseudomonads have been found to use a common strategy to coordinate 'social' gliding of groups of cells. Social motility depends on the assembly of cell-length pili by a type IV secretion mechanism, as well as on the extrusion of exopolysaccharide slime. For the pseudomonads, the genes required for social motility are also critical determinants of pathogenesis, suggesting that gliding motility plays a key role in the adaptation of a subset of bacterial pathogens to their hosts. The sequences of a variety of genes required for gliding reveal a common theme: specialized secretory pathways are involved in the assembly of filaments that may provide the motive force for a variety of different gliding mechanisms.

Myxococcus xanthus: dual gliding mechanisms

The investigation of bacterial gliding began in earnest with a genetic approach in the 1970s. Hodgkin and Kaiser [1] pioneered this enterprise, by isolating and characterizing non-motile mutants of *M. xanthus*. They found that two large, distinct subsets of genes control gliding in this behaviorally complex prokaryote. Both subsets of *M. xanthus* gliding genes support its wolf-like predation of other bacteria. Social motility enables cells to hunt as packs, whereas so-called 'adventurous' motility enables individual cells to explore a pack's territory.

No single mutation in *M. xanthus* abolishes movement; rather, with only one notable exception, it takes the combination of two mutations, one that disrupts social motility (an *S* mutation), and one that disrupts adventurous motility (an *A* mutation), to make a double-mutant, non-motile cell (Figure 1). Single mutations in the distinctive *mglA* (*mutual gliding*) gene can abolish both social and adventurous motility simultaneously. Subsets of both *S* and *A* mutants can be complemented extracellularly for movement by wild-type cells, showing that both the social and adventurous mechanisms involve extracellular components produced by donor cells to stimulate the movement of recipient cells [1,2].

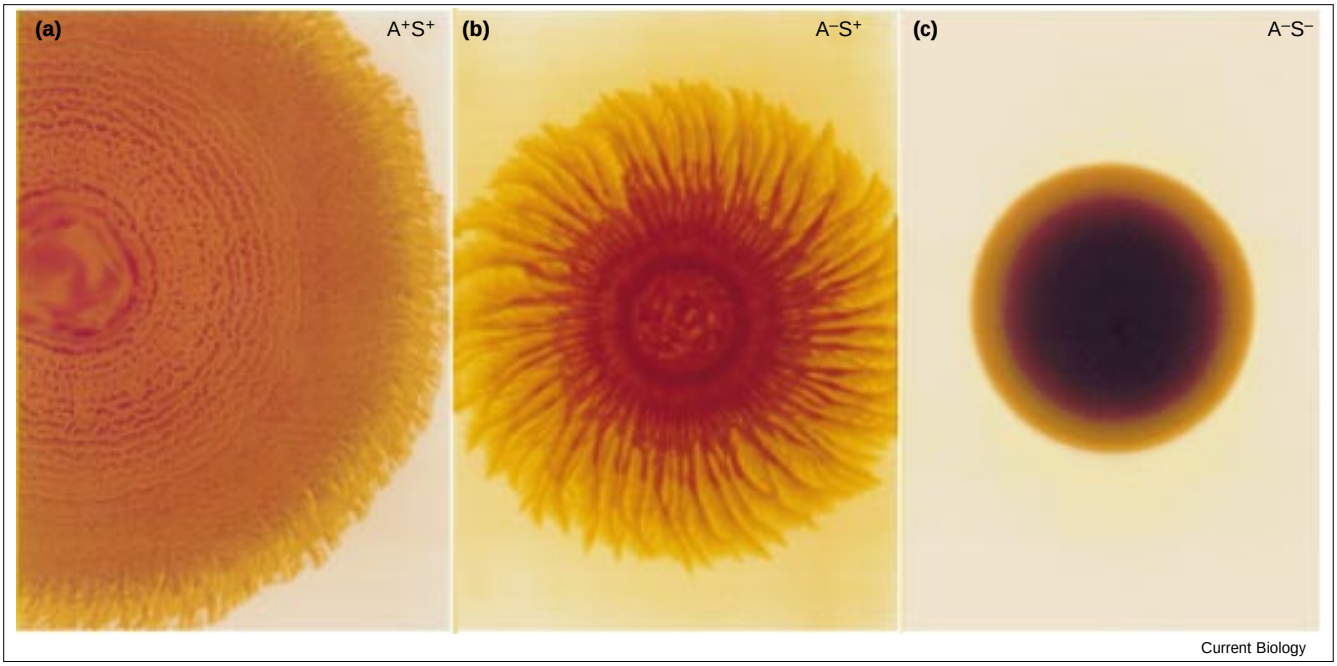
To date, *M. xanthus* has remained the main focus of gliding studies in bacteria. The Kaiser laboratory has led the effort to understand the roles that individual genes play in motility at the molecular level. We now know that more than 1% of the large, 9.5 megabase *M. xanthus* genome is dedicated to gliding. Each of the two mechanisms of gliding in this organism is at least as genetically complex as the mechanism of flagellar motility in bacteria such as *Salmonella typhimurium* or *Escherichia coli*. Mutant hunts, combined with sequence analysis of the *M. xanthus* genome, have revealed more than 100 genes critical for social or adventurous motility, and these have by no means been exhaustive [3].

Social systems involve type IV pili, fibrils and O-antigen

Kaiser and colleagues uncovered the first secret of gliding motility: that social motility depends on the production of cell-length pili [4]. Analysis of the cluster of genes determining pili production in *M. xanthus* showed that this bacterium's pili are secreted by a type IV mechanism, strikingly similar to that required for the secretion of pili and virulence factors in *Pseudomonas aeruginosa* [4,5] (Figure 2). The *pilA* cluster is required for the 'twitching motility' of flagellated pseudomonads, their version of social motility. Indeed, the uses of twitching motility by *P. aeruginosa* to invade the lung cells of cystic fibrosis patients [6], and of social motility by *M. xanthus* to construct elaborate fruiting structures [1,7], may have a eukaryotic parallel in the use of gliding by some protozoan parasites to invade their host cells [8].

The production of pili is not sufficient for social motility. *M. xanthus* mutants with defects in the *pilT* gene of the pilin cluster can assemble what appear to be mature pili, yet cannot move [9]. These mutations appear to uncouple pilus assembly from the social motor, as do their counterparts in *P. aeruginosa* [10]. A genetic approach, involving

Figure 1



Myxococcus xanthus has two independent motility mechanisms. Elimination of gliding motility in *M. xanthus* generally requires a combination of two mutations, an *A* mutation that inactivates adventurous motility, and an *S* mutation that inactivates social motility. The photographs show the morphologies of colonies formed by *M. xanthus* cells on rich medium after growth for five days (5 × magnification). **(a)** Wild-type (A^+S^+) cells; **(b)** *aglB* (A^-S^+) mutant cells, which lack adventurous motility yet retain social motility; **(c)** *aglB sglK* (A^-S^-) double-mutant cells, which lack both adventurous and social motility. (Photographs courtesy P.L. Hartzell.)

the isolation of second-site suppressors of missense mutations in *pilT*, may identify genes encoding components of the elusive social motor. Furthermore, although *M. xanthus tgl* mutants cannot make pili, they can be stimulated to do so by the presence of wild-type cells [11], suggesting that pilus manufacture is controlled by an extracellular signal or by direct cell–cell contact.

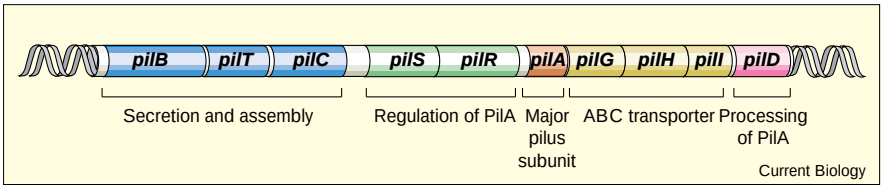
What is the nature of the social motor? Electron micrographs of thin sections of *M. xanthus* cells show bundles of envelope-associated intracellular filaments that bridge the cell poles [12]. These intracellular filaments could be extensions of the polar, filamentous pili, or they could be a separate component of the social motor. In either case, extrusion or retraction of pili through fixed portals in the cell envelope may help generate the motive force required for social motility.

The biochemical approach taken by Dworkin's group has uncovered a second secret of social motility. When *M. xanthus* moves, it deposits a trial of extracellular slime, from whence the myxobacteria earn their name. Slime fibrils comprise a polysaccharide backbone decorated with the multimeric fibrillin protein IFP-1 [13,14]. Mutants defective in slime production — '*dsp*' mutants — make pili, but are defective in social motility. The addition of purified extracellular fibrils restores motility to *dsp* mutants, showing that fibrils themselves are an extracellular component critical for social motility [15].

The *dsp* mutants are thus not defective in the receptor for fibrils, or in the transduction of the signal that fibril binding elicits. Additional mutations in the *dsp* genetic background are required to prevent extracellular complementation. A subset of these '*fbp*' mutations restore motility without restoring fibril production, suggesting that fibrils mediate the transduction of an extracellular signal required for social motility [16]. Presumably, the *fbp* mutants carry suppressor mutations that bypass the requirement for fibrils for social motility of wild-type cells, either by overproducing this fibril-mediated signal or by activating its receptor.

Hartzell's group has recently identified a gene required for social motility which encodes a homologue of the molecular chaperone heat-shock protein 70 (P.L. Hartzell, personal communication). Inactivation of this gene leads to a phenotype similar to that of the *dsp* mutants. The encoded protein protein is likely to be a component of a specialized, tripartite chaperone machine involved in the secretion of fibrils. Upstream of this gene is *fibR*, which encodes a repressor of fibril production similar in sequence

Figure 2



Organization of genes in the pilin gene cluster of *M. xanthus*. The findings that pilin biosynthesis is required for social motility in *M. xanthus* and for twitching motility in *P. aeruginosa* suggest that the social mechanism of motility may be widespread among Gram-negative gliders. The arrangement of *M. xanthus* genes is the same as in *P. aeruginosa*. (Courtesy of D. Wall and D. Kaiser.)

to the histone-like repressors of alginate biosynthesis in the pseudomonads (P.L. Hartzell, personal communication). The uronic-acid-containing polysaccharide alginate is a key virulence determinant in the chronic respiratory infection of cystic fibrosis patients, and *P. aeruginosa* mutants defective in the two-component activation of alginate production are defective in twitching motility [17]. Although *M. xanthus* fibrils do not appear to contain uronic acid [13], it is likely that the mechanism of fibril secretion is quite similar to that for *P. aeruginosa* alginate.

Kaplan's group has recently discovered that social motility depends on the synthesis of yet another extracellular polymer, the lipopolysaccharide O-antigen. They have found that many *M. xanthus* mutants that are resistant to phages that use O-antigen for adsorption are also defective in social motility, whereas mutants resistant to phage that do not use O-antigen are not (H.B. Kaplan, personal communication). The sequence of an *S* gene critical for both O-antigen production and development, *rfbA*, indicates that its product is an 'ATP-binding cassette' (ABC) transporter [18]. Again, O-antigen is also a key virulence determinant for Gram-negative pathogens such as *P. aeruginosa*. These results predict that pseudomonad mutants blocked in O-antigen biosynthesis may be defective in twitching motility.

Social motility plays a central role in the multicellular development of *M. xanthus*, one of the many members of the myxobacterial group whose most striking feature is the elaboration of fruiting structures to support the differentiation of a minority of starving cells into spores. It is not surprising that most of the cell–cell interactions used for social gliding also play essential roles in an intricate developmental process dependent on extracellular signals that mediate intercellular communication [19]. Given close relationship between social gliding and development in *M. xanthus*, one can envision the colonization of the lung by *P. aeruginosa* as yet another example of bacterial development, perhaps also accompanied by multiple pathways of cellular differentiation.

Adventurous motility appears to play a relatively minor role in the development of *M. xanthus*, as only a small subset of *A* mutants are defective in development. The first sequence of an *A* gene to become available shows that the gene encodes a transmembrane protein that is apparently unique in the sequence database and that may act as a receptor for an extracellular signal (A.M. Spormann, personal communication). The adventurous motility system awaits more adventuresome explorations.

A link between bacterial gliding and yeast vesicle budding

Only a single gene in *M. xanthus*, *mglA*, appears to be required for both adventurous and social motility, as well as for development. The MglA protein is the only known prokaryotic example of a Ras-family small GTPase, and its requirement for multicellular development of *M. xanthus* can be complemented by the Sar1 protein of the budding yeast *Saccharomyces cerevisiae* [20]. An additional mutation restores motility of an *M. xanthus* strain expressing yeast Sar1p; this *rpm* mutation may affect a GTPase-associated protein or GDP-release factor made by *M. xanthus* [20]. Sar1p is one of three cytosolic factors in yeast required, together with endoplasmic reticulum membrane protein Sec16p, for the assembly of the 'COPII' vesicles that transport proteins from the endoplasmic reticulum to the Golgi apparatus [21]. It would not be surprising if the *rpm* mutation were found to affect a protein related to one of these factors involved in yeast vesicle budding.

Rapid gliders: flavobacteria and cyanobacteria

Whereas the myxobacterial wolves can take minutes to move one cell length, other gliders include the flavobacterial and cyanobacterial gazelles, which race cell-lengths in seconds. The analysis of gliding in the chitin-devouring *Flavobacterium* (formerly *Cytophaga*) *johnsoniae*, like that in *M. xanthus*, began with the isolation of non-motile mutants [22]. These non-motility defects are associated with changes in the production of subsets of 17 different integral membrane proteins [23]. Like many of the *S* mutants of *M. xanthus*, these mutants are resistant to phage. The molecular analysis of these mutants has awaited the development of selectable markers, cloning vectors, transposons and established methods of gene transfer in this host.

McBride *et al.* [24] have succeeded in developing all of these genetic tools within the short span of the past five years. Using these tools, they have cloned the first of

many different gliding genes that complement non-motile mutants of *F. johnsoniae* [25]. Sequence analysis of this gene, *gldA*, shows that, like the *pilH* and *rfbA* genes of *M. xanthus*, its product is a component of an ABC transporter. A transposon insertion that results in the loss of motility defines a large cluster of genes likely involved in exopolysaccharide synthesis and secretion. Studies of the non-motile *F. johnsoniae* mutants promise to yield rapid advances in understanding the mechanism of rapid gliding in the rival flavobacterial model system.

The recent molecular dissection of gliding in the cyanobacterium *Phormidium unicatum* [26] suggests that there may be another mechanism of gliding, in addition to the social and adventurous mechanisms. This photosynthetic glider has an 'S-layer' sheathing its envelope, overlaid by parallel fibrils comprising a single rod-shaped protein, oscillin, which is essential for gliding. The *oscillin* gene is widely distributed throughout the cyanobacteria. Its glycoprotein product has a two-domain structure, with an amino terminus comprising multiple repeats of an 'EF-hand' motif for binding Ca^{2+}. Although oscillin likely plays a passive role in motility, oscillin filaments may have sliding-filament partners [26].

In the past few years, the complete genome sequences for several eubacteria have been determined. Although none of these species are gliders, we can anticipate that several gliders will soon have their genome sequences completed. When this has been achieved, the focus of gliding studies will shift from sequencing gliding genes to the more interesting functional analysis of gliding genes in different bacterial model systems.

Common gliding themes: secretion and filaments

Although there may be at three different mechanisms for gliding motility in prokaryotes, two common themes emerge from the available sequences of genes concerned with gliding. First, gliding involves specialized secretory machines that are required for the assembly of membrane-associated filaments consisting of polysaccharides, proteins, or a mixture of the two polymers. Second, gliding involves the recognition of extracellular components, and the activities of membrane-spanning transducers to trigger movement. These components appear to be involved directly in cell–cell communication, and lead the gliding drama.

The mechanisms of all other well-characterized forms of motility found in biological systems, with the exception of flagellar motility, involve sliding-filament motors. Filaments have been implicated in at least two of the three different mechanisms of bacterial gliding. It will not come as a surprise if the diverse mechanisms of bacterial gliding motility are found to depend on sliding filaments, and to resemble the more familiar actin–myosin and dynein–tubulin motors found in eukaryotic cells.

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