Motility in cyanobacteria: polysaccharide tracks and Type IV pilus motors

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Keywords

Cyanobacteria; Twitching motility; Gliding motility; Type IV pili; Hormogonia

Summary

Motility in cyanobacteria is useful for purposes that range from seeking out favourable light environments to establishing symbioses with plants and fungi. No known cyanobacterium is equipped with flagella, but a diverse range of species are able to “glide” or “twitch” across surfaces. Cyanobacteria with this capacity range from unicellular species to complex filamentous forms, including species such as *Nostoc punctiforme*, which can generate specialised motile filaments called hormogonia. Recent work on the model unicellular cyanobacterium *Synechocystis* sp. PCC 6803 has shown that its means of propulsion has much in common with the twitching motility of heterotrophs such as *Pseudomonas* and *Myxococcus*. Movement depends on Type IV pili, which are extended, adhere to the substrate and then retract to pull the cell across the surface. Previous work on filamentous cyanobacteria suggested a very different mechanism, with movement powered by the directional extrusion of polysaccharide from pores close to the cell junctions. Now a new report by Khayatan *et al.* (2015) suggests that the motility of *Nostoc* hormogonia has much more in common with *Synechocystis* than was previously thought. In both cases, polysaccharide secretion is

important for preparing the surface, but the directional motive force comes from Type IV pili.

Motility in unicellular and filamentous cyanobacteria

Cyanobacteria are a widespread and very diverse group of phototrophic prokaryotes, ranging from simple but incredibly abundant unicellular planktonic species to morphologically complex filamentous forms. Although all known cyanobacteria lack flagella, a number of species exhibit motility on surfaces (Rippka *et al*., 1979). The ability to move on surfaces may help cyanobacteria to locate favourable light and chemical environments, and in the case of certain filamentous species it is also known to be important for the establishment of symbioses with plants. Although cyanobacterial motility was first described more than a century ago (Hansgirg 1883; Drews 1959) the mechanism remained puzzling until now. Gliding motility, which is mechanistically poorly understood, involves very different and evolutionarily unrelated machineries in various bacteria. *Myxococcus xanthus (Myxococcus)*, for instance, uses Type IV pili for twitching motility as well as gliding motility, which involves a motility apparatus consisting of integral and periplasmic proteins and an outer membrane lipoprotein (Jakobczak *et al.*, 2015). Several mechanisms were suggested for gliding of filamentous cyanobacteria. Most hypotheses proposed that slow uniform gliding is mediated by the secretion of polysaccharide slime (Hosoi *et al*., 1951; Walsby *et al*., 1968). Hoiczyk and Baumeister (1998) characterised the structure of specific slime-secreting pores of two filamentous cyanobacteria, which they designated as junctional pore complexes. They suggested that polysaccharide secretion does not simply provide a suitable surface for gliding, as in other bacteria, but rather generates the directional force for movement. However, there were several arguments against this model, as discussed by Häder (1987). One problem is the enormous amount of slime which would be required for propulsion (Holton and Freeman, 1965). Furthermore, the considerable speeds achieved by some trichomes on harder surfaces such as 5% agar are difficult to reconcile with the slime propulsion model.

Polysaccharide pathways

In this issue of Molecular Microbiology, Khayatan *et al*. (2015) propose a new model for gliding motility of hormogonia of the filamentous cyanobacterium *Nostoc punctiforme (Nostoc)*. Hormogonia are differentiated motile filaments that are crucial for the establishment of symbioses with plants. The *hps* (hormogonium polysaccharide) locus was previously shown to be essential for slime production and motility in *Nostoc* hormogonia (Risser and Meeks, 2013; Risser *et al.*, 2014). This locus contains genes encoding putative glycosyl transferases and pseudopilins/minor pilins, suggesting that it encodes at least a part of a polysaccharide synthesis and secretion system. Khayatan *et al*. (2015) demonstrate that motility can be restored in an *hps*-deficient *Nostoc* mutant by addition of conditioned medium containing polysaccharide produced by the wild type. This simultaneously provides a demonstration that extracellular polysaccharide plays a role in motility and an elegant refutation of the “slime propulsion” model, which predicts that motility will only occur if the polysaccharide is actively extruded by the moving trichome. These results suggest that the role of the polysaccharide is simply to promote motility by modifying the surface properties of the trichome and/or the substrate. Earlier observations in *Oscillatoria* showed a tendency for trichomes to move back and forth within loose sheaths of slime, which effectively form pathways for movement of the trichome (Häder, 1987). Therefore polysaccharide slime seems to be required to provide a good surface for motility in several types of filamentous cyanobacteria.

Like most unicellular cyanobacteria, the model unicellular species *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) lacks the *hps* locus and must use a different, uncharacterised molecular machinery for polysaccharide export (Khayatan *et al.*, 2015). However, polysaccharide slime seems to play a comparable role in facilitating motility. Moving packs of *Synechocystis* cells secrete extracellular polymeric substances that facilitate the movement of other *Synechocystis* cells following on behind (Burriesci and Bhaya, 2008). Thus slime deposition by *Synechocystis* promotes the social movement of entire colonies, a phenomenon also well-known in heterotrophs such as *Myxococcus xanthus* (Li *et al.*, 2003).

The exact chemical nature of the polysaccharide secreted by *Nostoc* and *Synechocystis* remains uncertain. In addition, the export machinery is not yet characterised in *Synechocystis.* In *Nostoc*, there is good evidence for the involvement of the *hps* gene products and the junctional pore complexes (Risser *et al.*, 2014; Khayatan *et al.*, 2015), but questions remain concerning the detailed mechanism of polysaccharide export.

Type IV pili are the motility engines

Twitching motility in *Myxococcus*, *Pseudomonas* and *Neisseria gonorrheae* depends on the use of Type IV pili as “grappling hooks”. Pili are extended by the export and polymerisation of PilA subunits, powered by an ATPase known as PilB (Burrows, 2012). The extended pili then latch onto the surface and are retracted back into the cell, a process powered by a second ATPase known as PilT (Burrows, 2012). Pilus retraction drags the cell with a jerky motion across the surface. Motility in *Synechocystis* seems to be based on very similar principles. The Type IV pili are essential for motility (Bhaya *et al.*, 2000) and furthermore, the direction of motility correlates with the localisation of patches of PilB at the plasma membrane (Schuergers *et al.*, 2015).

An early indication that Type IV pili are also involved in *Nostoc* motility came from the work of Duggan *et al*. (2007). Recent studies provide convincing evidence that Type IV pili are the engines for *Nostoc* movement. Risser *et al*. (2014) used immunofluorescence microscopy to show that extracellular PilA is located in rings around the cell junctions in *Nostoc* hormogonia. Furthermore, PilA is concentrated at one end of the cell, and this bias in PilA localisation tends to be consistent in all cells of the hormogonium (Risser *et al.*, 2014; Khayatan *et al*., 2015). This could obviously provide the basis for coordinated movement of the trichome. Khayatan *et al*. (2015) further show that the motor ATPases PilB and PilT are localised close to the cell junctions, apparently as part of an integrated machinery for pilus extension, pilus retraction and also slime secretion. However, the demonstration by these authors that active slime secretion is *not* required for motility provides the final indication that the motive force comes from the Type IV pili.

Despite the fact that *Synechocystis* and *Nostoc* motility now seem to have so much in common, it is interesting that *Nostoc* hormogonia can move much faster than *Synechocystis* cells. *Nostoc* hormogonia can achieve speeds of 1-10 μm s^{-1} (Risser and Meeks, 2013), whereas *Synechocystis* is limited to a pedestrian 0.03-0.07 μm s^{-1} (Burriesci and Bhaya, 2008). It remains to be seen whether this discrepancy reflects a fundamental difference in mechanism, or a higher expression of the motility components in hormogonia, or whether it is simply an advantage achieved by the co-ordinated efforts of all the cells in the *Nostoc* trichome.

Do the pili pull or push?

Both the extension and the retraction of Type IV pili are powered by ATP hydrolysis, so the motive force could in principle come either from pilus extension (a pushing, or punting mechanism) or pilus retraction (a pulling, or grappling-hook mechanism). Which mechanism would be most effective depends on the mechanical properties of the pili and their interaction with the substrate. Merz *et al*. (2000) elegantly showed that motility is powered by pilus retraction in *Neisseria gonorrhoeae*. Motility in *Synechocystis* must similarly depend on pilus retraction, because a GFP-tagging study shows that the pilus extension motor PilB is located in patches at the leading edge of moving cells (Schuergers *et al.*, 2015). This suggests that the pili must be extruded only at the front of the cell, implying that the pili pull rather than push (Fig. 1). Similar arguments apply to twitching motility in *Pseudomonas aeruginosa* (Burrows, 2012). By contrast, Khayatan *et al*. (2015) favour a model in which the pili in *Nostoc* drive motility by pushing on the polysaccharide at the cell surface (Fig. 1). This model is suggested by the apparent tight co-ordination between polysaccharide secretion and pilus extension at the junctional pores. It is also notable that the *Nostoc* pili appear to be trapped in the slime layer surrounding the cell, rather than being free to extend away from the cell as in unicellular bacteria (Khayatan *et al.*, 2015). If this model is correct, it would be the first known instance in which motility is powered by pilus extension. However, they acknowledge that the question is unresolved, because they were not able to determine whether the pili are extended from the leading pole of each cell in the trichome, or from the lagging

pole as predicted by their model (Fig. 1). The Type IV pilus apparatus could be adapted to provide motility in different ways. Indeed, the archaellum of Archaea such as *Sulfolobus acidocaldarius* provides a striking instance of such adaptation, since this a modified Type IV pilus which generates motive force not by pulling, but by rotation (Shahapure *et al.*, 2014). Therefore it cannot necessarily be assumed that Type IV pili power motility in the same way in *Nostoc* as in the unicellular bacteria.

Direction switching and co-ordination

All the twitching and gliding bacteria that we have discussed are able to switch their direction of movement. *Pseudomonas* and *Myxococcus* switch direction by relocating the motor ATPases PilB and PilT between the two poles of the cell (Bulyha *et al.*, 2009). In *Synechocystis*, direction switching similarly involves relocation at least of PilB1, with the distinction that *Synechocystis* has spherical cells and therefore is not limited to a choice of two directions: it appears able to form patches of PilB at any point on the cell surface and thus to steer itself in any direction (Schuergers *et al*., 2015) (Fig. 1). By contrast, Khayatan *et al*. (2015) show that PilB does not relocate in *Nostoc* hormogonia. Unlike PilA, PilB is symmetrically located at both poles of the cell and its distribution does not change when the trichome reverses direction. Khayatan *et al*. (2015) do not exclude the possibility of dynamic relocation of PilT, since their GFP-tagged PilT is not functional. However, it is perhaps more likely that *Nostoc* has a way to selectively activate and inactivate the motor ATPases at the two poles of the cell without large-scale relocation.

Direction switching in *Myxococcus* is triggered by the Frz chemosensory system, and the bactofilin cytoskeleton and two small GTPases are all involved in controlling the localisation of PilB and PilT and hence the direction of movement (Bulyha *et al.*, 2013). In *Synechocystis*, the details of signal transduction are unclear but there is evidence for the involvement of the second messengers cAMP (Terauchi and Ohmori, 1999) and c-di-GMP (Savakis *et al.*, 2012), with phototactic signals triggered by a range of photoreceptors (Yoshihara and Ikeuchi, 2004). The signal transduction system in

Nostoc hormogonia is unknown, but it must have the fascinating ability to co-ordinate the direction of motility in all the cells of the trichome, with intercellular communication to co-ordinate rapid reversals in direction. Filamentous cyanobacteria possess arrays of “septal junction complexes” (Mullineaux and Nürnberg, 2014). These are pore structures that allow suitably rapid intercellular diffusion of small molecules, and they are active in hormogonia as well as in other filamentous forms (Nürnberg *et al.*, 2014). The septal junction complexes provide a likely route for exchange of messenger molecules that co-ordinate motility, but the molecular species involved remain to be determined. *Nostoc* hormogonia (Khayatan *et al.*, 2015), and *Oscillatoria* trichomes (Häder, 1987), frequently switch direction even when moving in apparently uniform environments, and the mechanism of this co-ordinated multicellular decision-making is a fascinating question for the future.

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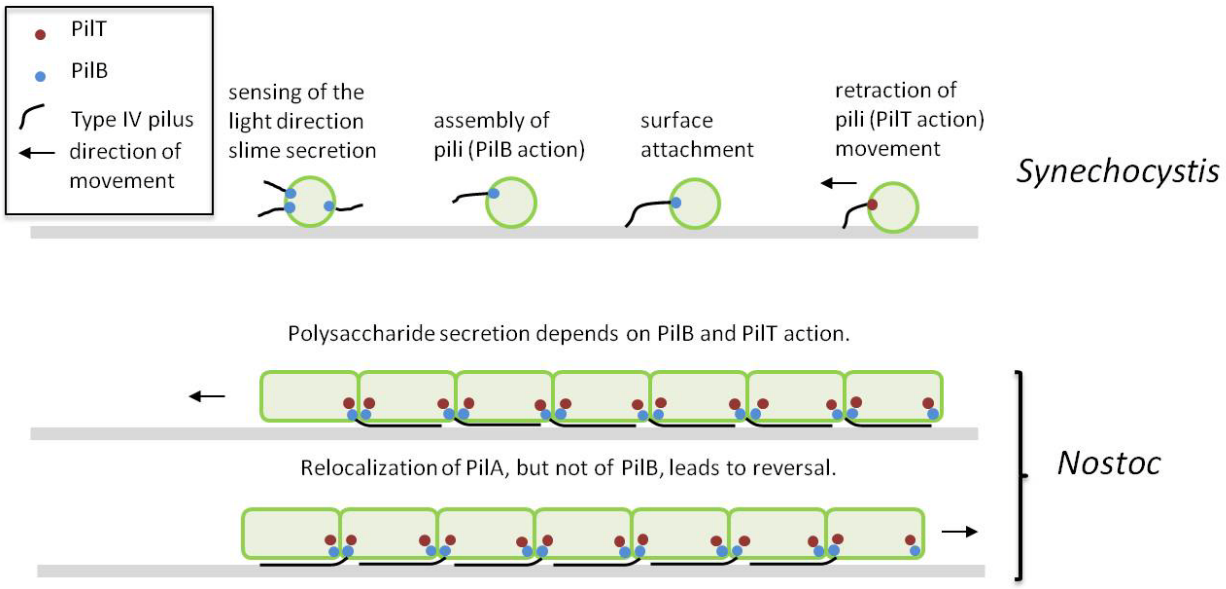

Fig. 1. Models for direction-switching in the Type IV pilus-based motility of cyanobacteria

In both species shown, motility is facilitated by the deposition of polysaccharide slime on the surface of the cell and/or the substrate, but the directional motive force comes from the Type IV pili.

Top: In the spherical unicellular cyanobacterium *Synechocystis* sp. PCC 6803, directional illumination triggers the relocalisation of the cytoplasmic pilus extension motor ATPase PilB, which can form patches at any part of the plasma membrane. Pili are extended at the site of the PilB patch, adhere to the substrate and then retract to pull the cell. Retraction is powered by the PilT ATPase, whose dynamic localisation is not yet established (Schuergers *et al*, 2015).

Bottom: In hormogonia of the filamentous cyanobacterium *Nostoc punctiforme*, pili are extended at one cell pole, with a consistent trend in the entire filament. Direction switches result from a co-ordinated switch in pilus extension to the other pole, but this does not involve any large-scale relocalisation of PilB. It is not yet certain whether motility is driven by pilus extension (as shown here) or pilus retraction; this could be established by determining whether pili are formed at the leading or the lagging poles of a moving trichome (Khayatan *et al*., 2015).