Keeping your lawn wet

Bacterial flagella can create, as well as sense, hydration in their external environment

Even before the existence of experimental proof, it was thought that the length of the bacterial flagellum was controlled by capillary action. Flagellin subunits travel through the hollow structure of the flagellum and assemble at the growing tip outside the cell body. The growth rate of a flagellum has been shown to decrease as its length increases, with growth stopping at a flagellum length of approximately 15 μm (Iino, 1969). The same growth rate and terminal length was observed for a flagellum that was sheared off by mechanical forces. This would suggest that length is indeed limited by capillary action.

Recent work by Wang and colleagues reports a relatively simple assay system showing that the length of *Salmonella* flagella is dependent directly on the degree of hydration in their external environment (Wang *et al.*, 2005). The authors take advantage of earlier discoveries by Harshey and colleagues that *Salmonella typhimurium*, *Escherichia coli* and *Serratia marcescens* can switch from a swimming phenotype, in which they use flagellar rotation to propel themselves (swim) through liquid environments, to a swarming phenotype, in which the bacteria use flagellar rotation to crawl (swarm) across surfaces (Alberti & Harshey, 1990; Harshey & Matsuyama, 1994). Furthermore, the bacteria undergo a morphogenetic change when they are taken from a swimming environment and placed on a hydrated surface. A low agar concentration in Petri dishes (0.3%) provides enough hydration to allow the bacteria to swim through the agar. Bacteria isolated from these 'motility' plates have the same physical appearance as bacteria taken from liquid culture: about 4–10 peritrichous flagella per cell that are typically 5–10 μm in length (Macnab, 1996). When the agar concentration is increased to the point at which they can no longer swim through it (0.5%), the bacteria double the density of flagella, elongate their bodies and begin to crawl along the surface. This morphogenetic change was no surprise, as it had been well established in systems such as *Proteus mirabilis* and *Vibrio parahaemolyticus* that bacteria switch from swimming cells to hyperflagellated and elongated swarming cells when they encounter solid surfaces (McCarter & Silverman, 1990; Mobley & Belas, 1995).

The Harshey group previously isolated swarming-defective mutants and found that the chemotaxis system, but not chemotaxis *per se*, was required for the differentiation to swarming cells

(Burkart *et al.*, 1998). In liquid environments, the rotation of flagellar motors in a counterclockwise direction produces flagellar filaments with a left-handed helical structure that bundle at one end of the cell and propel *Salmonella* or *E. coli* forwards, a process known as 'smooth swimming'. When the motor of any one or more of the 4–10 peritrichous flagella on a given bacterium changes rotation to a clockwise direction, the bundle is disrupted and the cells tumble. Resumption of counterclockwise rotation propels the cells in a new direction. If the new direction is favourable, and the bacterium moves towards an attractant or away from a repellent, the tumble signal is suppressed. This allows the bacterium to travel up or down a chemical gradient by what is essentially a biased 'random walk', or chemotaxis (Parkinson, 2003). Although unexpected, the discovery that mutants in the chemosensory system were unable to swarm shed no apparent light on what the swarm signal could be. Given that remarkably little is known about how cells move across surfaces or differentiate into swarming cells, this was just another interesting phenomenon that was associated with swarming.

Wang and colleagues have now shown that *Salmonella* can sense wetness in its external environment through the flagellum and that the chemosensory system is essential for this process and for the process of flagellar terminal length determination (Wang *et al.*, 2005). In liquid, the non-chemotactic mutants (*che*) grew flagella just like wild type, but when the cells were plated on the surface of swarm plates, the flagella were shorter and fewer in number. This corresponds directly with a decrease in the transcription of flagellar genes that are needed late in the assembly process. This was because the *che* mutant cells were less hydrated on swarm plates. The surface of lawns of wild-type cells produced a 'Swiss cheese' morphology that was absent in the *che* mutant lawns and it was shown that this swarm pattern retained water. So how does water retention allow the full differentiation to swarming cells? The flagellum is essentially a motor (known as the hook-basal body, HBB) with a propeller that consists of a polymer of about 20,000 subunits of flagellin protein. In peritrichously flagellated bacteria, a mechanism couples the expression of the genes that are needed late in assembly, such as the filament gene, to the completion of motor structures (Chilcott & Hughes, 2000). This is accomplished by the action of a flagellar-specific transcription factor, σ^{28} , and its inhibitor FlgM. Before completion of the HBB, FlgM binds σ^{28} and prevents σ^{28} -dependent transcription of late flagellar genes. On HBB completion, the flagellar secretory system changes specificity from HBB-type secretion substrates to late secretion substrates. FlgM is a late secretion substrate and, on HBB completion, it is secreted from the cell where it can no longer act to inhibit σ^{28} -dependent transcription of late flagellar genes. This mechanism ensures that the flagellar motors are built before the propeller genes are switched on. As the filament grows, FlgM is continuously secreted, presumably through the tip of the elongating filament, and the late genes remain fully on. However, because the *che* mutants are

less hydrated, FlgM secretion is reduced and accumulates in the cell to inhibit late flagellar gene transcription. As one would predict, a *che flgM* double mutant restores flagellar numbers on swarm plates because transcription of late genes is constitutive. However, the *che flgM* mutant still fails to swarm because the chemotaxis system is required to maintain colony hydration. Presumably this hydration is essential for the capillary action that is needed to allow the filament monomers to continue to travel to the tip of the elongating filament and assemble. Thus, although the absence of FlgM allowed the *che* mutants to produce high numbers of flagella, they remained short.

This all leads to a simple prediction: if the *che* mutants are defective in filament elongation, and hence defective in swarming, because they are less hydrated, adding water to the plates should lead to swarming. Indeed, Wang and colleagues performed this simple assay, and wild-type swarming was restored for the *che* mutants. The remaining puzzle is how chemotaxis produces the 'Swiss cheese' morphology that is associated with the lawns of swarming cells and the full hydration of these lawns. We know that the chemotaxis system controls motor reversals; *che* mutants that rotate their flagella constitutively in either a counterclockwise or clockwise direction fail to swarm, which suggests that motor reversal is essential for the 'Swiss cheese'-making process. Wang and colleagues have made the exciting and unanticipated discovery that flagella can create, as well as sense, wetness in their external environment, and that the regulation of flagellar gene expression and filament length is dependent on the level of wetness. So in the final analysis, growing flagella is a lot like growing grass: just keep your lawn wet.

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