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GUEST COMMENTARY

Multiple Modes of Motility: a Second Flagellar System in *Escherichia coli*

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Choosing transportation presents numerous decisions. Something fast has its attraction, as does something fuel efficient and economical. Then again, in inclement conditions and harsh terrain something more substantial seems indispensable. Bacteria must also solve this dilemma. Optimal locomotion under different circumstances requires different equipment. Thus, although costly, it may be advantageous to possess more than one mode of motility. In this issue of the Journal of Bacteriology, Mark Pallen and his colleagues at the University of Birmingham and the Sanger Institute report that even *Escherichia coli* has indulged in investment in two distinct flagellar systems (43).

FLAGELLA AS PROPULSIVE ORGANELLES IN LIQUID

Acting like helical propellers driven by reversible rotary motors, bacterial flagella are extremely effective organelles of locomotion (reviewed in references7 and 30). For example, *Salmonella enterica* serovar Typhimurium rotates its flagella at ~200 revolutions s^{-1} and swims at speeds of ~55 μm s^{-1} (31). This is a propulsion rate of many cell body lengths per second. *Salmonella* cells are approximately 0.73 μm wide and 1.4 μm long. Each cell possesses about five flagella, and these flagella are arranged peritrichously (or laterally) around the cell body. The measurements for peritrichous *E. coli* cells are similar, ~270 revolutions s^{-1} and 36 μm s^{-1} (29). In comparison, the marine bacterium *Vibrio alginolyticus*, whose cell size is similar to the cell size of *Salmonella* and *E. coli*, swims at speeds as fast as 116 μm s^{-1}, and its flagellar rotation rate, as measured by dark-field laser microscopy, is ~1,000 revolutions s^{-1} (32). The swimming speeds for *Pseudomonas* species are similarly fast, >70 μm s^{-1} (17, 44). Only a single flagellum, which is located at one cell pole, propels *V. alginolyticus*. *Pseudomonas* strains can be propelled by a single polar flagellum (like *Pseudomonas aeruginosa*) or multiple polar flagella (like *Pseudomonas putida*).

FLAGELLA AS PROPULSIVE ORGANELLES IN VISCOUS ENVIRONMENTS AND ON SURFACES

Although slight increases in viscosity enhance the swimming speed, high viscosity generally impedes flagellar performance (8, 44). As a consequence, bacteria implement additional strategies to maximize movement in viscous conditions and on surfaces. Different kinds, arrangements, or numbers of flagella can alter performance under high-viscosity conditions. Many peritrichous bacteria upregulate the number of flagella and alter extracellular components (such as polysaccharide and surfactant production) to enable movement on surfaces, which is called swarming. One of the most striking examples of a highly flagellated and robust swarmer is *Proteus mirabilis*, although most peritrichous bacteria, including *E. coli* and *Bacillus*, *Salmonella*, and *Serratia* species, have been found to increase the number of flagella and move on surfaces (reviewed in references 11, 14, and 15). Thus, overproduction of the same flagella used for swimming in liquid can be an effective means of translocation on surfaces.

Other bacteria induce completely new, alternate flagellar systems in response to growth in viscous environments and on surfaces. These bacteria include *Aeromonas* species (28), *Azospirillum* species (13, 25, 38), *Rhodobacter centenum* (41, 42), *V. alginolyticus*, and *Vibrio parahaemolyticus* (2, 45). They are polarly flagellated when they are grown in liquid and have mixed (polar and peritrichous) flagella when they are grown on surfaces. An example of surface-induced flagella is shown in Fig. 1. Although a superior organelle for propulsion in liquid, the polar flagellum of *V. alginolyticus* has been demonstrated to perform very poorly in viscous environments, whereas the peritrichous (lateral) flagella enable effective motility in highly viscous environments (e.g., 20 μm s^{-1} in a ~200-cP environment) (4). Thus, the two flagellar organelles of these bacteria seem to be adapted for optimal movement under distinct circumstances. For some of the bacteria that exhibit mixed flagellation, it is clear that the gene systems encoding the two flagellar systems are nonoverlapping (33), whereas for other bacteria there may be shared as well as distinct structural and/or regulatory components (3, 12, 21, 28, 34). Moreover, in the case of the marine *Vibrio*, not only does the organism switch its mode of motility on transition from liquid to surfaces, but it also switches the energy source driving motility; the polar system is powered by the sodium motive force and the lateral system is driven by the proton motive force (5).

The flagellar motor may also be equipped or configured differently to cope with fluctuations in viscosity and load or energy supply. Recent discoveries with *Bacillus subtilis* demonstrate that this organism possesses two types of flagellar motors driven by different energy sources (the sodium and proton motive forces), although the organism has only one set of flagellar genes (18). These motors must work interchangeably to power the single type of flagellar rotor. The relative force-generating capacities seem to differ as one type of motor sup-

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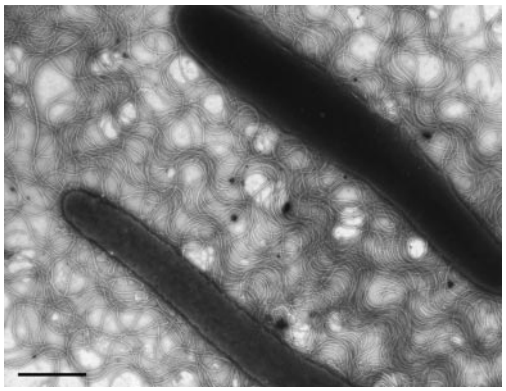

FIG. 1. The newly discovered Flag-2 locus of *E. coli* is very similar to the lateral gene system of *V. parahaemolyticus*. For this electron micrograph of peritrichous (or lateral) flagella of *V. parahaemolyticus*, the cells were grown on solidified medium, harvested, and stained with 0.5% phosphotungstic acid. The lateral flagella of *V. parahaemolyticus* propel the bacterium on surfaces and in viscous environments. Bar = ~1 μm.

ported swarming on high-agar-content surfaces better than the second type of motor. Torque generation to drive rotation of the single polar flagellum of *P. aeruginosa* seems to be similarly complex (10). Five motor genes (compared to the two genes required for *Salmonella* motility) contribute to rotation and enable movement in liquid and on surfaces.

Flag-2 LOCUS OF *E. COLI*

In this issue Ren et al. (43) report that analysis of the genome sequence of enteroaggregative *E. coli* strain 042 revealed a surprising difference from *E. coli* K-12 and other sequenced *E. coli* strains; they describe a new locus, designated Flag-2, that encodes a second flagellar system. PCR studies suggest that the Flag-2 locus can be found in 15 of 72 strains in the *E. coli* reference collection (ECOR strains).

The 44-gene locus potentially encodes all of the genes required for a flagellar motility system. The gene and potential operon organization and the gene product homologies are most similar to those of the lateral system of *V. parahaemolyticus* (33). The Flag-2 locus contains all potential orthologs of the *V. parahaemolyticus* lateral system except one, the *motY* gene encoding a motor component. Moreover, like the *V. parahaemolyticus* system and many other nonenteric flagellar systems, including those of *Caulobacter crescentus*, *Campylobacter jejuni*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, and other *Vibrio* species (1, 9, 19, 20, 22, 26, 37, 40, 46, 47), the Flag-2 system appears to have the capacity to be regulated in an RpoN-dependent manner. The locus encodes a potential σ^{54}-dependent flagellar regulator, similar to the $LafK_{vp}$ regulator (46), and consensus σ^{54} promoter regions can be found upstream of some of the potential operons.

E. COLI K-12 IS NOT THE ANCESTRAL *E. COLI*: LESSONS FOR COMPARATIVE GENOMICS

Comparative bioinformatic analysis revealed that *E. coli* K-12 possesses vestiges of the alternate flagellar system. In fact, pseudogenes representing remnants of an *lfhA-lafU* deletion of Flag-2 can be found in all available *Escherichia*/*Shigella* genome sequences. Therefore, it seems that the Flag-2 locus was present in the last common ancestor of the species and was subsequently lost by deletion in some strains. The Flag-2 locus was not discovered in the first 10 *Escherichia*/*Shigella* genomes studied. Thus, this work provides an important demonstration of the value of comparative genome sequencing. Moreover, it emphasizes that care should be taken in viewing genomes as fixed, common backbones supplemented by optional islands. *E. coli* genomes can vary by more than 1 Mb of DNA, and *E. coli* K-12 should not be regarded as the ancestral or archetypal strain. As Ren et al. note, genomes are like a palimpsest and may be better comprehended as changeable drafts that are subject to multiple instances of genomic expansion, deletion, and rearrangement.

Flag-2 LOCUS IN OTHER GENOMES

Flag-2-like flagellar genes were also identified in *Chromobacterium violaceum*, *Citrobacter rodentium*, and *Yersinia pseudotuberculosis*. The three sequenced strains of *Yersinia pestis* contain predicted Flag-2 genes, although none appear to encode functional flagellar systems, as frameshift mutations and deletions of key flagellar genes occur. In all cases, the Flag-2 flagellar genes are found in clusters. In *Citrobacter*, the Flag-2 cluster occurs in the same location as it occurs *E. coli*, whereas in *Yersinia* its position is different. Importantly, 11 *Salmonella* genomes completely lack Flag-2 genes and the *lfhA-lafU* gene remnants. On the basis of all the data, Ren et al. suggest that the Flag-2 locus was acquired in a single step by lateral gene transfers that occurred independently in *Yersinia* and *Citrobacter*/*Escherichia*. The Flag-2 locus was acquired by *E. coli* after divergence from *S. enterica*.

DOES Flag-2 ENCODE A FUNCTIONAL MOTILITY SYSTEM?

It seems likely that the Flag-2 locus of the 042 strain was recently operative. All of the genes required for a functional flagellar system are present in the Flag-2 locus. Moreover, the majority of these genes appear to be intact (i.e., they code for proteins with functional counterparts in other organisms). Only one gene, encoding a proximal rod protein, appears to be nonfunctional. Swarming behavior was tested, but it could not be demonstrated. However, swarming motility can be fastidious; it may be induced and observed under highly specific conditions, and motility is often lost during laboratory cultivation. For example, undomesticated strains of laboratory strains of *B. subtilis* exhibit robust swarming, although laboratory strains fail to swarm. This failure to swarm appears to be the result of multiple defects, at least one of which is created by phase variation (23, 24). Thus, it will be very interesting to determine what is necessary to reconstitute functional Flag-2 motility, as well as to examine the conditions under which it is maximally functional.

WHY TWO FLAGELLAR SYSTEMS?

Some bacteria may opt for a modest lifestyle, economizing with a single flagellar system that accommodates moderate swimming and swarming. Although *E. coli* K-12 can swarm, it

is not a particularly effective swarmer and requires specialized agar at a relatively low concentration (0.5%) to move on solidified medium (16). Other bacteria may inhabit changing environments, in which maximal speed and/or performance under different conditions is required for survival. In such a case, maintaining dual capacities for locomotion may have advantages. Flagella also play other roles (39). For example, the lateral flagella of *V. parahaemolyticus* enhance adhesion to chitin (6), and lateral flagella of *Aeromonas* species are important for adhesion to human cells and contribute to biofilm formation (12, 35, 36). Thus, different types of flagella may be key to survival in specific niches and/or hosts (27). The discovery reported by Ren et al. (43) of the Flag-2 locus in *E. coli* is most exciting, and future work on its function may have important implications for understanding colonization and pathogenesis.

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