

New and Notable

New Insights into the Workings of the Ultimate Swimming Machine

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Over the last few years, increasingly clever in vitro motility assays have allowed mechanical and kinetic measurements at the level of single molecular motors (see for example Block and Svoboda (1995), Finer et al. (1995), and Yanagida and Ishijima (1995) in a special issue of *Biophysical Journal* devoted to molecular motors). But to get a handle on complex phenomena such as cytokinesis or muscle contraction, we need to understand how molecular motors work in ensembles from a few to many millions. How do motors interact when they pull the same load along a substrate? Do they just follow an internal program, blind to the presence of other motors? Or do they fall into synchrony, like waltzing lovers? To address these questions that lie at the center of the problem of biological self-organization, experimental systems are needed in which a known number of molecules interact in the correct orientation with their substrates. In the world of eukaryotic motors, such as kinesin and myosin, we know how to study either exactly one or a poorly defined ensemble of motors. In contrast, for the molecular torque generators that power the bacterial flagellum of *Escherichia coli* we know how to study a finite ensemble from eight down to one. Samuel and Berg (this issue of *Biophysical Journal*) recognized this unique feature of the bacterial motility assay system and used it together with a powerful fluctuation analysis to show that individual molecular torque generators step essentially independently, and

much more. Before I describe their elegant experiments, some background is needed.

E. coli swim by screwing their helical flagella through the external medium. Flagella are powered at their base by a rotary engine that uses the energy provided by a proton gradient (Berg, 1995). The torque generators, each composed of MotA and MotB proteins, are attached to the cell wall and exert force on the rotor, which in turn is rigidly connected to the flagellum (Fig. 1). The movement of torque generators can be visualized by sticking a flagellum to a glass substrate and observing the rotation of the cell body about this flagellar tether under a microscope. Because of the large viscous drag on the cell body, the flagellar rotation rate is reduced by more than 10-fold compared to a free swimming cell, and the drag force is very close to stalling the motor. Cells lacking MotA or MotB perform free rotational Brownian motion about the flagellar tether, suggesting that the torque generators constitute the only link between rotor and cell wall (Fig. 1) (Berg, 1995). In what has been termed the “resurrection experiment,” producing the missing protein facilitated by a vector leads to the recovery of directed movement in eight equal velocity increments of ~ 1 Hz (Blair and Berg, 1988). As single torque generators are added one by one, they each provide a quantum of velocity. Samuel and Berg succeeded in prolonging the intervals between successive incorporation of torque generators, allowing the precise characterization of flagellar movement at different velocity levels and hence different torque generator numbers, n .

It has been recognized for some time that eukaryotic motors can be grouped into at least two classes with distinct ensemble behaviors, which have been termed “porters” and “rowers” (Leibler and Huse, 1993). This terminology derives from the fact that rowers on a galley dip their oars only transiently into the water, while porters have at least one foot on the ground at all times. Kinesin is an example of a porter, whereas muscle

myosin is thought to be a rower. To describe these classes in terms of molecular properties, we define the duty ratio, D , as the fraction of time a motor spends attached to its substrate; then porters correspond to $D \sim 1$, and rowers to $D \ll 1$. With this in mind, how might torque generators interact when pulling a load? In the simplest models we ignore possible correlations in the movement of neighboring torque generators. We also assume that torque generators are coupled to the cell wall with linear springs. A step can be modeled as a transient change in the rest length of the spring by δ , corresponding to an angle $\Delta\theta = \delta/r$, where r is the radius of the rotor. In the case of a single torque generator, the movement of the rotor will faithfully reflect the movement of the torque generator, apart from smoothing due to the compliances in the system. The consequences of additional torque generators will depend on the duty ratio (Leibler and Huse, 1993): 1) For rowers ($D \ll 1$), at most one torque generator will be bound at any instance and each step will move the rotor by $\Delta\theta$, irrespective of n . Angular velocities will increase in proportion to n at all loads, but the stall

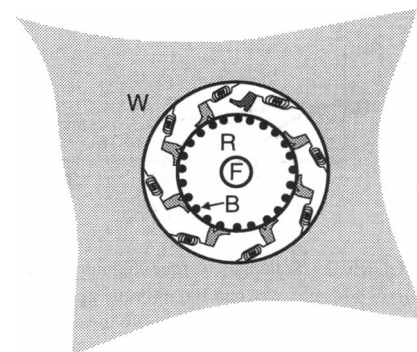


FIGURE 1 Minimal schematic of the bacterial flagellar motor with torque generators operating as porters. Torque generators are attached to the cell wall (W) and transiently unbind from the rotor (R) to move to the next binding site (B), leading to a change in the strain of the spring by δ (as illustrated by the dark boot). While attached to the rotor, the rest length of the spring again shortens, generating torque on the flagellum (F; perpendicular to the plane of the page).

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torque will be independent of n (Fig. 2, *dashed lines*). 2) For porters ($D \sim 1$), all but at most one torque generator will be bound at any instance and each step will move the rotor only through $\Delta\theta/n$. The reason is that the stretched spring moving the flagellum can only relax by compressing the springs of the other torque generators. In this case low load velocity will be independent of n (Fig. 2, *solid lines*). Because the torque on the flagellum is supported by equal tensions in all torque generators, the stall torque will be proportional to n .

Where do bacterial torque generators fit in? The quantal nature of the angular velocity increments in the resurrection experiment points toward porters, which can be understood as follows: At the low Reynolds numbers operative in this experiment, torque is strictly proportional to angular velocity (Purcell, 1977). This proportionality defines the load line, $T = \beta_\theta \dot{\theta}$, where β_θ is the rotational drag coefficient of the cell (Fig. 2, *dotted line*). The load line relates torque and velocity for a particular cell geometry and fluid viscosity. Stable rotation rates are given by the intersections of the load line and the torque-velocity curves (Fig. 2). For

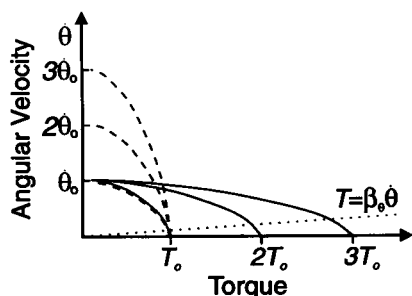


FIGURE 2 Schematic torque-angular velocity curves for one, two, and three torque generators. Torque-angular velocity curves are the analog of force-velocity curves for linear motors. The shape of the curves is loosely based on electro-rotation measurements (Berg and Turner, 1993). For porters (*solid lines*), stall torques will be multiples of the single torque generator stall torque, T_0 , and the curves will converge at low loads. The load line (*dotted line*) is a straight line with a slope given by the rotational drag coefficient of the cell (β_θ). For rowers (*dashed lines*), low load velocities will be multiples of the single torque generator velocity, θ_0 , and the curves will converge at high loads (*dashed lines*).

porters, torque-velocity curves for different n at large loads will be equally spaced along the torque axis (Fig. 2, *solid lines*). For the relatively large drag coefficients in this experiment the load line is shallow and thus intersects the porter (but not the rower) torque-velocity curves at approximately equally spaced velocity levels (Fig. 2), as observed in the resurrection experiment. Naively, one would expect that measurements of rotor step size as a function of n would provide further evidence: for rowers, the step size should be independent of n , whereas for porters the step size should vary as n^{-1} . Unfortunately, there is little hope of directly measuring the discrete steps underlying flagellar movement. These steps are expected to measure only one degree or so, and will be filtered by the soft compliance of the flagellum by which the cell body is attached to the microscope chamber (Block et al., 1989). Moreover, steps will be obscured by Brownian noise.

To bypass the difficulties with measuring molecular steps directly, Samuel and Berg turned to fluctuation analysis (Svoboda et al., 1994) instead, which works roughly as follows. Assume that each torque generator steps at stochastic times. The variance in angular position about the mean position will be proportional to the step size. Thus, if a given angular distance is covered by smaller steps, the variance will be correspondingly smaller. Samuel and Berg measured the fluctuations in angular position for different torque generator numbers and found unambiguously that these fluctuations decrease as $1/n$; the bacterial flagellum runs more smoothly with a larger number of torque generators in a manner exactly as expected for independently stepping porters coupled by linear springs.

The flagella of freely swimming *E. coli* move at hundreds of Hz (Berg and Turner, 1993) and those of its sodium-powered cousin *Vibrio alginolyticus* run at >1000 Hz (Muramoto et al., 1995). The step number per revolution is on the order of 100 and, therefore, for porters each torque generator would have to cycle at more than 10 kHz, two orders of magnitude faster than ATP-powered eu-

karyotic molecular motors. The evidence for porter-like behavior however has been accumulated only at high loads, and the interesting possibility exists that the duty ratio decreases with decreasing load, resulting in rowerlike behavior in the low load limit. Because rowers would produce a larger rotor movement per molecular cycle, the observed rotation velocities could be accounted for with more modest molecular cycle times of ~ 1 kHz. Designing a resurrection experiment at a variety of loads, perhaps using optical trapping techniques or electrorotation to counteract viscous loads, might shed light on the workings of this ultimate swimming machine over its complete operating range.

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