molecular genetics is underdeveloped, such as primates, this is almost out of the question. Even for organisms with a good collection of transcriptional promoters, such as mice and fruit flies, targeting a subpopulation of cells within a genetically and anatomically "homogeneous" cell population is a challenge. To address these issues, the authors resorted to *trans*-synaptic trafficking. They used two viruses: one encoding CRE recombinase fused to the transcellular tracer protein wheat germ agglutinin (WGA) and a second encoding a CRE-dependent opsin. They delivered these two viruses to a pair of remote but anatomically connected brain regions in rats or mice, one virus each to one of the two regions, and successfully labeled and optically controlled the subpopulation of neurons with projections connecting these two brain regions (Figure 1). This approach also raises an intriguing possibility that activation or inhibition may be targeted to specific axonal branches, rather than to the neuronal soma (cell body), potentially increasing the precision of optogenetic manipulation. Overall, *trans*-synaptic labeling of anatomically connected neurons with a WGA-CRE fusion protein enabled targeting of specific neurons on the basis of their synaptic connection patterns, thus opening new doors for the precise manipulation of neural circuits.

These optogenetic techniques described by Deisseroth and his team, as well as by others, provide powerful new tools for neuroscience research. Although these methods based on light-gated ion channels are effective only in cells (neurons, muscle, endocrine cells, etc.) that can be rendered excitable by these channels, some additional recent developments promise broadening of the range of target cell types that can be manipulated by optogenetics. For example, new light-sensitive G protein-coupled receptors (dubbed optoXRs) have the potential to influence signaling cascades in cell types other than neurons (Airan et al., 2009). Theoretically, light-gated calcium ion channels could also be useful, as calcium ions are a universal secondary messenger in all known cell types. Expanding optogenetic tools so that they can be applied more broadly is the goal of optogenetics 3.0 and beyond.

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Time for Bacteria to Slow down

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The speed of the bacterial flagellar motor is thought to be regulated by structural changes in the motor. Two new studies, Boehm et al. (2010) in this issue and Paul et al. (2010) in *Molecular Cell*, now show that cyclic di-GMP also regulates flagellar motor speed through interactions between the cyclic di-GMP binding protein YcgR and the motor proteins.

Cyclic di-GMP is the molecule of the moment in bacteriology. This ubiquitous secondary messenger has been implicated in myriad processes from pathogenicity to synthesis of pili (hairlike appendages involved in biofilm production) (reviewed in Hengge, 2009). Now two new papers, one in this issue of *Cell* (Boehm et al., 2010) and one in the upcoming issue of *Molecular Cell* (Paul et al., 2010), reveal the direct involvement of cyclic di-GMP in the regulation of flagellar movement and bacterial swimming.

Cyclic di-GMP is synthesized from two molecules of GTP by diguanylate cyclase domains and is broken down by phosphodiesterase domains. The

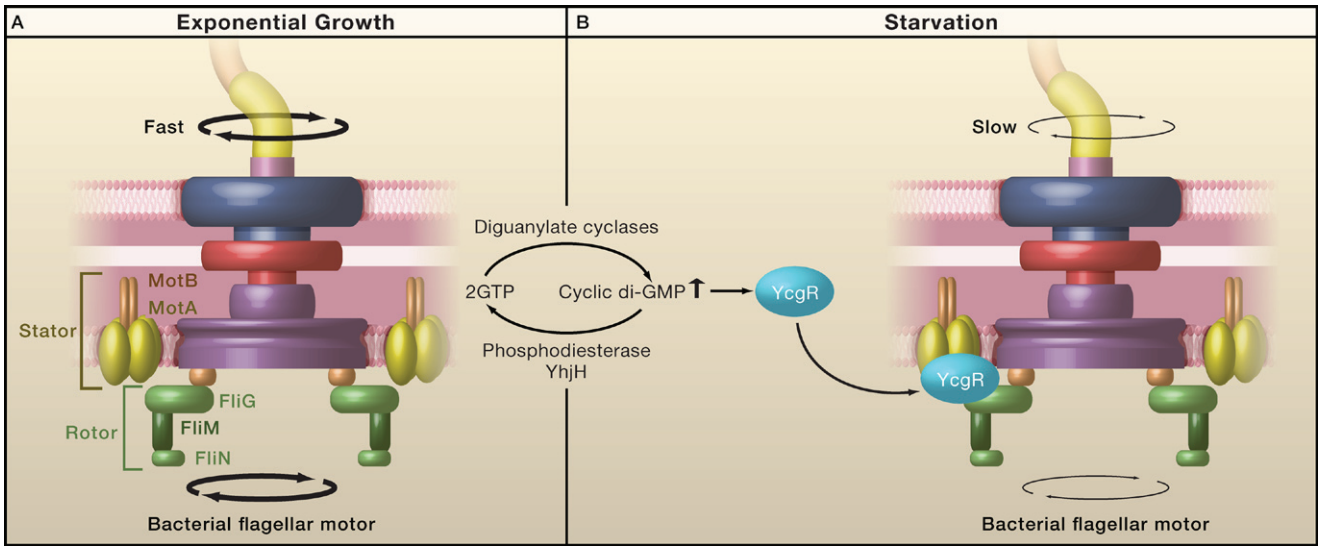

Figure 1. Cyclic di-GMP Regulates the Bacterial Flagellar Motor

(A) Bacterial swimming is powered by the rotary flagellar motor, which contains two major complexes: MotA and MotB form a ring of stationary complexes called stators; and FliG, FliM, and FliN form a rotor that rotates inside the ring. Levels of cyclic di-GMP are controlled by diguanylate cyclases and the phosphodiesterase YhjH. During exponential growth, the phosphodiesterase activity is favored, keeping cyclic di-GMP levels low and motor speed fast.

(B) During starvation, cyclic di-GMP concentrations increase. Boehm et al. (2010) and Paul et. al. (2010) now demonstrate that cyclic di-GMP binds and activates the protein YcgR, which in turn binds directly to the motor and causes it to slow down. The two studies identified the stator protein MotA and the rotor proteins FliG and FliM as probable binding sites for YcgR.

two domains, which are widely distributed across prokaryotes, are found in many proteins with a wide range of functions. Genome sequencing identified 98 putative diguanylate cyclase or phosphodiesterase domains in *Shewanella oneidensis* and 29 of these domains in *Escherichia coli*. How all of these cyclases and phosphodiesterases function together to produce a coherent output signal is still unclear. Some cyclase-phosphodiesterase pairs produce high local concentrations of cyclic di-GMP to regulate a specific local activity, as seen for stalk formation in *Caulobacter crescentus*, whereas others operate as riboswitches. The clearest role for cyclic di-GMP, which has been identified in a number of diverse bacterial species, is its ability to regulate the "decision" to change from a free-swimming bacterium to a surface-attached bacterium (that may be embedded in a protective polysaccharide biofilm).

E. coli cells swim with a variety of speeds, and the average speed decreases as cells enter the stationary phase of growth (Amsler et al., 1993). Swimming is powered by the rotary flagellar motor (Figure 1A): FliG, FliM, and FliN proteins form a rotor that rotates inside a stationary ring, which consists of up to ~11 stator complexes created by MotA and MotB proteins that are anchored to the cell wall. The motor is driven by proton flow through MotA and MotB (Sowa and Berry, 2008); when protons bind to MotB, they alter the conformation of MotA, causing it to push on the rotor protein FliG. Speed is proportional to proton motive force across the cell membrane, and it was assumed that changes in speed were due to changes in proton motive force.

Several years ago genetic studies identified an *E. coli* protein, YcgR, which seemed to reduce swimming speed when a specific phosphodiesterase, YhjH, was inactivated (Wolfe and Visick, 2008). YcgR contains a domain that is known to bind cyclic di-GMP (called the PilZ domain) (Amikam and Galperin, 2006). Therefore, YcgR is presumably a target of cyclic di-GMP that slows the flagellar motor when levels of cyclic di-GMP increase. How YcgR regulates motor speed has been a matter of speculation.

Boehm et al. (2010) and Paul et al. (2010) now show that cyclic di-GMP activates YcgR and that YcgR then binds directly to the flagellar motor, causing the motor to slow down (Figure 1B). Thus, the YcgR protein acts via direct interaction with the flagellar motor and not indirectly through motor assembly, as thought previously (Wolfe and Visick, 2008). The braking action of YcgR appears to occur as the bacteria enter starvation or stationary growth conditions, when it may be advantageous to slow down and locate a surface to attach to and initiate biofilm formation. Intriguingly, although both groups used the same bacterium, *E. coli*, and located the same cyclic di-GMP binding site on YcgR, the two studies identified different YgcR-binding targets in the flagellar motor: Boehm et al. (2010) pinpointed the stator protein MotA and Paul et al. (2010) identified the rotor proteins FliM and FliG (Figure 1B).

By altering the expression of YcgR, diguanylate cyclases, and the phosphodiesterase YhjH, both groups clearly demonstrate that bacteria swim slower when cyclic di-GMP levels increase and that YcgR is necessary for this effect. By fusing YcgR to fluorescent proteins, both groups also found that YcgR colocalized with the flagellar motor when the phosphodiesterase YhjH was inactivated. Boehm et al. (2010) found specific mutations in the stator protein MotA that resulted in fast swimming even in the presence of high levels of cyclic di-GMP. FRET study data supported interactions between fluorescently labeled MotA and YcgR proteins, with a strong signal in the

presence of cyclic di-GMP and a weak signal in its absence. On the other hand, Paul et al. (2010) measured directional switching of the motor and found that cyclic di-GMP increased the counterclockwise bias of the motor. Switching is regulated by binding of the signaling protein CheY to the rotor protein FliM, which in turn alters the interaction between MotA and FliG. Thus, the counterclockwise bias observed by Paul et al. (2010) implicated FliM and FliG as potential targets of cyclic di-GMP action. This result was supported by the coisolation of FliM and FliG with YcgR in pull-down assays. Mutations in YcgR and FliM, which suppressed the braking action of YcgR and the colocalization of fluorescent YcgR proteins with the motor proteins, suggested that YcgR interacts at the interface between FliM and FliG.

How can we reconcile the differences between these two data sets? Both groups found that activated YcgR alters the rotor-stator interface between FilG and MotA but via different proteins. Each initial set of experiments to identify the interaction sites highlighted different partner proteins, and subsequent studies centered on these proteins. Boehm et al. (2010) used a number of approaches to examine the effect of the local environment on rotational speed. Expressing wild-type YcgR protein with mutant YcgR proteins, which are defective in MotA binding, produced intermediate speeds at high cyclic di-GMP levels, suggesting that individual stator complexes are independently affected by YcgR. Further, high salt concentrations suppressed the braking effect of cyclic di-GMP without causing the release of YcgR from the motor. The authors interpreted this result as support for a model in which the cyclic di-GMP-bound YcgR interacts with the electrostatic rotor-stator interface to slow the motor but not stop it. Paul et al. (2010), on the other hand, concluded that the binding of YcgR to both FliM and FliG in the rotor disrupts interactions between FliG and MotA (at the rotor-stator interface), although the authors did not rule out a direct interaction between YcgR and MotA.

Thus, it remains possible that YcgR can interact with a number of sites within the motor to disrupt the electrostatic interaction between the rotor and the stator. More experiments will be required to fill in the details of exactly where YcgR interacts and how it slows rotation. However, it is becoming increasingly clear that the bacterial flagellar motor is not a structurally stable nanomachine but rather a very dynamic one. In response to changing physiological conditions, bacteria regulate both the rotation (Pilizota et al., 2009; Blair et al., 2008) and the structure (Reid et al., 2006) of the flagellar motor in ways previously unforeseen.

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