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MEETING REVIEW

Flagellar Stators Activate a Diguanylate Cyclase To Inhibit Flagellar Stators

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ABSTRACT The bacterial secondary metabolite cyclic di-GMP is a widespread, cytoplasmic signal that promotes a physiological transition in which motility is inhibited and biofilm formation is activated. A paper published in this issue (A. E. Baker, S. S. Webster, A. Diepold, S. L. Kuchma, E. Bordeleau, et al., J Bacteriol 201: e00741-18, 2019, https://doi.org/10.1128/JB.00741-18) makes an important connection between cyclic di-GMP and flagellar components. They show that stator units, which normally interact with the flagellum to power rotation, can alternatively interact with and activate an enzyme that synthesizes cyclic di-GMP in *Pseudomonas aeruginosa*. Moreover, the same stator units are also the target of cyclic-di-GMP-dependent inhibition such that the more the stators are inhibited, the more cyclic di-GMP is made. The resulting positive-feedback loop not only inhibits motility but also may initiate and stabilize biofilm formation.

KEYWORDS MotCD, flagella, motility, stators, swarming

Bacteria synthesize cyclic di-GMP (c-di-GMP) from two molecules of GTP catalyzed by enzymes called diguanylate cyclases (DGCs). Some bacteria encode dozens of DGCs and the redundancy is thought to permit a wide array of discrete, specific inputs to promote cyclic di-GMP synthesis. Whereas the demonstration of diguanylate cyclase activity is common, the identification of signals that directly stimulate DGC activity is rare (1–3). Baker et al. provide a "signal" in which the DGC activity of SadC is activated by interaction with the flagellar stator complex MotCD (4) (Fig. 1). Interaction between the two proteins takes place within the membrane, and the interaction seems to increase activity by promoting DGC dimerization. Moreover, SadC and MotCD interact when MotCD is not in contact with the flagellum, thereby providing information about the motility status of the cell.

Flagellar stator units are protein complexes that exist in two different states in the membrane: freely diffusing and stationary when docked with the flagellum (5). When docked with the flagellum, each stator unit conducts protons, changes conformation, and imparts torque to drive rotation. Freely diffusing units are thought to be inactive. Remarkably, Baker et al. (4) show that free stator units can instead interact with signaling proteins to take on a regulatory role and add enhanced complexity to stator dynamism. Moreover, it has been observed that mutation of stator proteins confers pleiotropic defects beyond the inability to rotate flagella in a number of systems, but how extra phenotypes manifest has been unknown (6–8). If the interaction between stator proteins and particular signaling systems is common, it might provide an explanation for stator mutant pleiotropy.

Pseudomonas aeruginosa is somewhat unusual in that it synthesizes two different pools of stator complexes that compete for association with a single polar flagellum. Whereas complexes of either MotAB or MotCD can drive swimming motility, only MotCD generates enough torque to power swarming over surfaces. Competition between stators is biased when high levels of c-di-GMP (e.g., by mutation of a

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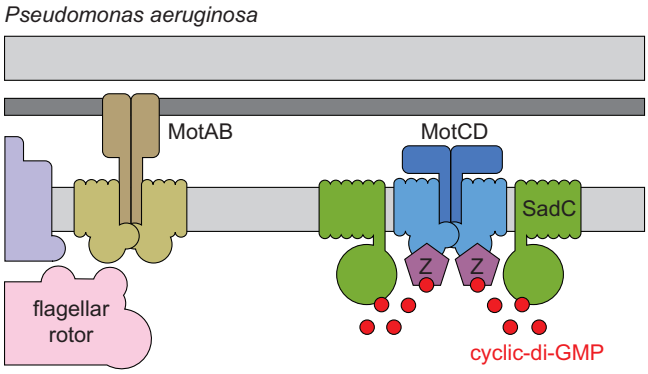

FIG 1 Model of the MotCD-SadC interaction. On the left is a cross section of the bacterial flagellum with the rotor (pink) and one torque-generating unit of MotAB (brown) associated. One MotCD complex (blue) is drawn free in the membrane associating with diguanylate cyclase SadC (green). SadC synthesizes c-di-GMP (red), which activates FlgZ (Z) to bind MotCD and sequester the complex from interacting with the flagellum.

phosphodiesterase) cause the protein FlgZ to bind to MotCD, inhibit MotCD association with the flagellum, and, as a consequence, inhibit swarming motility. In previous work, the authors showed that FlgZ-inhibited swarming could be rescued by mutating the MotAB complex and thus MotCD must have been relieved from inhibition (9). The present work by Baker et al. explains the swarming rescue in the absence of MotAB; MotCD fails to activate SadC, c-di-GMP levels drop, and FlgZ inhibition fails. Importantly, MotCD may fail to activate SadC because the absence of competition allows MotCD to interact with the flagellum and break the positive-feedback loop.

Positive feedback may occur because free MotCD in the membrane binds SadC, leading SadC to make c-di-GMP, which in turn activates the inhibitor FlgZ, which keeps MotCD free in the membrane. Under some conditions, the positive feedback may even make enough c-di-GMP to activate biofilm formation, but this evidently does not happen in planktonic swimming cells. While swimming cells likely have a pool of free MotCD due to competition between two sets of stators for a single flagellum, the level of free MotCD either does not initiate runaway feedback of SadC activation or the cytoplasmic levels of c-di-GMP simply do not rise to levels sufficient to inhibit swimming. Alternatively, some models suggest that c-di-GMP production, utilization and turnover are highly local, that MotCD may be a localizing factor, and thus that the positive feedback may occur but have limited range (10, 11) (Fig. 1). If c-di-GMP production, accumulation, and output are so local as to not leave a protein complex, why not inhibit stator function directly and omit the need for c-di-GMP as a soluble intermediate?

All told, the authors present a model for how stator occupancy at the flagellum indirectly impinges on c-di-GMP levels in a way that has the capacity to specifically inhibit swarming motility. To understand the dynamics of the system further, it will be important to determine the ratios of MotAB and MotCD relative to one another, relative to SadC, and relative to the flagellum. Moreover, if the system functions in wild-type cells, there is presumably a way to override the positive feedback and permit MotCD function under conditions that favor swarming. Perhaps these issues are related to why there are two different stators in the first place and how one stator set (MotCD) seems capable of generating greater torque despite the fact that both stator types operate on the same rotor. Whatever the details going forward, Baker et al., along with another recent report (8), illuminate the mechanisms by which flagella regulate cell physiology. The reports also highlight the difficulty in interpreting flagellum-related phenotypes because some phenotypes depend on which part within the structure/function of the machine is mutated.

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