

Insights into the Mechanism of Bacterial Flagellar Motor Rotation from the Structure and Dynamics

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The bacterial flagellum is a motility organelle, consisting of a long helical filament that extends in cell exterior to work as a propeller and a transmembrane basal body that functions as a rotary motor to drive high-speed rotation of the helical propeller. The flagellar motor consists of a rotor and a dozen stators and utilizes electrochemical gradient of cations, such as proton or Na^+ , across the cytoplasmic membrane for torque generation. The stator is a cation channel that couples ion flow with torque generation. Cyclic association and dissociation of the stator with the rotor has been observed as discrete stepwise rotation for artificially slowed motors rotating at 1 to 2 Hz, but its torque generation mechanism remains unknown because the dynamic process of torque generation observed as the step has been too fast to measure. We have established a nanophotometry system capable of determining the position of a 100 nm gold nanoprobe at 1 nm resolution for every 2.56 ms image frame so that stepping movements of a proton-driven flagellar motor rotating nearly at its maximum speed around 250 to 300 Hz can be measured. The step and dwell time are about 30 μs and 70 μs , respectively, and both of them distributed widely. A motor rotating slowly by a reduced intracellular pH to limit proton release rate from the stator channel to the cytoplasm shows prolonged dwell time but no significant change in step time, suggesting that proton translocation through the stator channel is coupled with association and dissociation of the stator and rotor but not directly with torque generation. Analysis of step time distribution and comparison of the rotational diffusion constant between the wild type motor and a stator-less mutant motor indicate the possibility that Brownian motion may be responsible for rotation period between consecutive dwells. We propose a thermal ratchet mechanism for flagellar motor rotation in which directionally asymmetric dissociation of the stator and rotor biases Brownian rotation of the rotor in one direction. A recent study on the structures of actomyosin in the strong and weak binding states gives a clue to directionally asymmetric dissociation.