

An electrostatic mechanism closely reproducing observed behavior in the bacterial flagellar motor

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A mechanism coupling the transmembrane flow of protons to the rotation of the bacterial flagellum is studied. The coupling is accomplished by means of an array of tilted rows of positive and negative charges around the circumference of the rotor, which interacts with a linear array of proton binding sites in channels. We present a rigorous treatment of the electrostatic interactions using minimal assumptions. Interactions with the transition states are included, as well as proton-proton interactions in and between channels. In assigning values to the parameters of the model, experimentally determined structural characteristics of

the motor have been used. According to the model, switching and pausing occur as a consequence of modest conformational changes in the rotor. In contrast to similar approaches developed earlier, this model closely reproduces a large number of experimental findings from different laboratories, including the nonlinear behavior of the torque-frequency relation in *Escherichia coli*, the stoichiometry of the system in *Streptococcus*, and the pH-dependence of swimming speed in *Bacillus subtilis*.

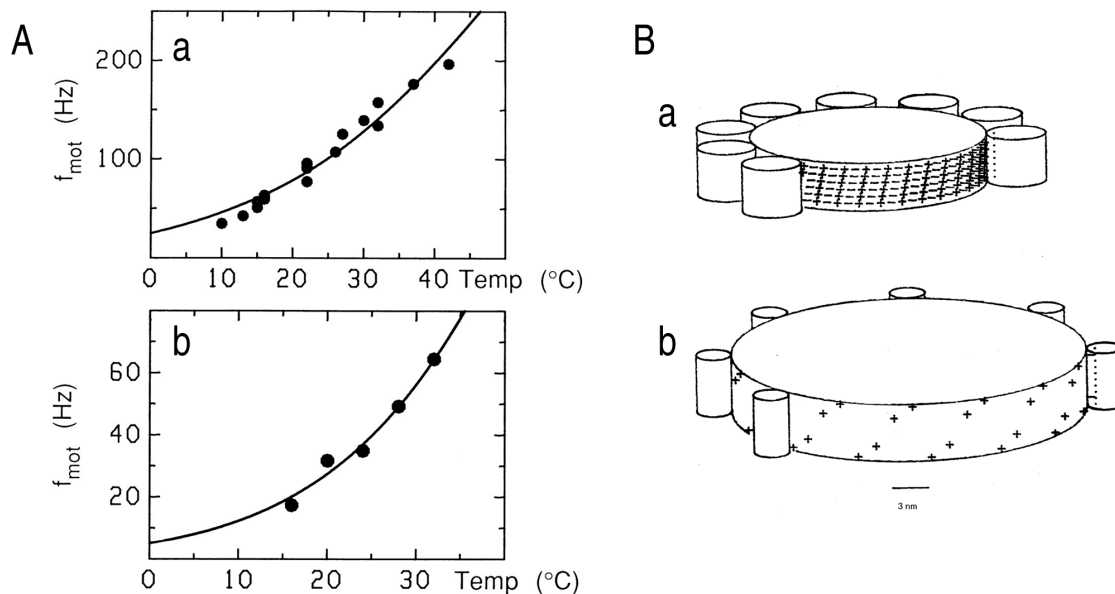


Fig. 1A An example of the model's capability. Motor frequency (f_{mot}) of freely swimming *Streptococcus* cells as a function of temperature. Data in (a) are taken from Lowe et al. (1987); $\text{pH}_{\text{ex}} = 7.5$, hence $\text{pH}_{\text{in}} = 7.5$ and $\psi_m = 132$ mV. Data in (b) are taken from Meister et al. (1987); $\text{pH}_{\text{ex}} = 7.02$, $\text{pH}_{\text{in}} = 7.62$, $\psi_m = 45$ mV.

The curves represent the results of simulation and analysis.

Fig. 1B A contrast: Two models of the electrostatic interactions in the flagellar motor of *E. coli*.

(a) Proton-driven model (Walz & Caplan, 2000). Two channels per force generating unit. Charge density on rotor is 1/3 of that available according to Kihara et al. (1989).

Tightly coupled. Predicts the measured stall torque and the stoichiometry.

(b) Proton-modulated ("ratchet") model (Elston and Oster, 1997). One channel per force generating unit.

Poorly coupled. Predicts a fraction of the measured stall torque and the stoichiometry.

Fig. 1B (a) above indicates the structure considered and Figs. 2A and 2B below the mode of calculation.

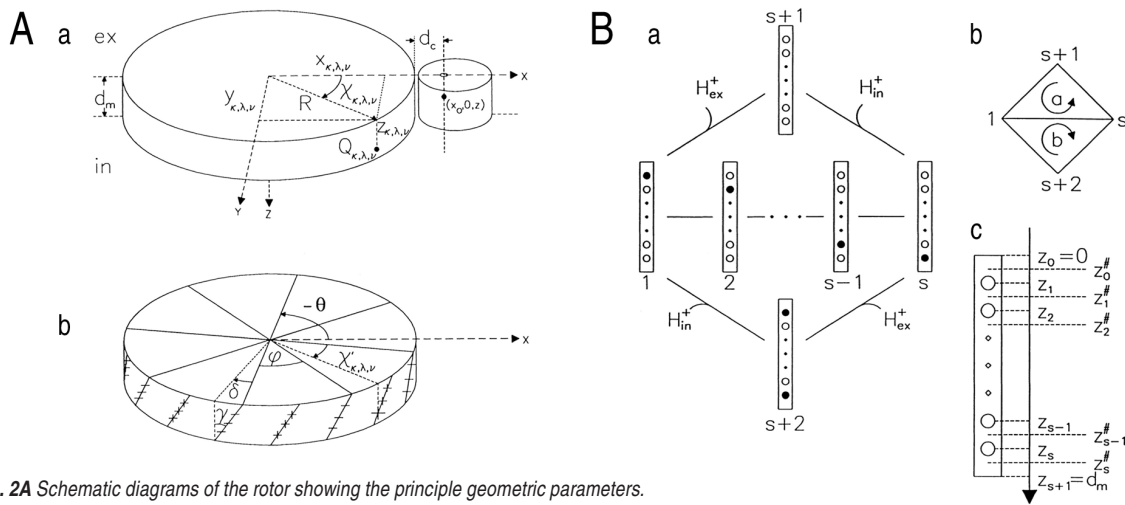


Fig. 2A Schematic diagrams of the rotor showing the principle geometric parameters.

(a) The rotor (radius R , thickness d_m), and one representative force-generating unit containing two channels (only one shown, at a distance d_c from the perimeter of the rotor), are situated within a rectangular coordinate system. The rod and hook to which the flagellar filament is attached project up from the center of the rotor in the $-z$ direction, toward the exterior of the bacterium. The coordinates of a particular position in the channel occupied by a proton are indicated, as well as those of a particular fixed charge $Q_{\kappa,\lambda,\nu}$ on the rotor (the κ th charge on a row in the λ th repeat with charge number ν).

(b) Eight representative repeat units are illustrated, with the angular position of the selected fixed charge relative to its repeat unit indicated. The angle of repeat is φ , the tilt and corresponding horizontal offset angles are γ and δ , respectively, and θ is the angle of rotation.

Fig. 2B Channel kinetic diagrams.

(a) Diagram showing the possible states of a channel following proton binding from, or release to, the periplasmic space or the cytoplasm, and internal proton transitions. Each line represents a forward and backward reaction to which a rate constant α_{ij} is assigned. The channel has s binding sites numbered in sequence from the cell exterior to the cell interior, and each state is labeled by the number of the site occupied. The empty state is designated $s+1$, and the only state considered having more than 1 bound proton is designated $s+2$.

(b) Schematic cycle diagram showing the two constituent cycles designated a and b, and the sign convention adopted for the cycle fluxes.

(c) The positions (along the z coordinate) of the i th proton binding site z_i and the i th transition state $z_i^{\#}$ in the channel.

Summary and conclusions

A ratchet-free electrostatic model of the bacterial rotor motor has been examined critically. The generality of the model, which is tightly coupled, is underscored by the large number of diverse experimental data that can be simulated using a single set of parameter values. The window in the parameter space is rather narrow, and characteristic features such as the plateau in the torque-frequency relationship or the dependence of motor torque on pH_{ex} are lost if values outside this window are chosen. Where possible, values of parameters were deduced from structural characteristics of the motor, thus taking species specific aspects into account. Switching the direction of rotation requires a conformational transition (20° change in the rotor tilt angle γ , from $+10^\circ$ to -10°). Values of the frictional drags computed from simulations of the measured torque-frequency curves of tethered cells are in line with published data. (In collaboration with Dr. Dieter Walz).

Selected Publications

- Prasad, K., Caplan, S.R., and Eisenbach, M. (1998) Fumarate modulates bacterial flagellar rotation by lowering the free energy difference between the clockwise and counterclockwise states of the motor. *J. Mol. Biol.* 280, 821-828.
- Walz, D. and Caplan, S.R. (1998) An electrostatic model of the bacterial flagellar motor. *Bioelectrochem. Bioenergetics* 47, 19-24.
- Eisenbach, M. and Caplan, S.R. (1998) Bacterial chemotaxis: Unsolved mystery of the flagellar switch. *Curr. Biol.* 8, R444-R446.
- Caplan, S.R. and Walz, D. (1999) The bacterial flagellar motor: a brief review of models, and a new electrostatic model. In: Bersani, F. (ed.) "Electricity and Magnetism in Biology and Medicine", Plenum, N.Y., pp. 251-254.
- Walz, D. and S.R. Caplan. (2000) An electrostatic mechanism closely reproducing observed behavior in the bacterial flagellar motor. *Biophys. J.* 78, 626-651. (Includes other references cited)