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Rotary macromolecular machines: is stepping necessarily a characteristic of rotational movement?

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Molecular motors are widely considered to function differently from ordinary motors, because of their supposedly universal use of ratchet mechanisms to convert momentum from random environmental influences into organized motion. Regarding rotary motors, consider the H^+/ATP synthase and the bacterial flagellar motor, both of which couple the flow of protons through transmembrane channels to a rotation. In the case of the H^+/ATP synthase smooth rotation is not observed, and here the most probable mechanism does seem to be a Brownian ratchet. But in the case of the flagellar motor we find that this type of mechanism is unlikely to be responsible for the rotation, in spite of existing experimental studies apparently demonstrating that the motor is a "Poisson stepper". These were based on a fluctuation analysis of the rotation: the variance in time taken for a given number of rotations was found to be inversely proportional to the second power of the frequency of rotation, which on theoretical grounds was considered to be a consequence of Poisson stepping. We have shown that this behavior is a necessary but not sufficient condition for a Poisson stepper, if indeed such a hypothetical mechanism exists at all. Hence it is not a suitable criterion for stepping; in fact a non-stepping mechanism can comply with this criterion, while stepping mechanisms mostly do not. A ratchet-free electrostatic model, which does not step and closely reproduces a wide variety of kinetic data, exhibits the identical stochastic behavior and hence may well reflect the real machinery.

Evidence for stepping in the bacterial flagellar motor

The evidence primarily derives from studies based on a model comprising a rate-limiting biochemical step linked to an elementary angular step ϕ of an unspecified element around the periphery of the rotor, which results in an *equal* increment in rotation angle $\Delta\theta$. Thus the Poissonian distribution of the biochemical events is directly translated into a Poissonian distribution of steps in rotation angle. It was shown theoretically that the variance in the time taken for n revolutions at a given rotational frequency f, $V(n,f)$, is then inversely proportional to the second power of f. Since the variance measurements obtained by imposing a variable external torque on tethered *Escherichia coli* cells conformed with this prediction, it was concluded that the observations were consistent with a Poissonian stepping mechanism using about 400 steps per revolution.

However, our studies have shown that a Poissonian stepper must be insensitive to external torque and these observations do not in fact exclude smooth rotation.

Analysis and simulations

This issue may be clarified by analysing a stepping model in which explicit consideration is given to the relaxation of the stepping units. The elastic behavior of the stepping units is described by

$$T_j = -\sigma \xi_j,$$

where σ is an elasticity coefficient reflecting bending of the stalk, and T_j is the torque exerted on the rotor by the jth unit due to its angular displacement ξ_j from its rest position (Hooke's Law). It emerges that Poissonian behavior can only occur if σ is larger than a critical value, so that relaxation of all units is completed after each step– but then $\Delta\theta$ becomes insensitive to the externally imposed torque T_{ex} .

For example, considering a rigid tether for simplicity, let ξ_{j0} denote the angular displacement *just prior* to the stepping of any one of the units. Then the average displacement just after the step is

$\langle\xi\rangle = \langle\xi\rangle_0 \pm \phi/u$,
where
$\langle\xi\rangle_0 = \Sigma_j \xi_{j0}/u$,

and u is the number of units. The change in rotation angle due to relaxation of $\langle\xi\rangle$ during the variable time interval Δt between steps is

$$\Delta\theta = [T_{ex}/(u\sigma) + \langle\xi\rangle]\,[1-\exp(-\Delta t/\tau)],$$

where $\tau = kT/(u\sigma D)$ is the rotational relaxation time and D the rotational diffusion coefficient of the cell body. Large σ means small τ *and* $T_{ex}/(u\sigma)$. (Note that measurements are usually made on tethered cells, and twisting of the tether does not alter these conclusions.)

The rate of stepping is given by an intrinsic rate constant α_0 , modified by a Boltzmann factor accounting for the electrochemical free energy and elastic changes involved in the step. Accordingly, it turns out that the jth unit can step in either direction with rate constants

$$\alpha_{j+} = \alpha_0 \exp\{\lambda[\Delta\tilde{\mu}_{H+} - \sigma\phi\ (\xi_{j0}+\phi/2)]/kT\}$$

and

$$\alpha_{j-} = \alpha_0 \exp\{(\lambda-1)[\Delta\tilde{\mu}_{H+} - \sigma\phi\ (\xi_{j0}-\phi/2)]/kT\},$$

which is consistent with recent studies of a 3-state stepping model. The coefficient λ reflects the position of the transition state in the step, and $\Delta\tilde{\mu}_{H+}$ is the protonmotive force. Poissonian stepping must be unidirectional, *which can only occur in the limit* $\lambda=0$.

Rotation angles as a function of time were obtained by Monte Carlo simulation, taking into account exclusion of attachment points due to steric hindrance. Variances were fitted to the relation (see Fig. 1)

$$V(n,f) = A/f^m.$$

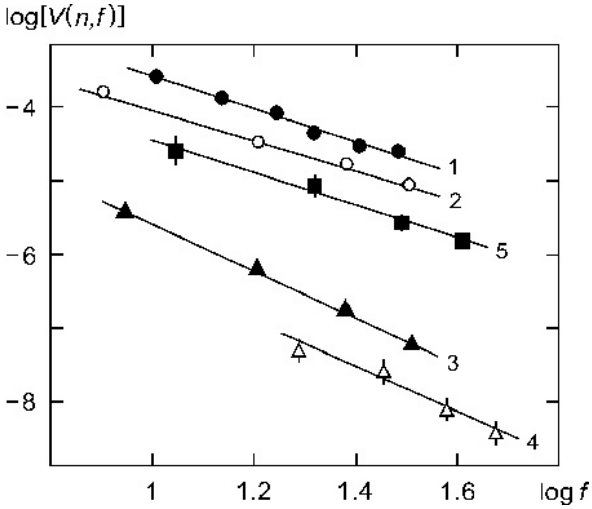
For a Poissonian stepper, $m=2$ *exactly* and $A=n/k$, where k denotes the number of elementary steps per revolution. Moreover, k is given directly by

$k = \langle t_n\rangle^2 / [nV(n,f)]$ at any value of f, where t_n is the time taken for n revolutions, and an average value $\langle k\rangle$ can then be calculated from measurements at several values of f.

Conclusion

The non-stepping electrostatic model of the flagellar rotary motor gives the best overall account of experimental data to date.

(In Collaboration with Dr. Dieter Walz, University of Basel.)



1	Experimental data*: m = 2.25, $\langle k\rangle$ = 414; Tex varied	–
2	Elastic model (limiting case): m = 2.03, $\langle k\rangle$ = 421; α_0 varied	+
3	Elastic model (general case): m = 3.22; T_{ex} varied	–
4	Three-state stepping model: m = 3.02; T_{ex} varied	–
5	Electrostatic model: m = 2.16; T_{ex} varied	–

Fig. 1 *Analysis of V(n,f), the variance in time for n revolutions at a frequency f, for experimental data and 4 models (Poissonian +, Non-Poissonian –)*

N.B. Curves 2 - 5 are vertically displaced to avoid overlap

*The most persuasive data set

Selected Publications

Walz, D. and Caplan S.R. (2000) An electrostatic mechanism closely reproducing observed behavior in the bacterial flagellar motor. Biophys. J., 78, 626-651.