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Bacterial flagellar motor and H^+/ATP synthase: two proton-driven rotary molecular devices with different functions

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Abstract

Both the bacterial flagellar motor and the H^+/ATP synthase are membrane-bound macromolecular complexes in which the movement of protons through channels across the membrane is coupled to the rotation of a part of the complex around an axis perpendicular to the membrane. Despite this similarity, the two devices are designed for quite different functions. The flagellar motor is responsible for a practically smooth rotation of the flagellar filament in order to propel the cell. Smooth rotation is not essential for the H^+/ATP synthase, which accumulates torque by twisting a rod-shaped structure. Possible mechanisms for generating torque in the two devices are presented, based on the models which have been proposed. The performances of the various mechanisms are discussed.

Keywords: Torque generation; Proton turbine; Brownian ratchet

1. Introduction

The bacterial flagellar motor and the H^+/ATP synthase (also known as F_0F_1 ATP synthase) are membrane-bound macromolecular complexes with rotatory components. In both cases, a transmembrane flow of protons driven by their electrochemical potential difference, $\Delta\tilde{\mu}_H$, gives rise to the rotation. Despite this similarity, the two devices are designed for quite different functions (see Table 1). The flagellar motor is responsible for a practically smooth rotation of the flagellar filament in order to propel the cell (for a review, see, e.g. Ref. [1]). Smooth rotation is not essential for the H^+/ATP synthase, which accumulates torque by twisting a rod-shaped structure. Discharging this torque by rotation over an angle of 120° then provides the energy for the synthesis of an ATP molecule (for recent reviews see Refs. [2,3]).

Fig. 1 shows a schematic representation of the two devices. The common feature of both complexes is a disk-like structure (the so-called rotor) which is known to rotate relative to an adjacent structure (the so-called stator). It is seen that the dimensions of the flagellar motor are larger than those of the H^+/ATP synthase. Moreover, the flagellar motor has an array of stator elements, while the H^+/ATP synthase has only one. In both cases, protons are assumed to flow through channels in the stator elements. The coupling of linear movement (proton flow) and rotational movement in a perpendicular plane requires an anisotropic configuration and an interaction between the coupled processes. The configurational requirements can be fulfilled either by a tilted arrangement of the interacting elements on the rotor, or by two laterally displaced half-channels for protons in the stator. The interaction could be either electrostatic (long-range) or molecular (short-range).

2. Bacterial flagellar motor

The two earliest models treated quantitatively were based on molecular interaction, i.e. binding of stator elements to the rotor; however, one of these used laterally displaced half-channels [4], while the other used a tilted arrangement of binding sites [5]. Neither model has ever been shown to be able to reproduce the wealth of diverse experimental data available at present (see, e.g. Refs. [5,6]). Other models that have been described have the same drawback (reviewed in

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Table 1
Comparison of proton driven rotary molecular devices; $\Delta\tilde{\mu}_H$ and A_{phos} are the thermodynamic forces for proton flow and ATP synthesis, respectively

	Bacterial flagellar motor	H^+/ATP synthase
Function	rotate flagellar filament	generate torque for ATP synthesis
Maximal torque (nN nm/rad)	1.2	0.04
Typical frequencies (Hz)	0–300 [1000]	30–70
Sense of rotation	in both directions with same polarity of $\Delta\tilde{\mu}_H$ (switching)	determined by $\Delta\tilde{\mu}_H$ and A_{phos}
Most likely mechanism	proton turbine	Brownian ratchet
Anisotropy	tilted rows of charges on rotor	displaced half-channels in stator
Interaction	electrostatic (generates torque)	electrostatic (barrier for ratchet)

Refs. [7,8]). The binding model has recently been revived [9], but need not be considered here because it is purely phenomenological and, thus, does not provide any mechanistic insight.

The high density of positive and negative charges found associated with one constituent of the rotor [10] suggests that the interaction may be electrostatic in nature. This is the basis of the two most recent attempts to model the flagellar motor [11,12]. Both assume a helical array of fixed charges on the rotor, thus fulfilling the configurational requirement of a tilted arrangement of interacting elements. However, in one case, the rotor carries alternating rows of positive and negative charges, while the stator need not carry any charges (Fig. 2A). Protons bound to any site in a channel interact with the electric field generated by the rotor charges, thus giving rise to a torque. This torque vanishes in certain configurations of rotor and stator which, however, is overcome by the continuous movement of the protons through the channels driven by $\Delta\tilde{\mu}_H$ [11]. In the other case, the rotor and the stator carry positive and negative charges, respectively, giving rise to a torque even in the absence of protons in the channels (Fig. 2B). Again, torque vanishes in certain configurations of rotor and stator. This is overcome by proton binding next to a negative charge on the stator, which is then neutralized and allows torque to be manifested again [12].

The electrostatic energy arising from the interaction between protons and fixed charges on the rotor and stator governs the kinetics of the proton transitions within the channels, and between bulk phases and channels. The pertinent energy profiles show that, in the first case, the proton has to cross only minimal barriers between binding sites [11]. As a consequence, the movement of protons is almost synchronous with the movement of the rotor. This is reminiscent of a hydraulic reaction turbine; electrostatic interaction performs the function of the blades of the propeller, and proton flow is equivalent to the water flow. Hence, we will refer to this model as a ‘‘proton turbine’’. In the second case, the proton has to cross a formidable barrier between the two binding sites [12]. As a consequence, proton and rotor movements are not necessarily synchronous. The role of the protons is to control interactions between the charges on the rotor and on the stator, thus modulating a ratchet effect, which governs a directed motion of the rotor. Hence, we may call this model an ‘‘electrostatic ratchet’’.

The performance of the two models is quite different. The proton turbine model is almost completely coupled, and yields the correct torque (about 0.3 and 1 nN nm/rad for swimming and tethered cells, respectively) and proton stoichiometry (1300 H^+/rev for *Streptococcus*). The electrostatic ratchet model is much less tightly coupled; it yields a torque and a stoichiometry which are twofold to fourfold too low and an order of magnitude too low, respectively.

3. H^+/ATP synthase

In the case of the H^+/ATP synthase, the most detailed model is based on a mechanism involving electrostatic processes [13]. The rotor carries negatively charged binding sites for protons, which are exposed to a region of low-dielectric constant (membrane lipids) except when they are juxtaposed against the single stator unit, which provides a zone of higher dielectric constant. Hence, only protonated binding sites can move into the low-dielectric region. The stator accommodates two binding sites simultaneously. Configurational anisotropy is introduced by offset half-channels in the stator. Accordingly, the two sites can only be deprotonated to (or protonated from) opposite sides of the membrane (see Figs. 2C and D).

If either or both of the binding sites facing the stator become deprotonated, movement of the rotor due to Brownian motion is restricted since deprotonated binding sites cannot leave the stator zone (Fig. 2C), a situation equivalent to an engaged ratchet. Protonation of a binding site allows the rotor to move that site out of the stator zone, thus releasing the ratchet (Fig. 2D). Unequal probabilities of protonation of the two sites due to $\Delta\tilde{\mu}_H$ introduces a bias in the otherwise random fluctuation of the rotor, which tends to wind up the rod attached to it (see Fig. 1B). The accumulated torque in the rod introduces a bias in the opposite direction. Since the proton-operated ratchet regulates Brownian motion, we will refer to this model as a ‘‘Brownian ratchet’’.

A detailed quantitative analysis [14] shows that this mechanism is feasible and able to accumulate the requisite

torque. This can be estimated by taking into account that the discharge of this torque by rotation over an angle of 120° must provide the energy for the synthesis of an ATP molecule. This sets an upper limit for the accumulated torque of about 0.04 nN nm/rad for an extreme value of the affinity of ATP synthesis $A_{phos} = -50$ kJ/mol. It should be added that there are additional fixed charges on the rotor and stator. These modulate proton binding (as in the case of the evidently essential stator Arg 210) and give rise to contributions to torque. Insufficient experimental data are available to permit comparison with simulations of this model. However, if a simplified version is combined with a kinetic description of the catalytic activity of the H^+/ATP synthase, experimental data can be successfully simulated [15,16].

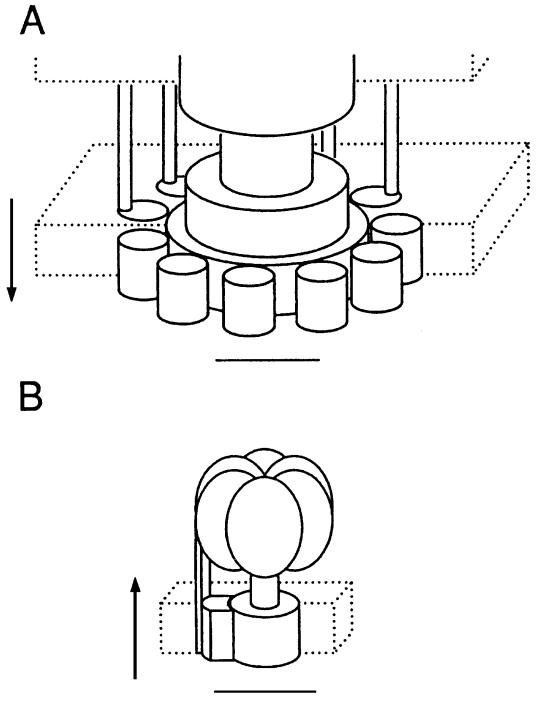

Fig. 1. Schematic representations of two rotary molecular devices. (A) Bacterial flagellar motor. The two disk-like structures called the S and M rings constitute the rotor. The rod attached to the S ring is the shaft of the motor. It passes through a structure consisting of the so-called P and L rings, which is anchored to the cell wall (dotted line) and acts as a bearing. Note that only part of the P ring and the peptidoglycan layer of the cell wall are shown; hence, the filament, which protrudes out of the cell and is connected to the shaft via a hook, cannot be seen. The M ring is adjacent to the cell membrane (dotted line) and surrounded by a number of cylindrical structures (11 in the case of *Escherichia. coli* shown). They are formed by the protein called MotA and are connected to the peptidoglycan layer of the cell wall by stalks consisting of the protein called MotB. Together, they form the stator of the motor. A bell-shaped structure responsible for switching, called the C ring, which is attached to the M ring and protrudes into the cytoplasm, is omitted for clarity. (B) The H^+/ATP synthase consists of two subcomplexes known as F_0 and F_1. F_0 is embedded in a membrane (dotted line), which can be the cell membrane of a bacterium, the inner membrane of a mitochondrion, or the thylakoid membrane of a chloroplast. It comprises a disk-like structure which acts as rotor and is built from several copies (usually 12) of the c-subunit. The stator is formed by the a-subunit adjacent to the rotor. F_1 contains the catalytic site for ATP synthesis and consists of three copies each of the α- and β-subunit in an alternating arrangement (symbolized by the ellipses). The rod attached to the rotor consists of the γ- and ε-subunit of F_1 and acts as the shaft of the motor; its bearing is in the top of the α,β-arrangement. Note that the a-subunit is connected by a stalk (b-subunit of F_0 and δ-subunit of F_1) to F_1, which is essential for its role as a stator. In (A) and (B), the bar represents 10 nm, and the arrow indicates the polarity of $\tilde{\mu}_H$ by pointing from the higher to the lower value of $\tilde{\mu}_H$.

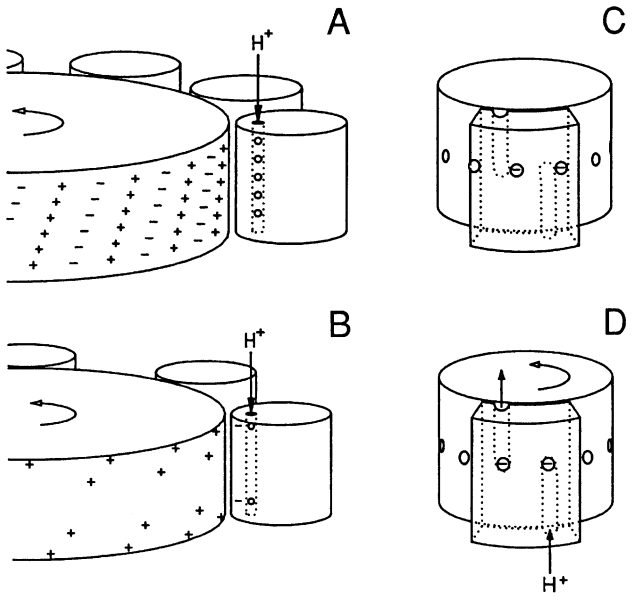

Fig. 2. Schemes illustrating molecular mechanisms. (A) Proton turbine model for flagellar motor. The rotor carries tilted alternating rows of positive and negative charges. Protons pass through channels (dotted line) in the stator via five binding sites (circles). Their interaction with the electric field generated by the rotor charges gives rise to torque. Note that, for graphical reasons, only one of the two channels per stator element and half of the tilted rows used in the model are shown. (B) Electrostatic ratchet model for flagellar motor. The rotor carries tilted rows of positive charges, while the stator contains two negative charges. Torque arises from electrostatic interaction, which is modulated by protons passing through channels with two binding sites close to the negative charges. (C, D) Brownian ratchet model for H^+/ATP synthase. (C) The rotor carries proton binding sites (circles) whose deprotonated form is negatively charged and, hence, cannot leave the interface between stator (shown in front) and rotor. This region has a higher dielectric constant than the membrane adjacent to the rest of the rotor surface. (D) Protonation and deprotonation occur through displaced half-channels (dotted lines) which open to opposite sides of the membrane.

4. Conclusions

The survey presented above leads us to conclude that the proton turbine model most likely reflects the molecular mechanism of the flagellar motor. So far as it has been tested, it closely reproduces a variety of experimental findings from different laboratories [11], and satisfies critical behavioral criteria set forth in Ref. [17]. On the basis of

the indirect evidence mentioned above, it seems that the mechanism of the Brownian ratchet model for the H^+/ATP synthase is probably correct.

One may ask why nature has developed two separate mechanisms for rotary devices which seemingly perform the same task. The answer may be found in the different functions they are called upon to fulfil. The flagellar motor is responsible for the rotation of the flagellar filament in order to propel the cell. This necessitates a maximal torque and frequencies which are considerably larger than those required for ATP synthesis (see Table 1). While the Brownian ratchet generates sufficient torque for ATP synthesis, it is highly unlikely that a flagellar motor operating on a Brownian ratchet principle could be developed. Thermodynamic considerations show that:

$$T_i \delta\phi_i \leq \Delta\tilde{\mu}_H \qquad i = \text{A or M}, \tag{1}$$

where T_i denotes the torque generated due to proton flow driven by $\Delta\tilde{\mu}_H$, and $\delta\phi_i$ is the angle between the radii pointing to adjacent binding sites. The index, *i*, indicates the H^+/ATP synthase (A) or a *hypothetical* Brownian ratchet motor (M). The molecular structure carrying a binding site extends over a length, *l*, on the circumference of the rotor (radius, R_i), which cannot be diminished and amounts to:

$$l = R_A \delta\phi_A = R_M \delta\phi_M. \tag{2}$$

Combining Eqs. (1) and (2) yields:

$$R_M \geq R_A \delta\phi_A T_M / \Delta\tilde{\mu}_H. \tag{3}$$

With the parameter values $\Delta\tilde{\mu}_H = 15$ kJ/mol = 0.025 nN nm/proton, $R_A = 3.5$ nm, and $\delta\ \phi_A = \pi/6$ (30°, 12 binding sites), we find $T_A \leq 0.048$ nN nm/rad (Eq. (1)), which indeed exceeds the maximal torque necessary for ATP synthesis (see Table 1). However, with $T_M = 1.2$ nN nm/rad, it follows that a hypothetical Brownian ratchet motor should have a radius $R_M \geq 88$ nm (Eq. (3)), which is six times larger than the radius of the largest motor known (*Salmonella typhimurium*), or $25 \times R_A$. Hence, Brownian motion would be about 600-fold reduced as compared to that of the rotor of the H^+/ATP synthase, and such a motor would be rather slow. Adding more stator elements would decrease the radius, but require that they act in synchrony. Such a motor would still be slow since protonation of all pertinent binding sites at the same time is not a frequent event. Moreover, the phenomenon of switching mentioned in Table 1, which is essential for bacterial motion, would be difficult to realize with a Brownian ratchet mechanism.

References

[1] C.S. Schuster, S. Khan, The bacterial flagellar motor, Annu. Rev. Biophys. Biomol. Struct. 23 (1994) 509–539.
[2] R.K. Nakamoto, C.J. Ketchum, M.K. Al-Shawi, Rotational coupling in the F_0F_1 ATP synthase, Annu. Rev. Biophys. Biomol. Struct. 28 (1999) 205–234.
[3] H. Noji, M. Yoshida, The rotary machine in the cell, ATP synthase, J. Biol. Chem. 276 (2001) 1665–1668.
[4] H.C. Berg, S. Khan, A model for the flagellar rotary motor, in: H. Sund, C. Veeger (Eds.), Mobility and Recognition in Cell Biology, DeGruyter, Berlin, 1983, pp. 485–497.
[5] P. Läuger, Torque and rotation rate of the bacterial flagellar motor, Biophys. J. 53 (1988) 53–65.
[6] M. Meister, S.R. Caplan, H.C. Berg, Dynamics of a tightly coupled mechanism for flagellar rotation, Biophys. J. 55 (1989) 905–914.
[7] S.R. Caplan, M. Kara-Ivanov, The bacterial flagellar motor, Int. Rev. Cytol. 147 (1993) 97–164.
[8] R.M. Berry, Theories of rotary motors, Philos. Trans. R. Soc. London, Ser. B 355 (2000) 503–509.
[9] W.S. Ryu, R.M. Berry, H.C. Berg, Torque-generating units of the flagellar motor of *Escherichia coli* have a high duty ratio, Nature 403 (2000) 444–447.
[10] M. Kihara, M. Homma, K. Kutsukake, R.M. Macnab, Flagellar switch of *Salmonella typhimurium*: gene sequence and deduced protein sequences, J. Bacteriol. 171 (1989) 3247–3257.
[11] D. Walz, S.R. Caplan, An electrostatic mechanism closely reproducing observed behavior in the bacterial flagellar motor, Biophys. J. 78 (2000) 626–651.
[12] T.C. Elston, G. Oster, Protein turbines: I. The bacterial flagellar motor, Biophys. J. 73 (1997) 703–721.
[13] W. Junge, H. Lill, S. Engelbrecht, ATP synthase: an electrochemical transducer with rotary mechanics, Trends Biochem. Sci. 22 (1997) 420–423.
[14] T. Elston, H. Wang, G. Oster, Energy transduction in ATP synthase, Nature 391 (1998) 510–513.
[15] D.A. Cherepanov, A.Y. Mulkidjanian, W. Junge, Transient accumulation of elastic energy in proton translocating ATP synthase, FEBS Lett. 449 (1999) 1–6.
[16] O. Pänke, B. Rumberg, Kinetic modeling of rotary CF_0F_1-ATP synthase: storage of elastic energy during energy transduction, Biochim. Biophys. Acta 1412 (1999) 118–128.
[17] H.C. Berg, Constraints on models for the flagellar rotary motor, Philos. Trans. R. Soc. London, Ser. B 355 (2000) 491–501.