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F_1-ATPase: A Rotary Motor Made of a Single Molecule

Minireview

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The bacterial flagellar motor (DeRosier, 1998) has long been the sole rotary mechanism known in the biological world. While we might call some movements in the body "rotation", the continuous nature of our joints does not allow true rotation, which requires separation of the two parts in order to achieve sliding of one part against the other over all angles. At the level of molecules, however, sliding is commonly encountered, and repeated use of identical subunits often leads to formation of helical structures, including cylinders and rings, which can support rotational motions. A helical actin filament sliding past myosin, for example, has been shown to rotate (Nishizaka et al., 1993), albeit inefficiently (Sase et al., 1997). It is quite possible that rotating molecular machines have simply been overlooked, due to the technical difficulties of detecting molecular rotations. Recently, a new face has joined the class of circularly rotating machines, second to the bacterial flagellar motor. A single molecule of F_1-ATPase, a portion of ATP synthase, is by itself a rotary motor in which a central rotor, made of a γ subunit, rotates over unlimited angles against a surrounding stator cylinder of an $\alpha_3\beta_3$ hexamer (Noji et al., 1997; Figure 1). At a size of ~10 nm, it is the smallest rotary motor ever found.

ATP Synthase

ATP, a major currency of energy, is synthesized by ATP synthase. This enzyme is composed of a membrane-embedded, proton-conducting portion, F_0, and a protruding portion, F_1 (Figure 1). When protons flow through F_0, ATP is synthesized in F_1. The synthase is fully reversible in that hydrolysis of ATP in F_1 drives reverse flow of protons through F_0. Isolated F_1 catalyzes only hydrolysis of ATP, and hence is called the F_1-ATPase.

How is the proton flow through F_0 coupled to the synthesis/hydrolysis of ATP in F_1? Almost 20 years ago Paul Boyer made a radical proposal that the two reactions are mechanically coupled by rotation of a common shaft penetrating F_0 and F_1 (see Boyer, 1997). Part of his reasoning was that F_1 contains three catalytic sites, one on each β, which participate on average equally in ATP synthesis/hydrolysis. The γ subunit, known to be adjacent to β, lacks 3-fold symmetry. For γ to touch the three β's impartially, therefore, it has to rotate. F_0 may also be a rotary motor if likened to the bacterial flagellar motor which is driven by the flow of protons. In this view, the ATP synthase comprises two motors, one ATP-driven and the other proton-driven, with a common shaft of which γ is a major part. Rotation in one direction produces ATP, and ATP hydrolysis causes reverse rotation.

Boyer's model gained support when a crystal structure of F_1 was solved by John Walker and colleagues (Abrahams et al., 1994). Importantly, the three β's carried different nucleotides in the crystal, AMPPNP (an ATP analog), ADP, and none in the clockwise order when viewed from the F_0 side (Figure 1, bottom). If hydrolysis were to proceed, the order in the next step would be ADP, none, and ATP. A face of γ opposing the empty β, for example, would thus turn counter-clockwise. In the crystal, the conformation of empty β was noticeably different from those bearing a nucleotide.

Cross-linking and spectroscopic studies have since given strong evidence favoring rotation of γ (Junge et al., 1997, and references therein): a residue on γ could be cross-linked to different β's only when ATP was hydrolyzed/synthesized, and an optical probe attached to

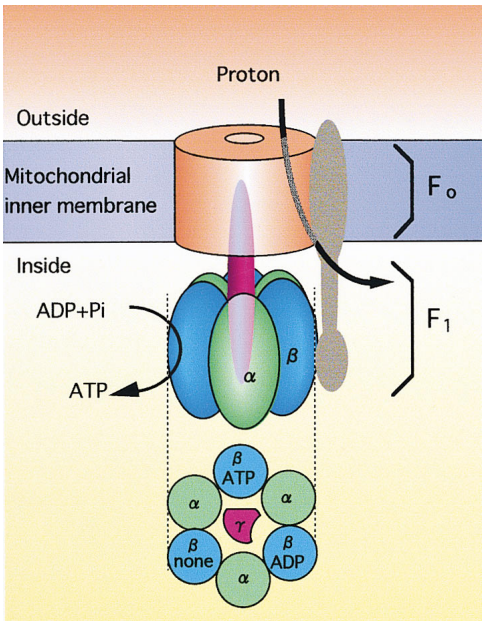

Figure 1. Simplified Structure of ATP Synthase

F_1 consists of $\alpha_3\beta_3\gamma\delta\epsilon$ (δ and ϵ are not shown), and the simplest composition of F_0 (not mitochondrial) is ab_2c_{9-12}. The gray portion indicates a suggested location of δab_2.

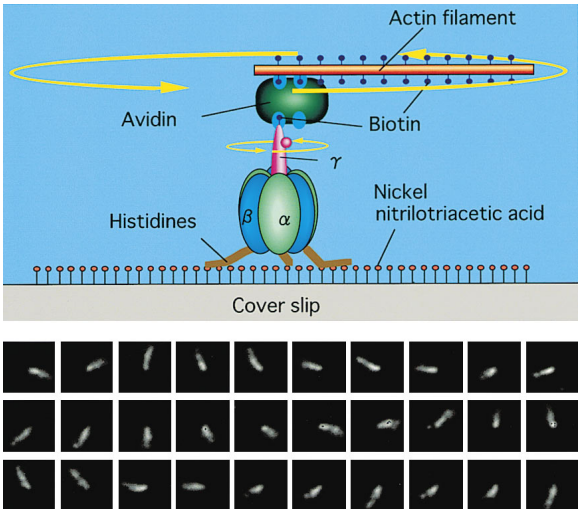

Figure 2. Observation of F_1 Rotation

Video images of a rotating actin filament at intervals of 133 ms are shown at the bottom.

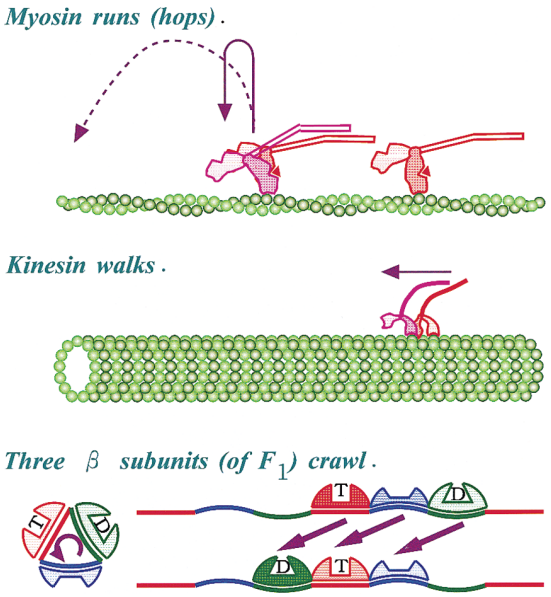

Figure 3. Mode of Operation of Various Molecular Motors

See text for further description. T, ATP; D, ADP. The β subunits of F_1 slide across unrolled γ with the forefoot in front and the hindfoot at back. Note that the different colors of the three β's indicate differences in the bound nucleotide and their nucleotide-dictated conformations (and not their identity). The surfaces of γ that would match the three β's conformations are colored accordingly. The heavy, medium, and light stippling of the β subunits indicate their respective identity. All three β's move simultaneously as shown by the arrows. The scheme here is simplified and the bound nucleotides on the three β's may not change simultaneously. In addition to its bound nucleotide, the conformation of each β should depend on the surface of γ it faces (and indirectly the conformations of the other two β's).

γ underwent large-amplitude rotations. However, the questions of whether γ continues to rotate in one direction, and, if so, its direction, were not addressed in these studies.

Rotation of F_1 Has Been Videotaped

One way of detecting molecular rotation is to attach a large tag that is readily visible under an optical microscope. Noji et al. (1997) attached a fluorescently labeled actin filament to the γ subunit of F_1 (lacking δ and ε subunits) through a streptavidin-biotin link (Figure 2). The β subunits were bound to a glass surface through histidine tags engineered at the N termini. When ATP was added, the filament rotated, invariably counterclockwise as anticipated from the crystal structure. The rotation continued for many minutes at a speed of several revolutions per second. Based on the rate of ATP hydrolysis measured in solution (on the order of 10^2/s), and the assumption of the hydrolysis of three ATP molecules per revolution, the rotational speed might have been predicted to be much higher.

The observed rotational speed was, in fact, quite high when taking into account the hydrodynamic friction against the rotating actin filament. If F_1 were scaled to the size of a person, the person would be standing at the bottom of a large swimming pool rotating an ~500 m rod at several revolutions per second! The F_1 was really working at full throttle. The torque the molecular F_1 produced to overcome the friction amounted to ~40 pN · nm over a broad range of rotational speed (Noji et al., 1997). This torque times $2\pi/3$ (=120°), ~80 pN · nm, is the mechanical work done in one third of a revolution. This work is comparable to the free energy of hydrolysis of one ATP molecule of ~80 pN · nm. If one ATP is consumed per 120° as one may anticipate from the make of this motor, the efficiency of our F_1 is nearly 100%, far superior to a Honda V6. A model by Oosawa and Hayashi (1986) has predicted such a high efficiency.

F_0 Awaits Experimental Proof of Rotation

Relatively little is known about the putative proton-driven motor F_0. In *E. coli*, the subunit composition of F_0 is believed to be ab_2c_{9-12} (the number of c subunits is yet uncertain). Models have been proposed (Junge et al., 1997; Elston et al., 1998) in which a ring of c_{9-12} is attached to γ and the two together constitute the common shaft; ab_2, bound to $\alpha_3\beta_3$ through δ, extends to the c ring and serves as the stator for ring rotation (Figure 1, gray part). Supportive (but not yet conclusive) evidence exists, but the models detract from the elegance in the Boyer's proposal in that the presumably symmetric ring of c's is attached to the asymmetric γ, and δab_2 rests on one side of the symmetric $\alpha_3\beta_3$. Whether the c ring really rotates with γ remains to be seen (videotaped?).

Comparison of Nucleotide-Driven Molecular Motors

In Figure 3, we compare the different ways that molecular motors use their multiple "feet." Myosin is a linear motor that "runs" along actin, in that its two feet (the two globular parts of myosin usually referred to as "heads") are detached from actin for most of the time (Sase et al., 1997). In fact, myosin can run, skipping many actin monomers in a step, if other myosin molecules pull the actin filament while the first one is detached. If only one myosin molecule interacts with actin, it simply hops and will not move relative to actin while detached (except for random diffusion). Kinesin, on the other hand, appears to "walk" along a microtubule, without detaching its two feet simultaneously and probably using

Table 1. Comparison of Nucleotide-Driven Motors

Motor/Rail	Step Size	Max. force	Max. Efficiency	Processivity	Mode
Myosin/Actin	variable	3–5 pN	~20%	none–poor	runs (hops)
Kinesin/Microtubule	8 nm	5 pN	~50%	good	walks
RNA polymerase/DNA	0.34 nm?	14 pN	~20%	excellent	crawls?
$F_1\beta/F_1\gamma$	120°	40 pN · nm	~100%	perfect	crawls
(at the radius of 1 nm)	(2 nm)	(40 pN)			

its two feet in an alternate fashion (Block, 1998, and references therein). F_1 could be unrolled, conceptually, to make it a linear motor (Figure 3). The three β's (and α's) then "crawl" on repeats of unrolled γ in that they never detach from γ (they would slide on γ by pushing and/or pulling actions) and, in contrast to the presumed walking of kinesin, the forefoot always remains in front and the hindfoot at the back. (If unrolled γ is considered to slide along repeats of unrolled $\alpha_3\beta_3$, the γ would "walk" in that it uses the three feet alternately. Figure 3 conforms to the prevailing custom of regarding only the molecule that hydrolyzes a nucleotide as the "motor" and its partner a passive rail.)

The three motors above and another linear motor, RNA polymerase (Gelles and Landick, 1998), are compared in Table 1. The F_1 motor most likely makes 120° steps, because the asymmetric conformations of the three β's, presumably dictating the orientation of γ, are stable in the crystal structure. Short pauses at 120° orientations were not resolved in the video images of Noji et al. (1997) at the resolution of 33 ms; however, measurements at low ATP concentrations could reveal such steps. RNA polymerase is expected to step by 0.34 nm, the distance between base pairs. Kinesin's 8 nm steps have been measured. All these step sizes represent intervals of the structural repeats. Myosin's so-called "unitary step" measured in vitro is a different quantity, in most cases representing movement made while a foot of myosin is attached to actin. It is believed by many to be related to the size of a conformational change that occurs in attached myosin (Goldman, 1998). Genuine steps of running myosin are expected to be multiples of 5.5 nm, the distance between neighboring actin monomers.

The four motors differ in efficiency, the mechanical work divided by the free energy of nucleotide hydrolysis. Because the motors can move without an external load (efficiency 0%), maximal efficiencies are quoted in Table 1. The near 100% efficiency of F_1 accords with the fully reversible nature of this motor; net synthesis of nucleotide triphosphate has not been reported for the other motors. The efficiency of the myosin/actin system quoted here is the work produced in a "unitary step" divided by the free energy of ATP hydrolysis. The myosin efficiency appears low in vitro, the quoted value being on the higher end in the literature (Ishijima et al., 1995), although the efficiency of intact muscle is generally considered to be higher.

Motor Mechanism: Bending versus Binding

Nucleotide-driven motors, including F_1, share common structural motifs near the nucleotide-binding site (Vale, 1996; Noji et al., 1996), suggesting that these motors might employ common principles in some aspects of their mechanisms. As a general principle, we propose that the distinction between bending and binding is important.

Bending (conformational change) of a motor protein alone could produce motion and force relative to its rail, the latter serving merely as a base that securely holds the "sole" of the "foot" of the motor. Myosin is considered to bend its leg forward when attached to actin, producing the "unitary step" (Figure 3, pink myosin on the left; Goldman, 1998). The machinery for bending could all be in myosin, because isolated myosin changes its conformation depending on the bound nucleotide (Gulick and Rayment, 1997). The free-energy changes associated with myosin ATPase, however, indicate that myosin alone would be unable to produce a large amount of work. Moreover, when myosin interacts with actin, as much as half of the free energy of ATP hydrolysis is used for unbinding of myosin from actin. Subsequent rebinding thus liberates energy. If myosin is to work at high efficiency, it should convert the energy gained during rebinding to mechanical output, by cooperation with actin.

A model by A. F. Huxley (1957) is on the other extreme: that binding alone produces motion and force. Myosin fluctuates thermally, and when it fluctuates in the correct direction, it binds to actin resulting in displacement and pull. In binding-alone models, thermal diffusion brings the motor and rail close to the binding configuration, and binding energy is used to stabilize that configuration. Work has to be done in the diffusion process, and can be done as shown below. Diffusive displacement of a particle of diameter d over a distance L takes a time of the order of $(L^2/2) \cdot (3\pi\eta d/k_BT)$, which is ~1 μs for $L = d = 10$ nm at room temperature (thermal energy $k_BT \approx 4$ pN · nm) in water (the viscosity $\eta \approx 10^{-3}$ N · s · m^{-2}). If this displacement is to produce work W (against a load), the time for displacement is multiplied by $\sim\exp(W/k_BT)$, which is 2×10^4 for $W = 10\ k_BT \approx 40$ pN · nm and 5×10^8 for $W = 20\ k_BT \approx 80$ pN · nm. Thus, work below $10\ k_BT$ can be done if the frequency of motor operation is below $\sim 10^2$/s. Binding models also require a mechanism that ensures correct choice of a binding site, or proper directional biasing of diffusion. The mechanism is not specified in the Huxley model.

An elegant interplay between bending and binding has been proposed for kinesin and its cousin ncd (Hirose et al., 1996). When one foot of kinesin (or ncd) is bound to a microtubule (rail), the other foot is unbound and undergoes thermal motion. They have shown that the unbound foot of kinesin, which walks toward the plus end of a microtubule, swings toward the plus end presumably by bending of the bound leg (Figure 3, pink

kinesin), and the unbound foot of minus-directed ncd swings toward the minus end. The bending biases the Brownian search of the unbound foot for the next binding site, for the plus direction for kinesin, and minus for ncd. The 8 nm step of kinesin (yet unresolved for ncd), and associated force, are produced when the foot lands on the binding site. A substep(s) and partial force may be produced by the bending, but the major mechanical output of this motor likely comes from the binding of the motor to its rail.

The three-foot F_1 (Figure 3) could in principle operate by binding alone, stepping among the three stable configurations with the correct direction being dictated by the bound nucleotide. ("Binding" for the case of F_1 should be interpreted as a transition to the most stable configuration between β's and γ, and might involve repulsive rather than attractive interactions.) The large mechanical output of $\sim$20 k_BT per step, however, cannot be achieved by a purely diffusive process because it would be too infrequent to account for the observed rate of rotation. Probably, the effective potential between β's and γ is downhill toward the next stable configuration, thus assisting the diffusion against an external load. The work per step would be determined by the total height of the potential slope, which is not dependent on the rotational speed. Bending of the three β's alone is unlikely to rotate γ by 120° because of the obstruction by intervening α subunits.

Of course the distinction between bending and binding becomes less obvious as one inquires more deeply into the mechanism. What we wish to stress here is that molecular motors must work through close cooperation of the two partners. The rail, in particular, is not a simple support, and binds and unbinds its nucleotide-hydrolyzing partner, supplying binding energy and controlling hydrolysis. The two aspects, bending and binding, should be useful in analyzing the mechanism of cooperation. The F_1 motor in which the two partners never detach from each other provides a wonderful opportunity to explore the details of the cooperation experimentally.

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