

F₁-ATPase: a highly efficient rotary ATP machine

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Introduction

Think of a single protein molecule that is by itself a rotary motor. Driven by three subunits each fuelled by ATP, the motor rotates in discrete 120° steps. The efficiency of energy conversion, from the free energy of ATP hydrolysis to mechanical output of the motor, amounts to nearly 100%. Mother Nature has created such a tiny yet powerful molecular machine, not for the purpose of producing mechanical work but for the synthesis of ATP in our body by reverse operation of the rotary motor.

This rotary motor is a part of the enzyme ATP synthase. In animals, the ATP synthase resides in the inner membrane of mitochondria. The food ingested by an animal is ‘burnt’ (oxidized) by protein machinery embedded in the inner membrane, and the energy obtained by the oxidation is used to eject protons from inside the mitochondrion to the external space. The protons eventually flow back into mitochondria through the ATP synthase, in which

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ATP is synthesized from ADP and P_i using the proton flow as the energy source. Similar systems occur in plants and bacteria.

That oxidation and ATP synthesis are coupled by the flow of protons across the mitochondrial membrane was proposed by Peter Mitchell [1], a radical concept at that time which took many years to be accepted. For the coupling between the proton flow and ATP synthesis in the ATP synthase, another revolutionary proposal was made by Boyer [2]: the proton flow and chemical reaction are coupled by the mechanical rotation of a subunit(s) within the protein molecule. This latter proposal, too, failed to arouse enthusiasm until, in 1994, John Walker and colleagues elucidated the atomic structure of part of the ATP synthase [3]. The structure strongly supported Boyer's idea, and also suggested many experiments that have led to the proof (at least in part) of Boyer's rotational catalysis model [4–8]. Here we briefly review some of the remarkable features of this molecular machine revealed in our laboratory, and discuss its possible mechanism.

The ATP synthase: two rotary motors with a common shaft

As shown in Figure 1(a), the ATP synthase consists of two parts, a membrane-embedded portion called F_0 and a protruding portion F_1 . When protons flow through F_0 from top to bottom in Figure 1(a), ATP is synthesized in F_1 . The ATP synthase is a completely reversible machine: when ATP is hydrolysed in F_1 , protons are pumped back in the reverse direction.

Boyer [2,9,10] proposed that F_0 is a rotary motor (or rather a turbine) driven by the proton flow, and that F_1 is another rotary motor driven by ATP hydrolysis. The two motors have a common rotary shaft (magenta in Figure 1a), but the genuine rotary directions of the two are different. When the free energy liberated by the downward flow of protons is greater than the free energy of ATP hydrolysis, the F_0 motor rotates the common shaft in the F_0 's genuine direction. The F_1 motor is forcibly rotated in its reverse direction, resulting in ATP production in its catalytic sites. If the free energy of ATP hydrolysis is higher, the F_1 motor gains control and rotates the shaft in its own direction. Protons are then pumped out by F_0 against an uphill potential.

Isolated F_1 catalyses only ATP hydrolysis, and hence is called F_1 -ATPase. Its subunit composition is $\alpha_3\beta_3\gamma\delta\epsilon$. One view of the crystal structure of bovine mitochondrial F_1 , determined by Walker and colleagues [3], and hereafter referred to as the Walker structure, is shown in Figure 1(b). The δ and ϵ subunits were not resolved, but these are not required for the rotation of F_1 . The $\alpha_3\beta_3$ cylinder forms the stator, and the central γ subunit, of which part of the protruding portion has not been resolved, would rotate in the cylinder. The catalytic sites in which ATP is synthesized/hydrolysed are on the three β subunits, each at an interface with a neighbouring α . Surprisingly, each of the three β subunits carried a different nucleotide in the crystal: one an analogue of ATP, another ADP, and the third carried none, in the clockwise order

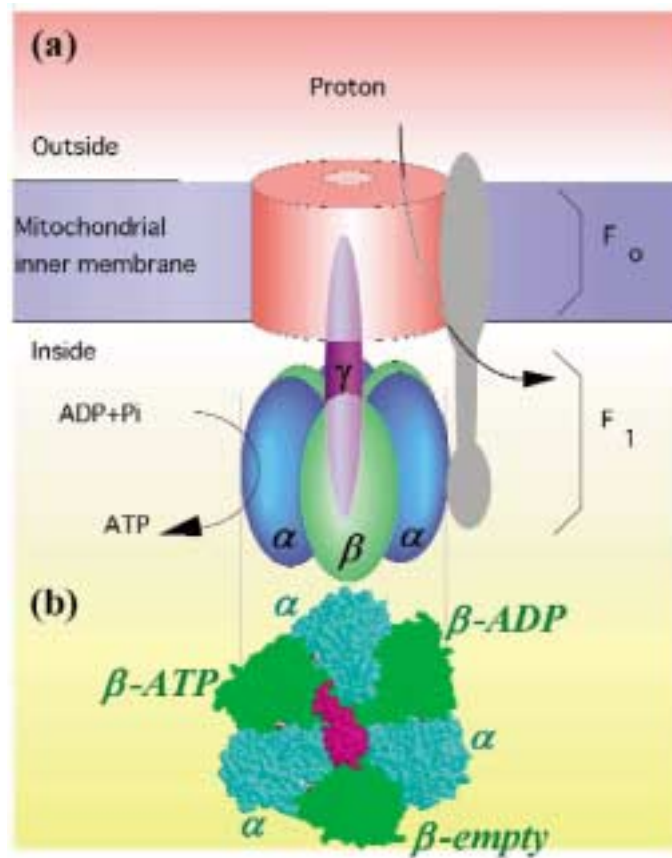


Figure 1. ATP synthase

(a) A schematic model. In a currently popular but unproven view, the red cylinder in the membrane rotates together with the γ shaft, and the grey part serves as the stator. Adapted from [12] with permission. ©1998 Cell Press. (b) Top view of the atomic structure of the F₁ part [3].

(Figure 1b). If hydrolysis were to proceed from this crystal structure, the ATP in the first β would be hydrolysed into ADP, the ADP in the second β would be released, and the third β would bind ATP from the medium. Thus the central γ is expected to rotate anticlockwise.

Proof that F₁ is indeed a rotary motor

Large-amplitude rotational motion of γ during ATP hydrolysis or synthesis had been shown by crosslinking and spectroscopic studies [4–7], but whether γ makes complete turns and does so in a unique direction was not clear until we observed the motion of γ directly under a microscope [8]. To visualize the rotation, Hiroyuki Noji prepared an $\alpha_3\beta_3\gamma$ subcomplex of bacterial origin. The $\alpha_3\beta_3$ cylinder was fixed to a glass surface, and a micrometre-sized actin filament was attached to γ via streptavidin (Figure 2a). The actin filament was

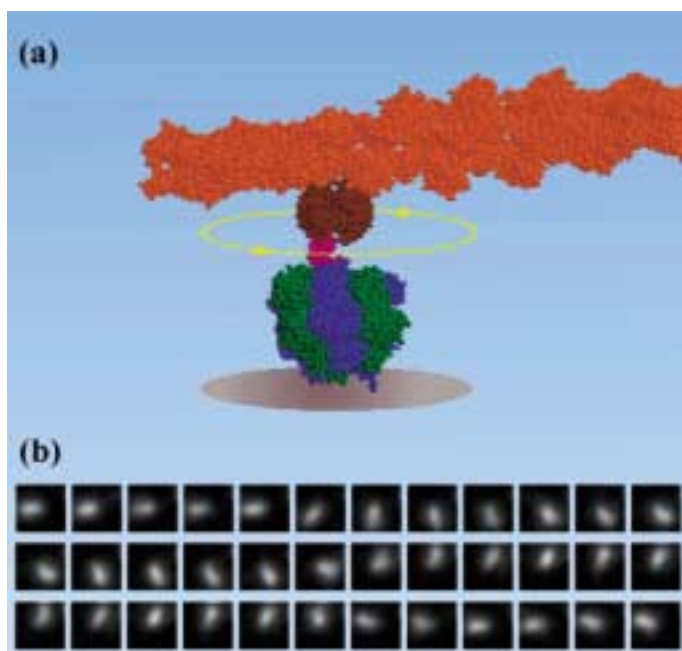


Figure 2. Visualization of F₁ rotation

(a) Orange rod at the top, actin filament; dark brown, streptavidin; magenta, γ subunit of F₁; green, β subunit; blue, α subunit. The diameter of the grey disk is ≈ 22 nm. (b) Photographs at 26 ms intervals of the actin filament rotating stepwise at $0.6 \mu\text{M}$ ATP.

fluorescently labelled. When Ryohei Yasuda looked into a fluorescence microscope, he saw a filament rotating continuously in one direction, anticlockwise as expected! The rotating filament was found on the very first day, within 30 min of the initial trial. The rotation was so beautiful that we were immediately convinced, almost, that F₁ was indeed a rotary motor. Soon a second rotating filament was found, and we were to toast Boyer after confirming the third. But the beer remained unopened; the third came only after a full month of struggle.

In our hands, at most a few percent of the actin filaments in a sample chamber rotate. A likely cause is surface obstructions. Note that the F₁ molecule is only ≈ 10 nm high, whereas the actin filaments are $\approx 1 \mu\text{m}$ or longer. Thus rotating the filament without touching the surface should be difficult. Indeed, rotating filaments often show a tendency to be stuck at a particular angle. Also, a significant fraction of F₁ is idle under normal assay conditions: MgADP, a product of the ATPase reaction, tends to bind tightly to a catalytic site and inhibit further ATP hydrolysis. Presumably, this MgADP inhibition prevents futile consumption of ATP in living cells.

Figure 3 shows typical (i.e. the most vigorously rotating) examples of rotation versus time. At a saturating concentration of ATP (2 mM), the rotation was essentially smooth and unidirectional. Rotation was slower for longer

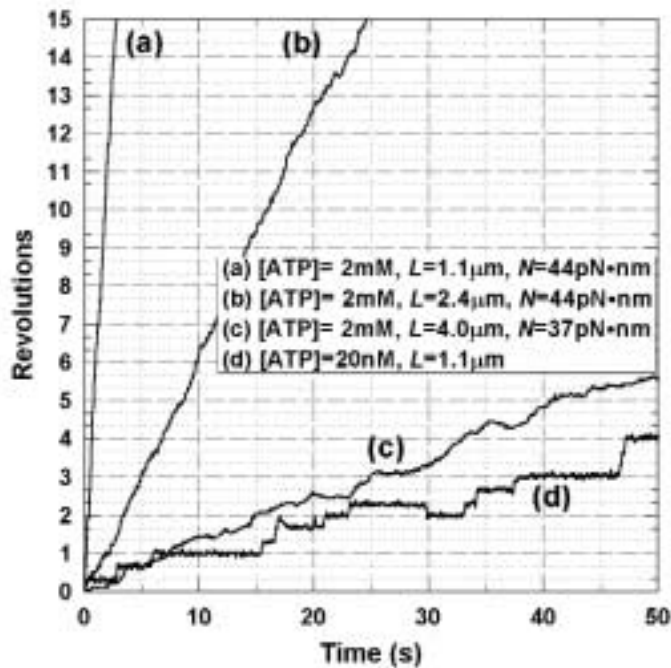


Figure 3. Rotation at high and low ATP concentrations

Traces (a–c) show the rotational rates for actin filaments of different lengths at 2 mM ATP. Analysis of the data [13] gives torque values of 44, 44 and 37 pN·nm, respectively. The rotational rates during individual steps at 20 nM ATP (trace d) are similar to the rate for the same filament length at 2 mM ATP (trace a).

actin filaments, because the viscous friction against the rotation is basically proportional to the cube of the actin length [11]. At very low ATP concentrations, the rotation became stepwise (Figures 2b and 3, trace d). The step size of 120° is precisely the one expected for the motor driven by the three β subunits separated by 120° . Note, in Figure 3 (trace d), that the motor made a back step at ≈ 30 s; a molecular machine must occasionally make mistakes.

The properties of the F_1 motor

Our studies [8,12–14] have revealed the following properties of F_1 -ATPase. These properties have been deduced from the observations of the most actively rotating F_1 , and do not necessarily represent the average behaviour of an ensemble.

(i) F_1 -ATPase is a rotary motor made of a single molecule

Occasionally an actin filament rotated around its centre, like a propeller [8]. If one were to hold a long rod at the middle and rotate it like a propeller, one would have to shift one's grip continually; true rotation requires slippage between the rotor and stator, compared with the pseudo-rotation that one can

make by holding the end of a rod and twisting (not really rotating) the wrist. Thus the γ subunit must slide against the surrounding $\alpha_3\beta_3$ cylinder over infinite angles. The propeller rotation of an actin filament cannot be supported by two different F_1 molecules, and therefore a single F_1 molecule must itself be a rotary motor. Its diameter and height being only ≈ 10 nm [3], the F_1 motor is the smallest rotary motor known.

(ii) $\alpha_3\beta_3\gamma$ subunits suffice for rotation

We have demonstrated rotation in the subcomplex $\alpha_3\beta_3\gamma$ [8]. Crosslinking studies [7] have indicated that the ϵ subunit also moves relative to α , and rotation of an actin filament attached to ϵ has been demonstrated [14]. Thus ϵ is likely to be part of the rotor, although it is not a necessary part of the rotary mechanism.

(iii) Rotation is anticlockwise when viewed from the F_0 side

Except for the occasional back steps, the sense of rotation is always anticlockwise when viewed from the top in Figures 1(a) and 2(a) [8]. This direction is in accord with the Walker structure (Figure 1b), suggesting that a structure similar to that in Figure 1(b) appears during rotation.

(iv) The F_1 motor is a 120° stepper

Stepwise rotation is seen at submicromolar ATP concentrations [13] (Figures 2b and 3, trace d). Between steps, F_1 waits for the next ATP molecule to arrive. At high ATP concentrations, the waiting time is shorter than the time required to rotate an actin filament through 120° , and hence the steps are not easily discerned. So far, substeps within the 120° step have not been resolved at our highest temporal resolution of 5 ms.

(v) The F_1 motor is designed to produce a constant torque

From the measured rate of rotation, we can calculate the torque the F_1 motor produces to move the actin filament [13]. At saturating ATP concentrations where ATP binding is not rate limiting, the torque, averaged over many revolutions, is ≈ 40 pN·nm irrespective of the viscous load or the rotational rate [13] (see also Figure 3). At low ATP concentrations where the 120° steps are resolved, the torque driving each step averages ≈ 44 pN·nm, again irrespective of the filament length [13] (see also Figure 4).

(vi) Work per step is also constant

Mechanical work done (against the viscous load) in a single step is given by the angular displacement, $2\pi/3$ radians (i.e. 120°), multiplied by the torque. Because the torque is constant, 40–44 pN·nm, the work done in a step is also constant and amounts to 80–90 pN·nm.

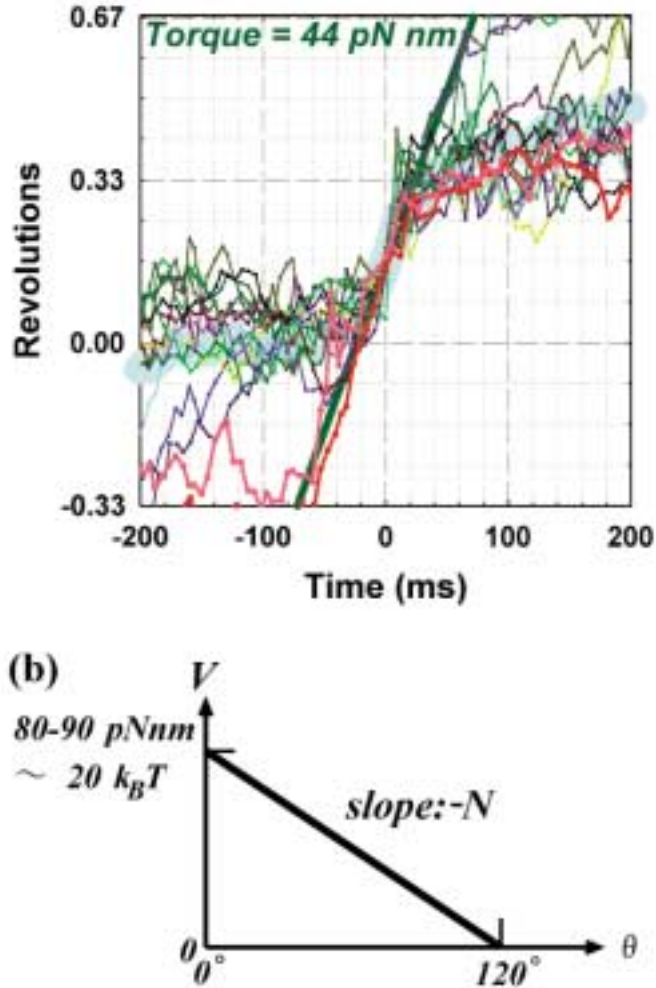


Figure 4. Time courses of individual steps

(a) Steps were measured at $0.2 \mu\text{M}$ ATP for F_1 bearing a $1.0 \mu\text{m}$ filament. Traces for individual steps are superimposed such that the middle of each step is located at time zero. Rapid succession of two steps are seen in two traces (red and pink). A thick cyan line shows the average of all traces. A thick green line shows a constant-speed rotation at 44 radians/s, corresponding to a torque of 44 pN·nm. (b) Rotational potential, $V(\theta)$, deduced from (a).

(vii) One ATP molecule is consumed per step

At low ATP concentrations where ATP binding is rate-limiting both for hydrolysis and rotation, the time-averaged rate of actin rotation was approximately equal to one-third of the number of ATP molecules hydrolysed per s [13]. This suggests that one ATP molecule is consumed per 120° step. Both the rate of rotation and the rate of ATP hydrolysis were proportional to the ATP concentration, indicating that neither of these processes required simultaneous consumption of two or more ATP molecules. At very low ATP

concentrations where the 120° steps were resolved, an analysis of the intervals between steps indicated that each step was fuelled by one ATP molecule [13].

(viii) The efficiency of energy conversion can reach $\approx 100\%$

Points (vi) and (vii) above indicate that the constant mechanical output of 80–90 pN·nm per step is produced by the consumption of one ATP molecule. The energy input, the free energy of ATP hydrolysis, ΔG , depends on the concentrations of ATP, ADP and P_i . In experiments at a controlled ΔG of 90 pN·nm, the work done per step was also ≈ 80 pN·nm [13]. Thus the F_1 motor can work at near 100% efficiency. The efficiency is lower at higher ΔG , because the mechanical output is constant. In cells where ΔG is 80–90 pN·nm, the efficiency could be $\approx 100\%$. This amazingly high efficiency, compared with other molecular motors [12], is probably related to the fully reversible nature of this molecular machine. In ATP synthase, the F_1 motor would pump protons at the energy conversion efficiency of $\approx 100\%$.

The rate of ATP hydrolysis quoted in point (vii) was measured in solution, and thus is an ensemble average. The hydrolysis rate of active F_1 might be higher, because some in the ensemble might have been inhibited. Hence, we cannot exclude the possibility that uncoupled, futile consumption of ATP occurs occasionally. Even so, each mechanical step is coupled to the hydrolysis of one and only one ATP molecule, and the free energy liberated in the coupled hydrolysis can be converted to mechanical work at $\approx 100\%$ efficiency.

(ix) Bi-site catalysis supports rotation

At submicromolar ATP concentrations, the F_1 -ATPase operates in the so-called bi-site mode [9,10], where at most two catalytic sites are filled with a nucleotide. Basically, one site binds tightly a nucleotide, ATP or ADP+ P_i in reversible equilibrium, and the other two sites are empty. When a second site binds ATP from the medium, ADP and P_i are rapidly released from the first site, resulting in net hydrolysis of one ATP molecule. Our results show that rotation can occur in this bi-site catalysis: there is no need to fill all the catalytic sites. The bi-site rotation is fundamental to the rotary mechanism, and there is no clear evidence that the motor adopts a different mechanism in the tri-site regime. Because torque generation requires broken symmetry, the use of an all-filled (or all-empty) state is not advantageous.

At extremely low ATP concentrations, uni-site catalysis occurs where the tightly bound products in one catalytic site are very slowly released in the medium without the binding of a second ATP. Whether the uni-site catalysis accompanies rotation is an important yet unsettled question. Crosslinking γ to a β did not inhibit uni-site catalysis [15], indicating that uni-site catalysis can occur without rotation. However, the possibility of rotation in the absence of crosslinking cannot be dismissed.

(x) Back steps occur, probably using ATP

Occasional back steps observed at low ATP concentrations were as rapid as the forward steps [13]. The torque driving the back steps, and thus the work per step, are as high as those of the forward steps, suggesting that the back steps are also driven by ATP hydrolysis. Presumably, ATP binding to the wrong site (one of the two empty sites) in the bi-site rotation produces a back step.

(xi) ATP hydrolysis is likely to introduce a linear downhill rotational potential

Point (vi) can be explained if an angle-dependent potential energy of height 80–90 pN·nm, downhill towards the position 120° ahead, is introduced for γ rotation upon the binding (and/or subsequent hydrolysis) of ATP. We can estimate the shape of this potential as follows.

In Figure 4(a), many steps in a rotation record are superimposed. Although individual traces are noisy, their average shown in the thick cyan line indicates that the slope, the rotational rate ω , is approximately constant throughout the 120° interval. The torque N ($=\omega\xi$; where ξ is the rotational frictional drag coefficient [11,13], $\xi=1.0$ pN·nm·s for the 1 μ m filament) is thus independent of the rotational angle θ , and is approximately 44 pN·nm throughout the 120° interval as shown in the thick green line. Because the potential $V(\theta)$ for γ rotation is related to N by $dV/d\theta=-N$, a linear potential profile is deduced, shown in Figure 4(b). Note that the actual potential profile should be dependent on the state of the bound nucleotides, which changes with time in each step. The profile shown in Figure 4(b) is the effective potential experienced by γ during the course of the chemical kinetics. Also, details of the actual potential profile may have been smoothed out by the combination of possible elastic linkage between γ and actin and the viscous friction on the latter.

How the F_1 motor may be designed

A rotary mechanism of human design is shown in Figure 5. This motor has three driving poles in the stator part, like the F_1 motor. The motor is powered by a unidirectional current source, while ATP hydrolysis is also practically unidirectional. Thanks to the three pairs of switches (commutators) on the shaft, the three poles change their polarities such that the shaft rotates continuously in the anticlockwise direction. The rotor is a permanent magnet, a static component. If the rotor is forcibly rotated in the reverse direction, this DC (direct current) motor becomes a DC generator and charges the external battery. The energy-conversion efficiency of modern electrical motors is quite high, often >95%. The DC motor operates in the bi-site mode in that the three driving poles never assume the same polarity. Thus there are similarities

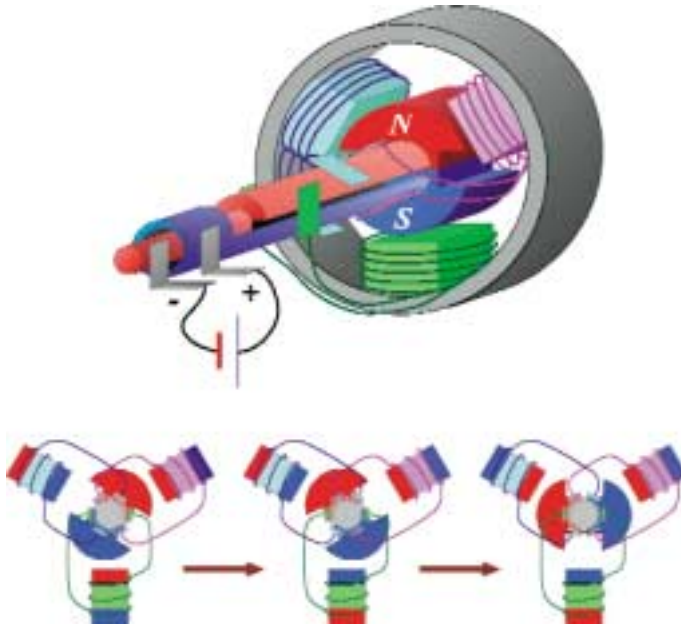


Figure 5. A three-pole DC motor

The commutators on the shaft change the polarity of stator magnets such that the shaft rotates anticlockwise continuously.

between the properties of this three-pole DC motor and those of the F_1 motor. Do these two share some operational principles?

The driving forces in the electrical motor in Figure 5 are the attraction between north and south poles and the repulsion between like poles. Such a push-pull mechanism may also operate between the γ and β subunits of the F_1 motor. Figure 6(a) shows side views of the three pairs of opposing β and α subunits, together with the central γ , in the Walker structure. The vertical black lines show the rotational axis suggested by Wang and Oster [16]: the bottom part of the $\alpha_3\beta_3$ stator has an approximate 3-fold symmetry around this axis, and thus the conformations of β and α in the bottom do not change greatly depending on the bound nucleotide. In the upper part, in contrast, the β subunits binding ATP or ADP are bent towards, and therefore push, γ , whereas the empty β retracts and pulls γ towards it. Wang and Oster [16] suggest that, because the central γ is slightly bent, co-operative push-pull actions of the three β subunits would rotate γ , as seen in Figure 6(b).

A simple F_1 model

Figure 7 shows a model for F_1 rotation based on this push-pull mechanism. The side of γ that faces the empty β in the Walker structure is designated the north pole, and thus an empty β is the south pole. A nucleotide-carrying β is north and repels the north face of γ and attracts its south face. By reciprocity, the south face of γ augments the affinity of the opposing β for a nucleotide,

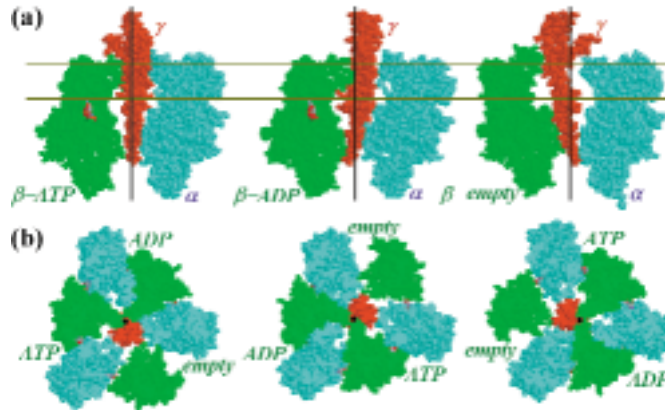


Figure 6. Nucleotide-dependent conformational changes in F_1

(a) The diagrams show the central γ subunit (orange), one β (green) to the left of γ , and one α (blue) to the right in the crystal structure [3]. The black lines indicate the rotation axis [16]. Nucleotides are shown in Corey-Pauling-Kutun (CPK) colours. (b) Top view of the cross sections of F_1 between the horizontal lines in (a).

and the north face decreases the affinity (the free energy is lowered when north and south oppose each other). To ensure rotation in a unique direction, additional control of nucleotide-binding kinetics via ‘commutators’ is required. A simple example is given in Figure 7(b): binding/release of ATP is allowed for β while it is on the pink side of γ , and ADP binding/release while on the green side. As shown in Figures 7(c) and 7(d), the switching ensures anticlockwise rotation of γ when ATP is hydrolysed; when the rotor is forced to rotate clockwise in the presence of ADP (and P_i), ATP is synthesized.

More elaborate switches have been proposed by Wang and Oster [16], and their model can account for many experimental observations, including the near 100% efficiency and 120° stepping with occasional back steps. In their model, as with the model in Figure 7, the motor tends to pause at angles 60° out of phase from the Walker structure (Figure 7a), an experimentally testable prediction. How the switching action is implemented in the protein structure is yet to be specified.

A switch-less F_1 model

The roles of the commutators in Figure 5 are to alternate the polarities of the stator magnets, and to do so at precise timings dictated by the rotational angle of the shaft. Unlike the magnet driven by direct current, the alternation of the polarity is inherent in the ATP-driven ‘magnet’, where bound ATP is eventually hydrolysed and released, restoring the south state spontaneously. Thus only co-ordination of nucleotide kinetics among the three stator magnets needs to be programmed. This could be done without switches, as shown in Figure 8, which is one version of the general model of Oosawa and Hayashi [17].

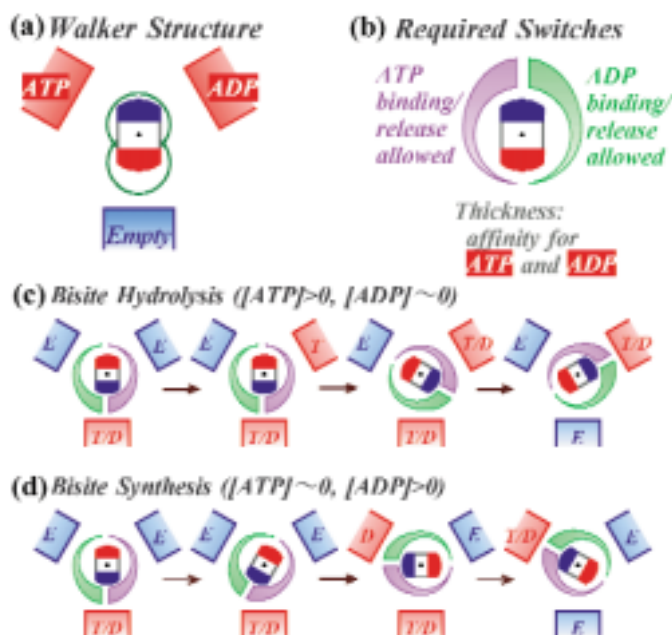


Figure 7. A simple model for the F_1 motor

(a) The γ subunit is regarded as a permanent magnet, the side of γ that faces the empty β in the Walker structure being the north pole. (b) The affinity for a nucleotide is higher when β is closer to the south pole of γ (strictly, the affinity for ATP is higher than that for ADP). Binding and release of ATP and ADP are kinetically inhibited on the green and pink sides, respectively. (c) Bound ATP (T) is in equilibrium with ADP (D) and P_i , and is released as ADP when a second ATP binds and rotates γ . E, empty. (d) Forced clockwise rotation of γ results in the uptake of ADP (and P_i) and release of ATP.

In Figure 8, the position of the ‘magnetic pole’ in β changes depending on the bound nucleotide. This dual-pole arrangement, combined with the higher affinity for nucleotides when the pole is closer to the south face of γ , ensures anticlockwise rotation in bi-site hydrolysis by inducing ATP binding primarily in the empty β in the anticlockwise direction (Figure 8c). Note that the angle-dependence of the nucleotide affinity results as a reaction to the nucleotide-dependent push–pull action, without requiring switches. An additional factor ensuring correct rotation is the higher affinity for ATP than for ADP. Because ATP hydrolysis on β is reversible (the free-energy difference between β binding ATP and β binding $ADP + P_i$ is small), the affinity for the hydrolysis products has to be lower in order for β to act as an ATPase. In the original model of Oosawa and Hayashi [17], near-100% efficiency was achieved for both hydrolysis and synthesis.

The essence of the dual-pole arrangement is that the mechanical interaction between γ and individual β subunits involves a nucleotide-dependent rotational component in addition to pushing/pulling. The Walker structure gives more emphasis to pushing/pulling rather than to direct rotation, but the

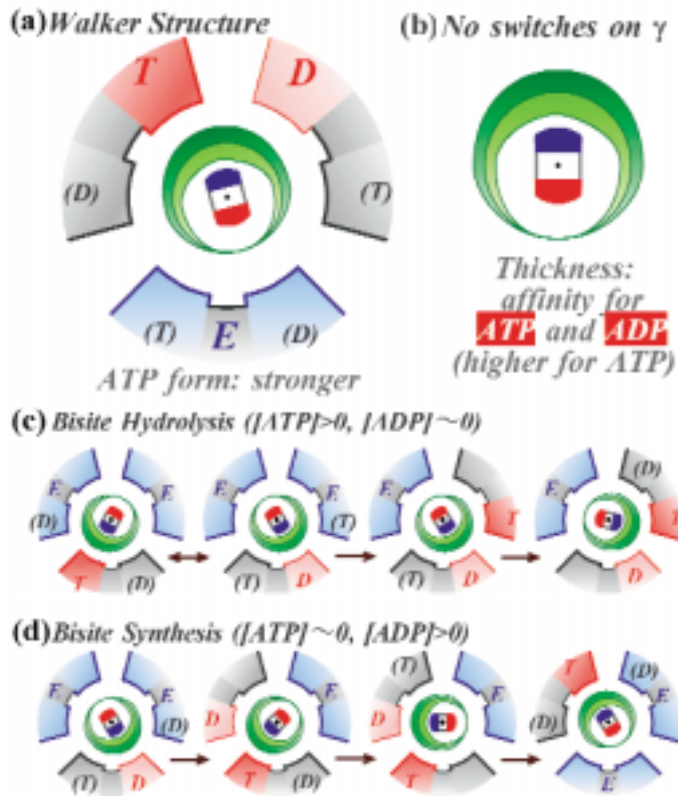


Figure 8. A switch-less model for the F_1 motor

(a) Location of the magnetic pole on β changes depending on the bound nucleotide. ATP (T) magnet is stronger than ADP (D) magnet and, hence, (b) the affinity for ATP is higher than that for ADP. (c) When only one nucleotide is bound it is reversibly interconverted between ATP and $ADP + P_i$. Comparison of affinities suggests that the most likely way of filling a second site is binding of ATP in the β in the anticlockwise direction. (d) When γ is forcibly rotated clockwise, the equilibrium between ATP and ADP is shifted towards ATP, which is eventually released while ADP is newly bound in the second site. E, empty.

structure of γ in the protruding portion has not been resolved. Also, key intermediates in actual rotation, particularly the state with only one bound nucleotide, may well have a different structure.

Models in Figures 7 and 8 have been introduced solely to point out several factors that may or may not be important in the mechanism of F_1 rotation. Neither is assumed to be the actual mechanism. Nor are all important factors explained in these models. For example, the magnet analogy, particularly that for γ , obscures the fact that neither γ nor β possesses reflection symmetry. The force between γ and individual β subunits must be more or less asymmetric, favouring one rotational direction over the other, as modelled by the dual poles in Figure 8. Whether the rotational potential can be approximated by a simple superposition of three pairwise interactions between individual β and γ ,

as implied by the magnetic analogy, remains unclear until structures of the different intermediates are revealed. Also, in Figures 7 and 8, nucleotide kinetics on different β s are co-ordinated only through the rotation of γ , but β subunits may also communicate through the intervening α subunits.

Problems to be solved

The key to understanding the rotational mechanism is to elucidate the structure of F_1 in which only one catalytic site is filled, the structure from which the 120° step begins. It is unlikely that the two empty β subunits in this structure both resemble the empty β in the Walker structure; presumably, the asymmetric γ would induce the β 120° ahead into a conformation closer to the nucleotide-carrying β in the Walker structure.

Also important is to establish the precise relation between the nucleotide binding/hydrolysis kinetics and the rotational potential. This could be done by imaging nucleotide turnover in a single motor molecule [18] while observing its rotation through an attached actin filament. Manipulation of the filament, e.g. with optical tweezers [19], will help establish the angle dependence of the nucleotide kinetics or, conversely, the nucleotide dependence of the rotational torque. Because an attached actin filament may not faithfully reflect the orientation of γ , assessment of the latter through imaging of polarized fluorescence [20,21] from a fluorophore rigidly attached to γ will also be useful.

What happens if the free energy of ATP hydrolysis is reduced below 80 pN·nm by manipulating nucleotide and P_i concentrations? This is a fundamental question for mechanisms of molecular machines in general. The predicted behaviours depend on the model. Answering the question experimentally is not easy, because MgADP inhibition is serious at high ADP concentrations.

Presumably, the MgADP-inhibited form is the most stable state of the F_1 motor, while rotation requires instability. The Walker crystal structure probably represents this stable inhibited form [3]. The anticlockwise rotation consistent with this structure, then, implies that slight destabilization of the Walker structure, e.g. by the presence of P_i next to ADP, would make an active intermediate. Interestingly, the inhibition does not occur in the synthesis mode [10], where proton-driven rotation of γ may destabilize the inhibited form. Many articles on F_1 -ATPase do not mention the degree of MgADP inhibition in the experiments described; in some cases, different interpretations could emerge if this almost inevitable inhibition was taken into account.

Will ATP be synthesized in F_1 without the aid of F_0 , if one mechanically rotates γ clockwise, e.g. by manipulating attached actin with optical tweezers? The answer should be yes, but experimental proof is still awaited. Such an experiment would demonstrate that mechanical energy can be directly transformed into chemical energy.

Relatively little is known about the F_0 part of the ATP synthase. Even whether F_0 is really a rotary motor is yet to be proved. If it is, which part is the rotor and how is it connected to γ ? Is the proton transport tightly coupled to the rotation as it seems to be between ATP hydrolysis and rotation in the F_1 motor? Many questions remain, demanding new experimental ideas. Boyer calls the ATP synthase a splendid molecular machine [10]. It is also a splendid toy for young, creative researchers.

Summary

- *A single molecule of F_1 -ATPase is by itself a rotary motor in which a central subunit, γ , rotates against a surrounding stator cylinder made of $\alpha_3\beta_3$ hexamer.*
- *Driven by the three β subunits that hydrolyse ATP sequentially, the motor runs with discrete 120° steps at low ATP concentrations.*
- *Over broad ranges of load and speed, the motor produces a constant torque of 40 pN·nm.*
- *The mechanical work the motor does in the 120° step, or the work per ATP hydrolysed, is also constant and amounts to 80–90 pN·nm, which is close to the free energy of ATP hydrolysis. Thus this motor can work at near 100% efficiency.*

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References

1. Mitchell, P. (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature (London)* **191**, 144–148
2. Boyer, P.D. & Kohlbrenner, W.E. (1981) The present status of the binding-change mechanism and its relation to ATP formation by chloroplasts, in *Energy Coupling in Photosynthesis* (Selman, B.R. & Selman-Reimer, S., eds.), pp. 231–240, Elsevier, Amsterdam
3. Abrahams, J.P., Leslie, A.G.W., Lutter, R. & Walker, J.E. (1994) Structure at 2.8 Å of F_1 -ATPase from bovine heart mitochondria. *Nature (London)* **370**, 621–628
4. Duncan, T.M., Bulygin, V.V., Zhou, Y., Hutcheon, M.L. & Cross, R.L. (1995) Rotation of subunits during catalysis by *Escherichia coli* F_1 -ATPase. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 10964–10968
5. Zhou, Y., Duncan, T.M., Bulygin, V.V., Hutcheon, M.L. & Cross, R.L. (1996) ATP hydrolysis by membrane-bound *Escherichia coli* F_0F_1 causes rotation of the γ subunit relative to the β subunits. *Biochim. Biophys. Acta* **1275**, 96–100
6. Sabbert, D., Engelbrecht, S. & Junge, W. (1996) Intersubunit rotation in active F-ATPase. *Nature (London)* **381**, 623–625
7. Aggeler, R., Ogilvie, I. & Capaldi, R.A. (1997) Rotation of a γ - ϵ subunit domain in the *Escherichia coli* F_1F_0 -ATP synthase complex. *J. Biol. Chem.* **272**, 19621–19624

8. Noji, H., Yasuda, R., Yoshida, M. & Kinoshita, Jr., K. (1997) Direct observation of the rotation of F_1 -ATPase. *Nature (London)* **386**, 299–302
9. Boyer, P.D. (1993) The binding change mechanism for ATP synthase — some probabilities and possibilities. *Biochim. Biophys. Acta* **1140**, 215–250
10. Boyer, P.D. (1997) The ATP synthase - a splendid molecular machine. *Annu. Rev. Biochem.* **66**, 717–749
11. Hunt, A.J., Gittes, F. & Howard, J. (1994) The force exerted by a single kinesin molecule against a viscous load. *Biophys. J.* **67**, 766–781
12. Kinoshita, Jr., K., Yasuda, R., Noji, H., Ishiwata, S. & Yoshida, M. (1998) F_1 -ATPase: a rotary motor made of a single molecule. *Cell* **93**, 21–24
13. Yasuda, R., Noji, H., Kinoshita, Jr., K. & Yoshida, M. (1998) F_1 -ATPase is a highly efficient molecular motor that rotates with discrete 120° steps. *Cell* **93**, 1117–1124
14. Kato-Yamada, Y., Noji, H., Yasuda, R., Kinoshita, Jr., K. & Yoshida, M. (1998) Direct observation of the rotation of γ subunit in F_1 -ATPase. *J. Biol. Chem.* **273**, 19375–19377
15. García, J. J. & Capaldi, R. A. (1998) Unisite catalysis without rotation of the γ - ϵ domain in *Escherichia coli* F_1 -ATPase. *J. Biol. Chem.* **273**, 15940–15945
16. Wang, H. & Oster, G. (1998) Energy transduction in the F_1 motor of ATP synthase. *Nature (London)* **396**, 279–282
17. Oosawa, F. & Hayashi, S. (1986) The loose coupling mechanism in molecular machines of living cells. *Adv. Biophys.* **22**, 151–183
18. Funatsu, T., Harada, Y., Tokunaga, M., Saito, K. & Yanagida, T. (1995) Imaging of single fluorescent molecules and individual ATP turnovers by single myosin molecules in aqueous solution. *Nature (London)* **374**, 555–559
19. Arai, Y., Yasuda, R., Akashi, K., Harada, Y., Miyata, H., Kinoshita, Jr., K. & Itoh, H. (1999) Tying a molecular knot with optical tweezers. *Nature (London)* **399**, 446–448
20. Sase, I., Miyata, H., Ishiwata, S. & Kinoshita, Jr., K. (1997) Axial rotation of sliding actin filaments revealed by single-fluorophore imaging. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 5646–5650
21. Hälsner, K., Engelbrecht, S. & Junge, W. (1998) Three-stepped rotation of subunits γ and ϵ in single molecules of F-ATPase as revealed by polarized, confocal fluorometry. *FEBS Lett.* **426**, 301–304