ATP Synthase

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Abstract

Oxygenic photosynthesis is the principal converter of sunlight into chemical energy. Cyanobacteria and plants provide aerobic life with oxygen, food, fuel, fibers, and platform chemicals. Four multisubunit membrane proteins are involved: photosystem I (PSI), photosystem II (PSII), cytochrome b₆f (cyt b₆f), and ATP synthase (F₀F₁). ATP synthase is likewise a key enzyme of cell respiration. Over three billion years, the basic machinery of oxygenic photosynthesis and respiration has been perfected to minimize wasteful reactions. The proton-driven ATP synthase is embedded in a proton-tight-coupling membrane. It is composed of two rotary motors/generators, F₀ and F₁, which do not slip against each other. The proton-driven F₀ and the ATP-synthesizing F₁ are coupled via elastic torque transmission. Elastic transmission decouples the two motors in kinetic detail but keeps them perfectly coupled in thermodynamic equilibrium and (time-averaged) under steady turnover. Elastic transmission enables operation with different gear ratios in different organisms.
INTRODUCTION

Solar Energy Capture by Oxygenic Photosynthesis

Oxygenic photosynthesis is the major energy source of life on Earth. Sunlight is converted into chemical energy and products that humans use as food, fiber, fuel, and platform chemicals. The primary processes of photosynthesis convert sunlight into two forms of chemical energy, namely the redox couple NADPH/O₂ and the couple ATP/ADP + P_i (inorganic phosphate). With a stoichiometric ratio of six to nine, these couples drive carbon fixation. NADPH and ATP are used in a six-to-nine ratio for carbon fixation. At the level of these primary products, and in the time domain of microseconds, the efficiency of solar energy conversion can reach up to 20%. Its efficiency is comparable to that of photovoltaic cells (1). In subsequent processes, the efficiency drops to 10% for carbon fixation and to 2% for crops in the field.

In this article and its companion review (2), we describe the structure and function of three major protein complexes, namely photosystem I (PSI), photosystem II (PSII), and ATP synthase (F-ATPase or F₀F₁). They are embedded in the same coupling membrane, composed from several subunits, and have high molecular mass (~600,000 au). Their mechanisms of action are fundamentally different. Photosystems are quantum-mechanical solid-state devices, and F₀F₁ is a mechanochemical machine. In the former, electromagnetic excitation and electrons migrate...
between fixed cofactors. The turnover rates are governed by the laws of quantum mechanics. In F₀F₁, intersubunit mobility is the prerequisite for its function as a proton-driven ATP synthase. The dynamics is governed by statistical mechanics, that is, the interplay of stochastic and directed forces.

PSI and PSII are present only in organisms that perform oxygenic photosynthesis (cyanobacteria, algae, and plants), whereas F₀F₁ is present in photoautotrophic and heterotrophic organisms (respiring and nonrespiring bacteria, plants, and animals). F₀F₁ shares its structure and operating principle with a large family of close relatives in chloroplasts, mitochondria, a wide range of bacteria, and yeast. The respective F₀F₁ varieties have different benefits for kinetic and structural examination. For this reason, this article merges data obtained on F₀F₁ from many different organisms.

ATP Production by Photosynthesis and Respiration

In 1958, Racker and colleagues (3) discovered in submitochondrial particles a soluble protein fraction required for coupling the phosphorylation of ADP to the oxidative electron transport chain. They coined it F₁. F₀F₁, or ATP synthase, refers to the combination of F₁ and its membrane-associated counterpart, named FO (the subscript stands for oligomycin sensitivity). In 1961, Mitchell (4) hypothesized that electron transport crosses the membrane and, being linked to proton transport, generates proton-motive force (PMF) that powers ATP synthesis. PMF is composed of the difference in pH (ΔpH) (4) and in electric potential (Δϕ) (5) across the respective coupling membrane:

\[
PMF = F \cdot Δϕ - 60 \cdot ΔpH \quad \text{in units of mV},
\]

\[
PMF = Δϕ - 60\cdot ΔpH \quad \text{in units of mV},
\]

\[
PMF = \frac{Δϕ}{60\text{mV}} - ΔpH \quad \text{in units of pH}.
\]

Here, F denotes the Faraday constant, R the gas constant, and T the temperature in Kelvin. For the in vivo distribution of the PMF over its two components, ΔpH and Δϕ, see References 6 and 7.

In thermodynamic equilibrium, the PMF is supposed to match the molar free energy of ATP synthesis, \(ΔG_{ATP}^0\):

\[
n \cdot PMF = ΔG_{ATP} = ΔG_{ATP}^0 + RT \ln \left( \frac{[ATP]}{[P_i] \cdot [H^+]} \right).
\]

Here, \(n\) denotes the stoichiometric ratio of protons to ATP, \([X]\) is the concentration over the respective (biochemical) standard, and \(ΔG_{ATP}^0 \approx +30.5 \text{ kJ/mol}\) is the standard free energy of ATP synthesis.

In the case of photophosphorylation, experimental evidence in favor of this hypothesis emerged soon after Mitchell’s paper (4) was published. Subjecting chloroplasts to an acid–base jump produced ATP (8). The photogeneration of transmembrane voltage was demonstrated by observations of electrochromic effects of intrinsic pigments, which served as a molecular voltmete (9). The decay of the voltage was quantitatively correlated with ATP production (10). Although Mitchell’s hypothesis had been established for photophosphorylation, its validity for mitochondria was heavily debated for quite a while until becoming generally accepted (11).
Boyer and colleagues found that F₁ hosts two or three catalytic sites (12) and might use stored conformational energy for ATP synthesis (13). Rotary action involving three reaction sites was considered as a possibility (14, 15). Several studies reported negative cooperativity for ATP binding with dissociation constants of 1 pM, 30 μM, and 150 μM in beef heart mitochondria (16) and <50 nM, 500 nM, and 25 μM in Escherichia coli (17).

F₁ contains five types of subunits in stoichiometric proportion α₃β₃γδε (18). For a long time, investigators did not know how to relate these properties to the enzyme structure nor, in particular, how protons interact with the protein to yield ATP (for this history, see Reference 19 and references therein). The function of F₁ could not be understood without knowledge of its crystal structure (see the section titled Structure of F₁, below). We now know that FOF₁ is composed of two rotary nanomotors: the ion-driven, electrochemical F₀ and the chemical F₁, which are mechanically coupled (reviewed in References 20–25).

Architecture of the Coupling Membrane

The coupling membranes of oxygenic photosynthesis contain PSI, PSII, the cytochrome b₆f complex (cyt b₆f), and F₀F₁ (Figure 1a). The primary photochemical electron transfer in both PSII and PSI, and the secondary transfer in cyt b₆f, is directed from the positively charged side
(p-side) to the negatively charged side (n-side) of the membrane (9, 26, 27). Proton uptake (n-side), oppositely directed hydrogen transfer, and proton release (p-side) acidify the p-side over the n-side and contribute further to the PMF, which drives ATP synthesis by $F_0F_1$.

The photosynthetic coupling membrane is highly crowded. With a specific membrane area of $\sim 2 \text{nm}^2$ per chlorophyll molecule (28) and $\sim 1000$ chlorophyll molecules per PSI:PSII:cyt $b_6f:F_0F_1$ in the approximate proportion 2:2:1:1, the mean area per large complex amounts to less than 200 nm$^2$. In chloroplasts of green plants, the coupling membrane is folded into flattened sacs, named thylakoids (29), that are piled up into grana and linked together by intergrana (or stroma) lamellae (Figure 1b). The whole membrane system inside a chloroplast can be unfolded into a large spherical bleb (28). Also, a single dimer of the powerful ionophore gramicidin accelerates the decay of the transmembrane voltage (30). In other words, this intricately folded system seems to form a single, simply connected sheet (in mathematical terms) such that more than $10^4$ electron transport chains and ATP synthase molecules are electrically coupled with one another. For decades, investigators have argued that stroma lamellae helically outgrow from grana stacks (31). Ongoing research by high-resolution electron tomography (32–34) supports this idea (Figure 1b). How such a structure can unfold into a large spherical bleb (either intrinsically or via fractionation and fusion) is still unknown. Biochemical analyses (35), freeze-fracture electron microscopy (36), and more recently electron tomography (with up-to-5-nm resolution) (33, 37) established the nonhomogeneous lateral distribution of the membrane proteins. PSI and F-ATPase are located mainly in the stroma lamellae, as well as in the top and bottom surfaces of grana. PSII is found almost exclusively inside grana, and the cyt $b_6f$ complex is found in grana and at grana margins (35, 37, 38). The lateral separation of PSII and PSI is beneficial for three reasons: It prevents their competition for light (39), facilitates regulation of the distribution of light (40), and facilitates repair of PSII after photodamage (2). How the lateral separation between the producers (PSII, PSI, cyt $b_6f$) and the consumer ($F_0F_1$) of PMF bears on the efficiency of ATP synthesis is discussed in the section titled The Enzyme in the Coupling Membrane, below.

**F$_1$, THE CHEMICAL MOTOR**

**Structure of F$_1$**

Walker and colleagues (41) used bovine mitochondria to obtain the first asymmetric crystal structure of F$_1$. The original resolution of 2.8 Å was later advanced to 1.9 Å (42). The crystal structure unveiled the determinants of rotary catalysis by F$_1$ (Figure 2b). The $\alpha$- and $\beta$-subunits are arranged in alternation into a pseudohexagon, ($\alpha\beta$)$_3$; the $\gamma$-subunit is a shaft in its center. One nucleotide is bound to each of the three $\alpha$-subunits without any known role in catalysis and regulation. The three $\beta$-subunits differ in that one is empty ($\beta_E$); one is loaded with MgADP ($\beta_{DP}$); and the third, named $\beta_{TP}$, is loaded with a nonhydrolyzable ATP analog. The central shaft of the $\gamma$-subunit is cranked. Its convex side pushes the helix-turn-helix motif at the C-terminal end of the $\beta$-subunit (hereafter referred to as the hinge) to expose and empty the respective nucleotide-binding site. This structural model may explain how the three (in principle) equivalent reaction sites cooperate in hydrolyzing ATP to yield ADP and P$_i$. The three sites perform different steps in the sequence involving the binding of ATP, ATP hydrolysis, and product release, and their roles change cyclically. The empty site, $\beta_E$, supposedly binds a new molecule of ATP; the cranked shaft is driven forward toward $\beta_{DP}$; and the convex side of the $\gamma$-subunit pushes the hinge on $\beta_{DP}$ to extrude the products of hydrolysis, ADP and P$_i$. ATP, which is bound to the $\beta_{TP}$ site, is split into bound ADP and P$_i$. 

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Figure 2
Structural models of the ATP synthase. (a) Homology model of FOF1 from *Escherichia coli*. The F1 and Fo portions and the subunit composition, (αβ)3γεδab2, are indicated. For (αβ)3 (orange), only one copy of the β-subunit, βE, and one copy of the α-subunit are shown. (b) The first asymmetric crystal structure of (αβ)γ from bovine mitochondria (41). The red dot marks the helix-turn-helix motif of the β-subunit, the hinge.

The Walker group has analyzed many other crystals grown in media with or without specific inhibitors and various nucleotide analogs (reviewed in Reference 24). Most of these crystals resemble the ground-state structure (42), in which one site, βE, is empty and the other two sites, βDP and βTP, are filled with nucleotides. The nucleotide-binding pockets of the β-subunit are lined by the so-called Walker motif, GxxxxGKT/S (where x refers to any residue) (43), which was later also found in other nucleotide-binding proteins. Another characteristic of the binding pocket is an arginine finger, which is contributed by the adjacent α-subunit (41). The arginine finger coordinates the γ-phosphate in βTP and turns away from this position in the absence of ATP. A related situation exists in Ras GTPase, in which phosphoryl transfer is electrostatically facilitated by an arginine finger, again provided by a companion protein (44).

Among several crystal structures of bovine F1, only two differ from this pattern. In these cases, all three sites contain nucleotides (45, 46). Both structures have been interpreted as intermediate states of ATP hydrolysis. In the first structure, the sites equivalent to βDP and βTP carry MgADP·AlF4−, supposedly a transition-state analog, and the third site, βE, carries MgADP. An immobilized water molecule, supposedly used for nucleophilic attack, appears on the γ-phosphate of ATP (45). In the second structure the βDP- and βTP-equivalent sites contain nucleotides, and a half-closed βE site contains only ADP (no magnesium or phosphate). It has been interpreted as a posthydrolysis, pre-nucleotide-release state (46). Evidence suggests that, of the two products of ATP hydrolysis, phosphate leaves first and ADP second (for a contrasting opinion, see the section titled Stepped Rotation Related to Crystal Structure, below).

The general discussion of the kinetics and mechanism of F1 from all organisms is based largely on structures of the bovine enzyme. The few structural models of (αβ)γ that come from other sources are basically similar to the bovine model but differ in detail. All three structures refer to nucleotide-free F1. In chloroplast F1 [at 3.2-Å resolution (47)], all three β-subunits are in a
closed conformation; in thermophilic Bacillus F₁ [at 3.1-Å resolution (48)], they are in an open conformation; and in yeast F₁ [at 3.6-Å resolution (49)], one nucleotide site is open and two are closed. The reason for the conformational differences between these nucleotide-free enzymes is not yet understood. The only structure of E. coli F₁ to date represents an ε-inhibited state of the (αβ)₃γε entity [at 3.26-Å resolution (50)]. In this structure, only one nucleotide of F₁ is bound—the two other sites are filled with SO₄²⁻—and the hinge points to an α-subunit rather than to a β-subunit. For the regulation of FOF₁ by the ε-subunit in bacteria, see the section titled Regulation, below.

ATP Hydrolysis Drives the Rotation of the Central Shaft

The first crystal structure described above prompted investigators to pursue real-time recording of rotary motion. Cross and colleagues (51) used a biochemical assay to show that the γ-subunit changes its position relative to the three β-subunits when hydrolyzing ATP, however, without time resolution. By using polarized photobleaching and recovery, Junge and colleagues (52, 53) demonstrated the rotation of the γ-subunit relative to (αβ)₃ in real time. Yoshida, Kinosita, and their colleagues (54) made a video recording of the rotation of the γ-subunit in single molecules of F₁. These authors fixed F₁ from Bacillus PS3 head down on a solid support and attached a fluorescent actin filament to the “foot” of the γ-subunit. They monitored the rotation of the probe with fluorescence microscopy. Their video-microscopic technique became the gold standard in the field. It demonstrated that hydrolysis of ATP turns the central shaft counterclockwise when viewed from the membrane. With a short probe (F-actin or bead) on the enzyme, the rate of rotation is not severely limited by viscous drag. Steps and dwells on the reaction path became apparent (55). The observed period of 120° reflects the threefold pseudosymmetry of (αβ)₃ and is subdivided by steps of 90° and 30° starting from the ATP-waiting dwell and the catalytic dwell, respectively. The catalytic dwell before the 30° step is composed of two mechanically silent subreactions, each ~1 ms long (55). The width of the substeps was later modified to 80° and 40° (56, 57). The nucleotide occupancy, conformational changes of the β-subunit, and rotation of the central shaft were monitored in parallel (58, 59). The 80° rotation is concomitant with (driven by) ATP binding and ADP release, and the 40° step is concomitant with (driven by) phosphate release and the hydrolysis of bound ATP. A freshly bound ATP molecule leaves the enzyme as ADP only after the central shaft has turned forward by 240° (58, 60–62). This stepwise motion has also been demonstrated in E. coli F₁ (63–66). In human F₁, an additional dwell and slightly different dwell angles have been reported (67).

The time resolution of stepped rotation has been greatly improved by use of a gold nanorod (typically 30 nm long) as a probe on the rotor. The rotation was detected at microsecond resolution via the polarization of scattered light (68–70).

Stepped Rotation Related to Crystal Structure

Stepped rotation of the γ-subunit in single-molecule experiments cannot be related a priori to the orientation of (αβ)₃ in lab coordinates and, thereby, to the crystal structure. Three research groups have overcome this drawback. Two of them (66, 71) recorded rotary stepping and then locked the rotor to the stator (by a disulfide bridge) in a position similar to the one in the crystallographic position aiming at the ground state of the bovine enzyme (Figure 3a,b). The third group used fluorescence resonance energy transfer (FRET) (59). All three groups found that the rotor orientation in the crystal structure of the bovine enzyme corresponds to the catalytic dwell in the
Figure 3
Stepping of F$_0$F$_1$ when driven by ATP hydrolysis. (a) Isolated and immobilized F$_0$F$_1$ with fluorescent actin filament attached to the c-ring of F$_0$ (135). (b) Engineered disulfide bridge (inside the blue circle) intended to trap the rotor orientation, as in crystals (see Figure 2b). (c) Histogram over 360° of the rotary stepping under ATP-limited conditions (hatched blue) and when dominated by catalysis (red). The position of the rotor in the inactive enzyme is blocked either by ADP (yellow) or by the disulfide bridge (green) (66). The term SS-locked denotes the closed disulfide bridge.

respective bacterial enzyme rather than to the ATP-waiting dwell. In E. coli F$_1$, this orientation coincides with that of the ADP-inhibited state (66). In the ATP-waiting dwell, the rotor is turned forward (counterclockwise) by more than 40° (Figure 3c).

The forward displacement of the ATP-waiting dwell was an unexpected result that prompted checks for possible packing deformations in crystals. The “foot” of the γ-subunit is susceptible for contortion by up to 35° (46), but the remainder is not (24). The distortion of the foot has led to the belief that the crystallographic results are difficult to reconcile to the discrete states observed in single-molecule biophysical experiments (24). This belief does not hold. Although the foot of the γ-subunit may be contorted in crystals, in active single molecules it thermally relaxes during dwells. [This relaxation has been used to determine the elastic parameters of F$_0$F$_1$ (25, 65, 66).]

In other words, a small probe that is directly (71) or indirectly (Figure 3a) (66) attached to the foot region can correctly indicate the orientation of the central stalk during dwells. The width of the fluctuations around the mean position monitors the torsion spring constant of the transiently resting enzyme during the dwell (Figure 3c) (66).

Figure 4 illustrates a model for the sequence of events over one full turn of the central stalk and the hydrolysis of three molecules of ATP. Whether phosphate or ADP is first released from the very reaction site where it is produced during the catalytic dwell is a matter of debate. Each view—namely phosphate first and ADP second (Figure 4a) (46, 72) or ADP first and phosphate second (Figure 4b) (61, 73)—is backed up by circumstantial structural and kinetic evidence. Recently, investigators used targeted molecular dynamics (MD) in an attempt to determine what makes
Figure 4
A full reaction cycle of ATP hydrolysis by three cooperative reaction sites in F1. The three sites cyclically change their role from binding ATP over hydrolysis to liberating the products ADP and inorganic phosphate. The occupation of the three reaction sites by ATP (T), ADP (D), and phosphate (P) is indicated. The reaction proceeds clockwise (green arrow). During a full reaction cycle, the central shaft (red arrow) turns counterclockwise. The tip of the red arrow denotes the apex position of the convex side of the γ-subunit (see Figure 1b). In the ATP-waiting dwell of the active enzyme, it is displaced by +40° (counterclockwise) forward (59, 66, 71) from the position in the first crystal structure (41). The oblique column in panel b parallels its neighbor to the left, except that the sequence of product release after hydrolysis is changed from phosphate first in the triangle to ADP first in the column.

These versions differ (74). The authors studied the energy profile of the release of PiE− from the binding pocket, with an emphasis on its interaction with the arginine finger. The release of PiE− from βE is kinetically feasible in the time range of milliseconds, whereas that from βDP is not. PiE− release from βE (∼1 ms) turns the γ-subunit by 40°. This transition might kinetically slave the catalytic step in βDP. In other words, waiting for the release of PiE− from βE, and not catalysis proper, might define the duration of the catalytic dwell. The binding of ATP occurs only after the 40° turn, when the βE site is unoccupied and in a semiopen conformation (Figure 4a).

How many nucleotide molecules occupy the enzyme at its three catalytic sites is under debate. Some researchers claim three (ATP-to-ADP ratio of one to two) on the basis of ensemble experiments in which engineered tryptophan in the binding pocket serves as a probe for occupancy (75, 76). Other authors claim two on the basis of single-molecule experiments with fluorescent ATP analogs (58, 77). The seemingly conflicting views are likely compatible with each other given that the former experiments used physiological (i.e., high) nucleotide concentrations versus low nucleotide concentrations in the latter studies. At physiological concentrations, βE may bind nucleotides with low affinity.

Investigators have established the reversibility of the chemomechanical coupling in F1 by magnetically reversing the rotation of the central shaft (with attached paramagnetic bead) and producing detectable amounts of ATP in femtoliter chambers (78, 79). Recently, the magnetic drive was used to first force the shaft into a given position, then release the constraint and monitor...
the relaxation of the cooperative reactions in \((\alpha\beta)_3\gamma\) to either side. This is an interesting way to further elucidate the energy landscape as a function of angle (62).

**Structural Determinants of Rotary Function**

The chiral activity of F1 may be determined by \((\alpha\beta)_3\), the coiled coil of the \(\gamma\)-subunit, and/or their interaction. The activity of bare \((\alpha\beta)_3\) (without the \(\gamma\)-subunit) was recently studied by time-resolved atomic force microscopy (80). In the presence of MgATP, the three copies of the \(\beta\)-subunit undergo cyclic conformational changes in the same direction as when the \(\gamma\)-subunit was present. Therefore, bare \((\alpha\beta)_3\) is intrinsically chiral and active, although at a much lower rate than in the presence of the \(\gamma\)-subunit. The \((\alpha\beta)_3\) of F1 is just one example of nature’s wide use of hexagonal nucleotide triphosphatases (A-, F-, and V-ATPases; helicases; bacterial DNA translocases), which rotate and/or translocate proteins, RNA, and DNA in their central cavity and probably share common ancestry with F1 (81).

The role of the coiled coil on the \(\gamma\)-subunit in the direction of the rotation, and the minimum structure of \(\gamma\)-subunit necessary to pick up torque, has been studied through successive truncation. The \(E.\ coli\) enzyme tolerates the truncation only of the C-terminal end of the \(\gamma\)-subunit, the shaft in the hydrophobic bearing of \((\alpha\beta)_3\) (82). F1 from *Bacillus* PS3 is more robust. It tolerates the elimination of 36 residues from the C terminus of the \(\gamma\)-subunit (83) and 50 residues from the N-terminal end (84), and it still produces sizable torque. Accordingly, the coiled coil of the \(\gamma\)-subunit is not essential for chiral operation. What about the remainder of the \(\gamma\)-subunit, which is in close contact with the C-terminal hinge? The partial truncation of the \(\beta\)-subunit’s hinge domain (85) and extensive mutations of the contact surfaces between the \(\gamma\)-subunit and the hinge on the \(\beta\)-subunit (86) reduce the magnitude of torque but do not fully eliminate its production.

All-atom 300-ns MD simulations of the nucleotide-free F1 (87) reveal that a fully open conformation of the hinge, as in \(\beta_E\) (41, 42), is not stable, as had been previously assumed (22, 88). Rather, this conformation is enforced by the interaction between the C-terminal helix-turn-helix motif of the \(\beta\)-subunit and the convex surface of the \(\gamma\)-subunit. The most stable conformation of \(\beta_E\) is half-open. The elastic relaxation of the hinge from the former into the latter conformation has a vector component perpendicular to the axis \((\alpha\beta)_3\) so that it turns the \(\gamma\)-subunit forward by \(>40^\circ\) in the hydrolysis direction (87). The relaxed, half-open conformation might represent the ATP-binding dwell, as indicated by kinetic single-molecule experiments on the active enzyme (59, 66, 71).

These observations suggest that the off-axial motion of the C-terminal helix-turn-helix motif of the \(\beta\)-subunit (the hinge) may be the major determinant of the chiral activity of F1. Its contact with the convex surface of the \(\gamma\)-subunit in F1—or with some other component in related hexagonal nucleotide triphosphatases—is required to transmit torque and, apparently, to speed up the enzyme activity.

**Torque and Efficiency**

The magnitude of the torque generated by the hydrolysis of ATP has been determined by single-molecule experiments in two ways. In the first approach, the rate of rotation of probe is recorded, and the torque, \(\tau\), is calculated from the angular velocity, \(\omega\), and the friction coefficient of the probe, \(f\), by the relation \(\tau = f \cdot \omega\). The friction coefficient is usually calculated for the viscosity of the bulk medium (54, 64). Thus, the torque is systematically underestimated. Viscous flow coupling between the rotating probe and the surface is difficult to assess but cannot be neglected. This problem is overcome by the second approach, which uses a long (typically 3-\(\mu\)m) actin filament.
attached to the central shaft to slow the rate of rotation by orders of magnitude. Thermodynamic equilibrium between the enzyme and the elastic probe is approximated. The curvature of the filament serves as a spring balance to gauge the torque without a need to know the viscous damping (89, 90). The filament’s spring constant is obtained from its thermal fluctuations. The mean torque, when determined by this method, is greater than that determined from the first approach. The magnitude, $\tau = 55 \text{ pN} \cdot \text{nm}$, matches the driving force of ATP hydrolysis. In quantitative terms,

$$3 \cdot \Delta G_{\text{ATP}} = 2\pi \cdot \tau \cdot N_A.$$ 

Here, $\Delta G_{\text{ATP}}$ denotes the molar free energy of ATP hydrolysis under the given conditions (70 kJ/mol), $\tau$ is the torque (55 pN · nm), $N_A$ is the Avogadro number, and the numerals account for the fact that three ATP molecules are hydrolyzed when the $\gamma$-subunit rotates by $2\pi$ (for the torque profile as a function of angle, see Reference 90 and the section titled Elastic Transmission of Torque, below). The finding that the chemical free energy matches the mechanical energy that is elastically stored in the actin filament implies that the chemical-to-mechanical energy transduction in $F_1$ has an efficiency of 100%. In other words, the $\gamma$-subunit does not slip relative to $(\alpha\beta)_3$. Slip has been observed only at very low nucleotide concentrations, when the $(\alpha\beta)_3$ hexagon apparently loosens its grip on the $\gamma$-subunit (91, 92). At physiological nucleotide concentrations, there is no slip in either $F_1$ or $F_{0}F_{1}$.

**Modeling $F_1$ Dynamics**

With a wealth of crystal structures at atomic resolution, detailed kinetic information at submillisecond time resolution, and video recordings of the rotary procession, $F_1$ can be considered a “hydrogen atom” of biological nanomotoring. Its dynamic behavior depends on the interplay between organizing forces and a disorganizing stochastic force. The former are either conservative (e.g., Coulomb force) or entropic (concentration dependent), and the latter results from thermal impact (Langevin force). Stochastic force affects the turnover rates, whereas the mean directionality of the reactions is fully determined by the organizing forces.

Several groups have attempted to describe $F_1$ in terms of atomic coordinates by all-atom explicit-solvent MD (see Reference 74 and references therein). These approaches have suffered from the need to apply a higher-than-physiological torque to the central stalk in order to squeeze the events into a narrow time frame (∼100 ns). Mukherjee & Warshel (93) overcame this drawback by using a coarse-grained approach. Using advanced electrostatic approximations, they reproduced the counterclockwise rotation with 80° and 40° steps in a reasonable time frame. Karplus and colleagues (see Reference 94 and references therein) merged a coarse-grained approach with biochemical data on nucleotide binding. So far, most theoretical attempts on $F_1$ have described what has already been observed; however, they can be predictive (for a predictive mutation in silico, see Reference 87).

**$F_{0}$, THE ELECTROCHEMICAL MOTOR**

**Structure of $F_{0}$**

In *E. coli*, $F_{0}$ is composed of three different subunits in the stoichiometric proportion $c_{10}ab_{2}$. A high-resolution structure of the entire $F_{0}$ is lacking. Common to the $F_{0}$ portion of the ATP synthase of all organisms is a homo-oligomeric ring of the $c$-subunit, the only portion of $F_{0}$ for which high-resolution structures are available (see Figure 5a,b and Reference 95 for the $\text{Na}^+$-binding ring of *Propionigenium modestum*, Reference 96 for yeast, and Reference 97 for chloroplasts). So far, the best resolution of the mutual arrangement of the $c$-ring and the $a$- and
b2-subunits (7 Å) has been obtained by cryo–electron microscopy of single F0 molecules from *Ilyobacter tartaricus* (Figure 5c,d) (98) and from the related V0 portion of the V-ATP synthase of *Thermus thermophilus* (9.7 Å) (99; for details, see below). The c-ring faces the a-subunit. In *E. coli*, the a-subunit is a four-helix bundle plus one transmembrane helix (TMH). The a-subunit associates with two TMHs of the b-subunits that connect F0 to the top of the (αβ)3 hexagon of F1. All three types of subunits are required in order to promote proton conduction by FO (100).

The c-subunit is highly hydrophobic and is referred to as a proteolipid. Consisting of a hairpin of two TMHs, with a molecular mass of ∼8 kDa, the c-subunit carries an ionizable residue (glutamic acid or aspartic acid, depending on the organism) in the middle of the membrane. The pocket around this residue determines the preference of F0 for either H+ or Na+ (101). Notably, the Na+-driven ATP synthase of certain bacteria can operate on H+ if the Na+ concentration falls below ∼100 μM (see Reference 23 for a review). The wide orifice in the c-ring is probably plugged by phospholipids (102). The copy number of the c-subunit in the ring, n, is constant for each species that has been tested. It numbers 8 in bovine mitochondria (103), 10 in yeast mitochondria (96), and 14 in spinach chloroplasts (97, 104), and among bacterial species it varies from 10 (105, 106) to 11 (107), 13 (108, 109), and 15 (110). The copy number is believed to determine the ion-to-ATP ratio, which ranges from 2.67 for n = 8 up to 5.00 for n = 15. Clearly, organisms that thrive at constant, high PMF (such as mammals) benefit from running at high-speed gear (low n), whereas those exposed to variable PMF (such as chloroplasts) and/or low PMF (alkaliphilic
bacteria (109)] benefit from high torque and low-speed gear (high \( n \)). Meier and colleagues (111) found that several engineered mutations in a conserved stretch of glycines are needed to enlarge the ring size from 11 (wild type) to 12, 13, 14, and more than 14. The relative ease of converting the ring size contrasts with the constant size found within each species. A possible benefit of invariant ring size is that it prevents congeneric individuals from competing in ATP production.

Whereas the symmetry of the c-rings (\( C_{8-15} \)) varies between species, the symmetry of \( F_1 \) (\( C_3 \)) does not, virtually excluding the possibility of fine-tuning the detailed reaction steps in one motor to those of the other. Instead, partial reactions in \( F_0 \) and \( F_1 \) are kinetically decoupled from one another by an elastic torque transmission between them (see the section titled Elastic Transmission of Torque, below, and reviews in References 25 and 112).

**Design Principle for Rotary, Single-File Ion Transport**

Both the ionizable glutamic acid or aspartic acid in the middle of helix 2 of the c-subunit (cG61 in *E. coli*) (113) and the arginine on the a-subunit (aR210 in *E. coli*) (114) are essential for ion translocation by \( F_0 \). For this reason, an ion pair is hypothesized to form between adjacent cD61\(^{-}\) and aR210\(^{+}\). With this information at hand, Junge et al. (21) presented a model for rotary ion transport and torque generation by \( F_0 \) in 1993. Vik & Antonio (115) proposed a similar model. These models are based on three assumptions: (a) rotary Brownian fluctuations of the c-ring against the a-subunit; (b) electrostatic constraints, namely the obligatory ionization of the very acid residue (in *E. coli*, cD61) that faces the basic residue on the a-subunit (aR210) and the obligatory protonation of the other copies on the c-ring that face the membrane; and (c) two staggered access half-channels for ions, left and right of the basic residue on the a-subunit (Figure 6) (for an animation, see Related Resources). The width of the fluctuations is limited as long as a given ion pair exists. If an ion hops toward the negative residue through one of the channels, the Coulomb penalty is relieved. The then-neutralized residue on the ring is free to move away from its former partner; that is, the ring may “step forward.” The arrival of one ion through one channel is paralleled by the exit of another ion through the other channel, such that a new ion pair is formed with the next copy of the c-subunit on the ring. The staggered access channels, placed left and right of the electropositive residue on the a-subunit, provide chirality to the rotary device. Depending on whether the ion concentration is greater in the phase above or below the membrane, the rotor turns around clockwise or counterclockwise. If torque is applied to the ring, the device pumps ions from one side of the membrane to the other. The interplay between random fluctuations and directed electrochemical force, known as Langevin dynamics, is a common feature of nature’s nanomotors (for Berg and colleagues’ pioneering work on the flagellar motor, see Reference 116). The quantitative treatment of the \( F_0 \) motor, which involved solving the Fokker–Planck equation for this model, has revealed its viability in principle (117).

Either residue of the ion pair can be shifted in the plane of the membrane to a neighboring helix of the same subunit (for cD61, see Reference 118; for aR210, see Reference 119) without total loss of the ion transport activity, whereas a perpendicular shift of either one is lethal (120). These observations are compatible with the above functional model.

The direction of rotation, counterclockwise or clockwise, depends on the probability of the ions’ presence at either side. In the middle of the membrane, the engine simply counts ions, or in thermodynamic terms, it operates on the entropic component of the electrochemical driving force. The transmembrane voltage as a driving force is invoked by Mitchell’s (121) concept of a proton well, in which the two access channels are monospecific for a given ion—say, the proton—and highly conducting (not rate limiting). Each half-channel converts one-half of the transmembrane voltage into its equivalent in terms of the respective local pH (the time-averaged proton activity).
Figure 6
Model of the function of Fo as a torque-generating, single-file ion channel (21). Green, Arg(+); red, Glu(−); blue, GluH.

If the lower phase is more positive by 120 mV than the upper one, it is more positive by 60 mV than the middle of the membrane, and the probability of finding a proton on the right of the ion pair is thus 10-fold greater than in the lower bulk phase; the local pH is more acidic by one unit. By the same argument, the local pH to the left of the ion pair is more alkaline by one unit than that in the upper bulk. This is why such a machine is equally sensitive to the chemical and electric components of the electrochemical driving force across the membrane.

The above discussion refers to the equilibrium state of the rotary ion transporter. In a situation far from equilibrium, that is, under kinetic control, the two components of the driving force are, of course, not a priori equivalent.

The a-Subunit and the Proton Access Channels
Extensive cysteine-mapping studies have revealed that the a-subunit is composed of five TMHs, including a four-helix bundle (see Reference 122 and references therein). At least aTM4, and perhaps aTM5, contacts the c-ring. The former carries the basic residue in E. coli aR210. The two copies of the b-subunit, which connects Fo to the upper portion of F1, are farther away from the c-ring, and they probably contact aTM2 and aTM5. This arrangement of the c-, a-, and b2-subunits, with a rather narrow contact surface between aTM4 and the c-ring of E. coli (123), is supported by structural models based on cryo–electron microscopy both for Fo from Ilyobacter tartaricus
(98) at 7-Å resolution (Figure 5b) and for the related V₀ of the proton-driven V-ATP synthase from Thermus thermophilus (99) at 9.7-Å resolution. Subunit I of the latter organism, whose eight TMHs are equivalent to the a-subunit of E. coli, makes a surprisingly small contact with the c₁₂-ring. Thus, the two closely apposed access channels for the proton have been tentatively attributed to the narrow contact surface between helices (99). On the basis of extensive cysteine mapping and monitoring of the Ag⁺/Cr⁺ accessibility of F₀ in E. coli, Fillingame & Steed (124) have associated the proton half-channel from the p-side with the center of the four-helix bundle. The location of the other half-channel, which connects the acid group on the c-subunit to the n-side, is still controversial. An interface between cTM2 and the a-subunit (124, 125) and an interface between the c-ring and the lipid membrane (126) have been proposed in this role.

Proton Conductance and Assembly

The proton conductance of F₀ is too small for detection by the patch-clamp technique. In an initial approach using isolated thylakoids, investigators inferred the electric conductance of F₀ from the decay of the transmembrane voltage after excitation of PSII and PSI with a short flash of light. The voltage decay was detected by the intrinsic “molecular voltmeter” in the thylakoid membrane (9, 127). F₁ was detached from the membrane and quantified by immunoassay. The electric conductance per free F₀ molecule was calculated to be at least 10 fS (128). A similar estimate was obtained for E. coli F₀ (129). When calculating the conductance, the authors assumed that every solubilized F₁ left a fully functional F₀ in the membrane, an assumption that was not trivial. The uncertainty over the proportion of actually conducting F₀ was eventually resolved by a single-molecule-per-vesicle approach (130). One can prepare very small (∼30-nm-diameter) chromatophores from Rhodobacter capsulatus such that they contain less than one (an average of 0.3) FOF₁ molecule per vesicle. The proton conductance of one exposed F₀F₁ molecule or, after removal of F₁, of a single F₀ molecule is determined via the relaxation of the light-induced voltage pulse. Only one-third of the vesicles show rapid voltage decay, which is attributable to a single exposed FOF₁ molecule or a single F₀ molecule (130). The magnitude of the unitary proton conductance per single F₀ molecule is 10 fS (130). The current–voltage relationship is linear; that is, the conductance is ohmic in the range between 7 and 70 mV. There is no evidence for voltage gating of the proton-driven F₀ of Rhodobacter capsulatus (130), in contrast to claims for sodium-driven F₀ (see Reference 131 and references therein). The pH dependence is surprisingly small. With a broad maximum at a pH value of 8, the conductance drops only twofold toward pH values of 6.3 and 10. Also, the hydrogen/deuterium (H/D) kinetic isotope effect, which is 2 at a pH value of 6, vanishes above a pH value of 8.5 (130). This behavior at alkaline pH might indicate proton supply to the rotary channel by water hydrolysis.

The 10-fS conductance corresponds to the translocation of 6,240 protons per second at a 100-mV electric driving force, or twice that, ∼12,500 at 200 mV. Thus, the turnover rate of a bacterial c₁₀-ring exceeds 1,000 revolutions per second, ∼10 times more than that of the coupled F₀F₁ at the same high driving force (130).

The properties of the rotary proton conduction by F₀—its magnitude of 10 fS, the low pH dependence between 6.5 and 10, and a linear current–voltage relationship—were kinetically modeled with a minimum set of three free parameters (130). The authors of this study assumed that one proton relay group per access channel was in very close proximity to the respective membrane–water interface. If so, each proton relay was exposed to a very small fraction of the transmembrane voltage, giving rise to a linear (ohmic) current–voltage relationship. To account for the shallow
pH dependence, the authors assigned the relay groups very different acid dissociation constants (pKs): one acid (pK 6.5) and the other alkaline (pK 10) (see Reference 130 and references therein). These features of the rotary channel were based solely on kinetic studies, and so far, they lack structural evidence.

During assembly of the holoenzyme from *E. coli*, EF_{O}F_{1}, the F_{O} portion is completed only under the control of F_{1} (132). In mutants of *E. coli* with delayed expression of δ-subunit, the stable subcomplexes ab_{2} and c_{10} (αβ)_{3}γε are integrated in the membrane. Their assembly into fully functional EF_{O}F_{1} necessitates the expression of the δ-subunit (133). The δ-subunit connects the b_{2}-subunit to the crown of (αβ)_{3} and couples the rotary proton transfer in F_{O} to rotary catalysis by F_{1}. This mode of assembly prevents bare c_{10}ab_{2} = F_{O} from acting as a proton leak.

**Rotation of the c-Ring**

Rotation of the c-ring, driven by ATP hydrolysis, was first detected in single F_{O}F_{1} molecules (134, 135). The rotary stepping, with steps of 40° and 80°, was dominated by F_{1} (65, 66). The expected finer steps of the c-ring in F_{O} have called for higher resolution of both time and angle. Börsch, Gräber, and their colleagues (136, 137) have relied on FRET between a pair of dyes attached to the rotor and the stator, a method that is also applicable to membrane-bound F_{O}F_{1}, whether driven by ATP hydrolysis or PMF. Thirty-six-degree steps of the c_{10}-ring from *E. coli* were detected at the limits of resolution (138).

Frasch and colleagues (70, 139) chose another approach. They attached gold nanorods to the c_{10}-ring of immobilized F_{O}F_{1} from *E. coli* and recorded the orientation-dependent color changes of the back-scattered light. Because of the small size of the rods, the rotation was not limited by viscosity, and it revealed the intrinsic substepping of the c-ring in F_{O}. These steps were superimposed onto those of the driving motor, F_{1}.

**Modeling F_{O} Dynamics**

Several research groups have extended this phenomenological scheme for rotary single-file ion conduction by F_{O} to explicitly incorporate further structural features of c_{10}ab_{2}. All these attempts have suffered from the lack of a detailed structural model of this ensemble. One team proposed a tentative energy landscape and simulated rotary trajectories by Langevin dynamics (131, 140); however, how the landscape relates to actual structural details is unclear. Two other groups used available structural information about the c-ring to carry out coarse-grained MD simulations (141, 142). When starting from a position where the ion pair (cAsp−/aArg+) is perfectly aligned, the energy profile to the right and the left of this position is asymmetric. It explains one rotational step but not the continuous rotation. Continuous rotation requires two staggered half-channels for ion access, which create chirality (21). More recently, Mukherjee & Warshel (143) used a coarse-grained approach to model both F_{O} rotation and proton transfer across the membrane. They calculated an (electrostatic) energy landscape based on the limited available structural information about the c_{a} moiety. They assumed two staggered half-channels used by the proton to access the essential anionic residue from either side of the membrane. By solving the statistical dynamics of proton motion and ring rotation over this energy profile, Mukherjee & Warshel (143) obtained a typical proton transfer rate of 42,000 H\(^{+}\) ions per second at a pH value of 8 (on both sides of the membrane) and an electric driving force of 200 mV. The order of magnitude compares well with the experimentally determined 12,500 H\(^{+}\) ions per second (130), considering the ambiguity of certain structural features and the sensitivity of kinetic constants to small differences in energy landscape.
**F₀F₁, THE PROTON-DRIVEN ATP SYNTHASE**

**Two Coupled Rotary Motors**

In the holoenzyme $F₀F₁$, two motors operate against each other. Net turnover stops when the thermodynamic driving forces are equal (Equation 1a). If the PMF prevails, then the enzyme synthesizes ATP, and if the molar free energy of ATP hydrolysis prevails, then the enzyme pumps protons and generates PMF. This simple picture holds if two conditions are met: (a) perfect coupling of the two motors and (b) full reversibility of the enzyme. The rotation of the c-ring is mechanically coupled to the turnover of the $F₁$-ATPase (90), and there is no leak conductance (i.e., proton slip) if the nucleotide concentrations are greater than 1 μM (91, 92). Various means of regulation, however, may convert the microscopically reversible enzyme into a seemingly irreversible device (see the section titled Regulation, below).

For a perfectly coupled dual motor, one expects the ratio of protons transported over ATP processed (the ratio of $H^+$ to ATP) to be determined by the ratio of the copy number of the c-subunit in the FO ring to the number of catalytic sites in $F₁$, which is 3 in all organisms. For the chloroplast enzyme, which has 14 copies of the c-subunit, the ratio should be 14 to 3, or 4.7. Why this expectation has not been met in kinetic and thermodynamic experiments despite considerable efforts is an open question (see Reference 144 and references therein). Instead of 4.7, a ratio of 4 has been consistently observed—this result implies “overcoupling,” which cannot exist.

**Elastic Transmission of Torque**

Circumstantial evidence suggests that the detailed reaction steps in $F₀$ are kinetically decoupled from those in $F₁$. Examples are the significant size variation of the c-ring (ranging from 8 to 15 copies) in different organisms and the functionality of a chimeric construct composed of the $Na^+$-translocating $F₀$ of *P. modestum* and the $F₁$ of *E. coli* (145). Hypotheses that kinetic decoupling occurs by elastic torque transmission between $F₀$ and $F₁$ (146, 147) were ultimately proven correct by single-molecule experiments with $F₀F₁$ from *E. coli*, in which the torque generated by $F₁$ and transmitted to $F₀$ was measured via the curvature of a 3-μm-long actin filament (90). Although torque production by $F₁$ was expected to have three steps (under the given conditions), the torque output to the actin filament on $F₀$ was almost constant (90). This surprising observation can be explained by solving the Fokker–Planck equation of a stepping nanomotor that is elastically coupled to a heavy load (90). Increasing the compliance of the elastic buffer has two consequences: a flattening of the torque profile at the output and an increase of the turnover rate under load. The latter effect is rather dramatic (Figure 7b). An elastic buffer increases the turnover rate by orders of magnitude over the rate obtained with a stiff transmission between motor and load. This benefit of a compliant transmission likewise applies to all of nature’s intrinsically stepping nanomotors that drive a heavy load.

The distribution of compliant and stiff domains over $F₀F₁$ was determined by a fluctuation analysis under selective stiffening by use of engineered disulfide bridges (65, 148). Broadly speaking, the most compliant domain in $F₀F₁$ (with a torsion spring constant of 70 pN·nm) is at the interface of $F₀$ and $F₁$ and involves the foot of the γ-subunit plus the ε-subunit (and perhaps the c-ring [149]) (Figure 7a). The C-terminal hinge of the β-subunits (with a stiffness of ~70 pN·nm) adds further compliance such that the total spring constant is ~35 pN·nm (see Reference 25 and references therein). Other domains of $E. coli$ $F₀F₁$ that are very stiff include the portion of the γ-subunit between the hinge and the C terminus (700 pN·nm) and the eccentric stator [1,500 pN·nm, involving the b-subunit (148)]. One fluctuation experiment (65) was simulated by MD
The elastic torque transmission between the two rotary motors in F₀F₁. (a) Distribution of stiff (gray) and compliant (pink) domains over the homology structure of *Escherichia coli* F₀F₁. Numbers indicate the torsion spring constants in piconewton nanometers (65, 148). (b) Calculated turnover rate of a nanosized stepper motor being elastically coupled to a heavy viscous load as a function of the torsion spring constant between motor and load (90).

The magnitude of the torsion stiffness of the γ-subunit between the C-terminal end and its contact area with the hinge on the β-subunit, 700 N·m, matched the experimentally determined figure. It may be difficult to accept that similar values result from two approaches addressing such different timescales, namely milliseconds in the experiment (65) and nanoseconds in the simulation (150). The reason for the equivalent values is the scaling of the viscous drag and, therefore, the rotational fluctuation time by the third power of the probe length (100 nm in the former approach and 1 nm in the latter).

**The Enzyme in the Coupling Membrane**

F₀F₁ is monomeric in the coupling membranes of photosynthetic organisms. In contrast, in mammalian mitochondria it forms bands of dimers lining the rim of cristae. The correlation time of rotational diffusion of F₀F₁ in the thylakoid membrane of chloroplasts (≈100 μs (53)) is approximately one-tenth as long as the rotation period of the c-ring in F₀ (≈1 ms) at high driving force (130). This observation implies that viscous drag of the lipid matrix on the rotating c-ring is small. The rate of proton-driven rotation of the c-ring in bare F₀ is much faster (≈1,000 revolutions per second) than when it is coupled to F₁ in the holoenzyme (100 revolutions per second). Thus, the proton transporting c-ring rapidly equilibrates with the elastic buffer, which provides F₁ with torque when needed for the reactive stepping of ATP synthesis.

Solitary F₀F₁ molecules are located at the top and bottom plates of grana stacks and on stroma lamellae. The generators of PMF (e.g., PSII) may be a distance of several hundred nanometers from the consumer, F₀F₁. Under steady turnover of the proton pumps and the proton-translocating ATP synthase, lateral losses of PMF are inevitable. The two components of the PMF propagate with different velocities along the membrane. A locally generated voltage pulse propagates fast and practically loss free because of the high electric conductance on both sides of the membrane.
As mentioned above, the whole thylakoid system, which contains $\sim 10^8$ chlorophyll and $10^5$ F$_0$F$_1$ molecules, may be considered electrically isopotential (30). The propagation of the chemical driving force, however, is slow and not loss free. The proton is a minority charge carrier, and its motion is electrically compensated by other ions. Protons diffuse from proton pumps to the ATP synthase at the p-side of the membrane and back at the n-side (Figure 1b). The driving force of proton diffusion between sources and sinks is a lateral $\Delta p$H difference. As a consequence, there is a lateral loss of the transmembrane $\Delta p$H difference between the pumps and F$_0$F$_1$. The magnitude of this loss of PMF was recently determined in active mitochondria (151), in which F$_0$F$_1$ dimers line the rim of disk-shaped cristae and the cytochrome oxidase (a proton pump) resides in the flat portions. The lateral drop of the transmembrane $\Delta p$H slightly modifies Mitchell’s original concept (5). Further modifications have been discussed, namely enhanced diffusion of the proton along the surface of the membrane and their delayed escape from the membrane surface into the adjacent bulk phase. Although there has been no experimental evidence for enhanced surface diffusion, the delayed escape of protons from the surface has been established (152) and may be important in enabling alkaliphilic bacteria to synthesize ATP at a seemingly too-low bulk-to-bulk PMF (19). In the narrow gap between thylakoid membranes, the terms surface and bulk lose their distinction. For protons, the bulk phase is defined more by buffering groups at the surface than by aqueous volume. Nevertheless, any charge crossing the dielectric membrane via F$_0$F$_1$ can be detected as a proton entering from the p-surface and leaving from the n-surface of the thylakoid membrane (153).

**Regulation**

In cyanobacteria, ATP synthase shares a coupling membrane with the electron transport chains of both photosynthesis and respiration. Whether generated in light or dark or under aerobic or anaerobic conditions, ATP molecules are powered by the PMF. In higher plants, photosynthesis and respiration are segregated from each other, the former in the thylakoid membrane of chloroplasts and the latter in the crista membrane of mitochondria. To prevent futile hydrolysis by the chloroplast enzyme of ATP made by mitochondria, the chloroplast enzyme is downregulated in the dark. Under oxidizing conditions, enzyme activity stops before the PMF drops below the thermodynamic compensation point. The apparent irreversibility of the auto-oxidized enzyme holds regardless of whether the electric component ($F \cdot \Delta \phi$) (10) or the chemical component ($-2.3 \cdot RT \cdot \Delta p$H) of the PMF is dominant (154). Whereas ATP hydrolysis is blocked at low PMF in the oxidized enzyme, at high PMF ATP synthesis proceeds at a high rate (155). The inhibition of ATP hydrolysis is relieved under illumination, when photosynthetic electron transport provides reducing equivalents. Chloroplast F$_0$F$_1$ is thiol-activated by thioredoxin (156). Two cysteine residues in the foot of the chloroplast $\gamma$-subunit are the target of modulation. They are absent in the cyanobacterial wild-type enzyme (157) and in the enzymes of *Bacillus* PS3, other $\alpha$-bacteria, and mitochondria. If the regulatory stretch of the chloroplast $\gamma$-subunit is engineered into $(\alpha\beta)\gamma$ from *Bacillus* PS3, its ATPase activity is enhanced upon reduction (158). In single-molecule experiments, longer pauses of rotation are observed if $(\alpha\beta)\gamma$ is oxidized (159). In addition to the two actuators ($\Delta \phi$ and $\Delta p$H) and the modulator pair (thioredoxin/dioxygen), further factors are involved in the activity regulation, namely tightly bound ADP and the $\varepsilon$-subunit. How these factors interact to prevent futile ATP hydrolysis in the chloroplast enzyme is the subject of active research (see Reference 160 and references therein). When relieving the inhibition, the actuator, PMF, is probably mechanically sensed via the directionality of F$_0$-induced rotation and/or the magnitude of torque, rather than electrostatically by a group of gating charges, as in cation channels (161).
Mammalian mitochondria host a special inhibitor protein of F-ATPase (162), known as IF1. Whereas IF1 inhibits ATP hydrolysis, it does not inhibit synthesis (163). The structural mechanisms underlying this unidirectional action have recently been determined (164). On the basis of crystal structures of the mitochondrial F-ATPase, with one, two, and three bound molecules of an N-terminal fragment of the inhibitor, investigators have proposed that, in solution, an unordered segment binds to the cleft in (αβ)E. This segment is then sequentially structured into an α-helical shape and is more deeply incorporated between the γ-subunit and (αβ)3 during the subsequent 120° steps. It then blocks the rotation of the central shaft. Reversal of the direction of rotation, as under proton-driven ATP synthesis, sequentially expels the inhibitor into the medium. The obvious physiological role of IF1 is to prevent mitochondria from hydrolyzing ATP when the PMF is low (e.g., during anoxia). It may also play a role in preventing apoptosis, in particular in tumor cells (see Reference 164 and references therein).

EPILOGUE

ATP synthase (F1F0-ATPase of F-ATPase) operates by the same electromechanochemical mechanism in numerous organisms, regardless of their evolutionary history and habitat. Its remote relatives, archaeal A-ATPase and eukaryotic vacuolar V-ATPase, probably operate by the same principle. They probably evolved from a common ancestor (81, 165). One difference between F-ATPase and V-ATPase is the ion-translocating ring in FO and VO. In the V-ATPase from yeast, each copy on the ring in VO contains four transmembrane helices (versus two in FO) and one or two negative charges in the middle of the membrane. Given an elastic torque transmission that is similar to that established for F-ATPase, this structural difference must not affect the efficient cooperation between the two rotary nanomotors of V-ATPase. V-ATPase operates as an ATP-driven proton pump in the plasma membrane of eukaryotic cells and in internal organelles such as vacuoles, lysosomes, synaptic vesicles, endosomes, secretory granules, and the Golgi apparatus, in which it can produce an internal pH lower than one. The tissue-specific regulation of V-ATPase in eukaryotes is a subject of great biomedical interest that calls for similarly detailed structural and kinetic data, as are available for F-ATPase.

DISCLOSURE STATEMENT

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RELATED RESOURCES

1. Original video recordings of the rotary motion of F_{1}: [http://www.k2.phys.waseda.ac.jp/Movies.html](http://www.k2.phys.waseda.ac.jp/Movies.html)
2. Original video recordings of F_{0}F_{1}: [http://www.home.uni-osnabrueck.de/wjunge/Media.html](http://www.home.uni-osnabrueck.de/wjunge/Media.html)
4. Website dedicated to this enzyme: [http://www.atpsynthase.info](http://www.atpsynthase.info)
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