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Electrical Power Fuels Rotary ATP Synthase

Minireview

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ATP synthesis by F-type ATP synthases consumes energy stored in a transmembrane electrochemical gradient of protons or sodium ions. The electric component of the ion motive force is crucial for ATP synthesis. Here, we incorporate recent results on structure and function of the F_0 domain and present a mechanism for torque generation with the fundamental nature of the membrane potential as driving force in the core.

Energy conversions are central to all life forms. The degradation of nutrients in animals or bacteria or photosynthesis in plants culminates in the production of ATP, the universal energy currency of living cells. The capacity of this process is impressive: the daily turnover of a human has been estimated to be 40 kg of ATP on average. The central metabolic role of ATP has stimulated much interest in how it is formed using the energy of oxidations or light. Research in this area has led to impressive progress, including some of the most spectacular discoveries in the history of biochemistry. One of the highlights was Peter Mitchell's unprecedented recognition that energy derived from light or oxidation could create an electrochemical proton gradient across the chloroplast or mitochondrial membrane in which the enzymes are embedded. The protonmotive force is then used to drive ATP synthesis from ADP and phosphate. This discovery shifted the focus to the question of how the movement of protons across the coupling membrane induced the formation of ATP and thus into the core of the mechanism of the ATP-synthesizing enzyme.

The enzyme responsible for ATP synthesis is the F_1F_0 ATP synthase, which is ubiquitous from bacteria to plants and animals. Our current knowledge of structure and function of an ATP synthase is shown in cartoon form in Figure 1. The multisubunit enzyme consists of two domains, F_0 and F_1, which are connected by a central and a peripheral stalk. Each of these domains functions as a reversible rotary motor and exchanges energy with the opposite motor through mechanical rotation of the central stalk (Capaldi and Aggeler, 2002). During ATP synthesis, the electrochemical ion gradient fuels the membrane-embedded F_0 motor to rotate the central stalk in its intrinsic direction. This rotation causes sequential binding changes at the peripheral F_1 domain so that one catalytic site binds ADP and phosphate, the second makes tightly bound ATP, and the third releases the ATP (Boyer, 1993). Important mechanistic details of these binding changes are still under active investigation (Weber and Senior, 2003; Menz et al., 2001). Conversely, ATP hydrolysis by the F_1 motor causes reverse rotation of the shaft, which converts the F_0 motor into an ion pump. Under normal circumstances, the F_0 motor generates the larger torque and drives the F_1 motor in ATP synthesis direction. However, in anaerobically growing bacteria, when the respiratory enzymes are not active, the F_1 motor hydrolyzes ATP to use the F_0 motor as the generator of the indispensable membrane potential.

The rotational model has gained impressive support from the crystal structure of F_1, which shows a marked asymmetry in the conformations and nucleotide occupancy of the catalytic β subunits (Abrahams et al., 1994). These structural features suggest that the different conformations interconvert by rotation of the central γ subunit relative to the $(\alpha\beta)_3$ subcomplex. Once this structure was available, it guided experimental approaches to establish the rotational catalysis by a variety of biochemical and spectroscopic techniques. Most convincingly, the rotation of a micrometer-sized fluorescent actin filament attached to the central shaft γ subunit has been directly visualized by video microscopy of single F_1 molecules (Noji et al., 1997).

Construction of the F_0 Motor

Structurally, the least well-defined part of the ATP synthase is F_0. So far, no high-resolution structures of this domain are known, which would undoubtedly be needed to explain the energy transduction mechanism in molecular terms. However, a wealth of biochemical knowledge about the driving forces used for rotation, the ion path across the membrane, and the function of key amino acid residues is available to draw a good picture of the F_0 motor. Based on moderate-resolution structural data, the overall architecture of F_0 consists of an oligomeric ring of c subunits that is flanked laterally by a single a subunit (Mellwig and Böttcher, 2003). Subunit a is connected by the peripheral stalk b_2 subunits to F_1. The c ring together with the γ and ϵ stalk comprises the rotor. As ions traverse the membrane through the a-c interface, the rotor turns against the residual parts of the assembly that is termed stator by convention. Most structural studies of F_0 were performed with subunit c. Each c subunit is folded as two transmembrane α helices connected by a loop (Girvin et al., 1998). In the oligomeric assembly, the N-terminal helices pack very tightly into an inner ring, and the C-terminal helices form a more loosely packed outer ring (Stock et al., 1999; Vonck et al., 2002). In this construction, enough space may be left between neighboring outer helices and the connecting inner helix for access channels from the cytoplasm to the binding sites in the center of the bilayer.

Interestingly, the number of c subunits forming the ring is not fixed but varies among species: numbers of 10, 11, and 14 have been found for the ATP synthases from yeast, *Propionigenium modestum*, and spinach chloroplasts, respectively. Hence, there is obviously a

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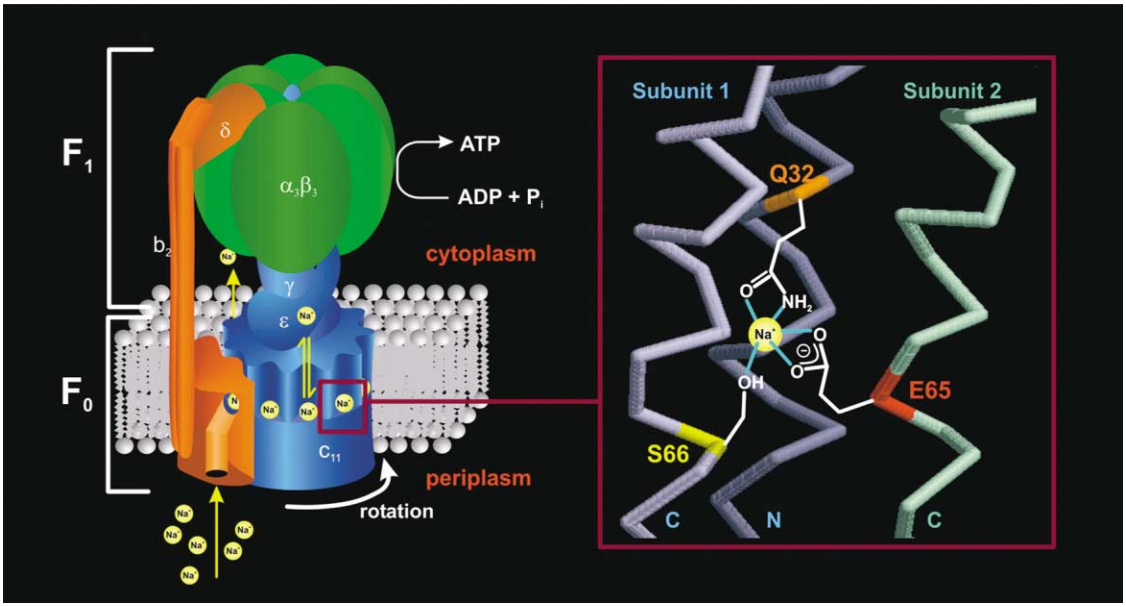

Figure 1. Schematic Model of Structure and Function of a Sodium F_1F_0 ATP Synthase

Left: The water-soluble F_1 domain with catalytic sites on the three β subunits is connected via the central stalk subunits γ and ϵ to the c ring (c_{11}) of the F_0 domain and via the peripheral stalk subunits $b_2\delta$ with subunit a of F_0. The rotor (blue) consists of subunits $c_{11}\gamma\epsilon$, and the stator (green, orange) consists of subunits $ab_2\alpha_3\beta_3\delta$. The path of Na^+ during ATP synthesis is from the periplasm through the subunit a channel onto an empty binding site on subunit c at the rotor-stator interface. After turning the rotor site out of the interface with the stator, the ion can freely exchange through its rotor channel with the cytoplasmic reservoir.

Right: The Na^+ binding site in the center of the membrane is formed in the space defined by three helices, with Glu65 from one c subunit and Ser66 from the C-terminal and Gln32 from the N-terminal helix of the neighboring c subunit. The acidic amino acid is conserved in all species and serves as universal binding site for Na^+ and H^+. The additional polar residues required for Na^+ binding (Gln32 and Ser66) are missing in proton translocating ATP synthases.

mismatch between the 3-fold symmetry of F_1 and the number of c subunits in F_0. In one complete rotation of the rotor, as many ions as c subunits present traverse the membrane and lead to the formation of three molecules of ATP. The H^+ (Na^+) to ATP stoichiometry is therefore not integer but varies between 3.3, 3.7, and 4.7.

Ion Path through the F_0 Motor

Most ATP synthases are proton-coupled enzymes. Certain anaerobic bacteria such as *Propionigenium modestum* contain a variant, which uses Na^+ as the coupling ion and can switch to Li^+ or H^+ if Na^+ is absent. These properties proved to be very advantageous to trace ion translocation across the membrane. Figure 1 summarizes numerous experimental results in a model for the ion path through the sodium F_0 motor. Each c subunit contains a Na^+ binding site contributed by ligands from one negatively charged and two polar residues (Gln32, Glu65, and Ser66). Glutamate 65 is at a conserved location in the C-terminal helix of subunit c near the center of the membrane (von Ballmoos et al., 2002). Proton-translocating ATP synthases have either glutamate or aspartate at this position but lack the two polar Na^+ binding site residues.

The carboxylate side chain of the acidic amino acid reacts covalently with dicyclohexylcarbodiimide (DCCD). This modification inhibits ion translocation through F_0 in agreement with the anticipated role of this residue in ion binding. The membrane-buried location of the ion binding site requires specific access channels to accommodate ion transport between this site and the two different reservoirs separated by the membrane. Based on experiments with the isolated c ring from the sodium F_0 motor, the site is accessible to Na^+ or H^+ by rotor-intrinsic ion channels from the cytoplasmic surface (Meier et al., 2003). Ions from the periplasm reach the site through a channel in the a subunit (stator channel). In the F_0 motor, an ion arriving from the periplasm via the stator channel binds to an empty rotor site, from where it is dislodged outside the subunit a-c interface into the cytoplasm via its rotor-intrinsic ion channel.

In principle, the ion path could also involve two half channels in subunit a in opposite orientation as proposed in the two-channel model for the proton-translocating F_0 motor of *E. coli* (Junge et al., 1997; Vik and Antonio, 1994). A proton arriving from the periplasm via the inlet channel would bind to a rotor site and would ride on it for almost a complete revolution before it dissociates through the outlet channel into the cytoplasm. Recent cysteine mutagenesis and accessibility studies with the proton F_0 motor of *E. coli* provide good evidence for an inlet channel in the stator a subunit corresponding to the inlet channel in the sodium F_0 motor (Angevine and Fillingame, 2003). The main difference in the models of the proton or sodium F_0 motor is therefore the location of the outlet channel, being part of subunit a in the proton motor and part of the c ring in the sodium motor, respectively.

Driving Force for the F_0 Motor

According to Mitchell's chemiosmotic model, energy stored in a transmembrane electrochemical ion gradient

is utilized by the ATP synthase to produce ATP from ADP and phosphate. A long-standing tenet of this model is the assumption that membrane potential ($\Delta\psi$) and transmembrane ion gradients are thermodynamically equivalent:

$$\text{Proton motive force } (\Delta p) = \Delta\psi + (2.3\ RT/F) \times \Delta pH.$$

While this relationship accurately describes the equilibrium energetic coupling, there is evidence that the transmembrane electrical potential controls the kinetics of the rotary motion in a manner that is independent from the ionic gradient (Kaim and Dimroth, 1999). In the absence of the electrical driving force, the F_0 motor is in an idling mode, which allows sodium ions to exchange across the membrane. The idling mode persists in the presence of a transmembrane Na^+ gradient (ΔpNa^+) but is converted into unidirectional Na^+ transport if a membrane potential $\Delta\psi > -40$ mV is applied. The pivotal role of the membrane potential as driving force for the ATP synthase is reinforced in ATP synthesis experiments with the reconstituted enzymes from *P. modestum*, *E. coli*, or spinach chloroplasts. These do not show any ATP formation in the absence of $\Delta\psi$. The rates of ATP synthesis increase with increasing membrane potential, approaching saturation at $\Delta\psi > 60$ mV for the chloroplast ATP synthase and at $\Delta\psi > 120$ mV for both bacterial ATP synthases (Kaim and Dimroth, 1999).

These coherent results demand to revisit the classical acid base transition experiment performed almost 40 years ago (Jagendorf and Uribe, 1966). Thylakoids equilibrated with succinate buffer (pH 5) synthesize ATP when the outside pH is increased rapidly to pH 8. It was therefore concluded that ATP synthesis could be driven by ΔpH only. However, in more recent investigations, the succinate monoanion, which is the most abundant form at pH 5, is shown to be membrane permeable, generating a diffusion potential of approximately 140 mV under the experimental conditions employed. ATP synthesis is observed only when the diffusion potential is created, but not in parallel experiments with membrane-impermeable buffers creating ΔpH only. The rationale for the permeability of the succinate monoanion is a delocalized and therefore shielded negative charge shared by both carboxylic groups in an energetically favored ring structure. Accordingly, maleinate (*cis*) but not fumarate (*trans*) induces a diffusion potential and supports ATP synthesis in similar experiments (Kaim and Dimroth, 1999).

Model of the F_0 Motor

Figure 2 gives a schematic account on the current model for torque generation in the *P. modestum* F_0 motor that is based on results summarized above and on additional mutagenesis and molecular modeling studies (Dimroth et al., 1999).

Without energization, the F_1F_0 ATP synthase is resting in its idling mode and catalyzes Na^+ exchange across the membrane. The rotor is not locked in a fixed position but performs thermal fluctuations in both directions against the stator within a narrow angle. Outside the subunit a/c interface, the ion binding site is accessible from the cytoplasm, and within the subunit a/c interface, the binding site is accessible from the periplasm. Hence, upon oscillations of an occupied site between these two positions and diffusion of the cargo through the appropriate channels, Na^+ ions are exchanged between the two different reservoirs.

The fundamental question that has to be answered is how the electric potential drives the ions through the F_0 motor components and how this ion flux is linked to the generation of torque. It is important to recognize that the rotor sites switch between the empty and the ion bound states, which endow them with strikingly different properties. It can be assumed that the binding sites outside of the a-c interface are occupied by Na^+ ions. Within the interface, however, the empty binding site is negatively charged and therefore electrostatically attracted by the universally conserved stator arginine (R227 in *P. modestum*) or by the membrane potential. After ion binding from the periplasmic channel, the net charge of the site is reduced. This lowers the energy barrier for its diffusion through the hydrophobic rotor-stator part and prevents it from getting attracted backward by the arginine.

The positive stator charge (R227) plays a fundamental role in the function of the F_0 motor. Any mutation of this residue—even the most conservative exchange to a lysine—abolishes the function of the *E. coli* enzyme completely (Vik and Antonio, 1994; Cain, 2000). The key role of R227 is strongly supported by mutational investigations with the *P. modestum* ATP synthase, which further establish its necessity for the dissociation of ions from approaching rotor sites (Wehrle et al., 2002).

When driving ATP synthesis, an ion arrives from the periplasm via the stator channel at an empty rotor site and binds to it. The pertinent rotor channel is closed at this position to prevent ion leakage through the membrane along this route. After the site has moved out of the interface with the stator, the rotor channel reopens and allows the bound ion to freely exchange with the cytoplasmic reservoir. However, at physiological pH and Na^+ concentrations, the site is occupied until it reaches the stator from the opposite side, where it encounters the positive stator charge, causing dissociation of the ion and release through its intrinsic rotor channel into the cytoplasm. Now negatively charged, the site is electrostatically attracted by the stator charge and guided into the a-c interface. Without an external driving force, there is an equal probability for the rotor site to move in either direction and the motor resides in its idling mode. After applying sufficient transmembrane voltage, however, the diffusion becomes biased toward the stator channel. Attraction of the site toward the channel can be rationalized by a horizontal component of the membrane potential (Dimroth et al., 1999). This will be present between two aqueous channels, reaching the center of the membrane from opposing sides. Given that the conductive channels have essentially the same potential as the connected reservoirs, the potential drop will occur laterally between them if their termini overlap in the center of the bilayer. When the rotor site has picked up an ion from the stator channel, reducing its net charge to that of a dipole, it moves onward through the hydrophobic part of the stator, while the arginine attracts the next empty rotor site.

When the ATP synthase operates in reverse as an

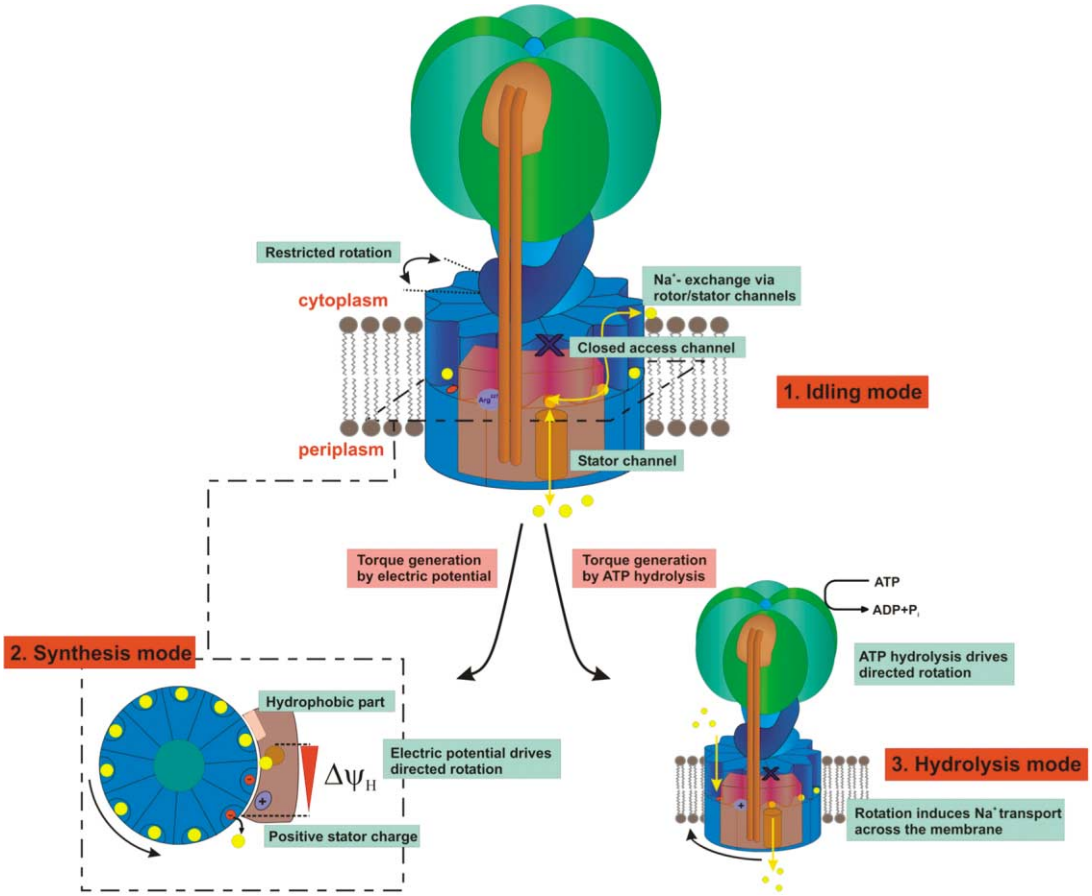

Figure 2. Model of Three Operation Modes of the ATP Synthase

(1) Idling mode. In the absence of a suitable energy source, the ATP synthase is in idling mode. The rotor moves back and forth against the stator within a narrow angle, exchanging Na^+ ions between the cytoplasmic and periplasmic reservoirs. A rotor site at the stator channel exchanges Na^+ with the periplasm, and a rotor site outside the a-c interface exchanges Na^+ with the cytoplasm. Upon moving the occupied site from the stator channel through the hydrophobic part to the outside of the a-c interface and backward, Na^+ ions exchange across the membrane. Please note that the rotor channel within the a-c interface is blocked to avoid leakage across the membrane. Rotor sites moving through the Arg227-containing part are obligatorily empty and therefore do not contribute to the exchange of Na^+ ions.

(2) Synthesis mode. Top view on the section through the ac_{11} assembly at the level of the binding sites. The positive stator charge (Arg227) is known to trigger the dissociation of an ion from the binding site approaching the a-c interface. The ion is subsequently released through the appropriate rotor channel into the cytoplasm. Now negatively charged, the ion binding site is electrostatically attracted by the positive stator charge and moves into the a-c interface. In the absence of a membrane potential, the binding site performs thermal fluctuations into either direction from the stator charge with equal probability (idling mode). During respiration, a membrane potential ($\Delta\psi$) is generated that is positive at the periplasmic surface. We envision a horizontal component of the potential ($\Delta\psi_H$) between a water-filled inlet and outlet channel within the center of the membrane. This component pulls the negatively charged rotor site toward the stator channel, where it quickly picks up a Na^+ ion. Now neutralized to a dipole, the site is no longer attracted backward by the stator charge but continues its movement through the hydrophobic part of the stator while the next empty rotor site is attracted by the membrane potential. Hence, the membrane potential is the crucial driving force to induce the torque required for ATP synthesis.

(3) Hydrolysis mode. At low electrochemical potential, the ATP synthase can operate in reverse as an ATPase, whereupon it functions as an electrogenic ion pump. ATP hydrolysis by the F_1 motor induces torque that drives the rotor into the opposite direction. Hence, rotor sites approach the stator channel from the hydrophobic portion of the a subunit. The sites must be occupied, since an unbalanced negative charge is unable to penetrate. Upon approaching Arg227, the ion dissociates and diffuses through the stator channel into the periplasm. The torque derived from ATP hydrolysis easily rotates the negatively charged site through the hydrophilic portion of the stator passing Arg227. Outside the a-c interface, the site quickly picks up a Na^+ from the cytoplasm through its rotor channel.

ion pump, rotor sites are forced to move through the hydrophobic portion of the stator first and must therefore have previously been loaded with ions. The size of the barrier to rotate an empty binding site even exceeds the free energy of ATP hydrolysis, and the enzyme is therefore not capable of hydrolyzing ATP in the absence of Na^+ ions. Upon approaching the stator arginine, the ion dissociates due to electrostatic repulsion and escapes through the stator channel. Unlike during synthesis, torque generation originates from ATP hydrolysis in the F_1 part and is used to overcome the electrostatic interaction between the negatively charged rotor site and the positive stator charge to rotate the site out of the a-c interface.

A rather different model for the rotation of the c ring in the proton F_0 motor of *E. coli* derives from NMR studies

of the isolated c subunit in solution. It has been found that protonation of the key carboxylate group causes the C-terminal α helix to rotate by 140° around its helix axis. This conformational change was proposed to be a key element in the rotational mechanism of the proton F_0 motor (Rastogi and Girvin, 1999).

Impressive progress has undoubtedly been made in recent years on the operation principles of the ATP synthase. The obligatory role of the electric potential to drive rotation in the F_0 motor toward ATP synthesis is a key feature that must be taken into account in any mechanistic model. Mutational and molecular modeling studies have contributed significantly to this knowledge, which will be greatly expanded by a high-resolution structure of F_0 and the entire F_1F_0 complex.

Intriguingly, the size of the membrane potential in a particular species may be correlated to the number of c subunits in the ATP synthase from this species. In chloroplasts, where the membrane potential is low and the ATP synthase operates at maximal rates at $\Delta\psi >$ 60 mV (see above), the c ring consists of 14 subunits. In contrast, in mitochondria or bacteria, where the membrane potential is higher and the ATP synthase requires $\Delta\psi >$ 120 mV for maximal activity, the c ring consists of only 10–11 subunits. In the ATP synthase with the larger c ring, more steps are needed for a complete revolution and the increment of a single step in torque generation is smaller. To investigate whether this is a general principle and to address the interesting question how the two motors, which operate with a different number of steps, are synchronized, sophisticated biochemical investigations of the enzyme's performance are required.

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