Minireview

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 276, No. 3, Issue of January 19, pp. 1665–1668, 2001

The Rotary Machine in the Cell, ATP Synthase*[S]

Published, JBC Papers in Press, November 15, 2000,
DOI 10.1074/jbc.R000021200

Hiroyuki Noji‡ and Masasuke Yoshida§¶

From the ‡CREST (Core Research for Evolutional Science and Technology) "Genetic Programming" Team 13, Teikyo University Biotechnology Research Center 3F, Nogawa 907, Miyamae-ku, Kawasaki 216-0001, Japan and the §Chemical Resource Laboratory, Tokyo Institute of Technology, Yokohama 226-8503, Japan

ATP synthase, a major ATP supplier in the cell, is a rotary machine found next to the bacterial flagella motor in the biological world. This enzyme is composed of two motors, F_0 and F_1, connected by a common rotor shaft to exchange the energy of proton translocation and ATP synthesis/hydrolysis through mechanical rotation. Rotation of the isolated F_1 motor driven by ATP hydrolysis was directly observed with an optical microscope, and its marvelous performance has been revealed. The motor rotates with discrete 120° steps, each driven by hydrolysis of one ATP molecule with nearly perfect energy efficiency. Apparently a cooperative domain bending motion of the catalytic β subunits initiated by ATP binding generates the torque. In the F_0 motor, which we know less about, it has been proposed that torque may be generated by the large twist of one helix of F_0c subunits or by the change in electrostatic forces between rigid subunits.

ATP Synthase

ATP synthase is a ubiquitous enzyme that is located in the inner membranes of mitochondria, thylakoid membranes of chloroplasts, or the plasma membranes of bacteria. As implicated by the binding change mechanism proposed by Boyer (1), ATP synthase employs mechanical rotation to convert the electrochemical potential energy of protons across the membranes ($\Delta\tilde{\mu}_{H^+}$), built up by respiration or a photoreaction, to the chemical energy of ATP synthesis. This enzyme is comprised of two motors sharing a common rotor shaft (Fig. 1*A*). The F_1 motor, a subcomplex of the ATP synthase corresponding to the protruding portion from the membrane, can generate rotary torque using the energy of ATP hydrolysis (Fig. 1*B*). Its subunit composition is $\alpha_3\beta_3\gamma_1\delta_1\epsilon_1$ and the M_r is ~380,000. The F_0 motor, a membrane-embedded subcomplex, generates torque coupled with proton movement down ($\Delta\tilde{\mu}_{H^+}$) (Fig. 1*C*). Bacterial F_0 has the simplest subunit structure ($a_1b_2c_{10-14(?)}$) with an M_r of ~150,000. The eukaryotic F_0 contains several kinds of subunits. The γ and ϵ of F_1 constitute a rotor shaft and are attached to the F_0c subunits. A stator stalk, made up of δ and F_0b_2, also connects F_1 and F_0 keeping the stators ($\alpha_3\beta_3$ and F_0a) from spinning with the rotor. Under physiological conditions where the driving force for the F_0 motor is larger than that for the F_1 motor, the F_0 motor rotates the common shaft in its intrinsic direction so as to reverse the F_1 motor enforcing the ATP synthesis (Fig. 1*A*). When the driving force for the F_1 motor is larger, the F_1 motor reverses the F_0 motor to pump protons to the opposite side of a membrane.

Structure of F_1

F_1 can be easily and reversibly dissociated from F_0 as a soluble enzyme that only hydrolyzes ATP and is often called F_1-ATPase. The catalytic sites are mainly located on the β subunit, but the minimum stable ATPase-active complex is the $\alpha_3\beta_3\gamma$ subcomplex (2). The crystal structures of $\alpha_3\beta_3\gamma$ of the bovine mitochondrial F_1 show that three αs and βs are alternatively arranged in a hexamer ring forming a large central cavity in which half of the long coiled-coil structure of γ is inserted (3). According to the recently reported structure of the F_1-F_0c complex of yeast ATP synthase (4), the other half of the coiled-coil of γ extends to touch the F_0c subunits. The ϵ subunit binds to the side surface of the lowest part of the coiled-coil. In ATP synthase, ϵ also has close contact with F_0c. The δ subunit, the last subunit whose atomic structure is not known, is likely to sit on top of the $\alpha_3\beta_3$ ring (5). Three catalytic sites on the βs are different in nucleotide binding states; the first is occupied by Mg·AMP-PNP[1] (an analog of ATP), the second is occupied by Mg·ADP, and the third is empty (no bound nucleotide); these sites are termed β_T, β_D, and β_E, respectively. These structural features are quite consistent with what the binding change mechanism predicted; the three catalytic sites should be in three different nucleotide states at a given moment, and cooperative interconversion of the states causes the rotation of γ.

Observing the Rotation

To visualize the rotation, F_1 molecules from a thermophilic bacterium (*Bacillus* strain PS3) were fixed on the glass surface of a coverslip, and a large marker, a fluorescently labeled actin filament, was attached to γ (Fig. 2*A*) (6). Dependent on ATP, the rotation of the actin filaments with a length of 1–4 μm at 0.2–10 revolutions per s was seen under an optical microscope. The rotation continued for several minutes with hundreds of revolutions. The direction of the rotation was always anticlockwise viewed from the F_0 side, consistent with the crystal structure in which one β undergoes transition from β_T to β_D to β_E. The F_1s from *Escherichia coli* (7, 8) and the chloroplast (9) are also shown to be a rotary motor by applying the same technique. No obvious difference among the γ rotations was observed. The mechanical properties of the F_1 motor described below seem to be conserved among species.

One ATP Drives 120° Step Rotation

Because of the hydrodynamic friction, at high ATP concentrations the rotation of an actin filament is the slowest step in the catalytic turnover. The rates of rotation of the filaments with the same length were, therefore, leveled off above 2 μM ATP. At ATP concentrations below 600 nM, the slowest step is the ATP binding and actin filaments showed a stepwise rotation; F_1 waits for ATP at the fixed position, makes a 120° rotation upon arrival of the ATP, and waits for the next ATP (10). Obviously, a 120° step is a reflection of the 3-fold arrange-

* This minireview will be reprinted in the 2001 Minireview Compendium, which will be available in December, 2001.

[S] The on-line version of this article (available at *http://www.jbc.org*) contains a supplemental video.

¶ To whom correspondence should be addressed: Chemical Resource Laboratory, Tokyo Institute of Technology, Yokohama 226-8503, Japan. Tel.: 81 45 924 5233; Fax: 81 45 924 5277; E-mail: myoshida@res.titech.ac.jp.

[1] The abbreviations used are: AMP-PNP, adenosine 5′-(β,γ-imino)-triphosphate; pN, piconewtons; DCCD, dicyclohexylcarbodiimide.

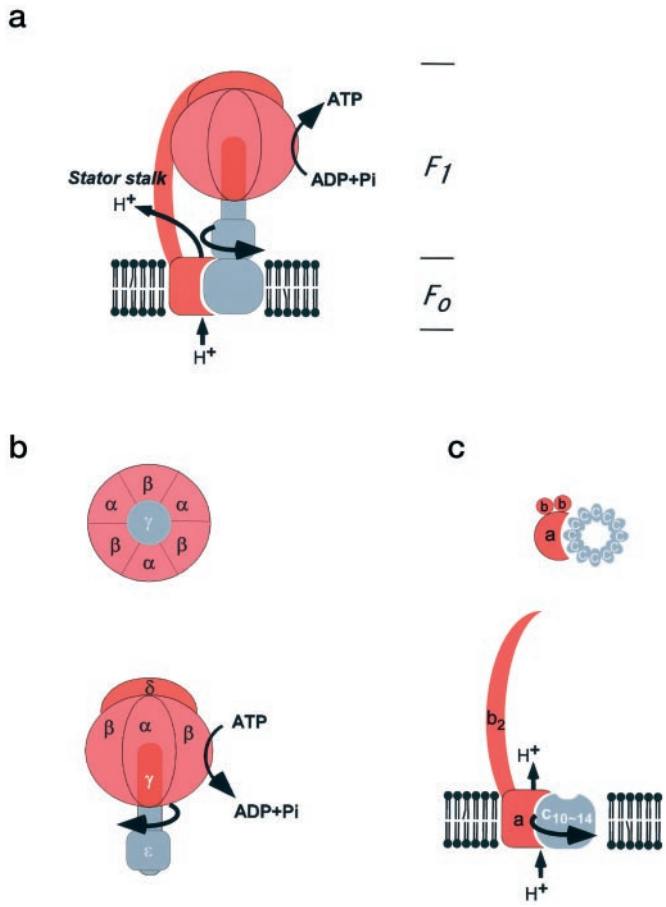

FIG. 1. **Schematic diagram of the ATP synthase.** *A,* side view of the ATP synthase. ATP synthase is composed of the F_1 and F_0 motor sharing a common rotary shaft (*gray*). A stator stalk connects two motors (*red*) that do not slip. The F_0 motor generates a rotary torque powered by the proton flow-enforcing F_1 motor to synthesize ATP. The rotational direction is clockwise viewed from the membrane side. *B,* cross-section and side view of F_1 motor. The $\alpha_3\beta_3$ cylinder hydrolyzing ATP makes an anti-clockwise rotation of the rotor part composed of the γ and ϵ subunits. *C,* cross-section and side view of the F_0 motor. Proton flow accompanies a clockwise rotation of the ring structure made of 10–14 copies of the c subunit.

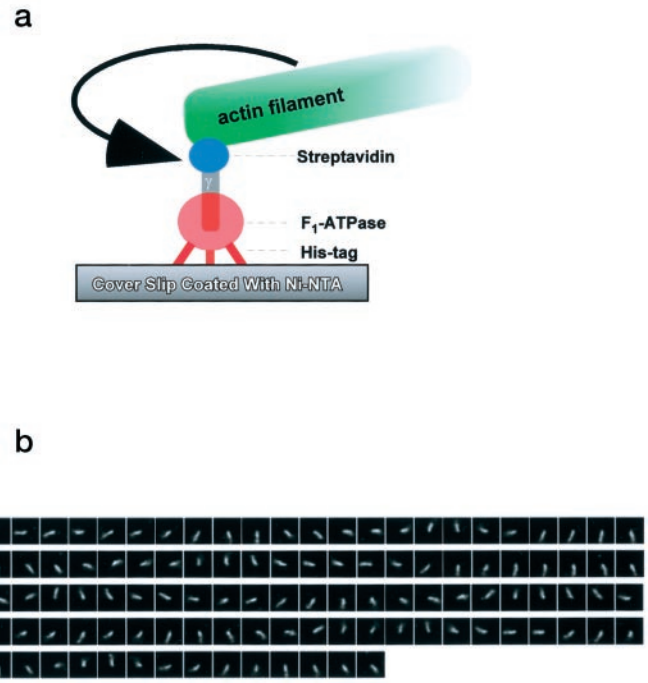

FIG. 2. **The direct observation of the γ rotation in the F_1 motor.** *A,* experimental system for the observation of the γ rotation using an optical microscope. The F_1 motor tagged with 10 His residues at the N terminus of the β subunit was immobilized upside down on a coverslip coated with nickel-nitrilotriacetic acid (*Ni-NTA*). An actin filament (*green*) labeled with fluorescent dyes and biotins was attached to the biotinylated γ subunit (*gray*) through streptavidin (*blue*). *B,* rotary movement of an actin filament observed from the bottom, the membrane side, with an epifluorescent microscope. Length from the axis to tip, 2.6 μm; rotary rate, 0.5 revolution per s; time interval between images, 133 ms.

ment of the catalytic β subunits in the $\alpha_3\beta_3$ hexamer. The histogram of the duration time between 120° steps obeys an exponential function, and the estimated apparent rate constant of ATP binding to F_1 agrees well with the rate obtained in a bulk F_1 solution. This confirms that the hydrolysis of one ATP molecule suffices for making one 120° step. Interestingly, F_1 occasionally makes a backward step as fast as forward steps and too fast to be ascribed to a thermal fluctuation. Presumably, the molecular machine makes a mistake in the order of ATP binding or product release.

Torque and Energy of Rotation

The rotational rate became slower with an increasing filament length because of the increased viscous friction. However, when the rotary torque is calculated from the frictional drag coefficient and the rotation rate, it becomes clear that the F_1 motor generates a constant torque of 40 pN·nm irrespective of the length of the actin filament (10). If the torque is produced at the β-γ interface at a radius of ~1 nm from the central axis of the $\alpha_3\beta_3$ hexamer, the force would amount to 40 pN. This is the highest value among reported nucleotide-driven motor proteins (3–5 pN for myosin/actin, 5 pN for kinesin/microtubule, and 14 pN for RNA polymerase/DNA) (11). The torque of 40 pN·nm $\times$ $2\pi/3$ radians (120°) or 80 pN·nm is the work done in a step against the viscous load. To define the free energy of the ATP hydrolysis, we purposely included 10 μM ADP and 10 mM P_i in addition to 2 mM ATP and measured the rotation rates (10). The free energy of the ATP hydrolysis under the condition is 90 pN·nm per one ATP molecule, and the energy for the observed rotation was 80 pN·nm per 120° step. Therefore, F_1 works with almost perfect efficiency. The high efficiency accords with the fully reversible nature of this motor.

Rotation without Load

As another probe to visualize the F_1 rotation, a single fluorophore was attached to γ, and its orientation was monitored (12). With this small marker, F_1 can rotate almost without a load. Under this condition, F_1 showed the 120° stepwise rotation at low ATP concentrations as seen in the experiment using an actin filament. Furthermore, the apparent rate of ATP binding is the same as that observed with actin filaments. This suggests that the torque-generating step in the ATPase catalytic cycle of F_1 is not the ATP binding but step(s) *after* it, including the interconversion of the β subunit that initially accommodates ATP from the "loose" binding state to the "tight" one.

Kinetic Framework; Bi-site or Tri-site?

Knowledge of the exact kinetic sequence in the catalytic turnover of F_1 is the prerequisite for any models of the rotation mechanism. Three catalytic modes are recognized when F_1 hydrolyzes ATP. At extremely low ATP (less than 1 nM) or a stoichiometric amount of ATP, only one ATP binds to the first catalytic site, and the hydrolyzed products are only released slowly (uni-site catalysis) (13). Uni-site catalysis is not inhibited by the cross-link between γ and β (14) and is probably, if

not exclusively, a process that does not couple with rotation. Binding of a second ATP to the next catalytic site significantly promotes the release of the products at the first site (13). The apparent K_m for this process is in the micromolar range (bi-site catalysis). Boyer's binding change mechanism has adopted the bi-site catalysis. We observed rotation in this ATP concentration range. It has been proposed that the third catalytic sites bind ATP to attain the maximum hydrolysis rate (tri-site catalysis). Actually, the ATPase activity of F_1 is usually saturated above 100 μM ATP. Using the fluorescence of tryptophan introduced near the catalytic site of *E. coli* F_1 as the signal of nucleotide binding, Senior's group (15) found that the change was saturated at an ATP concentration above ~100 μM and suggested that all three catalytic sites were filled by nucleotides. A similar observation has been made for the thermophilic F_1 (16). However, the interpretation has been complicated by the "Mg-ADP-inhibited form," a state observed for F_1s from any sources in which one of the catalytic sites is stuck with a tightly bound Mg-ADP. F_1 exerting the steady state catalysis is a dynamic mixture of the inhibited and active forms, and this equilibrium is dragged to the active form by the Mg-ATP binding to the noncatalytic α subunit of which the affinity is below 100 μM (17). Boyer has raised a question of whether deviation from simple kinetics at high ATP concentrations could be because of the Mg-ADP-inhibited form rather than the tri-site catalysis (18). Contrary to this, a mutant whose α subunits lost the nucleotide binding ability still showed kinetics best interpreted by tri-site catalysis (19). The current results favor the tri-site catalysis as a physiological mode, but exclusive evidence is still needed to settle the argument. Noticeably, no obvious shift in the properties of the γ rotation was observed from 2 μM to 2 mM ATP where the transition from the bi- to tri-site catalysis should occur (10).

Bending Motion of β May Drive the γ Rotation

The source of energy for the γ rotation is ATP hydrolysis on the β subunits. The conformation changes occurring in β during the ATPase cycle should then be responsible for (or at least closely related to) the torque generation. In the crystal structure of the mitochondrial F_1, both β_T and β_D are in the closed conformation in which the C-terminal domain is lifted to the nucleotide-binding domain (3). The β_E employs the open conformation with a wide crevice between the two domains. The crystal of the isolated β subunit takes the open conformation,[2] and the addition of a nucleotide caused the transition from the open to closed conformation (NMR) (20). The binding energy of ATP to the β subunit facilitates an energetically unfavorable transition from the empty to closed conformation of the β subunit. When β in F_1 is fixed in the closed conformation by cross-linking, ATP hydrolysis stops (21). Thus, β appears to undergo a bending motion upon binding and the release of the nucleotide during catalysis. Like an automobile engine, the reciprocal motion of β in F_1 is converted to the rotary motion of γ. For this to occur, three βs in F_1 coordinate the motion, pushing and pulling the eccentric γ (22). A real time recording of the motion of the βs simultaneously with ATP hydrolysis and γ rotation is a challenge to prove the above contention. Residues playing key roles in the torque generation have been sought by mutagenesis (23, 24). However, the F_1 motor seems fairly robust against the mutations of the β subunit at the "hinge region" of the bending motion (23) and the conserved "DELSEED region" that has a contact surface with γ in the closed conformation (24).

[2] K. Miki and M. Yoshida, unpublished result.

Structure of F_o

F_0 conducts proton movement across a membrane. F_0a is embedded in the membrane by five transmembrane helices. A dimer of F_0b is anchored to the membrane by a single transmembrane helix (25). F_0c is a small hydrophobic protein with a hairpin structure, two transmembrane helices connected by a short polar loop (26). The F_0c subunits are arranged in a ring structure, but agreement has not been established for the number of subunits in a ring; 10, 12, 14, and variable copies have been proposed (4, 27–29). F_0a and F_0b_2 most likely exist outside of the F_0c ring. A carboxyl group located in the middle of the C-terminal helix of F_0c (glutamate in most cases but aspartate (Asp-61) in the case of *E. coli*) is proven to be essential for proton translocation (30). This carboxyl group is specifically labeled with dicyclohexylcarbodiimide (DCCD), and the labeled ATP synthase loses the activity of the ATP hydrolysis/synthesis coupled with proton movement (31). Genetic studies indicated that several charged residues of F_0a are also essential and assumed to be components of a putative proton path of F_0 (reviewed in Ref. 32).

Is the F_oc Ring a Rotor?

Although the assumption is widely accepted that the F_0c ring rotates together with γ and ϵ, it has not been proven yet by experiment. Actually, we observed the ATP-driven rotation of the actin filaments attached to the F_0c ring of the immobilized ATP synthase (33). However, the detergent used in the experiments impaired the integrity of the enzyme, and the DCCD-labeled enzyme showed uninhibited rotation and ATP hydrolysis. The F_0c ring of the detergent-impaired ATP synthase could simply rotate by being dragged by the rotating γ without regard to whether the F_0c ring works as a stator in the native enzyme. Other groups also reported the same results using DCCD-insensitive preparations, but they thought that the rotation of the F_0c ring was proven (34). The loss of structural integrity of the ATP synthase in the detergent was unambiguously shown by the structure of the yeast ATP synthase crystals grown in detergent; the enzyme lost at least F_0a and F_0b_2. Whether the F_0c ring is a rotor or stator will be decided by demonstration of, for example, the DCCD-sensitive rotation of F_0c or by a clear biochemical result such as DCCD-sensitive proton translocation by ATP synthase containing a γ-ϵ-F_0c cross-link.[3]

Hints and Problems of F_o Motor

A monomer structure of F_0c in a water-saturated organic solvent, which mimics well the native structure, was determined by NMR (26). Using this method, a large conformational change of F_0c induced by deprotonation of essential Asp-61 was detected; the C-terminal helix rotates 140° as a unit with respect to the N-terminal helix, and the conformation of the loop region between two helices significantly changes (35). If a deprotonated F_0c subunit is sandwiched by protonated F_0c subunits in the F_0c ring, the deprotonated Asp-61 comes close to the protonated Asp-61 of the adjacent F_0c subunit, and proton transfer among the F_0c subunits and essential Arg of F_0a will occur. Although the details are unknown, the process by which the protons drive the F_0 motor may be more mechan-

[3] Very recently, unequivocal evidence that F_0c belongs to the rotor part was obtained by linking the γ, ϵ, and F_0c subunits by disulfide bridges between cysteine residues introduced genetically at the interfaces (Tsunoda, S. P., Aggeler, R., Yoshida, M., and Capaldi, R. A. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** in press). This fixing of the three subunits together had no significant effect on ATP hydrolysis, proton translocation, or ATP synthesis, and each of these functions retained sensitivity to DCCD.

ical than a simple rotational diffusion of the rigid F_0c ring driven by electrostatic forces.

Related to the above contention, evolutional variation of F_0c family is worth noting. Members of the F_0c family in V-type ATPases, found in membranes of archaebacteria, some eubacteria, and inside-acidic vacuolar systems in eukaryotic cells, are mostly a tandemly fused dimer of prototype F_0c units composed of four transmembrane helices (reviewed in Ref. 36). Interestingly, the dimer contains only a single essential carboxylate in the second helix. Because the ring structure of these double-sized *c* subunits in V-type ATPases is made most likely using two helices as a unit, the question arises as to how these homologues make a rotary motion with essential carboxylates having two times longer intervals. This places the restraint on any models trying to explain the common function of the ATP synthase and V-type ATPases.

The $\Delta\tilde{\mu}_{H^+}$ value has two components; the concentration difference (ΔpH) and the transmembrane voltage ($\Delta\psi$). Although they are energetically equivalent, they can be kinetically different. Each proton in F_0 receives the force by $\Delta\psi$ and can possibly drive the F_0 motor. On the contrary, ΔpH does not apply any force to each proton in F_0. Using the ATP synthase from *Propionegenium modestum*, which utilizes Na^+ instead of H^+ as a coupling ion, Dimroth's group (37, 38) indicated that a certain magnitude of $\Delta\psi$ is always required for ATP synthesis even when ΔpNa is a major component of $\Delta\tilde{\mu}_{Na^+}$. They further suggested that the ATP synthesis in the classic acid-base transition experiment cannot exclude contribution of the induced $\Delta\psi$ (39). It is possible to think that β subunits of F_1 resist the torque by the F_0 motor as a strong spring, and therefore only $\Delta\psi$ can wind up the strong spring of the β as quickly as observed.

Perspectives

ATP synthase is a rotary motor enzyme. The decisive evidence for the F_1 rotation has justified Boyer's prediction in the past few years. This is not the goal but the start of new exciting studies. The central questions are how the motor generates force and how the motor is regulated. Models have been proposed. However, we think more facts are required to develop a vivid model. We know relatively little about F_0. The proton-driven F_0 motor remains a matter of unproved rational prediction. The direct observation of proton-driven rotation in a membrane using the lipid bilayer membrane will be a challenge but probably is not an impossible task. Of course, more atomic structures are a prerequisite to understanding the F_1 and F_0 motors.

REFERENCES

1. Boyer, P. D. (1993) *Biochim. Biophys. Acta* **1140,** 215–250
2. Matsui, T., and Yoshida, M. (1995) *Biochim. Biophys. Acta* **1231,** 139–146
3. Abrahams, J. P., Leslie, A. G., Lutter, R., and Walker, J. E. (1994) *Nature* **370,** 621–628
4. Stock, D., Leslie, A. G., and Walker, J. E. (1999) *Science* **286,** 1700–1705
5. Wilkens, S., Zhou, J., Nakayama, R., Dunn, S. D., and Capaldi, R. A. (2000) *J. Mol. Biol.* **295,** 387–391
6. Noji, H., Yasuda, R., Yoshida, M., and Kinosita, K., Jr. (1997) *Nature* **386,** 299–302
7. Noji, H., Hasler, K., Junge, W., Kinosita, K., Jr., Yoshida, M., and Engelbrecht, S. (1999) *Biochem. Biophys. Res. Commun.* **260,** 597–599
8. Omote, H., Sambonmatsu, N., Saito, K., Sambongi, Y., Iwamoto-Kihara, A., Yanagida, T., Wada, Y., and Futai, M. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 7780–7784
9. Hisabori, T., Kondoh, A., and Yoshida, M. (1999) *FEBS Lett.* **463,** 35–38
10. Yasuda, R., Noji, H., Kinosita, K., Jr., and Yoshida, M. (1998) *Cell* **93,** 1117–1124
11. Kinosita, K., Jr., Yasuda, R., Noji, H., Ishiwata, S., and Yoshida, M. (1998) *Cell* **93,** 21–24
12. Adachi, K., Yasuda, R., Noji, H., Itoh, H., Harada, Y., Yoshida, M., and Kinosita, K., Jr. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 7243–7247
13. Grubmeyer, C., Cross, R. L., and Penefsky, H. S. (1982) *J. Biol. Chem.* **257,** 12092–12100
14. Garcia, J. J., and Capaldi, R. A. (1998) *J. Biol. Chem.* **273,** 15940–15945
15. Weber, J., and Senior, A. E. (2000) *Biochim. Biophys. Acta* **1458,** 300–309
16. Dou, C., Fortes, P. A., and Allison, W. S. (1998) *Biochemistry* **37,** 16757–16764
17. Jault, J. M., Matsui, T., Jault, F. M., Kaibara, C., Muneyuki, E., Yoshida, M., Kagawa, Y., and Allison, W. S. (1995) *Biochemistry* **34,** 16412–16418
18. Milgrom, Y. M., Murataliev, M. B., and Boyer, P. D. (1998) *Biochem. J.* **330,** 1037–1043
19. Matsui, T., Muneyuki, E., Honda, M., Allison, W. S., Dou, C., and Yoshida, M. (1997) *J. Biol. Chem.* **272,** 8215–8221
20. Yagi, H., Tozawa, K., Sekino, N., Iwabuchi, T., Yoshida, M., and Akutsu, H. (1999) *Biophys. J.* **77,** 2175–2183
21. Ren, H., Dou, C., Stelzer, M. S., and Allison, W. S. (1999) *J. Biol. Chem.* **274,** 31366–31372
22. Wang, H., and Oster, G. (1998) *Nature* **396,** 279–282
23. Masaike, T., Mitome, N., Noji, H., Muneyuki, E., Yasuda, R., Kinosita, K., Jr., and Yoshida, M. (2000) *J. Exp. Biol.* **203,** 1–8
24. Hara, K., Noji, H., Yasuda, R., Kinosita, K., Jr., and Yoshida, M. (2000) *J. Biol. Chem.* **275,** 14260–14263
25. Dunn, S. D., McLachlin, D. T., and Revington, M. (2000) *Biochim. Biophys. Acta* **1458,** 356–363
26. Girvin, M. E., and Fillingame, R. H. (1993) *Biochemistry* **32,** 12167–12177
27. Jones, P. C., and Fillingame, R. H. (1998) *J. Biol. Chem.* **273,** 29701–29705
28. Seelert, H., Poetsch, A., Dencher, N. A., Engel, A., Stahlberg, H., and Muller, D. J. (2000) *Nature* **405,** 418–419
29. Schemidt, R. A., Qu, J., Williams, J. R., and Brusilow, W. S. A. (1998) *J. Bacteriol.* **180,** 3205–3208
30. Sebald, W., Machleidt, W., and Wachter, E. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 785–789
31. Hermolin, J., and Fillingame, R. H. (1989) *J. Biol. Chem.* **264,** 3896–3903
32. Deckers-Hebestreit, G., Greie, J., Stalz, W., and Altendorf, K. (2000) *Biochim. Biophys. Acta* **1458,** 364–373
33. Tsunoda, S. P., Aggeler, R., Noji, H., Kinosita, K., Jr., Yoshida, M., and Capaldi, R. A. (2000) *FEBS Lett.* **470,** 244–248
34. Sambongi, Y., Iko, Y., Tanabe, M., Omote, H., Iwamoto-Kihara, A., Ueda, I., Yanagida, T., Wada, Y., and Futai, M. (1999) *Science* **286,** 1722–1724
35. Rastogi, V. K., and Girvin, M. E. (1999) *Nature* **402,** 263–268
36. Forgac, M. (1999) *J. Biol. Chem.* **274,** 12951–12954
37. Kaim, G., and Dimroth, P. (1998) *EMBO J.* **17,** 5887–5895
38. Dimroth, P., Kaim, G., and Matthey, U. (2000) *J. Exp. Biol.* **203,** 51–59
39. Kaim, G., and Dimroth, P. (1999) *EMBO J.* **18,** 4118–4127