Rotary catalysis of F$_o$F$_1$-ATP synthase

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The synthesis of ATP, the key reaction of biological energy metabolism, is accomplished by the rotary motor protein: F$_o$F$_1$-ATP synthase (F$_o$F$_1$). In vivo, F$_o$F$_1$, located on the cell membrane, carries out ATP synthesis by using the proton motive force. This heterologous energy conversion is supposed to be mediated by the mechanical rotation of F$_o$F$_1$; however, it still remained unclear. Recently, we developed the novel experimental setup to reproduce the proton motive force in vitro and succeeded in directly observing the proton-driven rotation of F$_o$F$_1$. In this review, we describe the interesting working principles determined so far for F$_o$F$_1$ and then introduce results from our recent study.

Key words: ATP synthase, molecular motor, heterogeneous energy conversion

F$_o$F$_1$ ATP synthase (F$_o$F$_1$) is a molecular energy-converter which catalyzes physiologically important synthesis of ATP from ADP and inorganic phosphate (P$_i$) by using the electrochemical energy in proton gradient; the proton motive force (pmf) across bio-membranes. The prominent feature of F$_o$F$_1$ is that mechanical rotation of the inner rotor complex mediates the aforementioned heterologous energy conversion with high efficiency and reversibility, which is not found in other biological systems, and therefore, F$_o$F$_1$ attracts great interest from many researchers across a wide range of research fields. F$_o$F$_1$ comprises 2 rotary motors, F$_1$ and F$_o$ (Fig. 1a). F$_1$ ($\alpha$$\beta$$\gamma$$\delta$), a water-soluble part of F$_o$F$_1$, is an ATP-driven rotary motor, which couples ATP hydrolysis or synthesis at $\gamma$$\delta$ stator ring to the mechanical rotation of $\gamma$ complex in counterclockwise direction (Fig. 1b). Each $\alpha$$\beta$ interface possesses a catalytic site for ATP hydrolysis; F$_1$ hydrolyzes 3 ATP molecules per turn. Extensive studies have been done to understand the chemo-mechanical coupling mechanism of F$_1$ [9–18], and therefore, currently, F$_1$ is one of the best characterized molecular motor proteins.

F$_o$, a membrane-embedded part of F$_o$F$_1$, is the proton-driven rotary motor, which couples proton translocation to mechanical rotation of the oligomer ring of the c-subunit against the ab$_2$ complex (Fig. 1b). Each c-subunit has a proton-binding site and mediates proton translocation: 1 proton per c-subunit per rotation. Thus, the number of c-subunits in the c-ring is thought to determine the total number of protons translocated per rotation. While the number of c-subunits varies among species from 8 to 15 [21,22], the bacterial F$_o$, such as E-coli and thermophilic Bacillus PS3, has 10 c-subunits that form a c$_{10}$ ring [23,24]. It is difficult to handle F$_o$ since it is embedded in the membrane, and therefore, the rotary mechanism of F$_o$ remains elusive.

In a cell, F$_1$ and F$_o$ are connected via central and peripheral stalks (Fig. 1a), which allow the torque transmission between the 2 motors. Under physiological conditions, F$_o$ generates a larger torque than F$_1$ and reverses the rotary direction of F$_1$, thereby inducing the reverse reaction of ATP hydrolysis, i.e., ATP synthesis (Fig. 2a). In contrast, when pmf diminishes, F$_1$ hydrolyzes ATP and reverses the rotary direction of F$_o$, thereby enforcing pumping of protons by F$_o$ in order to generate pmf (Fig. 2b). Thus, F$_o$F$_1$ manifests reversibility with regard to the process of energy conversion, and the rotation of inner rotor complex plays an important role in this reversibility.

To understand the precise role of the rotation on the energy conversion of F$_o$F$_1$, some single-molecule studies have been carried out [9,19,25–27]. Due to ease of handling, ATP-driven rotation of detergent-solubilized F$_o$F$_1$, which is...
course of pmf generated by F, most studies pertained to the measurement of ATP synthesis activity under a given pmf. In general, pmf mainly comprises 2 components: the trans-membrane proton gradient (ΔpH) and the potential difference (Δψ). At first, biochemical studies examined ATP synthesis activity by changing the amplitude of each of these components of pmf, and examined the component-dependence of ATP synthesis activity of F, 28-34. In recent studies, F, was purified and reconstituted into liposomes, and the ATP synthesis rate was quantitatively measured by the acid–base transition or valinomycin-mediated K+ diffusion potential for producing ΔpH or Δψ. It was found that ΔpH and Δψ contribute equally to ATP synthesis rate of F, and, moreover, either ΔpH or Δψ alone can drive synthesis of ATP. Kinetic equivalence implies that F, can use ΔpH or Δψ to drive rotation at an equal efficiency.

Next, to determine the coupling efficiency in F, biochemical studies examined the number of protons translocated per synthesis of 1 molecule of ATP; the H+/ATP ratio, by enzymes from various organisms, e.g., Escherichia coli, yeast, and chloroplasts3,5,36. In view of the rotary catalysis model of F, the H+/ATP ratio should coincide with the ratio of the number of proton-binding c-subunits to the 3 catalytic nucleotide-binding β-subunits when proton-translocation and ATP synthesis are highly coupled. Analyses of the equilibrium point, where the free energy of ATP synthesis is balanced with that of proton translocation, allowed determination of the H+/ATP ratio. The determined H+/ATP ratios were dependent on the stoichiometry of the c and β subunits, although they were not identical to the c/β ratios. In particular, H+/ATP ratios in a recent study were smaller than the c/β ratios36, which implies that proton translocation is stochastically coupled to the synthesis of ATP; however, the coupling efficiency is extremely high.

**Energy conversion mechanism**

As mentioned above, ATP synthesis/hydrolysis reaction is reversibly coupled with proton translocation across the membrane. To understand the reversible energy conversion mechanism of F, extensive biochemical studies have been performed. Since it is difficult to measure the time

![Figure 1](image_url)
for a lipid bilayer membrane, and attempted to visualize the rotary motion of membrane-constituted E. coli F₁F₅₃ driven by ATP hydrolysis. Their setup, the gold nanorod was attached to the ε-subunit as a rotation probe. The intensity of scattered red light from a nanorod changes in a sinusoidal manner as a function of the rotary position, and therefore, the rotary motion of F₁F₅₃ could be visualized from the analysis of red light scattered from the nanorod. On the other hand, the F₅₃ module was buried within the phospholipid bilayer nanodisc, which is large enough to allow incorporation of F₅₃, but which is on the same scale as the F₁F₅₃ complex, and thus, it was difficult to generate a pmf across the nanodisc. Using this experimental setup, the 36° stepping rotation of F₁F₅₃ in the presence of a high concentration of polyethylene glycol was observed for the first time. This reflects the structural symmetry of the F₅₃ module; 10 proton binding sites are located on a single molecule of F₅₃. In this setup, as mentioned above, pmf was not imposed on F₁F₅₃, and F₁ did not generate rotary torque; and therefore, it could not be confirmed whether the 36° step was coupled to the translocation of protons.

**pmf-driven rotation**

Diez et al. developed a method to indirectly visualize the rotation of E. coli F₁F₅₃ that had been reconstituted in liposomes by using single molecule Förster resonance energy transfer (sm-FRET). In their method, they introduced a pair of FRET probes at a stator and rotor subunit of F₁F₅₃ for visualization of the rotary motion of F₁F₅₃, and generated the pmf by using the acid–base transition or valinomycin-mediated K⁺ diffusion potential method, as mentioned above. By using this method, they observed a 120° stepping rotation driven by ATP hydrolysis, and moreover, for the first time observed the obscure 36° stepping rotation driven by pmf. However, due to the low signal-to-noise ratio and fast photobleaching of the fluorescent dyes used in sm-FRET, high resolution tracking and long-term recording of the rotational dynamics of F₁F₅₃ has not yet been achieved (recording time < approximately 300 ms). Therefore, the
fundamental features of the pmf-driven rotation of \( F_{o}F_{1} \), such as the exact step size, unidirectionality of the rotation, and stochasticity of the steps, has remained elusive to date.

To solve this problem, we recently developed a novel experimental setup that allows long-term direct observation of the pmf-driven rotation of \( F_{o}F_{1} \), with a high spatiotemporal resolution (Fig. 4a). In this setup, the \( F_{o}F_{1} \)-reconstituted, supported membrane was expanded on a coverslip covered with Ni-NTA-modified agarose, where \( F_{o}F_{1} \) molecules were anchored via His-tags that had been introduced to the periplasmic side of the c-subunits. The 80-nm gold colloid was attached as a rotation probe onto the \( \beta \) subunits of \( F_{1} \) to allow visualization using a total internal reflection dark-field illumination system, which facilitated the long-term recording of rotation (approximately 10 s) with a high spatiotemporal resolution of about 5 nm and < 0.5 ms.

In addition, pmf across the supported lipid bilayer was generated by photolysis of caged protons [1-(2-Nitrophenyl)ethyl sulfate] with a total internal reflection illumination of UV light (\( \lambda = 404 \) nm) that selectively acidified the space between the coverslip and the lipid bilayer (the interspace). This novel setup can stably generate \( \Delta \text{pH} \) of 1.8–3.7 for several tens of seconds, while the conventional method, i.e., acid–base transition, can generate \( \Delta \text{pH} \) for only a few seconds. The magnitude of \( \Delta \text{pH} \) upon photolysis of the caged protons was measured using a pH-sensitive fluorescent dye,
pHrodo-Red (pHrodo)\textsuperscript{39}, which increased the fluorescent signal upon acidification.

By using this experimental setup, we for the first time observed the clockwise rotary motion of F\textsubscript{1}F\textsubscript{o} upon UV irradiation (averaged velocity was 2.7 rps.), while no rotating particles were observed prior to UV irradiation (Fig. 4b). To investigate the correlation between the rotational rate and $\Delta \psi$, we measured $\Delta \psi$ by using the pHrodo located at the region where F\textsubscript{1}F\textsubscript{o} showed rotations. Although F\textsubscript{1}F\textsubscript{o} showed a large variation in velocity, it was still evident that faster rotation occurred at higher $\Delta \psi$ (Fig. 4c). In addition, the data points of higher velocity at a given $\Delta \psi$ qualitatively agreed with the aforementioned biochemical measurement of ATP synthesis activity\textsuperscript{28}, showing that the rotation observed in this study was coupled to the proton translocation and ATP synthesis, and vice versa, the rotation of F\textsubscript{1}F\textsubscript{o} can mediate the energy conversion with high efficiency.

We also observed a 120° stepping rotation of F\textsubscript{1}F\textsubscript{o} driven by pmf (Fig. 4b). The step size of rotation, viz., 120°, implies that the rotary potential of F\textsubscript{1} with 3-fold symmetry dominated the overall rotary potential of F\textsubscript{1}F\textsubscript{o}. In other words, the kinetic bottleneck of the pmf-driven rotation was not proton-translocation in F\textsubscript{o} but a catalytic event(s) on F\textsubscript{1}, such as ATP release or $P_i$ binding\textsuperscript{4,40}. This result is consistent with a previous sm-FRET measurement of rotation of F\textsubscript{1}F\textsubscript{o} in ATP synthesis condition where a pair of FRET probes were introduced at a stator of F\textsubscript{o} and a rotor subunit of F\textsubscript{1}, while small steps that was estimated to be 36° were recorded when FRET probes were introduced into F\textsubscript{o}\textsuperscript{19}. In the present study, we immobilized the rotor part of F\textsubscript{o} on a coverslip and attached the rotation probe at the stator part of F\textsubscript{1}. Therefore, the observed rotation reflects the stepping behavior both of F\textsubscript{1} and F\textsubscript{o} as shown in the other works, in which 36°-steps of ATP-driven rotation of F\textsubscript{1}F\textsubscript{o} were observed in a similar experimental setup\textsuperscript{27}. However, we do not exclude the possibility that the difference in the probe position caused the apparently different step size of the rotation. Another possible reason for the inconsistency in step size is the difference in the components of the pmf; while pmf in the sm-FRET measurements was composed of both $\Delta \psi$ and $\Delta \phi$, pmf was essentially composed only of $\Delta \psi$ in our study. To confirm this, a method for direct observation of the proton-driven rotation of F\textsubscript{1}F\textsubscript{o} by applying $\Delta \psi$ is crucial.

On the other hand, noted that the stepping rotation of F\textsubscript{1}F\textsubscript{o} was highly stochastic; F\textsubscript{1}F\textsubscript{o} showed forward-and-backward (clockwise-and-counterclockwise) steps during rotation (Fig. 4b). This is a prominent feature of the pmf-driven rotation of F\textsubscript{1}F\textsubscript{o} that is not seen in the ATP-driven rotation of F\textsubscript{1} or F\textsubscript{1}F\textsubscript{o}. Surprisingly, the stepping was also observed in the absence of pmf, suggesting that pmf biased the rotary diffusion of F\textsubscript{1}F\textsubscript{o} to the clockwise direction. To confirm this, we analyzed the pause durations between the 120°-steps. In the absence of pmf, the histograms of the pause duration before clockwise or counterclockwise steps showed single exponential decay. The rate constants of the clockwise and counterclockwise steps were determined to be 65 and 61 s\textsuperscript{-1}, respectively. The equilibrium constant of clockwise rotation was, thus, almost 1. In the presence of pmf, the rate constant of clockwise stepping markedly increased about two-fold, while that of counterclockwise step decreased slightly. Thus, the equilibrium constant in the presence of pmf increased to 2, showing that pmf actually biased step direction. This result also suggests that chemical equilibrium was slightly biased toward ATP synthesis by pmf. The stochastic rotation of F\textsubscript{1}F\textsubscript{o} would represent rotation under physiological conditions, where free energy of ATP synthesis almost balances pmf.

Future prospects

Owing to the progress of single molecule observation techniques, we can directly observe the rotary motion of F\textsubscript{1}F\textsubscript{o} both in ATP hydrolysis and synthesis conditions. In particular, the introduced novel experimental setup that allows us to stably apply, under an optical microscope, pmf to the membrane will push forward to understand the rotary catalysis mechanism of F\textsubscript{1}F\textsubscript{o} in ATP synthesis condition, which had been unclear for long time. This experimental strategy is fundamentally applicable to the study on the dynamics of other membrane proteins driven by electro-chemical potential. The most promising experiment is the application of this protocol to transporters and ion channels since there are several caged compounds that release specific ions or chemicals. Such studies would reveal the generality and uniqueness of the finding in single-molecule studies on F\textsubscript{1}F\textsubscript{o}.

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References

1. Yoshida, M., Muneyuki, E. & Hisabori, T. ATP synthase—a marvellous rotary engine of the cell. Nat. Rev. Mol. Cell Biol. 2, 669–677 (2001).
2. Junge, W., Stielaff, H. & Engelbrecht, S. Torque generation and elastic power transmission in the rotary F\textsubscript{1}F\textsubscript{o}-ATPase. Nature 459, 364–370 (2009).
3. Weber, J. Structural biology: Toward the ATP synthase mechanism. Nat. Chem. Biol. 6, 794–795 (2010).
4. Turina, P., Samoray, D. & Gräber, P. H+/ATP ratio of proton transport-coupled ATP synthesis and hydrolysis catalysed by CF\textsubscript{1}-liposomes. EMBO J. 22, 418–426 (2003).
5. Diez, M., Zimmermann, B., Börsch, M., König, M., Schweinberger, E., Steigmiller, S., Reuter, R., Felekyan, S., Kudryavtsev, V., Seidel, C. A. & Gräber, P. Proton-powered...
subunit rotation in single membrane-bound F$_{o}$F$_{1}$-ATP synthase. Nature Struct. Mol. Biol. 11, 135–141 (2004).
6. Noji, H., Yasuda, R., Yoshida, M. & Kinosita, K., Jr. Direct observation of the rotation of F$_{o}$-ATPase. Nature 386, 299–302 (1997).
7. Abrahams, J. P., Leslie, A. G., Lutter, R. & Walker, J. E. Structure at 2.8 Å resolution of F$_{o}$-ATPase from bovine heart mitochondria. Nature 370, 621–628 (1994).
8. Yasuda, R., Noji, H., Kinosita, K., Jr. & Yoshida, M. F$_{o}$-ATPase is a highly efficient molecular motor that rotates with discrete 120 degree steps. Cell 93, 1117–1124 (1998).
9. Ariga, T., Muneyuki, E. & Yoshida, M. F$_{o}$-ATPase rotates by an asymmetric, sequential mechanism using all three catalytic subunits. Nat. Struct. Mol. Biol. 14, 841–846 (2007).
10. Yasuda, R., Noji, H., Yoshida, M., Kinosita, K., Jr. & Itoh, H. Resolution of distinct rotational substeps by submillisecond kinetic analysis of F$_{o}$-ATPase. Nature 410, 898–904 (2001).
11. Shimabukuro, K., Yasuda, R., Muneyuki, E., Haraguchi, M., Kinosita, K., Jr. & Yoshida, M. Catalysis of rotation of F$_{o}$ motor: cleavage of ATP at the catalytic site occurs in 1 ms before 40 degree substep rotation. Proc. Natl. Acad. Sci. USA 100, 14731–14736 (2003).
12. Nishizaka, T., Oiwa, K., Noji, H., Kimura, S., Muneyuki, E., Yoshida, M. & Kinosita, K., Jr. Chromomechanical coupling in F$_{o}$-ATPase revealed by simultaneous observation of nucleotide kinetics and rotation. Nat. Struct. Mol. Biol. 11, 142–148 (2004).
13. Adachi, K., Oiwa, K., Nishizaka, T., Furukawa, S., Noji, H., Itoh, H., Yoshida, M. & Kinosita, K., Jr. Coupling of rotation and catalysis in F$_{o}$-ATPase revealed by single-molecule imaging and manipulation. Cell 130, 309–321 (2007).
14. Watanabe, R., Itoh, H. Phosphate release in F$_{o}$-ATPase catalytic cycle follows ADP release. Nat. Chem. Biol. 6, 814–820 (2010).
15. Watanabe, R., Okuno, D., Sakakihara, S., Shimabukuro, K., Iino, R., Yoshida, M. & Noji, H. Mechanical modulation of catalytic power on F$_{1}$-ATPase. Nat. Chem. Biol. 8, 86–92 (2011).
16. Adachi, K., Oiwa, K., Yoshida, M., Nishizaka, T. & Kinosita, K., Jr. Controlled rotation of the F$_{1}$-ATPase reveals differential and continuous binding changes for ATP synthesis. Nat. Commun. 3, 1022 (2012).
17. Rondalez, Y., Tresset, G., Nakashima, T., Kato-Yamada, Y., Fujita, H., Takeuchi, S. & Noji, H. Highly coupled ATP synthesis by F$_{o}$-ATPase single molecules. Nature 433, 773–777 (2005).
18. Itoh, H., Takahashi, A., Adachi, K., Noji, H., Yasuda, R., Yoshida, M. & Kinosita, K. Mechanically driven ATP synthesis by F$_{o}$-ATPase. Nature 427, 465–468 (2004).
19. Düsser, M. G., Zarrabi, N., Ciripiano, D. J., Ernst, S., Glick, G. D., Dunn, S. D. & Börsch, M. 36 degrees step size of proton-driven c-ring rotation in F$_{o}$F$_{1}$-ATP synthase. EMBO J. 28, 2689–2696 (2009).
20. Watanabe, R., Tabata, K. V., Iino, R., Ueno, H., Iwamoto, M., Oiki, S. & Noji, H. Biased Brownian stepping rotation of F$_{o}$F$_{1}$-ATP synthase driven by proton motive force. Nat. Commun. 4, 1631 (2013).
21. Dimroth, P., von Ballmoos, C. & Meier, T. Catalytic and mechanical cycles in F-ATP synthases. Fourth in the cycles review series. EMBO Rep. 7, 276–282 (2006).
22. von Ballmoos, C., Cook, G. M. & Dimroth, P. Unique rotary ATP synthase and its biological diversity. Annu. Rev. Biophys. 37, 43–64 (2008).
23. Mitome, N., Suzuki, T., Hayashi, S. & Yoshida, M. Thermostable ATP synthase has a decamer c-ring: indication of non-differential 10:3 H'/ATP ratio and permissive elastic coupling. Proc. Natl. Acad. Sci. USA 101, 12159–12164 (2004).
24. Jiang, W., Hermolin, J. & Fillingame, R. H. The preferred stoichiometry of c subunits in the rotary motor sector of Escherichia coli ATP synthase is 10. Proc. Natl. Acad. Sci. USA 98, 4966–4971 (2001).
25. Sambongi, Y., Iko, Y., Tanabe, M., Omote, H., Iwamoto-Kihara, A., Ueda, I., Yanagida, T., Wada, Y. & Futai, M. Mechanical rotation of the c subunit oligomer in ATP synthase (F$_{o}$F$_{1}$): direct observation. Science 286, 1722–1724 (1999).
26. Ueno, H., Suzuki, T., Kinosita, K., Jr. & Yoshida, M. ATP-driven stepwise rotation of F$_{o}$F$_{1}$-ATP synthase. Proc. Natl. Acad. Sci. USA 102, 1333–1338 (2005).
27. Ishmukhametov, R., Hornung, T., Spetzler, D. & Frasch, W. D. Direct observation of stepped proteolipid ring rotation in E. coli F$_{o}$-ATP synthase. EMBO J. 29, 3911–3923 (2010).
28. Iino, R., Hasegawa, R., Tabata, K. V. & Noji, H. Mechanism of inhibition by C-terminal alpha-helices of the epsilon subunit of Escherichia coli F$_{o}$F$_{1}$-ATP synthase. J. Biol. Chem. 284, 17457–17464 (2009).
29. Soga, N., Kinosita, K., Yoshida, M. & Suzuki, T. Kinetic Equivalence of Transmembrane pH and Electrical Potential Differences in ATP Synthesis. J. Biol. Chem. 287, 9633–9639 (2012).
30. Bokranz, M., Morschel, E. & Kroger, A. Phosphorylation and phosphate-ATP exchange catalyzed by the ATP synthase isolated from Wolinella succinogenes. Biochim. Biophys. Acta 810, 332–339 (1985).
31. Siootlen, L. & Vandenvanden, S. ATP-synthesis by proteoliposomes incorporating Rhodospirillum rubrum F$_{o}$, as measured with firefly luciferase: dependence on delta psi and delta pH. Biochim. Biophys. Acta 976, 150–160 (1989).
32. Junesch, U. & Gräber, P. The rate of ATP-synthesis as a function of delta psi and delta psi catalyzed by the active, reduced H-ATPase from chloroplasts. FEBS Lett. 294, 275–278 (1991).
33. Wiedenmann, A., Dimroth, P. & von Ballmoos, C. Functional asymmetry of the F$_{o}$ motor in bacterial ATP synthases. Mol. Microbiol. 72, 479–490 (2009).
34. Fischler, S. & Gräber, P. Comparison of Aphi- and Δψ-driven ATP synthesis catalyzed by the H-ATPases from Escherichia coli or chloroplasts reconstituted into liposomes. FEBS Lett. 457, 327–332 (1999).
35. Steigmiller, S., Turina, P. & Gräber, P. The thermodynamic H'/ATP ratios of the H'-ATP synthases from chloroplasts and Escherichia coli. Proc. Natl. Acad. Sci. USA 105, 3745–3750 (2008).
36. Petersen, J., Forster, K., Turina, P. & Gräber, P. Comparison of the H'/ATP ratios of the H'-ATP synthases from yeast and from chloroplast. Proc. Natl. Acad. Sci. USA 109, 11150–11155 (2012).
37. Tsunoda, S. P., Aggerer, R., Yoshida, M. & Capaldi, R. A. Rotation of the c subunit oligomer in fully functional F$_{o}$F$_{1}$ ATP synthase. Proc. Natl. Acad. Sci. USA 98, 898–902 (2001).
38. Ueno, H., Nishikawa, S., Iino, R., Tabata, K. V., Sakakihara, S., Yanagida, T. & Noji, H. Simple dark-field microscopy with nanometer spatial precision and microsecond temporal resolution. Biophys. J. 98, 2014–2023 (2010).
39. Han, J. & Burgess, K. Fluorescent indicators for intracellular pH. Chem. Rev. 110, 2709–2728 (2010).
40. Rosing, J., Kayalar, C. & Boyer, P. D. Evidence for energy-dependent change in phosphate binding for mitochondrial oxidative-phosphorylation based on measurements of medium and intermediate phosphate-water exchanges. J. Biol. Chem. 252, 2478–2485 (1977).