Operating principles of rotary molecular motors: differences between \( F_1 \) and \( V_1 \) motors

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Among the many types of bioenergy-transducing machineries, \( F_1 \)- and \( V_1 \)-ATPases are unique bio- and nanomolecular rotary motors. The rotational catalysis of \( F_1 \)-ATPase has been investigated in detail, and molecular mechanisms have been proposed based on the crystal structures of the complex and on extensive single-molecule rotational observations. Recently, we obtained crystal structures of bacterial \( V_1 \)-ATPase (\( A_3 B_3 \) and \( A_3 B_3 DF \) complexes) in the presence and absence of nucleotides. Based on these new structures, we present a novel model for the rotational catalysis mechanism of \( V_1 \)-ATPase, which is different from that of \( F_1 \)-ATPases.

Key words: V-ATPase, F-ATPase, ATP, Crystal structure, molecular mechanism

Several machineries of bio- and nanomolecular rotary motors are present in living cells. These include linear motors such as myosins that function in muscle contraction and kinesins/dyneins that utilize the energy from ATP hydrolysis for vesicle transport. Molecular rotary motors, which include \( F_1 \)-ATPases and \( V_1 \)-ATPases, are another class of motors. This class utilizes the energy from ATP hydrolysis and the ionic current for axis rotation; flagellar motors also utilize ionic currents for rotation. \( F_1 \)- and \( V_1 \)-ATPases resemble one another and consist of a hydrophilic rotary motor region (\( F_1 \) and \( V_1 \)) and a hydrophobic membrane embedded region (\( F_o \) and \( V_o \)). Rotation of the motor axis is coupled to ATP hydrolysis, whereas rotation of the embedded region drives proton transport across membranes. In this review, we focus on the rotary motor regions (\( F_1 \) and \( V_1 \)). Although numerous structural and biophysical studies have been conducted, the molecular rotational mechanism of the \( F_1 \) motor is not fully understood. We also introduce our recent achievements in the three-dimensional structural analysis of the \( V_1 \) motor and discuss the unresolved questions related to the molecular mechanism of \( F_1 \) motors. This review is an extension of a previous Japanese review by Murata (2014) [1], which discusses recent progress in determining the rotation mechanism of mammalian \( F_1 \) motors [2,3] in addition.

Studies on \( F_1 \) motors

\( F_1 \)-ATPases (or ATP synthases) are found in the inner membranes of mitochondria, thylakoid membranes of chloroplasts, and cytoplasmic membranes of bacteria. ATP is synthesized by the \( F_1 \) part of \( F_1 \)-ATPase, which utilizes rotational energy generated from the \( F_o \) part, which itself is driven by electro-chemical proton gradients across membranes (Fig. 1a). In the absence of an electro-chemical proton gradient and by utilizing the chemical energy derived from ATP hydrolysis, \( F_1 \)-ATPases also function reversibly as proton-pumping motors. The \( F_1 \) part is also called \( F_1 \)-ATPase, as the purified motor alone works as a rotary motor when it exhibits high ATP hydrolysis activity. It has been
recognized that the three catalytic sites of this ATPase work cooperatively to hydrolyze ATP; however, the rotational movement of its rotary motor was the subject of long-term intensive study. Based on careful experimentation using [18O]phosphate isotopes, Boyer [4] proposed a new model in 1980, suggesting that each of the three catalytic sites of the ATPase alternates its hydrolyzing activities and drives rotational movements. At the time, it seemed implausible that such a small protein could rotate and function as a rotary motor. However, in 1994, Abrahams et al. [5] determined the crystal structure of the bovine mitochondrial F$_i$-ATPase, demonstrating the plausibility of the rotational catalysis model. The structure of the F$_i$ motor showed an α$_3$β$_3$ ring structure containing three non-catalytic subunits (α) and three catalytic subunits (β), alternately arranged around a central axis γ subunit. Furthermore, the three catalytic sites showed asymmetrical structures with ATP bound (TP form), ADP bound (DP form), and empty (E form) (Fig. 1c, d). Three years later, using a fluorescently labeled actin filament attached to the γ-axis of a thermophilic bacterial F$_i$ motor, Noji et al. [6] directly visualized the ATP-driven rotation of the γ-axis by fluorescence microscopy. For their work, Boyer and Walker became Nobel Laureates of Chemistry in 1997.

During this time, the leading research on the molecular mechanism of the F$_i$ motor was conducted by crystallography research groups in England and single-molecule observation research groups in Japan. Crystallographic studies revealed various F$_i$-ATPase structures in complex with various nucleotides and inhibitors [7–11]. The use of various newly developed single molecule techniques revealed detailed molecular and rotational characteristics of ATP synthesis and hydrolysis reactions of the F$_i$ motor [12–15]. However, the rotation mechanism models, generated based on two research approaches, differed in several aspects [11,14,16]. Recently, a model was proposed that may explain both the structural and single-molecule data obtained from mammalian F$_i$-ATPase [2,3]. In this model, the central axis rotates by 120° per ATP molecule and has three intermediate states: a state “waiting for ATP binding” at 0° (and 120°), a state “waiting for Pi release” at 65°, and a state “waiting for ATP hydrolysis” at 90° [2]. Crystal structures corresponding to the states “waiting for Pi release” and “waiting for ATP hydrolysis” were obtained, which suggested how Pi is released in the rotary substeps [3].

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**Figure 1** (a) Structural model of the F$_i$-ATPase; (b) structural model of the V$_i$-ATPase; (c, d) crystal structures of the F$_i$ motor. (c) Side view of the F$_i$ motor. The C-terminal domain is shown as a transparent surface representation. The AMP-PNP molecules, bound in two catalytic and three non-catalytic sites, are shown as red spheres. (d) Top view of the F$_i$ motor generated by 90° rotation around the axis (dotted line) of (c). Red arrows show the locations of the catalytic sites.
ever, the crystal structure corresponding to the state “waiting for ATP binding” of this model of F$_1$·ATPases has not yet been obtained.

High-speed atomic force microscopy was developed by Ando et al., enabling real-time single-molecular imaging. This technique was used to reveal that the thermophilic F$_{1}$ motor without the γ-axis (α,β$_3$) rotates and changes its conformation in one decisive orientation and follows the correct order of ADP and Pi release after ATP hydrolysis [17]. Based on these observations, the unidirectional rotation of the F$_1$ motor is driven by conformational changes originating from the α-β$_3$ unit in an ATP hydrolysis-dependent manner. However, since the crystal structure of α-β$_3$ without bound nucleotide showed symmetrical arrangement of its subunits [18], the mechanism of the unidirectional movement of α-β$_3$ remains unknown. This is the first question that will be addressed in this review (Question I). Furthermore, it is not clear whether the γ-axis induces conformational changes in α-β$_3$, which will be addressed in our second question (Question II). These questions must be answered to fully understand the rotational mechanism of these motor proteins.

Studies on V$_1$ motors

The energy produced by the ATP-hydrolyzing activity of F-ATPase drives many cellular metabolic processes. V-ATPase (vacuolar type ATPase) also utilizes this energy to transport protons across organelle membranes in eukaryotes, acidifying their membranous compartments. Thus, as a proton pump, the function of the V-ATPase is opposite to that of F-ATPase. V-ATPases are found in the plasma membranes of osteoblasts and cancer cells, which function to acidify the metastasis of cancer cells [19]. Therefore, V-ATPases are an important target for drug design and prevention of osteoporosis and cancer. V-ATPase is thought to have evolved from a common ancestor with F-ATPase [20] and thus shares common structural features. These include a hydrophilic ATP-hydrolyzing part (V$_o$) and a hydrophobic membrane-embedded ion pump (V$_i$), which are connected by a central axis and peripheral stalks. However, their subunit compositions exhibit several differences (Fig. 1b).

Studies on V$_1$-ATPase were conducted using a thermophilic bacterial enzyme, which has subunits resembling that of V-ATPase but functions as an ATP synthase similar to the eukaryotic V-ATPase. We studied the structure and function of the V$_o$ part of this enzyme, and based on its Na$^+$-transporting activity, we proposed a model for its ion-transporting mechanism [24–26]. Beginning in 1996, we attempted crystallization trials of the V$_o$ motor part in order to obtain X-ray crystal structures. We initially attempted to purify and isolate the V$_o$ part from the whole V-ATPase complex. Our crystal did not diffract to high resolution, and our preparation of V$_o$ may have been contaminated with the motor without the axis (DF complex). Recently, using an E. coli cell-free protein synthesis system [27], we established expression and purification procedures for the motor part (A$_3$B$_3$ complex) without the DF complex, and subsequently solved its X-ray crystal structures [28].

Asymmetrical crystal structures of A$_3$B$_3$ complex

A crystal structure of the apo A$_3$B$_3$ complex obtained without the nucleotides ATP or ADP was solved to 2.8 Å resolution. The overall structure resembles that of the F$_1$ motor α-β$_3$ complex, revealing a hetero-hexameric ring composed of three catalytic subunits A and three non-catalytic subunits B arranged in an alternating configuration (Fig. 2a). Each subunit consists of an N-terminal β-barrel, middle α/β domain, and C-terminal helical domain. Since the hexameric ring is joined at the N-terminal β-barrel part, this region was fixed during structural comparison of the three A subunits. Superimposition revealed that all subunits adopt different conformations from one another. One of the A subunits is in the closed form (A$_c$) and is located closer to the ring center of the A$_3$B$_3$ complex, while the other two A subunits showed similar open forms (A$_o$ and A$_o’$) (Fig. 2b). Similarly, the three B subunits showed different conformations from one another; one exhibited a closed form (B$_c$), while the other two exhibited open forms (B$_o$ and B$_o’$) (Fig. 2b). Three nucleotide binding (catalytic) sites are located at the boundaries between the A/B pairs A$_3$B$_3$, A$_3$B$_3$ and A$_3$B$_3$ (red arrowheads in Fig. 2b). Surprisingly, even in the absence of nucleotide, the three catalytic sites formed by the same AB pair types show different conformations from one another. Previous reports of the apo structures of the thermophilic α-β$_3$ F$_1$ motor [18] and the A$_3$B$_3$ unit of the V$_o$ motor [21] both showed 3-fold rotational symmetry. Therefore, our structure is the first report of a motor protein structure with asymmetrical arrangement at the catalytic head.

Next, we obtained a crystal structure of the A$_3$B$_3$ complex in the presence of AMP-PNP, a non-hydrolysable analogue of ATP, at 3.4 Å resolution. In this structure, two of the three catalytic sites are occupied with electron density corresponding to AMP-PNP (Fig. 2c, d). The AB pair without bound AMP-PNP resembles the structure of the A$_3$B$_3$ pair in the apo A$_3$B$_3$ complex. We named this the empty form, as it appears to have low affinity for the nucleotide. The two other AMP-PNP-binding AB subunits show similar conforma-
As described above, the apo A3B3 complex appears to be composed of three different AB pairs adopting three conformations: an empty form that cannot bind ATP, a bindable form that can bind ATP, and a bound form that has the same conformation as the bound form. In the presence of ATP, the complex is thought to adopt two bound forms derived from binding ATP in the bindable and bound forms. When ATP in the bound form, originally the bound form in the apo structure, is hydrolyzed, the ADP and Pi products are likely released. The A3B3 complex may then resemble the structure of the initial stable apo-structure, as the ATP on the bound form derived from the original bindable form may remain without being hydrolyzed and maintain the bound form, which resembles the original bound form in the apo-structure. Thus, since the apo-structure of the A3B3 complex is stable when AB pairs adopt the empty, bindable, and bound forms, it is conceivable that when one AB pair adopts a bound form, its neighbor to the right takes the empty form, while the next to right neighbor takes the bindable form.

This description suggests that after ATP binding, hydrolysis, and release of ADP at the bound form, the complex rotates by 120° and each form takes on the next form in the turn. This is considered the principle of the unidirectional (or clockwise) rotational mechanism of the motor, driven by ATP hydrolysis. This may provide answers to Question I.

**Crystal structures of V1-ATPase**

Given the proposed mechanism for the unidirectional movement (Question I) described in the above section, we next address the role of the DF complex in the conformational change and rotation of the A3B3 complex (Question II). To answer this question, it is necessary to compare the structures in the presence and absence of the DF complex. Therefore, we reconstituted the V1-ATPase (A3B3 DF complex) from A3B3 and DF and obtained high-resolution diffraction crystals [28].

We determined the crystal structure of apo V1-ATPase to 2.2 Å resolution. As expected, the structure showed the DF complex penetrating through the center of the A3B3 ring (Fig. 2e, f). We compared the structures of the apo A3B3 complex with A3B3DF to examine the structural changes induced by DF binding. Similarly to the A3B3 complex, the A3B3DF complex also showed an empty form (A0B0′ pair) and bound form (A0B0, pair) (Fig. 2f). However, when the structures were superposed at the empty form positions, the bindable form in the A3B3 complex aligned with the bound form in the A3B3DF complex (Fig. 2b, f). This finding indicates that the conformational change from the bindable to the bound form, induced by nucleotide binding, can also be induced by DF binding. The third AB pair in V1-ATPase exhibited a closer conformation not found in the A3B3 complex. This pair (A0B0, pair) consisting of the closed A0B0 and B0A0 structures, was subsequently named the tight form (Figs. 2f, 3e, 3f). Thus, upon binding of DF, the bound form ob-

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**Figure 2** Crystal structures of the *E. hirae* V1 motor. The figures are drawn as described in Fig. 1c, d. (a, b) apo A3B3; (c, d) A3B3 with bound AMP-PNP; (e, f) apo A3B3DF; (g, h) A3B3DF with bound nucleotide.
Comparison of the tight and bound forms in the V$_1$-ATPase-nucleotide complex showed that the R-finger in the former structure is 1.6 Å closer to the γ-phosphate. This phosphate group is in turn 0.7 Å closer to E$_{261}$ in subunit A (Fig. 4b). E$_{261}$ was shown to be essential for ATPase activity in Saccharomyces cerevisiae V$_1$-ATPase [29]. Additionally, the corresponding E$_{188}$ residue in bovine F$_{1}$-ATPase is an important residue for ATP hydrolysis and is known to interact with the oxygen atom of the γ-phosphate and with an intermittent water molecule [30]. The closer proximity of the R-finger to the γ-phosphate, brought about by DF binding, may enhance the ATP hydrolysis reaction. Thus, we propose that this crystal structure corresponds to the state “waiting for ATP hydrolysis”.

Further experimental results; demonstration of V$_1$-ATPase rotation by single-molecule observation

ATP hydrolysis generates torque for the rotation of the DF axis. Single-molecule observation of the rotation is useful for obtaining direct evidence of rotational movement. In collaboration with Iino et al., we demonstrated 120° rotation of the DF axis in our V$_1$ complex [31,32]. At 4 mM ATP, the axis rotated continuously at a rotational speed of approximately 100 rps. The $K_M$ was approximately 100 μM. The angle distribution was analyzed, showing a 3-step rotation for each cycle or 120° rotation per step, at all ATP concentrations. Torque generation in the continuous ATP hydrolysis state was estimated to be approximately 20 pNnm [32], which is lower than values reported for F$_1$-ATPases.

Model of rotation mechanism of V$_1$-ATPase [difference from F$_1$ model [2,3]]

The following is a model for the rotation mechanism for V$_1$-ATPase based on crystal structures. This model resembles that of the F$_1$ motor proposed according to single-molecule observation studies, but several points are different and are highlighted in brackets [ ].
The rotation mechanism model starts with the structure of V1-ATPase with ATP at the bound and tight forms. Since the R-finger in the tight form is in close proximity to the ATP γ-phosphate, this ATP is “waiting for hydrolysis”. The process starts with hydrolysis of this ATP (Fig. 5a) [similar in the F1 motor]. Next, as the empty form shows low affinity for nucleotide, it is unable to bind ATP [similar to F1 motor]. After ATP is hydrolyzed to ADP and Pi, the tight form may take on another conformation, and the whole A3B3 complex may undergo a large conformational change. Based on single-molecule analysis of F1-ATPase, ATP binding and ADP release appear to occur after ATP hydrolysis, causing a large conformational change that induces axis rotation; therefore, the ATP binding conformation is expected to appear at this step. If the effects of the DF axis are ignored, the ATP hydrolysis reaction may induce the conformational change into the apo A3B3 structure, and the empty and tight forms appear to transform into bindable and empty forms, respectively (Fig. 5b) [no such step is predicted in the F1 motor model]. However, strong interactions between the DF and the tight form prevent the conformational change of the tight form into the empty form, and another intermediate state is likely to exist in place of Fig. 5b [this conformational change is not present in the model of the F1 motor]. This unidentified structure may be the “waiting for ATP binding” state and resemble the structure of the A3B3 apo-structure, which has a bindable form that can bind ATP [In the F1 motor, a structure corresponding to the bindable form has not been obtained]. In the next step, ATP binding to the bindable form induces a conformational change of this AB pair to the bound form and 120° rotation of the DF axis. Upon ATP binding and DF rotation, the bindable form changes into the bound form and a nucleotide-bound A3B3-like structure transiently appears (Fig. 5c). Finally, the original bound form is induced to change its conformation to the tight form by interacting with the DF complex [this is not thought to be the case in the F1 motor]. The R-finger approaches the γ-phosphate of ATP and resumes the state of “waiting for ATP hydrolysis” of V1-ATPase (Fig. 5d = a). This cycle is repeated. Because of the asymmetrical structure of the A3B3 ring, it is easy to conceive that the order of ATP hydrolysis and direction of rotation are intrinsically determined by the structure.

General discussion regarding bioenergy transduction

A more fundamental or basic question is about the energy transduction mechanism, referred to as the affinity change model by Yamato [34] or as the thermal ratchet model for muscle contraction by Vale and Oosawa [35]. Does the DF axis rotate by thermal fluctuation or by real force (torque) generated in the A3B3 motor complex coupled with ATP hydrolysis? In thermal fluctuation, the hydrolysis energy utilized to change the affinity of a protein or sub-complex for a substrate or other complex (i.e., DF complex or actin filament) is converted into directional information of movement. In real force model, actin filament movement or DF complex rotational movement is driven by force energy (also proposed as velocity change). In active transport systems, depending on the reaction direction, the energy utilized to alter substrate affinity may be converted into directional information. In the actomyosin system, the nucleotide-dependent change in affinity of myosin for the actin filament has been demonstrated [36]. Here, the energy of movement is likely derived from thermal fluctuation energy. We have not yet characterized the detailed affinity of the DF complex with the A3B3 motor, but the affinity appears to be quite high [37]. Therefore, without dissociating the axis, it would be difficult to generate torque through mechanical rotation. If the detaching/rotation/attaching movement of DF occurs to mechanically generate torque (force), the detaching/attaching steps should be seamless. However, in the crystal structures obtained so far, it is not conceivable for the motor to make large conformational changes to enable seamless detachment and reattachment. The DF binding sites between the tight form (or intermediary conformation) and the next bound form are too far apart (> 10 Å) to allow for seamless and efficient exchange of the binding residues between the motor and DF complex. Thus, the DF axis should be released, allowing for thermal fluctuation and searching for the correct landing site in both directions. Without information input into the direction of rotation, this will result in poor efficiency of energy utilization. Therefore, using an energy input, the affinity change model allows the motor...
molecule to select its direction of rotation. The conversion of free chemical energy from ATP hydrolysis into selective information decreases informational entropy. In this case, the selective information is the directional movement of the DF axis, which is guided by changes in affinity. A model system of such information-to-energy conversion has been demonstrated [38]. In a few instances, such informational entropy may be quantitatively correlated with thermodynamic free energy. However, this should be evaluated in future studies.

Conclusions

As described above, the model of rotation mechanism of E. hirae V$_1$-ATPase resembles that proposed by the single-molecule observation of F$_1$. However, there are several differences between the two models because of their fundamental differences in function; one functions in ATP synthesis, while the other in ATP hydrolysis. Although our model describes structural differences on the atomic level, it cannot predict the dynamic nature of structural changes, as time-dependent and reaction scheme information has not been fully determined. Thus, we have obtained supporting information from single-molecule observation of our enzyme, which will be useful for elucidating the reaction mechanism [31,32]. Furthermore, we are attempting to obtain other crystal structures to reflect intermediate rotational structures. We are also collaborating with a research group to perform molecular dynamics simulations to model the structural changes between the snapshot structures obtained from crystallography. In this respect, the MD results of Nam et al. indicate a F$_1$ motor conformation corresponding to the state “waiting for ATP binding” [39]. Using techniques that differ in spatiotemporal resolution and sample environment, we hope to clarify the detailed rotational mechanism of these rotary motors.

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Conflict of Interest

The authors declare no conflict of interests.

Author Contributions

I. Y., Y. K., and T. M. reviewed the field of rotary motors. I. Y. and T. M. wrote the manuscript.

References

[1] Murata, T. Operating principles of rotary molecular motors: differences between F$_i$ and V$_1$ motors. Seibutsu Butsuri 54, 79–84 (2014).
[2] Suzuki, T., Tanaka, K., Wakabayashi, C., Saita, E. & Yoshida, M. Chemomechanical coupling of human mitochondrial F$_{1}$-ATPase motor. Nat. Chem. Biol. 10, 930–936 (2014).
[3] Bason, V., Montgomery, M. G., Leslie, A. G. W. & Walker, J. E. How release of phosphate from mammalian F$_{1}$-ATPase generates a rotary step. Proc. Natl. Acad. Sci. USA 112, 6009–6014 (2015).
[4] Boyer, P. D. The binding change mechanism for ATP synthase—Some probabilities and possibilities. Biochim. Biophys. Acta 1140, 215–250 (1993).
[5] Abrahams, J. P., Leslie, A. G. W., Lutter, R. & Walker, E. Structure at 2.8 Å resolution of F$_{1}$-ATPase from bovine heart mitochondria. Nature 370, 621–628 (1994).
[6] Noji, H., Yasuda, R., Yoshida, M. & Kinoshita Jr., K. Direct observation of the rotation of F$_{1}$-ATPase. Nature 386, 299–302 (1997).
[7] Gibbons, C., Montgomery, M. G., Leslie, A. G. W. & Walker, J. E. The structure of the central stalk in bovine F$_{1}$-ATPase at 2.4 Å resolution. Nat. Struct. Biol. 7, 1055–1061 (2000).
[8] Menz, R. I., Walker, J. E. & Leslie, A. G. W. Structure of bovine mitochondrial F$_{1}$-ATPase with nucleotide bound to all three catalytic sites: Implications for the mechanism of rotary catalysis. Cell 106, 331–341 (2001).
[9] Kagawa, R., Montgomery, M. G., Braig, K., Leslie, A. G. W. & Walker, J. E. The structure of bovine F$_{1}$-ATPase inhibited by ADP and beryllium fluoride. EMBO J. 23, 2734–2744 (2004).
[10] Bowler, M. W., Montgomery, M. G., Leslie, A. G. W. & Walker, J. E. How azide inhibits ATP hydrolysis by the F$_{1}$-ATPases. Proc. Natl. Acad. Sci. USA 103, 8646–8649 (2006).
[11] Rees, D. M., Montgomery, M. G., Leslie, A. G. W. & Walker, J. E. Structural evidence of a new catalytic intermediate in the pathway of ATP hydrolysis by F$_{1}$-ATPase from bovine heart mitochondria. Proc. Natl. Acad. Sci. USA 109, 11139–11143 (2012).
[12] Yasuda, R., Noji, H., Yoshida, M., Kinoshita Jr., K. & Itoh, H. Resolution of distinct rotational substeps by submillisecond kinetic analysis of F$_{1}$-ATPase. Nature 410, 898–904 (2001).
[13] Adachi, K., Oiwa, K., Nishizaka, T., Furuike, S., Noji, H., Itoh, H., et al. Coupling of rotation and catalysis in F$_{1}$-ATPase revealed by single-molecule imaging and manipulation. Cell 130, 309–321 (2007).
Mulkidjanian, A. Y., Makarova, K. S., Galperin, M. Y. & Forgac, M. Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. Nat. Rev. Mol. Cell Biol. 10, 1347–1349 (2009).

Watanabe, R., Okuno, D., Sakakihara, S., Shimabukuro, K., Iino, R., Yoshida, M., et al. Mechanical modulation of catalytic power on F$_1$-ATPase. Nat. Chem. Biol. 8, 86–92 (2012).

Okuno, D., Fujisawa, R., Iino, R., Hirono-Hara, Y., Imamura, H. & Noji, H. Correlation between the conformational states of F$_1$-ATPase as determined from its crystal structure and single-molecule rotation. Proc. Natl. Acad. Sci. USA 105, 20722–20727 (2008).

Uchihashi, T., Iino, R., Ando, T. & Noji, H. High-speed atomic force microscopy reveals rotary catalysis of rotorless F$_1$-ATPase. Science 333, 755–758 (2011).

Shirakihara, Y., Leslie, A. G. W., Abrahams, J. P., Walker, J. E., Ueda, T., Sekimoto, Y., et al. The crystal structure of the nucleotide-free α3β3 subcomplex of F$_1$-ATPase from the thermophilic Bacillus PS3 is a symmetric trimer. Structure 5, 825–836 (1997).

Forgac, M. Vacular ATPases: rotary proton pumps in physiology and pathophysiology. Nat. Rev. Mol. Cell Biol. 8, 917–929 (2007).

Mulldjianian, A. Y., Makarova, K. S., Galperin, M. Y. & Koonin, E. V. Inventing the dynamo machine: the evolution of the F-type and V-type ATPases. Nat. Rev. Microbiol. 5, 892–899 (2007).

Maher, M. J., Akimoto, S., Iwata, M., Nagata, K., Hori, Y., Yoshida, M., et al. Crystal structure of A,B complex of V-ATPase from Thermus thermophilus. EMBO J. 28, 3771–3779 (2009).

Numoto, N., Hasegawa, Y., Takeda, K. & Miki, K. Inter-subunit interaction and quaternary rearrangement defined by the central stalk of prokaryotic V$_1$-ATPase. EMBO rep. 10, 1228–1234 (2009).

Imamura, H., Takeda, M., Funamoto, S., Shimabukuro, K., Yoshida, M. & Yokoyama, K. Rotation scheme of V$_1$-motor is different from that of F$_1$-motor. Proc. Natl. Acad. Sci. USA 102, 17929–17933 (2005).

Murata, T., Yamato, I., Kakunuma, Y., Leslie, A. G. W. & Walker, J. E. Structure of the rotor of the V-type Na$^+$-ATPase from Enterococcus hirae. Science 308, 654–659 (2005).

Murata, T., Yamato, I., Kakunuma, Y., Shirouzu, M., Walker, J. E., Yokoyama, S., et al. Ion binding and selectivity of the rotor ring of the Na$^+$-transporting V-ATPase. Proc. Natl. Acad. Sci. USA 105, 8607–8612 (2008).

Mizutani, K., Yamamoto, M., Suzuki, K., Yamato, I., Kakunuma, Y., Shirouzu, M., et al. Structure of the rotor ring modified with N,N'-dicyclohexylcarbodiimide of the Na$^+$-transporting vacular ATPase. Proc. Natl. Acad. Sci. USA 108, 13474–13479 (2011).

Saijo, S., Arai, S., Hosain, K. M. M., Yamato, I., Suzuki, K., Kakunuma, Y., et al. Crystal structure of the central axis DF complex of the prokaryotic V-ATPase. Proc. Natl. Acad. Sci. USA 108, 19955–19960 (2011).

Arai, S., Saijo, S., Suzuki, K., Mizutani, K., Kakunuma, Y., Ishizuka-Katsura, Y., et al. Rotation mechanism of Enterococcus hirae V$_1$-ATPase based on asymmetric crystal structures. Nature 493, 703–707 (2013).

Liu, Q., Leng, X.-H., Newman, P. R., Vasilyeva, E., Kane, P. M. & Forgac, M. Site-directed Mutagenesis of the Yeast V-ATPase A Subunit. J. Biol. Chem. 272, 11750–11756 (1997).

Dittrich, M., Hayashi, S. & Schulten, K. On the Mechanism of ATP Hydrolysis in F$_1$-ATPase. Biophys. J. 85, 2253–2266 (2003).

Minagawa, Y., Ueno, H., Haruta, M., Ishizuka-Katsura, Y., Ohsawa, N., et al. Basic properties of rotary dynamics of Enterococcus hirae V$_1$-ATPase. J. Biol. Chem. 288, 32700–32707 (2013).

Ueno, H., Minagawa, Y., Haruta, M., Rahman, S., Yamato, I., Muneyuki, E., et al. Torque generation of Enterococcus hirae V-ATPase. J. Biol. Chem. 289, 31212–31223 (2014).

Adachi, K., Iiwa, K., Yoshida, M., Nishizaka, T. & Kinosita Jr., K. Controlled rotation of the F$_1$-ATPase reveals differential and continuous binding changes for ATP synthesis. Nat. Commun. 3, 1022 (2012).

Yamato, I. Ordered binding model as a general tight coupling mechanism for bioenergy transduction—A hypothesis. Proc. Jpn. Acad. 69, 218–223 (1993).

Eisenberg, E. & Hill, T. L. Muscle contraction and free energy transduction in biological systems. Science 272, 999–1006 (1985).

Alam, Md. J., Arai, S., Saijo, S., Suzuki, K., Mizutani, K., Ishizuka-Katsura, Y., et al. Loose binding of the DF axis with the A,B complex stimulates the initial activity of Enterococcus hirae V$_1$-ATPase. PLoS One 8, e74291 (2013).

Toyabe, S., Sagawa, T., Ueda, M., Muneyuki, E. & Sano, M. Experimental demonstration of information-to-energy conversion and validation of the generalized Jarzynski equality. Nat. Phys. 6, 988–992 (2010).

Nam, K., Pu, J. & Karplus, M. Trapping the ATP binding state leads to a detailed understanding of the F$_1$-ATPase mechanism. Proc. Natl. Acad. Sci. USA 111, 17851–17856 (2014).