Structure and dynamics of rotary V₁ motor

Hiroshi Ueno · Kano Suzuki · Takeshi Murata

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Abstract
Rotary ATPases are unique rotary molecular motors that function as energy conversion machines. Among all known rotary ATPases, F₁-ATPase is the best characterized rotary molecular motor. There are many high-resolution crystal structures and the rotation dynamics have been investigated in detail by extensive single-molecule studies. In contrast, knowledge on the structure and rotation dynamics of V₁-ATPase, another rotary ATPase, has been limited. However, recent high-resolution structural studies and single-molecule studies on V₁-ATPase have provided new insights on how the catalytic sites in this molecular motor change their conformation during rotation driven by ATP hydrolysis. In this review, we summarize recent information on the structural features and rotary dynamics of V₁-ATPase revealed from structural and single-molecule approaches and discuss the possible chemomechanical coupling scheme of V₁-ATPase with a focus on differences between rotary molecular motors.

Keywords
Rotary catalysis · Ion pump · Enterococcus hirae · Crystal structure · Single-molecule technique

Introduction
All living cells use a chemical fuel called adenosine triphosphate (ATP) to maintain the functions required for life, such as protein biosynthesis, muscle contraction, and brain activity. Therefore, ATP is often referred to as the energy currency for the cell. Under aerobic conditions, the majority of ATP is produced by the F-type ATP synthase [1]. F-type ATP synthases (also known as F-ATPases) are ubiquitous rotary motor enzymes found in the inner membrane of mitochondria, the thylakoid membrane of chloroplasts, and the plasma membranes of bacteria. They catalyze ATP synthesis from ADP and inorganic phosphate using the energy of ion (proton or sodium) translocation caused by transmembrane electrochemical potential (proton or sodium ions) and, when operating in reverse, they also generate an electrochemical potential difference of ions using the energy released by ATP hydrolysis [2–4].

Eukaryotic vacuolar-type ATPases (V-ATPases) are also rotary motor enzymes; they are evolutionarily and functionally related to F-type ATP synthases [5, 6]. They function in reverse of F-type ATP synthases, that is, they transport ions (protons or sodium ions) across the membrane using the energy derived from ATP hydrolysis. Acidification of vesicles by intracellular V-ATPases is important for various cellular processes, including receptor-mediated endocytosis, membrane trafficking, and protein processing and degradation. They also function on the plasma membrane of certain cells, such as tumor cells, renal intercalated cells, and osteoclasts. Aberrant function of V-ATPase is associated with a number of human diseases including tumor metastasis, distal renal tubular acidosis, and osteoporosis. Therefore, they are considered as potential drug targets [5, 7]. V-ATPases are found in some bacteria, such as Thermus thermophilus (T. thermophilus) and Enterococcus hirae (E. hirae). V-ATPase from T. thermophilus functions as an ATP synthase under physiological conditions [8, 9]. Therefore, this enzyme is sometimes called A-type ATPase found in Archeae (A-ATPase). A-ATPases function as ATP synthases similar to the F-type ATP synthase, although the structure and subunit composition of A-ATPases are more similar to...
those of V-ATPases (Fig. 1a) [10–12]. V-ATPase from E. hirae functions as a sodium ion pump similar in nature to eukaryotic V-ATPase, and plays an important role in maintaining sodium homeostasis in cells under an alkaline environment [13–15].

All rotary ATPases are unique rotary molecular motors that function as energy conversion machines with a similar architecture and rotary catalytic mechanism [5, 11, 12, 16]. They are large, multi-subunit complexes composed of a hydrophilic F1/V1/A1 motor for ATP synthesis/hydrolysis and a membrane-embedded F0/V0/A0 motor for ion transport. The bacterial F1/V1/A1 and F0/V0/A0 motors are connected by one central stalk and one or two peripheral stalks (Fig. 1). Interestingly, V-ATPases in eukaryotes have three peripheral stalks, although there is only one peripheral stalk in eukaryotic F-ATPases [16–18]. The peripheral stalks of eukaryotic V-ATPases play an important role in reversible dissociation of V1 motor from Vo motor with silencing of the ATP hydrolysis activity of the free V1 motor [18], in contrast to F1 motor which will rapidly hydrolyze ATP when isolated. This implies the fact that it was necessary for V-ATPases to evolve a regulatory mechanism for keeping the dissociated V1 motors catalytically inactive to prevent wasteful energy consumption.

Among all known rotary ATPases, the hydrophilic portion of F-ATPase (F1-motor or F1-ATPase) is the best characterized; there are high-resolution crystal structures of several rotational states [19–28] and the chemomechanical coupling scheme has been revealed in detail by extensive single-molecule studies [29–40]. Relatively little is known about the structure and rotation scheme of V1-motors (V1-ATPase) [41–43]. Recently, high-resolution crystal structures of several rotational states of V1-ATPase from E. hirae have been determined [44, 45]. Furthermore, basic rotary dynamics of this V1-ATPase have been revealed by single-molecule studies [46–49]. These studies have provided new insights into the rotation mechanism of V1-ATPase. In the present review, we discuss recent findings on the structural features and rotary dynamics of bacterial V1-ATPase revealed from structural and single-molecule studies with a focus on differences from the properties of F1-ATPase.

### V1-ATPase: structural studies

In the bacterial V-ATPase, the catalytic V1 moiety is composed of A, B, D, and F subunits, in which three alternately arranged A and B subunits form a hexameric stator A3B3 ring. The central rotary shaft of D and F subunits penetrates the central cavity of the A3B3 ring and rotates using the energy of ATP hydrolysis [41–44]. Unlike the isolated eukaryotic V1 moiety in which subunit H inhibits its activity [18], the isolated bacterial V1 moiety can generally catalyze ATP hydrolysis and hence is called V1-ATPase. Structural studies have been conducted using a V1-ATPase from the thermophilic eubacterium T. thermophilus, which has high stability. The crystal structure of the A3B3 subcomplex from T. thermophilus was determined at 2.8 Å resolution [41]. The diameter of the A3B3 subcomplex is larger than that of the α3β3 subcomplex in F1-ATPase, because it includes an outward protrusion domain in the A subunit (Fig. 2a, green circles), termed the “non-homologous region” that is absent from β subunit; the structure also provides molecular information

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![Fig. 1 Schematic illustrations of bacterial rotary ATPases.](image-url)

**Fig. 1** Schematic illustrations of bacterial rotary ATPases. **a** A/V-type ATPases/synthases are found in Archeae and some bacterial taxa. They are composed of a soluble catalytic core A/V1 motor (A3B3DF) and a membrane integral A/V0 motor (ac, where n is the copy number of the c subunits). The A1/V1 and A0/V0 motors are connected by the two peripheral stalks (EG). The N- and C-terminal domains of a subunit are shown as aNT and aCT, respectively. **b** Bacterial F-type ATPases/synthases are structurally different from bacterial A/V-type ATPases/synthases. The F0 (aβγδε) and F1 motors (ab2c) are connected by only one peripheral stalk (b2).
about the B–A interface. The catalytic sites are located at the interfaces of the A and B subunits (Fig. 2a, red arrows), with the majority of the catalytic residues residing in the A subunits, similar to the catalytic β subunits and the non-catalytic α subunits in F1-ATPase [19, 27]. The overall structure of V1-ATPase (A3B3DF complex) from T. thermophilus was first determined at 4.5–4.8 Å resolution (Fig. 2b) [42]. This structure provided the initial information about the position and orientation of the rotor DF subunits in the A3B3 ring and revealed structural similarities and differences between V1- and F1-ATPase. However, the lack of high-resolution structural information for the overall V1-ATPase from T. thermophilus limits our understanding of its molecular architecture and operation. Meanwhile, high-resolution crystal structures of the A3B3 ring and entire V1-ATPase from E. hirae have recently been solved with and without bound nucleotides [44, 45].

**Structure of A3B3 ring from E. hirae: asymmetric structure**

The high-resolution crystal structures of the A3B3 ring from E. hirae were solved with and without a non-hydrolyzable ATP analog [adenosine 5′-(β,γ-imido)triphosphate or AMPPNP] at 3.4 and 2.8 Å resolution, respectively (Fig. 3) [44]. The overall architecture of A3B3 from E. hirae is similar to that of αβ3 in F1-ATPase, but the structures show some differences.

Each A and B subunit consists of an N-terminal β-barrel, central α/β domain, and C-terminal helical domain (Fig. 3a, right). Superimposition of the N-terminal β-barrel part of three A or B subunits shows that the conformations of each A and B subunit are not identical. If one of the A subunits is in the closed conformation (C),
the other two A subunits show open conformations (O or O'). Similarly, one of the B subunits takes the closed form (C) and the other two take open conformations (O or O') (Fig. 3a), resulting in the asymmetry of the A₃B₃ ring. Interestingly, even in the absence of nucleotides and central rotor DF subunits, each catalytic site shows the three distinct states. In contrast, F₁-ATPase shows a threefold symmetric structure with three identical catalytic sites in the absence of bound nucleotides [50, 51]; accordingly, the asymmetric structure of the stator ring is not observed in the case of F₁-ATPase. In the presence of high concentration of AMPPNP (5 mM), the A₃B₃ ring binds two AMPPNP molecules (Fig. 3b), resulting in changes in the conformation of A (O' to C) and B (O to O') subunits, but the A₃B₃+AMPPNP pair shows little conformational change upon AMPPNP binding. Therefore, the three catalytic sites (AₒBₒ, Aₒ'Bₒ, and Aₒ'Bₒ') are termed ‘empty’, ‘bindable’, and ‘bound’ sites, respectively (Fig. 3a, b).

Two AMPPNP-bound structure of V₁-ATPase: catalytic dwell state

In addition to the A₃B₃ ring structures, the nucleotide-free entire V₁-ATPase from E. hirae (eV₁) was also determined at 2.2 Å resolution [44]. Insertion of the rotor DF subunits into the stator A₃B₃ ring induces conformational changes in the A and B subunits, even in the absence of bound nucleotides.

Fig. 3 Crystal structures of A₃B₃ subcomplex from E. hirae. a Crystal structure of the nucleotide-free A₃B₃ subcomplex from E. hirae determined at 2.8 Å resolution (PDB ID: 3VR2) [44]. Each A and B subunit consists of the N-terminal β-barrel (N), the central α/β domain (α/β), and the C-terminal helical domain (C) as seen in A₃B₃ from T. thermophilus (Fig. 2a). b Crystal structure of the nucleotide-bound A₃B₃ subcomplex from E. hirae determined at 3.4 Å resolution (PDB ID: 3VR3) [44]. Two AMPPNP molecules are bound to the ‘bound’ sites (indicated by red arrowheads). Top views from the membrane side are shown on the left and center. Only the C-terminal domains of the A₃B₃ rings are shown in the center to clarify the distinct conformations of individual A or B subunits and the different structures of the three catalytic sites. On the right, conformations of the individual A and B subunits superimposed at the N-terminal β-barrel domain (white) are shown. O and O' open conformation, C closed conformation.
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(Fig. 4a). This results in changes in the conformations of A and B subunits from the closed (C) to the more closed ‘closer’ (CR) conformation and from the open (O') to closer (CR) conformation, respectively. Consequently, e$V_1$ shows three different catalytic sites termed ‘empty’, ‘bound’, and ‘tight’ sites (Fig. 4a). Furthermore, by soaking e$V_1$ in AMPPNP, the structure of nucleotide-bound $V_1$-ATPase (b$V_1$ or $2_{ATP}V_1$) was also determined at 2.7 Å resolution (Fig. 4b) [44, 45]. Two AMPPNP molecules were bound to the binding sites of the ‘bound’ and ‘tight’ sites of e$V_1$, but the overall structure was very similar to that of e$V_1$ (Fig. 4a, b). Even in the presence of high AMPPNP (2 mM), no electron density peak for AMPPNP was found in the ‘empty’ site, indicating that it has a very low affinity for AMPPNP. Comparison of the ‘tight’ and ‘bound’ sites in b$V_1$ revealed the movement of the Arg-finger (Arg-350) in the ‘tight’ site closer to the γ-phosphate relative to the ‘bound’ site (Fig. 4c). This γ-phosphate moved closer to Glu-261 in the A subunit which is essential for ATPase activity in yeast $V_1$-ATPase [52]. The corresponding Glu-188 of the β subunit in bovine mitochondrial $F_1$-ATPase is an essential residue for ATP hydrolysis and interacts with the γ-phosphate of the nucleotide and lytic water molecules [19, 28, 53]. The closer proximity of the Arg-finger to the γ-phosphate may enhance the ATP hydrolysis reaction. Therefore, it is possible that the ‘tight’ site corresponds to the catalytic site waiting for ATP hydrolysis and this structure corresponds to the catalytic dwell state (Fig. 5a). Similar nucleotide-free and nucleotide-bound structures of yeast mitochondrial $F_1$-ATPase have been reported [25]. These results suggest that interactions between the rotor and stator are as crucial as nucleotide binding for determining the structure of the catalytic sites of rotary ATPases.

Two ADP-bound structure of $V_1$-ATPase: ATP-binding dwell state

More recently, the crystal structures of two other nucleotide-bound states were reported [45], i.e., the two ADP-bound structure ($2_{ADP}V_1$) and the three ADP-bound structure ($3_{ADP}V_1$). When soaking the e$V_1$ crystals in 20 µM ADP, it binds to the ‘bound’ and ‘tight’ sites of e$V_1$, as in the case of b$V_1$, and the two ADP-bound structure was solved at 3.3 Å resolution (Fig. 5b). ADP binding to the ‘tight’ site in e$V_1$ induces changes in the A and B subunits to more open conformations (CR to C in A subunit; CR to C' in B subunit), but the ‘bound’ site in e$V_1$ shows no conformational change upon ADP binding. The observed conformational changes result in the tilting of rotor DF subunits towards the catalytic dwell state (Fig. 5a). Interestingly, the ‘empty’ site shows a cooperative conformational change without ADP binding. The newly found catalytic sites ($A_{c}B_{C'}$ and $A_{B}O_{C''}$) are termed ‘ADP-bound’ and ‘bindable-like’ sites, respectively. The ‘bindable-like’ site is similar to the ‘bindable’ site in the A$_3$B$_3$ structure (Fig. 3a) and takes a more open conformation.
Fig. 5 Crystal structures of two AMPPNP-bound $V_1$-ATPase ($bV_1$), two ADP-bound $V_1$-ATPase ($2_{ADP}V_1$), and three ADP-bound $V_1$-ATPase ($3_{ADP}V_1$) from $E. hirae$. a Crystal structure of two AMPPNP-bound $V_1$-ATPase ($bV_1$, 2.7 Å resolution, PDB ID: 3VR6) [44]. b Crystal structure of two ADP-bound $V_1$-ATPase ($2_{ADP}V_1$, 3.3 Å resolution, PDB ID: 5KNB) [45]. c Crystal structure of three ADP-bound $V_1$-ATPase ($3_{ADP}V_1$, 3.0 Å resolution, PDB ID: 5KNC) [45]. Top views of the C-terminal domain of A$_3$B$_3$ rings and central rotor D subunit (green) viewed from the membrane side are shown on the left. Red, orange, and magenta arrowheads indicate the catalytic sites that bind to AMPPNP, ADP, and sulfate, respectively. Conformations of the individual A and B subunits superimposed at the N-terminal β-barrel domain (white) are shown on the right. $O$ and $O'$ open conformation, $HC$ half-closed conformation, $C$ closed conformation, $CR$ more closed ‘closer’ conformation.
than that of the ‘empty’ site. Therefore, the ‘bindable-like’ site is considered to be the catalytic site waiting for ATP binding, and the $2_{\text{ADP}}V_1$ structure is regarded as the ATP-binding dwell state.

**Three ADP-bound structure of $V_1$-ATPase: ADP-release dwell state**

The $3_{\text{ADP}}V_1$ structure was solved at 3.0 Å resolution by soaking the e$V_1$ crystals in 2 mM ADP [45]. In this structure, all three catalytic sites are occupied by ADP and, in addition, a sulfate is bound to one catalytic site (Fig. 5c, magenta arrowhead). A comparison between $2_{\text{ADP}}V_1$ and $3_{\text{ADP}}V_1$ structures shows that ADP (and sulfate) binding to the ‘bindable-like’ site in $2_{\text{ADP}}V_1$ induces a conformational change in the A subunit (O$'$) to the ‘half-closed’ conformation (HC), whereas the B subunit (O$''$) shows no conformational change. This catalytic site (A$_{HC}B_{O''}$) in $3_{\text{ADP}}V_1$ is termed a ‘half-closed’ site. The conformational change in the catalytic site from the ‘bindable-like’ site to the ‘half-closed’ site induces a conformational change of the ‘ADP-bound’ site to a more tight-like conformation (ACR$'$BCR$'$). This shifted ‘ADP-bound’ site in $3_{\text{ADP}}V_1$ is termed a ‘tight-like’ site, because the nucleotide-binding site is more similar to that of the ‘tight’ site than to that of the ‘ADP-bound’ site (Fig. 6). The β-phosphate of ADP in the ‘tight-like’ site is more distant from the surrounding interacting residues compared to that in the ‘ADP-bound’ site, suggesting that an ADP will be easily released from the ‘tight-like’ site (Fig. 6) [45]. Therefore, the “tight-like” site is considered to be a dwelling state before ADP release and the $3_{\text{ADP}}V_1$ structure is regarded as the ADP-release dwell state.

**Structural difference between $F_1$- and $V_1$-ATPase: conformational features**

A comparison of the two AMPPNP-bound structures of $V_1$-ATPase from *E. hirae* [44, 45] and $F_1$-ATPase from bovine mitochondria [28] is shown in Fig. 7. Both nucleotide-binding sites show very similar arrangements of catalytically important residues and nucleotides (Fig. 7a). However, the overall structures of A and B subunits in $V_1$-ATPase show some differences from those of the β and α subunits in $F_1$-ATPase. The non-catalytic B subunit of this $V_1$-ATPase does not bind to a nucleotide, whereas the non-catalytic α subunit of $F_1$-ATPase binds to a nucleotide (Fig. 7b). Superimposition of the N-terminal β-barrel part of three A and B subunits in $V_1$-ATPase and β and α subunits in $F_1$-ATPase reveals conformational differences between A$_C$ and A$_{CR}$, and B$_C$ and B$_{CR}$ of $V_1$-ATPase, but the conformations of β$_{TP}$ and β$_{DP}$ as well as α$_{TP}$ and α$_{DP}$ of $F_1$-ATPase are very similar (Fig. 7c). These differences are also evidenced by the positional displacement of residues between two of the three A and B subunits in $V_1$-ATPase and β and α subunits in $F_1$-ATPase (Fig. 8), which also shows that the structures of A$_C$ and A$_{CR}$ are largely different from that of A$_B$ (Fig. 8, top left). In contrast, the central portions (residues 180–320) of the β$_{TP}$ and β$_{DP}$ structures show very similar conformations to that of the β$_E$ structure (Fig. 8, top right). These results suggest that the A subunit in $V_1$-ATPase from *E. hirae* undergoes the whole conformational change upon nucleotide binding, whereas the β subunit in $F_1$-ATPase undergoes the conformational change mainly in the P-loop and C-terminal domains.

Thus, the conformational features of the A and B subunits in $V_1$-ATPase from *E. hirae* are apparently different from those of the β and α subunits in bovine $F_1$-ATPase, despite the highly similar nucleotide-binding sites of these ATPases. These structural differences and similarities may be related.
to the 'unique' and 'common' mechanisms of rotary catalysis between these rotary ATPases, such as the chemomechanical coupling scheme of the rotation [40, 46].

**Dynamics of rotary V₁-ATPase: single-molecule studies**

After the establishment of the single-molecule rotation assay of F₁-ATPase in 1997 [29], the dynamics of rotary ATPases from various species have been studied at the single-molecule level [38–40, 46, 54–57]. The first rotation of V₁-ATPase has been directly visualized under an optical microscope by the attachment of large beads to the rotor DF subunits in V₁-ATPase from *T. thermophilus* [58]. V₁-ATPase from *T. thermophilus* rotates stepwise in a counterclockwise direction, consuming one ATP molecule at each step when viewed from the membrane side. The basic step size is 120°, which is similar to that of F₁-ATPase, and no substeps have been resolved in the rotation, even when using a slowly hydrolyzable ATP analog (adenosine 5′-O-(3-thio)triphosphate or ATPγS) and high-speed imaging of gold nanoparticles [48, 59]. These results indicate that ATP binding and ATP cleavage (and/or phosphate release) occur at the same angle in this V₁-ATPase. In the case of F₁-ATPases, some or all of these elementary reaction steps occur at different angles and the basic 120° step is further divided into two or three substeps [31, 32, 39, 40], i.e., 80° and 40° substeps in thermophilic *Bacillus* PS3 F₁-ATPase [31, 32], 85° and 35° substeps in *Escherichia coli* F₁-ATPase [39], and
65°, 25°, and 30° substeps in human F1-ATPase [40]. The 80°, 85°, and 65° substeps are triggered by ATP binding and ADP release, while the 40° and 35° substeps are triggered by ATP cleavage and phosphate release. In human F1-ATPase, it is proposed that ATP cleavage and phosphate release trigger different substeps of 30° and 25°, respectively.

Recently, rotary dynamics of V1-ATPase from E. hirae have been characterized using single-molecule analyses at a submillisecond temporal resolution employing gold nanoparticles and an objective-type total internal reflection dark field microscope (Fig. 9a) [46–48]. V1-ATPase from E. hirae rotates in basically the same manner as that from T. thermophilus which functions as ATP synthase [55, 59]. This V1-ATPase also shows only three pauses separated by 120° at all concentrations ranging from below to above the Michaelis constant (K_m), where distinct elementary reaction steps of ATP hydrolysis (ATP binding, ATP cleavage, or product release) become the rate-limiting step (Fig. 9b).
suggesting that $120^\circ$ stepping rotation without substeps is a common property of $V_1$-ATPase [46], despite the difference in physiological function between V-ATPase from *E. hirae* (ion pump) and that from *T. thermophilus* (ATP synthesis). These results imply that the basic properties of rotary dynamics of F-ATPases and V-ATPases are determined by their overall structures and that the difference in physiological function derives from regulatory mechanisms such as Mg$^{2+}$ ADP inhibition and inhibitor proteins [9, 18, 23, 26].

### Chemomechanical coupling scheme of $V_1$-ATPase

Figure 10a shows a proposed chemomechanical coupling scheme of $V_1$-ATPase from *E. hirae* based on recently determined structural features and rotary dynamics [44–47]. As mentioned above, three distinct structures of *E. hirae* $V_1$-ATPase have been solved (b$V_1 = 2_{ATP}V_1$, 2$_{ADP}V_1$, and 3$_{ADP}V_1$) [44, 45]. These three structures are regarded as the three different dwelling states in the rotation waiting for the elementary reaction steps of ATP hydrolysis corresponding, respectively, to the catalytic (ATP cleavage) dwell (b$V_1 = 2_{ATP}V_1$), ATP-binding dwell (2$_{ADP}V_1$), and ADP-release dwell (3$_{ADP}V_1$) states.

In this model, ATP binds to the ‘bindable-like’ site in 2$_{ADP}V_1$, which induces conformational changes of ‘bindable-like’ and ‘ADP-bound’ sites to ‘half-closed’ and ‘tight-like’ sites, respectively. This results in a slight shift of the rotor DF subunits toward the ‘tight-like’ site, but does not induce the obvious rotation of the rotor DF subunits. Then, ADP release occurs at the ‘tight-like’ site in 3$_{ADP}V_1$, and all three catalytic sites change the conformations from ‘half-closed’ to ‘bound’, from ‘bound’ to ‘tight’, and from ‘tight-like’ to ‘empty’. Coupled with these conformational changes, the DF subunits rotate $120^\circ$. After this rotation, ATP bound at the
Fig. 10  Chemomechanical coupling scheme of V₁-ATPase. a Proposed rotation model of E. hirae V₁-ATPase for 120° rotation [45]. The structure models are based on the crystal structures of 2ADPV₁ (ATP-binding dwell), 3ADPV₁ (ADP-release dwell), and bV₁ (catalytic dwell). The catalytic sites that bind to nucleotides are indicated by arrowheads (top). Each blue circle represents the chemical state of each catalytic site, and the central red arrow represents the orientation of the rotor DF subunits. ATP* represents the pre-or post-hydrolysis state of ATP (middle). Correspondence table for all catalytic sites observed in the crystal structures of the A₃B₃ and V₁-ATPase (bottom). b Possible chemomechanical coupling scheme of E. hirae V₁-ATPase [44–46, 48, 49] (left) and human F₁-ATPase [40] (right) for 360° rotation. 0° is set as the ATP-binding angle for the catalytic site at the 12 O’clock position (green). In the model of E. hirae V₁-ATPase, ATP bound at 0° is cleaved into ADP and Pi at 240°. Among these, phosphate first dissociates at 240°, and then, ADP release occurs at 240°. Other catalytic sites also obey the same reaction scheme offset by 120° and 240°. In the model of human F₁-ATPase, ATP bound at 0° is cleaved into ADP and Pi at 210°, ADP dissociates at 240°, and then, phosphate release occurs at 305°.
‘tight’ site in $bV_1 (= 2_{\text{ATP}V_1})$ is cleaved into ADP and phosphate. The release of phosphate, which has a lower affinity than ADP, is coupled with a conformational change from a ‘tight’ site to ‘ADP-bound’ site and from an ‘empty’ site to ‘bindable-like’ site. Consequently, the rotor DF subunits tilt toward the ‘ADP-bound’ site, without showing obvious rotation. Finally, it returns to the initial rotational state with 120° rotation (Fig. 10a).

Currently, there are no single-molecule studies on $V_1$-ATPase from $E. hirae$ that directly demonstrate the dwell angles for ATP cleavage, ADP release, and phosphate release at a single catalytic site over one revolution. However, considering the results of single-molecule studies and structural studies, the model for 360° rotation cycle is conceivable [48].

If the ATP-binding angle is defined as 0° in the 360° rotation cycle (Fig. 10b, left), ATP is cleaved at 240°. Then, phosphate is released first at 240°, and finally, ADP is released at 240°. In comparison, the proposed chemomechanical coupling scheme of $F_1$-ATPase is more complicated. In the case of thermophilic Bacillus PS3 $F_1$-ATPase, ATP cleavage, ADP release, and phosphate release occur at 200°, 240°, and 320° [37], respectively, although the timing of phosphate release is controversial. Furthermore, in the case of human $F_1$-ATPase, ATP cleavage, ADP release, and phosphate release occur at 210°, 240°, and 305°, respectively (Fig. 10b, right) [40]. The coupling scheme of human $F_1$-ATPase can be considered a variation of that of thermophilic Bacillus PS3 $F_1$-ATPase, in which the ATP cleavage and the phosphate-release dwells are split into different angles. Of course, it is possible that $V_1$-ATPase also shows the substeps, because the angles waiting for ADP release and phosphate release have not been directly demonstrated by advanced single-molecule experiments, as has been performed in the case of $F_1$-ATPase [34, 35, 37]. Furthermore, multiscale molecular dynamics simulations predict the formation of rotational intermediate states of this $V_1$-ATPase that have not yet been resolved [60]. Interestingly, the recent information-based soft clustering method revealed that thermophilic Bacillus PS3 $F_1$-ATPase makes a small rotational movement during the catalytic dwell triggered by the ATP hydrolysis reaction; this had not been previously resolved using the conventional analysis methods [61]. Such advanced single-molecule techniques and analysis methods may reveal the unresolved reaction scheme and movements of $V_1$-ATPase.

Future prospects

The recent high-resolution structural studies and single-molecule studies reviewed have begun to clarify the rotation mechanism of $V_1$-ATPase. By comparing the differences and similarities in the rotation mechanism between $V_1$-ATPase and $F_1$-ATPase, we can determine the ‘unique’ and ‘common’ mechanisms by which these rotary ATPases function and thereby establish the working principle of rotary ATPases. However, to fully understand the rotation mechanism of rotary ATPases, it is necessary to further improve our ‘knowledge and understanding’ by designing rotary ATPases with improved, modified, or novel functions based on our current ‘knowledge and understanding,’ and by experimentally verifying designed proteins. Such a synthetic approach has become a trend in biology and nanobiotechnology [62–67], and this approach will be extremely helpful to understand the mechanisms by which rotary ATPases operate.

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References

1. Mitchell P (1966) Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol Rev Camb Philos Soc 41:445–502
2. Boyer PD (1997) The ATP synthase—a splendid molecular machine. Annu Rev Biochem 66:717–749
3. Yoshida M, Muneyuki E, Hisabori T (2001) ATP synthase—a marvellous rotary engine of the cell. Nat Rev Mol Cell Biol 2:669–677
4. von Ballmoos C, Wiedenmann A, Dimroth P (2009) Essentials for ATP synthesis by F1F0 ATP synthases. Annu Rev Biochem 78:649–672
5. Forgac M (2007) Vacular ATPases: rotary proton pumps in physiology and pathophysiology. Nat Rev Mol Cell Biol 8:917–929
6. Mulkidjian AN, MAKARova KS, Galperin MY, Koonin EV (2007) Inventing the dynamo machine: the evolution of the F-type and V-type ATPases. Nat Rev Microbiol 5:892–899
7. Bowman EJ, Bowman BJ (2005) V-ATPases as drug targets. J Bioenerg Biomembr 37:431–435
8. Nakano M, Imamura H, Toei M, Tamakoshi M, Yoshida M, Yokoyama K (2008) ATP hydrolysis and synthesis of a rotary motor V-ATPase from Thermus thermophilus. J Biol Chem 283:20789–20796
9. Yokoyama K, Muneyuki E, Amano T, Mizutani S, Yoshida M, Ishida M, Ohkuma S (1998) V-ATPase of Thermus thermophilus. J Biol Chem 273:20504–20510
10. Gruber G, Manimekalai MS, Mayer F, Muller V (2014) ATP synthases from archaea: the beauty of a molecular motor. Biochim Biophys Acta 1837:940–952
11. Cross RL, Muller V (2004) The evolution of A-, F-, and V-type ATP synthases and ATPases: reversals in function and changes in the H+/ATP coupling ratio. FEBS Lett 576:1–4
12. Muench SP, Trinick J, Harrison MA (2011) Structural divergence of the rotary ATPases. Q Rev Biophys 44:311–356
13. Murata T, Igarashi K, Kakinuma Y, Yamato I (2000) Na+ binding of V-type Na+-ATPase in Enterococcus hirae. J Biol Chem 275:13415–13419
14. Murata T, Yamato I, Kakinuma Y, Leslie AG, Walker JE (2005) Structure of the rotor of the V-Type Na+-ATPase. Science 308:654–659
15. Murata T, Yamato I, Kakinuma Y, Shirouzu M, Walker JE, Yokoyama S, Iwata S (2008) Ion binding and selectivity of the rotor ring of the Na+-transporting V-ATPase. Proc Natl Acad Sci USA 105:8607–8612
16. Stewart AG, Sobti M, Harvey RP, Stock D (2013) Rotary ATPases: models, machine elements and technical specifications. Bioarchitecture 3:2–12
17. Kühlbrandt W, Davies KM (2016) Rotary ATPases: a new twist to an ancient machine. Trends Biochem Sci 41:106–116
18. Oot RA, Kane PM, Berry EA, Wilkens S (2016) Crystal structure of yeast V1-ATPase in the autoinhibited state. EMBO J 35:1694–1706
19. Abrahams JP, Leslie AG, Lutter R, Walker JE (1994) Structure at 2.8 A resolution of F1-ATPase from bovine heart mitochondria. Nature 370:621–628
20. Menz RI, Walker JE, Leslie AG (2001) 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
21. Kagawa R, Montgomery MG, Braig K, Leslie AG, Walker JE (2004) The structure of bovine F1-ATPase inhibited by ADP and beryllium fluoride. EMBO J 23:2734–2744
22. Rees DM, Montgomery MG, Leslie AG, Walker JE (2012) Structural evidence of a new catalytic intermediate in the pathway of ATP hydrolysis by F1-ATPase from bovine heart mitochondria. Proc Natl Acad Sci USA 109:11139–11143
23. Gledhill JR, Montgomery MG, Leslie AG, Walker JE (2007) How the regulatory protein, IF(1), inhibits F(1)-ATPase from bovine mitochondria. Proc Natl Acad Sci USA 104:15671–15676
24. Kabalescewaran V, Puri N, Walker JE, Leslie AG, Mueller DM (2006) Novel features of the rotary catalytic mechanism revealed in the structure of yeast F1 ATPase. EMBO J 25:5433–5442
25. Kabalescewaran V, Shen H, Symersky J, Walker JE, Leslie AG, Mueller DM (2009) Asymmetric structure of the yeast F1 ATPase in the absence of bound nucleotides. J Biol Chem 284:10546–10551
26. Cingolani G, Duncan TM (2011) Structure of the ATP synthase catalytic complex (F(1)) from Escherichia coli in an autoinhibited conformation. Nat Struct Mol Biol 18:701–707
27. Shirakihara Y, Shiratori A, Tanikawa H, Nakasako M, Yoshida M, Suzuki T (2015) Structure of a thermophilic F1-ATPase inhibited by an epsilon-subunit: deeper insight into the epsilon-inhibition mechanism. FEBS J 282:2895–2913
28. Bowlar MW, Montgomery MG, Leslie AG, Walker JE (2007) Ground state structure of F1-ATPase from bovine heart mitochondria at 1.9 A resolution. J Biol Chem 282:14238–14242
29. Noji H, Yasuda R, Yoshida M, Kinosita K Jr (1997) Direct observation of the rotation of F1-ATPase. Nature 386:299–302
30. Yasuda R, Noji H, Kinosita K Jr, Yoshida M (1998) F1-ATPase is a highly efficient molecular motor that rotates with discrete 120 degree steps. Cell 93:1117–1124
31. Yasuda R, Noji H, Yoshida M, Kinosita K Jr, Itoh H (2001) Resolution of distinct rotational substeps by submillisecond kinetic analysis of F1-ATPase. Nature 410:898–904
32. Shimabukuro K, Yasuda R, Muneyuki E, Hara KY, Kinosita K Jr, Yoshida M (2003) Catalysis and rotation of F1 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
33. Ariga T, Muneyuki E, Yoshida M (2007) F1-ATPase rotates by an asymmetric, sequential mechanism using all three catalytic subunits. Nat Struct Mol Biol 14:841–846
34. Adachi K, Oiwa K, Nishizaka T, Funuki S, Noji H, Itoh H, Yoshida M, Kinosita K Jr (2007) Coupling of rotation and catalysis in F(1)-ATPase revealed by single-molecule imaging and manipulation. Cell 130:309–321
35. Nishizaka T, Oiwa K, Noji H, Kimura S, Muneyuki E, Yoshida M, Kinosita K Jr (2004) Chemomechanical coupling in F1-ATPase revealed by simultaneous observation of nucleotide kinetics and rotation. Nat Struct Mol Biol 11:142–148
36. Masaite K, Koyama-Horibe F, Oiwa K, Yoshida M, Nishizaka T (2008) Cooperative three-step motions in catalytic subunits of F1-ATPase correlate with 80 degrees and 40 degrees substep rotations. Nat Struct Mol Biol 15:1326–1333
37. Watanabe R, Iino R, Noji H (2010) Phosphatase release in F1-ATPase catalytic cycle follows ADP release. Nat Chem Biol 6:814–820
38. Martin JL, Ishmukhametov R, Hornung T, Ahmad Z, Frasch WD (2014) Anatomy of F1-ATPase powered rotation. Proc Natl Acad Sci USA 111:3715–3720
39. Bilyard T, Nakanishi-Matsui M, Steel BC, Pilizota T, Nord AL, Hosokawa H, Futai M, Berry RM (2013) High-resolution single-molecule characterization of the enzymatic states in Escherichia coli F1-ATPase. Philos Trans R Soc Lond B Biol Sci 368:20120023
40. Suzuki T, Tanaka K, Wakabayashi C, Saita E, Yoshida M (2014) Chemomechanical coupling of human mitochondrial F1-ATPase motor. Nat Chem Biol 10:930–936
41. Mahler MJ et al (2009) Crystal structure of A3B3 complex of V-ATPase from Thermus thermophilus. EMBO J 28:3771–3779
42. Numoto N, Hasegawa Y, Takeda K, Miki K (2009) Inter-subunit interaction and quaternary rearrangement defined by the central stalk of prokaryotic V1-ATPase. EMBO Rep 10:1228–1234
43. Nagamatsu Y, Takeda K, Kuranaga T, Numoto N, Miki K (2013) Origin of asymmetry at the intersubunit interfaces of V1-ATPase from Thermus thermophilus. J Mol Biol 425:2699–2708
44. Arai S et al (2013) Rotation mechanism of Enterococcus hirae V1-ATPase based on asymmetric crystal structures. Nature 493:703–707
45. Suzuki K et al (2016) Crystal structures of the ATP-binding and ADP-release dwells of the V1 rotary motor. Nat Commun 7:13235
46. Minagawa Y et al (2013) Basic properties of rotary dynamics of Enterococcus hirae V1-ATPase by crystallar structure and rotary dynamics of Enterococcus hirae. J Mol Biol 425:2699–2708
47. Yasuda R et al (2014) Torque generation of Enterococcus hirae V1-ATPase. J Biol Chem 289:31212–31223
48. Iino R, Minagawa Y, Ueno H, Hara M, Murata T (2014) Molecular structure and rotary dynamics of Enterococcus hirae V1(1)-ATPase. IUBMB Life 66:624–630
49. Iino R, Ueno H, Minagawa Y, Suzuki K, Murata T (2015) Rotational mechanism of Enterococcus hirae V1-ATPase by crystal-structure and single-molecule analyses. Curr Opin Struct Biol 31:49–56
50. Shirakihara Y et al (1997) The crystal structure of the nucleotide-free alpha 3 beta 3 subcomplex of F1-ATPase
from the thermophilic Bacillus PS3 is a symmetric trimer. Structure 5:825–836

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

52. Liu Q, Leng XH, Newman PR, Vasilyeva E, Kane PM, Forgac M (1997) Site-directed mutagenesis of the yeast V-ATPase A subunit. J Biol Chem 272:11750–11756

53. Hayashi S et al (2012) Molecular mechanism of ATP hydrolysis in F1-ATPase revealed by molecular simulations and single-molecule observations. J Am Chem Soc 134:8447–8454

54. Noji H, Hasler K, Junge W, Kinoshita K Jr, Yoshida M, Engelbrecht S (1999) Rotation of Escherichia coli F(1)-ATPase. Biochem Biophys Res Commun 260:597–599

55. Imamura H, Takeda M, Funamoto S, Shimabukuro K, Yoshida M, Yokoyama K (2005) Rotation scheme of V1-motor is different from that of F1-motor. Proc Natl Acad Sci USA 102:17929–17933

56. McMillan DGG, Watanabe R, Ueno H, Cook GM, Noji H (2016) Biophysical characterization of a thermoalkaliphilic molecular motor with a high stepping torque gives insight into evolutionary ATP synthase adaptation. J Biol Chem 291:23965–23977

57. Hirata T, Iwamoto-Kihara A, Sun-Wada GH, Okajima T, Wada Y, Futai M (2003) Subunit rotation of vacuolar-type proton pumping ATPase: relative rotation of the G and C subunits. J Biol Chem 278:23714–23719

58. Imamura H, Nakano M, Noji H, Muneyuki E, Ohkuma S, Yoshida M, Yokoyama K (2003) Evidence for rotation of V1-ATPase. Proc Natl Acad Sci USA 100:2312–2315

59. Furuike S, Nakano M, Adachi K, Noji H, Kinoshita K Jr, Yokoyama K (2011) Resolving stepping rotation in Thermus thermophilus H(+)-ATPase/synthase with an essentially drag-free probe. Nat Commun 2:233

60. Isaka Y, Ekimoto T, Kokabu Y, Yamato I, Murata T, Ikekuchi M (2017) Rotation mechanism of molecular motor V1-ATPase studied by multiscale molecular dynamics simulation. Biophys J 112:911–920

61. Li CB, Ueno H, Watanabe R, Noji H, Komatsu-Saki T (2015) ATP hydrolysis assists phosphate release and promotes reaction ordering in F1-ATPase. Nat Commun 6:10223

62. Schwille P, Diez S (2009) Synthetic biology of minimal systems. Crit Rev Biochem Mol Biol 44:223–242

63. Kay ER, Leigh DA, Zerbetto F (2007) Synthetic molecular motors and mechanical machines. Angew Chem Int Ed Engl 46:72–191

64. Chen L, Nakamura M, Schindler TD, Parker D, Bryant Z (2012) Engineering controllable bidirectional molecular motors based on myosin. Nat Nanotechnol 7:252–256

65. Nakamura M, Chen L, Howes SC, Schindler TD, Nogales E, Bryant Z (2014) Remote control of myosin and kinesin motors using light-activated gearshifting. Nat Nanotechnol 9:693–697

66. Furuta A, Amino M, Yoshi M, Oiwa K, Kojima H, Furuta K (2017) Creating biomolecular motors based on dynein and actin-binding proteins. Nat Nanotechnol 12:233–237

67. DelRosso NV, Derr ND (2017) Exploiting molecular motors as nanomachines: the mechanisms of de novo and re-engineered cytoskeletal motors. Curr Opin Biotechnol 46:20–26

68. Ueno H, Nishikawa S, Iino R, Tabata KV, Sakakihara S, Yanagida T, Noji H (2010) Simple dark-field microscopy with nanometer spatial precision and microsecond temporal resolution. Biophys J 98:2014–2023