Insights into the mechanism of ATP-driven rotary motors from direct torque measurement

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Abstract

Motor proteins are molecular machines that convert chemical energy into mechanical work. In addition to existing studies performed on the linear motors found in eukaryotic cells, researchers in biophysics have also focused on rotary motors such as F_1 -ATPase. Detailed studies on the rotary F_1 -ATPase motor have correlated all chemical states to specific mechanical events at the single-molecule level. Recent studies showed that there exists another ATP-driven protein motor in life: the rotary machinery that rotates archaeal flagella (archaella). Rotation speed, stepwise movement, and variable directionality of the motor of *Halobacterium salinarum* were described in previous studies. Here we review recent experimental work discerning the molecular mechanism underlying how the archaellar motor protein FlaI drives rotation by generation of motor torque. In combination, those studies found that rotation slows as the viscous drag of markers increases, but torque remains constant at 160 pN·nm independent of rotation speed. Unexpectedly, the estimated work done in a single rotation is twice the expected energy that would come from hydrolysis of six ATP molecules in the FlaI hexamer. To reconcile the apparent contradiction, a new and general model for the mechanism of ATP-driven rotary motors is discussed.

Keywords Motor protein · Archaella · FlaI hexamer · F_1 -ATPase

All creatures on the earth are categorized into one of three *domains*, the eukarya, prokarya, and archaea (Woese et al. 1990). Through the course of evolution, life has developed a variety of strategies to transport cargos in cells to their required locations with high efficiency, and *biological motors* are key elements in realizing this function. In past decades, researchers have extensively studied three conventional linear motor proteins found in eukarya (Howard 2001), which are driven by the chemical energy of the hydrolysis of adenosine-tri-phosphate (ATP). These linear motors are based on two components, the motor and a filament along which the motor “walks,” i.e., actin-myosin, microtubule-dynein (MT-dynein),

and microtubule-kinesin (MT-kinesin). Hydrolytic cleavage reactions occurring in either single or multiple domains of the motor part are promoted by interaction with the filament component. As a consequence of ATP cleavage, conformational changes occurring within the motor domain are accompanied by twisting (Fujimura et al. 2017; Nishizaka et al. 1993; Yajima et al. 2008) resulting in a one-directed movement of the motor along the filament.

In contrast, motility machineries found in bacteria have more complex architectures and are comprised of multiple subunit assemblies. Motors are used to move a cell body to more beneficial (with regard to survival) environments and so it is necessary that some part of the assembly needs to protrude from the cell membrane in order to either attach to the environmental surface directly or to induce a thrust force against fluid. The gliding machinery of *Mycoplasma mobile*, for example, is an orchestrated ensemble of about ten proteins, including ATPase (Miyata and Hamaguchi 2016), in which a huge protein of 350 kDa, Gli349, extends from the membrane to the surface to transmit the force (Adan-Kubo et al. 2006). The step of the machine was directly measured as ~70 nm, which is the largest among all natural motor proteins (Kinosita et al. 2014). Another type of motor is the bacterial flagella motor that is a rotary, not a linear, motor. Over 30 different

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subunits are coordinated to induce both clockwise (CW) and counterclockwise (CCW) rotation using either proton or sodium-ion gradients across the membrane (Sowa and Berry 2008). The resultant behavior of the whole flagella structure is cell swimming—a phenomenon which has been a major topic of investigation in the microbiology field for decades. An interesting, if somewhat confusing aspect of flagella research, stems from the fact that eukaryotic motile flagella, although representing functionally equivalent subcellular organelles, are driven by a different motor principle that involves thousands of precisely oriented linear dynein motors surrounding the MT bundles. It has been an unfortunate aspect of nomenclature that these two biological components (bacterial and eukaryotic) happened to be denoted with the same name *flagellum*.

In addition to bacterial and eukaryotic motors, another motility machinery has been recently found in archaea. Most archaea also have a surface appendage that is similar to that of bacteria, but none of the components of the archaeal motility structure are homologous to any flagellum protein found in bacteria. To avoid additional confusion among another similar organelle in yet another different domain, archaeal flagellum was proposed to be denoted as “archaellum” (Jarrell and Albers 2012). The archaellum is built from as few as seven proteins with recent structural and functional studies providing a model of archaellar architecture (Albers and Jarrell 2015, 2018; Chaudhury et al. 2018; Jarrell and Albers 2012). To probe the mechanism of archaellar motility, the research team (including the current authors) have applied microscope techniques originally developed for single-molecule biophysics to the model swimming archaeon, *Halobacterium salinarum* (*Hbt. salinarum*). In 2016, almost all parameters to describe archaeal swimming were revealed (Kinosita et al. 2016). Swimming was $\sim 3 \mu\text{m s}^{-1}$ speed, which was more than ten times slower than that of *E. coli*, and was accompanied by

continuous precession because archaellum protrude obliquely, $\sim 20^\circ$, from the cell pole. The structure of archaellum was found to always be right-handed helical, having a $2.1 \mu\text{m}$ pitch, which is in stark contrast with the bacterial flagellum. The rotation rate of a cell body was estimated from 3-D tracking microscope as $\sim 3 \text{ Hz}$; both CCW and CW rotations of single archaella were directly quantified as $\sim 20 \text{ Hz}$ under the illumination of evanescent wave. Finally, stepwise rotation, proceeded by mainly six discrete steps per revolution, was possibly driven by a single motor.

Although the above achievements were previously quantified for the archaellar motor, the most important parameter for the description of a rotary motor, the “torque” as a measure of the energy output, was not reported. Our research team therefore addressed this subject and the torque was finally determined to be $160 \text{ pN}\cdot\text{nm}$ (Iwata et al. 2019). For this estimation, we used a bead assay to measure the rotation rate and radius of single polystyrene beads with different sizes attached to single archaella protruded from surface-immobilized archaeon. Torque was calculated as (viscous friction) $\times$ (angular velocity) after subtraction of background friction originating from the archaellum itself (Fig. 1). The torque is constant independent of rotation rate in the range we measured, a property similarly displayed by the F_1 -ATPase (Yasuda et al. 1998).

To provide greater insight into the mechanism underlying how archaellum motor induced rotation, we need to take a closer look at the world’s smallest rotatory motor made from a single protein molecule, the F_1 -ATPase. This enzyme is a soluble portion of ATP synthase and the subunit composition is $\alpha_3\beta_3\gamma\delta\epsilon$. Isolated $\alpha_3\beta_3\gamma$ works as a minimum unit to induce unidirectional rotation of the γ shaft using the chemical energy of ATP hydrolysis. Ordered and sequential reactions in the hydrolysis of ATP, occurring mainly in the three β subunits, are the key steps underlying the rotation mechanism

Fig. 1 Torque measurement of archaellar motor. **a** Schematic of the rotary motor driven by ATP in archaea. The hexameric ATPase motor Flal is one of candidates. **b** Experimental setup as a bead assay. **c** Relationship between torque and rotation rate on the different markers. The dataset contains the correction taking into account the effect of the interaction

of the bead with the surface after the subtraction of the viscous friction of single archaellum (Iwata et al. 2019). Dashed lines represent $T_a = 1.6 \times 10^2 \text{ pN}\cdot\text{nm}$. Red, green, and blue were taken with 210-nm, 490-nm, and 1.0- μm bead under tethered-cell assay

Fig. 2 Rotation mechanism of F_1 -ATPase revealed by single-molecule observation. Three β s are marked as $\beta 1$, $\beta 2$, and $\beta 3$. Left, counterclockwise (CCW) 80° substep rotation of the γ shaft. Binding of ATP to $\beta 1$ and release of ADP from $\beta 2$ accompany 40° and -20° bending motions, respectively, in the C-terminal domain of β s.

Meanwhile, $\sim 4^\circ$ outward tilting of the shaft is experimentally indicated. Right, 40° CCW substep rotation that completes 120° unitary step of the shaft. Hydrolysis of ATP at the catalytic site of $\beta 3$ correlates with -20° conformational change in the C-terminal domain

(Nishizaka et al. 2004), and were directly confirmed as angular conformational changes in β s in an active enzyme (Masaie et al. 2008; Sugawa et al. 2018). Additionally, tilting of the γ shaft was deduced from the rotational orbit of spherical markers attached to the shaft (Sugawa et al. 2011), which cannot be settled from studies based on X-ray crystallography models. These models are summarized in Fig. 2, in which a unitary 120° rotation is resolved into two substeps; a 40° substep and an 80° substep. Notably, in the unitary step, single complete turnover of ATP hydrolysis is beautifully divided among the β subunits: while $\beta 1$ recruits new ATP from surrounding environment, hydrolysis occurs

in $\beta 3$, and ADP is released from the remaining site, $\beta 2$. Likewise, the correlation between various chemical states and specific mechanical events is settled in detail in this tiny motor. Key to the mechanism is the strong cooperativity of the three catalytic subunits. If you look at one specific β subunit, its chemical state returns to the original one after the completion of a single rotation of the rotor. As a consequence, three ATPs are hydrolyzed per turn of the rotor, which approaches $\sim 100\%$ efficiency of the energy conversion (Toyabe et al. 2010; Yasuda et al. 1998).

If we simply apply the rule of coincidence between “the number of the catalytic subunits of the cylinder” and “the

Fig. 3 A general model for the mechanism of ATP-driven rotary motors. Rotor (gray) has n -fold rotational symmetry against the cylinder part (colored hollow circle). The number of catalytic sites in the cylinder is N . As an example, $n = 2$ and $N = 6$ are represented in the schematic. The different colors indicate subunits having different chemical states, and ϕ is empty sites that wait the recruitment of new ATP from surroundings. When ATP binds, chemical states in all subunits shift as “binding-change mechanism” that was originally proposed by Paul Boyer (Boyer 1993), which accompanies $[360/N]^\circ$ unitary step (gray arrow). Notably, n ATP molecules are required to terminate this process in one site, which leads

the fact that $n \times N$ ATPs are required to terminate one revolution of the rotor. If you watch specific one catalytic site out of N , the site returns to original chemical state with the completion of $[360/n]^\circ$ rotation of the rotor (green arrow). The model encompasses the mechanism of the F_1 -ATPase (Adachi et al. 2007; Masaie et al. 2008; Nishizaka et al. 2004; Sugawa et al. 2016) with a denotation of $n = 1$ and $N = 3$, and is also indicated by the estimation of energy conversion of archaeal motor based on direct torque measurement (Iwata et al. 2019) where $N = 6$ and n is possibly 2

number of ATPs hydrolyzed per rotor-turn” to the archaeal rotary motor, an apparent paradox emerges, i.e., energy output exceeds input (Iwata et al. 2019). In the archaeellar motor, the ATPase FlaI forms a hexamer (Ghosh et al. 2011; Reindl et al. 2013) that likely drives rotation, and thus the work from hydrolysis of six ATP molecules is calculated to be $6 \times \Delta G_{\text{ATP}} \sim 5 \times 10^2$ pN·nm, given that ΔG_{ATP} is 8×10 pN·nm in cells (Yasuda et al. 1998). In contrast, our estimated torque of 160 pN·nm requires work of $2\pi \times 160$ pN·nm $\sim 10 \times 10^2$ pN·nm for a single rotation. Therefore, with the premise that each FlaI subunit hydrolyzed a single ATP molecule per full archaeellar rotation, the motor would have a paradoxical apparent efficiency of nearly 200%.

To reconcile the paradox, we here propose a general model that encompasses rotation mechanisms of two rotary motors (Fig. 3). The idea is that the cylinder part, which has N catalytic sites, uses more than N ATP molecules to power a single rotation if the shaft has a rotational symmetry. Suppose the case that the order of shaft symmetry is n . Although the angle of single stepping of the shaft is $360^\circ/N$, a particular side of the shaft should face the same catalytic subunit in every $360^\circ/n$ revolution. This directly indicates the completion of a single turnover of ATP in one catalytic site with $1/n$ rotation of the shaft. Notably, this strict cooperativity was directly confirmed in F_1 -ATPase, where $N = 3$ and $n = 1$, through single-molecule observations using fluorescently labeled ATP (see Fig. 2e in Nishizaka et al. (2004)). As a consequence, a 360° shaft rotation would induce hydrolysis of $N \times n$ ATPs by the motor, which is three in F_1 -ATPase as the F_1 shaft has asymmetric structure (Abrahams et al. 1994) ($n = 1$). In the archaeellar motor, the component FlaJ (Albers and Jarrell 2015) may form a dimer, and the FlaI hexamer has twofold symmetry instead of a pure sixfold symmetry (Reindl et al. 2013), as does its homolog PilB from the related type IV pilus (McCallum et al. 2017). These findings suggest $n = 2$ in archaeellar motors, and therefore, $N \times n = 6 \times 2 = 12$, i.e., the energy input should be doubled, 10^3 pN·nm. With the above model, the efficiency of the energy conversion of archaeellar motor is now re-estimated to be 100% (Iwata et al. 2019), which is similar to that of F_1 -ATPase (Kinosita Jr. et al. 1998; Yasuda et al. 1998).

The new model proposed here is convincing from structural and energetic comparisons between the two motors. Notably, these motors are used in totally different ways in specific species: the protein that resembles F_1 -ATPase works as a core engine to induce one-way directed movement in *Mycoplasma mobile* (Nakane and Miyata 2007; Tulum et al. 2014), possibly with the similar conversion between a piston and cylinder in a steam train (Kinosita et al. 2014), whereas the homolog of FlaI, PilB, enhances the polymerization of globular PilA monomers to assist twitching motility in type IV pili system (Nakane and Nishizaka 2017). Type IV pili are evolutionally and structurally related to archaeellum, and PilB is essential for the assembly of helical pilus fibers (Chang et al.

2016; Korotkov et al. 2012). Whereas the motor itself is suggested to be a part of a rotary motor (McCallum et al. 2017), the pilus fibers move linearly by the repeated cycle of extension and retraction (Craig et al. 2019; Nakane and Nishizaka 2017). Remarkably, this type of cell surface appendage also performs critical function in DNA uptake, protein secretion, and biofilm formation in similar fashion (Craig et al. 2019). Deciphering the essential modifications of basic function in rotary motors will be the next challenge to understanding the rules of these fascinating molecular machines.

Compliance with ethical standards

Conflict of interest Takayuki Nishizaka declares that he has no conflict of interest. Tomoko Masaike declares that she has no conflict of interest. Daisuke Nakane declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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