PERSPECTIVE

Structural basis for power stroke vs. Brownian ratchet mechanisms of motor proteins

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Two mechanisms have been proposed for the function of motor proteins: The power stroke and the Brownian ratchet. The former refers to generation of a large downhill free energy gradient over which the motor protein moves nearly irreversibly in making a step, whereas the latter refers to biasing or rectifying the diffusive motion of the motor. Both mechanisms require input of free energy, which generally involves the processing of an ATP (adenosine 5′-triphosphate) molecule. Recent advances in experiments that reveal the details of the stepping motion of motor proteins, together with computer simulations of atomistic structures, have provided greater insights into the mechanisms. Here, we compare the various models of the power stroke and the Brownian ratchet that have been proposed. The 2 mechanisms are not mutually exclusive, and various motor proteins employ them to different extents to perform their biological function. As examples, we discuss linear motor proteins Kinesin-1 and myosin-V, and the rotary motor F_1-ATPase, all of which involve a power stroke as the essential element of their stepping mechanism.

kinesin | myosin | F1-ATPase | power stroke | Brownian ratchet

The function of motor proteins is to generate unidirectional motion, such as translation or rotation, making use of molecules, primarily ATP (adenosine 5′-triphosphate), as the energy source. Study of these molecular "machines" dates back to the late 19th century, when the name "myosin" was coined for the molecular constituents responsible for muscle contraction (1). With advances in modern statistical mechanics, it became feasible to ask about the physical mechanisms by which motor proteins operate on energy scales not much greater than that of thermal energy (2). For example, the motor protein kinesin generates about 5-pN force in its 8-nm walking step along the microtubule (MT), while hydrolyzing 1 ATP molecule. Thus, the amount of work in making a step is 40 pN·nm $\simeq$ 10 k_BT (k_BT: Thermal energy at 300 K), which is about 50% of the 20 to 25 k_BT free energy of ATP hydrolysis (2). It should be noted, however, that the hydrolysis energy is not used directly; it is thermalized too rapidly (in picoseconds to nanoseconds) to contribute to the stepping, which takes place on the microsecond to millisecond time scale (3). In other words, the length and time scales of thermalization due to ATP hydrolysis (angstroms and picoseconds) are very short compared to those of the walking motion (typically nanometers and longer than microseconds).

By consuming energy, the motors can overcome the randomizing effect of thermal fluctuations so as to be able to generate unidirectional motion. Since the 1990s (4), single-molecule experiments have revealed a rich variety of motility behavior that depends on the type of motor and the experimental conditions. For example, although all members of the kinesin family share a conserved motor domain possessing an ATP binding pocket (5, 6), their motility behavior varies over a wide range (7) (Fig. 1). Kinesin-1 is a processive motor that makes hundreds of steps on MTs, while Kinesin-14 motors individually diffuse relatively slowly along the MT, but when linked together in a group consisting of 2 or more motors the motion becomes processive, and they move robustly with a finite velocity in a direction opposite to that of Kinesin-1 (8, 9).

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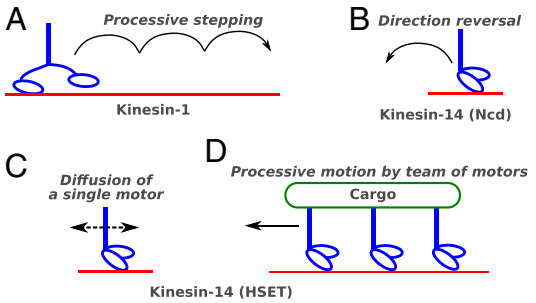

Fig. 1. Diversity in motor behaviors with a conserved ATP processor domain. Select features for the kinesin family are shown. (*A*) Processivity of kinesins (14). (*B*) Walking direction reversal (15, 16). (*C*) Diffusion of a single motor (8, 9). (*D*) Group of motors. Further diversity in motile behavior is achieved through variations in motor subdomains (17), oligomerization (18), and by nonmotor domains distal to the motor domain (19, 20). Other motor proteins such as myosins (11, 21) and AAA+ motors (12, 13) also exhibit diverse behaviors while using conserved ATP binding domains.

Myosins share a similar ATP binding pocket with kinesin (5) and they also exhibit diverse motility characteristics (10, 11). Another broad family of AAA+ motors, including ClpX, dynein, and F_1-ATPase, use a conserved ATPase domain to achieve diverse motile behavior such as pulling polypeptide chains (ClpX), walking on the MT (dynein), and rotary motion (F_1-ATPase) (12, 13).

A fundamental question is how the relatively conserved fuel processing (ATP binding, hydrolysis, and release of hydrolysis products), which are localized events, are harnessed to obtain unidirectional motion and diverse motility behavior. There are 2 contrasting views concerning the origin of the motion. One is via a power stroke and the other via a Brownian ratchet (Fig. 2); it is possible also that the motion involves a combination of the 2. Despite much discussion about what they are and how they apply to specific systems, a clear consensus has yet to be established. As discussed below, we define the power stroke as the generation of a large free energy gradient over a distance comparable to the step size, so that the transition to the forward position occurs nearly irreversibly. In the Brownian ratchet mechanism, the motor visits previous and forward positions through thermal motion, and stabilization in the forward position occurs by conformational changes triggered by the fuel processing event.

Recent advances in structural information and single-molecule experiments, plus the increasing power of atomistic molecular dynamics (MD) simulations, provide data that make possible a detailed examination of the 2 mechanisms and a determination of their role in specific motors. In what follows, we briefly review some of the proposals concerning the 2 types of mechanisms. We then focus on 2 well-studied types of biological motors, linear (kinesin and myosin) and rotary (F_1-ATPase), for which extensive experiments and simulations have contributed to our understanding.

Power Stroke

In his succinct review (22), Howard gives an observational definition of the power stroke as "large, rapid structural change in a protein that can be used to do mechanical work." The magnitude of the change is several nanometers, a distance that is comparable to the dimension of the protein itself. It is much larger than the angstrom-level changes of the motor domain that occurs in the fuel processing event. A well-known example of the large conformational change is the swinging motion of myosin's lever arm (11, 23). Since such a large displacement against load is difficult to achieve by random thermal motion alone (discussed below), this definition of the power stroke is of practical utility.

A widely held view regarding the mechanism is that the motor is strained like a spring and that a chemical event triggers relaxation from this state, resulting in the power stroke (22, 24–26) (Fig. 2*A*). This would require that elastic energy stored in the strained state is sufficient to drive the nanometer-scale conformational change involved in the power stroke. However, such an elastic element has not been identified. ATP-driven motors including kinesin, myosin, and the AAA+ family possess central β-sheets in their motor domain that show nucleotide-dependent twist changes. Hence, they have been proposed to store the twist-dependent elastic energy (27, 28). While experimentally probing the contribution of the β-sheet's twist changes to force generation is difficult, MD simulations indicate that changes in elastic energy is not significant. In the case of kinesin, the central β-sheet in the nucleotide-free prestroke state has a higher curvature than in the ATP-bound poststroke state, where the former is only 0.84 k_BT higher in elastic energy. Moreover, the energy barrier between these 2 states is low, so that the β-sheet visits both states in any given nucleotide state, which makes it even less likely as an elastic element responsible for the power stroke (29). For myosin-V, a normal mode analysis based on atomistic structures revealed that the changes in the twist of the central β-sheet is to accommodate the rearrangements in the surrounding domains (30). But no evidence was found that it can store significant elastic energy. As a related system, the β-sheet sandwich of the lectin domain in FimH changes its twist to allosterically control its affinity for mannose (31). The twist changes alter positions of the mannose-binding loops located on the edges of the β-sheets. Such a steric or conformational role of the β sandwich is analogous to the case for myosin-V.

The elastic network model that approximates a protein as a network of harmonic springs with identical spring constants was used to examine vibrational motions of kinesin, myosin, and F_1-ATPase (32, 33). For the latter 2, low-frequency vibrational modes from this simple model align well with the nucleotide-dependent conformational changes observed in X-ray structures. The authors argued that these results support a power stroke mechanism. In contrast, no strong correlation between the conformational changes and low-frequency normal modes was observed for kinesin (32, 33). Compared to myosin or F_1-ATPase, kinesin's motor head is more globular, so that a simple elastic network model may not be able to capture its conformational changes.

Although the idea of power stroke by spring-like action is simple and appealing, it is unlikely to be applicable to motor proteins. To see why, we use known elastic properties of proteins to estimate the amount of deformation required to store the energy used by motor proteins. For the purpose of estimation, we assume the motor protein is a linearly elastic material with Young's modulus $Y \sim 10$ GPa (34). Consider a beam of radius $r = 2$ nm and length $2r$ (dimension comparable to kinesin). To store an energy $\epsilon = 10\ k_BT$ (typical value for a motor protein), the beam has to bend by an angle $\theta \simeq \sqrt{\epsilon/Yr^3} = 1.3°$. If the energy is instead stored by compression of the beam, the same expression estimates a 2.3% linear strain, which is 0.9 Å for a length of $2r$, based on the theory of linear elasticity (35). Hence, given the size of the motor domain, the magnitude of the elastic deformation required to store the free energy involved is comparable to or smaller than the amplitude of thermal fluctuation, suggesting that it is impractical

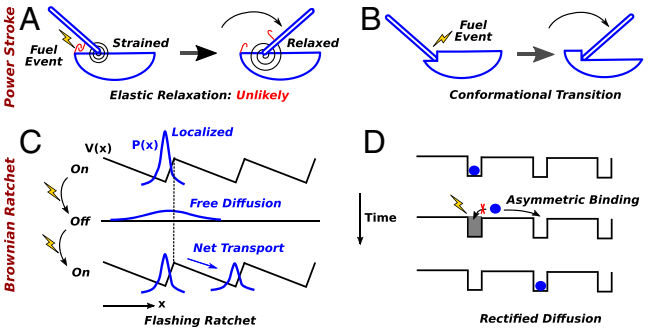

Fig. 2. Proposals for motility generation mechanisms of motor proteins. (*A* and *B*) Power stroke and (*C* and *D*) Brownian ratchet. (*A*) Elastic relaxation. A fuel processing event (e.g., binding of an ATP or release of a hydrolysis product, denoted by a lightning symbol) leads to release of elastic energy. This is an unlikely scenario given the size and mechanical properties of typical motor proteins. (*B*) Conformational transition. A fuel processing event causes conformational change of the motor head, which changes the equilibrium position of the mechanical element (denoted by swinging rod). Before and after the stroke, the motor is not strained. (*C*) Flashing ratchet model. Top: The potential $V(x)$ (x: position of the motor) is asymmetric, and the probability distribution $P(x)$ is localized at a minimum. Middle: When $V(x)$ is off, the motor performs free diffusion. Bottom: After $V(x)$ switches back, the motor to the right of the barrier (vertical dashed line) undergoes biased diffusion to the minimum on the right side. This generates to a net current. Switching of $V(x)$ on and off is mediated by a fuel. (*D*) Rectified diffusion model. A fuel processing event releases the motor from its initial position (top to middle), and the motor diffuses. Conformational change in the motor makes its affinity to the binding sites asymmetric, which results in preferential binding to the forward site (bottom).

to use elastic strain to generate a power stroke. Furthermore, motor proteins operate in the strongly overdamped viscous environment (36) where elastic relaxation slows down exponentially (2). Thus, additional interactions are required to speed up the moving element and complete a step. Then the process is no longer driven solely by elastic relaxation, where stabilization of the poststroke state by formation of contacts resembles a Brownian ratchet. Elastic strain may develop for domains with high aspect ratio where small local changes can accumulate along the length of the domain, such as bending of the lever arm of myosin-V (37), and twist of the γ-subunit (axle) of F_1-ATPase (38). They can be partly responsible for the power stroke.

It has also been proposed that the power stroke results from a conformational transition (24) (Fig. 2*B*). This is possible since angstrom-level changes of the motor resulting from a chemical event can shift the equilibrium between 2 conformational states. It also reconciles the aforementioned difference in the time scale between the power stroke and chemical events associated with the fuel molecule where the former proceeds more slowly. Thermodynamically, the conformational transition through a chemical event engendered by the fuel molecule creates a downhill free energy gradient. If the gradient is sufficiently large, the protein will possess a strong bias to move even against load. A mathematical model of a motor possessing both power stroke and Brownian ratchet components has been developed, where the former was defined via the work done by the "effective driving potential," whose difference from the total free energy change after a motility cycle was attributed to Brownian ratchet (39).

Brownian Ratchet

In contrast to power stroke where the fuel-mediated local changes of the motor head results in the large conformational transition responsible for making a step, in the Brownian ratchet mechanism thermal forces lead to Brownian motion of the motor head over a distance comparable to the step size. Beyond biological motors (40), there have been extensive studies on Brownian motors, both theoretically (41) and experimentally (42). Among the earliest theories of Brownian ratchets, the study of muscle contraction by Huxley (43) provided a foundation for structural and biochemical investigations of myosin's mechanochemical cycle (1, 11). The concept of asymmetric binding to the substrate of a thermally fluctuating motor powered by an external energy source was the basis of many Brownian motor theories (41, 44, 45). Feynman's celebrated lecture "Ratchet and pawl" (46) introduced a conceptual device coupled to 2 heat reservoirs at different temperatures and examined how useful work can be extracted out of random thermal fluctuation. Despite some criticisms (47), it nevertheless spurred the treatment of Brownian motor from the broader perspective of statistical thermodynamics (41, 48).

Among models of Brownian motors (25, 41, 49), the flashing ratchet model is perhaps the most extensively studied (Fig. 2*C*). A ratchet potential in the shape of an asymmetric sawtooth is turned on and off periodically. Adjacent minima of the potential are separated by a distance equal to the motor's step size. When the potential is off, the motor diffuses freely; when it is on, the motor settles into a minimum. Due to the asymmetry of the potential, this causes a biased diffusion (Fig. 2*C*, bottom). This model highlights key ingredients of the Brownian ratchet mechanism (i.e., the potential is periodic and asymmetric, and energy is externally supplied by switching the potential on and off). However, in a certain sense, turning on the ratchet potential creates a downhill free energy gradient, which is a power stroke (39). This suggests that a power stroke and a Brownian ratchet are not conceptually distinct (26, 39, 50). Here, time and length scales should be considered to distinguish between the 2 mechanisms. If the motor moves nearly irreversibly over a length comparable to the step size without noticeable diffusive behavior, it is a power stroke. By using illustrative examples, Wang and Oster (39) showed that ratchet-like actions in small increments compared to the motor's step size are difficult to distinguish from a power stroke. Since there is no diffusion on the length scale of the step, such a case can be regarded as a power stroke.

If the motor diffuses without a bias before binding to the next site, the mechanism is close to the "rectified diffusion" model (51) [Fig. 2*D*; also called "information ratchet" (52, 53)]. A problem with applying this model to motor proteins is that the motor can withstand much lower levels of force than experimental values (49, 51, 54). For conventional kinesin, its stepping event occurs within 30 μs over a distance of 8 nm (54). If the stepping occurs via unbiased diffusion, applying just a 2-pN hindering force, which is lower than the 5- to 7-pN stall force, will slow down stepping by 48-fold ($=e^{2\text{pN}\cdot 8\text{nm}/k_BT}$), to 1.4 ms. Conversely, an assisting load should make the motor move faster. Neither of these have been observed (55, 56), suggesting that a more active force generation is necessary (54). Similar analyses showed that neither biased nor rectified diffusion can account for the force generation ability observed in translocating motor proteins that have nanometer-sized steps (49, 51). In an earlier study, Huxley and Simmons (57) also proposed that myosin undergoes a "substantial displacement" while being attached to the actin filament

rather than diffusing and attaching to a single position, which is indicative of a power stroke. A model incorporating both Brownian ratchet and power stroke demonstrated that the latter "outperforms" the former, although the comparison was within the bounds of the model (26). Brownian ratchet may play a more significant role for motors with smaller step sizes such as RNA polymerase that has a 3.7-Å step (distance between bases), so that the forward site is readily visited by thermal motion (22, 58).

Astumian et al. (59) argued that power stroke defined as a free-energy releasing conformational change is "incorrect as an explanation of how chemical energy is used by a molecular machine to do mechanical work." This view may be valid if "free energy release" is assumed to be carried out by elastic relaxation. Their model considered transition between states with given transition rates, where only the position (or rotational angle) of the motor is used as the reaction coordinate. This is essentially a phenomenological description which is not suited for answering the question of how a force is generated. For the force generation mechanism, structural aspects must be considered. Yet, carefully constructed phenomenological or kinetic models can be useful for understanding trajectories of individual motor proteins (7, 60, 61). Most of these models assume the presence of irreversible transitions, although it has also been claimed that motor proteins must work with the constraint of microscopic reversibility (53, 59). We explain the nonequilibrium nature of the motility of motor proteins in *Concluding Remarks*.

Molecular Mechanisms of Motor Proteins

To determine how the power stroke and the Brownian ratchet mechanisms manifest in motor proteins, we discuss 2 linear motors and a rotary motor, namely kinesin and myosin, and F_1-ATPase.

Kinesin. The diverse motile characteristics of kinesins (Fig. 1) are achieved through variations of peripheral subdomains that control the fuel processing events (29) and harness the resulting conformational changes to produce different mechanical behavior (62). How power stroke or Brownian ratchet mechanisms apply depends on the kinesin family in question. Here we consider primarily the most widely studied conventional kinesin (Kinesin-1; unless noted otherwise, herein we call Kinesin-1 "kinesin"). There are several excellent reviews on its overall motility cycle (7, 54, 63). Our focus is on making a step in the one-head bound state, where the other "moving" head is not bound strongly to the MT. To generate an 8-nm step in a bipedal motion, the moving head must travel 16 nm, about the distance of 2 tubulin dimers (56).

First consider the thermal motion of the moving head. Its radius of gyration is $r = 2$ nm and mass $m = 5$ kDa. Applying the theory of Brownian motion (70), the drag coefficient of the motor head in an aqueous environment is $\zeta = 6\pi\eta r = 3.4 \times 10^{-11}$ N·s/m ($\eta = 8.9 \times 10^{-4}$ Pa·s; dynamic viscosity of water). During the relaxation time $\tau = \frac{m}{\zeta} = 0.24$ ps, the head moves with instantaneous speed $v = \sqrt{k_B T/m} = 23$ m/s, reaching a distance $v\tau = 5.5$ PM before a thermal "kick" of magnitude $\sqrt{2\zeta k_B T/\tau} = 1{,}100$ pN resets its motion into a random direction. The large and rapidly fluctuating thermal force averages out over the time scale of stepping. If the moving head were to undergo pure diffusion, the first passage time to move 16 nm is on the order of several microseconds, which is slightly shorter than the time resolution of a present-day optical trap apparatus (54). Thus, even if kinesin were to walk entirely by a Brownian ratchet mechanism (a freely diffusing head captured at the forward position), an unloaded motor could appear to make discrete steps, as experimentally observed (4). However, the first passage time exponentially increases with a load (71). Most kinetic models of kinesin motility contain a Boltzmann factor, $e^{F\delta/k_B T}$, where F is the applied load and δ is the characteristic distance for the load-dependent part of the motility cycle. Without explicitly incorporating structural aspects, one needs to be careful in interpreting δ. Nevertheless, given that its value is smaller than the 8-nm step size (55, 60, 61, 72), a ratchet-like behavior, if any, can only be a part of the stepping mechanism.

Single-molecule experiments revealed more details of the behavior of the moving head. By using fluorescently tagged motor heads, it was shown that in the one-head bound, ATP-waiting state, the moving head stays in the rear position without diffusing past the MT-bound head (73) (Fig. 3 *A* and *B*). This was supported by another experiment where DNA tethers are linked to a motor head, thus allowing direct manipulation of the head (56). More recently, the motion of a head in the dimer tagged with a gold nanoparticle could be visualized (64, 65). Here too, forward movement was rarely observed before a step was made, which suggests that Brownian motion is not sufficient to push the head forward. The moving head spends most of the time located on the right side of the MT-bound head relative to the walking direction. This is because the neck linker that connects the motor head and the neck stalk is located on the right side of the motor (Fig. 3*B*) (74).

The moving head does not explore the front position partly due to a steric block. In the nucleotide-free MT-bound head, the base of its neck linker is located behind the α4 helix, which prevents forward motion (Fig. 3*C*). Binding of an ATP causes the head to tilt leftward, so that the base of the neck linker is lifted over α4, allowing access to the forward-pointing state (75, 76) (Fig. 3*D*; a schematic illustration is in Fig. 2*B*). In this state, although forward diffusion may be possible, the exponential load dependence requires an active force-generation mechanism, that is, a power stroke. Furthermore, since the head stays on the right side of the walking direction, a rightward force will impede the stepping motion whereas a leftward load can assist it. Experimentally, rightward load slowed the motor less than the leftward load did (55), which again indicates that an active force generation is required for the "docking" of the neck linker. Kinesin also generates torque (77–79), which is unlikely for a freely diffusing head. As for the structural basis for the absence of free diffusion, our multimicrosecond MD simulation of a kinesin dimer showed that the moving head makes numerous nonspecific contacts with the MT surface that hinder diffusive motion (*SI Appendix*). In contrast, a coarse-grained simulation of the kinesin dimer where the moving head was made to diffuse freely (80) exhibited behaviors that did not agree with experiments (64, 65).

Simulations have shown that kinesin generates force by the folding of a domain called the cover-neck bundle (CNB) (75). The neck linker is attached to the C-terminal end of the motor head, and there is a flexible "cover strand" at the N-terminal end (Fig. 3*C*). Binding of an ATP triggers folding of a β-sheet between the 2, the CNB (Fig. 3*D*). It has sufficient forward conformational bias to overcome typical loads that kinesin experiences. The force generated by the CNB is greater on the right than on the left side of the motor, which is consistent with the response of the motor to sideways forces (55, 75). X-ray (67) and cryo-electron microscopy (EM) structures (81, 82) of kinesin–MT complexes have reported the presence of the CNB in ATP-analog states. In addition to

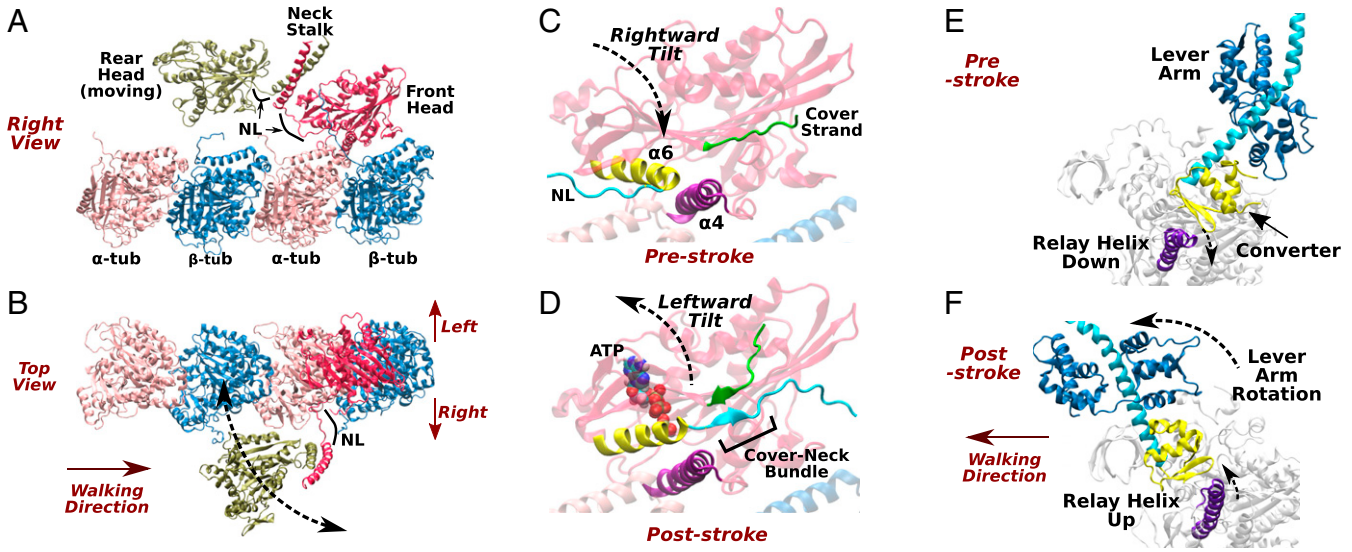

Fig. 3. Stepping mechanisms of linear motors. (*A–D*) Kinesin and (*E* and *F*) myosin. (*A*) One-head bound state (view from the right side relative to the walking direction). The front head waits for an incoming ATP and the rear head is detached from the MT. Two $\alpha\beta$-tubulin dimers on an MT protofilament (α-tub and β-tub) are shown. NL: neck linker connecting between the motor head and the neck stalk. (*B*) Top view. In the one-head bound state, the moving head is mostly on the right side (64, 65) because the NL is located on the right side of the front head. (*C* and *D*) Nucleotide-dependent conformational change of kinesin (cf. Fig. 2*B*). Right view. (*C*) In the nucleotide-free, ATP-waiting state, the motor head tilts to the right, and the end of α6 is behind α4. (*D*) In the ATP-bound state, α6 moves above α4 due to leftward tilting of the motor head. This allows the cover-neck bundle (CNB) to form. Structural models are based on Protein Data Bank (PDB) structures 4LNU (prestroke) (66) and 4HNA (poststroke) (67). (*E* and *F*) Power stroke of myosin. (*E*) Prepowerstroke state (PDB ID code 1QVI) (68). The motor binds to the F-actin in the ADP·P_i state. (*F*) Poststroke rigor state (PDB ID code 1SR6; nucleotide-free) (69). Corresponding domains between kinesin and myosin are shown in the same colors, that is, α4 vs. relay helix, α6 vs. converter, and NL vs. lever arm.

in vitro experiments (72, 83), perturbation of the cover strand impaired kinesin motility in cells (84). The CNB mechanism is also critical for kinesin's directionality. The MT minus end-directed Ncd (a Kinesin-14 motor) becomes plus end-directed when the cover strand and the neck linker of Kinesin-1 replace the corresponding domains of Ncd. Conversely, a chimeric Kinesin-1 possessing the corresponding domains of Ncd becomes minus end-directed (15, 16). Thus, the neck linker is not a free-standing strand, but its direction of motion and the magnitude of force generated is regulated through interaction with the cover strand.

After the moving head is thrown forward by a power stroke, it must land on the next MT site to complete a step. A Brownian dynamics simulation showed the MT binding is electrostatically guided as the kinesin–MT interfacial domains possess complementary charges (85). Thus, after ATP binds to the MT-bound front head, the stepping motion of the moving head consists of the CNB formation followed by electrostatic guiding (i.e., there is no free diffusion).

Kinesin stalls under a large load. If a ratchet potential were involved (see *Brownian Ratchet*), a large load would "flatten" it (2, 71), so that a more diffusive behavior would be observed rather than stalling. The extent of stall depends on the kinesin family. For example, a Kinesin-12 motor Kif15 detaches abruptly from the MT at high loads without showing a clear stall (20). Thus, rather than operating with a single mechanism, kinesin families likely use power stroke and Brownian ratchet mechanisms to different extents, which is not unexpected given their functional diversity.

Myosin. Myosin and kinesin are believed to have evolved from a common ancestral protein (5, 86, 87). They have a similar nucleotide binding pocket structure and, as for kinesin, different myosins use the nucleotide processing event to achieve diverse motile behaviors (11, 21). The arrangements of domains around the moving element are also similar; the neck linker of kinesin is attached to α6, while the lever arm of myosin is attached to the converter domain. Motion of these domains is controlled by structurally related α4 and the relay helix, respectively (5) (Fig. 3 *C–F*). However, kinesin and myosin differ in the stage of the ATPase cycle when the power stroke is generated; for kinesin it occurs upon ATP binding, while for myosin the release of an inorganic phosphate (P_i) is involved (88, 89). Similar to the CNB formation in kinesin as a disorder-to-order transition, the power stroke in myosin, which is initiated by its binding to F-actin, is a transient process that has been difficult to characterize in atomistic detail. Although atomistic MD simulations investigated conformational changes of the motor head (30, 90) or nucleotide processing (91, 92), connection of such results to the mechanism of power stroke remains to be established. Additional difficulties arise as the position of the lever arm is finely adjusted after power stroke, as revealed by recent cryo-EM studies (93, 94).

Measurements for the processively walking myosin-V have provided direct evidence for the presence of a power stroke (95, 96). After the power stroke, binding of the moving head to the next leading site on F-actin is more diffusive than is the case for kinesin (97–101). It becomes more prominent in the presence of a load, which also causes more frequent backward stepping (102). A more recent measurement suggests that the diffusion is spatially constrained (103). Thus, stepping of myosin-V appears to involve both power stroke and Brownian ratchet mechanisms. Interestingly, as for kinesin, the moving head of myosin-V tends to stay on one side, although it can be either the left or right side (cf. Fig. 3*B*); the choice of which may be determined by the initial engagement with the F-actin (103).

A dimer of myosin-VI walks processively toward the pointed end of F-actin, opposite to myosin-V (10). A coarse-grained simulation demonstrated a power stroke followed by diffusive motion of its lever arm (104), similar to myosin-V. In the model, the pre-to-poststroke transition was made to occur via a distance-based switching potential; hence, the force-generation mechanism was not investigated. The recovery stroke of myosin-VI was studied in another paper where a new structure called pretransition state (PTS) was observed (105). One interpretation of its role was to allow thermal fluctuations with ratchet-like trapping in a functional state during the recovery stroke. However, whether the PTS is indeed an intermediate or whether the ratchet-like behavior is a necessary consequence need further study (105). As noted earlier for the Ncd kinesin (106), the recovery stroke of myosin VI occurs while the motor is detached from the track without any need to generate force. Thus, it can be more diffusive than the forward stepping.

The parallels between kinesin and myosin in domain organization and stepping behavior may originate from the common requirement of achieving bipedal motility on a linear track, in addition to their common evolutionary origin.

F_1-*ATPase.* F_oF_1-ATP synthase is a major energy conversion protein complex of the cell (107). It uses a transmembrane proton gradient to synthesize ATP, or conversely it can hydrolyze ATP to serve as a proton pump. It is thus a reversible motor. The membrane-bound F_o subunit mediates proton transfer while the ectodomain F_1 synthesizes ATP (Fig. 4*A*). The F_1 part alone can hydrolyze ATP, hence it is called F_1-ATPase. The minimal complex of F_1 consists of hexagonally arranged $\alpha_3\beta_3$ subunits and the γ-subunit. The latter has a coiled-coil "rotor" located at the center of the $\alpha_3\beta_3$ ring and a globular portion below it (Fig. 4*B*). Conformational changes accompanying the ATPase cycle in the β-subunits of the ring rotate the γ-subunit counterclockwise, as viewed from the membrane, by 120° per 1 ATP used (108) (Fig. 4 *B–D*). For a given subunit (e.g., β_E in Fig. 4*B*), a full ATPase cycle (i.e., binding of an ATP, hydrolysis, and release of hydrolysis products) involves a 360° rotation of the γ-subunit.

Analysis of nucleotide-dependent conformational changes suggests that rotation of the γ-subunit is driven by a power stroke. Atomistic free-energy simulations have shown that ATP binding to a subunit produces the largest free energy change, which is responsible for 80° to 90° rotation of the γ-subunit (110, 111). This rotation assists with conformational changes in a neighboring subunit containing ADP (adenosine 5′-diphosphate) and P_i, where ADP is released during the 80° to 90° rotation and P_i release generates the remaining 30° to 40° rotation, completing a 120° rotation per ATP hydrolyzed (Fig. 4 *B–D*) (112–114). The substeps have been observed in single-molecule experiments (115, 116). The γ-subunit acts as a crankshaft that converts conformational changes in the $\alpha_3\beta_3$ ring to a unidirectional rotation and it also transmits conformational changes in an $\alpha\beta$ pair to another. However, the γ-subunit is not required for the ATPase cycle of the ring. Mutant F_1-ATPases with a series of γ-subunit truncations still show rotation in the correct direction, although it is slower and more irregular (117). Even a motor lacking the entire γ-subunit showed conformational changes in the $\alpha_3\beta_3$ ring in the correct order (118). These results indicate that the intersubunit communication is guided by the topology of the ring (13).

A coarse-grained simulation, which imposed conformational changes to the γ-subunit of the ring, showed how repulsive van der Waals interactions act on the γ-subunit to generate its rotation in the correct direction (112). This indicates that the γ-subunit rotation is essentially a power stroke, which is supported by experiments where constructs with mutant γ-subunits lacking specific contacts with the $\alpha_3\beta_3$ ring still generated a torque (119). However, the presence of specific contacts does augment torque generation (120).

Due to the steric coupling, the γ-subunit cannot perform free rotational diffusion over a significant fraction of the 120° step. ATP binding to a β subunit generates about 10 kcal/mol (111), which is nearly 80% of the free energy of ATP in cells. A large free energy gradient driving torque generation is by definition a power stroke. The power stroke nature of the γ-subunit rotation is supported by a single-molecule experiment that observed defined phases of angular acceleration and deceleration during the 120° rotation (116). This is incompatible with a Brownian ratchet mechanism involving rotational diffusion. A study of the temperature dependence of the angular velocity profile suggested that the initial rotation is driven by the elastic relaxation of the γ-subunit (38). As explained above in our analysis of protein elasticity, the γ-subunit has a high aspect ratio so that a torsional strain can develop. Torsional strain has also been noted for the α-helical coiled-coil stalk of the Ncd kinesin between the pre- and poststroke conformations (106). However, as a simulation (112) and experiments involving mutant γ-subunits demonstrated, the main source for turning the γ-subunit by the $\alpha_3\beta_3$ ring is via "steric push." Elastic relaxation, at best, augments the process. Moreover, by selective immobilization of the rotor via strategically placed disulfide bridges and monitoring rotational fluctuation, it was found that the γ-subunit coiled-coil is stiffer than the globular domain interfacing with the F_o subunit, which transmits the discrete rotation in F_1 into a smoother rotation in F_o (Fig. 4*A*) (121). The more compliant globular domain thus compensates for the incommensurate rotational symmetries of F_o and F_1 (122). The *OSCP* subunit supporting the top part of F_1 (Fig. 4*A*) also possesses a flexible hinge and facilitates the coupling between F_o and F_1 (123).

Concluding Remarks

Time and length scales are important when considering force-generation mechanisms of motor proteins. With nanometer-sized steps, thermal fluctuations alone are generally insufficient for generating a step within the time scale of the stepping motion. The free energy of ATP, the fuel molecule, is used to generate a directional force, most importantly when the motor moves against a hindering load. The free energy released upon ATP hydrolysis is rapidly dissipated and it is the differential binding of ATP and its hydrolysis products ADP and P_i that leads to the slow conformational transitions of the motor responsible for making a step. The power stroke is a transient generation of a large free energy gradient so that forward motion occurs in a nearly irreversible manner. After the major translocation, completion of a step can be assisted by a diffusive search over a limited range. Thus, rather than designating a motor protein either as a power stroke or as a Brownian ratchet motor, one should consider which stages during the motility cycle involve these mechanisms. For example, the rotation of the γ-subunit of F_1-ATPase is accomplished primarily by a power stroke. An interesting mapping of the 2 mechanisms from the protein–protein interaction viewpoint has been proposed to relate Brownian ratchet to conformational selection and power stroke to induced fit (50).

Another fundamental aspect is that the motility of motor proteins is a nonequilibrium process. In addition to the obvious fact that they consume fuel molecules, recent single-molecule experiments

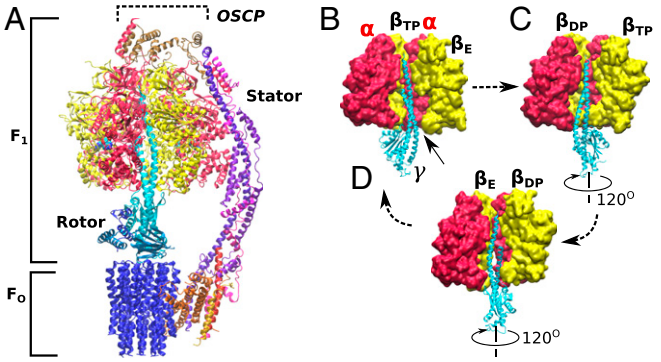

Fig. 4. Structural mechanism of F_1-ATPase. (*A*) Overview of mitochondrial F_oF_1-ATP synthase (PDB 6CP6) (109). The F_o part is embedded in membrane. (*B–D*) Rotational cycle of F_1-ATPase. Only β-subunits carry out ATP hydrolysis. Its 3 major conformational states are denoted by β_{TP} (ATP), β_{DP} (after ATP hydrolysis), and β_E (after product release). In each panel, a pair of $\alpha\beta$ subunits in front of the page are not shown, to reveal the γ-subunit. (*B*) Due to the asymmetry in the γ-subunit (arrow), the nucleotide-free β_E adopts an open conformation. (*C*) ATP binds to the right ($\beta_E \rightarrow \beta_{TP}$), and the β-subunit behind hydrolyzes ATP ($\beta_{TP} \rightarrow \beta_{DP}$). The β-subunit in front (not shown) releases hydrolysis products. The γ-subunit rotates by 120°. (*D*) The $\beta_{DP} \rightarrow \beta_E$ transition of the subunit behind γ and binding of an ATP to β_E in front (not shown) cause another 120° rotation. The subunit on the right hydrolyzes ATP, $\beta_{TP} \rightarrow \beta_{DP}$.

have directly measured the energy dissipation involved in a step (124–126). For kinesin, under a 2-pN hindering load, 80% of the input energy by ATP was dissipated in sources other than work against the load or viscous drag (126). The authors concluded this "hidden" dissipation to be internal, which should be due to the conformational fluctuation of the motor head itself. This is consistent with our finding that the flexible subdomains of kinesin play specific roles for processing an ATP molecule (29), whose motion would cause dissipation not accounted for by translocation of the motor. By contrast, for F_1-ATPase, the differential binding mechanism used to generate the torque on the γ-subunit results in high efficiency (124); conversely, mechanical work supplied to turn it in the opposite direction is used to synthesize ATP with little dissipation (125). Although its efficiency may not be 100% under all conditions (38, 116), lack of internal dissipation (126) reflects the tight coupling between the γ-subunit and the $\alpha_3\beta_3$ ring.

To generate a net transport, bulk concentrations of ATP and its hydrolysis products must be maintained out of equilibrium. Apart from such a global condition, actions of individual motor proteins are nonequilibrium in nature, even though a motor protein does not "know" bulk concentrations when making a step. Note that energy-consuming transitions differ from equilibrium transitions between states. The latter obey detailed balance (equal fluxes of forward and backward transitions), which is not the case for certain states of motor proteins. For example, even for the "reversible" F_oF_1 motor, ATP hydrolysis and synthesis cycles are not simple inverses and they involve different structural pathways (111). Most kinetic models contain irreversible transitions (7, 61). In Brownian motors, a broken symmetry in detailed balance is also needed (41). Detailed balance is not a universal principle, but it is a "physical property" that holds only for certain cases (see remarks on pp. 82 and 110 of ref. 70). By contrast, it has also been claimed that microscopic reversibility must hold for motor proteins (53, 59). Although smaller length and time-scale phenomena such as collision of water molecules and vibration of covalent bonds may be described locally as equilibrium processes, conformational states integrate atomic degrees of freedom, for which there is no guarantee that microscopic reversibility holds. Which transitions during the motility cycle can be considered reversible or irreversible depends on the specific motor protein in question.

Given the nonequilibrium and structure-dependent nature of the motility of motor proteins, atomistic MD simulations have been instrumental in elucidating their force-generation mechanisms (29, 30, 75, 85, 106, 110, 111). Coarse-grained simulations based on the atomistic properties have provided additional insight into the overall operation of the motor on time and length scales inaccessible to atomistic MD simulation (74, 112, 127). Further up in scale, kinetic models, with careful selection of states and collective variables (reaction coordinates), have been successful in interpreting experimental motility data and provided insights into underlying processes (60, 61, 111, 128).

The mechanisms of motor proteins discussed in the present study are based on increasingly quantitative experimental measurements, high-resolution structures of motors in different states, computer simulations, and theory. Elucidating the similarities and differences in the physical mechanisms of motor proteins, either within a given motor family or between different types of motors, is essential for understanding their biological function.

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