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REVIEW PAPER

Mechanical Design of Translocating Motor Proteins

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Abstract Translocating motors generate force and move along a biofilament track to achieve diverse functions including gene transcription, translation, intracellular cargo transport, protein degradation, and muscle contraction. Advances in single molecule manipulation experiments, structural biology, and computational analysis are making it possible to consider common mechanical design principles of these diverse families of motors. Here, we propose a mechanical parts list that include track, energy conversion machinery, and moving parts. Energy is supplied not just by burning of a fuel molecule, but there are other sources or sinks of free energy, by binding and release of a fuel or products, or similarly between the motor and the track. Dynamic conformational changes of the motor domain can be regarded as controlling the flow of free energy to and from the surrounding heat reservoir. Multiple motor domains are organized in distinct ways to achieve motility under imposed physical constraints. Transcending amino acid sequence and structure, physically and functionally similar mechanical parts may have evolved as nature's design strategy for these molecular engines.

Keywords Kinesin · Myosin · DNA motor · Chaperone · Ribosome · Mechanochemical cycle · Fish model

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Introduction

Motor proteins form distinct classes in the protein universe as they can convert chemical energy directly into mechanical work. Among them, translocating motors move along biopolymer tracks, such as nucleic acids, polypeptides, or quaternary biofilament structures like F-actin or microtubule (Kolomeisky and Fisher proposed the term "translocase" for these motors [41]. However, we prefer to use "translocating motor," since translocase refers to membrane-bound motors such as SecA, whose function is to translocate a protein across the membrane [24]). Movement is an essential part of their function. For example, RNA polymerase (RNAP) walks along the DNA molecule and transcribes the genetic code into RNA [25]. Lambda-exonuclease walks along DNA and simultaneously degrades it [45]. Subunits of molecular chaperones such as ClpX or HslU pull and unfold a protein to prepare it for degradation [28, 30]. Kinesins and dyneins walk along the microtubule for intracellular cargo transport and cell division, while myosins walk along F-actins and perform transport as well as muscle contraction [79]. Figure 1 shows diversity in sizes and shapes of translocating motors and their tracks. Although they include different families of proteins, they share common features so that they convert chemical energy derived from fuel molecules to generate mechanical forces and move along a track in a cyclic manner.

While studies on biological functions of these motors are diverse and increasing, little is known about their basic operation mechanisms: How the chemical energy of a fuel molecule, e.g., upon binding, unbinding, or hydrolysis of an adenosine triphosphate (ATP), powers force generation and motion? What are the structural elements involved in this process and their common design principles? Recent developments elucidating atomic structures and single

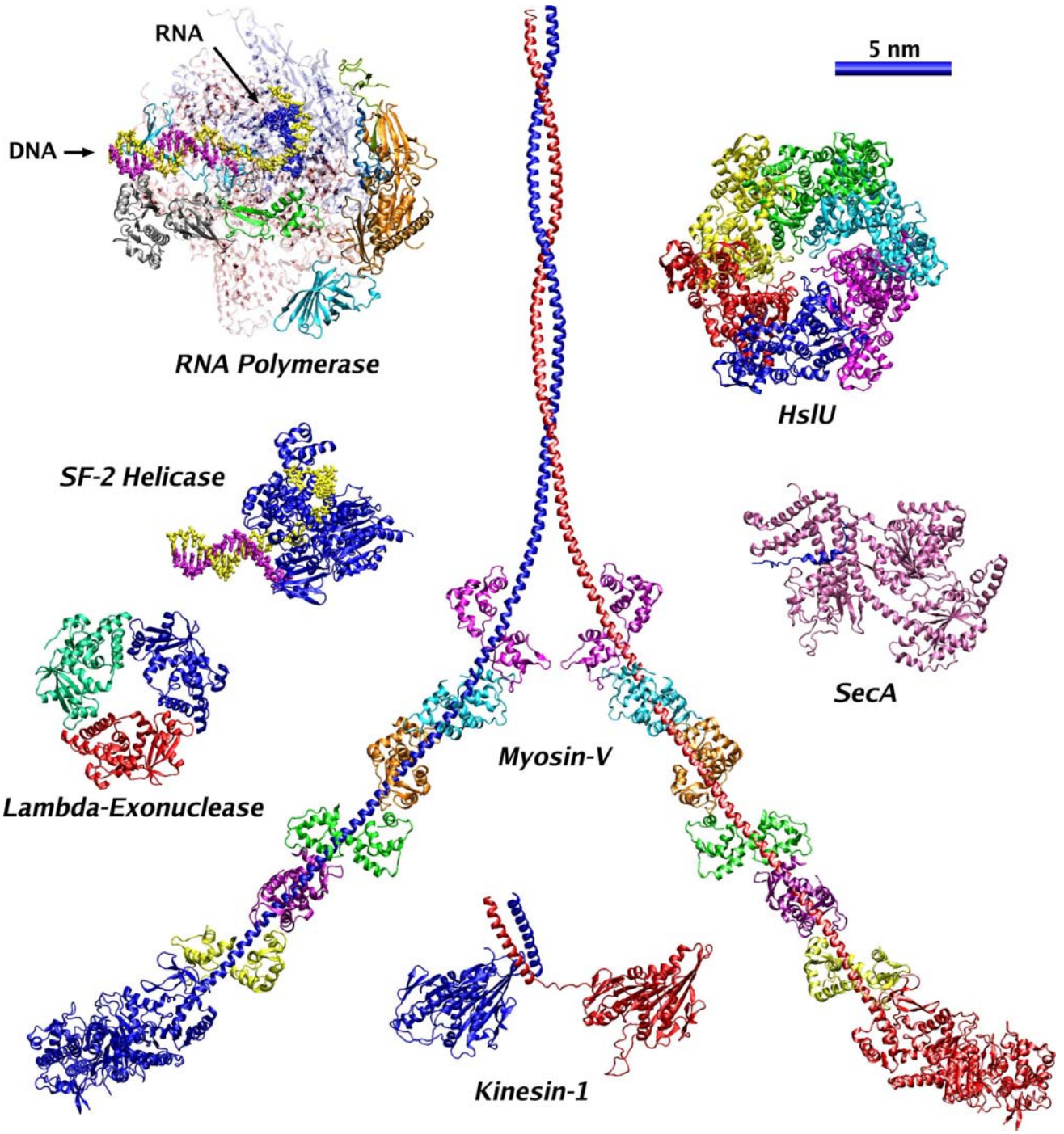

Fig. 1 Diversity of translocating motor proteins. Sizes are to scale (note the scale bar). The direction of motion is horizontal except for λ-exonuclease and HslU (heat shock locus U), which is perpendicular to the plane. Subunits are colored differently. In RNAP, the largest subunits (*red* and *blue*) are rendered transparent to reveal the DNA substrate and the nascent RNA chain (marked by *arrows*). Opposite page: Ribosome (a ribozyme), showing bound mRNA (thick yellow tube) and the ribosomal RNA backbones in light blue and pink. Three major tracks are also shown, 12-residue poly-alanine in an extended conformation, a 13-base pair DNA, F-actin (14 G-actins; 0.5 super-helical turn), and microtubule (13 protofilaments, each containing three pairs of α (pink) and β (light blue) tubulins). Structures are based on Protein Data Bank coordinates: 2E2I (RNAP) [85]; 2P6R (SF-2 helicase) [13]; 1AVQ (λ-exonuclease) [42]; 1DO0 (HslU) [9]; 2VDA (SecA) [24]; 2DFS (myosin-V) [48]; 1BG2/1MKJ [69, 43] (Kinesin-1); 2J02/2J03 (ribosome) [68]; 1LAQ (DNA) [90]; 1M8Q (F-actin) [15]. Microtubule structure was constructed based on a microtubule sheath structure provided by K. Downing [47]. Images were rendered using the VMD program [34]

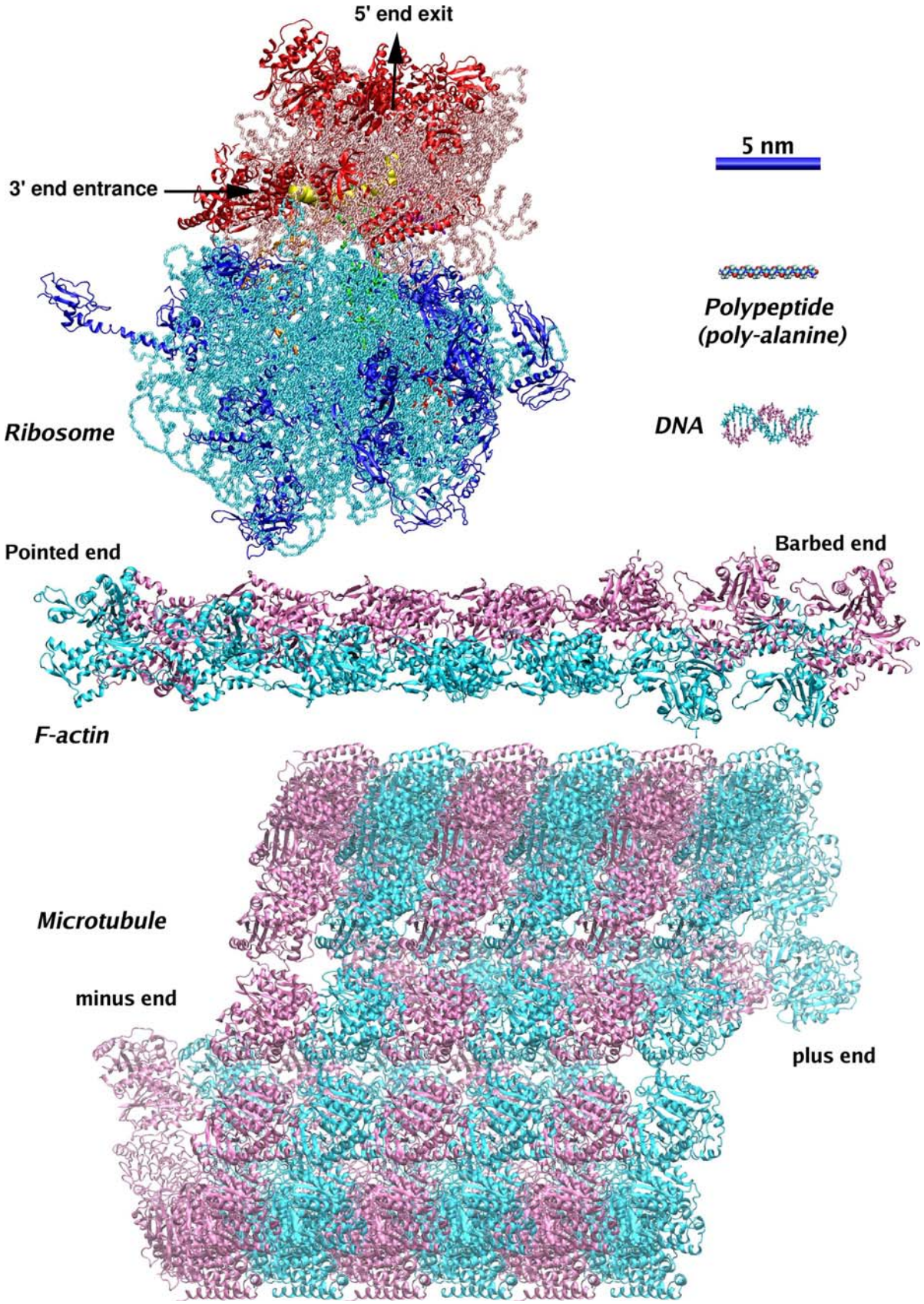

Fig. 1 continued

molecule analysis have made it possible to address these questions in a greater detail. Yet the dynamic and non-equilibrium nature of force generation and subsequent motion makes it difficult to establish a structural and physical basis, for which theoretical and computational modeling is also vital. In the case of Kinesin-1, it had been known for some time that the motion of its $\sim$12 residue neck linker connecting the motor head to the coiled-coil stalk is essential for the stepping event (cf. Fig. 4a) [60, 54]. However, the mechanism by which kinesin generates force remained unknown despite the availability of various kinesin crystal structures. Our recent computational [35] and experimental [38] investigations revealed that force is generated by the dynamic folding transition of a domain referred to as the cover–neck bundle (CNB), which is a β-sheet formed between the neck linker and the N-terminal dangling end of the motor head that we named the cover strand. This illustrates the difficulty of finding the force generation mechanism based on static structures alone. For most other translocating motors, the motility mechanism is even less understood.

All translocating motors necessarily share a common physical feature, i.e., to generate force and move. We thus submit that there are a set mechanical elements, or design criteria, which transcend chemical or structural details such as the fuel type, amino acid sequence, and tertiary/quaternary structures. In this study, we propose a "mechanical parts list" of translocating motors and illustrate them mainly using the better-known examples of kinesin and myosin. Elucidating such parts will help develop a more physical description of the working mechanism as well as establish common mechanical design principles of these diverse and apparently unrelated motor families.

Measurable Characteristics of Translocating Motors

We first describe general characteristics of a motor that are experimentally measurable. This list is by no means exhaustive, but it provides a quick glimpse of the mechanical and physical characteristics of a given motor. Some of the measured values of these parameters are summarized for nucleic acid motors in Ref. [67], and for F-actin and microtubule motors, in Table 13.1 of the book by Howard [33].

Unloaded Velocity

This is the speed of the motor when there is no obstacle, such as from a cargo attached to kinesin or a supercoiled DNA that hampers the motion of an RNAP. Even in an unloaded velocity measurement, there is still an inherent load, albeit small, due to the viscous drag of the part moving relative to the surrounding medium, which can be either the motor or the track, or both. But as illustrated by the fluctuation–dissipation theorem [81], the drag is in turn caused by thermal fluctuation, an essential driving force for motility. Typical unloaded velocity ranges from tens of nm/s, $\sim$700 nm/s for Kinesin-1 [8], and to an amazing 60 μm/s for green algae myosin-XI, although the latter is the actin-gliding velocity and not the velocity of a single motor [31].

Stall Force

The stall force F_s is the magnitude of the resisting force at which the motor ceases to move. It depends on the direction of the applied force as well as the location at which it is applied. However, since experimental setups for a given motor protein are similar, F_s as a scalar quantity is commonly used. Since the motor slows down as load increases, it is difficult to know exactly at what level of force the motor stops completely [86]. Thus experimentally measured F_s may be regarded as a lower bound. Stall force ranges from a few pN to 5–7 pN for Kinesin-1 [8], 25 pN for RNAP [89], and to 57 pN for the DNA packaging motor of the bacteriophage ϕ29 [71].

Step Size

This is the distance of travel when the translocation event occurs. For *tightly coupled* motors, translocation occurs once per fuel processing cycle, while different scenarios are also possible, e.g., one translocation event over multiple fuel processing cycles, or vice versa [37]. The step size is typically an integer multiple of the size of the underling lattice of the track on which the motor moves. It ranges from the length of one base pair (3.7 Å) of a DNA molecule for RNAP [1] to 8 nm (size of a tubulin dimer) in kinesin [74], to 36 nm (half of the helical periodicity of F-actin; see Fig. 1) for myosin-V [52]. Some motors have multiple step sizes. For example, the microtubule minus-end directed motor, cytoplasmic dynein, has step sizes ranging between 4 and 32 nm, and it takes smaller steps under load, even changing its walking direction to the plus end, as if having a gear mechanism [51, 64, 26]. A distribution of step sizes has also been reported for the F-actin pointed-end directed motor, myosin-VI [61].

Processivity

Processive motors make multiple steps or undergo multiple mechanochemical cycles in one encounter with the track, while non-processive ones only make one step and detach from the track. Processivity is measured as the unloaded *run length* of the motor. As a relevant concept, the *duty*

ratio is the fraction of time that the motor is attached to the filament during the mechanochemical cycle [32]. For a single motor to move processively, it must have a high duty ratio, such as Kinesin-1 or myosin-VI [18]. A key to high processivity in such dimeric motors is the coordination between the motor heads that keeps their mechanochemical cycles to be out of phase. This is further discussed below in the section "Motor Domain Organization." Low duty ratio motors such as myosin-II work as a group in order to prevent disengagement from the filament. Working as a group can also enhance processivity, which can be achieved by forming pairs, binding to the same cargo, or by forming a toroid to encapsulate the track.

Efficiency

Thermodynamic efficiency can be defined as the maximum work that can be done divided by the net free energy change per cycle [57]. Maximum work done is the stall force times the step size—the efficiency is the highest when the motor works near stall [12]. As an example of fuel, ATP provides about 20–25 k_BT of energy (k_B: Boltzmann constant, and temperature $T = 300$ K is assumed), given the standard free energy for hydrolysis modified by the physiological concentrations of ATP, adenosine diphosphate (ADP) and phosphate [33]. For kinesin, its stall force is $\sim$6 pN with a step size of 8.2 nm, which gives an efficiency of 48–60% (See Table 2 in Ref. [12] for efficiency of other motors).

Kinetics of Substeps

Each step of the motor involves transitions through a number of states (substeps), which generally include: binding to or unbinding from the track; force exertion (power stroke); binding of a fuel molecule; breakdown (burning) of fuel, typically via breakage of a covalent bond; release of the products of fuel breakdown; and relevant conformational changes of the motor and the track. Knowledge of the ordering of these events and their relationships to motor and track structures are a major step in understanding how the motor works.

Transitions between one or more substeps can be observed using methods such as stopped flow [50, 55], by tracking donor-acceptor distances in fluorescence resonance energy transfer (FRET) [62, 63], and polarization states of fluorophore-labeled segments of the motor [21, 72]. Structures in certain sub-states can be observed through cryo-electron microscopy (cryo-EM) [60, 70, 39] and crystallography [69, 56, 23, 85]. For single molecule motility data, the randomness analysis is used to find the number of rate determining substeps within the mechanochemical cycle. It is a dimensionless ratio between the variance of the motor position and the product of the average position and the step size [8, 73]. Randomness is inversely related to the number of rate-limiting steps in the cycle. For example, in the low ATP case where the kinesin cycle is dominated by ATP binding over other load-dependent transitions, randomness approaches a value of 1, indicative of one rate-limiting step governing the cycle. For multiple rate-limiting steps, randomness is less than one.

Mechanical Parts List

Now we take an engineering approach and propose a list of generic components, or modular domains, in these molecular machines. A generalized schematic model highlighting these parts is proposed in Fig. 2 and example mechanochemical cycles in myosin and kinesin are shown in Figs. 3 and 4.

Track

There are mainly three types of tracks; nucleic acids, polypeptides, and quaternary biofilaments. When either or both of the motor or the track move, there is hydrodynamic drag. But mechanical load can come from the track itself. For example, DNA-bound motors must walk against DNA supercoiling, nucleosome rearrangement or other DNA-

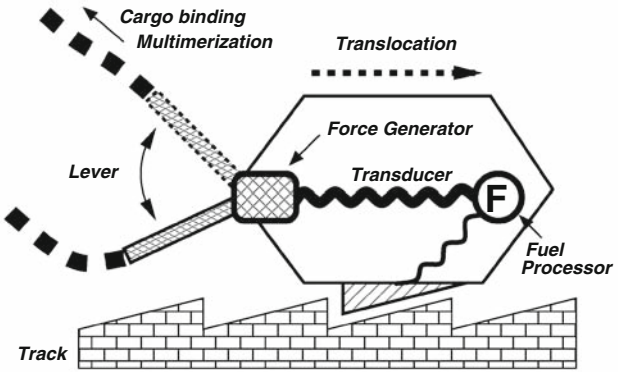

Fig. 2 The "fish model" of a translocating motor, illustrating its mechanical parts. Conformational change of the fuel processor (typically on the order of Ångströms) is relayed by the transducer to the force generator, where mechanical work is produced. This leads to motion of the lever that can be larger than 10 nm depending on the motor. The subsequent translocation event usually occurs only in one direction. Binding of the motor to the track is often controlled by the fuel processor and it can have a ratchet-like character. Track can change conformations as it interacts with the motor and may actively participate in the motility cycle, rather than just being a passive road. For cargo-carrying motors such as kinesin and myosin, levers are connected to cargo-binding and/or multimerization domains. Details of this schema varies among different motors. For example, see Figs. 3 and 4

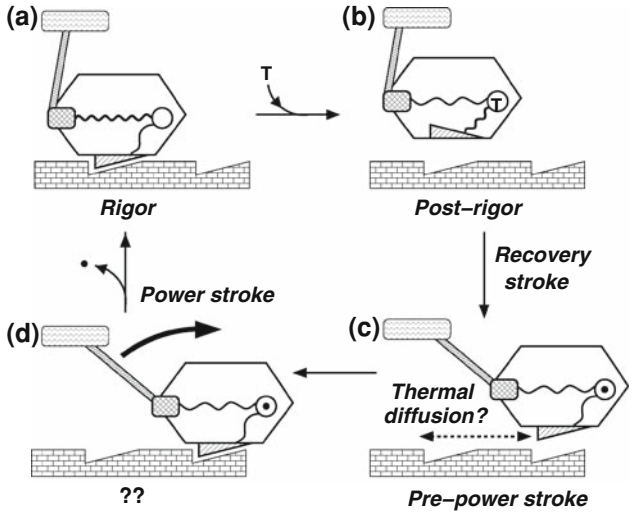

Fig. 3 The Lymn–Taylor cycle of myosin [49, 23]. **a** In the rigor state, the myosin motor head (nucleotide free) is strongly bound to F-actin. **b** ATP binding ("T" in the fuel processor) leads to dissociation from F-actin (the "fin" of the fish folds). **c** Hydrolysis of ATP (• in the fuel processor denotes ADP·P_i) leads to the recovery stroke. In the ADP·P_i state, the motor head may sample between the post-rigor and pre-power stroke states, but it can bind to F-actin tightly only in the latter state (the fin unfolds). This is likely the stage during the mechanochemical cycle where the ratchet-like behavior is exhibited. **d** Binding to F-actin releases P_i, which leads to force generation (power stroke) and the forward movement of the lever arm. ADP release completes the cycle and the motor returns to the rigor state, translated by one step. The transition from the pre-power stroke to the rigor state is transient, and the exact sequence of events and structural details (marked by '??') are yet to be known

bound proteins [25]. Clp family of motors must unfold a protein as they pull on the polypeptide backbone [66]. On the other hand, motors on quaternary biofilaments, such as kinesins or myosins, move on a relatively stationary and straight track, microtubule or F-actin. Loads in this case are mainly from drag that motors or attached cargo experience in the crowded intracellular environment. There may be other obstacles due to binding of accessory proteins on tracks or cross linking among tracks. However, since these stepping motors require binding of motor heads on the surface of tracks, they either detach or change path rather than by forcibly removing obstacles [3, 19]. In the case of myosins and kinesins, a cross-talk has been observed when multiple motors are bound to the same cargo. For example, although myosin-V walks on F-actin, it non-specifically binds to the microtubule via electrostatic interactions and either diffuses on the microtubule or enhances processivity of kinesins when they are bound to the same cargo. Conversely, kinesins play a similar role for transport along F-actin [4]. Such a cross-talk may enhance intracellular transport by utilizing both F-actins and microtubules as tracks. It has also been suggested that kinesins and dyneins walking in opposite directions on the microtubule coordinate motility for cargo transport, rather than engaging in a tug-of-war [44].

Fuel

As translocating motors, or motor proteins in general, are non-equilibrium systems that dissipate energy and produce mechanical work, energy must be supplied. Nucleic acid-based motors can use tracks or their substrates directly as fuel. For example, the energy source for RNAP are nucleotide triphosphates (NTPs) that are hydrolyzed with an average energy output of 12 k_BT [87], and added to the growing RNA chain that RNAP produces [89]. Another motor, λ-exonuclease moves on a double-stranded DNA molecule, degrade the backbone of one strand, leaving a single-stranded DNA behind, where energy released by degrading the DNA track ($\sim 9\ k_BT$) powers the motor [82]. Motors on polypeptide or quaternary biofilaments, on the other hand, use a separate fuel, mostly ATP. However, how ATP is used varies among motors. In the case of myosin, γ-phosphate (P_i) release after ATP hydrolysis is believed to lead to force generation (Fig. 3) [23], while in kinesin, binding of ATP results in force generation (Fig. 4) [80]. Note that ATP hydrolysis is not the only source of energy. Free energy associated with binding of a fuel and release of hydrolysis products may also contribute, although the *net* change in free energy after one mechanochemical cycle is equal to that of ATP hydrolysis. This is because after one mechanochemical cycle, the motor returns to the same state (except for a step advance along a periodic track) while the fuel molecule is broken down into products. However, states of the motor in successive cycles may differ slightly, as observed in the asymmetric stepping motion (limping) of kinesin [5]. More structural information and a deeper understanding of the stochastic nature of the motility cycle are required to explain such a behavior. In the section "Balancing the Energy," we discuss various energy sources in more detail.

Transducer

Once chemical events occur, such as ATP binding or phosphate release, the resultant conformational change of the catalytic site must be transmitted to the force generator or other parts of the motor. Without the transducer, chemical events involving the fuel have to be directly used to generate force, which would be a difficult task, since the same set of domains should process both chemical and mechanical events. This would be especially so for motors with large step sizes. The allostery between the fuel processor and the force generator coupled by the transducer makes it easier to control the direction and magnitude of the generated force as well as organize temporal sequence

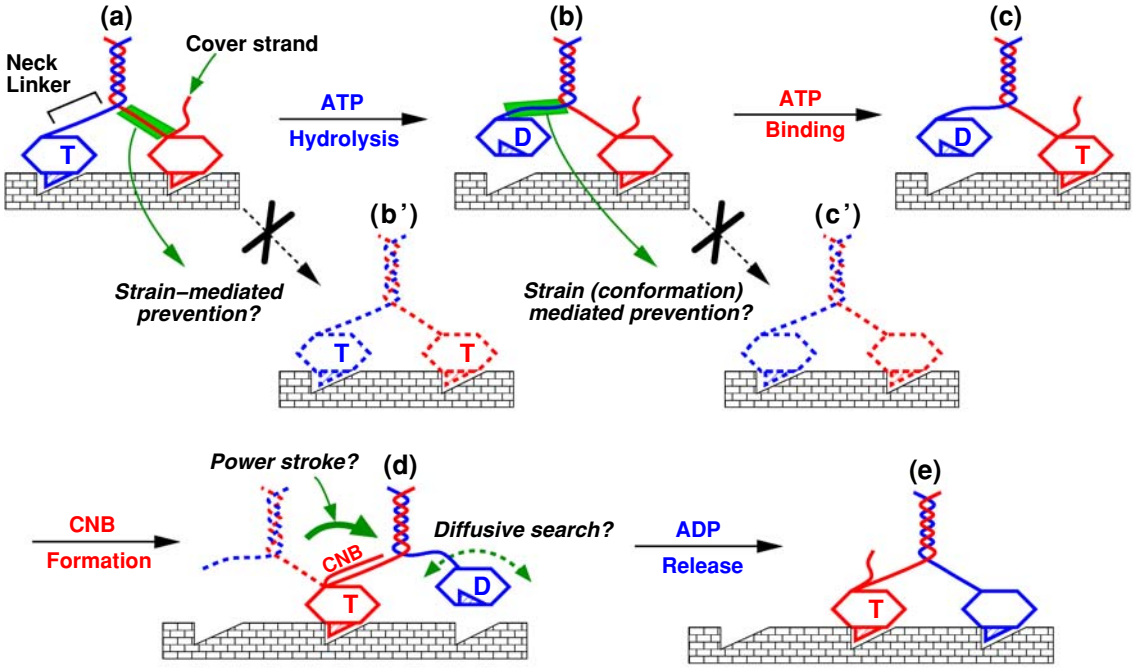

Fig. 4 The kinesin mechanochemical cycle. To distinguish events occurring at leading or lagging heads, legends associated with blue or red heads are colored accordingly (the cover strand is shown only on the red head). (**a**–**e**) One cycle during a processive run. Motor head coordination prevents states in (b′,c′), to ensure processivity. **a** Kinesin heads with a bound ATP or without any nucleotide have high affinity for the microtubule. Binding of an ATP to the leading head into the state (b′) is thought to be prevented through the strain on the rearward-pointing neck linker [7, 91], although structural details for this mechanical allostery is unknown. **b** Hydrolysis of ATP on the trailing head reduces its microtubule affinity. ADP release and subsequent re-binding of the head in the lagging position, as shown in (c′), is suppressed possibly due to the conformation of the forward-pointing neck linker [54]. **c** Reduced strain in the neck linker on the leading head allows ATP binding, which is followed by the cover–neck bundle (CNB) formation. **d** The power stroke by the CNB likely moves the detached head in the forward position [35, 38]. How much diffusion has to take place for this head to find the next microtubule binding site is not known. **e** The newly leading head releases ADP and enters a strong microtubule bound state, completing one cycle. Note that in addition to normal processive run as shown in Fig. 3 and here, there are other events such as pauses, entering and exiting the cycle

of events. Yet counter-examples exist—although not a translocating, but a rotary motor, in F_1ATPase, binding of an ATP to the hinge domain of the β subunit results in a conformational change that directly generates torque [22, 57].

Force Generator

Conformational changes of the transducer are linked to the force-generator to produce a "power stroke," through which the motor moves by a finite distance against load. The link: fuel processor → transducer → force generator is not necessarily mechanical, meaning that free energy associated with conformational changes of the fuel processor or the transducer may not be directly used for mechanical work by the force generator. Rather, as exemplified by kinesin below, conformational change of the transducer may only *trigger* an event that leads to force generation, but the energy source for the mechanical work may come from elsewhere. This is possible as these motors are not isolated systems, and can absorb or release energy from the surrounding heat reservoir (heat bath). In this case, energy released by burning the fuel is used to control flow of free energy between the motor and the reservoir, and is not directly connected to mechanical work. Alternatively, conformational energy stored in a prior state of the cycle may be released by the fuel. This contrasts with the design of a combustion engine, where a part of the free energy released by burning gas directly powers rotation of the wheel.

Another important concept that often contrasts with power stroke is the Brownian ratchet mechanism [57, 6]. In a Brownian ratchet motor, conformational changes in the motor activates a "pawl" that rectifies rapid thermal fluctuation in one direction. Known examples are myosin-II [17] and nucleic acid motors such as RNA polymerase [1, 67]. Myosin-II is a low duty ratio motor and ratchet-like jump to the next binding site on F-actin is possible because it operates as a group where individual motors are organized and a close distance between the motor and the track is maintained even in a detached state. On the other hand, it would be easier to utilize the Brownian ratchet mechanism in nucleic-acid motors and possibly in polypeptide motors, since their step sizes are much smaller (e.g., a few Å),

which is comparable to the size of thermal fluctuations of relevant sub-domains.

It is unclear how much external load a pure Brownian ratchet motor can withstand. Power stroke, on the other hand, can be regarded as a motion occurring over a downhill free energy gradient created by chemical events during the motility cycle. Although conceptually simple, power stroke and Brownian ratchet mechanisms are difficult to distinguish rigorously, as rectification of thermal fluctuation in an incremental manner looks like a power stroke [86]. Most real translocating proteins are expected to have both power stroke and Brownian ratchet components. So a more appropriate question to ask would be which substeps during the mechanochemical cycle are more ratchet-like or power stroke-like. In the fish model (Fig. 2), the power stroke substep corresponds to the motion of the lever, while the Brownian ratchet part controls the "fin" as a pawl in appropriate states during the mechanochemical cycle. When the motor head is detached from the track, it may undergo thermal diffusion, during which the head may undergo conformational change, such as the "recovery stroke" in myosin (Fig. 3) [23]. This prevents the motor head from binding to the same location on the track where it was previously attached to, and biases binding to its next binding site. A nice theoretical illustration of this process is in Ref. [17]. In the case of Kinesin-1, a difference in entropy by 6 k_B between forward and backward steps has been reported [75]. While conformational change of the moving head may rectify its Brownian motion, in the dimeric setting, power stroke produced by the other head attached to the track may provide additional forward bias (Fig. 4d).

Lever

While the force generator provides the source of mechanical work, the actual moving part can be different, which we call the lever. Myosin has a well-defined lever arm composed of an α-helix surrounded by calmodulin-like domains, as for myosin-V in Fig. 1. Its motion is controlled at its base by the rotation of the converter domain in the motor head [23]. Thus the converter domain and the lever arm may correspond respectively to the force generator and the lever, although details of force generation in myosin is still not well-understood. On the other hand, kinesin's neck linker acts as a part of the lever extending to the neck coiled-coil [60], at the same time being a part of the force generator, since it is a component of the force-generating cover–neck bundle (Fig. 4d; also see the section "Balancing the Energy") [35, 38]. In the case of the microtubule minus-end directed motor Ncd (Kinesin-14), the neck coiled-coil has a clearer role as a lever, similar to the lever arm of myosin [20].

The stall force of a motor measured in a single molecule optical trapping measurement is determined by the combination of the force generator and the lever. A long lever will lead to a greater step size, but the force generating element will experience a larger torque, resulting in a lower stall force. A good example is kinesin: replacing the "soft" neck coiled-coil with one with the perfect heptad repeat does not change the unloaded velocity but the stall force is reduced [36]. While this has been attributed to the change in the axial elasticity of the coiled-coil [36], recent studies suggest that filaments held by hydrogen bonds in the axial direction, such as α-helices [16, 2] and β-sheets [58], are in general longitudinally very stiff in an amino-acid sequence-independent manner. On the other hand, our recent study reveals that a leucine zipper coiled-coil (a "perfect" heptad) is about 1.5 times as stiff in bending compared to coiled-coils with non-ideal heptad sequences [46]. Since elements such as fuel processor and force generator are not directly affected by making the lever stiffer, there is no change in unloaded velocity or run length. However, when there is a load, a stiffer neck coiled-coil will lead to a decrease in stall force due to an increased torque. In the case of myosin-V, its stall force is 3 pN [52], lower than 5–7 pN stall force of Kinesin-1 [8]. Assuming a similar energy output by using the ATP, it is expected that myosin-V, with a longer lever and step size (36 nm) than Kinesin-1 (8 nm), has lower stall force [52]. Myosins with longer lever arm resulted in faster actin gliding velocity [77]. As the unit that actually carries out physical movement, the lever may also control the direction of motion [84, 29, 53].

Balancing the Energy

We consider how various inputs and outputs of free energy are organized by the mechanical elements listed above (below we simply use the term "energy" to refer to free energy). First, there is energy associated with the motor head binding to the track. Its magnitude (i.e., affinity) depends on the motor head conformation, which is in turn controlled by the state of the fuel molecule bound to the motor head. In the case of Kinesin-1, it is strongly bound when nucleotide-free or with an ATP, and weakly bound or detach from the microtubule in the ADP state (Fig. 4a,b) [56]. Considering the cycle: no nucleotide → ATP bound → ADP + P_i → ADP → no nucleotide, it is likely that energy released by ATP hydrolysis and subsequent P_i release is used to detach the kinesin motor head from the track, rather than to actually generate force.

Other than breakdown of the fuel (such as ATP hydrolysis), there are two other sources of fuel-related energy: fuel binding and product release. For example, ATP

binding leads to force generation in kinesin (Fig. 4c, d) [60], while it detaches a myosin motor head from the F-actin (Fig. 3a, b) [23]. In the case of myosin, the release of P_i is believed to lead to power stroke [23]. The DNA packaging motor of the *Bacillus subtilis* phage ϕ29 has also been suggested to translocate upon P_i release [14]. For Kinesin-1, we have recently discovered a likely mechanism by which ATP binding leads to force generation [35, 38]: The binding energy of ATP is used for a conformational change of the motor head, which involves about 20° rotation of the transducer (the switch II domain) [40, 69, 65]. This movement of the transducer is not extensive enough to generate any major stepping motion. Instead, its small conformational change triggers the folding of the cover–neck bundle, which may be regarded as the force generator and the lever together. The cover–neck bundle possesses a forward bias, so that folding of this domain results in a power stroke.

It should be noted that, the binding energy of ATP is not a direct source of kinesin's power stroke. It only results in the conformational change of the motor head that in turn allows the cover–neck bundle formation [35]. The actual energy for the stepping motion may be supplied by the folding energy of the cover–neck bundle, whose amount may depend on the applied external load. In addition, subsequent specific binding motion of the neck linker domain to the motor head (latching) [35], and also the interaction between the moving head and the microtubule track [76] could supply additional sources of energy for the mechanical step. Likewise, energy of ATP hydrolysis or product release may be used either to merely trigger a larger conformational event driven by thermal fluctuations, or release strain that is stored in the motor or the track. It is also expected that free energy change during the force generating substep depends on the external load [38], which would be minimal in the absence of load [59]. Details of how mechanical work is generated in kinesin as well as most other motors is still not clearly understood.

Motor Domain Organization

Among diverse families of translocating motors, there are some commonly used domains. Kinesins and myosins share a similar nucleotide sensing machinery (fuel processor and part of the transducer in Fig. 2) which is also found in G proteins and appears to have evolved from the same ancestor protein [80]. Another microtubule-bound motor, dynein, belongs to the ATPase associated with various cellular activities+ (AAA+) family [79]. AAA+ proteins in turn belong to the RecA superfamily that share the RecA fold nucleotide binding domain, which include DNA-bound motors (e.g., DNA helicase), peptide-bound motors (e.g., HslU), and even the membrane-bound rotary motor F_1-ATPase [88].

Although kinesins, myosins, and dyneins belong to different families, their motor domains operate as a monomer or as multimers, notably as a dimer [79]. For a bipedal dimer, motor domains are not in direct contact and are connected by additional domains such as the lever (Fig. 2). A key requirement for processive movement in this arrangement is that the two motor heads ensure their mechanochemical cycles to be out of phase, so that one head stays bound while the other head moves forward to make a step. Being more than a few nanometers apart, the most likely way of communication between the two heads is via mechanical strain transmitted through linker domains connecting them when both heads are bound to the track. Mechanical strain can modulate the nucleotide binding affinity of the motor head, so that its state when bound to the track can be different depending on whether the other head is also bound or not. Such a mechanism has been proposed for Kinesin-1 (Fig. 4a vs. c) [7, 91], cytoplasmic dynein [26], and myosin-V [83]. By contrast, RecA family of motors typically form hexameric or heptameric rings [78]. Subunits in this case are in tighter contact. Dynein has an interesting combination of these two designs. Its two AAA+ rings contact each other, but microtubule-binding domains are separate and connect to the AAA+ rings by 15-nm long stalks [11], displaying an overall bipedal structure. Generally, each subunit within the ring of the RecA superfamily is oriented such that its major conformational change upon nucleotide binding or release (rather than hydrolysis) occurs roughly in the direction of movement or force generation [88]. The toroidal organization of multiple motor domains may also allow the conformation of one domain to affect the state of its neighboring domains, so that motor activity may occur in a coordinated cyclic manner across the ring [14].

To date, bipeds have been found only for translocating motors that walk on quaternary biofilaments (F-actin and microtubule). This may be due to the fact that, to support mechanical tension between the two bound heads, the track must be sufficiently stiff. Compared to F-actin or microtubule, nucleic acid or polypeptide chains are more flexible thus would not allow two separately bound motor heads to develop tension along the linker connecting the heads. However, it is conceivable that large multi-subunit nucleic acid or polypeptide motors achieve internal communication by binding multiple base pairs of a DNA or a polypeptide chain in a slightly mismatched manner, so that tension or strain developed along the track is used as a signal. The large size of the motor compared to nucleotide or polypeptide tracks (Fig. 1) would also make it easier to achieve processivity simply by wrapping around the track and prevent diffusing away even when the motor temporarily

disengages from the track. Conversely, there are actin or microtubule-associated motors with domain organizations completely different from kinesins or myosins. The microtubule associated protein XMAP215 has a kite-like shape (a ring-shaped domain that fits a tubulin dimer and an attached tail) and it has been suggested to processively move at the growing tip of the microtubule, accelerating both microtubule polymerization and depolymerization [10]. It does not contain any ATPase or guanosine triphosphatase (GTPase) domains. Similarly as some nucleic acid motors, XMAP215 thus might harness the free energy of its track. Similarly, the actin tip tracker formin forms a ring-shaped dimer and promotes polymerization at the barbed end of F-actin without requiring a dedicated fuel [27].

Conclusion

While cellular functions of translocating motors are extremely diverse, as mechanochemical amplifiers, they should share common design features. Here we discuss such features and propose generic mechanical elements that comprise the motor machinery. Note, however, that these elements need not have static structures. A good example is kinesin's cover–neck bundle, that generates force by folding rather than by switching between well-defined conformational states [35, 38]. Furthermore, as explained above in motor domain organization, these parts are combined in various ways to achieve diversity of translocating motors on different tracks. Our fish model (Fig. 2) shows connection among these elements and also combines both ratchet-like and power stroke-like characters, which may be applicable to a large fraction of subcellular motors. With a more quantitative and physical understanding of how these elements operate, it will even be possible to classify translocating motors based on their working mechanism, rather than by their tracks or amino acid sequence, which would be a clear advance in understanding the cellular hardware.

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