Motor Proteins

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SUMMARY

Myosin motors power movements on actin filaments, whereas dynein and kinesin motors power movements on microtubules. The mechanisms of these motor proteins differ, but, in all cases, ATP hydrolysis and subsequent release of the hydrolysis products drives a cycle of interactions with the track (either an actin filament or a microtubule), resulting in force generation and directed movement.

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1 INTRODUCTION

Three superfamilies of motor proteins power directed movements on microtubules or actin filaments. Myosin motors move on actin filaments, whereas kinesin and dynein motors move on microtubules. These molecular motor proteins all convert the energy from ATP into force and movement on either the actin or microtubule tracks. Myosin and kinesin had a common ancestor related to GTPases, but dynein is an AAA ATPase. They power cellular functions as diverse as muscle contraction, cytokinesis, chromosomal movements, membrane trafficking, organelle movements, and cellular migration. The functions of myosin motors have been reviewed recently elsewhere (Leube et al. 2016; Pegoraro et al. 2016; Svitkina 2016), as have the functions of dynein and kinesin motors (Barlan and Gelfand 2016; Cheng and Eriksson 2016; Ishikawa and Marshall 2016; McIntosh 2016; Sweeney and Hammers 2016).

2 MYOSIN

Myosins form a superfamily of molecular motor proteins that power muscle contraction, as well as movement on actin filaments in all eukaryotic cells. All known myosin superfamily members follow the blueprint shown in Figure 1 (Odrionitz and Kollmar 2007). The myosin head can be subdivided into the motor domain, which includes the catalytic site for ATP hydrolysis and a converter subdomain, which is attached to an extended helix reinforced by a variable number of calmodulins or calmodulin-like light chains to form a “lever arm.” The lever arm amplifies the movements within the myosin motor domain. This is followed by a region of coiled coil in two-headed myosins, and this can contain sequences that act as elements for protein folding. Last is the targeting domain, which binds the myosin to its cellular target (Fig. 1).

All myosin motors hydrolyze ATP, but, when not bound to actin, only slowly release the hydrolysis products, MgADP and inorganic phosphate (P_i). Binding to an actin filament stimulates myosin to release P_i , followed by the release of Mg and ADP. Rebinding ATP dissociates myosin

from the actin filament, allowing hydrolysis to occur again, and the cycle to repeat. The structural changes required for hydrolysis bring about a large movement of the myosin lever arm, which is reversed when the products are released while the myosin head is bound to actin. The movement of the lever arm on actin is known as the myosin power stroke, and generates force and movement. The movement of the lever arm associated with hydrolysis is known as the recovery stroke, and involves a rapid isomerization with a low free-energy barrier.

2.1 Myosin Structure

The first high-resolution myosin structure (Fig. 2A) was of the entire head fragment of chicken fast skeletal myosin (Rayment et al. 1993). This structure was later recognized to correspond to the ATP state populated after MgATP binds to and dissociates myosin from actin.

This structure revealed two key features of the myosin motor mechanism. First was the presence of a large cleft (the so-called 50-kDa cleft) in the middle of the head that ran from the nucleotide-binding site to the actin interface. Rayment et al. (1993) suggested that this cleft likely closes when the products of ATP hydrolysis dissociate from myosin upon strong binding to actin. A high-resolution structure of the myosin V motor without nucleotide showed how this cleft closes and, in doing so, forms a new interface that allows for strong binding to actin (Coureux et al. 2003).

The second striking feature was the carboxy-terminal portion of the heavy chain, which formed an extended α -helix associated with two light chains. This region appeared to be a lever arm that could amplify small movements occurring within the rest of the head. This “swinging lever arm” hypothesis has been validated by multiple lines of experimental evidence (Whittaker et al. 1995; Uyeda et al. 1996; Tyska and Warshaw 2002).

Additional structures confirmed the swinging lever arm hypothesis by revealing the conformation of the myosin head before the power stroke of the lever arm (Dominguez et al. 1998; Houdusse et al. 2000). The key structures represented the pre-power stroke state with the hydrolysis

Figure 1. Domain structure of members of the myosin superfamily of proteins. This blueprint of the myosins shows the conserved motor domain and the divergence in other domains that mediate movement amplification, self-association, and cargo binding. CaM, calmodulin; IQ, isoleucine glutamine.

Figure 2. Structure of the myosin motor. (A) Ribbon diagram of the first myosin state (ATP state) for which there was a high-resolution crystal structure (Rayment et al. 1993). The structure represents the S1 fragment of skeletal muscle myosin II, which precedes the coiled coil. In different colors are the major subdomains of the motor and the myosin lever arm with its associated light chains. (B) Diagrammatic interpretation of the subdomains that move relative to each other as relatively rigid bodies as myosin undergoes state transitions during its ATPase cycle. The converter subdomain is the most flexible and mobile of the subdomains and amplifies the movements of the SH1 helix and relay into large movements of the lever arm, of which it is the most proximal component. The color-coded loops and connectors are individually identified at the bottom of the figure. The double-headed arrow indicates closure of the cleft between the upper and lower 50K subdomains that occur on actin binding, which results in a strong binding interface with actin and loss of the ATP hydrolysis products (P_i followed by ADP). Rebinding of ATP following the dissociation of ADP reopens this cleft, causing myosin to dissociate from actin. N term, amino terminal; ELC, essential light chain; 50K, 50-kDa subdomain; RLC, regulatory light chain.

products trapped in the active site. The power stroke on actin begins with the pre-power stroke conformation and ends with rigor, the state without nucleotide bound to myosin. The term rigor comes from rigor mortis, the stiff state of dead skeletal muscle with myosin bound irreversibly to actin once ATP is depleted. The lever arm position in post-rigor, following detachment from actin, is approximately the same as in rigor. Consistent with the lever arm hypothesis, the lever arm position is largely reversed with respect to the converter subdomain in class VI myosins, the one class of myosins that move in the reverse direction (Ménétrey et al. 2005). Additional specialized adaptations optimize this reverse movement (Sweeney and Houdusse 2010).

Comparison of the known myosin structures shows that the myosin motor domain consists of four major subdomains linked by four flexible structural connectors with highly conserved sequences (Fig. 2B). The connectors located on the periphery of the subdomains can readily change conformation, in coordination with movements of the subdomains relative to one another. The converter (which leads directly to the lever arm) has by far the greatest potential for movement among the subdomains as it is connected to the lower 50-kDa and amino-terminal subdomains by only two deformable connectors (the relay and

the SH1 helix, respectively). These features enable the converter to amplify movements of the subdomains, which are further amplified by the light chain domain acting as a lever arm (Fig. 2). Internal coupled rearrangements of the subdomains allow direct communication between the nucleotide-binding site, the actin-binding interface, and the lever arm. Coupling between the actin- and nucleotide-binding sites is mediated through the large cleft between the upper and lower 50-kDa subdomains that separates the actin-binding interface into two distinct sites and communicates with the γ -phosphate pocket through a connector called Switch II (see Fig. 2). In the pre-power stroke state, partial closure of the 50-kDa cleft involves only the inner part of the cleft (near Switch II) trapping the hydrolyzed phosphate. In the nucleotide-free myosin V rigor-like state, total closure of the outer and inner clefts generates a new actin-binding interface with a strong affinity for actin.

More recent structures showed that closure of the cleft when myosin binds an actin filament requires additional subdomain movements, including distortion of the seven-stranded β -sheet and associated loops (including the much studied loop 1) and linkers (referred to as the “transducer” region of the myosin motor) underlying the nucleotide pocket. These changes allow a relative movement of the amino-terminal and upper 50-kDa subdomains and the

associated elements of the nucleotide-binding site (P-loop and Switch I; see Fig. 2). These changes also lead to release of MgADP and movement of the lever arm, resulting in the nucleotide-free (rigor) structure.

A recently described structural state reveals that the initial binding of myosin to actin in the pre-power stroke state rearranges the interface of myosin with actin, opening an escape route for the trapped γ -phosphate (Llinas et al. 2015). Dissociation of phosphate from the active site allows the actin interface to rearrange further, likely closing the cleft near actin, coupled to a large movement of the lever arm. The lever moves further when MgADP is released, completing the power stroke on actin.

2.2 Myosin Motor Mechanism

The conformational changes in the myosin head during the ATPase cycle are the key to understanding the mechanism of

movement (Fig. 3). Myosin rapidly hydrolyzes ATP in the absence of actin, but rapid release of the γ -phosphate and ADP requires interaction with an actin filament. Once phosphate and ADP have been released, ATP rapidly rebinds to the actin-bound myosin, causing rapid dissociation from actin. Although all classes of myosin have the same basic kinetic cycle, the rates of transitions between the states are highly variable. This allows myosin motors to be “kinetically tuned” for a variety of cellular functions by not only setting the rate of the ATPase cycle, but also the fraction of the cycle that the myosin spends in strong actin-binding (force generating) states. The fraction of the ATPase cycle in the strongly bound states is called the “duty ratio.”

Fast skeletal muscle myosin forms thick filaments, and so the motors function in large ensembles. These myosins have a low duty ratio to maximize speed of shortening and power output. The cross-bridges spend most of the ATPase cycle detached or weakly attached to an actin filament be-

Figure 3. Myosin ATPase cycle on actin. The myosin states that bind weakly to actin and, thus, do not bear load or generate force and movement, are shown as noncolored. The myosin states that bind strongly to actin and generate force and movement are colored in shades of blue, becoming darker as the binding affinity increases. The binding of ATP to the rigor state (1) terminates the power stroke by causing myosin to dissociate from actin, forming the postrigor (PR) state (2). The repriming of the lever arm, known as the recovery stroke (3), occurs when myosin dissociated from actin in the PR state (or ATP state) undergoes an isomerization and hydrolyzes ATP to form the pre-power stroke state (PPS). Once the ATP is hydrolyzed, weak binding of the PPS myosin to actin can trigger a transition to the phosphate release (P_i R) state (4), which likely involves some movement of the lever arm and formation of higher-affinity, stereo-specific binding to actin. Release of phosphate then drives the reaction forward (essentially unidirectional) with a large movement of the lever arm to form the strong ADP state (5). A further isomerization of the motor leads to the release of phosphate and is coupled to a further movement of the lever arm, completing the myosin power stroke on actin (6).

cause of rapid detachment of the strongly bound states. This allows the actin filament to move rapidly with drag from cross-bridges that are not producing force, although it limits the maximum force produced when the resistance limits shortening.

High duty ratios allow some two-headed myosins to walk processively on an actin filament. This depends on the myosin making multiple interactions with actin without releasing from the actin filament. The first examples of processive myosins were myosin Va (De La Cruz et al. 1999; Mehta et al. 1999) and myosin VI (De La Cruz et al. 2001; Rock et al. 2001). Each head has a duty ratio that approaches unity at high actin concentrations. This ensures that, when ATP binding detaches one head from actin, the other head will remain in a strong binding state long enough for the detached head to hydrolyze the ATP and rebind to a more distal actin-binding site. In this manner, myosin V and VI can processively “walk” along an actin filament in a “hand-over-hand” fashion, as shown directly by observing fluorescent tags on single myosin molecules (Yildiz et al. 2003, 2004; Okten et al. 2004).

Two features of the ATPase cycle make myosin Va a processive motor with a high duty ratio (De La Cruz et al. 1999). First, actin-activated phosphate release is fast rather than slow and rate limiting, like myosin II. Second, myosin Va releases ADP slowly, so myosin–MgADP strongly bound to the actin filament is the predominant steady-state intermediate of the cycle. The rate of the ADP release step is highly dependent on the strain on the cross-bridge, and so load can slow ADP release and increase the duty ratio of myosin V and other myosins.

2.3 Myosins with Divergent Properties

The more than 40 known classes of myosin (Odrionitz and Kollmar 2007) all follow the blueprint given in Figure 1. There are 13 classes of myosin expressed by humans, which are encoded by 40 genes for myosin heavy chains. The features of these myosins and their known and/or putative functions are summarized in Table 1.

The first myosin discovered was isolated from the thick filaments of muscle and is now called myosin II, or conventional myosin. Fungi, amoebas, and animals have genes for nonmuscle isoforms of class II myosins, but the eukaryotes on other branches from the last eukaryotic common ancestor lack myosin II, including algae, plants, ciliates, and many single-celled organisms. Myosin II powers cytokinesis (Glotzer 2016) and plays roles in cellular locomotion and the establishment of cellular polarity during development (Bresnick 1999).

The second class of myosin discovered, myosin I, was isolated from *Acanthamoeba* (Pollard and Korn 1973) and

later shown to be broadly distributed among and within eukaryotic organisms. Thus, the gene arose early in eukaryotic evolution (Odrionitz and Kollmar 2007). Myosin I has a single head and lacks a coiled coil. The nomenclature of myosin I and II referred to the number of myosin heads per molecule, whereas all subsequent myosins discovered are numbered in their order of discovery.

Some members of the myosin family of motor proteins can move cargo as single (two-headed) molecules. The first example was myosin V (Mehta et al. 1999), which moves vesicles and other cargo processively along actin filaments, analogous to kinesin moving vesicles on a microtubule. The term “processivity” describes the ability of these two-headed motor proteins to move continuously along an actin filament (in the case of myosin V) or a microtubule (in the case of kinesin) with at least one head always bound to the filament. If both heads ever dissociated simultaneously, then the motor and cargo would diffuse away. This design is optimal for moving a cargo with a small number of motors but not for muscle contraction, in which many asynchronous motors work together. In the sarcomere of striated muscles, brief interactions of myosin heads with an actin filament under low loads allows for much higher shortening velocities and power output.

The motor domain is the most conserved feature of the myosin superfamily. The differences within the motor domains of myosins are primarily restricted to additions/deletions at the amino terminus of the motor and variations in surface loops, some of which interact with actin.

Myosin VI is an exception to this generality as it has two unique insertions in the motor domain: Insert 1 alters ATP binding under load, and insert 2 reverses the directionality of the motor (Fig. 4). This makes myosin VI the only myosin known to move toward the pointed end of the actin filament (Wells et al. 1999). Repositioning the lever arm allows myosin VI to move in this unconventional direction (Fig. 4).

The length of the light chain/calmodulin-binding component of the lever arm varies within the myosin superfamily, and even within the same class of myosin. These domains comprise an α -helix of the heavy chain with one to six IQ (isoleucine glutamine) motifs that bind calmodulin or related light chains. All myosin II isoforms have two IQ motifs that bind essential and regulatory light chains. Calmodulin occupies most IQ motifs in unconventional myosins, but some unconventional myosins use the same light chains as myosin II. For example, each head of chicken myosin V contains two light chains and four calmodulins (Espindola et al. 2000).

The IQ motifs with associated calmodulins/light chains function as a lever arm for the myosin motor. Al-

Table 1. Myosin superfamily members in humans

MYOSIN CLASS	Heads	Lever arm	Tail composition	Possible function
CLASS I				
Myo1 (short-tailed) MYO1A MYO1B MYO1C MYO1D MYO1G MYO1H	Monomers	2–6 IQ motifs	Extended PH domain (includes TH1 motif)	Lipid membrane binding; force sensors or anchors; slow transporters; membrane tensioning; membrane remodeling
Myo1 (long-tailed) MYO1E MYO1F	Monomer	1 IQ motif	Extended PH domain (includes TH1 motif), plus TH2 domain: proline-rich, actin binding, plus TH3 domain: SH3 domain	Binds actin-polymerization regulators; promotes cell motility; regulates intracellular transport
CLASS II				
Striated muscle MYR1-fast IIX MYR2-fast IIA MYR3-embryonic MYR4-fast IIB MYR6-Cardiac α MYR7-Cardiac β /Slow MYR7B/14-Slow tonic MYR8-neonatal MYR13-Fast extraocular MYR15-Slow extraocular MYR16-Superfast (not expressed in humans) Smooth/nonmuscle MYR9-NMIIa MYR10-NMIIb MYR11-Smooth MYR14-NMIIc	Dimer	2 IQ motifs	Long coiled coil—forms myosin filaments	Contraction of actin filaments in muscle and nonmuscle cells
CLASS III				
MYO3A	Monomer	2 IQ motifs	THDI motif: espin1 binding; THDII motif: actin binding motif THDI motif: espin1 binding	Amino-terminal extension kinase domain; cargo carrier
MYO3B				
CLASS V				
MYO5A MYO5B MYO5C	Dimer	6 IQ motifs	Long coiled coil—does not form filaments	Cargo transporter and tether; trafficking; recycling pathways
CLASS VI				
MYO6	Regulated dimer	1 Reverse gear CaM site, 1 IQ CaM, 1 weak CaM site (dimer)	Dimerization involving unfolded helical bundle; SAH domain follows bundle; carboxy-terminal cargo-binding domain	Reverse-direction cargo transporter and actin anchoring; trafficking; recycling pathways
CLASS VII				
MYO7A MYO7B	Regulated dimer	5 IQ motifs	MyTH4–FERM–SH3–MyTH4–FERM	Vesicle transporter and force sensor (?)
CLASS IX				
MYO9A MYO9B	Monomer	4 IQ motifs	Zn-binding domains, RhoGAP domain	Amino-terminal Ras-binding domain; membrane signaling
CLASS X				
MYO10	Regulated dimer	3 IQ motifs SAH domain	Antiparallel coiled coil; PH domain; MyTH4–FERM	Filopodia formation; spindle orientation; mediates migration, adhesion and invasion

Continued

Table 1. Continued

MYOSIN CLASS	Heads	Lever arm	Tail composition	Possible function
CLASS XV				
MYO15A	Regulated dimer?	3 IQ motifs	Possible weak coiled coil; MyTH4–FERM–SH3–MyTH4–FERM	Amino-terminal extension; plus end transport to tips of stereocilia
MYO15B				
CLASS XVI				
MYO16	Monomer	1 IQ motif	Proline-rich, but poorly defined	Amino-terminal extension with ankyrin repeats (binds PP1c); undefined signaling roles
CLASS XVIII				
MYO18	Dimer	2 IQ motifs	Coiled coil that forms antiparallel tetramer	Amino-terminal extension containing actin-binding region and PDZ domain; binds nonmuscle myosin II and might organize myosin II filament formation
CLASS XIX				
MYO19	Monomer	3 IQ motifs	Globular tail that targets mitochondria	Movement and partitioning of mitochondria

CaM, Calmodulin; FERM, 4.1 protein, ezrin radixin moesin; GAP, GTPase-activating protein; IQ, isoleucine glutamine (calmodulin-binding motif); MyTh, myosin tail homology; PH, pleckstrin homology; SAH, single α -helical; SH3, Src-homology 3; TH, tail homology; THD1, tail homology D1.

tering the length of the lever arm changes the step size of the myosin movement, as well as the ratio between speed of movement and ATP consumed. The lever arm region of some myosin classes, such as myosin X, is extended by a single, stable α -helix that follows the IQ motifs. Myosin VI has a three-helix bundle that unfolds to form a lever arm extension when two myosin VI subunits dimerize. The amount of flexibility within these lever arms (creating flexible “joints”) is likely quite variable and might be crucial for the cellular functions of different myosin classes. Relatively

stiff lever arms might make movement through dense actin networks difficult.

The regions of the myosin heavy chain carboxy-terminal to the light chain-binding domain are more variable than the heads. All two-headed myosins have a coiled-coil region, but the most carboxy-terminal region is different in each class of myosin, likely owing to different roles in cargo binding. Only the myosin II class appears to have a carboxy-terminal coiled-coil domain that promotes filament formation.

Figure 4. Reversal of directionality by repositioning the myosin lever arm. On the *left*, the movement of the myosin V lever arm on two actin monomers of a filament is depicted. The rigor position is depicted as an opaque arm, whereas the swing began near the pre-power stroke position (PPS), indicated by a transparent lever arm, on actin. The converter subdomain, which is the last subdomain of the motor, as well as the first component of the lever arm, is indicated in red. On the *right*, the myosin VI motor and its two calmodulins are indicated in rigor on actin. The converter is indicated in green, and note that its lever arm points in the opposite direction (toward the pointed end) of the actin filament as compared with myosin V. In the *middle* is a comparison of the high-resolution structures of the myosin V and VI converters, shown in red and green, respectively. Note that an insert (insert 2, shown in dark purple) immediately following the last helix of the myosin VI converter redirects the lever arm and binds a calmodulin (CaM; pale purple). This insert is solely responsible for reversing the direction of myosin VI.

2.4 Myosin Regulation and Cargo Binding

A number of different mechanisms regulate myosin motor activity. The primary regulation of the actin–myosin II interaction in vertebrate striated muscle is through a calcium-dependent troponin regulatory complex bound to the actin filament (Sweeney and Hammers 2016). Other myosins are autoinhibited by internal interactions that can be overcome by phosphorylation of the regulatory light chain or cargo binding. Some systems use two of these mechanisms.

Dimeric motors such as myosin II and V can fold into autoinhibited inactive states. The tails of nonmuscle and smooth muscle isoforms of myosin II interact with the heads, inactivating free dimers. The heads are trapped in the pre–power stroke state with bound MgADP and P_i unable to leave the active site (Wendt et al. 2001). Phosphorylation of the regulatory light chains frees the heads from intramolecular interactions and allows the myosin to polymerize and hydrolyze ATP during cycles of interaction with actin filaments.

The heads of myosin II isoforms of vertebrate striated muscle appear to pack in thick filaments similar to the inhibited smooth muscle myosin dimer (Woodhead et al. 2005). This packing modulates but does not turn off their activity. As the packing traps the myosin heads in the pre–power stroke state, ATP turnover is very slow, so this has been called a “superrelaxed” state. As in smooth muscle, phosphorylation of the regulatory light chains frees these heads for the ATPase cycle and interaction with actin filaments. In many invertebrate striated muscles, packing of the myosin heads on the thick filament backbone is tight enough to require phosphorylation of the regulatory light chains for activity. Troponin and tropomyosin on the thin filaments also regulate these invertebrate striated muscles and, thus, they have a dual regulatory system.

Myosin V is autoinhibited by interactions of the tails with the heads (Liu et al. 2006; Thirumurugan et al. 2006). The folding appears to stabilize the postrigor state of the head, which cannot hydrolyze ATP and interacts weakly with actin. Cargo binding to the tails frees the heads to first hydrolyze ATP and form the pre–power stroke state, which can bind to actin filaments and produce force.

Interaction with cargo also appears to activate myosins VI, VII, and X, but through a rather different mechanism than for myosin V. Unlike myosin V, myosins VI, VII, and X are monomeric in cells unless they interact with cargo. All three motors contain weak coiled coils and intramolecular interactions that result in a folded monomer. On interaction with cargo, the monomer unfolds and dimerizes with another unfolded monomer bound to the same cargo. This cargo-mediated dimerization allows

the motor to then perform its function on actin (Phichith et al. 2009).

3 KINESINS

Kinesin, now known as kinesin-1, was the first molecular motor found to move cargo along intracellular microtubules (Vale et al. 1985). A highly conserved, ~340-amino-acid residue motor domain was identified at the amino terminus of kinesin-1 (Fig. 2). This domain is now known to be shared by a broad superfamily of kinesin-related proteins, divided up into 14 families based on sequence comparisons (Lawrence et al. 2004). Up to 45 distinct kinesin-related proteins are expressed in humans (Miki et al. 2001), providing cells with a broad and diversified kinesin motor proteome.

Within the kinesin superfamily, the conserved motor domain is fused to an array of distinct protein association and cargo-binding domains. Most kinesins are oligomeric, with interactions mediated by diverse protein-association domains, most commonly coiled-coil motifs. Many kinesins are dimeric, such as the canonical kinesin-1 motor, but both hetero-trimeric (kinesin-2) and tetrameric kinesins (kinesin-5) have been characterized. Unique tail domains allow the motor domain to be targeted specifically to different cargos in the cell. Additional domains mediate either intramolecular or intermolecular interactions that allow for specific regulatory control, for example, by autoinhibition.

Although all members of the kinesin superfamily share significant sequence identity in their motor domains, not all superfamily members function as motors in cells. Instead, the primary cellular function of some kinesins is to regulate microtubule dynamics. Some of these kinesins can both move processively along microtubules and selectively modulate microtubule dynamics (kinesin-8), whereas others show no motor activity, and instead act solely to destabilize or depolymerize microtubule plus ends (kinesin-13).

3.1 Kinesin-1 Structure

Figure 1 shows the modular organization of the prototypical kinesin-1 motor in comparison with other members of the kinesin superfamily (Fig. 5A). There is a highly conserved motor domain at the amino terminus of kinesin-1 (Fig. 5B), followed by stalk and tail domains. Two heavy chains dimerize through their extended coiled-coil domains to form a dimeric complex (Fig. 5C). Two kinesin light chains (KLCs) often, but not always, associate with the carboxy-terminal ends of the heavy chains. These light chains contribute to cargo association and regulation of motor activity, but are not necessary for motor function.

Figure 5. Structure and mechanochemistry of the kinesin superfamily. (A) Kinesin superfamily members share a conserved motor domain but diverge in domains mediating self-association, such as coiled-coil domains and cargo-binding domains. (B) Ribbon diagram of the crystal structure of the kinesin-1 motor domain (from Kull et al. 1996). (C) Kinesin-1 is formed from the homodimerization of two heavy chains. The motor domains are regulated by autoinhibition; the tail domain binds to and inhibits the motor domains. Autoinhibition is relieved by association with specific binding partners. Kinesin-5 (Eg5) forms a bipolar, tetrameric motor. (D) Kinesin-1 moves processively along the microtubule through the coordinated stepping of the two motor domains. Binding of ATP (T) induces a high-affinity association of the head with the microtubule; release of the products of ATP hydrolysis, ADP (D) and P_i , allows dissociation of the head from the microtubule track. (Modified from Milic et al. 2014.)

The kinesin-1 motor domain binds to microtubules in a nucleotide-dependent manner (Fig. 5D). Structural analyses of the motor domain (Fig. 5B) have provided insights into mechanisms of force production (Kull et al. 1996). These studies also produced the rather surprising insight that the kinesin motor domain shares structural homology with myosin motors, although there is little apparent sequence similarity. The motor domains of both kinesins and myosins are built around a core seven-stranded β -sheet flanked by three α -helices on either side (Kull and Endow 2013). Within this common core, similar elements can be identified, including a nucleotide-binding P-loop, Switch I and II motifs, and a relay or α 4-helix (Kull and Endow 2013). Similar rearrangements in these elements occur in both kinesin and myosin motors as they alternate between

open and closed conformations during the catalytic cycle of nucleotide hydrolysis and force production.

3.2 Kinesin Motor Mechanism

Although some of the structural elements in kinesin resemble those identified in myosins, the kinetic cycles of the motor families are distinct. For kinesin-1, ATP binding induces a state that binds tightly to the microtubule, and subsequent ATP hydrolysis allows the formation of a more weakly bound state (Fig. 5D). There are mechanical differences as well, as kinesin-1 lacks the lever arms characteristic of myosins. Instead, a neck-linker element of ~ 15 amino acid residues at the carboxyl terminus of the motor domain undergoes a nucleotide-dependent conformational change

between a structured, docked state in the ATP-bound form and a more mobile conformation attained following phosphate release (Rice et al. 1999). Neck-linker docking biases directional movement toward the plus end of the microtubule (Clancy et al. 2011; Rice et al. 1999).

Kinesin-1 moves along the microtubule with an alternating, hand-over-hand mechanism that depends on the dimerization of the two heavy chains. The alternating stepping of the two heads is coordinated by the intramolecular strain generated during active translocation along the microtubule, with docking and undocking of the neck-linker element also key (Rice et al. 1999; Rosenfeld et al. 2003; Yildiz et al. 2008; Clancy et al. 2011; Milic et al. 2014). These mechanisms lead to highly processive motility—once bound to its track, a dimeric kinesin-1 motor will move approximately 100 steps before detaching from the microtubule (Block et al. 1990).

Kinesin-1 effectively uses the energy released by ATP hydrolysis to drive movement. Hydrolysis of a single ATP molecule drives an 8-nm step along the microtubule (Schnitzer and Block 1997). This 8-nm step corresponds to the distance between neighboring tubulin dimers within the microtubule lattice (reviewed by Herrmann and Aebi 2016). Kinesin-1 moves unidirectionally toward the microtubule plus end, tracking along a single protofilament of the microtubule (microtubule structure is discussed by Herrmann and Aebi 2016).

Kinesin-1 is a relatively fast motor, moving at 0.5–1 $\mu\text{m}/\text{sec}$ in vitro and in cells, and produces a unitary stall force of 6 pN (Svoboda and Block 1994; Mallik et al. 2013). This force is sufficient to move vesicular cargo through the cell. Kinesin-1 is more effective as a single motor than functioning in teams. Experiments both in vitro and in cells indicate that, even when multiple kinesin-1 motors are bound to cargo, a single kinesin-1 productively interacts with the microtubule at any given time (reviewed in Mallik et al. 2013). This contrasts with dynein motors, discussed below, which are weaker but can function more productively to generate force by working in teams.

3.3 Motor Regulation and Cargo Binding

The tail domain of kinesin-1 has two major functions—association with cargos and autoregulation. Structural studies have shown that the tail domain of kinesin-1 can fold back (Fig. 5C), allowing an IAK motif to bind between the two heads (Kaan et al. 2011). With the tail bound between the heads, the motor is effectively turned off. Release of the IAK motif allows the motor to unfold to form an extended, active conformation; the transition between the inhibited and active states is tightly regulated in vivo.

Both the tail domain of kinesin heavy chain and the associated KLCs are implicated in cargo binding. Kinesin-1 transports a wide variety of cargos through the cell, including mitochondria, late endosomes, and lysosomes. Multiple interactions between the motor and cargo-bound proteins might mediate the specificity of motor recruitment. Cargo binding to the kinesin tail domain was initially proposed to activate the motor by competitively inhibiting the head–tail interaction, but more-recent work suggests that cargo-bound kinesin-1 motors might remain autoinhibited. Rather, activation of autoinhibited kinesin motors is likely to be tightly regulated through interactions with specific adaptor or scaffolding proteins, including JIP1 and JIP3 (reviewed by Fu and Holzbaur 2014).

3.4 Kinesin Superfamily Motors: Kinesin-2 and -3

Motors from two additional kinesin families have major roles in driving vesicular and organelle transport in the cell. Kinesin-2 and -3 motors broadly share structural and mechanistic similarities with kinesin-1, but differences among these motors suggest that they are adapted to specific cellular functions.

For example, although kinesin-1 motors function as obligate dimers, kinesin-2 motors can form either homodimers or heterotrimers (Scholey 2013). Kinesin-2 motors drive intraflagellar transport (IFT; reviewed by Barlan and Gelfand 2016), and also power the movement of diverse intracellular cargos, including melanosomes, late endosomes/lysosomes, and fodrin- and choline-acetyltransferase-positive vesicles in neurons (reviewed in Scholey 2013). Biophysical studies revealed that kinesin-2 motors exert forces along the microtubule of similar magnitude to those generated by kinesin-1 motors, but might be more susceptible to detachment in response to opposing forces (Schroeder et al. 2012). Kinesin-2 motors are more likely to switch protofilaments during movement along the microtubule, whereas kinesin-1 motors tend to detach rather than switch tracks on encountering roadblocks such as microtubule-associated proteins (MAPs) (reviewed by Herrmann and Aebi 2016) bound to the microtubule (Dixit et al. 2008; Hoeprich et al. 2014). These more flexible responses might represent selective adaptations of kinesin-2 to specific functions in the cell.

Motors in the kinesin-3 family can be essential for intracellular transport; for example, loss of the kinesin-3 motor Unc-104 leads to a dramatic inhibition of synaptic vesicle transport in the nematode *Caenorhabditis elegans* (Hall and Hedgecock 1991). Kinesin-3 motors without bound cargo are monomeric owing to an autoinhibitory interaction of a neck-coil motif that associates intramolecularly with a coiled-coil domain in the carboxy-terminal

part of the molecule. Binding to cargo increases the local concentration of the motor, favoring the formation of intermolecular dimers. The resulting kinesin-3 dimers are active and, in fact, superprocessive, capable of runs of up to 10 μm along microtubules, leading to the suggestion that they are the “marathon runners” of intracellular transport (Soppina et al. 2014).

3.5 Kinesins with Divergent Properties

Most kinesin motors are plus end–directed, meaning that they move in a processive manner toward the more dynamic plus end of the microtubule (see Herrmann and Aebi 2016). However, the kinesin-14 motor Ncd moves toward microtubule minus ends (McDonald et al. 1990). Ncd also has a distinct primary structure, with the conserved kinesin motor domain localized to the carboxy-terminal, rather than amino-terminal, end of the polypeptide. Structural studies suggest that the key determinant of directionality is a coiled-coil element in the neck that rotates toward the microtubule minus end on ATP binding to the motor domain and, thus, biases movement in this direction (Endres et al. 2006).

Motors from the kinesin-5 family are also structurally and functionally distinct from other types of kinesins. Rather than forming a dimer, the kinesin-5 motor Eg5 forms a tetramer that can cross-link and slide overlapping microtubules (reviewed by Goulet and Moores 2013). Importantly, both Ncd and Eg5 function in cell division, and so their divergent properties reflect structural and functional adaptations to specific cellular roles in mediating the dynamics of the highly ordered meiotic and mitotic spindles (see Cheng and Eriksson 2016).

3.6 Kinesins Regulating Microtubule Dynamics

The most unexpected properties identified for kinesin superfamily proteins to date are the abilities of kinesin-8, -13, and -4 motors to actively regulate microtubule dynamics (Desai et al. 1999; Varga et al. 2006; Walczak et al. 2013). Cells use these activities in diverse functions, including spindle assembly and dynamics, axon growth and regeneration, and regulation of ciliary length (reviewed by Walczak et al. 2013).

Members of the kinesin-8 family function as both motors and microtubule depolymerases (Varga et al. 2006). Kinesin-8s are highly processive, with long runs toward the plus end of the microtubule. The enhanced processivity shown by motors in this family is because of a secondary microtubule-binding site in the tail domain that prevents release from the track. Once the motors reach the plus end of the microtubule, they catalyze the removal of the termi-

nal tubulin subunits and/or block the binding of new subunits (reviewed by Walczak et al. 2013).

In contrast, members of the kinesin-13 family, including MCAK and Kif2A, lack motor activity (Desai et al. 1999; Walczak et al. 2013); instead these proteins diffuse along the lattice of the microtubule. Once reaching either the plus end or minus end of the microtubule, kinesin-13s increase the frequency of catastrophes (Herrmann and Aebi 2016) by inducing protofilament peeling (reviewed in Walczak et al. 2013). Kinesin-13 motors play key roles in cell division, and also in axon outgrowth and regeneration. Because they are effective depolymerases, their activities are carefully regulated, for example, by the upstream kinase Aurora B (Walczak et al. 2013).

Members of the kinesin-4 family also modulate microtubule dynamics, but their mechanisms appear to vary. For example, Kif21A is a processive plus end–directed motor that stabilizes microtubule ends by suppressing catastrophe (van der Vaart et al. 2013). In contrast, the related Kif7 motor neither moves processively nor diffuses along the microtubule (He et al. 2014) but instead binds preferentially to GTP-bound tubulin subunits at dynamic microtubule ends and increases the frequency of catastrophes. In vivo, Kif7 is important for maintaining the structure of immotile primary cilia. Although the underlying mechanism is not yet clear, Kif7 function is essential for normal embryonic development.

3.7 Summary of the Diverse Kinesin Superfamily

Molecular, biophysical, and cellular experiments to date fully support a modular “Lego” model for the kinesin superfamily—with a relatively small, highly conserved motor domain fused to a diverse array of tail domains, allowing motors to bind to and move a broad array of cargo. Furthermore, the signature “motor” domain that defines the kinesin superfamily has diverged over time, leading to family members that no longer function as motors. This loss of motor function has been coupled to a surprising gain of function, in that several members of the kinesin superfamily can actively remodel the microtubule tracks that they move on. This elegant model of diversification of structure and function over time parallels the development of the myosin superfamily but differs significantly from the second major group of microtubule motors, the dyneins, discussed below.

4 DYNEINS

Dynein motors were initially identified and purified from the axonemes of eukaryotic cilia and flagella (Gibbons and Rowe 1965; reviewed by Sweeney and Hammers 2016).

Axonemal dyneins drive the sliding of adjacent microtubule doublets in axonemes; this sliding is coordinated to produce waveforms that drive the swimming of single-celled eukaryotes such as *Chlamydomonas* (Sweeney and Hammers 2016). The diverse family of axonemal dyneins shares both structural and sequence similarity with cytoplasmic dynein, which drives motility along intracellular microtubules. However, unlike the diverse set of axonemal dyneins and the extended kinesin superfamily described above, there is a single cytoplasmic dynein motor that functions as the major minus end-directed microtubule motor in the cell, driving the movement of vesicles, organelles,

proteins, and RNA particles in interphase and spindle assembly and dynamics in dividing cells.

4.1 Dynein Structure

Dyneins are large and complicated cellular motors (Fig. 6A–C). The motor domain is found within dynein heavy chain (DHC), a polypeptide of ~500 kDa (Fig. 6A). The carboxyl terminus of DHC includes six tandem repeats of the AAA (“ATPases associated with diverse cellular activities”) domain, a conserved module found in a divergent array of proteins, including enzymes that remodel mem-

Figure 6. Structure and mechanochemistry of dynein. (A,B) The dynein heavy chain, the motor subunit of dynein family motors, is a ~500-kDa polypeptide with a conserved structure in which an amino-terminal tail domain is followed by a linker domain and six concatenated AAA domains. AAA1 is the site of catalytic ATP hydrolysis; nucleotide binding to other AAA domains might exert a regulatory effect on motor properties. The microtubule-binding domain is located at the tip of the protruding stalk. C-term, carboxyl terminal. (C) Ribbon diagram of the crystal structure of cytoplasmic dynein-2 (Schmidt et al. 2015). (D) ATPase pathway for dynein (D), emphasizing the role of nucleotide binding and hydrolysis in the transition between states that bind either strongly or weakly to the microtubule (M). (Modified from Holzbaur and Johnson 1989, and Imamura et al. 2007.) (E) The bend in the linker element that spans the AAA-ring of dynein plays a key role in the structural transitions between the apo (no nucleotide bound) and pre-power stroke states (modified from Carter et al. 2016).

branes and sever microtubules (Roberts et al. 2013). Most AAA proteins consist of six separate polypeptides that associate to form a hexameric ring. In dynein, these six modules (AAAs 1–6) are concatenated into a single polypeptide that folds to form a hexameric ring (Carter et al. 2011; Kon et al. 2011). The first AAA domain in the dynein sequence, AAA1, is the primary site of ATP binding and hydrolysis required for motor function (Gibbons et al. 1987). Nucleotide binding at AAA3 and AAA4 allosterically affects dynein activity (Kon et al. 2004).

The hexameric structure of the dynein head is large, with a diameter of ~11 nm. Somewhat surprisingly, the microtubule-binding domain is not part of this ring. Instead, it is located to the tip of a ~15 nm stalk (Fig. 6A,B), an antenna-like structure that extends from the ring, projecting between AAAs 4 and 5 (Gee et al. 1997). During the ATP hydrolysis cycle, nucleotide-induced changes in the conformation of the ring are transmitted to this microtubule-binding site, localized ~25 nm away, via both the stalk and an element termed either “buttress” or “strut” that further connects the stalk to the ring (Roberts et al. 2013). A linker domain (Fig. 6B), crucial to the force-generation mechanism, arches over the ring, connecting AAA1 to the tail domain (Kon et al. 2012; Roberts et al. 2012).

The amino terminus of DHC is a tail domain that homodimerizes to form the two-headed cytoplasmic dynein motor, or can mediate association with other DHCs to form two- and three-headed axonemal dynein motors. The amino terminus of DHC also interacts with other dynein subunits; for cytoplasmic dynein, these subunits include dynein intermediate chains, light intermediate chains, and associated light chains.

4.2 Dynein Motor Mechanism

Although the structure of dynein resembles neither myosin nor kinesin, the kinetic cycle leading to ATP hydrolysis and force production by dynein is remarkably similar to that of the actin-myosin ATPase (Fig. 6D) (Holzbaur and Johnson 1989; Johnson 1985). ATP binding to AAA1 in the dynein ring induces a transition from a tight microtubule-binding state to a weak binding state, releasing the microtubule binding domain from its cytoskeletal track (Fig. 6D). ATP hydrolysis on the unbound head is followed by rebinding to the microtubule and product release; the power stroke leading to movement along the microtubule likely accompanies either isomerization of the ADP-bound intermediate or ADP release (Holzbaur and Johnson 1989).

Three-dimensional structures of axonemal dynein motors in situ obtained by cryo-electron tomography show three distinct states of the dynein–microtubule cycle (Lin et al. 2014). In the rigor-bound state before ATP binding,

the dynein head is tightly bound to the microtubule. In the initial pre–power stroke state, the microtubule-binding stalk is in a primed position likely resulting from ATP hydrolysis. A second pre–power stroke state with the head rebound to the microtubule likely corresponds to the ADP-P_i state or a tightly bound ADP state. On release of ADP, the stalk returns to the post–power stroke state. Comparisons of these structures suggest that rotation of the head relative to the linker drives the movement of dynein along the microtubule (Lin et al. 2014). This interpretation is supported by recent structural studies on human cytoplasmic dynein-2 (the motor that powers IFT) in its primed, pre–power stroke state (Fig. 6C,E; see also Schmidt et al. 2015).

4.3 Axonemal Dyneins

In the axoneme—the highly conserved structural array at the core of eukaryotic cilia and flagella (Barlan and Gelfand 2016; Sweeney and Hammers 2016)—dynein motors are bound in a nucleotide-independent manner by their cargo-binding domain to one of the nine parallel outer doublet microtubules. Dynein heads transiently interact with adjacent microtubule doublets in an ATP-dependent manner: ATP binding releases the dynein head from its track, ATP hydrolysis induces a conformational change, allowing the head to rebind, and force production accompanies release of the hydrolysis products ADP and P_i (Fig. 6D). This cross-bridge cycle of ATP hydrolysis results in the sliding of one microtubule doublet versus another. Tight regulation of sliding results in the characteristic waveforms of beating cilia and flagella (Sweeney and Hammers 2016).

Fourteen of the 16 human DHC genes encode axonemal dyneins (Roberts et al. 2013). Axonemal DHCs share a similar primary structure (Fig. 6A), but can assemble into one-, two-, or three-headed motors, along with a complex group of associated intermediate and light chains. Generation of this diversity occurred through gene duplication followed by evolutionary divergence to produce the extended axonemal dynein family, but the overall functional unit is highly conserved.

4.4 Cytoplasmic Dynein (DYNC1H1)

In contrast to the multigene family encoding axonemal dyneins, a single human gene (*DYNC1H1*) encodes the heavy chain of cytoplasmic dynein. This dynein serves as the major minus end–directed motor in the cell, driving the motility of a bewildering array of cellular cargo, including organelles, vesicles, and RNA particles. Cytoplasmic dynein is also required for multiple distinct steps in cell division, including assembly of the mitotic spindle, microtubule–

kinetochore interactions, and the spindle checkpoint (Cheng and Eriksson 2016). A second gene (*DYNC2H1*) encodes a distinct form of dynein adapted for the specialized function of IFT (Barlan and Gelfand 2016).

Five different types of subunits interact with the heavy chain of cytoplasmic dynein to form the active motor complex, with a combined molecular mass of ~ 1.5 MDa. These associated subunits are called dynein intermediate chains, light intermediate chains (LICs), and light chains (Tctex1, LC8, and roadblock). Variations in the specific isoforms of these subunits bound to the heavy chain are thought to provide specialization in regard to intracellular targeting and/or regulation. For example, there are both ubiquitous and neuron-specific isoforms of dynein intermediate chain expressed in mammals. Dynein intermediate chain1, or *DYNC1I1*, is expressed primarily in neurons, and preferentially associates with Trk receptors, driving the motility of signaling complexes required for normal neuronal development and function (Mitchell et al. 2012).

4.5 Dynein Effectors: Dynactin, LIS1, BICD2

A broad range of interacting proteins regulates cytoplasmic dynein activity in vivo. The dynactin complex is required for most dynein functions in the cell (Schroer 2004). Dynactin is a large, multisubunit complex, with a dynein-binding sidearm formed from an extended dimer of the coiled-coil protein p150^{Glued}, which binds to and projects from a 37-nm actin-like filament at the base of the complex formed by a polymer of the actin-related protein Arp1. The p150^{Glued} subunit binds directly to the intermediate chain of cytoplasmic dynein and also binds microtubules in an ATP-insensitive attachment (Waterman-Storer et al. 1995). The microtubule-binding domain of dynactin enhances the association of dynein with microtubules (Waterman-Storer et al. 1995; King and Schroer 2000; Ross et al. 2006; Ayloo et al. 2014), whereas the Arp1 filament and additional dynactin subunits contribute to cargo-binding interactions of the dynein–dynactin motor complex (Schroer 2004).

Lis1 is another dynein-binding protein that regulates motor activity in vivo. Lis1 binds directly to the dynein motor domain and acts as a clutch to uncouple the allosteric communication between the site of ATP hydrolysis at AA1 within the dynein ring and the microtubule-binding domain localized to the projecting stalk (Huang et al. 2012). The binding of Lis1 to the dynein ring promotes the strongly bound state (Fig. 6D), resulting in persistent attachment (Huang et al. 2012). Cellular studies in both fungi and neurons indicate that Lis1 plays an essential role in the initiation of dynein-driven transport (Lenz et al. 2006; Moughamian et al. 2013).

Additional effectors such as BICD2 bind to and stabilize the dynein–dynactin complex, enhancing processive motility along microtubules (McKenney et al. 2014; Schlagger et al. 2014; Urnavicius et al. 2015). These effectors might contribute to cargo-specific motility within the cell as they appear to be differentially targeted to intracellular organelles.

4.6 Dynein Biophysics

Single-molecule analysis has revealed that mammalian cytoplasmic dyneins can take steps ranging from 8 to 32 nm on microtubules, much more variable than the uniform 8-nm stepsize observed for kinesin-1. These steps do not follow a single protofilament like kinesin does but can wander across the microtubule lattice. Single dynein motors also frequently step backward (Wang et al. 1995; Mallik et al. 2005; Ross et al. 2006). Although stepping backward might sound counterproductive, the flexible stepping of the dynein motor has some positive consequences. Dynein motors can nimbly respond to obstacles along the microtubule track. For example, upon encountering a MAP bound to its microtubule track, a kinesin-1 motor is likely to detach, whereas dynein motors are more likely to back-step and sidestep and, thus, avoid the obstacle without becoming released from their tracks (Dixit et al. 2008).

Dynein motors also work much better in teams than kinesin motors (Mallik et al. 2013). Single cytoplasmic dynein motors move somewhat erratically along the microtubule and show a low unitary stall force (~ 1 pN), significantly weaker than that of kinesin-1 (~ 6 pN). However, two or more dynein motors bound to the same cargo display unidirectional and highly processive motility, indicating that dynein motors can function effectively in teams (Mallik et al. 2013). Binding partners also enhance the processivity of dynein. For example, the binding of adaptor proteins such as BICD2 enhance the stability of the dynein–dynactin interaction and induce robustly processive motility of the tripartite complex along microtubules (McKenney et al. 2014; Schlagger et al. 2014; Urnavicius et al. 2015).

To date, the most detailed biophysical characterization has been performed on cytoplasmic dynein from yeast, which is a slower, more powerful motor than the mammalian cytoplasmic dyneins discussed above (Reck-Peterson et al. 2006). These studies are fully consistent with previous biochemical studies showing that the two heads of dynein are not kinetically coupled, distinct from the tightly coupled stepping of alternating heads that characterizes kinesin-1 motility (Fig. 5D). Instead, the two dynein heads can move relatively independently along the microtubule until the head-to-head distance reaches its maximum, at which

point intramolecular tension leads to more coordinated stepping of the two heads (DeWitt et al. 2012; Qiu et al. 2012).

Finally, although dynein functions primarily as a motor in both yeast and mammalian cells, dynein can also actively tether microtubule plus ends, leading to selective modulation of polymerization dynamics near the cortex of both dividing and interphase cells (Hendricks et al. 2012; Laan et al. 2012).

5 CONCLUSION

Directed movements and forces in cells are generated by the actions of molecular motor proteins. The three superfamilies of cytoskeletal motor proteins are myosin (which moves on actin filaments) and kinesin and dynein (which move on microtubules). These molecular motors hydrolyze ATP and move on “tracks” within the cell. The tracks myosin uses comprise actin filaments, whereas kinesin and dynein move on microtubules. The basic structures and kinetic properties of these cytoskeletal molecular motors have been described in great detail, but we are still expanding our knowledge of the fine-tuning of these designs that generate different motor classes serving a wide variety of cellular functions. Indeed, we lack a detailed understanding of how many of the cytoskeletal motors function in cells. We also lack a detailed understanding of the structural changes induced by interaction with their respective motor tracks (i.e., actin filaments for myosin, and microtubules for kinesin and dynein). Understanding these induced structural changes is at the heart of the fundamental mechanism of chemo-mechanical transduction, which for the cytoskeletal motors consists of the production of force and movement from the hydrolysis of ATP and the release of the ATP hydrolysis products, coupled to interactions with the motor track.

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