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The Myosin Family of Mechanoenzymes: From Mechanisms to Therapeutic Approaches

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Abstract

Myosins are among the most fascinating enzymes in biology. As extremely allosteric chemomechanical molecular machines, myosins are involved in myriad pivotal cellular functions and are frequently sites of mutations leading to disease phenotypes. Human β-cardiac myosin has proved to be an excellent target for small-molecule therapeutics for heart muscle diseases, and, as we describe here, other myosin family members are likely to be potentially unique targets for treating other diseases as well. The first part of this review focuses on how myosins convert the chemical energy of ATP hydrolysis into mechanical movement, followed by a description of existing therapeutic approaches to target human β-cardiac myosin. The next section focuses on the possibility of targeting nonmuscle members of the human myosin family for several diseases. We end the review by describing the roles of myosin in parasites and the therapeutic potential of targeting them to block parasitic invasion of their hosts.

Contents

INTRODUCTION

Every step we take, every heartbeat, every movement of different muscular organs in our body, and many brilliant innovations of nature such as cell division, cell migration, and vesicular transport are only possible because of force generated by the motor protein myosin as it travels over cytoskeletal actin filaments. The discovery of conventional muscle myosin in the nineteenth century (1) laid the foundation for what is now a vast field of its own: molecular motors, which include a large family of myosins, a similarly large family of kinesins, and the dyneins (2, 3).

The myosin family of molecular motors is found in most eukaryotic organisms, from parasites to humans (2). In humans, there are more than 20 distinct classes and as many as 40 different myosin genes (4). Examples of some myosins across organisms are shown in **Figure 1**. Notable are the variety of tail domains that allow for many of the discrete functions that a particular myosin carries out, including anchoring of myosin molecules to specific sites for movement relative to actin filaments and specifying their binding to different cargoes (4). The tail also determines whether a myosin is single-headed or two-headed, usually by forming a coiled coil of two myosin heavy-chain α-helices in the tail region.

All myosins travel along actin filaments to carry out their functions. The actin cytoskeleton, with dozens of associated accessory proteins, provides the cell with structure and organization. Actin tracks are polarized (**Figure 2**), with barbed or plus ends and pointed or minus ends, and the plus ends are directed toward the plasma membrane or, in the case of striated muscle, the Z-lines of the sarcomere. All known myosins except myosin-VI (5) move along actin toward the plus end.

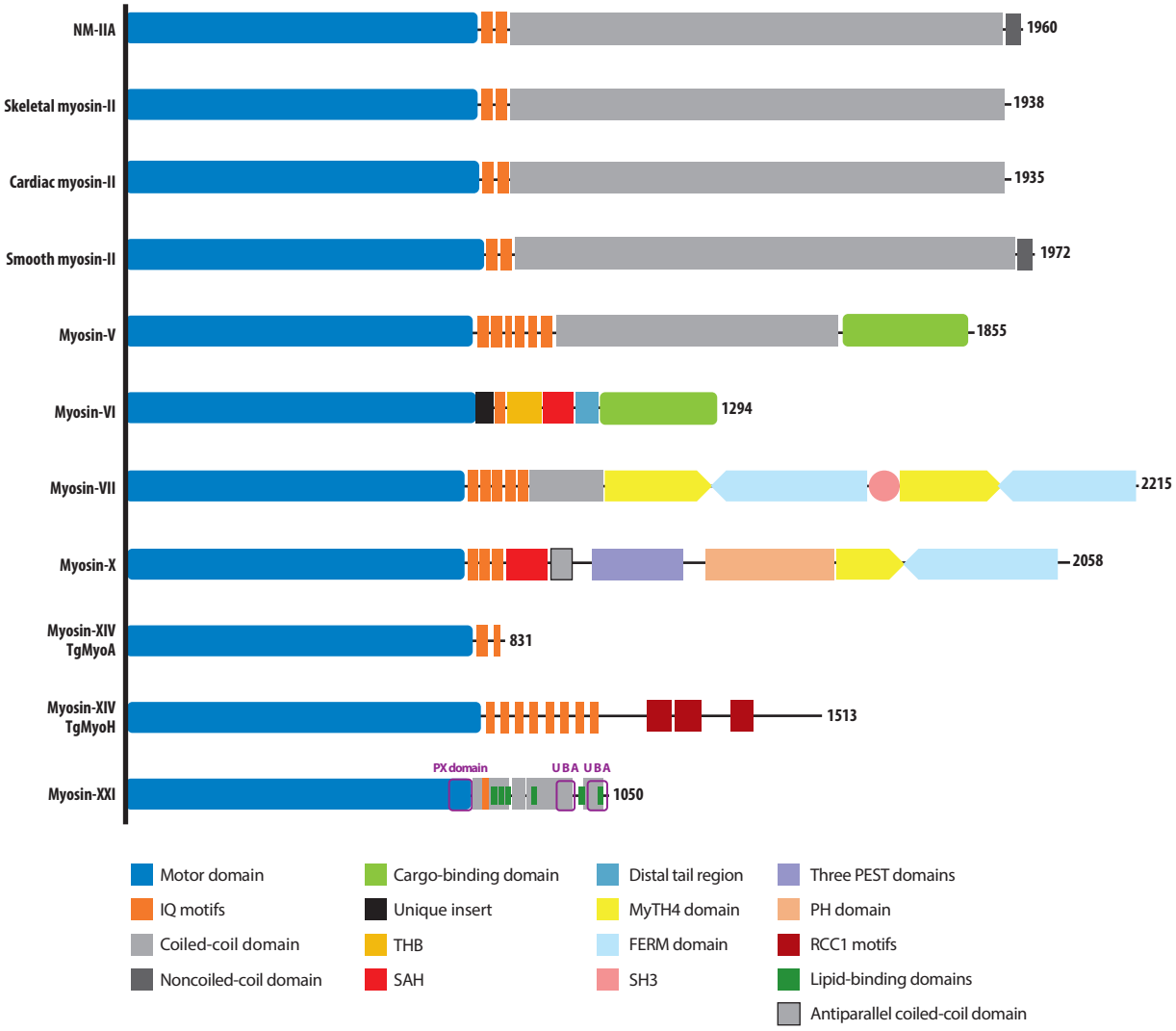

Figure 1

Linear structure depictions of representative members of the myosin family of molecular motors. The N-terminal domain is a globular motor domain (*blue*), followed generally by one or more IQ motifs (*dark orange*) that bind either calmodulin molecules or specialized light chains similar in size to calmodulin. Myosin-VI has a unique insert (*black*) between the motor domain and the IQ domain that redirects the motor movement toward the minus end of the actin filament. Many myosins are dimeric owing to the assembly of two α-helices into a coiled coil (*gray*). Other specialized domains are highlighted by different colors: THB (*light orange*), SAH (*red*), and others as listed in the key. Abbreviations: FERM, 4.1 protein, ezrin, radixin, moesin; IQ, isoleucine-glutamine; MyTH4, myosin tail homology 4; PEST, sequence rich in proline, glutamic acid, serine, and threonine residues; PH, pleckstrin homology; PX, phox homology; RCC1, related regulator of chromosome condensation 1; SAH, single α-helix; SH3, Src homology region type 3; TgMyo, *Toxoplasma gondii* myosin; THB, three-helix bundle; UBA, ubiquitin-associated.

The motor domain of all myosins is the approximately 80-kDa globular myosin head (6) (approximately 780 amino acids), which has an actin-binding domain that is about 4 nm from the nucleotide-binding site, as revealed by the first crystal structure of a myosin head domain (7) (**Figure 3*a***). This basic structure is common to all classes of myosins that have been crystallized

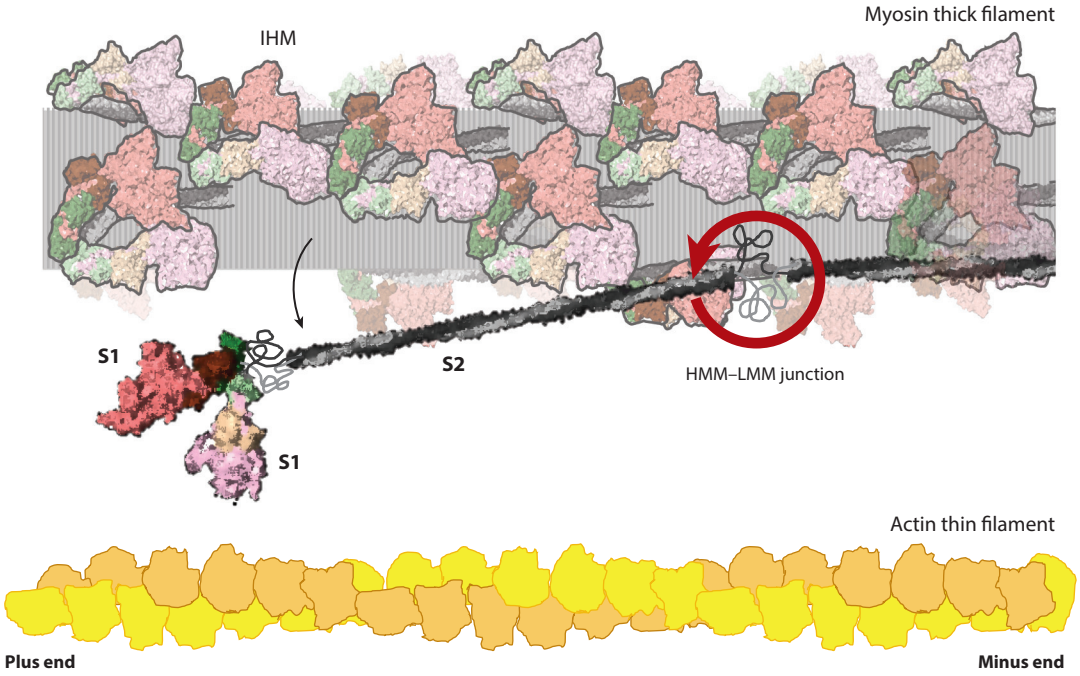

Figure 2

Structure of cardiac myosin and arrangements in the muscle sarcomere drawn to scale. The HMM domain of the myosin consists of the two S1 domains and the S2 domain. LMM is the C-terminal portion of the coiled-coil tail to the right of the HMM–LMM junction. Interactions between the LMM portions of myosin molecules form the shaft of the thick filament. The relative sizes and spacings of the myosin-containing thick filament and the actin-containing thin filament within the muscle sarcomere are shown. The heads folded back onto the shaft of the thick filament are illustrated in their IHM state, with the S1 heads folded back onto their S2 tails (*translucent myosins*). A single myosin molecule released from its IHM state is shown swinging away from the thick filament, swiveling about its HMM–LMM junction. Abbreviations: HMM, heavy meromyosin; IHM, interacting heads motif; LMM, light meromyosin; S1, subfragment 1; S2, subfragment 2.

(8). At the opposite end of the globular head domain from the actin-binding site is the converter, which is immediately distal to two cysteine-containing helices called the SH1 and SH2 helices (**Figure 3*b***). These two helices change their configurations dramatically, associated with the movement of the converter through an extensive angle while bound to actin (**Figure 3*c*,*d***). This movement is accompanied by the release of one of the ATP hydrolysis products, inorganic phosphate (P_i) (**Figure 4*a***). Thus, myosin is a P_i sensor, and the converter rotation associated with P_i release is the basis of all movements of myosins along actin. The step size the myosin takes (its movement along actin per ATP hydrolyzed) is amplified by a long α-helical extension of the globular heavy chain, which is stabilized by association with calmodulin molecules or, in the case of muscle and some nonmuscle myosins, calmodulin-like light chains (approximately 20 kDa each). The example shown in **Figure 2** is the muscle myosin globular head domain known as subfragment 1 (S1), where the lever arm length is about 10 nm, with two light chains bound: the essential light chain (ELC) and the regulatory light chain (RLC) (**Figure 4*b***). Other myosins have longer or shorter lever arms and rotate about different angles to accommodate different functions they serve. However, the essential elements of all myosins are depicted in **Figure 3**.

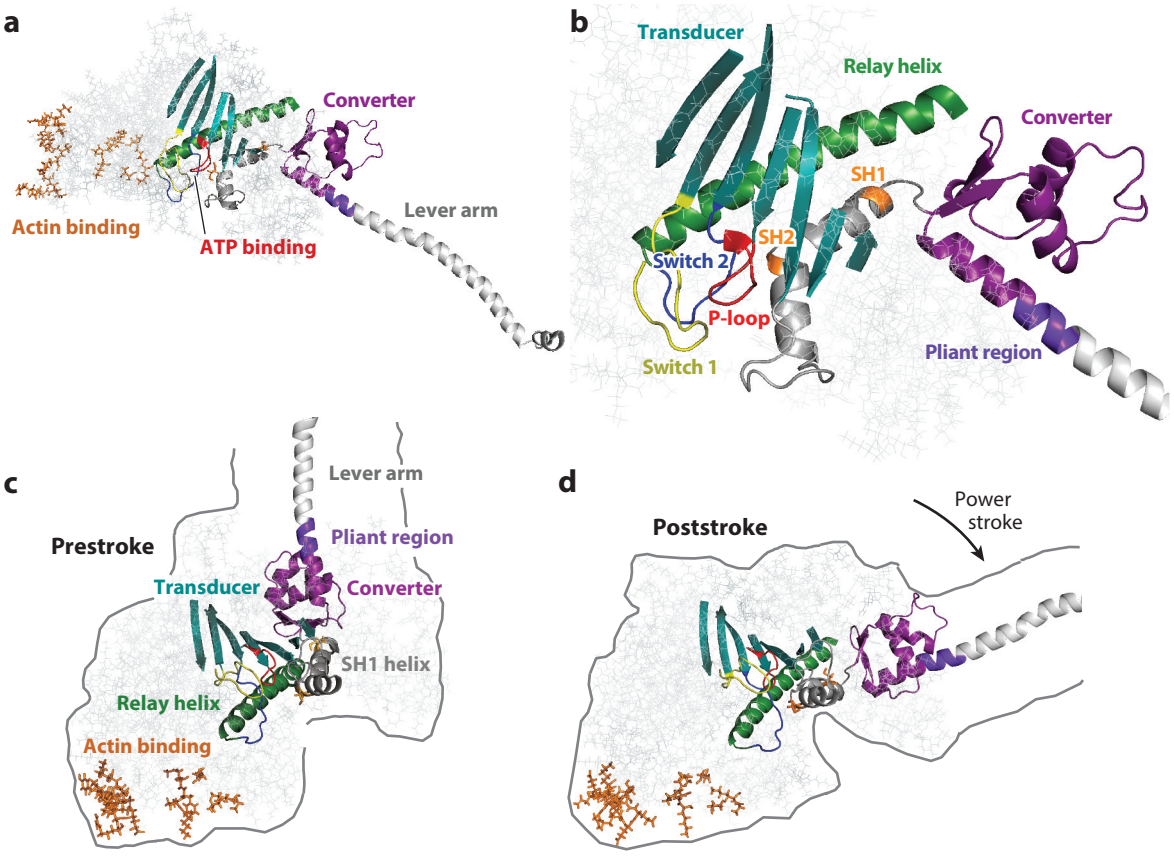

Figure 3

Structures of pivotal sites and structural elements relevant to all myosin motors. (*a*) PyMOL visualization of the myosin head (S1 domain) without the light chains bound to the heavy-chain α-helix (lever arm). Actin-binding residues (*orange*) are in stick mode. The ATP-binding pocket (*red*) is about 4 nm away from the actin-binding surface. The converter (*purple*) links the lever arm (*light gray*, without light chains shown) to the motor domain. (*b*) Enlarged view of the structure shown in panel *a*. The switch 1 (*yellow*), switch 2 (*blue*), and P-loop (*red*) form the nucleotide-binding pocket. The relay helix (*green*), transducer (*dark cyan*), and SH1 and SH2 (*orange residues*) helices (*gray*) are between the nucleotide-binding pocket and the converter (*purple*). A short pliant region (*purple-blue*) links the converter to the lever arm (*light gray*). (*c*) Arrangement of structural elements in the prestroke state of the S1. (*d*) Arrangement of structural elements in the poststroke state of the S1. Abbreviation: S1, subfragment 1.

The transition of the head from a prestroke to a poststroke configuration results from an ensemble of structural changes that add up to a new, lower-energy state of the S1, associated with P_i and ADP release. The S1 returns to its higher-energy prestroke state upon ATP binding and hydrolysis, with the ADP and P_i products remaining bound at the active site. There are many noteworthy changes in the structure of the S1 associated with prestroke to poststroke transition. An important structural element in the motor domain is a long α-helix known as the relay helix (**Figure 3*b***). A primary structural difference between the prestroke state and poststroke states of myosin is the presence of a kink in the relay helix at the beginning of the power stroke (**Figure 3*c***), and upon transitioning to the poststroke state, subsequent straightening of the relay helix accommodates a 60–70° rotation of the converter domain and the attached lever arm for muscle

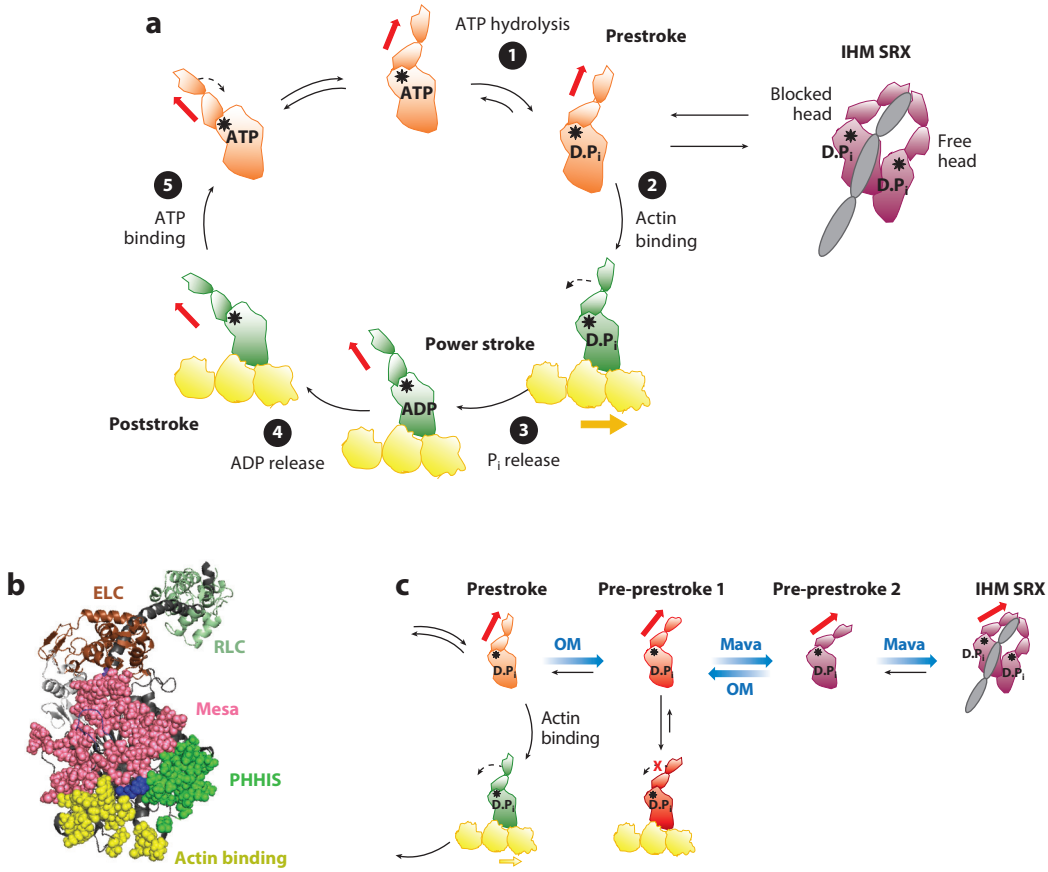

Figure 4

S1 heads of myosin-II in various states. (*a*) Chemomechanical cycle of the interaction of myosin heads with actin. ❶ The S1, free of actin, hydrolyzes ATP, and the products remain bound to the active site. ❷ The prestroke S1 with bound ADP and P_i binds to actin (*yellow*). ❸ While bound to actin, associated with P_i release, the lever arm swings to the left (*black dashed arrow*) about a fulcrum point (*black star*), moving the actin filament to the right (*bold yellow arrow*) with respect to the myosin thick filament. ❹ ADP release allows a further small stroke to the poststroke position and frees the active site for binding of ATP. ❺ ATP binding weakens the interaction of the S1 with actin. The prestroke state can be pulled into the folded IHM SRX state (*purple*). Actin-bound and actin-unbound myosin heads are shown in green and orange, respectively. Red arrow denotes direction of the lever arm. (*b*) PyMOL structure showing three critical surfaces of the S1: the actin-binding face (*yellow*), the mesa (*pink*), and the PHHIS (*green*). At the apex of the pyramid formed by these three surface domains is residue Arg403 (*blue*), which, when mutated to a glutamine, causes hypertrophic cardiomyopathy. The ELC (*brown*) and RLC (*pale green*) are shown attached to the lever arm heavy-chain α-helix (*dark gray*). This homology model of the prestroke state of human β-cardiac S1 can be downloaded from our website (**http://spudlab.stanford.edu/homology-models**). (*c*) Predicted different conformational states for OM-bound (*red*) and Mava-bound (*purple*) S1 heads. Abbreviations: D.P$_i$, ADP.P$_i$; ELC, essential light chain; IHM, interacting heads motif; Mava, mavacamten; OM, omecamtiv mecarbil; PHHIS, primary head-head interaction site; P_i, inorganic phosphate; RLC, regulatory light chain; S1, subfragment 1; SRX, super relaxed.

myosin-II (**Figure 3*d***). Associated with the prestroke to poststroke transition is a change in the nucleotide pocket that allows P_i to be released from the active site. The changes in the affinities of ATP, ADP, and P_i for the active site are associated with movements of a P-loop, a switch 1 loop, and a switch 2 loop, which are critical elements of the nucleotide-binding site (**Figure 3*b***). These same three elements are found in the G proteins, which are evolutionarily related to myosin (9, 10).

Muscle myosin and nonmuscle myosin-IIs (NM-IIs) self-assemble into bipolar thick filaments with heads arranged in a helical pattern (**Figure 2**). This self-assembly involves interactions between the light meromyosin (LMM) portion of the coiled-coil tail. The subfragment 2 (S2) portion of the tail is free to swing away from the shaft of the thick filament to allow the S1 heads to interact with actin. **Figure 2** shows the structure of the arrangement of myosin with respect to actin in human cardiac muscle drawn to scale. The myosin heads will not reach the actin filament without the S2 swinging away from the thick filament. The ability to swing out derives from a hinge region at the heavy meromyosin (HMM)–LMM junction, presumably a somewhat melted-out region of the coiled-coil tail (11) (**Figure 2**).

While the HMM–LMM hinge is specific to the class II type of myosins, a critical feature for all myosins is a similar disordered hinge at the S1–tail junction (**Figure 2**). This hinge region, which, like the HMM–LMM junction, is protease sensitive in muscle myosins (12, 13), provides the S1 heads the needed rotational freedom to more easily make productive interactions with actin.

During muscle relaxation, many of the myosin heads are believed to be flush against the shaft of the thick filament. These heads are thought to be mainly in a state that involves the two S1 heads asymmetrically folded back onto their S2 tail and interacting with one another in the interacting heads motif (IHM) (14–22) (**Figures 2** and **4*a***). The blocked head (its actin-binding site is buried in the structure) has a mesa surface (15, 23, 24) (**Figure 4*b***) that interacts with the proximal S2 tail and a primary head-head interaction site, which is distinct from the actin-binding interface and binds to the converter domain on the free head (actin-binding site exposed). Both heads in the IHM are believed to be in an OFF state. Heads in this state are effectively pulled out of the cycle of interaction between myosin and actin (**Figure 4*a***). This level of control of myosin function is believed to operate across all myosin-IIs (25, 26) and probably applies to other members of the myosin family as well. While they do not explicitly fold into an IHM configuration, myosin-Va (27), a type I myosin (28), and myosin-VIIa (29, 30) appear to involve the folding of the tail domain to interact with the head and inhibit its ATPase activity.

Many molecules of the single-headed myosins, such as myosin-I (31), have to act in concert to ensure continuous association with actin during movement. Myosin molecules need to let go of the actin to hydrolyze another ATP for another cycle of interaction with actin, and other molecules need to be actin-associated during this time to keep the actin and myosin ensemble engaged. This is also true of some two-headed myosins, such as muscle myosin-II. To achieve the high velocities needed during muscle contraction, each myosin head must spend only a very short time (1–10 ms) in a strongly bound force-producing state with actin. Thus, muscle contraction involves many myosin heads assembled into a thick filament so that during sliding of the myosin-containing thick filaments past the actin-containing thin filaments, there is at least one head, and in reality more, bound to actin at any moment during contraction to keep the two filaments engaged. Such myosins are called nonprocessive motors, meaning that a single molecule cannot take multiple steps along actin before dissociating. They generally spend $<10\%$ of their ATPase cycle time in a state strongly bound to actin (that is, their duty ratio is <0.1).

Some two-headed myosin molecules are processive, meaning that one molecule can carry cargo along an actin filament over considerable distances. Thus, myosin-V, which carries cargo from one place to another in nonmuscle cells, can take more than 20 steps of about 36 nm each (a total distance of >0.7 μm) toward the plus end of actin filaments, and myosin-VI can carry cargo similar distances toward the minus end. Myosin-VI reverses the normal directionality of myosins by inserting a new mechanical element between the converter and the light chain–binding domain (32–34) (**Figure 1**). Processive myosin motors generally spend $>50\%$ of their ATPase cycle time in a state strongly bound to actin (that is, their duty ratio is >0.5).

ALL MYOSINS ARE HIGHLY ALLOSTERIC SENSORS

The binding of ATP is associated with three significant changes in the myosin molecule that point to the highly developed allostery used by this magnificent enzyme. First, the switch elements close and interact with the γ-phosphate of ATP. This conformational change positions the ATP for hydrolysis to ADP and P_i (35, 36), which remain bound at the active site (**Figure 4*a***). Second, the changes at the active site upon ATP binding are allosterically associated with changes in the actin-binding domain approximately 4 nm away, which dramatically weakens the affinity of myosin for actin (37) (**Figure 4*a***). Third, the ATP and ADP.P_i configurations at the active site are associated with changes in the converter and lever arm, again several nanometers away from the active site. The binding of ATP and ADP.P_i results in a prestroke configuration of the converter and associated lever arm, accommodated by the kink in the relay helix. Thus, the formation of the prestroke state of myosin occurs after the myosin is dissociated from actin.

Upon strong binding to actin, changes in the conformation of myosin are again allosterically transmitted to the nucleotide site and the converter–lever arm (38–40). An important structural analysis of the nucleotide-free myosin-V head by Coureux et al. (41) shows that the relay helix can straighten with the converter–lever arm now positioned in the poststroke state while the switch 2 element remains closed. In this structure, the straightening of the relay helix is associated with a bending of a seven-stranded β-sheet known as the transducer (42) (**Figure 2*c*,*d***), and the active site is structurally altered to enhance the release first of P_i then of ADP. Thus, the power stroke occurs, appropriately, only after actin is bound (**Figure 4*a***).

BINDING OF THERAPEUTIC SMALL-MOLECULE EFFECTORS OF CARDIAC MYOSIN ACTIVITY REFLECTS MYOSIN'S ALLOSTERY

Small molecules binding to the catalytic head domain of cardiac myosin favor different conformational states of myosin and alter the number of myosin molecules functionally accessible for interaction with actin. These effects are also highly allosteric, with none of the small-molecule effectors binding at the active nucleotide site. Different small molecules have different effects on the functional parameters of myosin and trap the molecule in different conformational states. Besides the conventional prestroke and poststroke states, several myosin head crystal structures show the lever arm in positions that are different from these conventional configurations (for a review, see 43), revealing considerable flexibility of the lever arm position. At least one intermediate position between the conventional prestroke and poststroke configurations was also defined from dynamic studies on myosin using time-resolved fluorescence energy transfer (44).

Omecamtiv mecarbil (OM), developed by Cytokinetics and now in phase 3 clinical trials for heart failure, is an activator of cardiac muscle contraction (45–48) that binds near the SH1 helix and close to the surface of the S1 near the converter domain (49). OM accelerates P_i release in the presence of actin, and this is proposed to result in more heads entering the strongly bound force-producing state, as discussed by Planelles-Herrero et al. (49). Consistent with this is the finding that OM positions the lever arm in a conformation close to the prestroke state (49), thus pulling the equilibrium from the poststroke configuration to the ADP.P_i-bound prestroke configuration (**Figure 3*c*,*d***). Increasing the number of heads in the ADP.P_i prestroke state should provide more heads ready to interact with actin (49). In addition, the binding of OM may move heads out of their sequestered OFF state, causing an additional increase in the number of heads in their ADP.P_i state, ready to interact with actin (43, 50). Furthermore, cardiac contraction is regulated by the Ca^{2+}-sensitizing tropomyosin–troponin complex bound to the actin filaments. An additional activating effect of the OM-bound heads could be that their more rapid P_i release and hence more rapid than

normal binding to actin helps push tropomyosin out of its position that blocks myosin binding. This may result in the recruitment of more normal, OM-free heads.

Interestingly, OM-bound heads have a reduced rate of the prestroke to poststroke transition (approximately 2 s^{-1} rather than the normal approximately 15 s^{-1}) (51) and a reduced stroke size (52). Since stroke completion is associated with ADP release followed by ATP rebinding and release from actin, the OM-bound heads remain bound to actin for a much longer time than normal heads (approximately 80 ms rather than approximately 10 ms) (52, 53). This longer binding time and the reduced stroke size reduces the velocity of actin filament movement in vitro (53–56). If the OM-bound heads did not stroke at all while bound, they would produce zero work. Since the OM-bound heads do still undergo a small stroke, however, work is performed, adding to the force production by the normal, OM-free heads. Systole is observed to be extended (45), probably due to the increased number of myosin heads bound as well as the longer binding time of the OM-bound heads, giving an overall longer force-producing period.

During cardiac muscle contraction, any given myosin head is likely used only once during a particular systolic/diastolic contraction cycle (57). This means that a primed head with ADP.P_i bound is ready to go onto actin in a force-producing state, bleeding off that force as the lever arm swings, providing an amount of work that is the average force produced times the distance moved. Then that head releases, not to be used again in that heartbeat. This means it has plenty of time to hydrolyze its newly bound ATP and get ready for binding again to actin before the next heartbeat occurs.

Another allosteric effector, this time an inhibitor of contractility, is mavacamten, developed by MyoKardia for treatment of hypertrophic cardiomyopathy (HCM). Mavacamten, also in phase 3 clinical trials, appears to position the S1 heads in a structural state that easily fits into a folded-back sequestered state (58), possibly the IHM configuration. Either the mavacamten-bound S1 takes up a head configuration that is very near the head configurations in the folded-back sequestered state, or it has the compliance to easily adopt the conformation(s) of the heads in the folded-back sequestered state (43, 59). Mavacamten binding to bovine cardiac HMM (60) and human β-cardiac 25-hep HMM (58) causes inhibition of the basal ATPase of myosin as well as its actin-activated ATPase activity (61).

MAMMALIAN NONMUSCLE MYOSIN-IIs HAVE BEEN PROPOSED AS A POSSIBLE THERAPEUTIC TARGET

NM-IIs have very similar structures to muscle myosin (2). In mammalian cells, there are three different isoforms—NM-IIA, NM-IIB, and NM-IIC—with similar head domains and shared ELCs and RLCs. The head domains of these three isoforms show 90–92% similarity (62). As in muscle myosin, the tail regions of the NM-IIs are responsible for both dimerization of the heavy chains and formation of functional filaments (63). The C-terminal, short, nonhelical tailpiece of these three isoforms exhibits the most sequence divergence (75–77% similarity) and is thought to be responsible for their differential subcellular localizations.

Two different domains on NM-II can be phosphorylated: the RLC and the tail. Phosphorylation of the RLC by myosin light-chain kinase and Rho-associated protein kinase (ROCK) transforms the molecule from a folded, assembly-incompetent, inactive conformation into an asymmetric, unfolded active form that can form bipolar thick filaments, hydrolyze MgATP, and slide actin filaments (64, 65). However, phosphorylation of the coiled-coiled region of the tail and the nonhelical tailpiece by kinases such as protein kinase C, casein kinase II, and transient receptor potential melastatin 7 dissociate the bipolar filaments and prevent further filament formation (66).

In NM-IIA, phosphorylation of the tail domain is associated with the epithelial to mesenchymal transition in mouse epithelial cells (67).

In general, NM-II acts as a principal controller of cell morphology, with roles in several vital processes, including cellular migration and postsynaptic dendritic plasticity in neurons. NM-II also generates forces that remodel biochemical signaling by inducing changes in interactions between actin-associated proteins that can eventually regulate gene transcription. Additionally, NM-II has recently surfaced as a critical regulator of diverse, genetically complex diseases, including neuronal disorders, cancers, and vascular disease. Many of these functions have been discussed in detail in a review by Newell-Litwa et al. (68). Here, we discuss the emerging role of NM-II activity in cancer and briefly touch on its role in neurological and vascular disorders.

Nonmuscle Myosin-II and Cancer

NM-II plays an essential role in cytokinesis by forming a contractile ring at the furrow region of the dividing cell and applying forces that drive cell division. Over the past decade, many different types of cancers have been associated with altered NM-II regulation and localization. In these cancer models, NM-II is thought to be a crucial driver for processes that are necessary for tumor invasion and metastasis (reviewed in 69). Among the different types of cancer, breast cancer, glioblastoma, and squamous cell carcinoma have been the most studied. These cancer cells are thought to have differential expression and activation of NM-II isoforms, which lead to cell division and migration patterns that underlie tumorigenesis and invasion (68).

The current hypothesis is that many upstream regulatory signaling pathways converge down to the NM-II family of molecular motors, and hence NM-II has been proposed as a potential therapeutic target. For example, downregulation of the tumor suppressor gene *BRCA2* leads to mislocalization of NM-II in mitosis, leading to chromosome instability and aneuploidy (70, 71). Similarly, the oncogene *Ras* and its downstream target *BRAF*, which regulate cell survival and propagation, also increase NM-II activation, leading to tumor invasion in vitro and in a mouse melanoma model in vivo (72–75). In both in vitro and ex vivo models, it has been shown that gliomas use NM-II–dependent contractility to migrate within the brain, and NM-II inhibition by blebbistatin prevents glioma invasion (76, 77). In a rodent glioblastoma model, genetic deletion of both NM-IIA and NM-IIB genes decreases tumorigenesis and weakens tumor proliferation (78). Targeting NM-IIA alone impairs glioblastoma invasion but, in fact, enhances glioblastoma growth and lethality (78). In conjunction with a previous report (79), the Picariello et al. (78) study suggests that effective treatment of glioblastoma will require inhibiting targets that drive both invasion and proliferation separately. However, two reports have shown that targeting NM-IIA induces squamous cell carcinoma in mice (80, 81); while one report (80) found that NM-IIA stabilizes the tumor suppressor p53, a second did not observe this (81).

Another study (82) showed that stress-sensitive proteins such as NM-IIA and NM-IIC are highly upregulated in pancreatic ductal adenocarcinoma cancer and patient-derived pancreatic cancer cell lines. NM-IIB, by contrast, virtually disappears with pancreatic ductal adenocarcinoma cancer. In such disease models, NM-IIC is involved in the formation of actin arcs in single cells and cortical actin belts in tissue spheroids. The authors used this to their advantage by targeting NM-IIC with a small-molecule activator, 4-hydroxyacetophenone, which increases NM-IIC assembly and stiffens cells. This decreases propagation, induces cortical actin belts, and slows retrograde actin flow in spheroids. Similar treatment in a pancreatic cancer mouse model shows a reduction in liver metastases. The authors argue that increasing the activity of these mechanoresponsive proteins by increasing NM-IIC assembly to overcome the capacity of cells to polarize and penetrate may be an effective strategy to improve the survival rate of patients with pancreatic cancer.

Initial stages of tumor cell metastasis require an epithelial to mesenchymal transition that involves activation of amoeboid migration and loss of cell–cell adhesion. NM-II has roles in cell body translocation and retraction of the posterior of the cell during migration. In a study involving MDA-MB-231 breast cancer cells, both NM-IIA and NM-IIB were recruited to the lamellar margin during active spreading on fibronectin (83). There was also a transient increase in RLC phosphorylation associated with the recruitment. Furthermore, pharmacological or small interfering RNA–mediated inhibition of NM-II and myosin light-chain kinase indicates that both NM-IIA and NM-IIB contribute to overall cell migration but that the NM-IIB isoform contributes explicitly to the mechanics of lamellar spreading on extracellular matrix.

Given these observations and others, as reviewed in Reference 68, NM-II has been suggested as a possible target to arrest tumor metastasis. However, based on the roles of NM-IIs in other aspects of cell biology (e.g., see the sections titled Nonmuscle Myosin-II in Neurological Disorders and Nonmuscle Myosin-II in Other Disorders) and the widespread localization of NM-IIs in most cells in the body, toxicity from small-molecule effectors may be an important issue to consider.

Nonmuscle Myosin-II in Neurological Disorders

NM-II plays significant roles in neuronal cell biology by mediating growth cones, which are actin-enriched structures in neurites, axons, or dendrites that drive their motility toward desired targets in response to chemical cues. Among the three isoforms, NM-IIB is highly enriched in the brain, where it is located at both the presynaptic terminals and postsynaptic dendritic spines (84). The findings that NM-IIB mediates synaptic vesicle recycling and regulates the maturation of spines and the clustering of glutamate receptors in the postsynaptic terminals led researchers to label it as an essential regulator of stimuli-induced changes in spine morphology that underlie learning and memory formation (85–87). In addition to a direct role in neuronal cell biology, NM-II also plays a vital role in glial cell function (76, 88), the integrity of the blood-brain barrier (89, 90), and microglia activation in neuroinflammation (91).

Unsurprisingly, NM-II has been implicated in diverse neurological disorders (reviewed in 92). These include neurodevelopmental disorders such as autism, neurodegenerative disorders such as Alzheimer's disease, and neuronal migration disorders such as lissencephaly and disorders of axon regeneration following central nervous system injuries (93). De novo mutations in *MYH9*, which codes for NM-IIA, are associated with autism, schizophrenia, and intellectual disability, and de novo mutations in *MYH10*, which codes for NM-IIB, are related to both schizophrenia and autism (94). Direct targeting of NM-II using blebbistatin and inhibiting the NM-II ROCK restored axon growth on inhibitory substrates, in chick dorsal root and retinal ganglion cells, in rat cerebellar granule neurons (93, 95–99), and in an in vivo rat spinal cord injury model. These observations have led to the suggestion that NM-II may be a potential therapeutic target for axon regeneration in devastating spinal cord injuries and central nervous system lesions.

In glial biology, NM-II plays a significant role in preserving the function of astrocytes, oligodendrocytes, and microglia (reviewed in 100). In microglia, NM-II contributes to neuroinflammation resulting from microglia activation and release of inflammatory cytokines (reviewed in 101). In a rodent model of Parkinson's disease, ROCK inhibition prevents microglia activation and the phagocytosis of disintegrating dopaminergic neurons (102). Similarly, amyotrophic lateral sclerosis results in increased proinflammatory cytokine production, which is reduced by ROCK inhibition, leading to increased mouse motoneuron survival (103–105), also suggesting a role of NM-II in such disease progression.

The above complex roles of NM-IIs in neuronal functions suggest that toxicity from small-molecule effectors may be a hurdle when considering NM-IIs as a target for disease.

Nonmuscle Myosin-II in Other Disorders

NM-II activity in endothelial and smooth muscle cells is essential for blood vessel integrity of arteries, veins, and capillaries and therefore may contribute to many vascular disorders, such as atherosclerosis and edema. Autosomal dominant NM-IIA mutations have also been reported in many macrothrombocytopenia disorders such as May-Hegglin anomaly, Epstein syndrome, Fechtner syndrome, and Sebastian syndrome. Other than those, NM-IIA-related disorders also often present with deafness, cataracts, and nephropathy in affected individuals (106). Given the widespread localization of NM-IIs in most cells of the body, more work will be required to assess the toxicity profile of targeting NM-IIs.

THE UNCONVENTIONAL MOLECULAR MOTOR MYOSIN-X HAS ALSO BEEN PROPOSED AS A POSSIBLE THERAPEUTIC TARGET

Myosin-X, an unconventional molecular motor located at the tips of filopodia, is essential for wound healing (107), filopodia formation (108), angiogenesis (109), the regulation of growth cone formation (110), and invadopodia formation (111). Myosin-X is widely expressed in low abundance in vertebrate tissues. It is prominent in the developing brain, endothelial tissues, and many epithelial tissues (112). Like other myosins, myosin-X has a head domain that binds actin, hydrolyzes ATP, and generates force. Its lever arm consists of three isoleucine-glutamine (IQ) binding motifs, and it has a tail that endows class-specific properties such as dimerization and cargo binding. Myosin-X is unusual among actin-based motor proteins in that its tail can provide a direct link to microtubules. A unique feature of the myosin-X tail is its three pleckstrin homology domains, one of which binds to the vital signaling lipid phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) (113, 114). In the absence of PIP_3 binding, myosin-X can exist as a monomer in which the tail folds back onto the head to form a compact and auto-inhibited OFF state (115). Once activated to dimerize, myosin-X can undertake its biological roles, including transporting cargo and forming filopodia.

Myosin-X in Cancer

One of the first pieces of evidence of filopodia involvement in cancer biology was the discovery of both upregulated fascin, a filopodial crosslinking protein, and filopodial structures themselves in aggressive metastatic cancer cases (116). Both fascin and myosin-X have been identified as critical components in a different type of structures called invadopodia, which are actin-rich protrusions with proteolytic activity (111) capable of digesting surrounding extracellular matrix, an escape mechanism for primary tumor cells. Myosin-X knockdown and silencing results in the blockage of invadopodia function by inhibiting matrix digestion ability (111). Similarly, suppression of myosin-X in filopodium-like protrusions significantly decreases proliferation rates and invasiveness of cancer cells in breast cancer cell models (117).

Myosin-X in Infectious Disease

Recent evidence from filoviruses such as Marburg virus suggests that myosin-X may also be involved in filopodia-dependent viral budding. Expression of a dominant-negative myosin-X construct caused a significant reduction in virus-like particle production (118). The spread of the diarrhea-causing bacterium *Shigella flexneri* is also myosin-X driven (119).

In thinking about myosin-X as a potential therapeutic target, one runs into the same problem as for the NM-II family. Myosin-X is widely distributed and plays multiple roles in cell biology. Small-molecule effectors against myosin-X are therefore likely to lead to significant toxicity.

MAMMALIAN MYOSIN-V, MYOSIN-VI, AND MYOSIN-VII ARE ALSO DISEASE ASSOCIATED

Many other unconventional mammalian myosins such as myosin-V, myosin-VI, and myosin-VII localize to several intracellular compartments and participate in transporting and anchoring events (2). While the catalytic heads of these myosins share significant similarities with conventional myosins, their tail regions are highly divergent (120) (**Figure 1**). The diversity in the tail domain of these myosins is in the specific domains that bind to different adaptor binding proteins, which in turn can bind to different cargoes, such as an organelle, vesicle, or protein complex (121).

Mutations in such unconventional myosins have been linked to many human diseases. For example, different classes of myosins are associated with hearing through their contribution to the structure of the stereocilia of the inner ear (122). Mutations in class Ia, IIc, IIIa, VI, VIIa, and XV myosins can lead to Usher syndrome, the leading cause of genetic deaf-blindness (2). Similarly, myosin-Vb might transport apical endosomes in brush border cells (123), which could explain the association of mutations in this gene with microvillus inclusion disease (124). Mutations in myosin-Va are linked to Griscelli syndrome, which is characterized by defects in pigmentation and neuronal malfunction (125). Many of these myosins—other than myosin-XV, which is specifically known to be present only in the stereocilia of the inner ear—are distributed throughout different organs of the body, and therefore targeting these myosins therapeutically could lead to toxic effects.

THE SPECIALIZED MYOSINS FOUND IN PARASITES MAY REPRESENT EFFECTIVE THERAPEUTIC TARGETS

Myosin-XIV is a Class of Specialized Myosin Found in Many Mammalian Parasites

Class XIV myosins are present in the protist group Alveolata, and specifically the phylum Apicomplexa, which includes a broad variety of obligate intracellular parasites that infect many different animal species. Some of these parasites cause devastating human diseases such as malaria (*Plasmodium* spp.), toxoplasmosis (*Toxoplasma gondii*), or enteric infections (*Cryptosporidium*), notably in the developing world. Malaria is responsible for half a million deaths each year worldwide, and toxoplasmosis affects around 33% of the human population. A 2011 study (126) put toxoplasmosis as the second leading cause of foodborne-related deaths and the fourth leading cause of foodborne-related hospitalizations in the United States. Apart from infecting humans, these parasites infect livestock and cause significant economic losses.

Host cell penetration, an essential step in the infection process, is considered to be an active process governed by a unique actomyosin-based structure in the parasite called the glideosome (127). The glideosome, located between the plasma membrane and the inner membrane complex of the parasite, is critical for the gliding motility in these parasites that propels them across biological barriers and helps them invade and egress from host cells. Several apicomplexans fail to glide or invade when treated with drugs that interfere with actin dynamics (e.g., latrunculin B, cytochalasins, jasplakinolide) or with myosin activity (e.g., 2,3-butanedione monoxime). Knockdown of some critical actomyosin components of the glideosome, using a tetracycline-inducible transactivation system, led to a reduction in host-cell invasion (128). However, knockout of some parasite invasion machinery genes also led to the discovery of other redundant pathways the parasite can employ for host cell invasion (129).

Like every other myosin, myosin-XIVs have a conserved head domain that houses the actin- and ATP-binding sites and a lever arm that binds accessory light chains (**Figure 1**). Myosin-XIVs

lack a tail region that typically allows for dimerization. Hence, these are monomeric myosins. The highly studied MyoA of class XIV myosin is essential for maintaining the gliding motility and invasion of the *Plasmodium* parasites (127, 128, 130, 131). MyoA is a low-duty-ratio, fast, monomeric motor and is highly conserved in all apicomplexans (4, 132–134). Both *T. gondii* and *Plasmodium falciparum* appear to express MyoA through all life cycles of the parasite. The *T. gondii* MyoA binds a regulatory light chain called MLC1 and an essential light chain called ELC1. A functionally redundant ELC2 was also discovered, and both ELC1 and ELC2 were found to be important in invasion, egress, and motility of *T. gondii* (135). Recent work from Trybus and colleagues (136, 137) demonstrates cochaperone-mediated expression and purification of *T. gondii* and *P. falciparum* MyoA motors. The *T. gondii* MyoA moved actin at fast velocities in the in vitro motility assay when both the RLCs and ELCs were bound to the lever arm (136). The *P. falciparum* MyoA also moved actin at higher velocities only when the light chain called myosin A tail domain interacting protein and the *P. falciparum* specific ELC were bound (137).

Recently, a significant breakthrough was achieved by Powell et al. (138) when they crystalized the structure of the *T. gondii* MyoA motor domain at 2.6 Å. The structure demonstrates interesting strategies for chemomechanical coupling and efficient force transduction. The unique sequence elements discovered in this structure are located in known binding sites for inhibitors of myosin function and suggest that specific inhibitors may be able to be developed to target myosin-XIV motors of apicomplexan parasites. Following up, another breakthrough from Houdusse and colleagues (139) came when they determined the crystal structure of MyoA from *P. falciparum*. Combining structural, kinetic, and parasitological analyses, the authors proved that MyoA is essential for erythrocyte invasion by the parasite. They demonstrate that phosphorylation of the N-terminal extension of this MyoA tunes the motor either for high force (required for optimal invasion) or fast motility speed (required for parasite dissemination). This is a clear example of how the myosin family of motors shares the same global architecture but the evolutionary addition and deletion of structural elements makes them serve very distinct purposes. These evolutionary strategies can be exploited to design novel classes of therapeutics targeted to specific myosins in these parasites. The MyoA motor, along with its light chains and other proteins, forms a central part of the glideosome, which helps to propel the parasites and aids in infecting the host cells. The light chain and the associated proteins target the MyoA to the inner membrane complex of the *T. gondii* parasites. The *P. falciparum* species contain an analogous glideosome assembly with its different components (140, 141). Ca^{2+}-dependent phosphorylation of *T. gondii* MyoA has also been reported that aids in parasite motility and egress from the host cell (142, 143). Phosphorylation sites on the *T. gondii* myosin light chain 1 are near a site on this light chain that covalently binds a small molecule and inhibits parasite motility (144).

MyoB, another member of the myosin-XIV family, is not associated with the glideosome but rather is at the apical region of the *P. falciparum* cell. It has a very distinct light chain with a calmodulin-like domain at its C terminus and an extended N-terminal region, which is thought to help localize the motor to its appropriate cellular location (145). MyoC, a third member of the family, is a product of alternate splicing of a single gene that encodes both MyoB and MyoC in *T. gondii*. They are mostly similar except for their tail domains, which give them different solubilities and different subcellular localizations. MyoC in *T. gondii* assembles into distinct glideosomes from MyoA and can compensate for the MyoA glideosomes, and vice versa (146). This redundancy helps parasite survival.

From a potential therapeutic point of view, MyoH is particularly interesting. MyoH is indispensable for the survival of *T. gondii*, and its conditional depletion completely blocks gliding motility, invasion, and egress. This occurs despite a functional MyoA glideosome assembly. MyoH appears to be the principal initiator of the gliding motility, which is then communicated to the

MyoA-associated glideosome (147). It is found in all apicomplexan parasites except *P. falciparum* and *Gregarina polymorpha*. Its extended neck region has eight IQ domains bound to accessory light chains. The MyoH features tail domains with similarity to alpha-tubulin suppressor 1 (ATS1) and related regulator of chromosome condensation 1 (RCC1) proteins. RCC1 binds chromatin, and ATS1 may be involved in coordinating the cell cycle (148, 149) (**Figure 1**). The MyoH tail region establishes contact between the tubulin-based and actin-based cytoskeletons. This may hint at the role of this myosin during mitosis, cytokinesis, or other cell-cycle related events. The RLCs of MyoH regulate the activity of myosin in a Ca^{2+}-dependent way (150).

MyoD and MyoE are associated with *T. gondii*, but they are not essential for survival. MyoD is the smallest motor, structurally similar to MyoA, and functions as a monomeric, fast, low-duty-ratio myosin. Both MyoD and MyoE lack tail domains. An unclassified *T. gondii* motor, myosin-L, is also thought to belong to class XIV, and a recent CRISPR gene disruption study pointed out that the myosin-L gene was essential for the parasite viability (151).

Myosin-XIVs are also found in ciliates such as *Tetrahymena thermophila*. These myosins rely on phosphorylation-dependent changes in myosin activity. Myosin-XIVs diverge from other myosins in that they have a specific His-Tyr-Ala-Gly sequence near the SH1 and SH2 helices. While this sequence has a tyrosine or phenylalanine at the second position in other myosins, myosin-XIVs, as a hallmark, possess either serine or threonine at this location. The smaller serine or threonine residues here facilitate a tight coupling between the active site and the lever arm of myosin-XIVs, which imparts desirable functionalities for gliding and invasion (138).

Other Classes of Parasite Myosins

The apicomplexan MyoF, together with myosins from a particular diatom, forms a separate class of myosins called myosin-XXII (4). This broadly conserved apicomplexan class of myosins is predicted to feature multiple IQ motifs similar to those in class V, VIII, XI, and XIII. The apicomplexan myosin-XXIIs have a long tail predicted to contain four to six WD40 repeats at the C-terminal end. The WD40 repeats form β-propeller structures known to be involved in transcriptional regulation and signal transduction. These repeats are known to coordinate multiprotein assemblies and may target to centrosomes or the nucleolus (152, 153). The N-terminal regions of these tails are predicted to form coiled-coil structures. MyoF of *T. gondii* is important in centrosome positioning, apicoplast inheritance, and organelle trafficking, thus making it particularly crucial for parasite survival (154). *T. gondii* depends on intracellular protein secretion via secretory vesicles called dense granules, and the trafficking of these dense granules is undertaken by the MyoF motor (155).

Two myosins of *T. gondii* and some other apicomplexans are part of the myosin XXIII and XXIV classes. MyoG and MyoJ of *T. gondii* and *Eimeria tenella* form class XXIII, and MyoI from *T. gondii* and a homolog from a *Cryptosporidium* species belong to class XXIV (4). These sequences are predicted to feature one or two IQ motifs, and the MyoG tail contains a single myosin tail homology 4 (MyTH4) domain. MyTH4 domains, found in several myosin classes, bind microtubules. However, the exact role of this domain in the apicomplexan myosins is unknown. *T. gondii* MyoG is found in the parasite periphery, and its gene disruption led to no change in the parasite activity (156). MyoJ is found in the basal region of the parasite, and parasites lacking this motor displayed an overall reduced fitness. A mouse model of infection showed a loss of virulence when the parasites lacked MyoJ (156). *E. tenella* is an apicomplexan capable of causing coccidiosis in poultry, sheep, and goats. *Eimeria* infections are severely damaging to the poultry industry and cost the United States approximately $1.5 billion in annual losses (157). Several *Eimeria* species infect various fishes, reptiles, birds, and a wide variety of mammals.

Cryptosporidium is a genus of the apicomplexan family that causes cryptosporidiosis, which leads to severe gastrointestinal problems in both immunocompetent and immunocompromised individuals. It is one of the most common causes of waterborne disease in the United States. Between 2009 and 2017, cryptosporidium outbreaks in the United States increased by 13% annually (158). As of 2015, there is only one drug approved for its treatment. *Cryptosporidium parvum* is known to have two class XIV, one class XII, one class XXIV, and two class VI myosins. MyoI in *C. parvum* is predicted to dimerize based on coiled-coil–forming regions. MyoI, belonging to class XXIV in *T. gondii*, maintains cell–cell communication of the parasites within a vacuole and ensures proper cell division. It contains two IQ motifs, a coiled-coil domain (ensuring dimerization), and a long tail without any known protein domains (159). Deletion of MyoI in *T. gondii* showed no defects in the parasite lytic cycle, but it led to asynchronous division in the host cell vacuole (160). It is not known if MyoI plays an active role in the transport of material between parasites within the vacuole. Apicomplexans also contain myosins whose exact functions are unknown. These myosins are similar to the minus end–directed myosin-VI motors found in humans. A recent systematic gene deletion study demonstrated that MyoF, MyoK (class XXIII), and the MLC-B light chain of MyoB are likely essential in the asexual blood stages of *Plasmodium berghei*, a parasite used in rodent models of malaria (161).

The fungus-like oomycete family of plant pathogens also has myosin genes. For example, the pathogen *Phytophthora ramorum*, known to cause the disease sudden oak death, has 25 myosin genes that encode 13 different kinds of myosins (162). Some of these myosins appear to belong to class XXII, XXIII, or XXIV (4). *P. ramorum* leads to mortality in several oak tree species and also causes diseases in several native and nonnative ornamental plants, shrubs, and trees within the United States. Yeast species such as *Candida* also have a repertoire of myosins, with a recent study (163) demonstrating the efficacy of an actin-binding drug in murine vulvovaginal candidiasis. This study revealed the importance of the yeast cytoskeleton in maintaining its life cycle. The presence of myosins in the *Candida* species opens avenues for designing novel therapeutics for multidrug-resistant species of *Candida*, such as *Candida auris*. This newly discovered species is tagged as an emerging global health threat by the US Centers for Disease Control and Prevention, as it is nonresponsive to any of the well-known antifungals.

Additionally, an insect-specific myosin-XX is present in *Drosophila* and the malaria parasite vector mosquito *Anopheles gambiae*. This myosin contains a well-conserved IQ motif in the neck domain. Myosins are also critical in the functioning of parasitic nematodes that infect humans with intestinal and other diseases. A recent study (164) pointed at the presence of 13–18 myosin genes in the nematode family, which includes parasitic nematodes such as *Strongyloides ratti* (a member of the Strongyloides family of parasites that infect humans) and *Brugia malayi* (which causes elephantiasis in humans). Thus, developing novel therapeutics targeted to these myosins is an open arena of exploration.

In summary, several classes of myosins—specifically myosin-XIV—that are likely to be targetable in particular ways without affecting mammalian host myosins represent an attractive target for small-molecule therapeutics for parasitic invasions. Indeed, in a recent screen, several egress and invasion inhibitors of the blood stage of *P. falciparum* were discovered (165).

Myosin-XXI

A similarly attractive potential target for small-molecule therapeutics is myosin-XXI, found in parasitic kinetoplastids such as *Trypanosoma* and *Leishmania*. *Leishmania* is transmitted by sandflies, and the human infection is caused by 21 of the 30 species of *Leishmania* protozoa that infect mammals. It occurs in the cutaneous and visceral forms in humans, with the visceral form being

fatal. Tropical and subtropical regions are affected by leishmaniasis, including Mexico, Texas, and countries in Central and South America, southern Europe, Asia, the Middle East, and Africa. Myosin-XXI is present in both the motile and nonmotile stages of the *Leishmania* parasite life cycle. In the motile stage, these myosins are predominantly localized to the proximal part of the flagellum but are also found in other cell compartments. Two pools of myosin-XXI have been identified within the parasite: a membrane-bound fraction found at the base of the flagellum and a cytosolic fraction that is presumably involved in local transport (166).

Reducing the levels of *Leishmania* myosin-XXI expression in cultured motile stages of the parasite led to a loss of intracellular trafficking in the parasite and a loss of flagellar assembly. The heterozygotes appeared short and stumpy and had significantly shorter flagella, which were not enough to propel the parasite forward (167). Homozygous knockout of myosin-XXI in these parasites was lethal. Deletion of the myosin gene led to ploidy in these parasites, indicating that the presence of myosin-XXI is critical for the survival of *Leishmania*.

Recent studies have reported expression and purification of full-length constructs and a truncated minimal motor domain construct of *Leishmania* myosin-XXI (168, 169). The motor is activated by actin and binds calmodulin that is required for motility but not for ATPase. Myosin-XXI is structurally similar to all other myosins except that the neck region does not contain perfect IQ motifs. Rather, the converter is followed by an extended dimerization region until the C-terminal end of the molecule. However, close to the converter are binding sites of *Leishmania*-specific calmodulin-like proteins that bind the myosin in a Ca^{2+}-dependent manner (169) (**Figure 1**). This is very different from other myosins that bind calmodulin (such as myosin-V or myosin-VI), in which the dimerization region starts downstream of the calmodulin-binding sites. This ensures that dimerization does not disrupt calmodulin binding in these myosins, allowing the calmodulin-binding lever arm to transduce force and movement. However, in the case of myosin-XXI, binding of Ca^{2+}-calmodulin prevents dimerization and enables motility by forming a functional lever arm. In the absence of Ca^{2+}-calmodulin binding, the lever arm structure is lost, thus allowing dimerization, a loss in movement, and cross-linking of actin filaments. Negative-staining electron microscopy studies showed monomeric, full-length myosin-XXI bound to actin in the rigor state, while the dimeric myosin (lacking calmodulin) was shown to cross-link actin filaments (169). This motor binds lipids via its extended dimerization region; lipid binding and dimerization are mutually exclusive. The calmodulin-unbound motor is dimeric, nonmotile, and expected to be cytosolic, as it was unable to bind lipids. However, Ca^{2+}-calmodulin binding makes the motor monomeric and targets it to different lipid compartments. This monomeric motor can generate force and movement. Importantly, membrane binding of myosin-XXI is driven by a phospholipid-binding phox homology domain that overlaps with the converter region and is a unique specialty of this myosin. Thus, with lipid-binding regions scattered along the converter and tail region of this myosin, the myosin is well suited for specific targeting to the plasma membrane and membranes of specific organelles. Unlike myosin-XIV, myosin-XXI can adopt a monomeric or a dimeric state according to the dynamic needs of the parasite. The very C-terminal region of this myosin is also predicted to contain two ubiquitin-associated domains (**Figure 1**), whose role is not clear.

Trypanosomes are also reported to contain myosin-XXI along with myosin-I. *Trypanosoma cruzi*, the causative agent of Chagas disease, has expanded the kinetoplastid myosin family to several members with more diverse sequences (170, 171). It contains one myosin-I, six myosin-XXIs, and one unclassified myosin. Chagas disease is a major economic burden, with global costs of $7.1 billion per annum. More than 10% of these losses occur in the United States and Canada, where this disease is not even endemic. The losses are attributed to the loss in productivity by cardiovascular disease–induced early death (172). The chronic stage of this disease affects the nervous system, the digestive system, and the heart, where it causes dilated cardiomyopathy. Another

family member, *Trypanosoma brucei*, the causative agent of sleeping sickness and nagana (animal trypanosomiasis), also possesses myosins belonging to classes I and XXI. The myosin-I in *T. brucei* localizes to the polarized endocytic pathway in the bloodstream form of the parasite. Knockdown studies of this myosin in *T. brucei* led to a significant reduction in endocytic activity, an inhibition of cell division, and eventual cell death (173). The knockdown effects are consistent with an imbalance of cellular trafficking in these parasites. *T. brucei* myosin-I is an unusual member of the short-tailed myosin-I family. The motor domain is followed by a single, degenerate IQ motif followed by a WW domain, which is involved in protein–protein interactions. This is further followed by a tail homology 1 domain, which is disrupted by a putative zinc-binding FYVE domain (173). TH1 domains are rich in basic residues and are thought to be involved in membrane interactions.

CONCLUSIONS AND PERSPECTIVES

As emphasized here, the members of the large family of myosin motors all work primarily the same and are all highly allosteric, and cardiac myosin has proved to be a useful target for small-molecule therapy. There are three reasons the cardiac modulators OM and mavacamten are proving so successful. First, as described above, cardiac myosin can be either activated or inhibited with small-molecule effectors. Second, cardiac myosin is the most downstream component of the activation of contraction of the heart. Many current therapies rely on upstream modulations that change Ca^{2+} transients in cells, but these have many pleiotropic effects. Both OM and mavacamten have little or no effect on Ca^{2+} transients, but rather, they modulate the contractile machinery directly. In the case of HCM, nearly 40% of all HCM mutations occur in the β-cardiac myosin itself, and mavacamten targets the very protein that carries the mutation that is the source of the disease. Third, work from both Cytokinetics and MyoKardia has shown that one can target cardiac myosin very specifically without significantly affecting skeletal or smooth muscle cells. This specificity, combined with the downstream nature of the target, means very little toxicity is seen with these agents. However, owing to the ubiquitous presence of NM-IIs in the body, pleiotropic side effects may arise when targeting them for diseases such as cancer. However, while this is not ideal, many current cancer drugs target proteins that are critical for tumor survival but are also present in other cells of the body, leading to significant toxicity and side effects, but these are considered acceptable if the cancer progression is halted.

Parasite myosins, in contrast, could be promising targets. Parasite myosins are considerably more different from mammalian myosins than are the various mammalian myosins from one another (**Figure 5**), and as in the cases of malaria and leishmaniasis, some classes of these myosins are critical for the survival of these parasites, making them an attractive target for therapeutic intervention. Thus, it is very likely that small-molecule effectors can be designed that show high levels of specificity for parasite myosins, thereby causing little or no toxicity to the mammalian host cells. Furthermore, since many different parasites carry closely related myosin types, one could likely develop a single small-molecule effector that acts across several parasitic diseases. As the genomic revolution continues, and as we delve deeper into the genomes of these enigmatic parasites, we are bound to gain more insights into the presence of novel myosins in them that can be specifically targeted by small molecules. A recent work (174) reported on the rise of a multidrug-resistant colineage of *P. falciparum* that evolutionarily diversified into multiple subgroups and acquired new genetic features. From Cambodia, this genetically enhanced subgroup has rapidly spread into neighboring southeast Asian countries. This raises the terrifying prospect of such genetically fit parasites spreading into Africa, where most malaria cases occur. There is an urgent need for novel classes of antimalarials as these parasites develop resistance against current

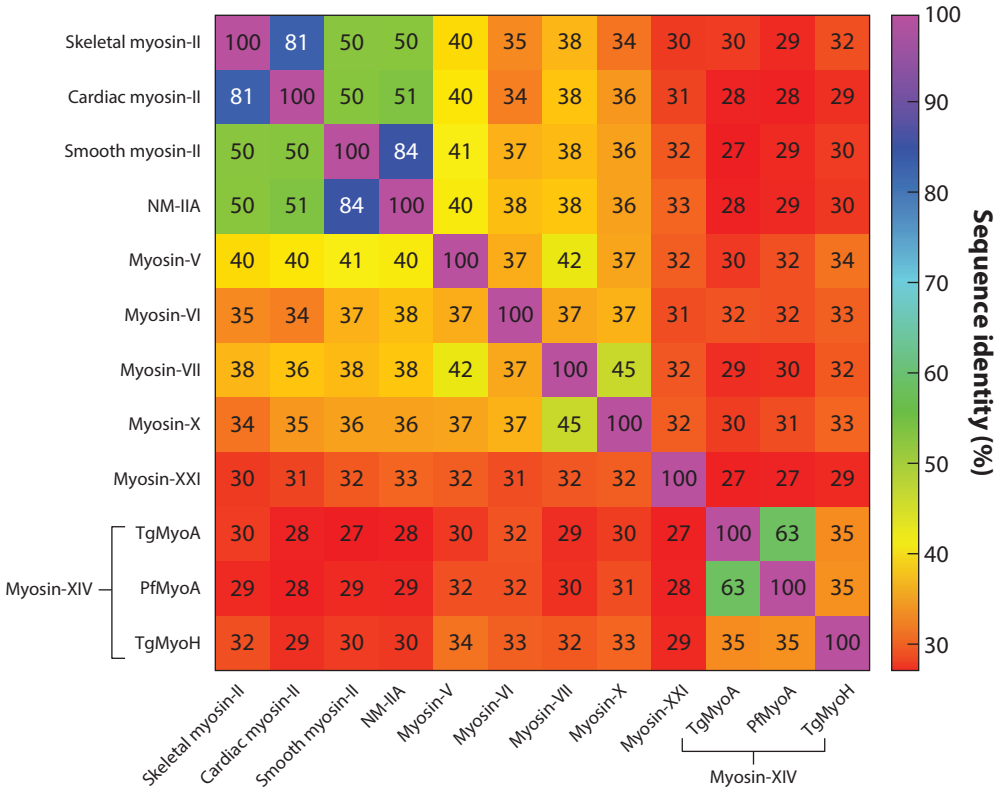

Figure 5

Heat map showing the percent sequence identity of the motor domain across different isoforms of myosin from a variety of organisms. Multiple sequence alignment was performed using the EMBL-EBI Clustal Omega tool. Myosin-XXI is the *Leishmania infantum* sequence, and the mammalian myosins are all human sequences. Abbreviations: Pf, *Plasmodium falciparum*; Tg, *Toxoplasma gondii*.

therapies (175, 176). As climate change warms the earth, typically tropical and subtropical parasitic diseases are expected to spread into now temperate areas.

Work over the past several years has led to extensive structural and mechanochemical understanding of the myosin motor. Given the promise that myosin has shown as a therapeutic target for the treatment of cardiac disease, it is likely that this understanding will be leveraged to design therapeutics for diseases that involve myosin at the core of their functioning.

DISCLOSURE STATEMENT

J.A.S. is a cofounder and member of the scientific advisory boards of Cytokinetics and MyoKardia, biotechnology companies developing small molecules that target the sarcomere for the treatment of various muscle diseases. K.M.R. is on the scientific advisory board at MyoKardia. S.N. is a scientist at MyoKardia.

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