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Kinetic Adaptations of Myosins for their Diverse Cellular Functions

Sarah M. Heissler[*] and **James R. Sellers**

Laboratory of Molecular Physiology, National Heart, Lung, and Blood Institute, National Institutes of Health, 50 South Drive, B50/3523, Bethesda, MD 20892-8015

Abstract

Members of the myosin superfamily are involved in all aspects of eukaryotic life. Their function ranges from the transport of organelles and cargos to the generation of membrane tension, and the contraction of muscle. The diversity of physiological functions is remarkable, given that all enzymatically active myosins follow a conserved mechanoenzymatic cycle in which the hydrolysis of ATP to ADP and inorganic phosphate is coupled to either actin-based transport or tethering of actin to defined cellular compartments. Kinetic capacities and limitations of a myosin are determined by the extent to with actin can accelerate the hydrolysis of ATP and the release of the hydrolysis products and are indispensably linked to its physiological tasks. This review focuses on kinetic competencies that – together with structural adaptations – result in myosins with unique mechanoenzymatic properties targeted to their diverse cellular function.

Keywords

Actin; Allostery; Cation; Cytoskeleton; Mechanoenzymology; Molecular Motor; Muscle; Myosin; Transporter

Introduction

Myosins constitute a superfamily of ubiquitous, multifunctional and structurally related motor proteins that catalyze the hydrolysis of ATP to power the directed movement on filamentous actin. Members of the ~31 myosin classes participate in all aspects of eukaryotic physiology, from the contraction of muscle, over the transport of organelles, the generation of cytoskeletal tension to the function of the mitotic spindle, and autophagy (1–4).

The defining feature of myosins is a motor domain that harbors a prototypic nucleotide binding pocket and a binding site for actin. The motor domain is followed by a neck domain that contains individual or multiple IQ motifs that bind characteristic light chains. Together, the neck and the light chains function as a myosin lever arm. Myosins terminate in a tail domain of variable length and class-specific domain composition (Fig. 1) (1–3, 5).

[*]Address correspondence to: Sarah M Heissler, Laboratory of Molecular Physiology, National Heart, Lung, and Blood Institute, National Institutes of Health, 50 South Drive, B50/3529, Bethesda, MD 20892-8015, USA, Tel.: (301) 496-8010; Fax: (301) 402-1519; sarah.heissler@nih.gov.

The kinetic properties of the motor domains of members of almost all myosin classes found in higher eukaryotes have been characterized *in vitro* with steady-state and pre-steady-state approaches. Kinetic studies were historically conducted on crude preparations from tissue- or cell-purified myosins and later on proteins that were recombinantly overproduced in *Dictyostelium* (6, 7). The recent introduction of heterologous viral expression systems for myosin heavy chains genes such as the baculovirus/*Sf*9 insect cell system and the adenovirus system enabled and greatly contributed to systematic studies of the mechanoenzymatic properties of less abundant myosins, their fragments and mutants (8–10). Remarkably, the studies uniformly indicate that all myosins follow a conserved kinetic cycle but differ in their maximum catalytic activity and the velocity on actin > 3000-fold (11). These kinetic differences, together with a class-specific multidomain architecture, enable myosins to participate in the aforementioned plethora of physiological functions and define myosin-specific features. The movement of all myosins for example is directed towards the barbed-end of actin at the cell periphery, with the exception of members of one myosin class (12, 13). This unique feature allows members of this class to be involved in several physiological tasks that can not be accomplished by any other myosin in the cell, for example transport during endocytosis (14, 15). Moreover, a myosin is commonly involved in multiple physiological processes including transport and anchoring. These apparently contradictory and dichotomous physiological challenges require a modulation of the kinetic signatures that allow it to generate or maintain force *in vivo*. Kinetic consequences of mutations in myosin are associated with the onset or progression of diseases of the sensory system including hearing impairment besides cancers and myopathies, underlining the importance of myosin motor function on cellular homeostasis (16–18).

Excellent reviews that cover kinetic, mechanic and thermodynamic aspects of myosin motor function have been published in the past years (19–22). Here we cover new aspects of how kinetic fine-tuning, structural adaptation, and intrinsic regulation modulate myosin's kinetic behavior to gear it for a particular physiological function to meet changing cellular requirements.

The Myosin Kinetic Cycle

All enzymatically active myosins follow the basic consensus kinetic scheme as depicted in Fig. 2A. This strongly simplified model of the catalytic cycle consists of six steps with the main pathway highlighted. (I) A strongly bound actomyosin complex, also referred to as rigor complex, is formed in the absence of nucleotide. (II) ATP binding to myosin dissociates the actomyosin complex. (III) Myosin hydrolyzes ATP in the actin-detached state. (IV) M.ADP.P_i weakly re-binds to actin. The subsequent release of the hydrolysis products (V) P_i and (VI) ADP shifts the myosin back into a strong actin-binding state and demarks the end of the catalytic cycle.

The catalytic cycle is paralleled by a mechanical cycle that utilizes the free energy derived from the hydrolysis of ATP to drive subtle conformational changes in the myosin motor domain (Fig. 2A). These conformational changes are allosterically communicated to the lever arm and amplified into mechanical energy in form of a force-producing powerstroke that drives the relative displacement of myosin on the actin filament. This

mechanoenzymatic coupling ensures the potent actin-activation of the myosin ATPase activity whereas kinetic details determine whether a myosin can generate force or movement on actin.

Kinetic Concepts

The distribution of the rate and equilibrium constants that define the flux through the kinetic cycle (Fig. 2A) vary considerably and set the functional signatures between different myosins. The slowest step in the kinetic cycle limits the overall ATPase activity and is commonly associated with transitions between strong and weak actin-binding states. For most myosins, the release rates for either P_i or ADP have been identified as rate-limiting. Myosins with a rate-limiting P_i release spend a large fraction of the kinetic cycle in the weak actin-binding M.ATP and M.ADP.P_i states and a small fraction of the enzymatic cycle in the strong actin-binding states (Fig. 2A, 2B). Conversely, myosins with a rate-limiting ADP release spend a long fraction of their kinetic cycle in the strong actin-binding AM and AM.ADP states and a short fraction in the M.ADP and M.ADP.P_i states (Fig. 2A, 2B). The predominant population of states with different actin affinities allows determining the duty ratio; the fraction of time during the ATPase cycle myosin spends in the strong actin-binding states (Fig.2B). The duty ratio is a function of the actin and ATP concentration (20, 23).

Other signatures that make a kinetic distinction between myosins is the thermodynamic coupling of actin and nucleotide binding to myosin, and the kinetic coupling, the efficiency of actin to increase the displacement of ADP (19, 22).

Duty ratio, thermodynamic and kinetic coupling are among the parameters used to classify myosins based on their kinetic signatures. More importantly, these parameters define other features of myosin performance such as processivity, run length, and different modes of motility (19). These kinetic concepts, together with the directionality of the movement of myosin on actin, the responsiveness of the kinetic cycle to external force, and the individual multidomain organization determine the physiological function of a myosin motor.

Classification

Myosins can be grouped into ~ 31 distinct classes based on the phylogenetic analysis of the conserved motor domain (24). Throughout this work, Arabic numerals are used to number myosin classes. Members of a myosin class are indicated by the respective class number and a letter code/identifier. The use of Arabic rather than the traditional Roman numerals has become common practice in the field as the number of myosin classes is steadily increasing with the sequencing of more and more genomes.

Phylogenetic classification of myosins does not allow attributing a physiological function to a myosin class, as individual members of a class are commonly involved in diverse cellular processes. A classification that is based on the functional properties rather than phylogeny suggest four types of myosins: (I) fast movers, (II) slow/efficient force holders, and (III) strain sensors in addition to (IV) gated and processive myosins (19). We propose to expand this classification by the type of (V) kinetically inactive myosins (Fig. 3). Expansion of the classification to five types allows assigning each newly characterized myosin to a designated type, thereby contributing to the functional classification of all myosins. The kinetic and

functional signatures of myosins from each group are briefly described and the reader is redirected to the excellent review by Bloemink and Geeves for an in depth discussion of type I-IV myosins (19). It is of note that the boundaries for each group are not rigid and regulatory mechanisms such as changes in the cation concentration and load could shift a particular myosin to a different functional group (Fig. 3).

Fast moving type I myosins are characterized by a low duty ratio, high thermodynamic coupling and a low load-dependence of their catalytic cycle. Prototypic type I myosins are mammalian skeletal muscle myosin-2 and *Dictyostelium* myosin-1B (19, 25–27). On the other end of the kinetic spectrum are gated and processive type IV myosins such as mammalian myosin-5A, myosin-6 and myosins-7 that exhibit a high duty ratio, a strong load-dependence of their kinetic cycle but a low thermodynamic coupling ratio (19, 28–33). Type II and III myosins have intermediate kinetic signatures compared to type I and IV myosins: As slow and efficient force holders, some type II myosins have a low but slightly elevated duty ratio, a high thermodynamic coupling ratio and a low load-dependence of their enzymatic cycles (19). These kinetic signatures are compatible with the function of cardiac and smooth muscle myosins-2 in the contraction of muscle (34, 35). Type III myosins, including the strain sensors mammalian myosin-1B, nonmuscle myosins-2, and myosin-3A have a higher duty ratio and are more susceptible to load when compared to type II myosins but exhibit a lower thermodynamic coupling (9, 19, 36–40). Type V myosins were recently described as kinetically inactive *in vitro*. These pseudomyosins have evolved from enzymatically active homologues and resemble them structurally (41–44). An estimation based on the analysis of catalytic sites indicates that a minimum of 10% of mammalian and 15% of *Drosophila* enzymes are catalytically inert (45). Pseudomyosins are proposed to have regulatory functions in cells, therefore kinetic inertness is not coupled to physiological insignificance (46, 47).

All kinetically characterized myosins from classes-18 and -20 are pseudomyosins (41–44). Their motor domains do not bind nucleotides or bind nucleotides weakly but are unable to hydrolyze ATP (42–44). In agreement, no conformational change is observed in the myosin-18A motor domain upon ATP binding in electron microscopic studies (42). In this respect myosin-18A “kinetically” resembles an unphosphorylated, inactive nonmuscle myosin-2 (48, 49). The motor domain also binds actin weakly and in a nucleotide-insensitive manner (42–44). Actin binding is strengthened by N-terminal extensions of the myosin motor domain in some myosin-18A isoforms that harbor an ATP-insensitive actin-binding site (44, 50). Kinetic inertness is attributed to the loss of critical catalytic residues involved in nucleotide binding and hydrolysis and distinctive variations within the motor domain that are predicted to interfere with tight actin binding (42–44). Different from class-18 and -20 myosins, class-3 myosin from *Limulus* does not exhibit actin-activated ATPase activity but binds actin more tightly than the other inactive pseudomyosins, but still weaker than kinetically active myosins (51).

Heterotypic Myosins

Recent studies showed that the three mammalian nonmuscle myosins-2 copolymerize to form heterotypic bipolar filaments *in vitro* and *in vivo* (52, 53). All paralogs have slightly

different kinetic features that include a low but elevated duty ratio, very high affinities for ADP even when bound to actin and very slow ATPase activity under steady-state conditions (9, 38, 39, 54). The properties of myosin-2 tails to form filaments with a characteristic number of 14–30 myosins-2 suggests that heterotypic filaments may contain both, a variable number of myosin-2 isoforms and a broad spectrum of mechanoenzymatic properties (55). Heterotypic filaments were described in various locations in the cell including the cytoplasm, stress fibers and the contractile ring (52, 53). The ratio of a nonmuscle myosin-2 paralog found in a heterotypic filament is reflected in the differential distribution of the paralog itself (52). The physiological significance, mechanoenzymatic consequences and regulatory aspects of the heterotypic filaments have not been explored in detail.

Nonmuscle myosins-2 also copolymerize with the structurally related, but catalytically inert myosin-18A that appears to be expressed ~100-fold less compared to nonmuscle myosin-2A (47). The incorporation of a low number of myosin-18A molecules in nonmuscle myosin-2 filaments should not significantly alter the mechanoenzymatic properties of the heterotypic filament, however, the incorporation of a higher number of myosin-18A molecules in the filament should result in a heterotypic filament that would be less active as a mechanical unit due to a decrease in the collective duty ratio (42–44). Excess myosin-18A results in nonmuscle myosin-2 filament disassembly and/or interferes with filament assembly *in vitro*. This may not represent a physiologically significant mechanism given the disparity in the cellular concentrations of the two myosin classes (47). The regulation of the enzymatic properties of the heterotypic myosin ensemble, and hence the collective production of contractile force by catalytically inactive pseudomyosins represents a novel, higher order form of kinetic adaptation of myosin motor activity. Besides the effect of the mechanoenzymology, incorporation of myosin-18A in the bipolar nonmuscle myosin-2 filaments may enhance its interactome and localizome due to class-18 specific domains such as PDZ domains.

Kinetic Adaptations of Myosins

Kinetic and structural adaptations are the prerequisite for myosins' functional capacities and limitations and are inherently linked to the interaction signatures with actin. The interaction of a myosin with actin can be (i) nonprocessive, (ii) processive, or (iii) conditionally processive (Fig. 2B).

Nonprocessive myosins bind to an actin filament and couple the hydrolysis of one molecule of ATP to force-generation on actin before dissociating. Nonprocessivity is commonly associated with low duty ratio, monomeric myosins but has also been described for dimeric myosins. An example for the former is *Acanthamoeba* myosin-1A, an example for the latter human myosin-5C (56, 57). Nonprocessive myosins often work as cytoplasmic anchors or dynamic tethers that link the actin cytoskeleton to a subcellular compartment. However, nonprocessive myosins may establish conditional processivity, as discussed below.

A processive myosin can take multiple ATP-dependent steps as a single molecule on actin before dissociating. Vertebrate myosin-5A for example is a dimeric, high duty ratio motor with a high ATPase activity implicated in the transport of vesicular cargos and organelles including the endoplasmic reticulum (58–64). The geometry of the myosin-5A dimer allows

both motor domains to bind to the same actin filament, 36 nm apart from each other (65). In this binding geometry, the lead motor of the walking dimer binds towards the barbed-end of actin, the rear motor 36 nm behind the lead motor. The high affinity of the actomyosin complex for ADP allows both motor domains to strongly bind in the ADP bound state to actin (29, 66, 67). Intermolecular communication between the two motor domains, also referred to as gating, ensures that their kinetic cycles are out of synchrony but coordinated in a way that the rear motor releases its ADP while the lead motor remains attached to the actin filament (66, 68–70). Binding of ATP to the rear motor causes its dissociation from the actin filament and triggers the lead motor to undergo its powerstroke which positions the former rear motor in a position to rebind 36 nm forward on the actin filament (65, 66, 68, 71, 72). This motor rapidly hydrolyses ATP while in the actin-detached state and rebinds in the ADP.P_i state as the new lead motor to actin (65, 72, 73). While in the detached state, the motor undergoes a fast diffusive search for its binding site on actin (68, 72, 74). P_i release establishes a strong actin-binding state of the new lead motor and facilitates the ADP release from the rear motor. This coordinated mechanism establishes processive and directed hand-over-hand movement of the myosin-5A dimer in discrete 36nm steps on actin to minimize dissociation from actin for maximum transport efficiency *in vitro* and *in vivo* (68, 71, 73, 75–77).

As a processive motor, a single molecule of myosin-5A can transport a polymer bead *in vitro*, a situation that is rarely found in cells where usually >100 myosins-5A cluster at the surface of cargo vesicles (78, 79). *In vitro* reconstitution of physiological transport complexes of motor proteins collectively reveal that multiple motors bound to a rigid cargo reduce the speed of the motor but increase the run length when compared to a cargo transported by a single motor (80–84). Reasons for this behavior may include negative interference and load-dependence, as outlined in greater detail below. Flexible attachment of myosin-5A to fluid vesicles results in increased transport velocities relative to a single motor or a motor that is coupled to a gel-like vesicle (83). This indicates that properties of the cargo such as size, membrane composition and myosin surface-density regulate movement by a mechanical coupling mechanism (83). Mathematical simulations support a model for the observed increased transport velocity of a myosin-5A multi-motor transport complex in which stepping of a lead myosin causes the enhanced detachment of a rear myosin from the actin filament. This triggers the rapid motion of the attached cargo towards the lead myosin (83). Clustering of myosin motors on the surface of a cargo represents a higher order form of processivity that might be commonly employed by processive myosins to increase the intracellular transport efficiency.

Structural features of myosin tail domains may support processivity in monomeric high duty ratio myosins. For example, human myosin-3A is thought to be monomeric, yet it localizes to the tips of stereocilia in hair cells and traffics out to the tips of filopodia when overexpressed in cultured cells suggesting that it moves processively (85). Myosin-3A contains a second, ATP-insensitive actin-binding site in the tail domain (86). The coordinated action of tail attachment to actin and ATPase activity of the motor domain suggests an inchworming mechanism for this monomeric myosin rather than a hand-over-hand mechanism as has been shown for dimeric myosin-5A (86). Inchworming allows myosin-3A to function as cargo transporter in the auditory system where it is implicated in

the elongation of stereocilia (37, 85). Moreover, inchworming suggests a coevolution of kinetic and structural features of the myosin heavy chain and underlines the importance of monomeric high duty ratio motors in cellular transport. Note, however, that the proposed inchworming mechanism has not been proven by *in vitro* single molecule experiments.

A conditional processive myosin is a nonprocessive myosin that can establish processivity in certain settings for example by oligomerization, local clustering, binding partners, or driven by changes in the free Mg^{2+} concentration. Prototypic examples for conditional processive myosins are class-2 myosins. As low duty ratio, dimeric motors, myosins-2 must oligomerize into filaments consisting of a variable number of molecules to establish a continuous interaction with actin (87–89). Kinetic and electron microscopic studies revealed that the number of myosins-2 per filament increases with decreasing duty ratio. Sarcomeric and smooth muscle myosins-2 for example have low duty ratios of ~0.04 and assemble in large filaments composed of several hundred molecules (25). Cytoplasmic nonmuscle myosins-2 with a low, but elevated duty ratio of ~0.1–0.35 assemble into small filaments consisting of 14–30 molecules (9, 38, 39, 55). This ensures that a fraction of motor domains of the filament can interact simultaneously with actin to generate force and processive movement of the ensemble. The collective, continuous interaction powers the sliding of actin filaments past each other, as seen in the fast contraction of the sarcomeres in skeletal and cardiac muscle and cytoplasmic contractility in nonmuscle cells (87–89).

The first *in vitro* evidence for generating processivity by clustering came from the motility assay in which high densities of skeletal muscle myosins-2 processively moved actin filaments (90). Processive movement could also be *in vitro* reconstituted in optical-trapping experiments with skeletal muscle myosins-2 when myosin densities beyond the single motor regime were used (91).

In vivo, clustering of myosins occurs on the surface of a membrane or a cargo vesicle is required for intracellular transport processes. The tail domain of myosin-19, a monomeric myosin with a high duty ratio, associates with mitochondria in cells (92–95). The myosin-dependent transport of mitochondria along the cellular actin cytoskeleton requires the presence of multiple myosins on the organelle and is important for the distribution and mitochondrial partitioning during cell division (92, 93). A model suggests that at a high local concentration of ATP, actomyosin-based transport relocates mitochondria in the cell. A drop in the intracellular ATP concentration would slow transport function and the motor would become a cytoplasmic anchor as its ATPase activity decreases with decreasing ATP concentration. At the same time, the low ATP concentration would trigger an increase in mitochondrial ATP production that in turn could locally increase the ATP concentration in the cytoplasm. The combined action of transport and anchor function of different myosins-19 on mitochondria could result the mitochondrial network shape and geometry changes observed in cells (95).

Clustering of monomeric myosins on organelles, vesicles or adaptor proteins has also been described for myosins from classes-5, -6, -7 and -10. (81, 96–99). Different from myosin-19, clustering at the surface of vesicles or organelles induces the dimerization of the high duty ratio myosin monomers by means of class-specific tail domains (81, 96, 97, 100). Clustering

might not only induce dimerization of two monomeric myosins but also change the mechanoenzymatic signatures of a myosin as reported for nonprocessive myosin-5C (57). Clustering of two myosin-5C dimers via a DNA scaffold renders the ensemble conditionally processive. Velocity, run length, and step size of the ensemble decrease with decreasing motor distance, a mechanism that may be of physiological significance in cells where myosin-5C ensembles transport structurally diverse cargos with variable diameters from 40–800 nm (57, 101–103).

Another strategy to establish conditional processivity is to employ biochemical properties of a binding partner or cargo to maintain contact with actin. This facilitated transport has been reported for myosin-3B, which in contrast to its paralog myosin-3A lacks a second actin-binding site in its tail domain. Myosin-3B can only target the tip of stereocilia when associated with its cargo protein espin-1. Espin-1 is an actin-bundling protein that, by the virtue of its actin-binding site is proposed to increase the effective actin affinity of the transport complex, tether the myosin-3B tail domain to the actin filament and limit its diffusion from actin (86). This tethered motility establishes processivity and switches the nonprocessive myosin-3B to a conditionally processive motor that inchworms to the tip of actin protrusions. It is of note that the concerted effect of espin-1 and myosin-3B to establish processivity of a monomeric myosin has not been verified with *in vitro* studies.

Tethered motility is also described for dimeric myosins-5A: Unlike in myosin-3B, tethered motility of myosin-5A is not a prerequisite to establish processivity but rather maximizes transport efficiency (104). Tethered motility of myosin-5A established by its cargo adapter melanophilin not only serves as a functional link between myosin-5A and Rab27-melanosomes but also tethers the transport complex to the actin filament (104). This additional actin-tethering enhances processive runs, but reduces the speed of the transport complex relative to the behavior of myosin-5A. This mechanoenzymatic adaptation may be physiologically beneficial for the correct localization of melanosomes in cells (104).

Dictyostelium myosin-5B is a dimeric conditional processive motor (105). Unlike the previously discussed examples, switching from nonprocessivity to processivity may be exogenously induced by the concentration of free Mg^{2+}. A duty ratio of 0.23 at low concentrations of free Mg^{2+} does not support processive movement of the motor (105). High concentrations of free Mg^{2+} decrease the actin-activated ADP release and the ATP-induced dissociation of the actomyosin complex, thereby increasing the duty ratio to 0.74 which should allow the molecule to move processively (105). Physiological relevance of the conditional switching between nonprocessivity and processivity is in the context of the contractile vacuole system, an osmoregulatory organelle that is transported by myosin-5B along the actin cytoskeleton in *Dictyostelium* (105, 106). For myosin-6 it was shown that increasing free Mg^{2+} decreases the rate of the actin-activated ADP release which decreases sliding velocities and increases processivity (31, 107). A similar effect is expected for all Mg^{2+}-sensitive myosins (30, 38, 108–110).

May The Force Be With Myosin: Load-Sensitivity

Exogenous forces regulate structure and function of cells and the actomyosin cytoskeleton and trigger responses to the resulting mechanical load, for example during cell migration

and morphogenesis (111, 112). Nytrai and Geeves propose a universal strain-sensitive mechanism that affects kinetic and mechanical properties in a myosin-specific manner (22). Strain-sensitivity is not obvious from the analysis of transient and steady-state kinetic data which are obtained under unloaded conditions and therefore not considered in the classification of myosins according to their kinetic and physiological function shown in Fig. 3 (19).

The activity of a myosin at the single-molecule level can be measured with optical-trapping experiments to obtain information on how myosins adjust their mechanochemical behavior in response to external force applied to the lever arm. Specifically, optical-trapping experiments provide valuable information on (i) the displacement of myosin on actin (power-stroke size), (ii) the number of steps myosin takes on actin before it detaches (processivity), (iii) the amount of force a myosin exerts on actin, and (iv) the mechanoenzymatic details of the kinetic cycle (113, 114). A feature of all myosins studied in optical-trapping experiments is that resisting loads prolong and assisting loads reduce force-sensitive transition rates in or out of the strong actin-binding states of the catalytic cycle (Fig. 2A). A powerstroke or step is only possible if the resisting load is smaller than the stall force, the force at which no net movement is observed since forwards and backwards steps occur at the same rate. This change in directionality is independent from ATP binding and hydrolysis and solely mechanically induced (115). Directionality changes may be of importance in tug-of-war scenarios in which different myosins that move on the same actin filaments but in opposite directions transport a common cargo (116). The responsiveness to external force and the identity of force-sensitive transitions in the kinetic cycle are myosin-specific and gear a myosin for its cellular function which may be dual load-dependent.

Well-studied examples of how strain-sensitivity impacts the kinetic cycles of related enzymes are mammalian myosins-1B and -1C. As reviewed by Greenberg and Ostap, both myosins are low duty ratio motors that have similar unloaded kinetics with a rate-limiting P_i release step (117). However, their mechanoenzymatic output varies considerably under load that resists their powerstrokes (36, 118–120). Low resisting forces > 0.5 pN applied to myosin-1B slow down the ADP release rate to become rate-limiting thereby increasing the duty ratio of the motor. Further increasing the applied force > 1pN inhibits its detachment rate and myosin-1B remains attached to actin over time periods in the second-minute range (121, 122). Myosin-1C in contrast is less force-sensitive and becomes a high duty ratio motor only at forces > 2.5 pN. Unlike myosin-1B, the ATP-induced dissociation is the force-sensitive step in the kinetic cycle and decreases > 75-fold under high loads (118, 123). These mechanoenzymatic adaptions are reflected in the functional activities of both class-1 myosins: Myosin-1B is a force-sensitive anchor or tether that links actin filaments to a membrane, thereby controlling membrane tension and deformation which is important during the formation of tubular post-Golgi carriers. Myosin-1C in contrast is a slow transporter, for example, of the glucose transporter GLUT4 to the plasma membrane of adipocytes after insulin-stimulation (117, 124, 125). Conversely, both myosins could not participate in their physiological functions if they were more or less susceptible to load or if they had swapped load-dependent kinetics (117). Interestingly, intrinsic and extrinsic regulatory mechanisms such as alternate splicing in the lever arm which determines its

length, disease-causing mutation in the motor domain and the concentration of Ca^{2+} have been shown to alter force-sensitivity in class-1 myosins (117, 119, 122, 126). The latter mechanism is of particular interest as Ca^{2+} potently desensitizes myosin-1B against resisting forces and may represent a physiological relief mechanism for the force-induced actin attachment (119). Transient increases in the intracellular Ca^{2+} concentration may therefore release a tethered vesicle from the actin cytoskeleton (117, 119). At the structural level, Ca^{2+}-binding to calmodulin, the light chain bound to the IQ motif closest to the myosin motor domain, is believed to allosterically uncouple conformational changes in the motor domain form the powerstroke (119). The structural prerequisites that renders a specific myosin more or less susceptible for strain are generally unknown but may involve an allosteric communication pathway between 10–15 amino acids at the very N-terminus of the motor domain and a hydrophobic site formed by the converter and the lever arm in some class-1 myosins (127).

Strain-sensitivity also coordinates the action of numerous myosin-2 motors. In muscle, this mechanism is known as Fenn effect where the muscle becomes more efficient as the load increases and the velocity of shortening decreases. This effect has been shown in optical trapping experiments for smooth muscle, cardiac muscle and skeletal muscle *in vitro* (128–130).

Strain-dependence of the kinetic pathway has been demonstrated by four different methods for myosin-5A. I) Optical trapping studies of a monomeric myosin-5A fragment demonstrated that resisting force dramatically slowed the rate of ADP release - whereas assisting forces increased it (68) Since the lead motor should be experiencing resisting strain from the bound trail motor, this would lead to a marked gaiting of the ADP release that from the lead motor and help confer directionality and promote processivity. II) Measurement of the unbinding force of a monomeric myosin-5A fragment to actin in the presence of ADP under assisting and resisting load was modeled to support the case that the ADP dissociation rate of the strained lead motor would be 20 times slower than that from an unstrained motor (131). III) Transient kinetic studies of dimeric myosin-5A initially bound to actin via both motors in complex with ADP showed that the bound rear motor exerted a resisting load on the lead motor that resulted in a much slower release of ADP from that motor compared to the unstrained release of ADP by a singly attached motor (69, 70). IV) This effect was also seen in a super-resolution single molecule tracking experiment where the movement of a dimeric myosin-5A was monitored along with the binding and dissociation of a fluorescent nucleotide analog. This experiment showed that nucleotide release from the lead motor always occurred before that of the rear motor (60). These experiments provide direct experimental support for the gating of the kinetics of the two motor domains of myosin-5A, which contributes towards the directionality and processivity of the molecule.

Myosin-6 is a high duty ratio motor that uniquely moves to the pointed-end of the actin filament (12, 13, 28, 31). Its oligomeric state is still controversial as recombinant and tissue-purified myosin-6 is monomeric and has a low tendency to dimerize, whereas its physiological function as transporter for endocytic vesicles and cytoplasmic anchor suggest that dimerization may occur in cells (14, 132–137). Myosin-6 dimerization has been shown to be enhanced in the presence of adapter proteins that link the motor to a cargo or by

monomer-clustering *in vitro* (96, 97, 134). Dimerization of myosin-6 by its adapter proteins optineurin and Dab2 reduces its steady-state ATPase activity around 2-fold, indicating gating between the two motor domains which ensures that the lead motor remains bound to actin until the rear motor detaches by reducing the ATP-induced dissociation of the lead motor from actin (138, 139). This mechanism renders myosin-6 processive under unloaded conditions and allows a processive hand-over-hand movement, consistent with its function as transporter during endocytosis (15, 97). Different from monomeric class-1 myosins where the very N-terminus of the myosin heavy chain allosterically modulates force sensitivity, the unique insert-1 in myosin-6 sterically interferes with the binding of ATP to the lead motor of the strained dimer (139).

Consistent with the observed processivity *in vitro* and *in vivo*, optical-trapping experiments revealed that an artificial myosin-6 dimer functions as transporter at low loads <1.5 pN. Increasing forces gradually slow down its processive movement on actin, which allows myosin-6 to maintain tension as cytoskeletal anchor. Forces >2.5 pN applied to myosin-6 result in detachment from the actin filament (140, 141). These dual force-dependent functions may have physiological significance in hair cell stereocilia where myosin-6 functions as a cargo transporter and an anchor that links the actin cytoskeleton to the membrane (16). A myosin-6 deafness mutation in the motor domain induces the premature release of P_i which prevents the initiation or favors the termination of processive movement of the dimer on actin as the detached motor can not rebind to actin before ATP induces the dissociation of the attached motor from actin (142). Manifestations of the impaired processivity include clustering of myosin-6 at the tip of hair cell stereocilia and a gradual distribution along the length of the stereocilium in the auditory system (16).

Other studies indicate that solely the high duty ratio and neither dimerization nor gating is required for myosin-6 to function as processive motor: An *in vitro* study shows that a monomeric myosin-6 is nonprocessive but can establish conditional processivity when coupled to a polystyrene bead that compares in the dimensions to a cellular vesicle. The monomer-driven movement of the bead is 2.5-fold shorter when compared to the movement of a bead coupled to dimeric myosin-6 and further characterized by a load-dependent step size and stall force. A model suggests that the bead acts as a diffusional anchor which supports the processive movement of the monomer by increasing the association with actin after detachment (81). In line with this interpretation, the coordinated mechanic interaction of individual myosin-6 motors coupled together through an endosome-sized nanosphere implies that dimerization is not required for processive movement under unloaded conditions over long distances (82). Multi-motor behavior of myosin-6 was also studied in an artificial myosin filament based on a DNA nanotube scaffold that allows controlling the number and spacing of myosin motors (143). In this setting, the collective transport of an actin filament by myosin-6 motors is independent from the spacing and myosin density, also indicating a mechanical coordination of myosins that are not physically coupled directly. The same has been described for cardiac myosin-2 and myosin-5A, suggesting that it is a generic feature of myosins across classes (143).

Both myosin-5A and myosin-6 are involved in the intracellular transport of a common cargo in neural growth cones (144). The observed directionality of cellular cargos suggest that this

multiple-motor transport resembles a tug-of-war as both myosins move in a hand-over-hand motion in opposite directions on a single actin filament, thereby imposing resistive forces on each other (116). *In vitro* reconstitution of this setting at the single molecule level reveals that myosin-5A is the stronger motor when equal numbers of both myosins are present on a common cargo, suggesting unidirectional transport towards the barbed-end of actin (116). Physical coupling of both motors causes the mutual coordination of their stepping behavior during the multi-motor transport in that the weaker motor coordinates its backwards steps with the forward steps of the stronger motor (116). Moreover, a generic feature of multi-motor transport is that the velocity of the stronger motor is reduced due to the resisting load of the weaker motor (116). In the presence of ADP and resistive forces, myosin-6 switches from transport to anchor function and mechanically stalls myosin-5A movement (116, 141). This observation is attributed to the distinct effect of ADP on the mechanoenzymatic properties of both myosins (140, 141, 145). It also implies that the metabolic state, in addition to other factors, may be a physiological regulator of the actin-based transport of a cargo to its cellular destination (116). The metabolic state of a cell is also suggested to be regulatory for the transport of mitochondria by myosin-19 as described above (94).

Myosin-10 is best known for its function as cargo transporter in intrafilopodial trafficking, filopodial growth and maintenance which exhibits long-range motility (146–148). Its oligomeric state is still controversial but unloaded solution kinetic studies indicate that the monomer is a high duty ratio motor with a rate-limiting ADP release rate (3, 149–151). Myosin-10 is nonprocessive as a monomer but works as a processive motor when artificially dimerized (152). Applied forces <1 pN allow an artificial myosin-10 dimer to walk processively in a hand-over-hand stepping motion on actin. Forces >1 pN abolish or terminate processive movement as the lead motor detaches before the rear motor binds to actin, causing the dissociation of the dimer from the actin filament (150). This force-relief mechanism of myosin-10 as cargo transporter in the dense environment of a filopodium may facilitate trafficking as the motor can quickly release from the actin filament when the cargo is tangled in the cytoskeleton and applies an increasing resistive force on myosin, and continue its processive movement toward the filopodial tip on another actin filament. In this cellular setting, the relatively low force-dependence of the myosin-10 kinetics might be advantageous as its stop-and-go mechanism ensures a net movement toward the filopodial tip whereas a stall-and-stop mechanism would require higher forces and decrease transport efficiency (150). Notably, the actin-activated ADP release from both myosin-10 motor domains is not gated, supporting the results of studies on myosin-6 and an artificial myosin-5 construct that demonstrate that gating is dispensable for processive movement (81, 150, 153).

Optical trapping experiments also revealed that the myosin powerstroke is commonly biphasic (68, 121, 130, 154–157). The first step is usually larger and is independent from the ATP concentration indicating that it represents a conformational change associated with Pi release. The lifetime of the second step decreases with increasing ATP concentration suggesting that it is coupled to ADP release (68, 154). For myosin-5A, the first step is more dependent on externally applied forces than is the second step (156). Analysis of small myosin-5A-coated beads moving along an actin filament in a single beam optical trap showed evidence for substeps of 12 and 24 nm. The 12 nm substep occurrs upon ATP

binding to the rear motor, the 24 nm substep is associated with an isomerization of the actin-bound lead motor in the ADP state (157). It is not clear how the ADP-dependent second step described earlier from studies of monomeric myosin-5A relate to the substeps observed during stepping.

Autoinhibition

To prevent futile ATP consumption and weaken the interaction with actin, some myosins were found to undergo conformational transitions that inhibit their catalytic activity. Myosins from classes-2, -5, -7, and -10 have been shown to be in equilibrium between an extended conformation and a compact conformation (158–161). The former is catalytically active, the latter catalytically inactive. Regulatory factors that shift the equilibrium from the active to the inactive conformer and *vice versa* are myosin-specific and may include phosphorylation events, the interaction with lipids, cations and binding partners (98, 104, 159, 162–164).

Structural details of the compact conformer are myosin-specific and strongly determined by the domain architecture of the tail. However, in all cases folds the tail back and interacts with the myosin motor domain. The compact and extended myosin conformer can be distinguished in analytical ultracentrifugation experiments that assign a sedimentation rate or S-value to each conformer. In the case of myosins-2, the compact conformer (10S) occurs for nonphosphorylated myosin and involves both, an asymmetric motor-motor interaction as well as folding of the long tail back upon the motor domains. The structural constrains imposed by motor-motor and motor-tail interaction in 10S compromises its catalytic activity by reducing the rate-limiting P_i release rate ~1000-fold (165–168) This inhibition does not strictly require the distal half of the tail domain since a heavy meromyosin fragment of smooth muscle myosin-2 is also well regulated by phosphorylation and displays the identical motor-motor interaction as seen in the full length myosin-2 (49, 167). The autoinhibited conformer of myosins-2 is still able to interact with actin (49, 167, 169). The physiological function of 10S in nonmuscle cells is still controversial but may, besides representing a myosin storage pool, expose binding sites for regulatory proteins to locally control actomyosin-based contractility (170). The asymmetric motor-motor interaction characteristic of the autoinhibited myosin-2 conformer is also seen in relaxed thick filaments from invertebrate muscles, indicating that autoinhibition is an evolutionary conserved regulatory mechanism for the enzymatic activity of myosins-2 (171–173).

Mammalian myosin-5A is in equilibrium between an extended (14S) and a compact conformation (11S). The globular tail domain contacts the motor domain in 11S and allosterically reduces the P_i release rate ~1000-fold to become rate-limiting (160, 174–176). The inactive conformer also has a reduced the duty ratio which virtually abolished the interaction with actin (175, 176). Autoinhibition relief can be artificially induced *in vitro* by Ca^{2+} and by binding partners such as melanophilin that bind to the globular tail domain *in vitro* and *in vivo* (104, 162, 164, 177). Unlike binding partners that shift the equilibrium to the extended, mechanoenzymatically competent 14S inside cells, Ca^{2+} is not regarded as a physiological regulator of myosin-5A function *in vivo* as it dissociates a subset of

calmodulin light chains from the heavy chain and renders the motor mechanically incompetent (160, 162, 177–179).

Myosin-7A is a monomeric high duty ratio motor that is important for the maintenance and development of actin based protrusions such as filopodia and stereocilia (30, 32, 33, 180, 181). It is implicated in the transport and localization of the Usher I scaffold proteins harmonin and sans which are important for hearing and vision (3, 182, 183). Myosin-7A adopts a compact conformation in which motifs of the tail domain fold back on the motor domain to inactivate the enzymatic activity (161, 184). One study concluded that the last MyTH4 domain of the tail contacts the myosin-7A motor domain, another study suggests that the tail domain contacts both, motor and neck domain (161, 184). The tail-motor interaction inactivates the myosin ATPase activity and weakens the interaction with actin at low concentrations (161). High actin concentrations relieve the autoinhibition and render the motor enzymatically active. The ability of actin to shift the equilibrium to the extended conformer is caused by the tail domain that additionally contains an ATP-insensitive, low affinity actin-binding site that may overlap with the MyTH4 domain (161). One activation model suggests that the compact myosin-7A conformer extends when the tail domains encounters a locally high concentration of actin as it is found in filopodia or hair cell stereocilia (161). The motor domain is then able to bind to the same or an adjacent actin filament and exert force on it. Another activation model suggests that micromolar concentrations of Ca^{2+} relieve autoinhibition by binding to the light chain calmodulin that is bound in close proximity to the motor domain (98, 184). A conformational change induced in calmodulin upon Ca^{2+} binding would abolish the interaction with the tail domain and activate the myosin ATPase activity. In striking contrast to myosin-5A in which Ca^{2+} binding to calmodulin causes the dissociation of the light chain from the neck domain *in vitro*, Ca^{2+} bound calmodulin remains bound to the neck of myosin-7A, suggesting that Ca^{2+} might be a physiological regulator of the enzymatic activity of myosin-7A (178, 179, 184). The extended conformer would be able to interact with binding partners that either may link myosin to cellular compartments or may induce myosin dimerization and render the motor into a processive transporter (32, 98, 161).

Another myosin that contains MyTH-FERM domains in the tail is myosin-10. Its tail domain additionally contains PH domains that change its cargo selectivity compared to myosin-7A (3). This structural distinction also changes the mode of autoinhibition relief: The compact conformer extends upon binding of phosphoinositides to the tail PH domains, a process that is independent from Ca^{2+}, and renders the motor enzymatically active and motile in the *in vitro* motility assay (159). Phosphoinositide binding further triggers myosin-10 dimerization that would allow the cargo associated motor to function as processive transporter toward the filopodial tip (159).

In summary, autoinhibition prevents futile ATP consumption for all myosins but also prevents the accumulation of unengaged myosins implicated in cellular transport at the barbed-ends of actin filaments at the cell periphery, and promotes their recycling by diffusion (63).

Quantitative Prediction of Kinetic Features

Despite a steadily increasing number of biochemically, kinetically, mechanically, and structurally characterized myosins and their mutants, a quantitative faithful prediction on how myosin kinetics and physiological functions respond to genetic or pharmacological perturbation is difficult to make. For example, a disease-causing single amino acid mutation in nonmuscle myosin-2A reduces the maximum actin-activated ATPase activity, the apparent affinity for actin in the presence of ATP, and the ability to slide actin filaments *in vitro*. The corresponding mutation in nonmuscle myosin-2B has a less severe phenotype but interferes with the movement of actin filaments, despite 86% identity and 95% homology between the motor domains of both enzymes at the amino acid level (185). Reasons for the observed kinetic and motile properties lie in the amino acid composition of the acceptor myosin that slightly differs from the donor and implies myosin-specific allosteric signatures that differently respond to amino acid substitutions.

Insights in the allosteric pathways and predictions of mechanoenzymatic phenotypes would be especially beneficial for *in vitro* and cell biological studies where a physiological function of a myosin needs to be probed with a mutant that confers a desired kinetic signature. These include but are not limited to rigor mutants that stay strongly attached to actin, mutants that do not bind actin or mutants that are enzymatically inactive or compromised to a certain extent. Unfortunately, a true rigor-mutation has neither been described nor laboratory-generated and the above mentioned disease-causing amino acid substitutions are associated with caveats when introduced in a heterologous myosin without a detailed *in vitro* study of its kinetic properties.

One structural feature that unifies all kinetically active myosins is a highly conserved salt bridge that is formed between switches-1 and -2 in the nucleotide binding pocket (Fig. 1C) (186–188). Structural, kinetic, and simulation studies uniformly indicate that the formation of this salt bridge is a key step during the ATPase cycle and pivotal for the ATP hydrolysis to occur. Its disruption drastically reduces the motor activity in myosins ranging from *Aspergillus* class-1 myosin MYOA to *Dictyostelium* myosin-2 and vertebrate smooth muscle myosin-2 (186, 189, 190). Intriguingly, the salt bridge is absent in all pseudomyosins from class-18, underlining its importance for myosin motor function (42–44). Sequence analysis further indicates that roughly 6% of the myosin heavy chain sequences deposited in Cymobase contain substitutions from the consensus sequence in switch-2 that may interfere with salt bridge formation and belong amongst others to myosin classes-17, -18, and -20 (191).

We propose that a good way to create inactive monomeric myosins for cell biological studies is to disrupt the salt bridge between the nucleotide switches-1 and -2 (Fig. 1C) rather than introducing disease-causing mutations in the myosin heavy chain. This approach has successfully been used in cell biological and *in vitro* studies on members of various myosin classes and on related molecular motors from the kinesin superfamily (192–196). Such mutant myosins would not have the ability to productively move or generate force on actin filaments, but should maintain a weak actin-binding state. Disruption of the salt bridge in filament forming class-2 myosins has only been studied with S1-like constructs *in vitro* and a potential effect on the 10S/filament equilibrium has not been ascertained.

Conclusion and Perspectives

Studies of kinetic and mechanical properties on isolated myosins, mutants, and complexes *in vitro* vastly expanded our understanding of myosin function *in vivo*. In the future, it will be pivotal to determine in more detail how force, structure and allostery influence kinetic properties with the long term goal to measure and understand enzymatic reaction and spatiotemporal response to regulatory factors in the in real time in cells, rather than in a test tube (197). A more detailed understanding on myosin allostery would also allow the *ab initio* design of myosins with desired functions that would be beneficial for *in vitro* and *in vivo* studies as well as therapeutic opportunities.

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Abbreviations

HMM	heavy meromyosin
P_i	inorganic phosphate
S1	myosin subfragment-1

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Synopsis

Myosins are ubiquitous actin-based molecular motors that can be grouped into five classes based on kinetic properties. The distinction between individual members of each class lie in subtle kinetic differences and the capability to adapt its kinetic features in response to factors including external force, dimerization and oligomerization, the cation concentration and binding partners that gear a myosin for its particular cellular function.

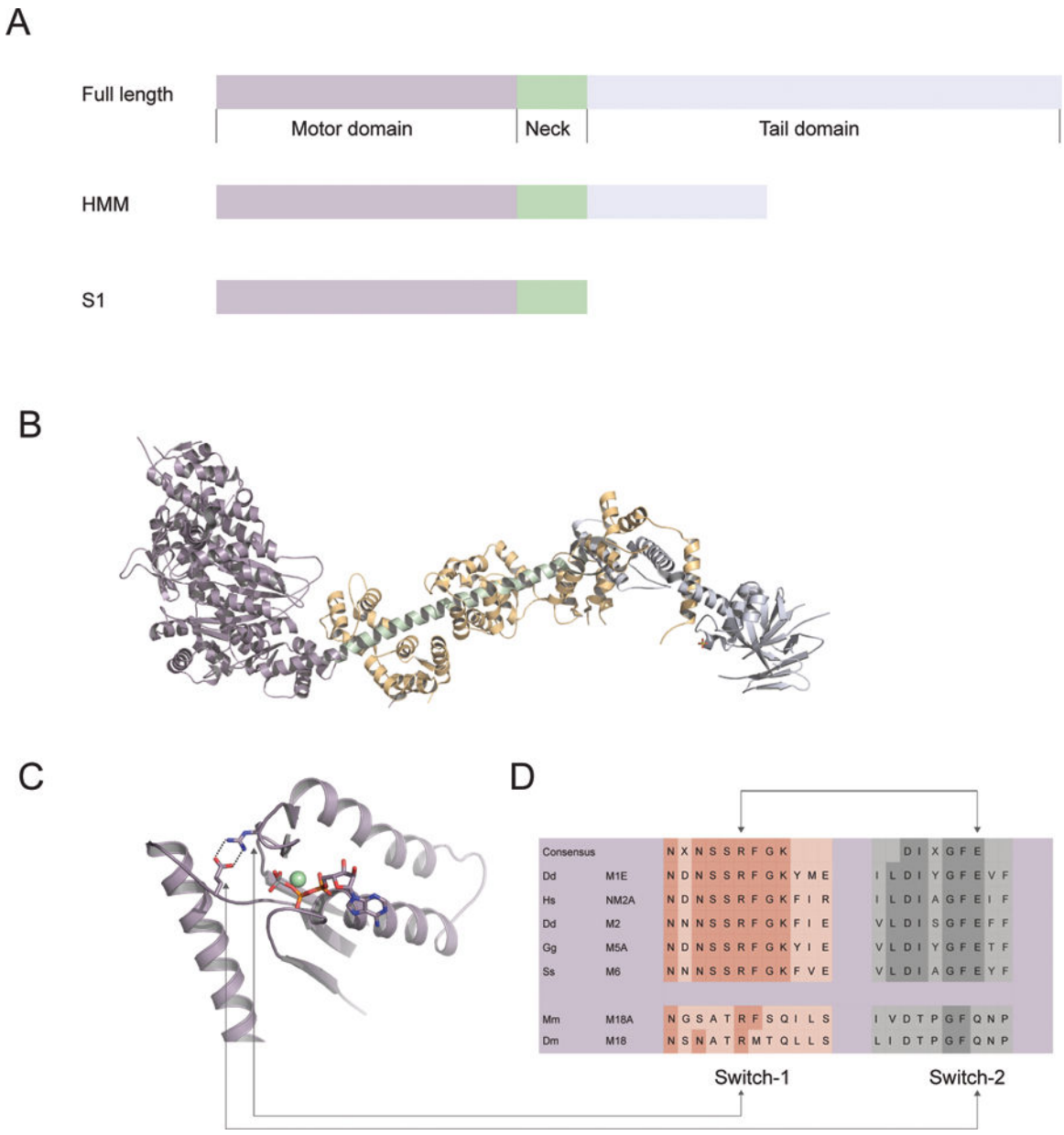

Fig. 1.

Myosin Topology. A) A generic myosin heavy chain consists of a motor domain (lilac), a light chain binding neck domain (pistachio) and a class-specific tail domain (blue). The motor domain is catalytically active and largely determines the interaction signatures of the motor with actin in a nucleotide-dependent manner. The neck domain and the associated light chains function as amplifiers for mechanical force. The tail domains may contain a large collection of functional subdomains which establish the interaction with binding partners and cargos, target a myosin to specific subcellular compartments (3). The tail domain may also dimerize two myosin heavy chains and promote their oligomerization. For kinetic studies, monomeric myosin subfragment-1 (S1)-like constructs comprising the myosin motor domain and the light chain binding neck region are commonly used. S1-like

constructs are in most cases constitutively active. To study regulatory aspects of the kinetic and functional activity of dimeric myosins, the heavy meromyosin (HMM) fragment comprising the motor domain, the neck domain, and parts of the coiled-coil forming tail domain are used. The schemes are not drawn to scale. B) 3-dimentional model of a myosin showing the globular motor domain (lilac), the light chain (lemon) binding neck domain (pistachio) and the tail domain (blue). For illustration purposes, the structures of the motor domain of myosin-1C (lilac, PDB entry 4BYF) and the light chain binding tail domain of myosin-1C (pistachio, PDB entry 4R8G) were merged. The neck domain of myosin-1C binds three calmodulins as light chains as shown in lemon. The tail domain harbors a C-terminal extended PH domain (blue). C) Overview of the myosin nucleotide binding pocket (PDB entry 2XEL) in which a salt bridge is formed between the nucleotide switches switch-1 and switch-2. The respective amino acids and the nucleotide are shown in stick representation. The cofactor Mg^{2+} is shown as green sphere. D) Sequence alignment of selected myosins from classes-1, -2, -5, -6 that follow consensus switch-1 and switch-2 sequences. Catalytically inactive class-18 myosins have highly degenerate switch-1 and switch-2 consensus sequences that do not allow for the formation of a salt bridge between both nucleotide switches. Abbreviations used are as follows: Dd M1E: *Dictyostelium discoideum* myosin-1E, Hs NM2A: *Homo sapiens* nonmuscle myosin-2A; Dd M2: *Dictyostelium discoideum* nonmuscle myosin-2; Gg M5A: *Gallus gallus* myosin-5A; Ss M6: *Sus scrofa* myosin-6; Mm M18A; *Mus musculus* myosin-18A; Dm M18: *Drosophila melanogaster* myosin-18.

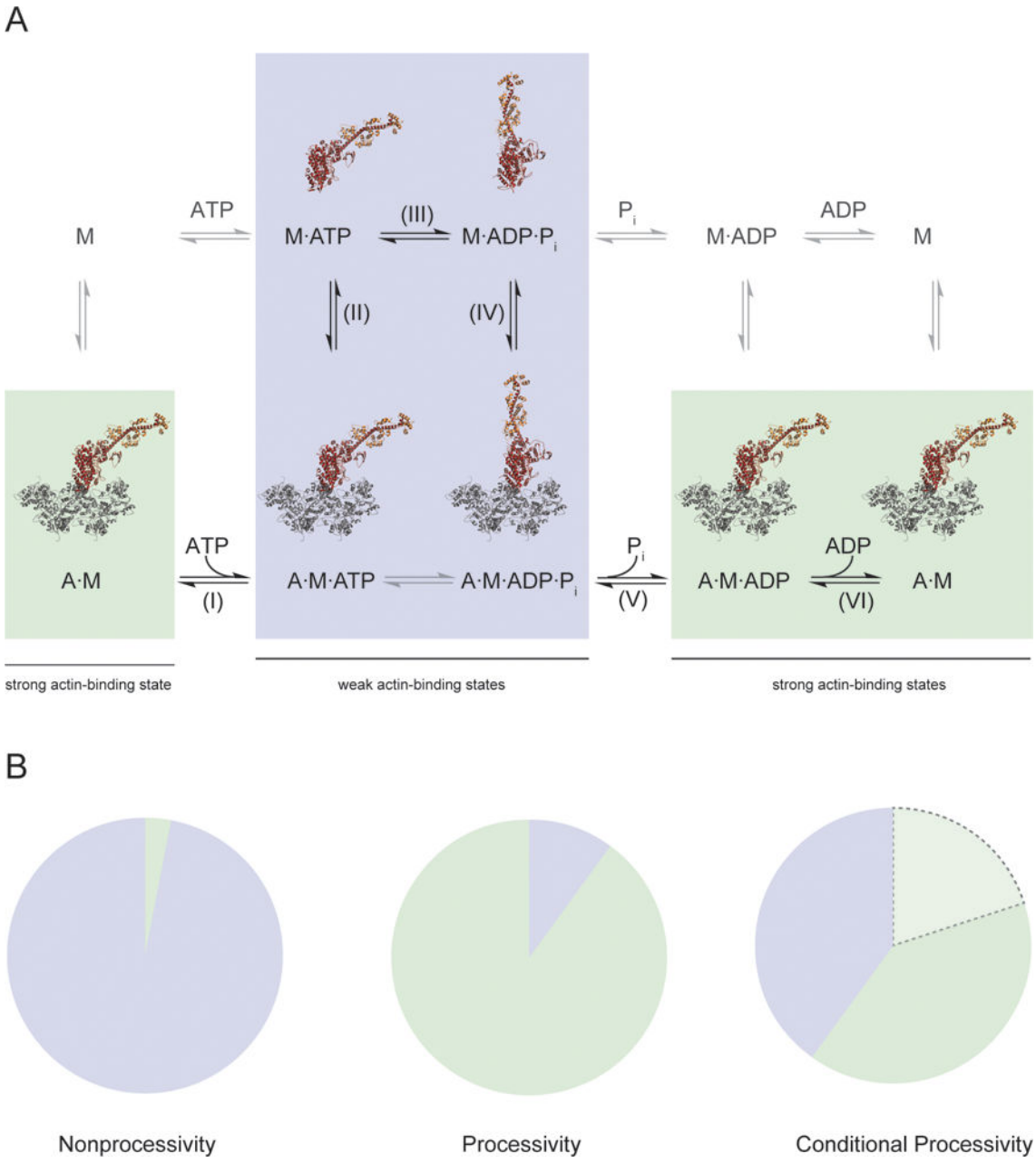

Fig. 2.

The myosin kinetic and mechanic cycle and kinetic concepts. A) Consensus scheme of the myosin and actomyosin ATPase cycle in conjunction with a mechanical model. The upper part represents the ATPase cycle in the absence of actin. The lower part represents the ATPase in the presence of actin. The main flux through the pathway is described as a generic series of sequential steps including (I) ATP binding, (II) ATP-induced dissociation of myosin from actin, (III) ATP hydrolysis, (IV) Rebinding of M.ADP.P_i to actin sets the starting point for the mechanical interaction. The release of the hydrolysis products (V) P_i and (VI) ADP are linked to conformational changes in the motor domain that result in the translocation of myosin on the actin filament. The (V) P_i release is rate-limiting in low duty ratio myosins to

extend the time myosin spends in the weak actin-binding states. The release of (VI) ADP is slow and rate-limiting in high duty ratio myosins to extend the time the motor spends in the strong actin-binding states. The main flux through the pathway is indicated by black font color. A green box indicates strong actin-binding states, a blue box weak actin-binding states. Abbreviations used: A: actin; M: myosin; P_i: inorganic phosphate. In the structural models, actin is represented in grey, the myosin motor and neck region in cherry and the light chains in orange color. B) Schematic representation of the duty ratio, the time myosin spends in the strong actin-binding states. The duty ratio is a function of the actin and ATP concentration. Both define the relative distribution of the strong and weak actin-binding states in the actomyosin ATPase cycle (20, 23). Nonprocessive myosins have a low duty ratio and spend a short fraction of the kinetic cycle bound to actin and a large fraction of time in the weak actin-binding states (left panel). Conversely, processive myosins spend a short fraction of the overall kinetic cycle in the weak actin-binding states and a long fraction in the strong actin-binding states (middle panel). Conditional processive myosins are kinetically nonprocessive myosins that can alter their kinetic properties to increase the time they spend strongly attached to actin to become processive (right panel). Weak actin-binding states are colored lilac, strong actin binding states in pistachio.

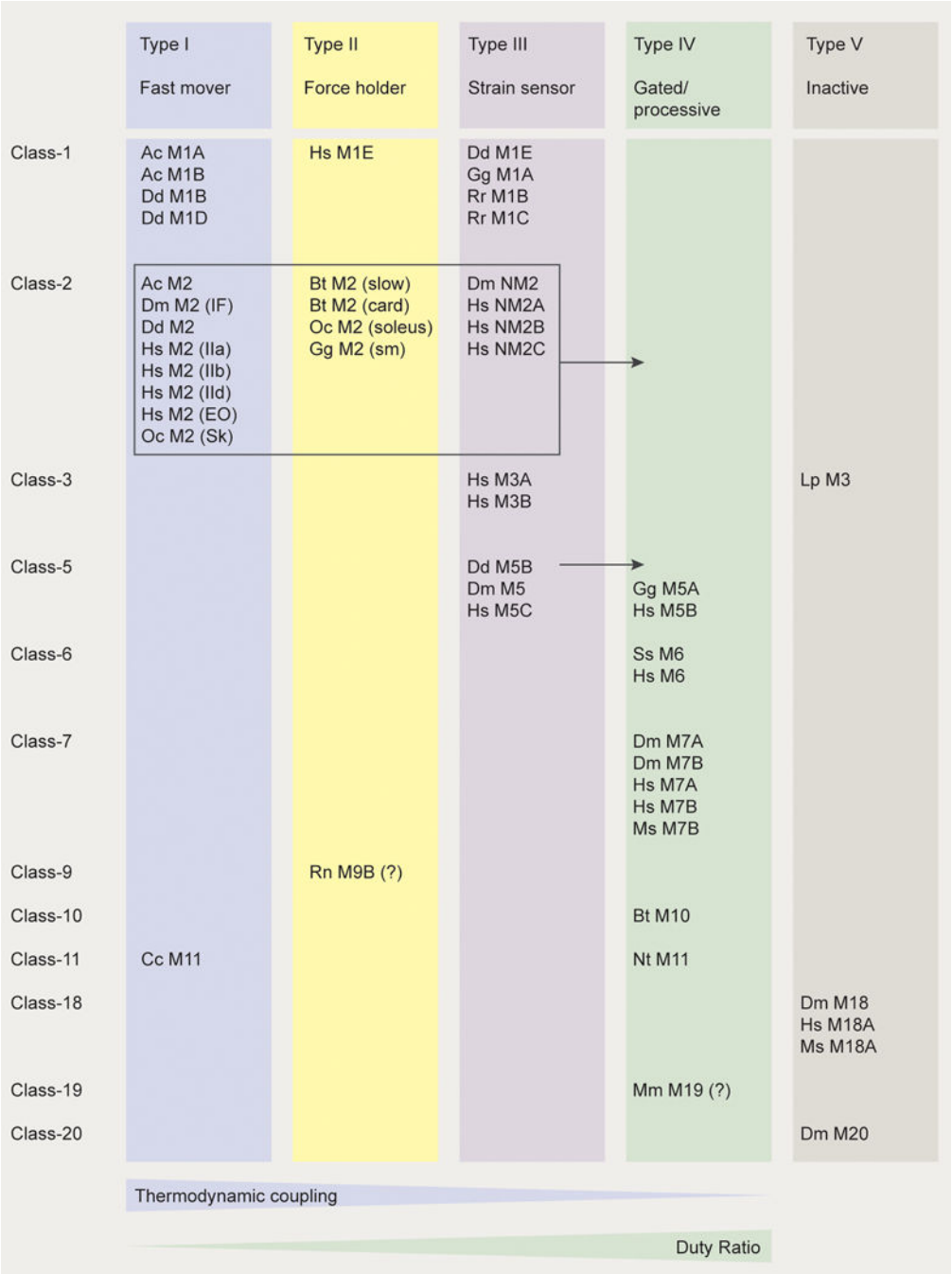

Fig. 3.

Myosin classification. The classification of myosin motors into five classes includes type I (fast movers), type II (force holders), type III (strain sensors), type IV (gated/processive myosins), and type V (catalytically inactive) myosins. The arrows indicate changes in the myosin type that may be induced by oligomerization of myosin-2 molecules into filaments or by increases in the free Mg^{2+} concentration for some class-5 myosins. The lack of detailed kinetic transient-kinetic information only allows for a provisional classification of class-9 and -19 myosins. Abbreviations used: Ac M1A: *Acanthamoeba castellanii*

myosin-1A; Ac M1B: *Acanthamoeba castellanii* myosin-1B; Ac M2: *Acanthamoeba castellanii* myosin-2; Bt M2 (card): *Bos taurus* cardiac myosin-2; Bt M2 (slow): *Bos taurus* slow muscle myosin-2; Bt M10: *Bos taurus* myosin-10; Cc M11: *Chara corallina* myosin-11; Dd M1B: *Dictyostelium discoideum* myosin-1B; Dd M1D: *Dictyostelium discoideum* myosin-1D; Dd M1E: *Dictyostelium discoideum* myosin-1E; Dd M2: *Dictyostelium discoideum* myosin-2; Dd M5B: *Dictyostelium discoideum* myosin-5B; Dm M2 (IF): *Drosophila melanogaster* indirect flight muscle myosin-2; Dm M2: *Drosophila melanogaster* nonmuscle myosin-2; Dm M5: *Drosophila melanogaster* myosin-5; Dm M7A: *Drosophila melanogaster* myosin-7A; Dm M7B: *Drosophila melanogaster* myosin-7B; Dm M18: *Drosophila melanogaster* myosin-18; Dm M20: *Drosophila melanogaster* myosin-20; Gg M1A: *Gallus gallus* myosin-1A; Gg M2 (sm): *Gallus gallus* smooth muscle myosin-2; Gg M5A: *Gallus gallus* myosin-5A; Hs M1E: *Homo sapiens* myosin-1E; Hs M2 (IIa): *Homo sapiens* striated muscle myosin-IIa; Hs M2 (IIb): *Homo sapiens* striated muscle myosin-IIb; Hs M2 (IId): *Homo sapiens* striated muscle myosin-IId; Hs M2 (EO): *Homo sapiens* extraocular muscle myosin-2; Hs NM2A: *Homo sapiens* nonmuscle myosin-2A; Hs NM2B: *Homo sapiens* nonmuscle myosin-2B; Hs NM2C: *Homo sapiens* nonmuscle myosin-2C; Hs M3A: *Homo sapiens* myosin-3A; Hs M3B: *Homo sapiens* myosin-3B; Hs M5B: *Homo sapiens* myosin-5B; Hs M5C: *Homo sapiens* myosin-5C; Hs M6: *Homo sapiens* myosin-6; Hs M7A: *Homo sapiens* myosin-7A; Hs M7B: *Homo sapiens* myosin-7B; Hs M18A: *Homo sapiens* myosin-18A; Lp M3: *Limulus polyphemus* myosin-3; Mm M7B; *Mus musculus* myosin-7B; Mm M18A; *Mus musculus* myosin-18A; Mm M19; *Mus musculus* myosin-19; Nt: *Nicotiana tabacum* myosin-11; Oc M2 (sk): *Oryctolagus cuniculus* skeletal muscle myosin-2; Oc M2 (soleus): *Oryctolagus cuniculus* soleus muscle myosin-2; Rr M1B: Rattus *rattus* myosin-1B; Rr M1C: Rattus *rattus* myosin-1C; Rr M9B: Rattus *rattus* myosin-9B; *Ss* M6: *Sus scrofa* myosin-6.