

Regulation of Class I and Class II Myosins by Heavy Chain Phosphorylation*

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Myosins have been traditionally viewed as mechanonchemical, actin-activated MgATPases that convert the energy of ATP hydrolysis into force between actin and myosin filaments exhibited as either movement (isotonic contraction) or tension (isometric contraction). "Conventional" myosins, i.e., myosins that form filaments, consist of a pair of heavy chains (~200 kDa) and two pairs of light chains, the regulatory light chain and the essential light chain. Each heavy chain has an N-terminal, globular head, where the actin-activated ATPase activity resides, and a C-terminal tail, through which the two heavy chains interact to form an $alpha$-helical coiled-coil rod. One pair of each of the light chains is bound to the neck region of the globular heads (subfragment-1) near the head-tail junction. The tail also mediates the self-association of multiple myosin molecules into bipolar thick filaments.

Ca$^{2+}$ activates muscle actomyosins predominantly in one of two general ways (1, 2): (i) actin-based regulation in which Ca$^{2+}$ binds to troponin C, a component of the troponin-troponin complex that lies in the groove of the $alpha$-helical coiled-coil actin thin filament (skeletal muscle); (ii) myosin light chain-based regulation in which either Ca$^{2+}$/calmodulin-dependent myosin light chain kinase phosphorlcpylation of a serine in the regulatory light chain (smooth muscle) or Ca$^{2+}$ binds directly to the regulatory light chain (molluscan muscle).

For many years, myosin filaments, as well as actin filaments, were thought to be essential for physiologically meaningful interactions of actin and myosin. The discovery of nonmieronic Acanthamoeba myosin I (3), however, proved that single-headed, nonfilamentous myosins, i.e., "unconventional" myosins, could exert force between, and translocate along, actin filaments. This expanded the potential physiological roles for myosins beyond those that might be performed by filamentous myosins and initiated a search for other unconventional myosins. We now recognize the existence of a myosin family presently comprising 11 classes defined by the extent of sequence homology within the subfragment-1 (S-1)$^4$ domain: 10 unconventional myosin classes and 1 conventional (so named because it was the first to be discovered) class. There are several excellent recent reviews of the sequence, structural diversity, and possible biological functions of the multiple members of this extended family (1, 4–6).

Most conventional (class I, by the current classification system) nonmuscle myosins are regulated by phosphorylation of their regulatory light chains, similarly to smooth muscle myosin (7). All unconventional myosins contain at least one light chain which, with the notable exception of the amoeba myosins, appears to be calmodulin (4, 8). In some cases, calmodulin has been shown to be associated with the purified myosin, but in many cases a calmodulin light chain is inferred from the heavy chain sequence, which can contain from one to six calmodulin-binding IQ motifs in the light chain-binding region (4, 8). The IQ motifs and the regulation of unconventional myosins by Ca$^{2+}$ interacting with calmodulin, which is reminiscent even if different in detail (e.g. in some cases, Ca$^{2+}$ causes calmodulin to dissociate from the heavy chain with loss of actomyosin activity) of the regulation of molluscan myosins, were reviewed recently (4, 8, 9) and will not be discussed here.

Early in the study of the Acanthamoeba myosins it became clear that they were regulated exclusively by a heretofore unknown mechanism: heavy chain phosphorylation (10). The phosphorylation occurs in the S-1 domain of the unconventional, class I myosins, as one might expect for a covalent modification that regulates MgATPase activity, but, and quite unexpectedly, near or at the end of the C-terminal tail of the conventional, class II myosin (6). The latter modification is quite remote from the regulated ATPase site in the S-1 domain and separated from it by a relatively rigid $alpha$-helical coiled-coil, thus posing some very interesting questions about the mechanism of signal transduction between the phosphorylation site and the catalytic site.

Thus far, only Acanthamoeba, Dictyostelium, and Physarum myosins have been shown definitively to be regulated by heavy chain phosphorylation. However, there are numerous examples of heavy chain phosphorylation of vertebrate nonmuscle class II myosins, in vivo as well as in vitro, with as yet no known substantial biochemical or physiological consequences (6, 11). Heavy chain phosphorylation of vertebrate nonmuscle class II myosins deserves more attention than it has received. Possibly this review will stimulate interest in those systems.

In addition to novel mechanisms for regulation of actomyosin MgATPase activity, an emerging feature from the study of unconventional myosins is the probable role of their nonfilamentous tails in regulating function. Tail domains may determine the intracellular localization of the myosin and the physiological task for which the mechanochemical activity of its S-1 domain is used. This aspect of unconventional myosins will be briefly addressed.

Regulation of Class II Ameoba Myosins by Heavy Chain Phosphorylation

Three conventional amoeba myosins have been studied, two in considerable detail. Acanthamoeba myosin II is regulated only by heavy chain phosphorylation while Dictyostelium and Physarum myosins II are regulated by phosphorylation of light and heavy chains. Heavy chain phosphorylation inhibits the activities of Acanthamoeba and Dictyostelium myosins (but by different mechanisms) while heavy chain phosphorylation activates Physarum myosin II. Because relatively little is known about the details of regulation of Physarum class II myosin by heavy chain phosphorylation (6), it will not be discussed further.

Acanthamoeba Myosin II—The relatively short (171 kDa) heavy chains of this conventional myosin (5) comprise a ~90-kDa S-1 domain and an ~80-kDa $alpha$-helical coiled-coil rod separated by a hinge region into a relatively long heavy meromyosin (HMM) domain and a short light meromyosin (LMM) domain ending in a 29-residue nonhelical C-terminal tail (Fig. 1). As for other conventional myosins, there are two pairs of light chains, but their functions are not known.

Phosphorylation of up to 3 serine residues at the tip of the nonhelical tail almost totally inactivates actomyosin MgATPase activity in the S-1 head by reduction of $V_{max}$ with no effect on $K_{app}$ (the actin concentration required for half-maximal activity) (13). Heavy chain phosphorylation also inhibits the in vitro motility activity of Acanthamoeba myosin II (14), the ability of myosin fixed to a substrate to translocate actin filaments. Under assay conditions in vitro (14) (and almost certainly also in situ (15)), Acanthamoeba myosin II is in short minifilaments (12) with the phosphorylation sites at the tip of the tail far removed from the S-1 domain of the same or other myosin molecules in the minifilament (Fig. 1). However, the phosphorylation sites and the hinge regions of most of the molecules in the filament are relatively tightly

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‡ The abbreviations used are: S-1, subfragment-1; HMM, heavy meromyosin; LMM, light meromyosin; MHC, myosin heavy chain; PKC, protein kinase C; MIHCK, myosin I heavy chain kinase; MHCK, myosin heavy chain kinase; PAK, p21-activated kinase.
Fig. 1. Schematic representation of a minifilament of Acanthamoeba myosin II. The angles and directions of the bends in the rods at the HMM/LMM junctions and the “packing” of the molecules are arbitrary, and the two-dimensional display cannot, of course, truly represent the three-dimensional structure. However, the overlaps of the tails in the longitudinal direction (12) and the positions of the hinge regions (6) are to scale, and therefore, the longitudinal relationships between the hinge regions and the phosphorylation sites at the end of the tails are approximately correct. Note that the hinge region of each of the 6 interior molecules lies near the phosphorylation sites at the tip of the nonhelical tail of an adjacent molecule. The light chains are not shown.

Clustered (Fig. 1). It was proposed, therefore, that the state of phosphorylation of the tails regulates the actomyosin MgATPase activity of the heads of all of the molecules in the filament by altering the conformation of the hinge regions (16), i.e., the filament is regulated coordinately and not the myosin molecules individually. Experimentally, observations consistent with this proposal include (16–18): (i) phosphorylation does not affect the actomyosin MgATPase activity of monomers (which can be monitored under special circumstances); (ii) phosphorylation of myosin in copolymers; and (iii) phosphorylated LMM inactivates unphosphorylated native myosin in copolymers (and, reciprocally, unphosphorylated LMM activates phosphorylated native myosin). Electric birefringence studies provide direct evidence that tail phosphorylation affects the conformation of the hinge region (19). The hinge regions of active, unphosphorylated myosin II filaments are much more rigid than the hinge regions of inactive, phosphorylated myosin II filaments (19). Moreover, the Mg$^{2+}$ dependency curves of enzymatic activity and rod flexibility are essentially identical (19). And, recently, binding of nucleotide to the globular heads was shown to increase substantially the flexibility of unphosphorylated myosin II filaments (20). This leads to the suggestions that cylical changes in the flexibility of the HMM/LMM hinge are tightly coupled to the S-1 ATPase activity of the heads. Indeed, phosphorylated myosin II filaments (20) but markedly inhibits filament formation by stabilizing the polymerization-incompetent bent dimer (30); this is reminiscent of the effect of light chain phosphorylation on vertebrate myosin II (1, 2). The biological relevance of this regulatory mechanism is evidenced by elegant experiments (31) in which myosin II with the phosphorylatable Thr residues replaced by either Ala or Asp residues was expressed in myosin II null mutants. The Ala mutant (behaving in vitro like unphosphorylated wild type myosin II) was overexpressed in vivo and supported relatively normal cell morphology and cell motility; the Asp mutant (behaving in vitro like phosphorylated wild type myosin II) underassembled in vivo and did not support normal function or morphology of myosin II null cells. Very similar results were obtained by disrupting or overexpressing the MHCK-A gene (32). In analogous experiments (33), elimination of the expression of MHCK-PKC abolished phosphorylation of the myosin II heavy chain in cAMP-stimulated cells resulting in overassembly of myosin II filaments and abnormal cell morphology and cell motility. Conversely, overexpression of MHCK-PKC resulted in abnormally high levels of heavy chain phosphorylation, impaired myosin II localization, and blocked cell polarization and chemotaxis.

### Regulation of Class I Amoeba Myosins by Heavy Chain Phosphorylation

Acanthamoeba myosin I has a current structure (8), a subclass-1 myosin) was the first unconventional myosin to be discovered (3, 10). The three known Acanthamoeba myosin I isozymes share the features illustrated in Fig. 2: a single heavy chain with an ~80-kDa S-1 head and a short, non-helical ~50-kDa tail (10, 34). The tail of this monomeric myosin can be divided into three regions (34): (i) a very basic, phospholipid- and membrane-binding region near the head/tail junction (35); (ii) a gly/Ala/Pro-rich region that contains an ATP-insensitive, actin-binding site (36); and (iii) a ~50-residue segment highly homologous to Src homology region 3 (34). These myosins have one or two light chains (different for each isozyme) of unknown function (10). Two of the five Dictyostelium class I myosins have similar structures while the other three are shorter, lacking the actin-binding region in the tail (4, 8, 34). Long
and short forms of myosin I also occur in many other species including vertebrates (4, 8).

The very high actomyosin MgATPase activity of the three Acanthamoeba myosin I isozymes (10) and their in vitro motility activity (37, 38) require phosphorylation of a single Ser or Thr residue (depending on the isozyme) in the heavy chain (39). Actomyosin MgATPase activity is enhanced 30–50-fold, entirely by an increase in the catalytic activity. The phosphorylated residue of Acanthamoeba myosin I corresponds to Glu-411 of chicken skeletal muscle myosin, which is phosphorylated in vivo as well as those assayed in vitro. Studies with isozyme and phosphorylation state-specific antibodies (44) demonstrate that (i) a substantial fraction of the myosin I is phosphorylated in vivo (20–80% depending on the isozyme), (ii) the phosphorylated forms are preferentially enriched at motile regions of the cell such as pseudopods and phagocytic cups, and (iii) phosphorylation of the myosin I C-terminal tail domain is not required for regulation of activity (40).

There is good evidence that phosphorylation of the heavy chain of the Acanthamoeba myosin I isozymes is essential for their activities in vivo as well as those assayed in vitro. Studies with MIHCK reveal a putative Cdc42-binding site.4 More recently, a very similar MIHCK has been purified from Dictyostelium (53). It phosphorylates and activates the actomyosin MgATPase activity of Dictyostelium myosin ID (53) and also phosphorlates synthetic peptide substrates with sequences corresponding to the phosphorylation sites of Dictyostelium and Acanthamoeba class I myosins (54). The Dictyostelium MIHCK is very similar to Acanthamoeba MIHCK. It has a similar mass (110 kDa), is activated 50-fold by autophosphorylation of up to 10 sites, and binds to acidic phospholipids, which accelerates its autophosphorylation, and enhancement of its autophosphorylation by phospholipids is inhibited by Ca2+/calmodulin (54). The only significant biochemical difference between the two kinases is that the Acanthamoeba kinase can autophosphorylate maximally (although slowly) in the absence of phospholipid whereas the Dictyostelium kinase incorporates only 1 phosphate in the absence of phospholipid. Amoeba Myosin I Heavy Chain Kinases Are Related to PAKs—Recent sequence data confirm the relationship between Acanthamoeba and Dictyostelium MIHCK and show that both are members of the PAK family. PAKs are activated by the small GTP-binding proteins Rac and Cdc42 (55) and subsequently autophosphorylate at multiple sites (56). The deduced amino acid sequence of the 35-kDa catalytic domain of Acanthamoeba MIHCK is 50% identical and 70% similar to the sequences of the catalytic domains of vertebrate PAK and yeast Ste20 and only about 25% identical to protein kinase A, PKC, and calmodulin-dependent kinases.3 Provocatively, the homologous sequence includes the consensus phosphorylation sequence for Acanthamoeba MIHCK.3 Equally exiting, the complete deduced amino acid sequence of Dictyostelium MIHCK reveals a putative Cdc42-binding site.3

The minimum consensus substrate sequence for Acanthamoeba MIHCK, as determined by studies with synthetic peptides and in vivo evidence, is KXp(S,T,D). Acanthamoeba MIHCK phophorylates synthetic peptides with sequences corresponding to the consensus phosphorylation sequence for Acanthamoeba MIHCK. As predicted, Acanthamoeba MIHCK does not phosphorylate Dictyostelium myosin I and also phosphorylates a synthetic peptide with the Aspergillus sequence.

3 H. Brzeska, J. Szczepanowska, J. Hoey, and E. D. Korn, manuscript in preparation; presented at the 1995 annual meeting of the Biophysical Society but not included in the published abstract.

4 S. F. Lee, T. T. Egelhoff, A. Mahasneh, and G. P. Côté, personal communication.

3 H. Brzeska, J. Szczepanowska, J. Hoey, and E. D. Korn, unpublished observation.
extensive biochemical studies are required to establish if the amoeba kinases and PAK are similarly regulated and have similar functions. Interestingly, the small GTPases that activate PAK also induce plasma membrane ruffling and reorganization of the cytoskeleton of mammalian cells in culture (55). However, the effects of small GTPases on cell morphology have not been shown to be the result of activation of PAK, and the physiological substrates of PAK and STE-20 are not known. Speculatively, a myosin VI might be a substrate for vertebrate PAK, and yeast MYO3 might be a substrate for STE-20 as each has a potential phosphorylation site (Fig. 3).

Regulation of Unconventional Myosins by Their Tails

In contrast to muscle myosins, the intracellular localization of non-muscle myosin is spatially and temporally regulated, and in contrast to filamentous class II myosins, nonmuscular myosins must be anchored to actin filaments or membranes to produce movement. The myosin tails are likely to be responsible for most of these dynamic interactions, and the conserved sequences within the tails, e.g., the GAP (GTPase-activating protein) domain of a class I myosin (Myr5), the PH (pleckstrin homology) domain of a class X myosin, and the SH3 domain of class I myosins (4, 8), are good candidates for binding to structural and/or regulatory proteins. Indeed, the exciting finding that the GAP domain of Myr5 stimulates GTP hydrolysis by small GTP-binding proteins (59) suggests possible new functions for myosins (it is not known how the properties of Myr5 are affected by binding to small G-proteins), and it is likely that the SH3 domain of long class I Myr3 may regulate ATP-sensitive actin binding (58).

The membrane-binding site in the tail of both long and short class I myosins allows them to associate with acidic phospholipids, membranes, and vesicles in vitro and translocate along actin filaments (10). We do not know why the three Acanthamoeba class I myosins localize to unique membrane sites in vivo (44), but the recent discovery of a protein that may bind specifically to the SH3 domain of one of the three Acanthamoeba myosin I isoforms is a promising lead (59). For some vertebrate class I myosins, Ca2+-induced loss of the calmodulin light chain stimulates binding to lipids (60, 61).

Concluding Remarks

The detailed studies of the amoeba myosins briefly summarized in this review have shown that myosins are regulated in diverse ways by heavy chain phosphorylation. The active site of Acanthamoeba myosin II is regulated at a distance through phosphorylation-dependent conformational changes in the supramolecular structure of the myosin filament. Dicysteomyl myosin II is regulated by phosphorylation-dependent filament polymerization and depolymerization, and the class I amoeba myosins are regulated by the phosphorylation state of a serine or threonine in an activatory binding loop. The regulatory sites are very different from the well studied Ca2+-/calmodulin-dependent myosin light chain kinase, and the same myosin can be regulated by more than one kinase: PAK-like kinases for amoeba class I myosins and an atypical kinase (MHCK-A) and a PKC-like kinase for Dicyostelium myosin II. Recent observations that PKC phosphorylates the tail of the heavy chains of brain myosin II (62) and some vertebrate class I myosins (61, 63) and that cell cycle-dependent Cdc2 kinase phosphorylates Xenopus myosin II B (but not myosin II A) near the ATP-binding site in the head (64) provide strong reasons for believing that heavy chain phosphorylation will have as important a regulatory role for vertebrate myosins as it does for amoeba myosins.

REFERENCES

1. Sellers, J. R., and Goodson, H. V. (1995) in Protein Profile (Sheterline, P., ed) Vol. 2, pp. 1323–1423, Academic Press Ltd., London.

2. Trybus, K. M. (1994) J. Muscle Res. Cell Motil. 15, 587–594.

3. Pollard, T. D., and Korn, E. D. (1973) J. Biol. Chem. 248, 4682–4690.

4. Moseley, M. S., and Cheney, R. E. (1995) Annu. Rev. Cell. Dev. Biol. 4, 633–675.

5. Hammer, J. A. (1990) J. Muscle Res. Cell Motil. 13, 1–10.

6. Korn, E. D., and Hammer, J. A., III (1988) Annu. Rev. Biophys. Biophys. Chem. 17, 23–45.

7. Tan, J. L., Ravid, S., and Sudar, J. A. (1992) Annu. Rev. Biochem. 61, 721–759.

8. Hasson, T., and Moeseker, M. S. (1996) J. Biol. Chem. 271, 16431–16434.

9. Wolenski, J. S. (1995) Trends Cell Biol. 5, 310–317.

5 References and recent papers are sometimes cited rather than the original paper.