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Myosin phosphatase target subunit: Many roles in cell function ⋆

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Abstract

Phosphorylation of myosin II is important in many aspects of cell function and involves a myosin kinase, e.g. myosin light chain kinase, and a myosin phosphatase (MP). MP is regulated by the myosin phosphatase target subunit (MYPT1). The domain structure, properties, and genetic analyses of MYPT1 and its isoforms are outlined. MYPT1 binds the catalytic subunit of type 1 phosphatase, δ isoform, and also acts as an interactive platform for many other proteins. A key reaction for MP is with phosphorylated myosin II and the first process shown to be regulated by MP was contractile activity of smooth muscle. In cell division and cell migration myosin II phosphorylation also plays a critical role and these are discussed. However, based on the wide range of partners for MYPT1 it is likely that MP is implicated with substrates other than myosin II. Open questions are whether the diverse functions of MP reflect different cellular locations and/or specific roles for the MYPT1 isoforms.

Keywords

Myosin II phosphorylation; Myosin phosphatase; Myosin phosphatase targeting subunit; Cell division; Cell migration

Background

The important role of myosin phosphorylation is established in many tissues. In smooth muscle and non-muscle cells, phosphorylation of the regulatory light chains (RLC) of myosin II is required to initiate the contractile or motile event. Early studies done with smooth muscle established the framework in which the level of RLC phosphorylation is determined by activities of two enzymes: the Ca^{2+}-calmodulin-dependent myosin light chain kinase (MLCK) and a myosin phosphatase (MP). This system was shown to occur in non-muscle cells and many aspects are similar.

As isolated from chicken gizzard the MP holoenzyme (review [1]) contained three subunits: a type 1 protein phosphatase catalytic subunit, δ isoform (PP1cδ); and two non-catalytic subunits —the myosin phosphatase target subunit, ~110 kDa (MYPT1; also termed myosin binding subunit [MBS], M_{110}, M130/M133), and a small subunit, ~20 kDa (M20) of unknown function. Most of the properties of the holoenzyme can be described by a complex of PP1cδ and MYPT1 and there is no compelling evidence to include M20 as a functional subunit of MP.

⋆This article is offered as tribute in honor of Professor Setsuro Ebashi's remarkable career. Prof. Ebashi's many contributions were essential in developing contemporary views on Ca^{2+} regulation, in general, and muscle contraction, in particular.

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MYPT1 is expressed from a single gene in many cell types and is present at higher concentration in smooth muscle ([phasic] > [tonic]). Several isoforms can be generated by cassette-type alternative splicing and these represent the presence or absence of central inserts (exons 13 and 14 in rat) and/or the C-terminal leucine zipper (LZ) motifs (splicing of exon 23). To some extent the expression pattern of these isoforms is tissue specific [2]. For the central insert isoforms, there is no clear distinction in function. However, the LZ+ isoform appears to be required for sensitivity to cGMP/PKG [3]. In addition to the splicing variants of MYPT1 there are other members of the MYPT family. These separate gene products include: MYPT2 (in striated muscle and brain); MBS 85; MYPT3; and TIMAP [1]. MYPT2 is assumed to play a major role in the dephosphorylation of striated muscle myosin but functions for the other family members are not established.

Properties of MYPT1

Features of the MYPT1 molecule (using the rat 1 isoform [2] as a model; this has no central exon deletions and is LZ+) show a PP1c-binding motif, often referred to as the RVXF motif (consensus sequence (R/K)X_1(V/I)X_2(F/W)) at the N-terminal edge (residues 35–38) of eight ankyrin repeats spanning residues 39–296. Two nuclear localization sequences (NLS) are present in both N- and C-terminal regions, residues 27–33 and 845–854, respectively. The N-terminal NLS appears to be more important [4]. It is interesting that both NLSs are flanked at the C-terminal edge by a phosphorylation site, T34 and T855. At the C-terminal ends of some MYPT1 isoforms (including rat 1, but not chicken M130/133) are four LZ motifs. In all isoforms at the C-terminal end are regions showing propensity to form coiled-coils, for example in the rat 1 isoform the sequence 970–1003 has a high probability to form an amphipathic α-helix (the LZ motifs cover the sequence 1009–1030). It is likely that the C-terminal end of MYPT1 is an important interactive site. In addition there are several phosphorylation sites: S696 and S854 are PKA/PKG sites; T697 and T855 are the inhibitory/regulatory sites phosphorylated by several kinases [1], notably Rho-kinase (ROK); and S473 is phosphorylated during mitosis, probably by cdc2 (Matsumura, unpublished). The influence, if any, of tyrosine phosphorylation has not been evaluated.

Obviously, a critical role for MP is dephosphorylation of phosphorylated myosin (P-myosin) and, in part, the diversity of MP reflects the many roles of myosin II in cell function, e.g. initiation of contraction, motile responses, organization of the cytoskeleton, and cytokinesis. (Two examples where myosin II plays a crucial role in cell function are given below in sections on cell division and cell migration.) Several kinases may phosphorylate the RLCs of myosin II, notably MLCK and ROK and possibly PKC (in general MLCK favors S19, ROK, S19 and T18, and PKC, T9), but the opposing reaction reflects only the activity of MP that can dephosphorylate RLC sites, S19, T18, and T9, but not S1/S2. To achieve this function there are two central requirements, i.e. binding of PP1cδ and the substrate, P-myosin (or, P-RLC) to MYPT1. PP1c is anchored by the RVXF PP1c-binding motif and forms secondary interactions with the N-terminal sequence, some of the ankyrin repeats and with a site within the sequence 304–501 [5]. It was suggested that the acidic patch (326–372) was involved in activation of phosphatase activity with P-RLC [6]. As shown by the crystal structure [7] of PP1c plus an N-terminal fragment of MYPT1 these hierarchical interactions extend the catalytic groove of PP1c and possibly modify its catalytic properties. The binding to P-myosin is more controversial. One view is that the P-RLC binds to the ankyrin repeats and facilitates interaction with the catalytic cleft of PP1c. Another view is that myosin binds to the C-terminal segment of MYPT1 (review [1]). Considering the size of the myosin molecule, both are possible and each interaction may reflect different aspects of MYPT1 function. The N-terminal interaction representing the essential dephosphorylation step and the C-terminal interaction might reflect part of the regulatory mechanism or be involved in MYPT1 localization. In this respect it is interesting that phosphorylation of T855 reduces binding of myosin [8]. Another view is that

the cell localization of myosin II could involve another protein, such as M-RIP (aka p116RIP) [9].

Diverse cell functions for MYPT1

In addition to P-myosin and PP1cδ many other proteins bind to MYPT1 and the MP holoenzyme has several additional substrates. It was expected that the N-terminal ankyrin repeats would form an interactive platform for protein interaction, but it became evident that the C-terminal part of MYPT1 also interacts with many proteins [1]. A partial list of proteins interacting with MYPT1 is given in Table 1. Some of these proteins are known to be substrates of MP and have putative roles in cell function, for others the interaction with MYPT1 merely raises that possibility. (It is assumed that if a protein is a substrate for MP it would interact with MYPT1, e.g. ERM, merlin, HDAC7.) Several of the MYPT1 partners are phosphorylated by ROK and thus become substrates for MP. Many of these proteins are involved in cytoskeletal organization, ERM, adducin, Tau, etc. (Table 1). Obviously, the initial idea that MP was restricted to P-myosin as substrate needs to be re-evaluated as MP is implicated in several diverse cell functions. How the different phosphorylation sites (and surrounding sequences) are accommodated in the catalytic cleft created by the PP1cδ-MYPT1 complex for P-RLC is not known.

Cellular localization of MYPT1

The initial expectation was that MYPT1 would colocalize with myosin II or, P-myosin II. While this is observed, several other cell locations have been shown and would be predicted based on the range of proteins known to interact with MYPT1 (Table 1). Obviously different cell locations for MP may indicate substrates in addition to P-myosin and would be an important component in the regulation of MP for a given substrate. In support of a broad cellular distribution, MYPT1 has been found in all subcellular fractions; for example in “resting” human hepatocarcinoma cells (HepG2) MYPT1 was at higher concentrations in the nuclear, cytoskeletal, and microsomal–mitochondrial fractions, but also present in the cytosol and plasmalemma fractions [18]. Other studies also identified MYPT1 in isolated nuclei [4,19].

There have been several immunofluorescence studies to investigate the cellular location of MYPT1 with permeabilized fibroblasts and smooth muscle cells (SMC) myosin and MYPT1 were colocalized on stress fibers. But in non-permeabilized cells, different locations were observed where myosin II was mostly at the cell periphery and MYPT1 was found in the cytoplasm and nucleus [20]. In migrating fibroblasts, the MYPT1 appeared to colocalize with P-myosin II at the trailing edge, not with dephosphorylated myosin II at the leading edge [20]. In rat embryo fibroblasts and non-confluent MDCK (epithelial) cells MYPT1 and myosin were observed with stress fibers, but in confluent MDCK cells distinct distributions were evident. MYPT1 was localized at cell–cell adhesions and overlapped with the location of β-catenin (a component of adherens junctions), whereas myosin was found mostly in the cytoplasm [21]. In another study, MYPT1 was colocalized with moesin at TPA-induced membrane ruffling areas and cell–cell contacts in MDCK cells and it was proposed that the C-terminal part of MYPT1 interacts with N-terminal domains of ezrin and moesin [22]. With isolated SMCs from ferret portal vein it was found that the distribution of MYPT1 depended on the agonist used for stimulation. In unstimulated cells the staining for MYPT1 and PP1c was diffuse throughout the cell and a similar pattern was retained on stimulation by the α-agonist, phenylephrine. However, stimulation by prostaglandin F2α induced a translocation of MP from the cytosol to the plasma membrane and this was followed by a dissociation of MP and the return of PP1c to the cytosol. The translocation to the membrane was accompanied by phosphorylation of MYPT1 at T697 and was prevented by inhibition of ROK. The dissociation of MP would reduce phosphatase activity toward P-myosin and this was consistent with a

sustained contractile response to PGF2α, compared to a more phasic response with phenylephrine [23]. This was the first report that documented the possible role of MYPT1 phosphorylation in translocation. This theme was developed further by Lontay et al. [18] using HepG2 cells. In non-stimulated "resting" cells, MYPT1 was dispersed in the cytoplasm and accumulated in the nucleus. The phosphorylation level of MYPT1 was increased by treatment with okadaic acid and it was found that phosphorylation at the two inhibitory sites induced different cell locations. T697 phosphorylation favored a plasmalemma location and T855 phosphorylation a perinuclear and nuclear location. Two additional points were made: the T697 kinase was probably not ROK as suggested for the T855 kinase; and the level of okadaic acid used inhibited only PP2A (thus implicating PP2A as a MYPT1 phosphatase). The location of MYPT1 phosphorylated at T855 in the nucleus (and on stress fibers) previously was shown with REF52 cells [24]. In colonic SMCs stimulation by acetylcholine increased phosphorylation at T697 by ROK and induced translocation of MYPT1 and HSP27 to the particulate fraction [16].

Thus there are several examples documenting different cell locations for MYPT1 and there is preliminary data on some requirements for translocation (i.e. phosphorylation at T697 and/or T855). However, the latter area is essentially unexplored and several obvious points must be established. For example: are other phosphorylation sites on MYPT1 important, both S/T and Y; what signaling pathways operate and which kinases are recruited; is there any distinction for different MYPT1 isoforms with respect to location and function; and for all cell locations which docking or interacting partners are involved?

MYPT1 in cell division

There are several pieces of evidence indicating that MP is involved in cytokinesis. They include (1) myosin II is a primary motor for cytokinesis [25,26]; (2) RLC phosphorylation rapidly increased in the midzone between the two separating sister chromatids just before the onset of, and throughout cytokinesis [27], and (3) MYPT1 phosphorylated at an inhibitory site is localized in cleavage furrows [24,28]. These observations suggest that MP is inhibited during cytokinesis, leading to an increase in RLC phosphorylation and myosin II activation. There are two important open questions.

First, to what extent is the inhibition of MP critical for cytokinesis? Because RLC kinases including MLCK [29,30], ROK [31,32], and citron kinase [33] are localized in cleavage furrows, and because regulation of RLC kinases could theoretically control RLC phosphorylation, MP regulation may not be essential for cytokinesis. It should be noted, however, that contractile rings are dynamic structures that must contract and, at the same time, disassemble during cytokinesis [34,35]. Because assembly of actomyosin is coupled with RLC phosphorylation [36], contractile rings would not be disassembled if MP activity was completely blocked throughout cytokinesis. Indeed, our preliminary data have shown that MYPT1 depletion leads to an increase in multinuclear cells. Further studies should be directed to elucidate at which stages of cytokinesis (such as initiation and/or abscission) MP activity is controlled.

The second question is which signaling pathway leads to the inhibition of MP during cytokinesis. Because MYPT1 appears to be phosphorylated at an inhibitory site at the cleavage furrows [24,28], and because RhoA is known to be critical for cytokinesis [37-39], the Rho/ROK pathway is likely to be involved in the regulation of MP. Recent studies suggest an involvement of PLK1 in this signaling pathway [40,41]. By specifically inhibiting PLK1 during anaphase and telophase, it was demonstrated that PLK1 is essential for the formation of an ECT2 (RhoGEF)/MgcRacGAP/MKLP1 complex, as well as the localization of ECT2 in the central spindle and cleavage furrow. The central spindle (microtubule bundles between two

separating chromatids, also called spindle midzone) is known to be the critical structure that transmits a signal to initiate cytokinesis in mammalian cells [42-44]. ECT2 (RhoGEF) would then activate RhoA at the cleavage furrows, increasing the activities of RhoA effectors including ROK and citron kinase. The activation of ROK would have two effects. One is to inhibit MP by phosphorylating MYPT1 at the inhibitory sites and the other is to directly phosphorylate RLC. The activation of RhoA would also increase citron kinase, which has been shown to phosphorylate RLC at the cleavage furrow. MgcRacGAP, another component of the complex, may function to inactivate RhoA at the end of cytokinesis, which may be necessary for the release of the inhibition of MP, leading to disassembly of contractile rings.

Other RhoGEFs may be involved in the RhoA/ROK pathway during cytokinesis. Using a biosensor that can detect active RhoA, Birkenfeld et al. [45] have recently shown that GEF-H1, not ECT2, is critical for RhoA activation. Wu et al. [46], have reported yet another RhoGEF that appears to be involved in cytokinesis. This GEF interacts with myosin II (thus called MyoGEF), and the depletion of MyoGEF causes multinuclear cells. Apparently, further work is required to clarify whether these GEFs have distinct or overlapping functions in the activation and localization of RhoA during cytokinesis.

In addition to its role in cytokinesis, MP appears to have a role in the regulation of mitotic progression. MYPT1 is phosphorylated at a central region in a mitosis-specific way [47]. We found that during mitosis mammalian MYPT1 is phosphorylated by proline-directed kinases including cdk1, allowing for MYPT1 to bind to polo-like kinase1 (PLK1) [unpublished result]. Depletion of PLK1 by small interfering RNAs is known to results in loss of γ-tubulin recruitment to the centrosomes, blocking centro-some maturation, leading to mitotic arrest. It was found that co-depletion of MYPT1 and PLK1 reinstated γ-tubulin at the centrosomes, rescuing mitotic arrest caused by PLK1 depletion. These results suggest a previously unrecognized role for MYPT1 in regulating mitosis by directly antagonizing PLK1. Whether the PLK1/MYPT1 association is involved in the PLK1-mediated regulation of cytokinesis awaits further investigation.

Role of MYPT1 in cell migration

Cell migration is a multi-step process, involving actin polymerization and actomyosin contraction [48-50]. The first step is extension of a membrane protrusion in the direction of migration, a process driven by actin polymerization [51]. The second step is the establishment of new adhesion sites (called focal complexes) by the extended membrane [52]. In fibroblasts, focal complexes mature into more stable adhesions called focal adhesions. The 3rd step is rear contraction driven by a contractile force of actin and myosin, which moves the cell body forward. Finally, the rear part of the cell is detached from the substrate. For effective migration, membrane protrusion must be restricted to the leading edge, and adhesions must show turnover.

Immunofluorescent localization using an antibody specific to Ser19-phosphorylated RLC [27], as well as a study using a biosensor approach [53], revealed that phosphorylated myosin is localized in both the posterior and anterior regions of motile fibroblasts. Whereas RLC phosphorylation at the posterior of the cell supports the well-accepted rear contraction role for myosin II, RLC phosphorylation at the anterior edge of migrating cells suggested additional functions of myosin II in regulating motile activities at the front. Recent work has demonstrated that myosin II is critical in controlling the membrane protrusions [54], as well as retrograde actin movement [55], at the leading edge of motile cells.

MYPT1 has been localized in membrane ruffles [24], suggesting its role in the control of myosin phosphorylation at the leading edge of motile cells. Several recent studies have revealed critical roles of MP in cell migration. For example, microinjection of a function-inhibiting antibody against MYPT1 blocks cell migration of fibroblasts [54]. These cells show greatly

increased RLC phosphorylation, resulting in thicker stress fibers, cortical fibers, and focal adhesions. Importantly, increased RLC phosphorylation inhibits membrane ruffling and blocks turnover of focal adhesions. These phenotypes can be reproduced by overexpression of phosphomimetic RLC, ruling out a possibility that increased phosphorylation of other substrates of MP, if any, may be involved in the phenotypic changes in the motile activities. These observations indicate that turnover of RLC phosphorylation is required for turnover of focal adhesions (it is known that assembly of focal adhesions requires myosin II activity [56]) and that myosin II activity counteracts membrane protrusive activity driven by actin polymerization. Similar inhibition of cell migration is also observed when MYPT1 is depleted by siRNA treatment though a role of MYPT1 in actin polymerization has also been suggested [57].

Which RLC kinase counteracts MP in regulating turnover of RLC phosphorylation at the leading edge? The study using kinase inhibitors has demonstrated a critical role of MLCK in antagonizing MP at the cell periphery [54,58,59]. MLCK inhibition decreases RLC phosphorylation at the cell periphery, whereas ROCK inhibition decreases RLC phosphorylation at the center of cells [54]. MLCK-inhibited cells, concomitant with the loss of RLC phosphorylation at the periphery, generated membrane protrusions all around themselves, lost polarity, and made more frequent turns. ROCK-inhibited cells, on the other hand, showed one major protrusion, and migrated straighter and faster. The generation of multiple membrane protrusions in MLCK-inhibited cells suggests that, if myosin II is inactivated (either by MLCK inhibition or MP activation), actin polymerization and resulting membrane protrusions can occur anywhere at the periphery, resulting in the loss of cell polarity and inhibition of directed cell migration. This notion is consistent with the observation made by the reciprocal approach described above: an increased RLC phosphorylation caused by MP inhibition blocks membrane protrusion all around cells.

We propose a working model for the roles of MLCK and MP for directional migration (Fig. 1): At the leading edge (A and B), MLCK activity may cycle between high and intermediate activity states while MP may show high activity. In an intermediate activity state of MLCK (A), RLC phosphorylation is reduced, thus allowing a membrane protrusion and turnover of adhesions. In a high activity state (B), RLC phosphorylation is increased, leading to assembly of new and transient adhesions at the periphery (indicated by small green dots). High RLC phosphorylation may also act as a retracting force for retrograde movements of actin. The resultant high turnover of RLC phosphorylation at the leading edge would cause cycles of protrusions, adhesions, and retraction, a characteristic feature of membrane ruffling (It should be noted that, instead of cycling MLCK activity, cycling MP activity would yield a similar effect.). Along the sides and at the trailing edge (Fig. 1C), on the other hand, MP may be inactivated, whereas MLCK may stay active. This will increase RLC phosphorylation and at the same time, block the turnover of RLC phosphorylation. High RLC phosphorylation together with low turnover rate of RLC phosphorylation would restrict membrane protrusions and stabilize adhesions in this area. This model is consistent with recent reports indicating critical roles of MLCK in the regulation of protrusions and adhesions [60,61].

Genetic analyses of MYPT1

MYPT is also found in model organisms including *Drosophila* and *C. elegans*, though it is apparently absent in yeast and *Dictyostelium*. In both *Drosophila* and *C. elegans*, MYPT mutation is embryonic lethal. Genetic analyses of MYPT in these organisms revealed critical roles of MYPT in cell migration, as well as morphogenesis and, in the case of *C. elegans*, in cytokinesis. *Drosophila* MYPT mutants show defects in late embryogenesis including a failure of dorsal closure due to a migration defect in an ectoderm cell sheet but apparently display no defects in cell division [62,63]. In *C. elegans*, MYPT mutation also causes defects in embryonic

morphogenesis [64]: *C. elegans* MYPT (MEL-11) mutants fail embryonic elongation due to hyper-contraction of epidermal cell layers of embryos. In addition, cytokinesis failure, though mild, has also been reported [65]. These phenotypes are alleviated by mutations of a worm ROK homologue (LET-502), indicating that actomyosin contraction is regulated by these two opposing enzymes. Indeed, RLC phosphorylation is increased by MYPT mutation, decreased by ROK mutation, and restored by double mutations during cytokinesis [65].

Mice lacking MYPT1 are also embryonic lethal [66]. However, in contrast to MYPT mutants in flies and worms, the phenotypes are more severe: these mice die at a very early stage of development before 7.5 dpc and no MYPT1 null cells have been so far isolated. While the molecular mechanisms underlying these differences in the phenotypes are not clear, MYPT1 may play more critical roles in early cell division in mammalian cells than does MYPT in flies and worms.

Perspectives

Unlike MLCK that is dedicated to phosphorylation of RLCs, MP is likely to have diverse functions. An important role, obviously is directed to dephosphorylation of P-RLC and, to an extent, the varied roles of MP is reflected by the breath of cell functions in which myosin II is involved. For each of these functions (e.g. smooth muscle contraction, cell division, cell migration) the activity of MP is regulated and an important objective is to identify the signal transduction pathways involved for different aspects of MP function. Some of the signaling mechanisms are known (e.g. the RhoA/ROK axis) but it is likely that other pathways are involved and must be integrated to converge at MYPT1 and coordinate its function. A relatively new possibility is that MP is implicated with a variety of substrates, other than myosin II. This is supported by the finding that several proteins bind to MYPT1. It is not known if and how the catalytic site, generated by the PP1c†-MYPT1 interaction, could be adapted to accept a range of substrates. Are they all similar to P-RLC? (clearly some flexibility is allowed since MP can act on P-T9, the PKC site) Another intriguing aspect is that the putative multiple functions of MP may reflect different cell locations that could involve various target proteins (for MYPT1) that may or may not be substrates for MP. MYPT1 is found at the plasmalemma, in the cytosol, attached to the cytoskeleton and in the nucleus and each location may be determined by different signals. In this respect, the influence of MYPT1 phosphorylation and roles of individual MYPT1 isoforms on translocation and targeting must be evaluated.

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References

1. Hartshorne DJ, Ito M, Erdodi F. Role of protein phosphatase type 1 in contractile functions: myosin phosphatase. J. Biol. Chem 2004;279:37211–37214. [PubMed: 15136561]
2. Dirksen WP, Vladic F, Fisher SA. A myosin phosphatase targeting subunit isoform transition defines a smooth muscle developmental phenotypic switch. Am. J. Physiol. Cell Physiol 2000;278:C589–C600. [PubMed: 10712248]
3. Payne MC, Zhang H-Y, Prosdocimo T, Joyce KM, Koga Y, Ikebe M, Fisher SA. Myosin phosphatase isoform switching in vascular smooth muscle development. J. Mol. Cell. Cardiol 2006;40:274–282. [PubMed: 16356512]
4. Wu Y, Muranyi A, Erdodi F, Hartshorne DJ. Localization of myosin phosphatase target subunit and its mutants. J. Mus. Res. Cell Motil 2005;26:123–134.
5. Toth A, Kiss E, Herberg FW, Hartshorne DJ, Erdodi F. Study of the subunit interactions in myosin phosphatase by surface plasmon resonance. Eur. J. Biochem 2000;267:1687–1697. [PubMed: 10712600]

6. Hirano K, Phan BC, Hartshorne DJ. Interactions of the subunits of smooth muscle myosin phosphatase. J. Biol. Chem 1997;272:3683–3688. [PubMed: 9013623]
7. Terrak M, Kerff F, Langsetmo K, Tao T, Dominguez R. Structural basis of protein phosphatase 1 regulation. Nature 2004;429:780–784. [PubMed: 15164081]
8. Velasco G, Armstrong C, Morrice N, Frame S, Cohen P. Phosphorylation of the regulatory subunit of smooth muscle protein phosphatase 1 M at Thr850 induces its dissociation from myosin. FEBS Lett 2002;527:101–104. [PubMed: 12220642]
9. Surks HK, Riddick N, Ohtani K-I. M-RIP targets myosin phosphatase to stress fibers to regulate myosin light chain phosphorylation in vascular smooth muscle cells. J.Biol.Chem 2005;280:42543–42551. [PubMed: 16257966]
10. Haystead TAJ. ZIP kinase, a key regulator of myosin protein phosphatase 1. Cell. Signal 2005;17:1313–1322. [PubMed: 16005610]
11. Mulder J, Poland M, Gebbink MFBG, Calafat J, Moolenaar WH, Kranenburg O. p116^{Rip} is a novel filamentous actin-binding protein. J. Biol. Chem 2003;278:27216–27223. [PubMed: 12732640]
12. Mulder J, Ariaens A, van Horck FPG, Moolenaar WH. Inhibition of RhoA-mediated SRF activation by p116^{Rip}. FEBS. Lett 2005;579:6121–6127. [PubMed: 16243315]
13. Koga Y, Ikebe M. p116^{Rip} decreases myosin II phosphorylation by activating myosin light chain phosphatase and by inactivating RhoA. J. Biol. Chem 2005;280:4983–4991. [PubMed: 15545284]
14. Jin H, Sperka T, Herrlich P, Morrison H. Tumorigenic transformation by CPI-17 through inhibition of merlin phosphatase. Nature 2006;442(3):576–579. [PubMed: 16885985]
15. Parra M, Mahmoudi T, Verdin E. Myosin phosphatase dephosphorylates HDAC7, controls its nucleocytoplasmic shuttling, and inhibits apoptosis in thymocytes. Genes Dev 2007;21:638–643. [PubMed: 17369396]
16. Patil SB, Bitar KN. RhoA- and PKC-α-mediated phosphorylation of MYPT and its association with HSP27 in colonic smooth muscle cells. Am. J. Physiol. Gastrointest. Liver Physiol 2005;290:G83–G95. [PubMed: 16179599]
17. Lontay B, Serfozo Z, Gergely P, Ito M, Hartshorne DJ, Erdodi F. Localization of myosin phosphatase target subunit 1 in rat brain and in primary cultures of neuronal cells. J. Comp. Neurol 2004;478:72–87. [PubMed: 15334650]
18. Lontay B, Kiss A, Gergely P, Hartshorne DJ, Erdodi F. Okadaic acid induces phosphorylation and translocation of myosin phosphatase target subunit 1 influencing myosin phosphorylation, stress fiber assembly and cell migration in HepG2 cells. Cell. Signal 2005;17:1265–1275. [PubMed: 16038801]
19. Tran HT, Ulke A, Morrice N, Johannes CJ, Moorhead GBG. Proteomic characterization of protein phosphatase complexes of the mammalian nucleus. Mol. Cell. Proteom 2004;3:257–265.
20. Murata K, Hirano K, Villa-Moruzzi E, Hartshorne DJ, Brautigan DL. Differential localization of myosin and myosin phosphatase subunits in smooth muscle cells and migrating fibroblasts. Mol. Biol. Cell 1997;8:663–673. [PubMed: 9247646]
21. Inagaki N, Nishizawa M, Ito M, Fujioka M, Nakano T, Tsujino S, Matsuzawa K, Kimura K, Kaibuchi K, Inagaki M. Myosin binding subunit of smooth muscle myosin phosphatase at the cell–cell adhesion sites in MDCK cells. Biochem. Biophys. Res. Commun 1997;230:552–556. [PubMed: 9015360]
22. Fukata Y, Kimura K, Oshiro N, Saya H, Matsuura Y, Kaibuchi K. Association of the myosin-binding subunit of myosin phosphatase and moesin: dual regulation of moesin phosphorylation by Rho-associated kinase and myosin phosphatase. J. Cell Biol 1998;141:409–418. [PubMed: 9548719]
23. Shin H-M, Je H-D, Gallant C, Tao TC, Hartshorne DJ, Ito M, Morgan KG. Differential association and localization of myosin phosphatase subunits during agonist-induced signal transduction in smooth muscle. Circ. Res 2002;90:546–553. [PubMed: 11909818]
24. Kawano Y, Fukata Y, Oshiro N, Amano M, Nakamura T, Ito M, Matsumura F, Inagaki M, Kaibuchi K. Phosphorylation of myosin-binding subunit (MBS) of myosin phosphatase by Rho-kinase in vivo. J. Cell Biol 1999;147:1023–1037. [PubMed: 10579722]
25. Mabuchi I, Okuno M. The effect of myosin antibody on the division of starfish blastomeres. J. Cell Biol 1977;74:251–263. [PubMed: 141455]
26. DeLozanne A, Spudich JA. Disruption of the Dictyostelium myosin heavy chain gene by homologous recombination. Science 1987;236:1086–1091. [PubMed: 3576222]

27. Matsumura F, Ono S, Yamakita Y, Totsukawa G, Yamashiro S. Specific localization of serine 19 phosphorylated myosin II during cell locomotion and mitosis of cultured cells. J. Cell Biol 1998;140:119–129. [PubMed: 9425160]
28. Yokoyama T, Goto H, Izawa I, Mizutani H, Inagaki M. Aurora-B and Rho-kinase/ROCK, the two cleavage furrow kinases, independently regulate the progression of cytokinesis: possible existence of a novel cleavage furrow kinase phosphorylates ezrin/radixin/moesin (ERM). Genes Cells 2005;10:127–137. [PubMed: 15676024]
29. Poperechnaya A, Varlamova O, Lin PJ, Stull JT, Bresnick AR. Localization and activity of myosin light chain kinase isoforms during the cell cycle. J. Cell Biol 2000;151:697–708. [PubMed: 11062269]
30. Chew TL, Wolf WA, Gallagher PJ, Matsumura F, Chisholm RL. A fluorescent resonant energy transfer-based biosensor reveals transient and regional myosin light chain kinase activation in lamella and cleavage furrows. J. Cell Biol 2002;156:543–553. [PubMed: 11815633]
31. Kosako H, Goto H, Yanagida M, Matsuzawa K, Fujita M, Tomono Y, Okigaki T, Odai H, Kaibuchi K, Inagaki M. Specific accumulation of Rho-associated kinase at the cleavage furrow during cytokinesis: cleavage furrow-specific phosphorylation of intermediate filaments. Oncogene 1999;18:2783–2788. [PubMed: 10348354]
32. Madaule P, Eda M, Watanabe N, Fujisawa K, Matsuoka T, Bito H, Ishizaki T, Narumiya S. Role of citron kinase as a target of the small GTPase Rho in cytokinesis. Nature 1998;394:491–494. [PubMed: 9697773]
33. Yamashiro S, Totsukawa G, Yamakita Y, Sasaki Y, Madaule P, Ishizaki T, Narumiya S, Matsumura F. Citron kinase, a Rho-dependent kinase, induces di-phosphorylation of regulatory light chain of myosin II. Mol. Biol. Cell 2003;14:1745–1756. [PubMed: 12802051]
34. Schroeder, TE. Cell Motility. Goldman, R.; Pollard, T.; Rosenbaum, J., editors. Cold Spring Harbor Laboratory; Cold Spring Harbor, NY: 1976. p. 265-278.
35. Rappaport, R. Cytokinesis in Animal Cells. first ed.. Cambridge University Press; Cambridge: 1996.
36. Scholey JM, Taylor KA, Kendrick-Jones J. Regulation of non-muscle myosin assembly by calmodulin-dependent light chain kinase. Nature 1980;287:233–235. [PubMed: 6893621]
37. Kishi K, Sasaki T, Kuroda S, Itoh T, Takai Y. Regulation of cytoplasmic division of Xenopus embryo by rho p21 and its inhibitory GDP/GTP exchange protein (rho GDI). J. Cell Biol 1993;120:1187–1195. [PubMed: 8436590]
38. Mabuchi I, Hamaguchi Y, Fujimoto H, Morii N, Mishima M, Narumiya S. A rho-like protein is involved in the organisation of the contractile ring in dividing sand dollar eggs. Zygote 1993;1:325–331. [PubMed: 8081830]
39. Drechsel DN, Hyman AA, Hall A, Glotzer M. A requirement for Rho and Cdc42 during cytokinesis in Xenopus embryos. Curr. Biol 1997;7:12–23. [PubMed: 8999996]
40. Petronczki M, Glotzer M, Kraut N, Peters JM. Polo-like kinase 1 triggers the initiation of cytokinesis in human cells by promoting recruitment of the RhoGEF Ect2 to the central spindle. Dev. Cell 2007;12:713–725. [PubMed: 17488623]
41. Burkard ME, Randall CL, Larochelle S, Zhang C, Shokat KM, Fisher RP, Jallepalli PV. Chemical genetics reveals the requirement for Polo-like kinase 1 activity in positioning RhoA and triggering cytokinesis in human cells. Proc. Natl. Acad. Sci. USA 2007;104:4383–4388. [PubMed: 17360533]
42. Balasubramanian MK, Bi E, Glotzer M. Comparative analysis of cytokinesis in budding yeast, fission yeast and animal cells. Curr. Biol 2004;14:R806–R818. [PubMed: 15380095]
43. Glotzer M. Cleavage furrow positioning. J. Cell Biol 2004;164:347–351. [PubMed: 14757750]
44. Barr FA, Sillje HH, Nigg EA. Polo-like kinases and the orchestration of cell division. Nat. Rev. Mol. Cell. Biol 2004;5:429–440. [PubMed: 15173822]
45. Birkenfeld J, Nalbant P, Bohl BP, Pertz O, Hahn KM, Bokoch GM. GEF-H1 modulates localized RhoA activation during cytokinesis under the control of mitotic kinases. Dev. Cell 2007;12:699–712. [PubMed: 17488622]
46. Wu D, Asiedu M, Adelstein RS, Wei Q. A novel guanine nucleotide exchange factor MyoGEF is required for cytokinesis. Cell Cycle 2006;5:1234–1239. [PubMed: 16721066]

47. Totsukawa G, Yamakita Y, Yamashiro S, Hosoya H, Hartshorne DJ, Matsumura F. Activation of myosin phosphatase targeting subunit by mitosis-specific phosphorylation. J. Cell Biol 1999;144:735–744. [PubMed: 10037794]
48. Lauffenburger DA, Horwitz AF. Cell migration: a physically integrated molecular process. Cell 1996;84:359–369. [PubMed: 8608589]
49. Mitchison TJ, Cramer LP. Actin-based cell motility and cell locomotion. Cell 1996;84:371–379. [PubMed: 8608590]
50. Ridley AJ. Rho GTPases and cell migration. J. Cell Sci 2001;114:2713–2722. [PubMed: 11683406]
51. Pollard TD, Blanchoin L, Mullins RD. Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. Annu. Rev. Biophys. Biomol. Struct 2000;29:545–576. [PubMed: 10940259]
52. Nobes CD, Hall A. Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell 1995;81:53–62. [PubMed: 7536630]
53. Post PL, DeBiasio RL, Taylor DL. A fluorescent protein biosensor of myosin II regulatory light chain phosphorylation reports a gradient of phosphorylated myosin II in migrating cells. Mol. Biol. Cell 1995;6:1755–1768. [PubMed: 8590803]
54. Totsukawa G, Wu Y, Sasaki Y, Hartshorne DJ, Yamakita Y, Yamashiro S, Matsumura F. Distinct roles of MLCK and ROCK in the regulation of membrane protrusions and focal adhesion dynamics during cell migration of fibroblasts. J. Cell Biol 2004;164:427–439. [PubMed: 14757754]
55. Cai Y, Biais N, Giannone G, Tanase M, Jiang G, Hofman JM, Wiggins CH, Silberzan P, Buguin A, Ladoux B, Sheetz MP. Nonmuscle myosin IIA-dependent force inhibits cell spreading and drives F-actin flow. Biophys. J 2006;91:3907–3920. [PubMed: 16920834]
56. Chrzanowska-Wodnicka M, Burridge K. Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. J. Cell Biol 1996;133:1403–1415. [PubMed: 8682874]
57. Xia D, Stull JT, Kamm KE. Myosin phosphatase targeting subunit 1 affects cell migration by regulating myosin phosphorylation and actin assembly. Exp. Cell Res 2005;304:506–517. [PubMed: 15748895]
58. Wu Y, Erdodi F, Muranyi A, Nullmeyer KD, Lynch RM, Hartshorne DJ. Myosin phosphatase and myosin phosphorylation in differentiating C2C12 cells. J. Muscle Res. Cell Motil 2003;24:499–511. [PubMed: 14870965]
59. Uehata M, Ishizaki T, Satoh H, Ono T, Kawahara T, Morishita T, Tamakawa H, Yamagami K, Inui J, Maekawa M, Narumiya S. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature 1997;389:990–994. [PubMed: 9353125]
60. Giannone G, Dubin-Thaler BJ, Dobereiner HG, Kieffer N, Bresnick AR, Sheetz MP. Periodic lamellipodial contractions correlate with rearward actin waves. Cell 2004;116:431–443. [PubMed: 15016377]
61. Webb DJ, Donais K, Whitmore LA, Thomas SM, Turner CE, Parsons JT, Horwitz AF. FAK-Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly. Nat. Cell Biol 2004;6:154–161. [PubMed: 14743221]
62. Mizuno T, Tsutsui K, Nishida Y. *Drosophila* myosin phosphatase and its role in dorsal closure. Development 2002;129:1215–1223. [PubMed: 11874917]
63. Tan C, Stronach B, Perrimon N. Roles of myosin phosphatase during *Drosophila* development. Development 2003;130:671–681. [PubMed: 12505998]
64. Wissmann A, Ingles J, McGhee JD, Mains PE. Caenorhabditis elegans LET-502 is related to Rho-binding kinases and human myotonic dystrophy kinase and interacts genetically with a homolog of the regulatory subunit of smooth muscle myosin phosphatase to affect cell shape. Genes Dev 1997;11:409–422. [PubMed: 9042856]
65. Piekny AJ, Mains PE. Rho-binding kinase (LET-502) and myosin phosphatase (MEL-11) regulate cytokinesis in the early Caenorhabditis elegans embryo. J. Cell Sci 2002;115:2271–2282. [PubMed: 12006612]
66. Okamoto R, Ito M, Suzuki N, Kongo M, Moriki N, Saito H, Tsumura H, Imanaka-Yoshida K, Kimura K, Mizoguchi A, Hartshorne DJ, Nakano T. The targeted disruption of the MYPT1 gene results in embryonic lethality. Transgenic Res 2005;14:337–340. [PubMed: 16145842]

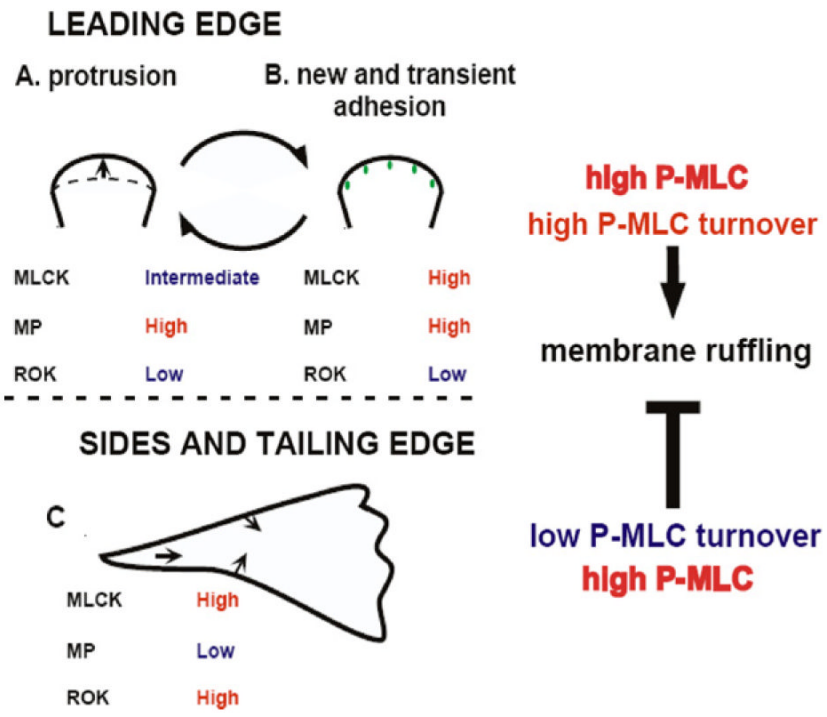

Fig. 1.
Model for the roles of MP and MLCK at the leading edge during protrusion (A) or retraction and adhesion (B) and along the side and trailing edge (C). For details see text.

Table 1

MYPT1—binding Proteins

Protein	Cell function	Substrate for ROK
Myosin II	Motor functions Cytoskeletal organization	Yes
Ezrin, radixin, moesin (ERM)	Cytoskeletal organization	Yes
α-Adducin	Cytoskeletal attachment	Yes
Tau, MAP2	Microtubule dynamics	Yes
Elongation factor-1α	Cofactor protein synthesis Cytoskeletal organization	Yes
ZIP kinase	Phosphorylation of myosin II and/or MYPT1 [10]	Yes
RhoA-GTP	Activates ROK	No
M20	MYPT2 gene product; unknown function	Yes
PKG (cGKIα)	Regulation of Ca^{2+} level; activation of MP	?
Interleukin-16 precursor	Cytokine precursor	?
p116 RIP	F-actin assembly; binds RhoA-GTP [11]	?
p116 RIP	Inhibits SRF activation [12]	
p116 RIP	Activates MP; RhoA-GTP [13]	
M-RIP (aka p116 RIP)	Targets MYPT1, RhoA, myosin [9]	
Merlin	Tumor suppressor protein [14]	?
HDAC 7	Transcriptional repressor [15]	?
HSP 27	Docking protein, binds RhoA [16]	?
Synaptophysin	Synaptic vesicles, neurotransmitter release [17]	?
Retinoblastoma protein	G1/S transition [4]	?

Several references are given in [1]. Others are cited.