Myotonic dystrophy kinase-related Cdc42-binding kinases (MRCK), the ROCK-like effectors of Cdc42 and Rac1

Zhuoshen Zhao¹ and Ed Manser^{1,2,3,*}

¹GSK Group; Institute of Molecular and Cell Biology (IMCB); Singapore; ²Institute of Medical Biology (IMB); Singapore; ³Department of Pharmacology; National University of Singapore; Singapore

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Cdc42 is a member of the Rho GTPase protein family that plays key roles in local F-actin organization through a number of kinase and non-kinase effector proteins. The myotonic dystrophy kinase-related Cdc42-binding kinases (MRCKs), and the RhoA binding coiled-coil containing kinases (ROCKs) are widely expressed members of the Dystrophia myotonica protein kinase (DMPK) family. The MRCK proteins are ~190 kDa multi-domain proteins expressed in all cells and coordinate certain acto-myosin networks. Notably MRCK is a key regulator of myosin18A and myosin IIA/B, and through phosphorylation of their common regulatory light chains (MYL9 or MLC2) to promote actin stress fiber contractility. The MRCK kinases are regulated by Cdc42, which is required for cell polarity and directional migration; MRCK links to the acto-myosin complex through interaction with a coiled-coil containing adaptor proteins LRAP35a/b. The biological activities of MRCK in model organisms such as worms and flies confirm it as a myosin II activator. In mammalian cell culture MRCK can be critical for cancer cell migration and neurite outgrowth. We review the current literatures regarding MRCK and highlight the similarities and differences between MRCK and ROCK kinases.

Background

The Rho family in mammals represents a group of 23 gene products¹ that in general are activated by guanine nucleotide exchange factors and inactivated by GTPase-activating proteins.² Target (effector) proteins for these Rho proteins often contain recognizable sequence motifs involved with GTPase interaction that allow identification via database searches.³ The Rho proteins are hubs for cytoskeletal regulation: as such they are known to regulate F-actin assembly through formins,⁴ F-actin branching through WAVE and WASP proteins,⁵ septin assembly through Borg proteins,⁶ and intermediate filament reorganization through kinases such as Pak1.⁷

One of the key contractile systems in non-muscle cells is the acto-myosin network comprising myosin II heavy chains (different genes encode A, B and C isoforms also termed MYH9, 10 and 14) which are regulated by a common regulatory light chain (MYL9 /, or MLC2).⁸ The myosin II system comprises alternating units of bipolar myosin filaments and actin filaments bundled by α -actinin. Recent evidence suggests that the different myosin isoforms can co-assemble into mixed bipolar filaments.⁹ MLC2 phosphorylation at Ser19 sometimes in combination with Thr18, controls the ATPase activity of the myosin II heavy chain, to generate contractile forces. Besides the classical myosin light chain kinases (MLCK) which is regulated by calcium/calmodulin,¹⁰ multiple Rho GTPase effector kinases are now implicated in this process, including Rho kinase (ROK/ROCK/Rho-kinase),^{11,12} citron kinase (CRIK),¹³ myotonic dystrophy kinase-related Cdc42-binding kinase (MRCK),¹⁴ and p21-activated kinase 1 (PAK1).¹⁵

The founding member of these MLC2 kinases is Dystrophia myotonica protein kinase (DMPK) which belongs to the AGC super-family and is most closely related by sequence to MRCK α , sharing ~60% sequence identity in the kinase domain (Fig. 1). There are 6 different DMPK isoforms¹⁶ of which 4 are expressed independently of tissue type, while 2 are predominantly present in smooth muscle. All have molecular weights of 68–74 kDa which are much smaller than other family members. Changes within in the DMPK gene locus give rise to myotonic dystrophy type 1 (DM1), the most prevalent muscular dystrophy in adults. Genetic defects in DM1 cause amplification of a trinucleotide repeat in the 3' untranslated region of DMPK. It is thought that the disease results from both gain-of-function of the DMPK RNA and from decreased DMPK expression in the cytoplasm, as previously reviewed.¹⁷

RhoA-ROCK activity is well established for maintaining actin stress fibers and stabilizing associated focal adhesion formation in cultured cell.¹⁸ ROCK also plays a key role in allowing tail retraction of migrating cells and prevention of cell rear protrusion.¹⁹ The roles of ROCK in this context has been recently reviewed in some detail.²⁰ In some cell types MLCK has been shown to regulate focal complex formation during lamellipodial extension,^{21,22} but subsequently MRCK was identified as a key regulator of lamellar acto-myosin dynamics independent of MLCK and ROCK.²³ The ability of these different MLC kinases to promote

*Correspondence to: Ed Manser; Email: ed.manser@imb.a-star.edu.sg
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Figure 1. Domain organization of MRCK and related kinases. The size of the human proteins (number of residues) is indicated on the right of the schematic. All DMPK-like kinases contain an N-terminal region that forms a tight dimer and packs against the hydrophobic motif found at the C-terminal of the kinase domain (see Fig. 2). The length of the coiled-coil region that lies C-terminal to the kinase domain is variable between the various kinases; in the case of MRCK this region contains the kinase-inhibitory motif (KIM) that serves to autoinhibit the kinase. The three human MRCK isoforms have very similar domain structures, with MRCKγ being the smallest and most divergent. ROCK and CRIK contain a RhoA binding domain (RBD) whose coiled-coil structure serves to interact with the GTP-bound form of RhoA. The Cdc42 and Rac1 binding region (CRIB) by contrast is located at the C-terminal region of the polypeptide. The C1 domain of MRCK (yellow) interacts with diacylglycerol; the related C1 domain of ROCK, which however does not bind diacylglycerol, splits the PH domain (green). The citron homology domain (CH, brown) is found in MRCK and CRIK.

protrusion, traction and retraction must relate to both their unique localization in the cell and different target selectivity.²⁴ The precise coordination of these kinases downstream of their respective Rho GTPase activators allows efficient cell movement.

Signaling pathways that arise from ‘classical’ phospholipase-C initiated signaling pathway allows Ca^{2+} mobilization to activate MLCK,²⁵ and diacylglycerol to activate MRCK.²⁶ In non-muscle cells there is a great deal known about the RhoA-ROCK pathway that drives MLC phosphorylation because of the existence of the potent and selective small molecule inhibitors of ROCKs such as Y27632.²⁷ The activity of this compound on ROCK was described in classic paper in 1997.²⁸ In addition to ROCK and CRIK, the Myotonic dystrophy-related Cdc42-binding kinases MRCKα and MRCKβ¹⁴ and less widely expressed MRCKγ²⁹ contribute to MLC phosphorylation downstream of Cdc42.

The structural and primary sequence features of MRCK

Full-length rat MRCKα and MRCKβ are ~180 kDa proteins that were identified through expression screening¹⁴ using a strategy that previously uncovered the Cdc42 effector ACK1.³⁰ Human MRCKα and MRCKβ kinases are closely related,^{31,32} and share the same functional domains with each other and MRCKγ.^{9,29} The MRCK proteins contain a C-terminally located Cdc42/Rac interaction binding (CRIB) domain (Fig. 1); MRCKα can also bind to Rac1.³³ Interestingly the CRIB region of MRCKγ, which is expressed predominantly in muscle cells binds preferentially to TC10 versus Cdc42.²⁹ This is consistent with TC10 being a muscle enriched Cdc42-like GTPase.³⁴ Although Cdc42 is clearly upstream of MRCK, the potential for

other Cdc42-like Rho GTPases (TCL, Chp, and Wrch) to recruit MRCK has not been studied in detail.

The organization of the kinase domain and flanking N-terminal and C-terminal sequences are similar to the myotonic dystrophy kinase (DMPK) family of kinases.³⁵ The region N-terminal to the kinase domain is conserved across the family; MRCKα contains a functional binding site for phorbol 12-myristate 13-acetate (PMA; an analog of diacylglycerol, DAG) that is similar to the zinc finger C1 domains of PKC and chimaerin.¹⁴ The human gene for MRCKα is located at chromosome band 1q42.1 and extends over approximately 250–300 kb³¹; no genetic lesions have been identified in this gene to date. The (C1) domains (Fig. 1) of MRCKα and MRCKβ are similar to conventional PKC isoforms and bind phorbol esters with nanomolar affinities.^{36,37} The binding of DAG to the C1 domains can drive kinase activation through a mechanism that appears to involve the flanking kinase-inhibitory

motif (KIM).³⁷ However the lower affinities of MRCK C1 domain for phorbol esters compared to homologous PKC C1 domains mean that this interaction alone not sufficient to translocate the MRCK to the plasma membrane.³⁶ The Pleckstrin Homology (PH) domains (Fig. 1) are generally used in targeting proteins various lipid compartments, but the nature and function of the MRCK PH domain is not understood. All three MRCK proteins have a Citron Homology domain, which derives its name from the citron kinase CRIK, where the domain is similarly located next to the PH domain.³⁸

Biochemical regulation of MRCK activity

The regulation of related ROCK kinases has been more extensively studied; auto-inhibition of the ROCK kinase domain (Fig. 1) via intramolecular interactions between the catalytic and the C-terminus of the kinase can be relieved via proteolytic cleavage, binding of lipids to a PH domain, or indeed binding of RhoA.GTP to the central coiled-coil region of ROCK.³⁹ Although auto-phosphorylation of MRCKα was suggested for activation loop on sites including Ser234 based on substitution,³⁷ activation-loop phosphorylation is not annotated in Phosphosite. The lack of documented auto-phosphorylation events (upon kinase activation) has prevented the development of phospho-specific antibodies capable of assessing MRCK activity *in vivo*. This has hampered proper analysis of the subcellular localization of active MRCK (the same situation prevails for ROCK and Citron kinases).

The crystal structure of the MRCKβ kinase domain has revealed a dimeric kinase structures highly related to ROCK1

and DMPK.⁴⁰ The kinase domains of MRCK β ⁴⁰ DMPK⁴¹ and ROCK1⁴² indicates its can adopt a compact 'active' conformation in an unphosphorylated state. The activation (A)-loop occupies a well-ordered conformation that does not impede access to the nucleotide or substrate binding sites (see red loop in Fig. 2). The MRCK β A-loop lies below the α C helix (yellow), which holds ATP in a conformation competent for catalysis.⁴⁰ As for the crystal structure of ROCK kinases,⁴² a C-terminal extension of the kinase domain termed the hydrophobic motif (dark blue in Fig. 2) serves to correctly position the α C helix (yellow). This motif contains 3 phenylalanine residues which contact the α C helix and also makes multiple contacts with the N-terminal extension of the kinase (MRCK residues 5–65) through both intramolecular (gray helices) and inter-molecular interactions (green helices) of the other subunit (b) of the dimer. Thus the N-terminal region likely plays a direct role in positioning the α C helix. Many AGC kinases (including DMPKs, the RhoA regulated PKNs and atypical PKCs contain the regulatory site termed the hydrophobic motif or "PIF-pocket" which in some kinases serves as a docking site for substrates. This site is also involved in the allosteric regulation of N-terminal domains of several AGC kinases. Recently it has been suggested that the 'master regulator' of AGC kinases PDK1 positively modulates MRCK α activity,⁴³ although this does not involve the phosphorylation activity of PDK1.

MRCK α forms oligomers which by gel filtration chromatography correspond primarily to inactive tetramers of ~900 kDa.³⁷ The coiled-coil domain arrangement of the region downstream of the kinase domain indicates parallel intermolecular interactions as for ROCK.⁴⁴ It is unclear whether Cdc42.

Figure 2. Structure of the dimeric MRCK β catalytic domain. The dimeric MRCK β is based on the coordinates in 3TKU. The catalytic domain reveals a typical structure, with the disposition of C- and N-lobe as indicated. The activation loop (purple) is fully ordered, and the α C-helix (yellow) lies in a position that would be compatible with catalysis. The position of ATP as indicated is based on binding of Fasudil in this crystal structure.⁴⁰ The hydrophobic motif (dark blue) binds to the N-lobe as is typical for other kinases, but this region makes substantial contacts also with the N-terminal helices that are involved in dimerization. Thus the dimerization domain may be required for holding the hydrophobic motif.

GTP binding to MRCK plays any direct role in kinase activation, whereas RhoA.GTP binding to ROCK directly regulates kinase activity.⁴⁵ The C-terminal domains of MRCK (including the CRIB) do interact with the kinase-containing N-terminal region.³⁷ An auto-inhibitory region positioned within the coiled-coil region can bind and inhibit the MRCK catalytic domain^{29,37}; this has been termed the kinase inhibitory motif (KIM, see Fig. 1). Removal of this sequence leads to elevated kinase activity.

Based on careful *in vitro* and *in vivo* analysis of MRCK α , kinase activation appears in part to require diacylglycerol (DAG) binding to the zinc finger region, leading to ~3-fold MRCK activation.³⁷ In co-transfection experiments no enhancement of MRCK activity is seen when Cdc42V12 is expressed with full-length kinase.³⁷ Nonetheless siRNA mediated knockdown of Cdc42 in cells causes a loss of MRCK association with the actomyosin network.²³ This is associated with loss of circumferential myosin II filaments at the lamella region that are regulated by Cdc42, while the ventral stress fibers are unaffected. In summary, the mechanism by which the (active) kinase domains of MRCK, ROCK and Citron are regulated by their cognate Rho proteins remains largely unresolved.

The intracellular localization of MRCK

The tight interaction of Cdc42.GTP with the CRIB domain (s) has been shown as promoting the proper localization of MRCK. Depletion of Cdc42 by siRNA treatment leads to a profound loss of MRCK clusters associated with actomyosin filaments in lamella of Hela cells.²³ The association of MRCK α with myosin at this site is dependent on a small adaptor protein LRAP35a which allows it to form a direct tripartite complex with the heavy chain of myosin 18a designated MYO18A.²³ The LRAP35a C-terminus is bound to the MYO18A PDZ domain, while its central coiled-coil region binds the KIM motif of MRCK. It seems likely therefore that oligomeric MRCK is localized through MYO18A to actomyosin clusters, which in turn regulates the more abundant myosin IIA isoform (although MRCK may not directly bind to these 2 proteins) which is found in the lamella.²³ Interestingly a smaller protein adaptor LRAP25, which shares sequence homology in the coiled-coil region with LRAP35a, has been reported to target MRCK to the cell edge or lamellipodium and allows it to interact with LIM kinase.⁴⁶ It seems likely that MRCK is therefore targeted primarily by LRAP-like adaptors, with additional contributions Cdc42 or Rac1 binding and diacylglycerol interaction with the C1 domain. The ability of PDK1 and MRCK α to associate with each other in lamellipodia⁴³ may require LRAP25.

The identification of Shroom3 as an adaptor for ROCK has been a key finding.⁴⁷ It seems likely that the various Shroom proteins are essential for cellular targeting of ROCK1/2. The Shroom binding site on ROCK lies within the coiled-coil domain (in a similar position to that for LRAP35a in MRCK), and N-terminal to the RhoA binding site.⁴⁸ This Shroom binding region is essential for ROCK-dependent apical constriction of epithelial cells. Interestingly the *Drosophila* CRICK ortholog (Sticky) localizes to the cleavage furrow of dividing cells

independent of RhoA,⁴⁹ while the coiled-coil region is necessary and sufficient for its localization. In post-mitotic neurons the mammalian Citron kinase similarly becomes concentrated at postsynaptic sites through interactions of the coiled-coil region.⁵⁰ Thus several strands of evidence point to the coiled-coil domain as critical for the localization and function of DMPK family kinases, mediated by selective adaptor proteins.

MRCK kinase inhibitors

The ROCK and MRCK kinase domains being highly related are able to phosphorylate common substrates when tested *in vitro*. The widely used ROCK inhibitor Y-27632 and related compounds have been crucial to the identification of ROCK targets *in vivo*.²⁷ Although Y-27632 is considered to be 10-fold less effective against MRCK isoforms than ROCK,⁴⁰ there are no robust bio-markers for these kinases (for example phospho-specific antibodies directed to the kinases themselves or specific substrates). Thus it is problematic to accurately assess MRCK *in vivo* selectivity. Even though Y-27632 is regarded as ROCK-selective, it inhibits the RhoA target PRK2 with similar affinity to ROCK.^{51,52} The ROCK inhibitor fasudil binds to MRCK, and crystals of the MRCK β kinase domain in complex with fasudil and TCPA-1⁴⁰ revealed how these act as ATP-mimetics. Recently a potent MRCK selective inhibitor has been reported (abbreviated as BDP5290) that is more than 50-fold selective over ROCK.⁵³ The X-ray structure of the MRCK β kinase domain in complex with BDP5290 indicates the basis of its selectivity.

The benzophen-anthridine alkaloid chelerythrine has been found to be selective pharmacological inhibitor for MRCK.²⁴ No significant activity was found for PKC α nor other MLC kinases such as ROCK and MLCK.²⁴ Chelerythrine does show some activity *in vitro* toward CRIK at 10 μ M. With an IC₅₀ for MRCK of 1.8 μ M *in vitro*, and an effective concentration of 5.0 μ M in cells, chelerythrine can pass through biological membranes and elicits cellular phenotypes that are consistent with MRCK inhibition.²⁴ Chelerythrine does not target the ATP binding pocket and is potentially more specific than ATP-competitive inhibitors. Remarkably >1000 publications have used chelerythrine as a PKC α inhibitor since it was first described.⁵⁴ However chelerythrine (at 10 μ M) does not inhibit PKC α family kinases *in vitro*.⁵¹ It should be noted that chelerythrine has a number of non-kinase targets can complicate interpretation of phenotypes (Brunhofer et al., 2012; Basu et al., 2013). The KIM domain of MRCK can inhibit MRCK kinases in trans; over-expressing the MRCK γ KIM domain suppresses MRCK α *in vivo*.³⁷ This genetically encoded pan-MRCK inhibitor has the potential to validate less selective chemical inhibitors, much like the PAK1 auto-inhibitory domain.⁵⁵

MRCK substrates

There has been no systematic search for MRCK substrates, but based on structural and primary similarities kinase between ROCK and MRCK one might assume they can share targets. It was established early on that ROCK directly regulates myosin II through phosphorylation of its regulatory light chain (MLC2).⁵⁶

Among the various DMPK family kinases, it is notable that ROCK and CRIK are capable of di-phosphorylation of MLC2 Thr18/Ser19, whereas MRCK (and MLCK) can only mono-phosphorylate Ser19²⁴; thus one can partly differentiate the various MLC2 kinase activities *in vivo*.²⁴ The double phosphorylation of MLC2 leads to changes in the contractility of the myosin II heavy chain.⁵⁷ Surprisingly DMPK itself has no activity toward MLC2, although it can modify MYPT1 at Thr654, which is a key site to inhibit its phosphatase activity.⁵⁸ Conversely in smooth muscle MLC2 is convincingly shown to be a direct target of the muscle enriched zipper-interacting protein kinase (ZIPK), but this kinase cannot phosphorylate MYPT1.⁵⁹

Changes in MLC2 phosphorylation status induced by MRCK α ⁶⁰ result from its direct phosphorylation, as well as inhibition of MYPT1^{61,62} which is part of the myosin light chain phosphatase (MLCP) complex (Fig. 3). MLCP is composed of 3 subunits: a 38-kDa catalytic subunit (PP1 δ), the 130-kDa myosin targeting subunit (MYPT1), and an additional 20-kDa subunit (M20) that has homology to the C-terminal portion of MYPT1. Both MYPT1 and the smaller p85 MYPT3 are substrates of MRCK.⁶³ Phosphorylation of a centrally located conserved motif termed the phosphatase inhibitory motif (PIM) allows it to bind and inhibit (the associated) PP1 δ .⁶⁴ Therefore inhibition of MLCP activity can result from MYPT phosphorylation by multiple DMPK family kinases potentially over 3 sites that are conserved across the various isoforms and equivalent to MYPT1 Ser472, Thr697 and Thr855.⁶⁵⁻⁶⁷ The phosphorylation of Ser472 allows binding of 14-3-3⁶⁸ which blocks MYPT1 interaction with myosin heavy chain (Fig. 3). Thr697 phosphorylation inhibits MLCP activity by inactivating the catalytic PP1 δ .⁶⁹ The role of Thr850 phosphorylation is less clear but this modification also down-regulates MLCP activity.^{67,70}

The situation is further complicated by the observation that the Thr697 and Thr855 sites containing flanking serine residues which can be co-phosphorylated.⁷¹ Dual phosphorylation of MYPT1 occurs *in vitro* and *in situ* at both Ser696–Thr697 and Ser854–Thr855 (Fig. 3). Pre-phosphorylation of Ser696 and Ser854 inhibits the ability of ROCK to phosphorylate the neighboring Thr697 and Thr855. The role of Ser696 phosphorylation has been studied for some time.^{72,73} The mono-phosphorylation of Thr697 and Thr855 (by ROCK and presumably MRCK and CRIK) precludes the ability of PKAc to phosphorylate the neighboring Ser residues. Thus the interplay of inhibitory (Thr) and dis-inhibitory (Ser) neighboring modifications of MYPT1 regulate its activity.

Genetic experiments in *Caenorhabditis elegans* revealed that MRCK and ROCK orthologues contributed to phosphorylation of MLC and MYPT1 proteins, though a constitutively-active form of MLC could complement only loss of MRCK, but not ROCK.⁷⁴ Thus the primary function of MRCK in this *C. elegans* developmental context is MLC regulation, while ROCK must have additional targets. In other situations MRCK α can act on LIM kinases 1 and 2, by phosphorylating their activation loop.^{75,76} This serves to stabilize F-actin by inactivating cofilin.⁷⁷ Cofilin serves to fragment and destabilize F-actin. Mammalian LIMK1 is required for synaptic architecture,⁷⁸ while

knocking out LIMK2 results in an eyelid defect in mice.⁷⁹ Recently an adaptor LRAP25 has been described which links MRCK to LIMK1⁴⁶ in a similar way to LRAP35 coupling MRCK with MYO18a. The lamellipodium-localized LRAP25-MRCK complex allows activation of LIMK1, which promotes F-actin stability and allows membrane protrusion. In B16 cells inhibition of either MRCK or LRAP25 resulted in suppression of LIMK1 activity.⁴⁶

Biological activities of MRCK

Since MRCK is a direct target of Cdc42, there has been strong interest uncovering those aspects of Cdc42 function that result from MRCK coupling. MRCK has been found in a number of contexts to promote cancer cell motility and invasiveness as recently reviewed.⁸⁰ Cdc42 plays an important role in establishing cell polarity in a number of contexts, and its dysregulation can be a factor in cancer progression.⁸¹ The actin-myosin contraction regulated by MRCK aids re-orientation of cell nuclei relative to microtubule-organizing center (MTOC), and this help establish the forward polarity of the Golgi apparatus in migrating cells.⁸² Cdc42 has other direct targets that are needed in this process including Par6 that recruits the atypical-PKC complex.⁸³ MRCK forms clusters at the leading edge of cells and undergoes retrograde flow as is typical for actin-myosin associated proteins.²³ This activity promotes re-orientation of cell nuclei relative to microtubule-organizing centers (MTOC) to establish polarity in migrating cells in a monolayer.⁸² In epithelial adherens junctions(AJs), circumferential actin bundles that align along the cell-cell junctions; non-muscle myosin IIA (NM-IIA) generates tension required for proper formation of AJs alignment.^{84,85} In contrast to epithelial cells, the junctional fibers of endothelial cells rely on NM-IIB recruited in a Rap1-dependent manner.⁸⁶ This pathway requires MRCK β to form circumferential actin bundles proximal to the plasma membrane that are important for its barrier function.⁸⁷ By contrast Rho-ROCK promotes radial stress fibers and adherens junction clustering which reduces endothelial barrier function.⁸⁷

To date no knockout mice targeting any of the 3 MRCK genes have been reported. In *Drosophila*, mutations of *gek* (a *Drosophila* homolog of MRCK) cause abnormal F-actin accumulation and, as expected, *gek* interacts genetically with *Drosophila* Cdc42.⁸⁸ MRCK α activity can enhance the ability of Cdc42 to induced filopodia¹⁴ which could relate to the ability of the kinase

Figure 3. Features of the MYPT1 (PPP1R12A) phosphatase complex and its activity toward myosin regulatory light chain (MLC2). The catalytic PP1 δ subunit binds MYPT1 at an RVxF motif found at amino acids 35–38 (red bar). Ankyrin repeats (x8) are further involved to bind the protein phosphatase catalytic subunit PP1c. When the complex is in the vicinity of the myosin complex, it can act on MLC2. MYPT1 can be phosphorylated at Thr696 (PIM, phosphatase inhibitory motif) by both ROCK and MRCK, which allows this region to inhibit the PP1c (dotted line). Although MYPT1 Thr853 phosphorylation is also inhibitory, the mechanism is not so well understood. Other important regulatory sites marked as blue spheres are regulated by cGMP- or cAMP-dependent kinases⁷¹; these help maintain MYPT1 activity by preventing ROCK phosphorylation of neighboring residues. Phosphorylation of Ser472 generates a site that allows for recruitment of 14–3–3, which prevents myosin interaction. The third subunit of the MYPT1 holoenzyme is termed M20 which binds to and indeed resembles MYPT1 residues 934–1006. This region of the complex is thought to allow targeting the bipolar myosin heavy chain complexes as indicated (arrow).

to activate ERM proteins.⁶¹ Interestingly MRCK (like Cdc42) is permissive for neurite outgrowth in PC12 cells,⁸⁹ an activity opposite to that of RhoA-ROCK, which is commonly down-regulated during neuriteogenesis in the central nervous system.⁹⁰

Among the multiple kinases that contribute to MLC phosphorylation, MRCK and ROCK are likely expressed in all non-muscle cells. Several studies have found somewhat overlapping functions for MRCK and ROCK kinases; for example the ability of ephrinB to induce endothelial cell retraction required the combined blockade of ROCK and MRCK.⁹¹ Similarly reducing the level of both ROCK and MRCK can block MDA MB 231 breast cancer cell invasion into 3-dimensional protein matrices.⁶² This study showed that single cells move through a 3-dimensional matrix with either a rounded morphology, characterized by Rho-ROCK dependence, or with an elongated morphology characterized by Cdc42-MRCK. The kinase is also implicated in collective cancer cell invasion.⁹² Thus Cdc42-MRCK and Rho-ROCK signals can drive *in vivo* cancer cell motility albeit via quite different modes of migration.⁸⁰

Conclusions

MRCK kinases belong to a relatively restricted group of kinases that are key regulators of actomyosin contractility; this activity in turn plays critical roles in cell adhesion, motility and division. It is notable that ROCK and MRCK are probably targeted by interaction with selective adaptor proteins such as Shroom and LRAP35, which serve to independently target these kinases in the

cell. The localization of such kinases also involves membrane-binding domains, notably the zinc finger C1-like and PH domains. The future development of tools to identify active forms of MRCK (and ROCK) will be essential to better understand these abundant and important kinases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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