Myosin Light Chain Phosphatase
It Gets Around
Frank V. Brozovich

The importance of Ca\textsuperscript{2+}/calmodulin-dependent myosin light chain kinase (MLCK) for smooth muscle contraction is well documented. Until recently, myosin light chain (MLC) phosphatase was thought to be unregulated and constitutively active. However, studies have demonstrated that smooth muscle contraction is dependent on regulation of MLC phosphatase activity. The inhibition of MLC phosphatase activity increases force at a constant [Ca\textsuperscript{2+}], whereas a stimulation of MLC phosphatase activity reduces force at a constant [Ca\textsuperscript{2+}].

**MLC Phosphatase**

MLC phosphatase isolated from smooth muscle is a holoenzyme consisting of 3 subunits: a small \(\approx\)20-kDa subunit, an \(\approx\)38-kDa catalytic subunit, and a myosin-targeting subunit (MYPT) of 110 to 133 kDa. The small subunit has no established function and is not required for either catalytic activity or activation of PPIc. The catalytic subunit is a PPIc phosphatase, and the \(\delta\) isoform is associated with the holoenzyme. The large subunit is the MYPT. Isoform diversity of MYPT was first shown in chicken where 2 isoforms (M130 and M133) were shown to differ by the presence of a central insert between aa residues 512 to 552. Similar isoform diversity has been demonstrated for MYPT isolated from other species. In addition to the isoform diversity produced by the central insert, alternative splicing of a COOH-terminal exon leads to MYPT isoforms, which differ by the presence or absence of a leucine zipper.

**Agonist-Induced Force Enhancement**

At any intracellular [Ca\textsuperscript{2+}], force for an agonist stimulated contraction is higher than for depolarization. This phenomenon has been termed agonist-induced force enhancement, or Ca\textsuperscript{2+} sensitization, and several mechanisms have been proposed. Although the signaling pathway(s) for Ca\textsuperscript{2+} sensitization have not been elucidated, most proposed mechanisms converge on an inhibition of MLC phosphatase activity.

In smooth muscle, G protein–coupled receptor activation increases MLC\textsubscript{20} phosphorylation and force. Trinkle-Mulcahy et al demonstrated that MYPT was thiophosphorylated by ATP\textsubscript{\gamma}S, resulting in a decrease of MLC phosphatase activity and force enhancement. These data are consistent with a signaling pathway involving heterotrimeric G protein–induced activation of Rho A and Rho kinase. Rho kinase has been shown to phosphorylate MYPT at Thr695. Phosphorylation at this site decreases MLC phosphatase activity to increase both MLC\textsubscript{20} phosphorylation and force, although MYPT phosphorylation and force enhancement do not always correlate. Another mechanism for Ca\textsuperscript{2+} sensitization involves an inhibitor protein for MLC phosphatase, CPI-17, which can be phosphorylated by protein kinase C and Rho kinase. CPI-17 inhibits MLC phosphatase activity. An additional mechanism is dissociation of the subunits of MLC phosphatase by arachidonic acid. Activation of the G proteins increases the activity of phospholipase A\textsubscript{2} and production of arachidonic acid, which binds to and dissociates MLC phosphatase to reduce phosphatase activity. The Rho kinase signaling pathway may also lead to a direct phosphorylation of MLC\textsubscript{20} by Zip-like kinase and/or integrin-linked kinase to produce a Ca\textsuperscript{2+} independent increase in force.

In this issue of *Circulation Research*, the study from Morgan’s laboratory has evidence for another mechanism for Ca\textsuperscript{2+} sensitization. Their data shows that there is an agonist-specific translocation of MLC phosphatase to the smooth muscle cell membrane. PGF\textsubscript{2\alpha} stimulation of freshly dispersed smooth muscle cells results in phosphorylation of MYPT at Thr697 (Rho kinase site of mammalian sequence) and a translocation of MLC phosphatase from the cytosol to the membrane. The Rho kinase inhibitor, Y27632, inhibited both MYPT phosphorylation and MLC phosphatase translocation, suggesting that the initial event in the signaling cascade is an activation of Rho kinase after MLC phosphatase translocates to the membrane, there is a dissociation of MYPT and PP1c; PP1c returns to the cytosol. Phenylephrine stimulation produced a distinctly different pattern of MLC phosphatase movement. MYPT did not translocate but remained homogenously distributed throughout the cell, whereas PP1c initially demonstrated a homogenous pattern that became transiently nonhomogenous after stimulation.

Agonist-induced protein translocation is not unprecedented. Translocation of PKC, calpain, and Rho A have all been reported. Translocation of Rho A from the cytosol to the cell membrane has been suggested to lead to spatial activation of Rho kinase, which would phosphorylate MYPT to inhibit MLC phosphatase activity at the cell membrane and produce a sustained increase in MLC\textsubscript{20} phosphorylation at the cortical region of the smooth muscle cell. The data of Shin et al show a translocation of MLC phosphatase from the cytosol to the cell membrane. The first

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event in this process is a Rho kinase–induced phosphorylation of MYPT, which would inhibit MLC phosphatase activity.5 Thus, their data23 are consistent with and could produce the sustained increase in MLC20 phosphorylation in the cortical region of the smooth muscle cell observed by others.26

Isolated PP1c has been shown to have a reduced phosphorylation activity toward phosphorylated MLC20.28 Thus, the results of Morgan’s group23 make it intriguing to speculate that dissociation of PP1c from MYPT decreases MLC phosphatase activity to produce the sustained increase in MLC20 phosphorylation observed during PGF2α stimulation. Phenylephrine stimulation does not lead to dissociation of MLC phosphatase, and MLC phosphatase activity would remain elevated to decrease the level of MLC20 phosphorylation. For phenylephrine, force would have to be maintained by a mechanism outside of actomyosin.29 However, one would expect both PGF2α and phenylephrine to activate Rho A and Rho kinase to lead to a phosphorylation of MYPT and force enhancement. Consistent with this hypothesis is the data demonstrating that the Rho kinase inhibitor Y27632 almost completely inhibited force for both agonists.30 This could suggest the effects of Y27632 are nonspecific.31 Rho kinase has more than one substrate,31 or the differential translocation of MYPT and PP1c is unrelated to inhibition of MLC phosphatase activity. There could be a difference in the PGF2α and phenylephrine signaling pathways, which led to this differential translocation of MLC phosphatase; ie, there could be an agonist-dependent activation of phospholipase A₂ and production of arachidonic acid. This would result in an agonist-specific dissociation of the MLC holoenzyme and be consistent with the results of Morgan’s group, but again, it is difficult to envision how Rho kinase inhibition produces a similar decrease in force for both agonists.

Summary

The study by Morgan’s group23 provides a provocative molecular mechanism for Ca2+ sensitization. Others have shown that both the dose response relationship and the magnitude of Ca2+ sensitization is tissue-specific.11,12,17,18 In addition, it has been shown that the distribution of the M130/M133 isoforms of the MYPT8,32 as well as CPI-17,33 are tissue-dependent and correlate with the magnitude of Ca2+ sensitization.15,34 Therefore, the role and physiological importance of differential translocation of MLC phosphatase, MYPT isoforms, CPI-17, and arachidonic acid for the molecular mechanism of force enhancement remains to be determined.

The molecular mechanism for Ca2+ sensitization has broad clinical implications. Ca2+ sensitization and desensitization have been implicated in the pathogenesis of hypertension,35 erectile dysfunction,36 and could lead to asthma, vasospasm, and both the resting vasoconstriction and resistance to nitric oxide–associated vasodilatation associated with congestive heart failure. Many cellular signaling pathways involve protein phosphorylation, and modulation of MLC phosphatase activity could also play a role in the metastasis and invasion of malignant cells,37 as well as the growth and development of neurons.38 Therefore, the molecular mechanism for the regulation of phosphatase activity is important for both normal physiology and pathophysiology of human disease, and agents designed to modulate MLC phosphatase activity may have broad therapeutic potential.

References


**Key Words:** agonist \[\text{smooth muscle}\] myosin targeting subunit \[\text{Rho A} \[\text{G protein}\]
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