Kinases, myosin phosphatase and Rho proteins: curiouser and curiouser

Andrew P. Somlyo

Department of Molecular Physiology and Biological Physics, University of Virginia School of Medicine, Charlottesville, VA 22906-0011, USA

The primary mechanism activating smooth muscle is phosphorylation of the myosin regulatory light chain (MLC_{20}) by a myosin light chain kinase (MLCK) activated by Ca^{2+}–calmodulin (CaM)-dependent phosphorylation; this allows actin to activate myosin ATPase, causing muscle to contract. Inactivation (relaxation) occurs as the result of dephosphorylation of MLC_{20} by a heterotrimeric smooth muscle myosin phosphatase, SMPP-1M (Hartshorne *et al.* 1998). Dephosphorylation of MLC_{20} was thought to be by an unregulated 'housekeeping' enzyme, until it was recognized that it can be regulated, independently of changes in $[Ca^{2+}]_i$, by G-protein-coupled cascades (Somlyo *et al.* 1989). These and related (Gallagher *et al.* 1997) signal transduction mechanisms play physiologically important roles in both smooth muscles and in non-muscle cells in which cytoplasmic myosin II motors are regulated by phosphorylation/dephosphorylation (Somlyo & Somlyo, 1994). Two proteins involved in such mechanisms are reported in this issue of *The Journal of Physiology*.

An enzyme that can phosphorylate MLC_{20} in the absence of Ca^{2+}–CaM is described by Walsh and co-workers (Weber *et al.* 1999). The activity of this Ca^{2+}-independent kinase in permeabilized smooth muscle is unmasked when the phosphatase is inhibited by microcystin, tautomycin or calyculin. The altered balance between the activities of Ca^{2+}-independent kinase and SMPP-1M increases MLC_{20} phosphorylation, causing contraction in the absence of Ca^{2+}. The myosin kinase responsible has not been identified, although MLCK activated by autophosphorylation in the absence of Ca^{2+} has been a suspect (Tokui *et al.* 1995; Walker *et al.* 1998). Walsh and colleagues now show that, in addition to this weakly active autophosphorylated MLCK, there is also another Ca^{2+}-independent myofibrillar kinase that is neither inhibited by MLCK inhibitors nor a protein kinase C. The physiological role of this 'new' enzyme is uncertain; its activity is low and the Ca^{2+}-independent contractions induced by microcystin are extremely slow. Therefore, unless it can be activated by an as yet unknown messenger, it is unlikely to be a major contractile regulator. It may perform other, possibly important, physiological functions, with its ability to phosphorylate MLC_{20} unmasked only in the laboratory. Answers to this question should come from sequencing and cloning it.

The other paper (Loirand *et al.* 1999) deals with a recently discovered protein that impacts on a major pathway of signal transduction: regulation of SMPP-1M and, through it, myosin II. Activation of a variety of G-protein-coupled excitatory receptors or direct activation of G-proteins by GTPγS or AlF_4^- results in Ca^{2+}-independent inhibition of MLC_{20} dephosphorylation and slowing of relaxation (Kitazawa *et al.* 1991) or, in the presence of MLC_{20} kinase activity, increased MLC_{20} phosphorylation and contraction (reviewed in Somlyo & Somlyo, 1994). The major upstream G-protein activating this Ca^{2+}-sensitizing cascade is the monomeric GTPase RhoA, and the depression of the tonic component of agonist-induced contractions of intact smooth muscle by toxins (bacterial exoenzymes) that inactivate RhoA (Fujihara *et al.* 1997; Lucius *et al.* 1998) indicates the physiological importance of this mechanism. The downstream Ca^{2+}-sensitizing effector of RhoA is Rho kinase, which phosphorylates the regulatory subunit of SMPP-1M and so inhibits the catalytic activity of the enzyme (Kimura *et al.* 1996). The highly selective Rho-kinase inhibitor Y-27632 reverses Ca^{2+} sensitization of smooth muscle (Uehata *et al.* 1997; Fu *et al.* 1998) and reduces blood pressure in hypertensive animals (Uehata *et al.* 1997), further indicating the pathophysiological importance of this pathway.

In resting cells, most RhoA is maintained as inactive cytoplasmic RhoA·GDP complexed with another protein, guanine nucleotide dissociation inhibitor (GDI). The discovery of another GTP-binding protein, Rnd1, that is an antagonist or negative regulator of the effects of RhoA on the cytoskeleton and is hormonally regulated (Loirand *et al.* 1999), was not expected. Rnd1 is a GTP-binding protein without detectable GTPase activity and occurs as Rnd1·GTP associated with the cell membrane through its farnesylated C-terminus. Its absence from the cytosol indicates that, unlike its geranylgeranylated relatives (rac, rho and CDC42), it does not form a complex with GDI. Prenylated Rnd1 inhibits agonist, GTPγS and recombinant RhoA induced Ca^{2+} sensitization of smooth muscle, while non-prenylated Rnd1 has very little or no such effect. Rnd1 inhibits Ca^{2+} sensitization by prenylated RhoA·GTP when added simultaneously, but does not reverse the effect of previously added RhoA, suggesting that inhibition occurs upstream: before or during activation of Rho-kinase. This and the fact that prenylation and, presumably, association with the membrane is required for Rnd1 to inhibit (Loirand *et al.* 1999) and RhoA to activate (Gong *et al.* 1996) the Rho-kinase pathway suggest that they compete for a common hydrophobic binding site. The Rnd1 content of aortic and ileum smooth muscle is increased by treatment with sex steroids, oestrogen and progesterone, and this increase is associated with a decrease in the Ca^{2+}-sensitizing effects of agonists. Sex steroids also increase the concentration of other Ca^{2+}-desensitizing messengers such as cAMP and cGMP, and of telokin, a protein that accelerates MLC_{20} dephosphorylation and relaxation of smooth muscle (Wu *et al.* 1998; Smith *et al.* 1998). These studies also underline the value of smooth muscle as an experimental paradigm of signalling systems operating in both muscle and non-muscle cells and modulating important mechanisms such as cell migration and cytokinesis, propelled by myosin II regulated by kinases and phosphatases. The complexity of these mechanisms is becoming more apparent as their studies delve deeper and deeper, like Alice into the rabbit hole.

Fu, X. *et al.* (1998). *FEBS Letters* **440**, 183–187.

Fujihara, H. *et al.* (1997). *Molecular Biology of the Cell* **8**, 2437–2447.

Gallagher, P. J. *et al.* (1997). *Journal of Muscle Research and Cell Motility* **18**, 1–16.

Gong, M. C. *et al.* (1996). *Proceedings of the National Academy of Sciences of the USA* **93**, 1340–1345.

Hartshorne, D. J. *et al.* (1998). *Journal of Muscle Research and Cell Motility* **19**, 325–341.

Kimura, K. *et al.* (1996). *Science* **273**, 245–248.

Kitazawa, T. *et al.* (1991). *Proceedings of the National Academy of Sciences of the USA* **88**, 9307–9310.

Loirand, G. *et al.* P. (1999). *Journal of Physiology* **516**, 825–834.

Lucius, C. *et al.* (1998). *Journal of Physiology* **506**, 83–93.

Smith, A. F. *et al.* (1998). *American Journal of Physiology* **274**, C1188–1195.

Somlyo, A. P. *et al.* (1989). *Advances in Protein Phosphatases* **5**, 181–195.

Somlyo, A. P. & Somlyo, A. V. (1994). *Nature* **372**, 231–236.

Tokui, T. *et al.* (1995). *Biochemistry* **34**, 5173–5179.

Uehata, M. *et al.* (1997). *Nature* **389**, 990–994.

Walker, L. A. *et al.* (1998). *FASEB Journal* **12**, 813–821.

Weber, L. P. *et al.* (1999). *Journal of Physiology* **516**, 805–824.

Wu, X. *et al.* (1998). *Journal of Biological Chemistry* **273**, 11362–11369.