Calponin and Mitogen-activated Protein Kinase Signaling in Differentiated Vascular Smooth Muscle*  

(Received for publication, May 22, 1997, and in revised form, July 29, 1997)  

Constance B. Menice‡§, Justin Hulvershorn‡, Leonard P. Adam‡§, C.-L. Albert Wang‡, and Kathleen G. Morgan‡¶  

From the ‡Signal Transduction Group, Boston Biomedical Research Institute, Boston, Massachusetts 02114 and the §Cardiovascular Division, Beth Israel Deaconess Medical Center, and Harvard Medical School, Boston, Massachusetts 02215  

Contraction of smooth muscle cells is generally assumed to require Ca\textsuperscript{2+}/calmodulin-dependent phosphorylation of the 20-kDa myosin light chains. However, we report here that in the absence of extracellular calcium, phenylephrine induces a contraction of freshly isolated ferret aorta cells in the absence of increases in intracellular ionized calcium or light chain phosphorylation levels but in the presence of activation of mitogen-activated protein kinase. A protein at 36 kDa co-immunoprecipitated with the mitogen-activated protein kinase and was identified as the actin-binding protein, calponin, by immunoblot. An overlay assay further confirmed an interaction between the kinase and calponin, even though the kinase did not phosphorylate calponin in vitro. Calponin also co-immunoprecipitated from smooth muscle cells with protein kinase C-e. High resolution digital confocal studies indicated that calponin redistributes to the cell membrane during phenylephrine stimulation at a time when mitogen-activated protein kinase and protein kinase C-e are targeted to the plasmalemma. These results suggest a role for calponin as a signaling molecule, possibly an adapter protein, linking the targeting of mitogen-activated protein kinase and protein kinase C-e to the surface membrane.

Although the importance of [Ca\textsuperscript{2+}], and LC20\textsuperscript{1} phosphorylation in the regulation of smooth muscle contraction is recognized, certain incongruous observations have indicated that additional factors may also regulate, particularly, sustained contractions of smooth muscle. During the initiation of smooth muscle contraction, a transient rise in [Ca\textsuperscript{2+}], generally occurs that in turn activates the Ca\textsuperscript{2+}/calmodulin-dependent myosin light chain kinase, causing phosphorylation of LC20 and a consequent increase in myosin ATPase activity and cross-bridge cycling velocity. The increase in intracellular calcium as well as LC20 phosphorylation is usually observed to decline with time during sustained agonist-induced contractions (1–4). An hypothesis has been put forward to explain this dissociation of [Ca\textsuperscript{2+}] and LC20 phosphorylation from force, which suggests that noncycling or slowly cycling cross-bridges termed “latch bridges” are responsible for maintaining force (5); however, it has been difficult to reconcile all reported data with this hypothesis, and the precise mechanism of regulation of latch bridges has been difficult to define. Others have suggested that the maintenance may be regulated by the actin-binding proteins, calponin and caldesmon (reviewed in Ref. 6).

A considerable and growing body of evidence has suggested that factors in addition to the level of LC20 phosphorylation are responsible for regulating sustained contraction in smooth muscle. The involvement of a second kinase, PKC, was first suggested by the observation that phorbol esters, known to activate PKC, induce slow sustained contractions in several types of vascular smooth muscle (4, 7–11). In some cases the phorbol ester-induced contractions were observed in the absence of changes in [Ca\textsuperscript{2+}], or phosphorylation of the myosin light chain kinase sites on LC20 (4, 10, 11). α-Agonist-induced PKC-dependent contractions have also been shown to be, in part, Ca\textsuperscript{2+}-independent in permeabilized ferret aorta cells (12). Studies reported by Khalil et al. (13) have documented a cytosol-to-plasmalemmal translocation of the Ca\textsuperscript{2+}-independent isoform PKC-ε during α-agonist-induced cell shortening in these cells. Ferret aorta cells contain both PKC-ε and PKC-ζ, another Ca\textsuperscript{2+}-independent PKC isoform, but only the addition of exogenous PKC-ε and not PKC-ζ induced contraction in these cells (14). These data suggest that the Ca\textsuperscript{2+}-independent contraction is associated with activation of PKC-ε.

Nonetheless, the signaling pathway by which PKC-ε translocation to the plasma membrane activates the contractile filaments remains unclear. The actin-binding proteins calponin and caldesmon can both be phosphorylated in vitro by PKC, but their direct involvement in the signaling cascade has not been demonstrated (reviewed in Ref. 6). Khalil et al. found MAPK to transiently co-distribute with membrane-associated PKC but then subsequently to be targeted to the contractile apparatus; thus, they suggested that MAPK links PKC activation to the measured contractile activity (15). Reports that caldesmon is phosphorylated during contraction (16) and that a caldesmon peptide antagonist contracted permeabilized cells (17) suggest that caldesmon may play a role in this pathway. Furthermore, the identification of the phosphorylation sites on caldesmon as MAPK sites (18) support a role for MAPK in this signaling cascade. The late targeting of MAPK to the contractile filaments was found to be dependent on tyrosine phosphorylation of MAPK, but the early targeting of MAPK to the plasmalemma occurred independent of tyrosine phosphorylation of MAPK (15), and its mechanism is unknown.

In the studies presented herein we provide evidence that in the absence of extracellular Ca\textsuperscript{2+}, a sustained α-agonist-induced contraction is associated with phosphorylation of a
p46 MAPK, but occurs in the absence of a detectable increase in the level of LC20 phosphorylation. Additionally, we present evidence supporting a role for calponin as a signaling molecule linking the targeting of PKC and MAPK to the plasmalemma.

MATERIALS AND METHODS

**Tissue Preparation**—Ferrets were anesthetized with chloroform in a ventilation hood, and the aorta were quickly removed to an oxygenated physiological saline solution (PSS) (4). The endothelium was removed by gentle abrasion with a rubber policeman. Circular muscle strips were then prepared as described previously (4) and attached to a force transducer. Muscle strips were incubated in PSS for at least 1 h and then challenged with 24 mM KCl PSS (made by replacing NaCl for KCl mole for mole) to test for viability. Muscles were frozen with Freon, precooled in liquid N₂ at the desired time points following agonist stimulation, and then stored at −80 °C until used.

**LC20 Phosphorylation**—Phosphorylated and unphosphorylated forms of the 20,000-Da myosin light chain were determined by two-dimensional polyacrylamide gel electrophoresis according to Jiang et al. (4). After homogenization, samples were transferred to an isoelectric focusing gel in which an 80%-20% mixture of pH 4.4–5.4 and pH 3–10 ampholytes were used. After SDS gel electrophoresis, all gels were processed for staining. Gels were incubated for 30 min in 30% methanol/10% acetic acid, 0.05% bromophenol blue, 0.05% Coomassie blue, and 0.003% 4-fluorobenzylamine. After being dried, the gels were placed in ImageQuant software for image analysis. Myosin phosphorylation levels were calculated by dividing the area of the phosphorylated spot by the area of the phosphorylated plus unphosphorylated spots.

**Calcium Measurements**—Muscle strips were loaded with the bioluminescent protein aequorin by a chemical loading procedure as described previously (2) to measure [Ca²⁺].

**Western Blot Protocol**—Previously frozen tissue samples were homogenized in a buffer containing 50 mM Tris, pH 7.4, 10% glycerol, 5 mM NaF, 20 mM β-glycerophosphate, and 2 μM phenylmethylsulfonyl fluoride. Protein matched samples (modified Lowry protein assay, DC Protein Assay kit, Bio-Rad) were subject to electrophoresis on SDS-polyacrylamide gels and transferred electrophoretically to polyvinylidene difluoride membranes. For each secondary antibody, control lanes were run that are incubated in the absence of primary antibody. Primary antibodies and dilutions were as follows: ERK-1, Upstate Biotechnology Inc. (Lake Placid, NY), ERK2 1:500 (Transduction Laboratories, Lexington, KY), Pan ERK 1:1 250 (Transduction Laboratories), JNK1 1:1000 (Santa Cruz Biotechnology, Santa Cruz, CA), GRB-2 1:500 (Transducer Laboratories), GRB-2 (Santa Cruz), human calponin 1:1000 (Sigma), and Actin 1:1000 (ICN, Costa Mesa, CA). The blots were visualized with Super Signal CL-HRP (Pierce) and mounted with Fluorosave (Calbiochem, San Diego, CA) before analysis.

Images were obtained using a Nikon Diaphot 300 inverted microscope equipped with a Nikon ×100 oil immersion objective (NA 1.3). Filters used were 560 ± 20 nm (excitation), 595 nm (dichroic), and 630 ± 30 nm (emission) for Texas Red. Images were recorded with a liquid cooled CCD camera (Photometrics CH250) via Photometrics Micro-vox Imaging-compatible Image-processing Software (PIMMS™), attached to a MS-DOS-based microcomputer. The digitized images were then processed and analyzed on a SPARC station 5 computer using deconvolution and analysis algorithms written in the Khoros image processing environment and based on Bayesian Maximal Entropy theory, using a priori information as described previously (22). This method gives a resolution of less than 140 nm in the x-y axis.

A modified version of a previously described ratio analysis (22) of fluorescence intensities was performed to determine the relative distribution of various labeled proteins within each cell and to normalize for possible differences in staining between cells. A ratio (R) for a central optical section was calculated (after deconvolution) by determining the mean pixel intensity (total intensity, divided by the number of pixels) of the outer 20% of the cell (surface cortex) and dividing this value by the mean pixel intensity of the remaining area (cell core). The part of the section containing the nuclear area was avoided when calculating R values.

**RESULTS**

**Contraction Occurs in the Absence of Changes in LC20**—As has been reported previously from this laboratory, in the presence of PSS containing 2.5 mM [Ca²⁺], force, [Ca²⁺], and LC20 phosphorylation levels increase in ferret aortic strips at 37 °C (Fig. 1A) upon addition of 10⁻³ M of the α-agonist phenylephrine. A sustained increase in isometric force was observed, whereas the increase in intracellular [Ca²⁺] and LC20 phosphorylation were observed to be largely transient during this time period. Isometric force reached a maximum level by 4 min and remained at this level throughout the period of activation. The resting level of LC20 phosphorylation was 8.2 ± 1.7 mol P/mol protein (n = 5). The level of LC20 phosphorylation reached a maximum of 44 ± 4.9 mol P/mol protein (n = 4) after 1 min of stimulation with 10⁻³ M phenylephrine and declined toward basal levels reaching 16 ± 2.2 mol P/mol protein (n = 6) at 10 min. Following stimulation with 10⁻³ M phenylephrine, the concentration of intracellular ionized calcium rose sharply from a basal level of 179 ± 18.5 nM to 502 ± 42.5 (n = 3) at 30 s, decreased to 225 ± 37.1 nM (n = 3) by 2 min, and decreased to 204 ± 34.6 nM (n = 3) by 10 min.

In contrast to the above results, when force, [Ca²⁺], and LC20 phosphorylation were measured during activation with 10⁻³ M phenylephrine in the absence of [Ca²⁺], (2 mM EGTA) (Fig. 1B), a smaller, sustained increase in force was observed; however, no increase in either [Ca²⁺], or LC20 phosphorylation was observed at any time point. These observations suggest that sustained force induced by phenylephrine in the absence of [Ca²⁺], is regulated by factors other than changes in [Ca²⁺], and LC20 phosphorylation.

The concentration dependence of ferret aorta for phenyleph-
addition to ERK-1 and -2. The polyclonal “ERK-1” antibody was the same antibody previously used by Khalil et al. (15, 26) to demonstrate the biphasic translocation of MAPK in ferret aorta cells. All anti-ERK antibodies detected a p42 and a p44 ERK and possibly a p38 isoform in ferret aorta homogenates. The Pan ERK antibody also detected proteins at 54 and ~87 kDa. Because members of the JNK family of MAPKs are found in the 50-kDa range as well as 46- and 87-kDa ranges, it is possible that some of the bands recognized by the Pan ERK antibody in Fig. 2 are part of the JNK family. Gerthoffer et al. (25) have also reported the presence of 38-, 50-, and 57-kDa proteins in tracheal smooth muscle recognized by a MAPK antibody that are capable of phosphorylating myelin basic protein. If a p46 JNK were present in ferret aorta, it would possibly be obscured by the presence of ERK-1, thus a p46 specific JNK-1 antibody (Santa Cruz) was also used (Fig. 2A), demonstrating that p46 JNK is present in ferret aorta. Additionally, we found that the ERK-1 antibody previously used to image the targeting of MAPK in the ferret aorta also reacts with expressed JNK-1 protein (Santa Cruz) (Fig. 2B). Thus, a number of members of the MAP kinase family are present in this vascular smooth muscle. Exactly what role(s) or specificities can be assigned to each type of MAP kinase remains to be determined.

MAPK activation was monitored by probing Western blots with an anti-phosphotyrosine antibody. Interestingly, in these freshly isolated tissues, in the absence of serum or agonist stimulation, only a single major band at 45–46 kDa was detected (Fig. 2C). This band increased in intensity after addition of phenylephrine. On over-exposed blots, a second band could also be detected at p38. Stimulation with a phorbol ester produced no greater signal at p46 but an increased signal at 38 kDa (Fig. 2C), and bands at higher molecular masses appeared. These data clearly show, however, that the major band tyrosine phosphorylated in response to the α-agonist is at 46 kDa, presumably either ERK-1 or JNK-1 MAPK.

**Calponin and MAPK Co-immunoprecipitate**—Immunoprecipitation of MAPK was performed using the polyclonal ERK-1 antibody that was previously used to demonstrate the transient plasmalemmal translocation followed by contractile filament targeting of MAPK in these cells (15). MAPK was identified as immunoprecipitating at 46 kDa by Western blot of the immunoprecipitate using the same ERK-1 antibody (Fig. 2D). The major protein occurring at 36 kDa was identified by Western blot as calponin, an actin-binding protein (Fig. 2D). As a negative control, an antibody to GRB-2 was also used in immunoprecipitation experiments on ferret aorta whole cell homogenate. The antibody recognizes and immunoprecipitates GRB-2 from these samples, but no co-immunoprecipitating calponin is detectable, supporting the specificity of the interaction between calponin and MAPK.

No detectable increase in calponin co-immunoprecipitating with MAPK was seen during agonist stimulation, but at 10 min after the addition of PE, the amount of calponin in the immunoprecipitates decreased to an average of 24% of the control level (n = 4). Actin was also identified in the MAPK immunoprecipitates; raising the question of whether calponin was directly binding to MAPK or indirectly, by binding to actin in the immunoprecipitates.

**PKC-e Immunoprecipitates with Calponin**—Even though PKC-e and MAPK appear to co-localize on high resolution fluorescence micrographs after 4 min of stimulation of tissue with PE (15), we were unsuccessful in detecting PKC-e in the MAPK immunoprecipitates, which suggests that they do not directly interact. Therefore, immunoprecipitation with an anti-PKC-e antibody after 4 min of stimulation with phenylephrine was performed on ferret aorta homogenates to further investi-
gate whether PKC-ε associates with MAPK. MAPK was not observed in the immunoprecipitate, but again calponin was determined to be present by Western blot (data not shown), and the amount of calponin in the immunoprecipitate increased by 2 min to an average of 456% of the unstimulated levels (n = 3).

Calponin Directly Binds Both MAPK and PKC-ε—To confirm that calponin binds directly to MAP kinase rather than simply being associated with actin in the immunoprecipitates, we performed an overlay assay using ERK-1K67R kinase. As shown in Fig. 2E, MAPK binds directly to isolated purified calponin, actin, caldesmon, and, as a positive control, myelin basic protein. Additionally, a band at the molecular mass of CaP in whole cell homogenates (Fig. 2E, arrow) also bound MAPK. As a negative control, MAPK did not bind to calmodulin (data not shown). These data indicate that the calponin can directly bind MAPK and argue against its being simply a contaminant in the immunoprecipitate. Additionally, the binding of MAPK to actin directly in the overlay assay is consistent with the targeting of MAPK to contractile filaments in situ.

Calponin has previously been reported to be a substrate of PKC-ε (14); however, an interaction between CaP and MAPK has not been previously reported. Therefore, we tested whether CaP could be used as a substrate by MAPK and found that it was not significantly phosphorylated (Fig. 2F). In contrast, under identical conditions, using one-third the concentration of caldesmon, phosphorylation by MAPK was readily detectable (Fig. 2F). These findings are consistent with there being no (S/T)P sequences in CaP.

Calponin Is Targeted to the Plasmalemma with MAPK and PKC-ε in Intact Cells—Calponin has previously been reported to undergo a dynamic subcellular redistribution during contraction/relaxation cycles in ferret portal vein cells (22), but it is not known if this occurs in other cell types. To determine if calponin is in the appropriate spatio-temporal location to interact with MAPK and PKC-ε in the intact ferret aorta cell, high resolution digital imaging studies were performed using a monoclonal antibody to calponin. In resting cells (Fig. 3A, top) calponin was distributed on filamentous bundles in the core of the cell. Following 2 min of stimulation with an α-agonist, calponin began to redistribute toward the subplasmalemmal cell cortex. After 4 min (Fig. 3A, middle) of stimulation with

![Image](image-url)

**Fig. 2.** A, immunoblots for MAPK in ferret aorta. Whole tissue homogenates were probed with MAPK using ERK-1, ERK2, Pan ERK, and JNK1 antibodies. B, immunoblots using an ERK-1 antibody against whole cell ferret aorta homogenates and recombinant JNK1 protein. C, immunoblots for phosphotyrosine in ferret aorta whole cell homogenates. Tissue strips were incubated for 30 min in the absence of [Ca²⁺], and then frozen in the absence of stimulation or in the presence of 10⁻⁵ m phenylephrine for 4 min or 3 × 10⁻⁶ m 12-deoxyphorbol 13-isobutyrate 20-acetate until steady state contraction was reached. D, immunoblots of ferret aorta tissue homogenates immunoprecipitated with an anti-ERK-1 antibody. Immunoprecipitates were immunoblotted with antibodies to ERK-1, CaP, and actin. E, overlay assay. Whole cell homogenates and pure proteins (calponin, myelin basic protein, actin, and caldesmon) were incubated with recombinant ERK-1 then immunoblotted with an anti-ERK-1 antibody to determine which proteins bound ERK-1. F, *in vitro* phosphorylation with MAPK. Caldesmon and calponin were phosphorylated by purified MAPK (ERK-1) that was activated by constitutively active GST-MEK.

![Image](image-url)

**Fig. 3.** A, cells were fixed at rest (top), or stimulated with phenylephrine (10⁻⁵ m) for 4 (middle) or 10 (bottom) min before fixation. Cells were labeled with anti-calponin antibody (Sigma) and imaged as described under “Materials and Methods.” B, quantitative time course of CaP translocation. C, comparison of CaP translocation time course to that of PKC epsilon (●), MAPK (○), calponin (□), or shortening of cells (□).
phenylephrine, calponin redistributed to the submembranous cortex where it remained through 10 min of stimulation (Fig. 3A, bottom). These results were quantitated (Fig. 3B) by the measurement of a surface-to-cytosolic ratio for the distribution of fluorescently labeled CaP, as described previously (22). In Fig. 3C, the time course of the redistribution CaP is compared with that of MAPK and PKC-ε and cell shortening as previously determined for this cell type (26). These data show that through 4 min of stimulation, calponin, MAPK, and PKC-ε follow an indistinguishable time course for targeting to the plasmalemma.

**DISCUSSION**

In the present study we have demonstrated that in the absence of a change in [Ca\(^{2+}\)] or LC20 phosphorylation, phenylephrine can cause a contractile response in ferret aorta smooth muscle. Others have suggested that inhibition of LC20 phosphatase can allow agonist-induced contraction of smooth muscle to occur in the absence of changes in [Ca\(^{2+}\)], (27). However, inhibition of the phosphatase would result in sustained increases of LC20 phosphorylation, and if this were the mechanism of the contraction, contractile force should parallel LC20 phosphorylation levels. Clearly, the results reported here are not consistent with such a mechanism. The fact that the EC\(_{50}\) for phenylephrine in the presence of [Ca\(^{2+}\)], was determined to be significantly different from the EC\(_{50}\) for phenylephrine in the absence of [Ca\(^{2+}\)], is consistent with there being two different mechanisms involved in the presence and absence of [Ca\(^{2+}\)]. In the presence of Ca\(^{2+}\), activation of myosin light chain kinase is expected to contribute to the generation of tone, but in the absence of Ca\(^{2+}\), other mechanisms must be involved. Past studies have implicated PKC-ε and MAPK in this alternative pathway (6), and this is consistent with the detection of MAPK activation under these Ca\(^{2+}\)-free conditions (Fig. 2C).

The signaling pathways involving MAP kinase in differentiated nonproliferating cells, such as contractile smooth muscle, are less well understood than those in proliferating cells. This laboratory (15) has previously reported that phenylephrine-induced Ca\(^{2+}\)-independent contraction of ferret aorta cells is completely abolished by PKC inhibitors and significantly inhibited by tyrosine kinase inhibitors suggesting that PKC activation and tyrosine phosphorylation of MAPK are involved in a signaling cascade leading to Ca\(^{2+}\)-independent smooth muscle contraction. The signals that link PKC activation to MAP kinase activation in differentiated smooth muscle remain unclear. Khalil et al. (15) described an initial translocation of MAPK to the membrane after 4 min of phenylephrine stimulation. This translocation was totally inhibited by PKC antago-

**REFERENCES**

1. Dillon, P. P., Akey, M. O., Driika, S. P., and Murphy, R. A. (1981) Science 211, 495–497
2. Morgan, J. P., and Morgan, K. G. (1982) Pflegers Arch. Eur. J. Physiol. 395, 75–77
3. Rembold, C. M. (1986) Am. J. Physiol. 253, C719–C723
4. Jiang, M. J., and Morgan, K. G. (1989) Pflegers Arch. Eur. J. Physiol. 413, 637–643
5. Hai, C.-M., and Murphy, R. A. (1989) Annu. Rev. Physiol. 51, 285–298
6. Horowitz, A., Menice, C. B., Laporte, R., and Morgan, K. G. (1996) Physiol. Rev. 76, 967–1003
7. Danthuluri, N. R., and Deth, R. C. (1984) Biochem. Biophys. Res. Commun. 125, 1103–1109
8. Chatterjee, M., and Tejada, M. (1986) Am. J. Physiol. 251, C356–C361
9. Rasmussen, H., Forder, J., Kojima, I., and Scariabe, A. (1984) Biochem. Biophys. Res. Commun. 122, 776–784
10. Morgan, J. M., and Morgan, K. G. (1987) Am. J. Physiol. 253, H1365–H1371
11. Singer, H. A., and Baker, K. M. (1987) J. Pharmacol. Exp. Ther. 243, 841–821
12. Collin, E., Walsh, M. P., and Morgan, K. G. (1992) Am. J. Physiol. 262, H754–H762
13. Khalil, R. A., Lajoie, C., Resnick, M. S., and Morgan, K. G. (1992) Am. J. Physiol. 263, C714–C719
14. Horowitz, A., Clement-Chomienne, O., Walsh, M. P., and Morgan, K. G. (1996) Am. J. Physiol. 271, C589–C594
15. Khalil, R. A., Menice, C. B., Wang, C.-L. A., and Morgan, K. G. (1995) Circ. Res. 76, 1101–1108
16. Adam, L. F., Haerberle, J. R., and Hathaway, D. R. (1989) J. Biol. Chem. 264, 7698–7703
17. Katsumura, H., Wang, C.-L. A., and Morgan, K. G. (1992) J. Biol. Chem. 267, 14555–14558
18. Adam, L. P., and Hathaway, D. R. (1993) FEBS Lett. 322, 56–60
19. Wu, J., Rossomando, A. J., Her, J.-H., Veecho, R. D., Weber, M. J., and Sturgill, T. W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9508–9512
20. Roos, A., and Morgan, K. G. (1994) J. Biol. Chem. 269, 25766–25772
21. Alesi, D. R., Saito, Y., Campbell, D. G., Cohen, P., Sithanandam, G., Rapp, U., Ashworth, A., Marshall, C. J., and Cowley, S. (1994) EMBO J. 13, 1610–1619
22. Parker, C. A., Takahashi, K., Tao, T., and Morgan, K. G. (1994) Am. J. Physiol. 267, C1162–C1170
23. Ruycke, A. L., and Morgan, K. G. (1989) Br. J. Pharmacol. 97, 391–400
24. Khalil, R. A., Lajoie, C., Resnick, M. S., and Morgan, K. G. (1992) Am. J. Physiol. 263, C714–C719
25. Gerthoffer, W. T., Yamboiley, I. A., Shearer, M., Pohl, J., Haynes, R., Sang, S., Sato, K., and Seligers, J. R. (1986) J. Physiol. 403, 557–609
26. Khalil, R. A., and Morgan, K. G. (1993) Am. J. Physiol. 265, C460–C461
27. Somlyo, A. P., and Somlyo, A. V. (1994) Nature 372, 231–236
28. Horowitz, A., Clement-Chomienne, O., Walsh, M. P., Tao, T. Katsumura, H., and Morgan, K. G. (1996) Am. J. Physiol. 270, H1585–H1603
29. Ishi, T., Suzuki, A., Watanebe, Y., Min, I., Naka, M., and Tanaka, T. (1995) J. Biol. Chem. 270, 20400–20403
30. Mabuchi, K., Li, Y., Tao, T., and Wang, C.-L. A. (1996) J. Muscle Res. Cell Motil. 17, 1–18