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Physiologic properties and regulation of the actin cytoskeleton in vascular smooth muscle

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Abstract

Vascular smooth muscle tone plays a fundamental role in regulating blood pressure, blood flow, microcirculation, and other cardiovascular functions. The cellular and molecular mechanisms by which vascular smooth muscle contractility is regulated are not completely elucidated. Recent studies show that the actin cytoskeleton in smooth muscle is dynamic, which regulates force development. In this review, evidence for actin polymerization in smooth muscle upon external stimulation is summarized. Protein kinases, such as Abl, FAK, Src, and MAP kinase, have been documented to coordinate actin polymerization in smooth muscle. Transmembrane integrins have also been reported to link to signaling pathways modulating actin dynamics. The roles of Rho family of the small GTPases and the actin-regulatory proteins including CAS, N-WASP, the Arp2/3 complex, profilin, and heat shock proteins in regulating actin assembly are discussed. These new findings promote our understanding on how smooth muscle contraction is regulated at cellular and molecular levels.

Keywords

Vascular smooth muscle; actin cytoskeleton; contraction

Introduction

Vascular smooth muscle tone plays a fundamental role in regulating blood pressure, blood flow, microcirculation, and other cardiovascular functions. Abnormal smooth muscle contraction may result in the development of many diseases such as primary hypertension, ischemic heart disease, pulmonary hypertension, and stroke.

Smooth muscles exhibit a unique capability to contract and relax in response to changes in the environment surrounding them. Smooth muscles may adapt their contractile status by remodeling cellular structures and by altering signaling cascades regarding contractile element activation.

The contractile apparatus of smooth muscle is comprised of thin filaments containing actin and thick filaments containing myosin. Actin filaments of smooth muscle cells connect with dense plaques on the membrane, and link to dense bodies in the myoplasm. The majority of actin filaments localize around thick filaments in a hexagonal array, forming the contractile apparatus. This pool of actin filaments is called “contractile actin”. Part of actin filaments is not structurally associated with myosin. It is called “cytoskeletal actin”, which plays a role in maintaining structural integrity of smooth muscle cells (Fig. 1). The detailed structure of smooth muscle cells has previously been reviewed¹⁻³.

A wealth of evidence has been documented to suggest that the actin cytoskeleton of smooth muscle undergoes a dynamic remodeling process, which is a key component of the structural adaptation upon changes in chemical and mechanical environments^{4, 5}. In this article, we will review our recent understanding on the role and regulation of the actin cytoskeleton system in smooth muscle.

Physiologic properties of the actin cytoskeleton in arterial smooth muscle

Actin filament polymerization occurs in response to contractile and mechanical stimulation

The actin cytoskeleton in differentiated smooth muscle cells and tissues has long been thought to be a fixed structure. Recent studies have documented that the actin architecture of smooth muscle is in a dynamic state; polymerization-depolymerization of actin filaments participates in the contraction-relaxation cycle of smooth muscle. Studies on carotid smooth muscle have shown that the ratio of filamentous (F) actin to globular (G) actin is approximately 1.8 in unstimulated arterial smooth muscle tissues, suggesting that approximately 25-30% of total actin exists in the form of actin monomers^{6, 7}. The F-actin/G-actin ratio increases to 5.8 in response to contractile stimulation, indicating that approximately 10-15% of total actin is G-actin. The F-actin/G-actin ratio decreases to basal level during the relaxation process.

The dynamic feature of the actin cytoskeleton has also been observed in other studies; the amount of actin monomers is decreased, or the fraction of filamentous actin is increased during contractile stimulation⁸⁻¹⁶. In cultured airway smooth muscle cells, treatment with carbachol and endothelin-1 leads to the increase in the ratio of F-actin to G-actin as estimated by cytofluorescence analysis, whereas treatment with smooth muscle relaxant (isoproterenol and forskolin) decreases the F-actin/G-actin ratio^{17, 18}. Consistent with these findings, Barany and colleagues¹⁹ have reported a high concentration of G-actin in vascular smooth muscle tissues by assessing the exchange rates of actin-bound nucleotide, and observed a decrease of the G-actin pool in response to contractile stimulation. These studies demonstrate that actin filament assembly occurs in various smooth muscle cell/tissue types, which may be a unique phenomenon in smooth muscle in response to contractile activation.

Changes in mechanical environments also affect actin filament reorganization. A single stretch imposed on the cell membrane alters the stiffness of the cell, which is intimately associated with the status of the actin cytoskeleton^{20, 21}. Passive tension also induces the tyrosine phosphorylation of the cytoskeletal protein paxillin, an index of the actin cytoskeleton reorganization^{8, 22, 23}. Furthermore, mechanical signals locally imposed on cells triggers structural reorganization of the actin architecture in smooth muscle cells^{4, 5, 24}.

Actin polymerization is believed to occur at the barbed end of actin filaments, which is located near the membrane of smooth muscle cells. At the barbed end, globular (G) actin is added onto existing filamentous (F) actin. This process is regulated by various actin associated proteins (See below). Actin depolymerization takes place at the point end of actin filaments, which positions the opposite of the barbed end (Fig. 1)^{1, 10, 12, 13, 25-27}.

The dynamic feature of actin filaments in smooth muscle is markedly distinctive as compared to striated muscle; a significant G-actin pool is not detected and the rate of actin-bound nucleotide exchange is very low in skeletal muscle^{19, 28}. Smooth muscle tissues also contain a much higher ratio of F-actin to myosin than skeletal muscle, and in contrast to skeletal muscle, they maintain a significant pool of F-actin that does not interact with myosin^{2, 29}.

Actin filament assembly participates in contractile force development

There is compelling evidence that the depression of actin polymerization by such inhibitors as cytochalasin or latrunculin attenuates force development in a variety of smooth muscle tissues

6-8, 30-32. The short-term treatment of smooth muscle with these inhibitors does not impair the actin cytoskeleton; no differences in contractile filament organization or ultrastructure are detected in smooth muscle strips treated with cytochalasin or latrunculin as compared to control tissues⁸. Latrunculin binds to actin monomers and prevents their assembly onto actin filaments whereas cytochalasin caps existing actin filaments, preventing their extension at the barbed end^{8, 33, 34}. More importantly, the depression of actin polymerization disrupts force development in smooth muscle with little or no effects on myosin phosphorylation, a cellular event that is crucial for smooth muscle contraction^{6, 8, 10}. These studies suggest: 1) new actin polymerization *per se* is a necessary step in the cellular process for force development; 2) actin filament assembly and myosin phosphorylation are independent cellular events; and 3) both actin filament polymerization and myosin activation are required for smooth muscle contraction.

There are several possibilities that actin polymerization may affect force development. First, the actin filaments of smooth muscle cells connect to the membrane at the membrane-associated dense plaques, which are structurally similar to focal adhesion sites of cultured cells. At these structures, the cytoplasmic domain of β integrins associates with linker proteins such as vinculin and talin that in turn attach to actin filaments. The extracellular portion of β integrins engages with extracellular matrix^{1, 2, 35, 36}. Thus, the membrane-associated dense plaques have been believed to mediate mechanical force transmission between actin filaments to extracellular matrix^{1, 4, 36}. Recent studies have shown that actin polymerization is initiated by the Arp2/3 (Actin Related Protein) complex in non-muscle cells as well as in smooth muscle, indicating that nascent actin polymerization may occur at cell cortex^{10, 12, 13, 37, 38}. Cortical actin assembly may strengthen the linkage of actin filaments to integrins and enhance the transmission of contractile force^{1, 4, 5, 10, 12, 13, 27, 35, 36, 39-42}.

Second, actin assembly has been shown to increase the number of contractile units and the length of actin filaments, providing more and efficient contractile elements for force development^{31, 43-46}. Third, newly polymerized filaments may be a part of reorganization processes that allow for rapid adjustment of stiffness and tension^{1, 4, 5, 10, 12, 13, 35, 47-51}. Fourth, actin filament assembly may participate in the “latch” formation of contractile elements, supporting force maintenance under the condition of lower crossbridge phosphorylation^{15, 16, 35, 52-54}.

Cellular processes regulating actin dynamics in smooth muscle

In the last several years, considerable efforts have been made from a number of laboratories to explore how actin filament assembly is regulated in smooth muscle. Thus far protein kinases, such as Abelson tyrosine kinase (Abl), focal adhesion kinase (FAK), Src, mitogen-activated protein (MAP) kinase and other kinases, have been documented to coordinate actin polymerization in smooth muscle. Transmembrane integrins have also been reported to link to signaling pathways modulating the actin cytoskeleton. Rho, Cdc42, and Rac are the major members of Rho family of the small GTPases that mediates actin dynamics in smooth muscle. The actin-regulatory proteins are effector molecules in the signaling cascades to mediate actin dynamics. Some of the proteins are neuronal Wiskott-Aldrich syndrome Protein (N-WASP), the Arp2/3 complex, profilin, cofilin, and heat shock proteins. In general, receptor activation and/or integrin ligation activates protein kinases and/or small GTPases, which in turn regulate the functional status of the actin regulatory proteins and eventually actin filament assembly or structural reorganization (Fig. 2).

Role of CAS-mediated process in actin filament assembly in smooth muscle

Crk-associated substrate (CAS) is a 130-kDa focal adhesion protein that was originally identified as a prominent tyrosine-phosphorylated protein in v-src and v-crk transformed cells

55, 56. Molecular analysis of CAS revealed a docking protein that contains an SH3 domain, proline-rich regions, and a substrate domain containing multiple Tyr-Xaa-Xaa-Pro (YXXP) 56, 57. CAS has been shown to regulate the actin cytoskeleton in smooth muscle. The downregulation of CAS by antisense oligonucleotides dramatically attenuates force development and actin polymerization in response to contractile stimulation⁷. CAS undergoes tyrosine phosphorylation in vascular smooth muscle cells in response to stimulation with angiotensin II and serotonin^{58, 59} as well as in non-muscle cells including rat-1 cells, COS-7 cells, and CE cells in response to growth factors and cell adhesion^{57, 60, 61}. Phosphorylation of CAS increases its binding to the adapter protein CrkII^{25, 57}. In smooth muscle, contractile stimulation leads to the increase in the association of CrkII with N-WASP. When unstimulated, the C-terminal portion of N-WASP binds to its GTP-binding domain, masking its binding motif for the Arp2/3 complex. The interaction of CrkII with N-WASP induces conformational changes, exposing the binding motif for the Arp2/3 complex and initiating actin polymerization and branching mediated by the Arp2/3 complex^{10, 12, 13, 25, 27, 39, 62}.

Profilin is an actin-associated protein that regulates actin dynamics, the organization of actin filaments and smooth muscle force development. *In vitro* studies have shown that profilin binds to actin monomers and transport monomers onto the barbed end of actin filaments. Profilin may also promote the nucleation of actin filament polymerization mediated by N-WASP and the Arp2/3 complex^{63, 64}. In carotid smooth muscle preparations, contractile activation promotes the association of profilin with G-actin. Moreover, the downregulation of CAS blocks the formation of profilin/G-actin coupling, actin polymerization and tension development. These results suggest that CAS may regulate actin dynamics by affecting the activity of profilin^{6, 7, 59}.

The tyrosine phosphorylation of CAS is mediated by Abl, a non-receptor tyrosine kinase. Studies from *in vitro* biochemical assay showed that CAS phosphorylation is catalyzed directly by Abl^{25, 65}. Silencing of Abl by short hairpin RNA dramatically depresses CAS phosphorylation in resistance arteries upon contractile stimulation^{25, 59}. Furthermore, Abl in vascular smooth muscle is activated by agonist stimulation; the phosphorylation of Abl at Tyr-412 (an index of kinase activation) is enhanced in stimulated arteries compared to unstimulated tissues²⁵. Abl tyrosine phosphorylation has also been reported in vascular smooth muscle cells upon angiotensin II stimulation⁶⁶. Interestingly, a selective inhibitor of Abl, imatinib (STI-571), has been recently shown to be effective for the treatment of pulmonary hypertension in clinical studies^{67, 68}.

FAK and Src family tyrosine kinase mediated cytoskeletal remodeling

FAK and c-Src are located at the integrin-associated membrane microdomain and are activated by integrin clustering as well as activation with contractile agonists and growth factors^{22, 23, 69-80}. FAK has been shown to catalyze phosphorylation of the cytoskeletal protein paxillin *in vitro* and in airway smooth muscle tissues^{23, 69, 81-83}. Although similar studies on vascular smooth muscle have not been pursued, it is generally believed that vascular and airway smooth muscles share similar intracellular regulatory mechanisms. Paxillin also undergoes tyrosine phosphorylation in vascular smooth muscle preparations during agonist stimulation¹⁶. Paxillin may be involved in the regulation of N-WASP that mediates actin dynamics and/or structural re-array of the actin cytoskeleton in various cell types including smooth muscle cells^{10-12, 16, 22, 35, 81, 84-87}.

c-Src has been reported to mediate CAS phosphorylation in vascular smooth muscle; inhibition of Src activity attenuates CAS tyrosine phosphorylation upon agonist activation⁵⁹. As described earlier, CAS phosphorylation regulates actin filament assembly mediated by the Arp2/3 complex. Src may modulate CAS phosphorylation by affecting the activity of Abl^{25, 66}. However, other studies suggest that Src may be responsible for integrin-mediated tyrosine

phosphorylation of CAS^{88, 89}. In addition, the activated c-Src interacts with FAK to mediate the regulation of basal Ca²⁺ channel activity and the platelet-derived growth factor (PDGF)-induced increase in L-type Ca²⁺ current^{4, 90}.

Integrin-mediated signaling cascades in the modulation of actin dynamics

Transmembrane integrins are cell surface receptors that have been shown to serve as mechanical sensors in cells. In smooth muscle cells, integrins are located in membrane-associated dense plaques that are structurally analogous to focal adhesions of cultured cells^{1, 4, 22}. As discussed earlier, β -integrins interact with extracellular matrix on the outside of the membrane and link to actin filaments in the inside of the membrane. The structural linkage of matrix to the actin cytoskeleton by integrins facilitate mechanical force transmission between the contractile apparatus and extracellular matrix^{1, 4, 22, 23, 35, 50}.

Integrins are able to sense mechanical signals in the environment, and to transduce the signals onto intracellular pathways that regulate remodeling of the actin cytoskeleton. FAK, c-Src, protein tyrosine kinase 2 (Pyk2), CAS, and paxillin are associated with integrins on the membrane. FAK is activated by autophosphorylation at Tyr-397 in response to ligand binding to integrins^{4, 91, 92}. Both FAK and c-Src become phosphorylated in nonmuscle cells (e.g. endothelial cells and leukocytes) stimulated by adhesion-mediated integrin activation^{4, 91-94}. Integrin activation may also stimulate FAK and c-Src in smooth muscle; the tyrosine phosphorylation of FAK and paxillin is mechanosensitive (integrin is a known mechanosensor) in smooth muscle^{22, 23}. Cyclic strain activates Pyk2 and FAK phosphorylation at focal adhesion sites in cells⁹⁵. The integrin-mediated activation of tyrosine kinases in turn modulates the functional status of downstream molecules such as paxillin and Hic-5 that are related to remodeling of the actin architecture^{4, 5, 41, 94, 95}.

Conversely, the reorganization of the actin cytoskeleton may influence the clustering of integrins and engagement of integrins with extracellular matrix. There is evidence to show that stress fiber formation affects the aggregation of integrins on the membrane⁹⁶. Treatment of smooth muscle cells with contractile agonists leads to the increase in resistance of integrin-bound beads, indicating enhancement of the matrix-integrin association^{4, 20}. The “inside-out” signaling may provide a positive feedback to dense plaques, render integrins poised to respond to next mechanical signals.

The integrin-activated FAK and c-Src have also been linked to the regulation of Ca²⁺ signaling in differentiated smooth muscle cells. The inhibition of c-Src attenuates the increase in Ca²⁺ current activated by α_5 -integrin antibody or fibronectin whereas the inhibition of tyrosine phosphatase enhances the integrin-mediated intracellular Ca²⁺⁹⁷. The integrin-stimulated enhancement of Ca²⁺ current is also depressed when cells are dialyzed with antibodies against FAK or c-Src^{4, 97}. In addition to activation of the contractile protein myosin, the FAK/Src-mediated increase in intracellular Ca²⁺ may also activate protein tyrosine kinase 2 (PYK2) that is able to catalyze CAS and/or paxillin phosphorylation and regulates the actin cytoskeleton^{4, 69, 95, 97-102}.

Small GTPases and actin architecture in smooth muscle

The Rho family of the small GTPases consists primarily of Rho, Cdc42, and Rac. Rho may mediate agonist-induced actin stress fiber formation in cultured smooth muscle cells. Treatment with C3 exoenzyme (inactivator of Rho) diminished the agonist-induced increase in F/G-actin ratios as estimated by cytofluorescence microscopy. This was reported to be mediated by G_q and G_i in smooth muscle cells^{5, 17, 103}. Rho may also regulate actin cytoskeleton remodeling via the Rho kinase/LIM kinase/cofilin pathway. Cofilin is an actin-regulatory protein that triggers actin filament depolymerization; phosphorylation of cofilin by

LIM kinase inhibits its ability to depolymerize actin filaments. Rho kinase induces the phosphorylation of LIM kinase, which enhances LIM kinase activity towards cofilin and promotes cofilin phosphorylation and actin filament assembly¹⁰⁴⁻¹⁰⁷. Rho may also modulate actin dynamics by affecting Mammalian Diaphanous-related (mDia) formins that nucleate, progressively elongate and bundle actin filaments¹⁰⁸⁻¹¹⁰. Further studies are needed to assess whether the Rho-mediated actin dynamics occurs in intact vascular smooth muscle tissues.

There is a wealth of evidence that Cdc42 regulates actin filament polymerization in various cell types including smooth muscle cells^{12, 111-115}. GTP bound form of Cdc42 (activated Cdc42) binds to the GTP binding domain of N-WASP, inducing a conformational change and activating N-WASP, and triggering the nucleation of actin polymerization and actin filament branching^{10, 12, 27, 40, 116}. In smooth muscle tissues, introduction of a dominant Cdc42 mutant depresses the activity of N-WASP concurrently with the decrease in actin polymerization and force development^{10, 12}, suggesting that Cdc42-mediated actin filament assembly is essential for smooth muscle contraction.

Cdc42 and Rac are known to activate p21-activated kinase (PAK) in mammalian cells¹¹⁷⁻¹²¹. Although 6 isoforms have been found thus far, PAK1 is a major isoform in smooth muscle cells^{117-119, 119, 120, 122, 123}. PAK has been shown to participate in the regulation of p38 MAP kinase in smooth muscle upon activation with the growth factor¹¹⁷. The activation of p38 MAP kinase may modulate the actin cytoskeleton and cell migration via heat shock protein (HSP)^{117, 124, 125}(See below).

The small GTPases are activated by contractile agonists as well as integrin aggregation. Cdc42 activation has been observed in smooth muscle tissues in response to contractile stimulation^{10, 12}. Chemical stimulation also induced Rho activation in vascular smooth muscle tissues¹²⁶⁻¹²⁸. Attachment of cells to fibronectin leads to activation of both Cdc42 and Rac. Rho is also activated by integrin ligation, resulting in the formation of stress fiber⁹⁶. In addition, cyclic strain induces reorganization of actin stress fibers, which may be mediated by the small GTPase Rho^{26, 129}.

Role of small heat shock proteins in smooth muscle

In addition to providing protection against physical and chemical stresses, HSP27 (heat shock protein 27) may participate in the regulation of the actin cytoskeleton^{4, 5}. *In vitro* biochemical studies have shown that unphosphorylated HSP25 (mouse and chicken homologs of HSP27) inhibits actin polymerization as assessed by fluorescence spectroscopy and electron microscopy. Phosphorylated HSP27 loses the ability to inhibit actin filament assembly^{4, 129-131}. Signaling cascades regulating HSP27 phosphorylation has been documented. In non-muscle cells, HSP27 is phosphorylated by MAP kinase-activated protein (MAPKAP) kinase 2 (MK2) that is activated by phosphorylation mediated by p38 MAP kinase. Upstream regulators of p38 MAP kinase includes PAK and other kinases^{4, 5}. Smooth muscle seems to share similar signaling pathways to phosphorylate HSP27 during contractile activation^{4, 124}.

HSP27 is thought to regulate the actin cytoskeleton in differentiated smooth muscle because of high-level expression of the protein in smooth muscle and spatial localization with contractile proteins^{4, 132}. There is evidence to show that HSP27 is associated with the cellular processes that orchestra smooth muscle contraction. In permeabilized smooth muscle preparations, agonist-induced contraction was inhibited by antibodies against HSP27^{5, 133, 134}. Moreover, agonist activation induced HSP27 phosphorylation in smooth muscle tissues, which was blocked by a p38 MAP kinase inhibitor. The depression of HSP27 phosphorylation inhibited angiotensin II-induced vasoconstriction⁴.

Another heat shock protein HSP20 was reported to be phosphorylated at Ser-16 in vascular smooth muscle tissues by the treatment with forskolin. Forskolin is known to increase the cellular concentration of cAMP that is able to activate protein kinase A (PKA). HSP20 phosphorylation was associated with the decrease in force development in vascular smooth muscle. However, phosphorylation of HSP20 seems not to depolymerize filamentous actin in swine carotid artery. Further studies are needed to elucidate the role of HSP20 phosphorylation in the actin cytoskeleton and smooth muscle contraction ^{4, 15}.

Perspectives

Understanding the molecular mechanisms by which chemical and mechanical stimuli initiate tension development in vascular smooth muscle may disclose new biological targets for the development of more effective treatment of cardiovascular diseases. Actin filament polymerization has recently emerged as an essential cellular event that substantially regulates force development in vascular smooth muscle. A pool of G-actin polymerizes onto existing actin filaments in smooth muscle in response to chemical and mechanical stimulation. There is compelling evidence that the inhibition of actin assembly depresses smooth muscle contraction. Changes in chemical and mechanical environments surrounding smooth muscle activate several important signaling pathways that involve protein kinases, integrins, small GTPases, and actin-associated molecules. These pathways are critical for the regulation of actin dynamics and force development; intervention of the signaling cascades affects smooth muscle contraction.

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References

1. Gunst SJ, Tang DD. The contractile apparatus and mechanical properties of airway smooth muscle. *Eur Respir J* 2000 April;15(3):600–16. [PubMed: 10759460]
2. Small JV. Structure-function relationships in smooth muscle: the missing links. *Bioessays* 1995 September;17(9):785–92. [PubMed: 8763831]
3. Small JV, Gimona M. The cytoskeleton of the vertebrate smooth muscle cell. *Acta Physiol Scand* 1998 December;164(4):341–8. [PubMed: 9887957]
4. Gerthoffer WT, Gunst SJ. Invited review: focal adhesion and small heat shock proteins in the regulation of actin remodeling and contractility in smooth muscle. *J Appl Physiol* 2001 August;91(2):963–72. [PubMed: 11457815]
5. Gerthoffer WT. Actin cytoskeletal dynamics in smooth muscle contraction. *Can J Physiol Pharmacol* 2005 October;83(10):851–6. [PubMed: 16333356]
6. Tang DD, Tan J. Downregulation of profilin with antisense oligodeoxynucleotides inhibits force development during stimulation of smooth muscle. *Am J Physiol Heart Circ Physiol* 2003 June 12;285:H1528–H1536. [PubMed: 12805028]
7. Tang DD, Tan J. Role of Crk-Associated Substrate in the Regulation of Vascular Smooth Muscle Contraction. *Hypertension* 2003 July 28;42:858–63. [PubMed: 12885796]
8. Mehta D, Gunst SJ. Actin polymerization stimulated by contractile activation regulates force development in canine tracheal smooth muscle. *J Physiol* 1999;519(Pt 3):829–40. [PubMed: 10457094]
9. Tang DD, Turner CE, Gunst SJ. Expression of non-phosphorylatable paxillin mutants in canine tracheal smooth muscle inhibits tension development. *J Physiol* 2003 August 29;553(1):21–35. [PubMed: 12949231]
10. Tang DD, Zhang W, Gunst SJ. The Adapter Protein CrkII Regulates Neuronal Wiskott-Aldrich Syndrome Protein, Actin Polymerization, and Tension Development during Contractile Stimulation of Smooth Muscle. *J Biol Chem* 2005 June 17;280(24):23380–9. [PubMed: 15834156]

11. Tang DD, Wu MF, Opazo Saez AM, Gunst SJ. The focal adhesion protein paxillin regulates contraction in canine tracheal smooth muscle. *J Physiol* 2002 July 15;542(Pt 2):501–13. [PubMed: 12122148]
12. Tang DD, Gunst SJ. The small GTPase Cdc42 regulates actin polymerization and tension development during contractile stimulation of smooth muscle. *J Biol Chem* 2004 December 10;279(50):51722–8. [PubMed: 15456777]
13. Zhang W, Wu Y, Du L, Tang DD, Gunst SJ. Activation of the Arp2/3 complex by N-WASp is required for actin polymerization and contraction in smooth muscle. *Am J Physiol Cell Physiol* 2005 May; 288(5):C1145–C1160. [PubMed: 15625304]
14. Chen X, Pavlish K, Zhang HY, Benoit JN. Effects of chronic portal hypertension on agonist-induced actin polymerization in small mesenteric arteries. *Am J Physiol Heart Circ Physiol* 2006 May;290 (5):H1915–H1921. [PubMed: 16339838]
15. Meeks MK, Ripley ML, Jin Z, Rembold CM. Heat shock protein 20-mediated force suppression in forskolin-relaxed swine carotid artery. *Am J Physiol Cell Physiol* 2005 March;288(3):C633–C639. [PubMed: 15509660]
16. Rembold CM, Tejani AD, Ripley ML, Han S. Paxillin phosphorylation, actin polymerization, noise temperature, and the sustained phase of swine carotid artery contraction. *Am J Physiol Cell Physiol* 2007 September;293(3):C993–C1002. [PubMed: 17596300]
17. Hirshman CA, Emala CW. Actin reorganization in airway smooth muscle cells involves Gq and Gi-2 activation of Rho. *Am J Physiol* 1999;277(3 Pt 1):L653–61. [PubMed: 10484474]
18. Hirshman CA, Zhu D, Panettieri RA, Emala CW. Actin depolymerization via the beta-adrenoceptor in airway smooth muscle cells: a novel PKA-independent pathway. *Am J Physiol Cell Physiol* 2001 November;281(5):C1468–C1476. [PubMed: 11600409]
19. Barany M, Barron JT, Gu L, Barany K. Exchange of the actin-bound nucleotide in intact arterial smooth muscle. *J Biol Chem* 2001 December 21;276(51):48398–403. [PubMed: 11602582]
20. Bursac P, Fabry B, Trepac X, et al. Cytoskeleton dynamics: fluctuations within the network. *Biochem Biophys Res Commun* 2007 April 6;355(2):324–30. [PubMed: 17303084]
21. Trepac X, Deng L, An SS, et al. Universal physical responses to stretch in the living cell. *Nature* 2007 May 31;447(7144):592–5. [PubMed: 17538621]
22. Tang D, Mehta D, Gunst SJ. Mechanosensitive tyrosine phosphorylation of paxillin and focal adhesion kinase in tracheal smooth muscle. *Am J Physiol* 1999 January;276(1 Pt 1):C250–C258. [PubMed: 9886941]
23. Tang DD, Gunst SJ. Selected contribution: roles of focal adhesion kinase and paxillin in the mechanosensitive regulation of myosin phosphorylation in smooth muscle. *J Appl Physiol* 2001 September;91(3):1452–9. [PubMed: 11509548]
24. Deng L, Fairbank NJ, Fabry B, Smith PG, Maksym GN. Localized mechanical stress induces time-dependent actin cytoskeletal remodeling and stiffening in cultured airway smooth muscle cells. *Am J Physiol Cell Physiol* 2004 August;287(2):C440–C448. [PubMed: 15070813]
25. Anfinogenova Y, Wang R, Li QF, Spinelli AM, Tang DD. Abl silencing inhibits CAS-mediated process and constriction in resistance arteries. *Circ Res* 2007 August 17;101(4):420–8. [PubMed: 17615370]
26. Mehta D, Tang DD, Wu MF, Atkinson S, Gunst SJ. Role of Rho in Ca(2+)-insensitive contraction and paxillin tyrosine phosphorylation in smooth muscle. *Am J Physiol* 2000;279(2):C308–C318.
27. Pollard TD. Regulation of actin filament assembly by Arp2/3 complex and formins. *Annu Rev Biophys Biomol Struct* 2007;36:451–77. [PubMed: 17477841]
28. Jones KA, Perkins WJ, Lorenz RR, Prakash YS, Sieck GC, Warner DO. F-actin stabilization increases tension cost during contraction of permeabilized airway smooth muscle in dogs. *J Physiol* 1999;519 (Pt 2):527–38. [PubMed: 10457068]
29. Small JV, Rottner K, Kaverina I. Functional design in the actin cytoskeleton. *Curr Opin Cell Biol* 1999 February;11(1):54–60. [PubMed: 10047522]
30. Adler KB, Krill J, Alberghini TV, Evans JN. Effect of cytochalasin D on smooth muscle contraction. *Cell Motility* 1983;3(56):545–51. [PubMed: 6686493]

31. Cipolla MJ, Gokina NI, Osol G. Pressure-induced actin polymerization in vascular smooth muscle as a mechanism underlying myogenic behavior. *FASEB J* 2002 January;16(1):72–6. [PubMed: 11772938]
32. Obara K, Yabu H. Effect of cytochalasin B on intestinal smooth muscle cells. *European Journal of Pharmacology* 1994;255(13):139–47. [PubMed: 8026540]
33. Coue M, Brenner SL, Spector I, Korn ED. Inhibition of actin polymerization by latrunculin A. *FEBS Lett* 1987;213(2):316–8. [PubMed: 3556584]
34. Cooper JA. Effects of cytochalasin and phalloidin on actin. *J Cell Biol* 1987 October;105(4):1473–8. [PubMed: 3312229]
35. Gunst SJ, Tang DD, Opazo SA. Cytoskeletal remodeling of the airway smooth muscle cell: a mechanism for adaptation to mechanical forces in the lung. *Respir Physiol Neurobiol* 2003 October 16;137(23):151–68. [PubMed: 14516723]
36. Burridge K, Chrzanowska-Wodnicka M. Focal adhesions, contractility, and signaling. *Annu Rev Cell Dev Biol* 1996;12:463–518. [PubMed: 8970735]
37. Rohatgi R, Ma L, Miki H, et al. The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. *Cell* 1999 April 16;97(2):221–31. [PubMed: 10219243]
38. Blanchoin L, Pollard TD, Mullins RD. Interactions of ADF/cofilin, Arp2/3 complex, capping protein and profilin in remodeling of branched actin filament networks. *Curr Biol* 2000 October 19;10(20):1273–82. [PubMed: 11069108]
39. Pollard TD, Blanchoin L, Mullins RD. Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *Annu Rev Biophys Biomol Struct* 2000;29:545–76. [PubMed: 10940259]
40. Higgs HN, Pollard TD. Regulation of actin filament network formation through ARP2/3 complex: activation by a diverse array of proteins. *Annu Rev Biochem* 2001;70:649–76. [PubMed: 11395419]
41. DeMali KA, Wennerberg K, Burridge K. Integrin signaling to the actin cytoskeleton. *Curr Opin Cell Biol* 2003 October;15(5):572–82. [PubMed: 14519392]
42. Kiselar JG, Mahaffy R, Pollard TD, Almo SC, Chance MR. Visualizing Arp2/3 complex activation mediated by binding of ATP and WASp using structural mass spectrometry. *Proc Natl Acad Sci U S A* 2007 January 30;104(5):1552–7. [PubMed: 17251352]
43. Cipolla MJ, Osol G. Vascular smooth muscle actin cytoskeleton in cerebral artery forced dilatation. *Stroke* 1998;29(6):1223–8. [PubMed: 9626298]
44. Langevin HM, Churchill DL, Cipolla MJ. Mechanical signaling through connective tissue: a mechanism for the therapeutic effect of acupuncture. *FASEB J* 2001 October;15(12):2275–82. [PubMed: 11641255]
45. Herrera AM, McParland BE, Bienkowska A, Tait R, Pare PD, Seow CY. ‘Sarcomeres’ of smooth muscle: functional characteristics and ultrastructural evidence. *J Cell Sci* 2005 June 1;118(Pt 11):2381–92. [PubMed: 15923651]
46. Herrera AM, Martinez EC, Seow CY. Electron microscopic study of actin polymerization in airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2004 June;286(6):L1161–L1168. [PubMed: 14751850]
47. Bai TR, Bates JH, Brusasco V, et al. On the terminology for describing the length-force relationship and its changes in airway smooth muscle. *J Appl Physiol* 2004 December;97(6):2029–34. [PubMed: 15531570]
48. Wang L, Pare PD, Seow CY. Effects of length oscillation on the subsequent force development in swine tracheal smooth muscle. *J Appl Physiol* 2000;88(6):2246–50. [PubMed: 10846042]
49. Gunst SJ, Wu MF. Plasticity of airway smooth muscle stiffness and extensibility: role of length-adaptive mechanisms. *J Appl Physiol* 2001;90:741–9. [PubMed: 11160077]
50. Gunst SJ, Fredberg JJ. The first three minutes: smooth muscle contraction, cytoskeletal events, and soft glasses. *J Appl Physiol* 2003 July;95(1):413–25. [PubMed: 12794100]
51. An SS, Bai TR, Bates JH, et al. Airway smooth muscle dynamics: a common pathway of airway obstruction in asthma. *Eur Respir J* 2007 May;29(5):834–60. [PubMed: 17470619]
52. Rembold CM, Kaufman E. Heat induced HSP20 phosphorylation without increased cyclic nucleotide levels in swine carotid media. *BMC Physiol* 2003 April 25;3(1):3. [PubMed: 12716456]

53. Murphy RA, Rembold CM. The latch-bridge hypothesis of smooth muscle contraction. *Can J Physiol Pharmacol* 2005 October;83(10):857–64. [PubMed: 16333357]
54. Rembold CM. Force suppression and the crossbridge cycle in swine carotid artery. *Am J Physiol Cell Physiol* 2007 September;293(3):C1003–C1009. [PubMed: 17522140]
55. Kanner SB, Reynolds AB, Wang HC, Vines RR, Parsons JT. The SH2 and SH3 domains of pp60src direct stable association with tyrosine phosphorylated proteins p130 and p110. *EMBO J* 1991 July; 10(7):1689–98. [PubMed: 1710979]
56. Sakai R, Iwamatsu A, Hirano N, et al. A novel signaling molecule, p130, forms stable complexes in vivo with v-Crk and v-Src in a tyrosine phosphorylation-dependent manner. *EMBO J* 1994 August 15;13(16):3748–56. [PubMed: 8070403]
57. Shin NY, Dise RS, Schneider-Mergener J, Ritchie MD, Kilkenny DM, Hanks SK. Subsets of the major tyrosine phosphorylation sites in Crk-associated substrate (CAS) are sufficient to promote cell migration. *J Biol Chem* 2004 September 10;279(37):38331–7. [PubMed: 15247284]
58. Takahashi T, Kawahara Y, Taniguchi T, Yokoyama M. Tyrosine phosphorylation and association of p130Cas and c-Crk II by ANG II in vascular smooth muscle cells. *Am J Physiol* 1998 April;274(4 Pt 2):H1059–H1065. [PubMed: 9575907]
59. Ogden K, Thompson JM, Hickner Z, Huang T, Tang DD, Watts SW. A new signaling paradigm for serotonin: use of Crk-associated substrate in arterial contraction. *Am J Physiol Heart Circ Physiol* 2006 December;291(6):H2857–H2863. [PubMed: 16861698]
60. Ojaniemi M, Vuori K. Epidermal growth factor modulates tyrosine phosphorylation of p130Cas. Involvement of phosphatidylinositol 3'-kinase and actin cytoskeleton. *J Biol Chem* 1997;272(41): 25993–8. [PubMed: 9325334]
61. Petch LA, Bockholt SM, Bouton A, Parsons JT, Burrridge K. Adhesion-induced tyrosine phosphorylation of the p130 src substrate. *J Cell Sci* 1995;108(Pt 4):1371–9. [PubMed: 7542255]
62. Rohatgi R, Nollau P, Ho HY, Kirschner MW, Mayer BJ. Nck and phosphatidylinositol 4,5-bisphosphate synergistically activate actin polymerization through the N-WASP-Arp2/3 pathway. *J Biol Chem* 2001 July 13;276(28):26448–52. [PubMed: 11340081]
63. Yang C, Huang M, DeBiasio J, et al. Profilin enhances Cdc42-induced nucleation of actin polymerization. *J Cell Biol* 2000 September 4;150(5):1001–12. [PubMed: 10973991]
64. Zigmond SH. Actin cytoskeleton: the Arp2/3 complex gets to the point. *Curr Biol* 1998 September 10;8(18):R654–R657. [PubMed: 9740796]
65. Mayer BJ, Hirai H, Sakai R. Evidence that SH2 domains promote processive phosphorylation by protein-tyrosine kinases. *Curr Biol* 1995 March 1;5(3):296–305. [PubMed: 7780740]
66. Ushio-Fukai M, Zuo L, Ikeda S, Tojo T, Patrushev NA, Alexander RW. cAbl tyrosine kinase mediates reactive oxygen species- and caveolin-dependent AT1 receptor signaling in vascular smooth muscle: role in vascular hypertrophy. *Circ Res* 2005 October 14;97(8):829–36. [PubMed: 16151024]
67. Hoeper MM, Rubin LJ. Update in pulmonary hypertension 2005. *Am J Respir Crit Care Med* 2006 March 1;173(5):499–505. [PubMed: 16493163]
68. Ghofrani HA, Seeger W, Grimminger F. Imatinib for the treatment of pulmonary arterial hypertension. *N Engl J Med* 2005 September 29;353(13):1412–3. [PubMed: 16192491]
69. Tang DD, Gunst SJ. Depletion of focal adhesion kinase by antisense depresses contractile activation of smooth muscle. *Am J Physiol Cell Physiol* 2001 April;280(4):C874–C883. [PubMed: 11245605]
70. Abedi H, Zachary I. Vascular endothelial growth factor stimulates tyrosine phosphorylation and recruitment to new focal adhesions of focal adhesion kinase and paxillin in endothelial cells. *J Biol Chem* 1997;272(24):15442–51. [PubMed: 9182576]
71. Baron V, Calleja V, Ferrari P, Alengrin F, Van Obberghen E. p125Fak focal adhesion kinase is a substrate for the insulin and insulin-like growth factor-I tyrosine kinase receptors. *J Biol Chem* 1998;273(12):7162–8. [PubMed: 9507031]
72. Guan JL. Focal adhesion kinase in integrin signaling. *Matrix Biology* 1997;16(4):195–200. [PubMed: 9402009]
73. Parsons JT. Focal adhesion kinase: the first ten years. *J Cell Sci* 2003 April 15;116(Pt 8):1409–16. [PubMed: 12640026]
74. Tapia JA, Camello C, Jensen RT, Garcia LJ. EGF stimulates tyrosine phosphorylation of focal adhesion kinase (p125FAK) and paxillin in rat pancreatic acini by a phospholipase C-independent

process that depends on phosphatidylinositol 3- kinase, the small GTP-binding protein, p21rho, and the integrity of the actin cytoskeleton. *Biochimica et Biophysica Acta* 1999;1448(3):486–99. [PubMed: 9990300]

75. Azar ZM, Mehdi MZ, Srivastava AK. Activation of insulin-like growth factor type-1 receptor is required for H₂O₂-induced PKB phosphorylation in vascular smooth muscle cells. *Can J Physiol Pharmacol* 2006 July;84(7):777–86. [PubMed: 16998541]
76. Angelucci A, Bologna M. Targeting vascular cell migration as a strategy for blocking angiogenesis: the central role of focal adhesion protein tyrosine kinase family. *Curr Pharm Des* 2007;13(21):2129–45. [PubMed: 17627545]
77. Gunst SJ. Does airway inflation stretch the bronchial mucosal membrane? *J Appl Physiol* 2005 December;99(6):2059–60. [PubMed: 16288098]
78. Berk BC, Corson MA. Angiotensin II signal transduction in vascular smooth muscle: role of tyrosine kinases. *Circ Res* 1997;80(5):607–16. [PubMed: 9130441]
79. Hirshman CA, Zhu D, Pertel T, Panettieri RA, Emala CW. Isoproterenol induces actin depolymerization in human airway smooth muscle cells via activation of an Src kinase and GS. *Am J Physiol Lung Cell Mol Physiol* 2005 May;288(5):L924–L931. [PubMed: 15821021]
80. Ishida T, Ishida M, Suero J, Takahashi M, Berk BC. Agonist-stimulated cytoskeletal reorganization and signal transduction at focal adhesions in vascular smooth muscle cells require c-Src. *J Clin Invest* 1999;103(6):789–97. [PubMed: 10079099]
81. Brown MC, Turner CE. Paxillin: adapting to change. *Physiol Rev* 2004 October;84(4):1315–39. [PubMed: 15383653]
82. Bellis SL, Miller JT, Turner CE. Characterization of tyrosine phosphorylation of paxillin in vitro by focal adhesion kinase. *J Biol Chem* 1995;270(29):17437–41. [PubMed: 7615549]
83. Brown MC, Perrotta JA, Turner CE. Identification of LIM3 as the principal determinant of paxillin focal adhesion localization and characterization of a novel motif on paxillin directing vinculin and focal adhesion kinase binding. *J Cell Biol* 1996;135(4):1109–23. [PubMed: 8922390]
84. Roy S, Ruest PJ, Hanks SK. FAK regulates tyrosine phosphorylation of CAS, paxillin, and PYK2 in cells expressing v-Src, but is not a critical determinant of v-Src transformation. *Journal of Cellular Biochemistry* 2002;84(2):377–88. [PubMed: 11787067]
85. Turner CE. Paxillin interactions. *J Cell Sci* 2000 December;113(Pt 23):4139–40. [PubMed: 11069756]
86. Wade R, Bohl J, Vande PS. Paxillin null embryonic stem cells are impaired in cell spreading and tyrosine phosphorylation of focal adhesion kinase. *Oncogene* 2002 January 3;21(1):96–107. [PubMed: 11791180]
87. Yano H, Uchida H, Iwasaki T, et al. Paxillin alpha and Crk-associated substrate exert opposing effects on cell migration and contact inhibition of growth through tyrosine phosphorylation. *Proc Natl Acad Sci U S A* 2000 August 1;97(16):9076–81. [PubMed: 10922062]
88. Vuori K, Hirai H, Aizawa S, Ruoslahti E. Introduction of p130cas signaling complex formation upon integrin-mediated cell adhesion: a role for Src family kinases. *Mol Cell Biol* 1996;16(6):2606–13. [PubMed: 8649368]
89. Nakamura I, Jimi E, Duong LT, et al. Tyrosine phosphorylation of p130Cas is involved in actin organization in osteoclasts. *J Biol Chem* 1998 May 1;273(18):11144–9. [PubMed: 9556601]
90. Hu XQ, Singh N, Mukhopadhyay D, Akbarali HI. Modulation of voltage-dependent Ca²⁺ channels in rabbit colonic smooth muscle cells by c-Src and focal adhesion kinase. *J Biol Chem* 1998;273(9):5337–42. [PubMed: 9478993]
91. Schlaepfer DD, Broome MA, Hunter T. Fibronectin-stimulated signaling from a focal adhesion kinase-c-Src complex: involvement of the Grb2, p130cas, and Nck adaptor proteins. *Mol Cell Biol* 1997;17(3):1702–13. [PubMed: 9032297]
92. Schlaepfer DD, Hanks SK, Hunter T, van der Geer P. Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. *Nature* 1994;372(6508):786–91. [PubMed: 7997267]
93. Eliceiri BP, Puente XS, Hood JD, et al. Src-mediated coupling of focal adhesion kinase to integrin alpha(v)beta5 in vascular endothelial growth factor signaling. *Journal of Cell Biology* 2002 April 1;157(1):149–60. [PubMed: 11927607]

94. Totani L, Piccoli A, Manarini S, et al. Src-family kinases mediate an outside-in signal necessary for beta2 integrins to achieve full activation and sustain firm adhesion of polymorphonuclear leucocytes tethered on E-selectin. *Biochem J* 2006 May 15;396(1):89–98. [PubMed: 16433632]
95. Guignandon A, Boutahar N, Rattner A, Vico L, Lafage-Proust MH. Cyclic strain promotes shuttling of PYK2/Hic-5 complex from focal contacts in osteoblast-like cells. *Biochem Biophys Res Commun* 2006 May 5;343(2):407–14. [PubMed: 16546139]
96. Schoenwaelder SM, Burridge K. Bidirectional signaling between the cytoskeleton and integrins. *Curr Opin Cell Biol* 1999;11(2):274–86. [PubMed: 10209151]
97. Wu X, Davis GE, Meininger GA, Wilson E, Davis MJ. Regulation of the L-type calcium channel by alpha 5beta 1 integrin requires signaling between focal adhesion proteins. *J Biol Chem* 2001 August 10;276(32):30285–92. [PubMed: 11382763]
98. Astier A, Avraham H, Manie SN, et al. The related adhesion focal tyrosine kinase is tyrosine-phosphorylated after beta1-integrin stimulation in B cells and binds to p130cas. *J Biol Chem* 1997;272(1):228–32. [PubMed: 8995252]
99. Keogh RJ, Houliston RA, Wheeler-Jones CP. Human endothelial Pyk2 is expressed in two isoforms and associates with paxillin and p130Cas. *Biochem Biophys Res Commun* 2002 February 8;290(5):1470–7. [PubMed: 11820787]
100. Li X, Earp HS. Paxillin is tyrosine-phosphorylated by and preferentially associates with the calcium-dependent tyrosine kinase in rat liver epithelial cells. *J Biol Chem* 1997 May 30;272(22):14341–8. [PubMed: 9162070]
101. D'Angelo G, Mogford JE, Davis GE, Davis MJ, Meininger GA. Integrin-mediated reduction in vascular smooth muscle [Ca²⁺]_i induced by RGD-containing peptide. *Am J Physiol* 1997;272(4 Pt 2):H2065–70. [PubMed: 9139994]
102. D'Angelo G, Davis MJ, Meininger GA. Calcium and mechanotransduction of the myogenic response. *Am J Physiol* 1997 July;273(1 Pt 2):H175–H182. [PubMed: 9249488]
103. Hirshman CA, Togashi H, Shao D, Emala CW. Galphai-2 is required for carbachol-induced stress fiber formation in human airway smooth muscle cells. *Am J Physiol* 1998;275(5 Pt 1):L911–6. [PubMed: 9815108]
104. Kuhn TB, Meberg PJ, Brown MD, et al. Regulating actin dynamics in neuronal growth cones by ADF/cofilin and rho family GTPases. *Journal of Neurobiology* 2000 August;44(2):126–44. [PubMed: 10934317]
105. Lee S, Helfman DM. Cytoplasmic p21Cip1 is involved in Ras-induced inhibition of the ROCK/LIMK/cofilin pathway. *J Biol Chem* 2004 January 16;279(3):1885–91. [PubMed: 14559914]
106. Maekawa M, Ishizaki T, Boku S, et al. Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. *Science* 1999;285(5429):895–8. [PubMed: 10436159]
107. Ohashi K, Nagata K, Maekawa M, Ishizaki T, Narumiya S, Mizuno K. Rho-associated kinase ROCK activates LIM-kinase 1 by phosphorylation at threonine 508 within the activation loop. *J Biol Chem* 2000;275(5):3577–82. [PubMed: 10652353]
108. Kaunas R, Nguyen P, Usami S, Chien S. Cooperative effects of Rho and mechanical stretch on stress fiber organization. *Proc Natl Acad Sci U S A* 2005 November 1;102(44):15895–900. [PubMed: 16247009]
109. Lammers M, Rose R, Scrima A, Wittinghofer A. The regulation of mDia1 by autoinhibition and its release by Rho*GTP. *EMBO J* 2005 December 7;24(23):4176–87. [PubMed: 16292343]
110. Gupton SL, Eisenmann K, Alberts AS, Waterman-Storer CM. mDia2 regulates actin and focal adhesion dynamics and organization in the lamella for efficient epithelial cell migration. *J Cell Sci* 2007 October 1;120(Pt 19):3475–87. [PubMed: 17855386]
111. Wu X, Li S, Chrostek-Grashoff A, et al. Cdc42 is crucial for the establishment of epithelial polarity during early mammalian development. *Dev Dyn* 2007 October;236(10):2767–78. [PubMed: 17849438]
112. El-Sibai M, Nalbant P, Pang H, et al. Cdc42 is required for EGF-stimulated protrusion and motility in MTLn3 carcinoma cells. *J Cell Sci* 2007 October 1;120(Pt 19):3465–74. [PubMed: 17855387]
113. Arozarena I, Aaronson DS, Matallanas D, et al. The Rho family GTPase Cdc42 regulates the activation of Ras/MAP kinase by the exchange factor Ras-GRF. *J Biol Chem* 2000 August 25;275(34):26441–8. [PubMed: 10840034]

114. Cau J, Hall A. Cdc42 controls the polarity of the actin and microtubule cytoskeletons through two distinct signal transduction pathways. *J Cell Sci* 2005 June 15;118(Pt 12):2579–87. [PubMed: 15928049]
115. Zigmond SH, Joyce M, Yang C, Brown K, Huang M, Pring M. Mechanism of Cdc42-induced actin polymerization in neutrophil extracts. *J Cell Biol* 1998;142(4):1001–12. [PubMed: 9722612]
116. Higgs HN, Pollard TD. Activation by Cdc42 and PIP(2) of Wiskott-Aldrich syndrome protein (WASP) stimulates actin nucleation by Arp2/3 complex. *J Cell Biol* 2000 September 18;150(6):1311–20. [PubMed: 10995437]
117. Dechert MA, Holder JM, Gerthoffer WT. p21-activated kinase 1 participates in tracheal smooth muscle cell migration by signaling to p38 Mapk. *Am J Physiol Cell Physiol* 2001 July;281(1):C123–C132. [PubMed: 11401834]
118. Woolfolk EA, Eguchi S, Ohtsu H, et al. Angiotensin II-induced activation of p21-activated kinase 1 requires Ca²⁺ and protein kinase C{delta} in vascular smooth muscle cells. *Am J Physiol Cell Physiol* 2005 November;289(5):C1286–C1294. [PubMed: 16033904]
119. Li QF, Spinelli AM, Wang R, Anfinogenova Y, Singer HA, Tang DD. Critical Role of Vimentin Phosphorylation at Ser-56 by p21-activated Kinase in Vimentin Cytoskeleton Signaling. *J Biol Chem* 2006 November 10;281(45):34716–24. [PubMed: 16990256]
120. Jaffer ZM, Chernoff J. p21-activated kinases: three more join the Pak. *Int J Biochem Cell Biol* 2002 July;34(7):713–7. [PubMed: 11950587]
121. Knaus UG, Bokoch GM. The p21Rac/Cdc42-activated kinases (PAKs). *Int J Biochem Cell Biol* 1998 August;30(8):857–62. [PubMed: 9744077]
122. Tang DD, Bai Y, Gunst SJ. Silencing of p21-activated kinase attenuates vimentin phosphorylation on Ser-56 and reorientation of the vimentin network during stimulation of smooth muscle cells by 5-hydroxytryptamine. *Biochem J* 2005 June 15;388(Pt 3):773–83. [PubMed: 15766329]
123. Wang R, Li QF, Anfinogenova Y, Tang DD. Dissociation of Crk-associated substrate from the vimentin network is regulated by p21-activated kinase on ACh activation of airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2007 January;292(1):L240–L248. [PubMed: 16997882]
124. Larsen JK, Yamboliev IA, Weber LA, Gerthoffer WT. Phosphorylation of the 27-kDa heat shock protein via p38 MAP kinase and MAPKAP kinase in smooth muscle. *Am J Physiol* 1997;273(5 Pt 1):L930–40. [PubMed: 9374719]
125. Yamboliev IA, Chen J, Gerthoffer WT. PI 3-kinases and Src kinases regulate spreading and migration of cultured VSMCs. *Am J Physiol Cell Physiol* 2001 August;281(2):C709–C718. [PubMed: 11443070]
126. Shakirova Y, Bonnevier J, Albinsson S, et al. Increased Rho activation and PKC-mediated smooth muscle contractility in the absence of caveolin-1. *Am J Physiol Cell Physiol* 2006 December;291(6):C1326–C1335. [PubMed: 17102036]
127. Shimizu T, Nakazawa T, Cho A, et al. Sphingosine 1-Phosphate Receptor 2 Negatively Regulates Neointimal Formation in Mouse Arteries. *Circ Res*. 2007 September 13;
128. Sakurada S, Takuwa N, Sugimoto N, et al. Ca²⁺-dependent activation of Rho and Rho kinase in membrane depolarization-induced and receptor stimulation-induced vascular smooth muscle contraction. *Circ Res* 2003 September 19;93(6):548–56. [PubMed: 12919947]
129. Smith PG, Garcia R, Kogerman L. Strain reorganizes focal adhesions and cytoskeleton in cultured airway smooth muscle cells. *Exp Cell Res* 1997;232(1):127–36. [PubMed: 9141629]
130. Benndorf R, Hayess K, Ryazantsev S, Wieske M, Behlke J, Lutsch G. Phosphorylation and supramolecular organization of murine small heat shock protein HSP25 abolish its actin polymerization- inhibiting activity. *J Biol Chem* 1994;269(32):20780–4. [PubMed: 8051180]
131. Miron T, Vancompernelle K, Vanderkerckhove J, Wilchek M, Geiger B. A 25-kD inhibitor of actin polymerization is a low molecular mass heat shock protein. *J Cell Biol* 1991;114:255–61. [PubMed: 2071672]
132. Ibitayo AI, Sladick J, Tuteja S, et al. HSP27 in signal transduction and association with contractile proteins in smooth muscle cells. *Am J Physiol* 1999;277:G445–G454. [PubMed: 10444459]
133. Bitar KN, Kaminski MS, Hailat N, Cease KB, Strahler JR. Hsp27 is a mediator of sustained smooth muscle contraction in response to bombesin. *Biochem Biophys Res Commun* 1991;181:1192–200. [PubMed: 1722399]

134. Yamboliev IA, Hedges JC, Mutnick JL, Adam LP, Gerthoffer WT. Evidence for modulation of smooth muscle force by the p38 MAP kinase/HSP27 pathway. *Am J Physiol Heart Circ Physiol* 2000 June;278(6):H1899–H1907. [PubMed: 10843887]

Figure 1. Schematic illustration of the actin cytoskeleton in smooth muscle cells
(A & B) Actin filaments connect with dense plaques (DP) on the membrane (M), and link to dense bodies (DB) in the myoplasm. The majority of actin filaments localize around myosin filaments (My) in a hexagonal array, forming the contractile apparatus. This pool of actin filaments is called “contractile actin (CA)”. Part of actin filaments is not structurally associated with myosin. It is called “cytoskeletal actin (CskA)”. **(C)** Actin polymerization occurs at the barbed end locating near the membrane whereas actin depolymerization takes place at the opposite of the barbed end. At the dense plaque, actin filaments attach to transmembrane integrins via linker proteins such as talin and vinculin.

Figure 2. Signaling cascades for the regulation of actin dynamics in smooth muscle

Contractile agonists and mechanical signals activate proteins kinases (Abl, FAK, Src, MAP kinase, etc) and small GTPases (Rho, Cdc42 and Rac) that regulate the downstream effectors (CAS/CrkII, N-WASP, the Arp2/3 complex, profilin, paxillin/Hic-5, HSP27, cofilin, etc.), actin polymerization, and cytoskeletal remodeling. Intracellular Ca^{2+} modulated by electrical signals (depolarization), receptor, and FAK/Src may mediate actin dynamics by regulating the PYK/paxillin/CAS pathway.