Published in final edited form as:
J Cardiovasc Pharmacol Ther. 2009 June ; 14(2): 89–98. doi:10.1177/1074248409333490.

p130 Crk-associated Substrate (CAS) in Vascular Smooth Muscle

Dale D. Tang, MD & PhD
The Center for Cardiovascular Sciences, Albany Medical College, 47 New Scotland Avenue, MC-8, Albany, NY 12208

Abstract

Vascular smooth muscle is a key effector in the wall of blood vessels during the pathogenesis of hypertension. Various factors directly elicit smooth muscle cell contraction, migration, growth, and hypertrophy, which lead to the progression of hypertension. CAS, the first discovered member of the adapter protein CAS family, has recently emerged as a critical cellular component that regulates smooth muscle functions. In this review, the molecular structure and protein interactions of the CAS family members are summarized. Evidence for the role of CAS in the regulation of vascular smooth muscle contractility, cell migration, hypertrophy, and growth are presented. Regulation of CAS by novel tyrosine kinases/phosphatases and unique downstream signaling partners of CAS are also discussed. These new findings establish the important role for CAS in regulating vascular smooth muscle functions. The CAS-associated processes may be new biological targets for the development of new treatment of cardiovascular diseases such as hypertension.

Keywords

Crk-associated substrate protein; cytoskeleton; muscle contraction; cell movement; cardiovascular diseases

Introduction

Hypertension is characterized by vascular smooth muscle constriction and vascular remodeling involving cell migration, hypertrophy, and growth. Various factors including neural transmitters, angiotensin II (AngII), endothelin, natriuretic peptides, nitric oxide (NO), oxidants, and hormones directly influence smooth muscle functions, which lead to the development and progression of high blood pressure.

The CAS family of adapter proteins has recently emerged as an important regulator of vascular smooth muscle cells (VSMC) [1–6]. Thus far, four members of the CAS family have been identified. The first member is 130-kDa CAS that was originally identified as a major tyrosine-phosphorylated protein in v-src and v-crk transformed cells [7, 8]. The second member is human enhancer of filamentation (HEF1), also referred to as Crk-associated substrate in lymphocyte (Cas-L), which was identified during screening for human proteins that modulate filamentous budding in yeast and as a tyrosine phosphorylated protein in lymphocytes. The third member of the CAS family, embryonal fyn substrate/Src interacting (Efs/Sin), was characterized as a fyn SH3-domain-binding protein. The fourth member, HEF1-Efs-p130CAS-like (HEPL), was recently identified [1, 2, 9].

Correspondence: Dale D. Tang, The Center for Cardiovascular Sciences, Albany Medical College, 47 New Scotland Avenue, MC-8, Albany, NY 12208, Tel: (518)-262-6416, Fax: (518)-262-8101, E-mail: E-mail: tangd@mail.amc.edu.

Members of the CAS proteins share a conserved molecular structure with several domains for protein-protein interactions (Figure 1). 1) Located at the N-terminus is a Src-homology 3 (SH3) domain that is able to interact with proteins containing the proline-rich motif. Thus far, the most intensively investigated partners for the SH3 domain are focal adhesion kinase (FAK), the autonomously expressed C-terminal domain of FAK (FRNK), proline-rich tyrosine kinase 2 (PYK2), protein tyrosine phosphatase-proline, glutamate, serine and threonine sequence protein (PTP-PEST); the association of CAS with these proteins modulates its phosphorylation state and subcellular localization. 2) Adjacent to the C-terminus of the SH3 domain is the proline-rich domain (RPLPSPP) that interacts with some members of the Src family including Lyn, Fyn, Yes, and Hck. 3) The substrate domain contains 15 YXXP sequences, which are phosphorylated by Abelson tyrosine kinase (Abl), c-Src, or FAK depending on cell types and external stimuli. Abl may also interact with CAS via this motif. 4) The C-terminal motif of the CAS proteins is characterized by a bipartite binding site containing a proline-rich domain (which binds the Src SH3 domain) and a tyrosine-containing sequence (YDYV) that interacts with the Src SH2 domain when phosphorylated. A helix-loop-helix (HLH) motif is also present in this region. The C-terminus is responsible for the formation of homodimers or heterodimers among the CAS family members. In addition, CAS may associate with vimentin filaments in smooth muscle via this region. 5) The serine-rich region is located between the substrate domain and the C-terminal motif. The adapter protein 14-3-3 and Grb2 are the interactive partners for this region. During mitosis, CAS serine phosphorylation occurs in a number of cells regulating cytokinesis [1, 2, 10–12].

Although these four CAS members possess conserved molecular structure, their expression, tissue distribution and functional roles are distinct. HEF1 is primarily studied in lymphocytes, epithelium and nerve tissues, which regulates cell migration, mitosis, and neurite outgrowth. Efs has been shown to regulate the functions of T-lymphocytes. HEPL is mainly distributed in the lung and the spleen as well as in cancer cells such as leukemia and ovarian cell lines. CAS is ubiquitously distributed in animal cells/tissues with high expression in vascular smooth muscle [1, 2, 10–12]. This review focuses on the functional role of CAS in vascular smooth muscle.

CAS and VSMC Contraction

Recent investigations show that CAS plays a fundamental role in orchestrating smooth muscle contraction [2–6, 13–16]. Treatment of smooth muscle strips with antisense oligodeoxynucleotides (ODNs) against CAS selectively downregulates CAS protein expression without affecting the protein levels of smooth muscle actin, myosin heavy chain, and paxillin. In contrast, sense ODNs do not affect the expression of these proteins. The downregulation of CAS dramatically attenuates force development and actin polymerization in response to contractile stimulation without affecting myosin regulatory light chain phosphorylation [4, 5]. Crossbridge cycling regulated by myosin activation has been thought to be a sole mechanism modulating smooth muscle contraction [17]. These studies suggest that CAS is a necessary element of cellular processes that regulate tension development in smooth muscle, and that CAS-regulated smooth muscle contraction is independent of myosin activation.

There is considerable evidence to suggest that CAS may modulate smooth muscle contraction via actin polymerization, which has recently documented to be an important cellular event in response to contractile stimulation [5, 6, 13, 14, 18–24]. CAS undergoes tyrosine phosphorylation in various cell types including smooth muscle cells in response to constrictor stimulation or other external signals [3, 4, 15, 25, 26]. Phosphorylation on CAS occurs in its substrate domain, which contains 15 YXXP phosphorylatable motifs [2, 15]. Tyr-410 is a major phosphorylation site on CAS in vascular smooth muscle upon contractile activation [3, 4].

Phosphorylation on the tyrosine residues creates docking sites for the SH2/SH3-containing signaling effector CrkII in various cell types including smooth muscle cells under contractile activation [3, 15, 27, 28]. Activated CAS/CrkII recruits N-WASP, an actin-regulatory protein. When unstimulated, the C-terminal portion of N-WASP binds to its GTP-binding domain, masking its binding motif for the Arp2/3 (Actin Related Protein) 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 [13, 14, 29].

In smooth muscle, actin polymerization transpires at focal adhesion sties (membrane-associated dense plaques) [13, 14, 18, 29–32]. Actin polymerization may enhance force development by the following mechanisms. First, the actin filaments of smooth muscle cells attach to the transmembrane β integrins, facilitating mechanical transduction between the contractile apparatus and extracellular matrix. Cortical actin assembly may strengthen the linkage of actin filaments to integrins and enhance the transmission of contractile force [13, 14, 18, 29–34]. 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 [35–37]. Third, newly polymerized filaments may be a part of reorganization processes that allow for rapid adjustment of stiffness and tension [13, 14, 18, 30–32, 38]. Fourth, actin filament assembly may participate in the “latch” formation of contractile elements, supporting force maintenance under the condition of lower crossbridge phosphorylation [21, 31, 39–41].

Spatial Localization of CAS in Smooth Muscle Cells

As described above, actin polymerization occurs at focal adhesions, which is important for smooth muscle contraction. Therefore, spatial localization of the actin regulatory molecules is crucial to the efficient regulation of localized actin filament assembly. Immunofluorescent analysis shows that in unstimulated smooth muscle cells, CAS is primarily distributed in the myoplasm. In response to contractile activation, CAS translocates to the cell periphery where actin polymerization may occur [11, 12, 33]. In addition, paxillin (a cytoskeletal protein) also displays similar translocation in smooth muscle [42].

This observation is further supported by studies on rat aortic smooth muscle cells. Activation with AngII promotes the focal adhesion accumulation of CAS along with c-Src and cell movement. This translocation may be mediated by the tyrosine phosphorylation of CAS and c-Src, and CAS/Src coupling. Overexpression of inactive Src, but not wild type Src, inhibits CAS phosphorylation and spatial redistribution as well as cell migration under AngII stimulation. Furthermore, a CAS mutant lacking its substrate domain blocks CAS association with c-Src and CAS redistrubtion toward focal adhesions [25]. These studies indicate that CAS spatial translocation is an essential cellular process for focal adhesion formation, which is known to regulate smooth muscle contraction and cell migration.

The spatial redistribution of CAS may be regulated by the vimentin intermediate filament network. The fractionation assay shows that a pool of CAS is found in cytoskeletal vimentin in smooth muscle cells/tissues. In addition, purified vimentin is detected in immobilized CAS in Far-Western analysis. Contractile stimulation induces the dissociation of CAS from cytoskeletal vimentin, which is regulated by vimentin phosphorylation at Ser-55 (a major phosphorylation site on vimentin in smooth muscle). Moreover, overexpression of the non-phosphorylatable vimentin mutant S56A in smooth muscle cells blocks the dissociation of CAS from the vimentin network upon agonist stimulation [4, 5, 10–12, 43, 44]. These results suggest that vimentin filaments are able to harbor the adapter protein. The site-specific phosphorylation

of vimentin regulates the redistribution of CAS in smooth muscle cells during agonist activation.

p21-activated kinase (PAK) modulates CAS translocation by affecting vimentin phosphorylation. In vitro studies demonstrate a direct role for PAK in catalyzing vimentin phosphorylation [12, 43, 45–47]. Downregulation of PAK by antisense oligonucleotides or RNAi inhibits vimentin phosphorylation at Ser-56, which initiates partial disassembly of intermediate filaments. Because CAS binds to filamentous vimentin, this depolymerization may be responsible for CAS dissociation from the vimentin cytoskeleton and the translocation from the cytoplasm to the membrane [11, 12, 45]. Similarly, vimentin filaments also regulate spatial redistribution of Ca^{2+}/calmodulin protein kinase II and Rho kinase in response to external stimulation [43, 48, 49].

CAS and VSMC Migration

One of the major pathologic processes of hypertension is vascular remodeling that is characterized by smooth muscle cell growth and hypotrophy in the medial layer. It is well recognized that cell migration plays a fundamental role in the proliferation of various cell types including smooth muscle cells. Cell migration includes the following major steps: 1) in response to chemotactic signals, cells form lamellipodia and filapodia at the leading edge, which is mediated by local actin filament assembly; 2) new focal adhesions are created in the front part of motile cells to strengthen the attachment to extracellular matrix; 3) actin-myosin motor assembly occurs to induce cell contraction; 4) detachment of focal contacts at the rear part of cells and 5) cells move towards chemoattractants [2, 50].

There is a wealth of evidence to suggest that CAS participates in the regulation of vascular smooth muscle cell migration. Angiotensin II (AngII), an octopeptide associated with renal hypertension, has been shown to stimulate motility of vascular smooth muscle cells [50]. Moreover, CAS-null cells display reduced cell spreading and migration along with decreases in filamentous actin in cell protrusion, which is restored by the expression of full-length CAS [2, 51]. In contrast, expression of CAS mutants lacking phosphorylation sites and the C-terminal Src-binding domain does not restore the migratory phenotype [2, 15]. These studies indicate an important role of phosphorylation and c-Src in CAS-mediated cell migration.

Dephosphorylation of CAS inhibits the motility of aortic smooth muscle cells during the activation of IGF-I, an important mediator for cardiovascular diseases [52, 53]. Exposure to IGF-I leads to an increase in the motility of aortic smooth muscle cells probably by stimulating CAS phosphorylation. Nitric oxide (NO) is able to induce dephosphorylation of CAS by increasing the activity of PTP-PEST. NO-mediated CAS dephosphorylation inhibits the activation of the small GTPase Rac1, the production of H_2O_2 (a second messenger) and cell migration. Overexpression of PTP-PEST, or dominant negative CAS mutant suppresses Rac 1 activation, H_2O_2 generation, actin filament assembly in the protrusion region, and cell motility, which is reversed by expression of inactive PTP-PEST, or constitutively active CAS [1, 2, 50, 52–54].

CAS regulates smooth muscle cell motility mainly by stimulating local actin filament assembly at focal adhesions. As described above, CAS phosphorylation on the substrate domain creates high affinity for the adapter protein CrkII and DOCK-180, which stimulates N-WASP mediated formation of F-actin in the leading edge. In addition, the assembly of CAS/CrkII/DOCK-180 at focal contacts enhances the activation of Rac1 facilitating actin polymerization in lamellipodia and filapodia [2, 10, 29, 43]. CAS may also regulate the association of profilin with G-actin (globular actin) transporting actin monomers to the barbed end and stimulating actin polymerization [5, 6].

CAS may mediate AngII-induced VSMC migration by affecting activation of the mitogen-activated protein kinase (MAPK) family. In a study, phosphorylation of c-Src and CAS, the activation of extracellular signal-regulated kinase 1/2 (Erk1/2) and c-Jun NH2-terminal kinase (JNK) (but not p38) upon AngII activation were depressed by Src inhibitors or in cells expressing inactive Src. In contrast, AngII-induced JNK activation, but not Erk1/2 activation, was inhibited in cells expressing an inactive CAS mutant. Motility upon AngII activation was also suppressed in VSMC expressing the inactive CAS mutant or inactive Src [25]. These results suggest that CAS mediates VSMC movement by regulating JNK activity whereas c-Src affects cell migration by modulating both Erk1/2 and JNK activity.

Signal transducer and activator of transcription 3 (STAT3) may antagonize the migratory functions of CAS in non-muscle cells. In STAT3-null keratinocytes, levels of CAS tyrosine phosphorylation were significantly higher than wild type cells. Furthermore, these STAT3-null cells displayed increased adhesive capability and fast spreading on a collagen matrix. Because STAT3 is a transcription factor, the results suggest that STAT3 may negatively regulate CAS phosphorylation and cell movement possibly by inhibiting expression of protein tyrosine phosphatases or increasing the production of tyrosine kinases [2]. In addition, Bmx/Etk is a member of the Tec/Btk family of non-receptor tyrosine kinases, which has been implicated in regulating CAS phosphorylation and cell movement. Expression of Bmx resulted in an increase in the association of Bmx with CAS at membrane ruffles, microstructures containing nascent actin filaments in motile cells. Expression of Bmx also increased the tyrosine phosphorylation of CAS and CAS/CrkII coupling, and coexpression of Bmx with CAS enhanced membrane ruffling and haptotacitc cell migration. In contrast, a Bmx mutant lacking the CAS binding domain did not induce cell migration. Moreover, an inactive CAS mutant inhibited Bmx-induced cell migration [2]. Nevertheless, these results were largely from studies on non-muscle cells. More investigations are needed to evaluate whether similar mechanisms are present in VSMC.

Role of CAS in VSMC Hypertrophy, Growth, and Survival

In the early stage of hypertension, increases in peripheral resistance are mainly caused by vasoconstriction that is reversibly when the local concentration of constrictors is reduced. In contrast, the increased arterial resistance in the late stage of the disease stems from VSMC hypertrophy/growth as well as contraction, which is usually not reversible [55].

The hypertrophic growth response is characterized by an increase in cell size and protein content without alterations in cell number and DNA replication. CAS may be involved in the development of VSMC hypertrophy. AngII induced CAS tyrosine phosphorylation and hypertrophic growth in cultured VSMC as well as in intact arterial tissues [2, 55]. CAS phosphorylation can also be induced by integrin activation in response to changes in mechanical environments. AngII-induced CAS phosphorylation initiated assembly of a multiprotein complex containing CAS, PYK2 and phosphatidylinositol 3-kinase (PI 3-kinase) as determined by co-immunoprecipitation analysis. Complex formation between CAS, PYK2 and PI 3-kinase was associated with a rapid phosphorylation of the ribosomal p70 S6 kinase (an enzyme critical for protein synthesis) in a Ca^{2+}- and tyrosine kinase-dependent manner [55]. In addition, agonist stimulation facilitates CAS/CrkII coupling that is able to activate MAP kinase via Rac1. MAP kinase has been shown to regulate phosphorylation of PHAS-1/eIF4E complex, a key regulator of translation initiation [15, 55, 56]. These studies suggest that CAS participates in the cellular processes which regulate the hypertrophic response in VSMC.

CAS has been implicated in the signaling cascades regulating VSMC growth and survival. CAS is required for cell survival mediated by integrin activation, growth factors, and peptides. Cell detachment or the withdrawal of growth factors as well as peptides initiates rapid

dephosphorylation of CAS and therefore cell death. FAK/Src mediated CAS phosphorylation activates the small GTPses Ras and Rac as well as JNK and Erk1/2, which are known to trigger gene expression for cell survival/growth. This implies a dual role for CAS in regulating survival/growth because it behaves as a critical effector in integrin and growth factor signaling and as a positive regulator of intergrin-growth factor receptor cross-talk [2, 57].

Regulation of CAS in VSMC

Because CAS is a tyrosine-phosphorylated protein, its regulation is largely dependent upon the activity of specific tyrosine kinases and/or protein tyrosine phosphatases in VSMC in response to a variety of changes in environments (Figure 2).

Tyrosine kinases

The tyrosine phosphorylation of CAS is mediated by Abl, a non-receptor tyrosine kinase. Studies from *in vitro* biochemical assay show that CAS phosphorylation is catalyzed directly by Abl [3, 58]. Silencing of Abl by short hairpin RNA dramatically depresses CAS phosphorylation in resistance arteries upon stimulation with the α-adrenergic receptor agonist phenylephrine [3]. Moreover, Abl silencing inhibits the AngII-mediated signaling event in VSMC [59]. 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 tissues [3]. Abl tyrosine phosphorylation has also been reported in vascular smooth muscle cells upon angiotensin II stimulation [59]. Tyr-412 is located at the activation loop of Abl kinase domain. When unstimulated, the activation loop of the kinase domain folds into the active site, thereby preventing binding of both the substrate and ATP. Phosphorylation at this residue induces conformation changes; the activation loop no longer blocks the active site, which leads to the increase in kinase activity [60].

Since Abl plays an important role in regulating CAS and arterial constriction, development of a strategy to block Abl kinase activity could be a new way to treat hypertension. Recent exciting studies support this theory; a selective inhibitor of Abl, imatinib (STI-571), is effective for the treatment of pulmonary hypertension in clinical studies [61, 62].

Integrin-associated signaling has also been implicated in the regulation of CAS in smooth muscle. FAK and c-Src, located at the integrin-associated membrane microdomain, are activated by integrin clustering as well as activation with contractile agonists and growth factors [10, 22, 23, 63–66]. Specifically, FAK tyrosine phosphorylation in smooth muscle is mechanosensitive, which is mediated by transmembrane integrins [22, 63]. Integrin activation may induce FAK autophosphorylation, creating a binding site for Src-family kinases and other proteins. CAS protein binds to the C-terminal FAK poly-proline region via their SH3 domain [1, 10]. In addition, activation of 5-HT receptor also induces the tyrosine phosphorylation of CAS in vascular smooth muscle. The inhibition of c-Src by the inhibitor PP1 abolished CAS phosphorylation on tyrosine residues, suggesting a role for c-Src in CAS activation [4]. Similar results were obtained from studies on non-muscle cells [67]. Since Abl activation is attenuated by a c-Src inhibitor in cultured VSMC [59], and integrin clustering and receptor activation initiate assembly of a multiprotein complex containing FAK, c-Src and Abl, it is possible that contractile activation may activate Abl via c-Src/FAK; activated Abl may directly catalyze CAS phosphorylation in vascular smooth muscle. However, other studies suggest that Src may be responsible for integrin-mediated tyrosine phosphorylation of CAS [68, 69].

PYK2 is another non-receptor tyrosine kinase that has been found in VSMC. Unlike FAK that is largely activated by integrin aggregation and is Ca^{2+}-independent, PYK2 activation is Ca^{2+}-dependent in response to changes in environments surrounding cells. Agonist stimulation induced PYK2 phosphorylation on Tyr-402 and Tyr-881, and increased the association of

PYK2 with CAS via its SH3 domain in VSMC, which was not inhibited by PI 3-kinase inhibitors. The interaction of PYK2 with CAS may facilitate CAS phosphorylation, or may facilitate formation of the PYK2-associated scaffold complex transducing extracellular signals to downstream effectors such as p70 S6K or Erk1/2 [10, 55, 57].

Tyrosine phosphatases

Thus far, two protein tyrosine phosphatases, PTP-PEST and SHP-2, have been shown to regulate CAS dephosphorylation in smooth muscle [52, 70]. PTP-PEST is a ubiquitously expressed non-membrane protein tyrosine phosphatase. IGF-I stimulation of rat VSMC induced the elevation of phosphotyrosine levels in CAS in association with the enhancement of Rac1 and H_2O_2 elevation; overexpression of PTP-PEST blocked the effect of IGF-I. Moreover, treatment with NO donors resulted in a decrease in CAS phosphorylation and cell migration via activation of PTP-PEST [53].

Exposure of VSMC to AngII triggers reorganization of the actin cytoskeleton, an important end point of CAS-mediated process. In a study, stable cell lines overexpressing wild type or constitutively inactive SHP-2 were utilized to evaluate the role of the tyrosine phosphatase in regulating AngII-induced actin cytoskeletal remodeling. Overexpression of wild type SHP-2 inhibited AngII mediated stress fiber formation whereas inactive SHP-2 increased actin cytoskeletal reorganization. Furthermore, the expression of wild type SHP-2 led to dephosphorylation of CAS and other focal adhesion associated proteins. The inactive SHP-2 mutant did not have similar effects. Dephosphorylation of the focal adhesion associated proteins caused by the overexpression of wild type SHP-2 was significantly accompanied by a decrease in focal adhesion sites within VSMC [70].

Regulation of CAS cellular localization

The functional role of CAS can be regulated by its spatial localization in cells because localized actin polymerization and focal adhesion formation occur in particular cellular compartments. As described above, PAK-mediated vimentin phosphorylation at Ser-56 and partial disassembly modulates CAS redistribution in smooth muscle cells. In addition, c-Src may regulate focal accumulation of CAS in rat smooth muscle cells during AngII activation [25].

The zyxin and Ajuba family of LIM proteins are components of cellular adhesive complexes, and associated with CAS as evidenced by coimmunoprecipitation analysis. Ajuba-null fibroblasts are inhibited in their motility without affecting cell adhesions, cells spreading, and integrin activation. However, CAS and FAK phosphorylation at nascent focal adhesions and lamellipodia production are defective in these cells. Interestingly, overexpression of CAS rescue the migratory defect of Ajuba-null cells [71]. These experiments indicate that Ajuba acts as an upstream regulator of CAS to position CAS to nascent focal adhesions in migrating cells thereby leading to the activation of Rac [71]. Future studies are needed to determine whether Ajuba recruits CAS in VSMC during contractile activation.

Conclusions and perspectives

The adapter protein CAS plays a critical role in regulating VSMC functions. CAS tyrosine phosphorylation mediated by Abl/Src/FAK as well as phosphatases profoundly influences contractility, movement, hypertrophy, and growth of VSMC. The association of CAS with other protein partners also modulates its spatial localization, focal adhesion formation, and actin cytoskeletal reorganization. Because CAS translocation is important in precisely mediating the assembly of actin filaments and other microstructures, understanding whether microtubules and/or intermediate filaments participate in the unique spatial redistribution of CAS is fundamental to our knowledge in this research area. In addition, we need to evaluate

whether CAS expression, spatial localization, and other molecular/biologic features are altered in the context of hypertension. Finally, promising results show that a selective inhibitor of Abl can lessen the symptoms of pulmonary hypertension. Thus, future studies are needed to develop new pharmacologic agents to specifically block Abl kinase and other kinases, or stimulate phosphatases for better treatment of essential and/or pulmonary hypertension.

Acknowledgments

This work was supported by The National Heart, Lung, and Blood Institute Grant HL-75388

References

1. O'Neill GM, Fashena SJ, Golemis EA. Integrin signalling: a new Cas(t) of characters enters the stage. Trends Cell Biol 2000 March;10(3):111–9. [PubMed: 10675905]
2. Defilippi P, Di SP, Cabodi S. p130Cas: a versatile scaffold in signaling networks. Trends Cell Biol 2006 May;16(5):257–63. [PubMed: 16581250]
3. 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]
4. 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]
5. 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]
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. 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]
8. 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]
9. Singh MK, Dadke D, Nicolas E, et al. A Novel Cas Family Member, HEPL, Regulates FAK and Cell Spreading. Mol Biol Cell 2008 April;19(4):1627–36. [PubMed: 18256281]
10. Tang DD, Anfinogenova Y. Physiologic properties and regulation of the actin cytoskeleton in vascular smooth muscle. J Cardiovasc Pharmacol Ther 2008 June;13(2):130–40. [PubMed: 18212360]
11. 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]
12. 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]
13. 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]
14. 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]
15. 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]
16. 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]

17. Somlyo AV, Khromov AS, Webb MR, et al. Smooth muscle myosin: regulation and properties. Philos Trans R Soc Lond B Biol Sci 2004 December 29;359(1452):1921–30. [PubMed: 15647168]
18. 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]
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. 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]
21. 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]
22. 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]
23. 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]
24. 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]
25. Kyaw M, Yoshizumi M, Tsuchiya K, et al. Src and Cas are essentially but differentially involved in angiotensin II-stimulated migration of vascular smooth muscle cells via extracellular signal-regulated kinase 1/2 and c-Jun NH2-terminal kinase activation. Mol Pharmacol 2004 April;65(4):832–41. [PubMed: 15044612]
26. 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]
27. Cho SY, Klemke RL. Purification of pseudopodia from polarized cells reveals redistribution and activation of Rac through assembly of a CAS/Crk scaffold. J Cell Biol 2002 February 18;156(4): 725–36. [PubMed: 11839772]
28. Chodniewicz D, Klemke RL. Regulation of integrin-mediated cellular responses through assembly of a CAS/Crk scaffold. Biochim Biophys Acta 2004 July 5;1692(2–3):63–76. [PubMed: 15246680]
29. Pollard TD. Regulation of actin filament assembly by Arp2/3 complex and formins. Annu Rev Biophys Biomol Struct 2007;36:451–77. [PubMed: 17477841]
30. 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]
31. 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(2–3):151–68. [PubMed: 14516723]
32. Gerthoffer WT. Actin cytoskeletal dynamics in smooth muscle contraction. Can J Physiol Pharmacol 2005 October;83(10):851–6. [PubMed: 16333356]
33. 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]
34. 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]
35. 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]
36. 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]

37. 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]
38. 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]
39. Murphy RA, Rembold CM. The latch-bridge hypothesis of smooth muscle contraction. Can J Physiol Pharmacol 2005 October;83(10):857–64. [PubMed: 16333357]
40. 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]
41. 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]
42. Opazo SA, Zhang W, Wu Y, Turner CE, Tang DD, Gunst SJ. Tension development during contractile stimulation of smooth muscle requires recruitment of paxillin and vinculin to the membrane. Am J Physiol Cell Physiol 2004 February;286(2):C433–C447. [PubMed: 14576084]
43. Tang DD. Invited review: intermediate filaments in smooth muscle. Am J Physiol Cell Physiol 2008 April;294(4):C869–C878. [PubMed: 18256275]
44. Wang R, Li Q, Tang DD. Role of vimentin in smooth muscle force development. Am J Physiol Cell Physiol 2006 September;291(3):C483–C489. [PubMed: 16571866]
45. 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]
46. Chan W, Kozma R, Yasui Y, et al. Vimentin intermediate filament reorganization by Cdc42: involvement of PAK and p70 S6 kinase. Eur J Cell Biol 2002 December;81(12):692–701. [PubMed: 12553669]
47. Goto H, Tanabe K, Manser E, Lim L, Yasui Y, Inagaki M. Phosphorylation and reorganization of vimentin by p21-activated kinase (PAK). Genes Cells 2002 February;7(2):91–7. [PubMed: 11895474]
48. Sin WC, Chen XQ, Leung T, Lim L. RhoA-binding kinase alpha translocation is facilitated by the collapse of the vimentin intermediate filament network. Mol Cell Biol 1998 November;18(11):6325–39. [PubMed: 9774649]
49. Marganski WA, Gangopadhyay SS, Je HD, Gallant C, Morgan KG. Targeting of a novel Ca+2/calmodulin-dependent protein kinase II is essential for extracellular signal-regulated kinase-mediated signaling in differentiated smooth muscle cells. Circ Res 2005 September 16;97(6):541–9. [PubMed: 16109919]
50. Gerthoffer WT. Mechanisms of vascular smooth muscle cell migration. Circ Res 2007 March 16;100 (5):607–21. [PubMed: 17363707]
51. Honda H, Nakamoto T, Sakai R, Hirai H. p130(Cas), an assembling molecule of actin filaments, promotes cell movement, cell migration, and cell spreading in fibroblasts. Biochem Biophys Res Commun 1999 August 19;262(1):25–30. [PubMed: 10448062]
52. Lin Y, Ceacareanu AC, Hassid A. Nitric oxide-induced inhibition of aortic smooth muscle cell motility: role of PTP-PEST and adaptor proteins p130cas and Crk. Am J Physiol Heart Circ Physiol 2003 August;285(2):H710–H721. [PubMed: 12714323]
53. Ceacareanu AC, Ceacareanu B, Zhuang D, et al. Nitric oxide attenuates IGF-I-induced aortic smooth muscle cell motility by decreasing Rac1 activity: essential role of PTP-PEST and p130cas. Am J Physiol Cell Physiol 2006 April;290(4):C1263–C1270. [PubMed: 16354758]
54. 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 March;103(6):789–97. [PubMed: 10079099]
55. Rocic P, Govindarajan G, Sabri A, Lucchesi PA. A role for PYK2 in regulation of ERK1/2 MAP kinases and PI 3-kinase by ANG II in vascular smooth muscle. Am J Physiol Cell Physiol 2001 January;280(1):C90–C99. [PubMed: 11121380]
56. Lawrence JC Jr, Brunn GJ. Insulin signaling and the control of PHAS-I phosphorylation. Prog Mol Subcell Biol 2001;26:1–31. [PubMed: 11575163]

57. Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol 2007 January;292(1):C82–C97. [PubMed: 16870827]
58. 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]
59. 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]
60. Wang JY. Controlling Abl: auto-inhibition and co-inhibition? Nat Cell Biol 2004 January;6(1):3–7. [PubMed: 14704671]
61. Hoeper MM, Rubin LJ. Update in pulmonary hypertension 2005. Am J Respir Crit Care Med 2006 March 1;173(5):499–505. [PubMed: 16493163]
62. 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]
63. 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]
64. Parsons JT. Focal adhesion kinase: the first ten years. J Cell Sci 2003 April 15;116(Pt 8):1409–16. [PubMed: 12640026]
65. 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]
66. 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]
67. Ruest PJ, Shin NY, Polte TR, Zhang X, Hanks SK. Mechanisms of CAS substrate domain tyrosine phosphorylation by FAK and Src. Mol Cell Biol 2001 November;21(22):7641–52. [PubMed: 11604500]
68. 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]
69. 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]
70. Fernstrom K, Farmer P, Ali MS. Cytoskeletal remodeling in vascular smooth muscle cells in response to angiotensin II-induced activation of the SHP-2 tyrosine phosphatase. J Cell Physiol 2005 December;205(3):402–13. [PubMed: 16021628]
71. Pratt SJ, Epple H, Ward M, Feng Y, Braga VM, Longmore GD. The LIM protein Ajuba influences p130Cas localization and Rac1 activity during cell migration. J Cell Biol 2005 February 28;168(5):813–24. [PubMed: 15728191]

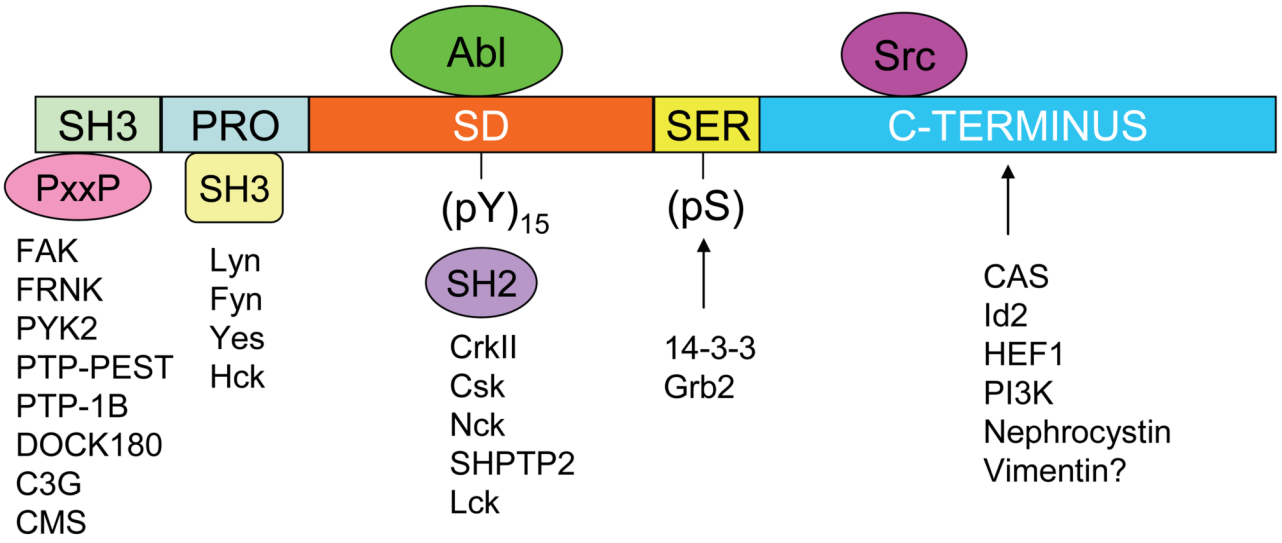

Figure 1. Molecular structure and protein interactions of CAS

The conserved molecular structure of the CAS proteins contains a SH3 domain, a proline-rich domain (PRO), a substrate domain (SD), a serine-rich motif (SER), and the C-terminus domain. Proteins able to interact with the CAS family members are depicted in the figure (see the text for details).

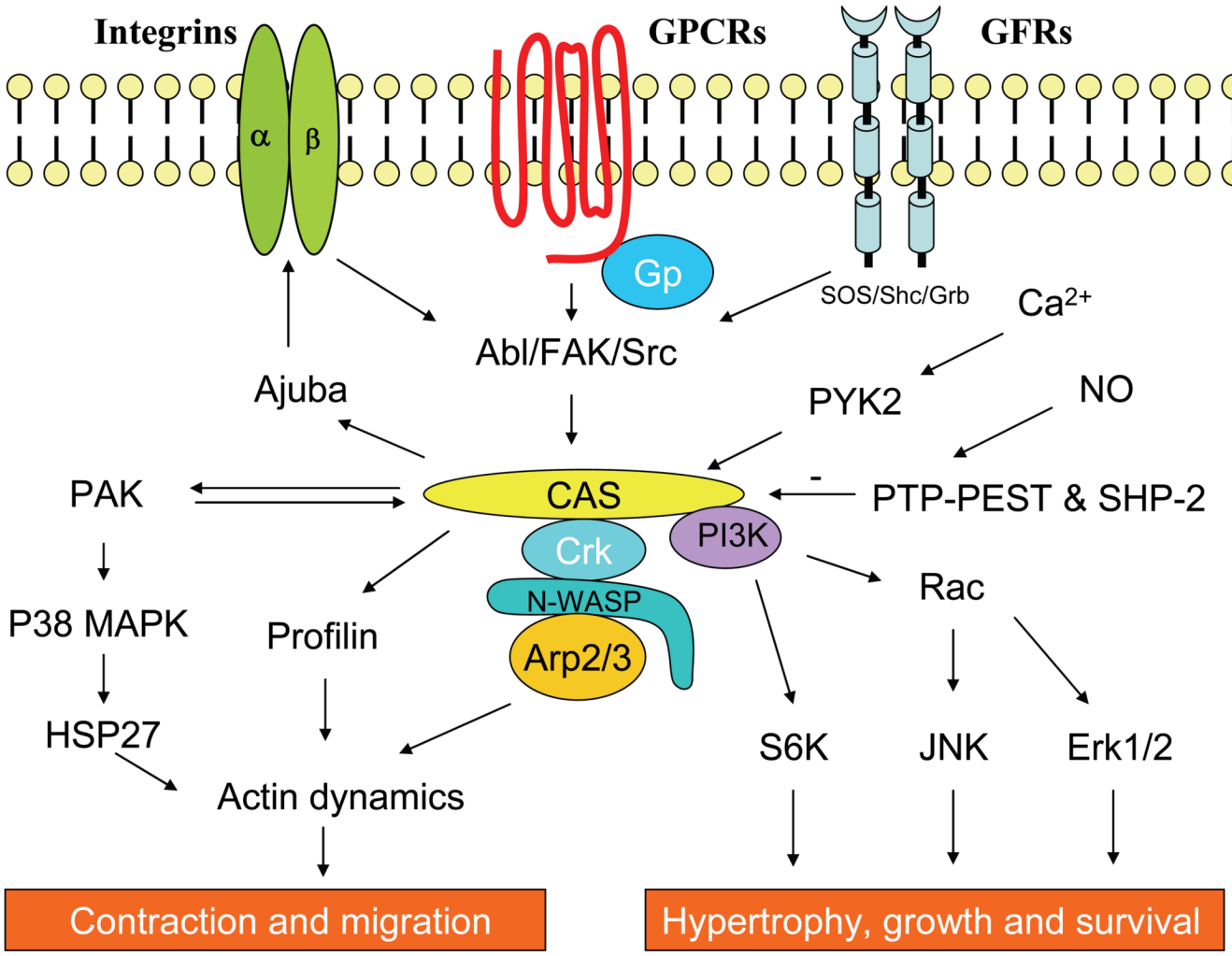

Figure 2. Regulation and downstream effects of CAS in smooth muscle

Activation of G protein-coupled receptors (GPCRs) or growth factor receptors (GFRs) and integrin aggregation may trigger autophosphorylation of c-Src and FAK, which may activate Abl in smooth muscle. Abl catalyzes the tyrosine phosphorylation of CAS, creating binding sites for CrkII and stimulating N-WASP actin assembly mediated by the Arp2/3 complex. Ca^{2+}-dependent PYK2 also activates CAS whereas PTP-PEST and SHP-2 inactivates CAS via dephosphorylation. PAK may regulate CAS via the vimentin cytoskeleton. Conversely, CAS may influence PAK activity by a multiprotein complex. Ajuba may recruit CAS to focal adhesion sites for localized actin polymerization. Activated CAS regulates p70S6K, JNK and MAP kinases via PI 3-kinases and small GTPases. CAS also regulates the actin-regulatory protein profilin and possibly HSP 27 via the PAK-p38 MAPK pathway. These downstream molecules of CAS may affect VSMC contraction, movement, hypotrophy, and growth.