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Caldesmon and the Regulation of Cytoskeletal Functions

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Abstract

Caldesmon (CaD) is an extraordinary actin-binding protein, because in addition to actin, it also binds myosin, calmodulin and tropomyosin. As a component of the smooth muscle and nonmuscle contractile apparatus CaD inhibits the actomyosin ATPase activity and its inhibitory action is modulated by both Ca^{2+} and phosphorylation. The multiplicity of binding partners and diverse biochemical properties suggest CaD is a potent and versatile regulatory protein both in contractility and cell motility. However, after decades of investigation in numerous laboratories, hard evidence is still lacking to unequivocally identify its *in vivo* functions, although indirect evidence is mounting to support an important role in connection with the actin cytoskeleton. This chapter reviews the highlights of the past findings and summarizes the current views on this protein, with emphasis of its interaction with tropomyosin.

Introduction

Many important cellular processes involve changes of the actin cytoskeleton, which maintains and controls the cell shape in concert with activities of the contractile apparatus as a result of cell signaling. It is well established that polymerization and depolymerization of actin filaments plays a central role in controlling a wide spectrum of cellular phenomena including cell division and migration, in addition to exo- and endocytosis, apoptosis and inflammation, as well as vesicle trafficking and gene expression.^{1,2} A large number of actin cytoskeleton proteins, including focal adhesion complex-associated adaptor proteins and actin-binding proteins (ABPs) are responsible for the organization and remodeling of the actin cytoskeleton. Malfunction of these processes leads to pathological consequences, but how actin-mediated motility is regulated is only beginning to be understood. With recent advances in the field, particularly in the elucidation of protein structures and their binding partners, the potential roles of the actin cytoskeleton in many diseases is becoming apparent. Two proteins, caldesmon (CaD) and tropomyosin (Tm), stand out as unique types of ABPs, because they are also integral components of the contractile apparatus. Both of them are the so-called “side-binders” of actin filaments, which stabilize the filamentous structure; their presence reinforces the stability of F-actin and therefore is intimately involved in the regulation of actin cytoskeleton assembly and organization. In this chapter we aim to describe the biochemical properties of CaD in the context of its relationship with Tm and discuss its biological functions. There is a vast amount of data in the literature on CaD. In an attempt to attain an overview and to derive new insights, some of the earlier observations, especially those controversial ones, will be discussed in comparison with more recent findings. However, it is not the intention of this article to make an exhaustive survey of the entire field. Interested readers are referred to many other excellent reviews that can be found elsewhere.^{3–7}

Genes and Isoforms

Vertebrate CaD has a single gene that is alternatively spliced⁸ to generate the smooth muscle h-CaD and the nonmuscle isoform (I-CaD),^{9,10} the latter being present in almost all types of

cells.¹¹ In the human CaD gene (7q33-q34) there is further isoform diversity in l-CaD. In addition to the first exon (exon1') shared by h-CaD (793 residues) and l-CaD (termed WI38 l-CaD; 538 residues), another exon (exon 1) further upstream can be used as an alternative initiation site; the resulting isoform (HeLa l-CaD) contains a different N-terminal segment that is 6 residues longer than the WI38-counterpart.¹² Moreover, depending on whether exon 4 (coding for 26 amino acid residues) is incorporated or not, both HeLa- and WI38-l-CaD further exist in type-I and type-II, respectively, giving rise to a total of 4 isoforms of human l-CaD.¹³ Exon 1, however, is never transcribed in h-CaD. During embryogenesis h-CaD appears at a later stage than do other smooth muscle markers such as smooth muscle actin,¹⁴ but more parallel to smooth muscle α -Tm (Tm6).¹⁵ It is generally assumed that h-CaD is a component of the contractile apparatus and l-CaD is a cytoskeleton protein.^{4,16,17} The difference between the two isoforms is a highly charged repeating sequence, encoded by exon 3b and exon 4, that is only present in h-CaD (Fig. 1). This region forms a 35-nm long single helix (see below) known as the "spacer", which separates the more compact N-terminal myosin-binding domain from the C-terminal actin-binding domain of CaD.¹⁸ It is curious why only h-CaD, but not l-CaD, needs such a spacer region. Presumably, the distance between the two end-domains in h-CaD is evolutionally optimized to *fit* the specific spatial arrangement of myosin molecules in the smooth muscle thick filament. Such a requirement may not be present in nonmuscle cells. Recently, the expression of smooth muscle CaD was successfully abrogated in mice without blocking the expression of nonmuscle CaD.¹⁹ Such a strategy was achieved by disrupting the CaD gene (on mouse chromosome 6) at exon 3 beyond the splicing site. The homozygous mice thus obtained indeed lack h-CaD, but still express l-CaD. The expression level varies with the tissue type: In tonic vascular smooth muscles the level of l-CaD is low in both the homozygote and the wild-type, whereas in the visceral smooth muscles of homozygous animals l-CaD is much more over-expressed. This appears to be true both at the protein level (by Western blot analysis; Fig. 2) and at the message level.¹⁹ The fact that nonmuscle CaD is apparently able, at least in certain tissues, to "substitute" for h-CaD blurs the boundary between the contractile and cytoskeletal nature of the two isoforms; it also raises questions about the function of CaD in nonmuscle cells.

Smooth muscle cells gradually lose the contractile phenotype upon culturing and become dedifferentiated, fibroblast-like cells; at the same time, h-CaD is replaced by l-CaD.^{20,21} Although a number of smooth muscle specific proteins undergo such a differentiation-dependent isoform switchover, including actin,²² myosin heavy chain,²³ calponin,²⁴ vinculin²⁵ and Tm,²⁶ the expression of CaD and Tm are probably most closely related to each other. In fact, the same splicing machinery appears to work on both CaD and Tm, as the two α -Tm isoforms (α -Tm-SM or Tm6 and α -Tm-F1/2 or Tm2; for Tm nomenclature, see Gunning et al²⁷) are expressed in a tightly coordinated fashion with the two isoforms of CaD both in vivo and in vitro.^{26,28} Interestingly, when cells were forced to express Tm1 but not Tm2, there was also an up-regulation of CaD expression.²⁹ It has been shown that a serum response factor (SRF) is necessary but not sufficient to transactivate the CaD promoter.³⁰ Whether the same or additional factors are recruited for the Tm promoter is not known.

Many actin-binding proteins, including CaD³¹ and Tm,^{32,33} are down-regulated in transformed cells and in certain, although not all, types of cancerous cells. In a sense, these ABPs may act as tumor suppressors. On the other hand, CaD has also been found to be a marker for other types of cancers.^{34,35} HeLa-CaD, for example, was found in a number of tumor cells.³⁶⁻³⁸ While the antibodies specific for the exon 1 of HeLa-CaD could serve as useful tools for diagnostic purpose, it is rather surprising that the replacement of the leading peptide in the N-terminus would make such a functional difference.

Cellular Content of h-CaD

There has been a controversy over whether the h-CaD content varies among different types of smooth muscles.^{39–42} Using immuno-precipitated whole muscle extracts, quantified by Coomassie Blue staining in combination with Western blot analysis, Haeberle et al³⁹ found that phasic smooth muscles (rat uterus and guinea pig taenia coli, where CaD : actin = 1 : 22–28) have more CaD (8- and 5-fold using actin and myosin as references, respectively) than do tonic vascular smooth muscles (bovine aorta and porcine carotid artery). Lehman et al⁴² on the other hand, reported that the two types of smooth muscles (sheep and rabbit aorta, versus rabbit stomach and uterus and chicken gizzard) have about the same level of CaD (~1 mol CaD per 34 mol actin or 5 mol Tm) in either isolated native thin filaments, or heat-treated or unfractionated tissue extracts. It was thought that immuno-quantification of CaD from different species could be difficult owing to differential reactivity toward the antibodies;⁴¹ in the meantime, fractionation could also have left some proteins unaccounted for.⁴⁰ Still, the discrepancy is puzzling, because both groups seem to have had adequate controls and exercised stringent precautions. A simple, although unlikely, explanation is that different tissues were used. Other potential causes for the apparent disagreement include the estimated amount of reference proteins (such as actin) and differential degradation of CaD.

It was reported that the h-CaD content was more than twice higher in phasic opossum esophageal body than in the tonic sphincter.⁴³ Mice also have a lower h-CaD level in tonic vascular smooth muscles (aorta) than in visceral smooth muscles (bladder; Fig. 2). By Western blot analysis the ratios of h-CaD to β -actin staining in bladder and aorta were found to be 2.1 ± 0.4 and 0.33 ± 0.02 , respectively. This would be more in agreement with Haeberle et al.³⁹ In addition, the nonmuscle isoform was also present in the smooth muscle tissues that were devoid of endothelial cells. In mouse aorta the amount of l-CaD was ~25% of that of h-CaD, but much less (<10% of h-CaD) in the urinary bladder. Previously, Gluhkova et al⁴⁴ had reported that 78.2% of the total immunoreactive CaD (both isoforms) in the media of human aorta was attributable to h-CaD, leaving 21.8% (or 27.8% of h-CaD) as l-CaD, in good agreement with these data. Interestingly, Lehman et al⁴² also noted that they found a “degradation fragment” of 75 kDa in an unfractionated aorta sample, which may very well be l-CaD. Whether such a peptide had contributed to their overall estimation of CaD content is an intriguing question.

Since h-CaD is known to play an inhibitory role during muscle contraction, the higher expression level of h-CaD in visceral smooth muscles may be necessary to suppress contractility after reaching peak force, giving rise to the characteristics of transient contraction (hence phasic). When the expression of h-CaD was abolished in mice, l-CaD became up-regulated only in phasic muscles.¹⁹ This may suggest that CaD (of either isoform) is “indispensable” in phasic smooth muscles and that l-CaD can at least partially substitute h-CaD for its function. On the other hand, tonic smooth muscles, which contain less h-CaD in the wild-type, did not show isoform switchover in the h-CaD-deficient animals, probably because the basal level of l-CaD was sufficient to carry out h-CaD’s function. Although it is conceivable that the actin cytoskeleton exists in all cell types, the exact role of l-CaD in terminally differentiated smooth muscle cells remains an interesting subject of further studies.

Tissue Distribution and in Situ Localization

Immunostaining of isolated thin filaments from chicken gizzard indicated that h-CaD binds actin filaments with a periodicity of 38 nm,⁴⁵ similar to that of Tm. However, since the total CaD content is much less than that of actin (actin:Tm:CaD = 28:4:1), a “nonsaturating” model was proposed with staggered binding of CaD to the smooth muscle thin filaments. Interestingly, in such an arrangement the N-terminal end of all CaD molecules would naturally appear on

the same side of an actin filament (Fig. 3).^{46,47} This type of binding mode may facilitate interactions with the neighboring myosin filament (see below), which is presumably assembled in a side-polar fashion.⁴⁸ Furthermore, in order for h-CaD to function as a regulatory protein, the CaD-containing thin filaments must be “appropriately” located on the actin lattice to interact with the much fewer thick filaments.⁴⁵ Indeed, *in situ* immunostaining of gizzard sections demonstrated that h-CaD is associated with a subset of actin filaments and there are regions containing both actin and Tm but void of CaD.⁴⁹ A closer examination of these images revealed that CaD is distributed in clusters.⁴⁶ Such clustering was seen both in the sections and in isolated native thin filaments, thus ruling out antibody inaccessibility and rebinding of dissociated CaD. The discontinuous distribution was also apparent both along the thin filaments and along the thick filaments. The simplest explanation is that the two kinds of filaments are not parallel to each other, but rather intersect at a small angle (Fig. 4).¹⁷ Since CaD is only needed in the regions that actin filaments overlap with myosin filaments, CaD would naturally appear in clusters along the actin filaments and, at the same time, along the myosin filaments as observed. This model accounts for the limited CaD needed to “saturate” actin and myosin present in much higher amounts and carry out its regulatory function. The obliquely arranged filaments also allow for a greater extent of shortening, which is characteristic for smooth muscles. The fact that smooth muscle myosin forms side-polar filaments^{48,50} that are torsionally flexible⁵⁰ further supports this model, as the nonparallel filaments could maximize their interactions and facilitate contraction. So far this model remains speculative, but should be testable by mathematical modeling based on the geometrical parameters and more precise force measurements.

Nonmuscle CaD also binds to actin filaments along with Tm. It was reported that both proteins exhibit the same periodicity (~36 nm) on the microfilaments isolated from cultured rat embryonic fibroblasts.⁵¹ Similar patterns of periodic distribution of CaD was also observed in human astroglia.⁵² It was noted that such “cross striation” was not seen in gizzard filaments because of the sparse distribution. The similarity of the periodicities for CaD and Tm is striking. This suggests the two proteins not only interact with each other, but are also functionally related. I-CaD and Tm (in most cases, the high molecular weight Tm, HMW-Tm, which includes Tm1, 2, 3 and 6)²⁷ are normally present in the stress fibers of nonmuscle cells, but characteristically excluded from stable focal adhesions. On the other hand, both proteins as well as myosin II are found in nascent focal contacts and more dynamic structures such as podosomes⁵³ and neuronal growth cones.⁵⁴ It was thought that these contractile proteins are involved in the regulation of actomyosin activities (see below) and their presence therefore indicates the loci of cellular contraction. In fibroblasts I-CaD distinguishes itself from myosin II and HMW-Tms by the fact that it is primarily associated with short actin filaments in the core of the podosomes,⁵³ as well as ruffling membranes.⁵⁵ Curiously, low molecular weight Tms (LMW-Tms),⁵⁶ along with other cytoskeleton proteins including myosin I,⁵⁷ are also found at cell leading edges and growth cones. More recently, it was reported that in osteoclasts LMW-Tms are present in the podosomes with Tm4 in the actin core and Tm5a/5b in the ring that encircles the podosome core.⁵⁸ These findings suggest that by targeting a separate pool of actin filaments I-CaD and LMW-Tm are involved in another type of contractile activity.

When the N-terminal fragment of CaD was expressed in CHO cells, the expressed protein appeared in the nucleus.⁵⁹ Whether the positively charged amino acid stretches in this region serve as a nuclear localization signal is an intriguing question that remains to be answered. More recently, HeLa-CaD was also found to be present in the nucleus of tumor cells.⁶⁰ This finding raises interesting questions, e.g., whether I-CaD is involved in the contractile activities or some other functions in the nucleus.

Direct Interaction between CaD and Tm

Colocalization of CaD and Tm implies these two proteins interact with each other. The direct interaction between CaD and Tm was shown by binding studies⁶¹ and by the salt-dependent enhancement in the viscosity of gizzard Tm in the presence of stoichiometric amounts of h-CaD.⁶² Such binding was reversed by calmodulin (CaM) in the presence of Ca^{2+} .^{63,64} This direct interaction may conceivably contribute to the observed increase in the inhibitory effect of CaD, although a more indirect route through actin cannot be ruled out.⁶⁵ Subsequently, it was determined that the Tm-binding sites were localized in the C-terminal region.^{66–70} This region of the last ~300 amino acid residues not only contains the actin- and the CaM-binding sites, but also harbors TnT-analogous sequences that interact with Tm.⁷¹ Apparently there are several peptide segments in this region that bind actin, Tm and CaM independently (see, for example, 72). The affinity between CaD and Tm at physiological ionic strength was estimated to be $2.5 \times 10^{-5} \text{ M}^{-1}$ and was enhanced by actin.⁷³ Based on further binding studies a model was proposed in which CaD binds Tm in an antiparallel manner at sites near Cys-190 (residue 201–227).⁷⁴ This region probably is fairly sticky, because it also interacts with calponin.⁷⁵

CaD and Tm enhance each other's affinity for actin. They also act synergistically on other actin binding proteins. For example, CaD and Tm together, but not separately, inhibit the actin-binding and actin-bundling activity of fascin, a protein involved in the formation of microspikes in cultured cells.⁷⁶ Clearly, the interaction between CaD and Tm plays a role in this effect, although the detailed mechanism requires further elucidation. In light of that I-CaD exhibits differential colocalizations with specific nonmuscle Tm isoforms (see above), it would also be interesting to compare the interactions between I-CaD and different Tm isoforms.

Molecular Shape and Domain Structure

Both h-CaD and Tm are elongated molecules. Hydrodynamic studies indicated that h-CaD is 74 nm in length,⁷⁷ about twice as long as Tm. While Tm forms an α -helical coiled-coil, h-CaD contains a long, single helix in the middle of the molecule.⁷⁸ It is interesting that this middle segment, which is encoded by exons 3b and 4 and is therefore missing in I-CaD, contains 11 repeats of a 15-amino acid residue motif of oppositely charged residues (Glu and Lys/Arg) and alanine. This unique sequence has long been thought to form a coiled-coil structure based on widely accepted algorithms (such as COILS⁷⁹ and Paircoil,⁸⁰) but the lack of cooperativity in the thermal unfolding that is characteristic to Tm⁸¹ clearly defies such a prediction. Instead, the periodically spaced charged residues form (i, i + 4) ion pairs and thus stabilize the single helical structure.⁸² Under the electron microscope, h-CaD appears to be a rather rigid dumbbell, with the two end-domains separated by a fixed distance of $60 \pm 3 \text{ nm}$.⁸³ The two domains at the N- and the C-termini are configurationally more compact, but lack well-defined secondary structures. They appear easily collapsible under rotary shadowing.⁸³ Indeed, structural studies showed the C-terminal region of CaD contains multiple β -turns, which allows for plenty of flexibility and facilitates docking on actin filaments.⁸⁴

Functionally, the C-terminal region of CaD harbors actin-, CaM- and Tm-binding sites,^{68,85–89} whereas the N-terminal domain contains the major myosin-binding sites^{90–92} and also interacts with CaM and actin.^{93,94} The actin- and myosin-binding capacity enables CaD to play a role in bringing the thin filaments close to the thick filaments (see below). Actin binding on both ends of CaD renders the molecule to lie down on the actin filament longitudinally. The fact that one of these two actin-binding sites is near the myosin-binding site is also consistent with the observation that myosin S-1 is able to change the orientation of bound CaD in the ternary complex without displacement.⁹⁵ However, the N-terminal actin-binding site apparently has a lower affinity for actin than the C-terminal ones and is heat labile.⁹⁶ After heat treatment, CaD no longer adheres to the actin filament, but only binds to the filament by

its C-terminal end, leaving the N-terminal end sticking out.⁹⁷ This type of binding mode, termed as the mosaic multiple-binding model,^{98,99} may also occur when CaD is added to actin in the absence of Tm under near saturating concentrations. That CaD contains multiple actin-binding sites (both in the full-length molecule and in the C-terminal fragment, see below) is consistent with its strong tendency to bundle actin filaments *in vitro*.^{100,101} Tm seems to promote the dissociation of such bundles,¹⁰² which could indicate that in the presence of Tm lengthwise binding is preferred.

CaD and Tm as Actin-Binding Proteins

The elongated shapes of CaD and Tm facilitate their binding to actin filaments as “side-binders”. Upon binding, they stabilize the filamentous structure, increase the rigidity of the filament¹⁰³ and synergistically protect against gelsolin-mediated severing.^{104,105} Tm is also known to enhance the affinity of CaD for actin by lowering the dissociation rate¹⁰⁶ and thereby to augment CaD’s inhibitory action on the actomyosin ATPase activity.

Binding of CaD on actin filaments was visualized by 3D reconstruction studies. Helical reconstruction of negatively stained filaments decorated with a C-terminal CaD fragment (H32K) demonstrated density attributable to H32K on subdomain 1 of actin, consistent with earlier reconstructions of full length CaD¹⁰⁷ and with biochemical data.⁸⁴ In the reconstructed image H32K is localized on the inner aspect of subdomain 1, but instead of binding along the same genetic strand, it traverses the upper surface of the subdomain towards actin’s C-terminus and forms a bridge to the neighboring actin monomer of the adjacent long-pitch helical strand, by connecting to its subdomain 3 (Fig. 5), thus “stapling” the two strands of actin filaments.¹⁰⁸ This “staple-like” binding mode suggests a mechanism by which CaD could stabilize actin filaments and resist F-actin severing or depolymerization in both smooth muscle and nonmuscle cells. In the image of ERK-phosphorylated H32K, which is less effective in inhibiting the ATPase (see below), the density over subdomain 1 is much less and the inter-strand connectivity is lost. The two-pronged attachment of CaD on actin was supported by intramolecular FRET coupled with mass spectrometric analysis.¹⁰⁹ It was shown that within the C-terminal region of CaD there exist multiple actin-binding segments that form two clusters, each constituting one actin-attaching site; when H32K is phosphorylated by ERK, only one of the two actin-binding clusters dissociates from F-actin.

The above model was further tested by crosslinking experiments using chicken mutants, H32Kqc (with Q766 replaced by Cys, thus containing 2 Cys) and H32Kqc/ca (a double mutant with the endogenous Cys595 also replaced by Ala; containing only one Cys at position 766). When both Cys residues were labeled with a photo-crosslinker, benzophenone maleimide, H32Kqc was capable of crosslinking actin subunits to form high molecular weight adducts, indicating that it spans at least 2 actin subunits when bound to F-actin. ERK-phosphorylated H32Kqc, however, no longer crosslinks actin to polymers.¹⁰⁸ Similar results were obtained by disulfide crosslinking between H32Kqc and actin (Fig. 6). The single-Cys mutant H32Kqc/ca only crosslinks to one actin monomer; such crosslinking is also diminished after phosphorylation by Erk. Furthermore, acrylamide quenching experiments showed that the solvent accessibility of probes attached to Cys766, but not to Cys595, was increased by ERK treatment.¹⁰⁸ These results are consistent with a phosphorylation-dependent conformational change that moves the C-terminal segment of CaD, but not the other binding site, away from F-actin, conferring the observed removal of its inhibitory effect.

The presence of multiple actin-binding sites in the C-terminal region is again consistent with the observation that even the CaD fragment bundles actin filaments.¹¹⁰ The bundles formed by the fragment, however, are tight and straight, unlike the loose bundles derived from the full-length CaD (Fig. 7), indicating the relatively close proximity between the actin-binding sites

in the fragment. Interestingly, it was noted that the bundling activity of the C-terminal half of CaD was diminished by the addition of the N-terminal fragment.¹¹¹ Although this may have been a charge effect exerted by the acidic N-fragment, as seen with polyanionic peptides,¹¹² it could also be due to the N-terminal actin-binding site. That the C-terminal fragment of CaD binds two neighboring actin subunits also explains CaD's ability to act as a nucleator that promotes actin polymerization under the conditions actin normally exists as monomers.¹¹³ ¹¹⁴ On the other hand, we (Huang and Wang, unpublished observations) and others (Matsumura, personal communication) have found that CaD imposes some inhibition on actin polymerization when monitored by the pyrene fluorescence of labeled actin, although only a minimal effect was reported earlier.¹¹⁵ This apparent paradox and how a "side-binder" inhibits the polymerization process remains to be reconciled.

Biochemical Properties and Regulatory Functions

Smooth muscle CaD inhibits the actomyosin ATPase activity¹¹⁶ and Tm enhances such an inhibitory effect. A similar inhibitory effect of nonmuscle CaD has also been observed in resting platelets.¹¹⁷ The molecular mechanism of the CaD-Tm inhibition has been a subject of great interest (see also Chapter 9). It has been shown that in the presence of Tm, the actomyosin ATPase activity was inhibited to a maximum (>80%) at a 1:10–13 molar ratio of CaD to actin monomer compared to only 50% inhibition for CaD alone.^{101,118} This is not only because the enzymatic activity of actomyosin is enhanced by Tm owing to its activating effect at the myosin concentration used,^{119–121} but more importantly, because there is a synergistic effect of Tm on CaD's action. CaD exerts its inhibitory effect by decreasing the $V_{\max}$ ¹²² and/or increasing the K_{ATPase} .¹²³ The latter is most likely due to a direct competition between CaD and weak myosin binding to actin (in the presence of ATP)¹²⁴ as their binding sites on the actin surface overlap.¹⁰⁷ It has been shown that competition between CaD and myosin alone can give rise to an apparent cooperative activation of the thin filament even in the absence of Tm,¹²⁵ although Tm enhances such a cooperativity.¹²⁶ This Competition Model, however, has been disputed, because there was a disagreement whether the concentration of CaD needed for the inhibition of myosin binding matches that for the inhibition of the ATPase activity.¹²⁷ Indeed it takes "unphysiologically" high ($[\text{CaD}] : [\text{actin}] > 1 : 7$) amounts of CaD to show a sigmoid-shaped binding isotherm, although there seems no such requirement to slow the myosin binding rate in the kinetic experiments.¹²⁶ On the other hand, it was argued that the reduced binding rate could be balanced out by a change in the dissociation rate, yielding a moderate (9%) overall maximum rate reduction that fails to account for the observed level of inhibition (75%) of the ATPase unless a higher concentration of CaD was used.¹²⁸ The discrepancy is difficult to reconcile, not only because of the subtle differences in the experimental conditions (such as ionic strength and temperature), but also due to the intrinsically complicated nature of the system that involves multiple, mutually interacting, protein species. However, it might be worthwhile to point out that the cellular contents of CaD and actin only give an averaged ratio between the two. Knowing that CaD has a clustered distribution in the smooth muscle,⁴⁶ one cannot rule out the possibility of a higher local concentration of CaD. Moreover, it is well known that there is a minimal level (10–15%) of myosin phosphorylation before tension is detected in smooth muscles.¹²⁹ Whether the blockage of myosin binding by CaD causes this threshold, which resembles the simulated cooperativity,¹²⁶ awaits further investigation.

An alternative interpretation of CaD's inhibition is primarily based on the observed change in the catalytic activity, which can be best explained by a switch of the thin filament state. Like troponin (Tn) in the striated muscle system, CaD at relatively low concentrations may alter the position of Tm on the actin filament depending on other regulatory factors such as Ca^{2+} /CaM or phosphorylation and thereby modulate the actomyosin interaction in a cooperative fashion.¹³⁰ So it can be Tm, instead of CaD, that blocks the binding of myosin. This Cooperative-Allosteric Model is supported by both structural¹⁰⁷ and biochemical data.^{127,128} Two important

pieces of evidence are that at low ratios (1:14) to actin, CaD was able to compete with not only weak, but also strong, myosin binding to actin¹³¹ and that CaD was able to decrease the population of moving filaments without much changing the velocity in the in vitro motility assay.¹³² These observations are indeed difficult to explain by the pure Competition Model. While in many cases CaD behaves similarly to Tn, there are differences. Kinetic experiments (using skeletal S1)¹²⁸ showed that CaD affects the cross-bridge cycling by mainly slowing down the rate of phosphate release, rather than that of the ADP release as in the case of Tn-Tm.¹³³ These results also suggested that CaD moves Tm between two states (open and closed), rather than three states (including a blocked state) as in the skeletal muscle system.¹³⁴ On the other hand, CaD itself may constitute a blocked state in smooth muscle as implied by the Competition Model and structural studies (see below).

Since CaD abolishes the effect of Tm to enhance the actomyosin ATPase activity (at high myosin concentrations) without dissociating bound Tm from F-actin,¹¹⁷ it was thought that CaD could change the state of bound Tm along the actin filament, a conclusion reached indirectly by several other investigators.^{61,118,135} This structural effect was later visualized in the 3D reconstruction from electron microscopic images of the smooth muscle thin filament. It was found that CaD “keeps” Tm at a position that is close to the inner domain of the actin subunit where it is away from the major myosin binding sites.¹³⁶ Upon addition of $\text{Ca}^{2+}/\text{CaM}$, CaD (the C-terminal part) is dissociated from the filaments and at the same time, Tm becomes dislodged and rolls to the outer domain of actin surface that usually is considered as the “closed” position.¹³⁷ While these studies confirm that CaD indeed alters the position of Tm on actin, such a movement is in sharp contrast to what has been observed for Tn-Tm in the striated muscle system,¹³⁸ suggesting that CaD regulates the actomyosin ATPase activity in a fashion different from that of Tn. The finding that Tm is situated in a position opposite to that predicted by the actomyosin ATPase activity in both activated and resting states is also surprising. This apparent paradox was reconciled as follows: In the resting state smooth muscle Tm lies in a position partially overlapping the strong myosin binding site, while CaD sterically blocks the weak myosin binding and results in inhibition.¹⁰⁷ When activated by $\text{Ca}^{2+}/\text{CaM}$, CaD is locally dissociated, allowing Tm to move to a closed position. In that position active myosin heads can move Tm to the open position, thereby activating neighboring actin subunits.¹³⁹ Smooth muscle Tm has a strong end-to-end interaction;¹⁴⁰ it would be easier for myosin to turn on more actin subunits than in the striated muscle, conferring a higher cooperativity. Thus it seems both the Competitive Model and Allosteric Model are partly correct. By either mechanism, CaD negatively regulates the actomyosin interaction and acts as a molecular brake.¹⁴¹

CaD also Binds to Myosin

Unlike Tm, CaD also interacts with myosin.^{142,143} The primary myosin-binding sites are localized in the N-terminal region of CaD,⁹² although a secondary site in the C-terminal region has also been reported.¹⁴⁴ The interaction site on myosin has been identified as being in the S-2 region.¹⁴² Smooth muscle myosin is known to undergo a conformational change upon phosphorylation.^{145,146} In the unphosphorylated state the myosin heads tend to fold back to the rod and assumes a compact configuration (the so-called 10S form). Upon phosphorylation the molecule prefers an extended configuration (the 6S form). The folded form of unphosphorylated myosin is enzymatically inactive and unable to form filaments. Electron microscopic imaging showed that the myosin heads in this inactive configuration “droop” down, whereas they are pointing more upward in the extended, active 6S configuration.¹⁴⁷ The heads-down configuration of the relaxed myosin was also demonstrated in a recent 3D image reconstruction of invertebrate thick filament,¹⁴⁸ where the unphosphorylated myosin heads appear to interact with the S2 region of the rod. Such potential interactions may be disrupted by the N-terminal region of CaD, shifting the equilibrium toward the “heads-up” configuration, thereby “activating” the myosin head without phosphorylation (Fig. 8). Indeed, it was observed

that CaD promotes filament assembly of unphosphorylated myosin under the conditions that myosin otherwise stays soluble.¹⁴⁹ We have found that the actin-activated ATPase activity of unphosphorylated smooth muscle heavy meromyosin is increased by the N-terminal CaD fragment (by 46%), but not by the C-fragment (Huang and Wang, unpublished results). However, this effect may not manifest itself as true activation, because the C-terminal region of CaD would inhibit the ATPase activity under a condition when myosin is unphosphorylated.

Binding of myosin via the N-terminal end of CaD and binding of actin via the C-terminal end enables cross-linking of actin filaments to myosin filaments.¹⁵⁰ This may be structurally important for the maintenance of the intracellular filamentous organization, considering that smooth muscle cells do not contain sarcomeric compartments. At a low CaD concentration the tethering effect actually recruits myosin molecules and promotes *in vitro* motility.¹⁵¹ The interaction between CaD and myosin is rather weak ($K_a \sim 10^5 \text{ M}^{-1}$),⁹² so that it does not impede the sliding movement of the filaments during contraction; on the other hand, the binding energy may be sufficient to dictate the localization of CaD and give rise to a discontinuous distribution.¹⁷ It is also conceivable that the latch phenomenon known for tonic type of smooth muscles could result from the simultaneous binding of CaD to both actin and myosin.¹⁵² That the unphosphorylated myosin exerts a mechanical load to shortening filaments suggests that tethering of thick and thin filaments by CaD might help in maintaining some basal force such as the vascular tone under resting conditions.¹⁵³ On the other hand, antisense knockdown of h-CaD in arterial smooth muscles showed that after the CaD content was decreased (by 78%) upon antisense oligodeoxynucleotide treatment, there was an apparent 62% decrease in contractility.¹⁵⁴ This was interpreted as an increased basal tone due to the loss of h-CaD. If true, this observation would imply that smooth muscle regulation includes a thin-filament-based disinhibition component by h-CaD. The *in vivo* function of CaD may thus be both inhibitory for the active tension and maintaining the passive tension. In non-muscle cells l-CaD most likely plays a similar role to regulate the cellular tension.¹⁵⁵ For example, CaD has been suggested to be involved in cell blebbing.¹⁵⁶ This interesting aspect also deserves more investigation.

Phosphorylation as a Regulatory Mechanism

Nearly all cytoskeleton proteins are substrates of one or more kinases. Phosphorylation by intracellular kinases downstream of Ras and Rac may in fact be one common feature shared by many ABPs. Through phosphorylation the actin-binding properties of these ABPs are altered, thus allowing actin cytoskeleton remodeling. For CaD, the inhibitory effect is diminished upon phosphorylation.¹⁵⁷ In intact smooth muscle the level of CaD phosphorylation indeed increases upon stimulation¹⁵⁸ and the responsible kinase has been identified to be MAPK.^{159,160} Phosphorylation of CaD was also detected in cultured smooth muscle cells upon serum stimulation.¹⁶¹ Such phosphorylation was markedly reduced by the MEK inhibitor PD98059, but not by the p38 MAPK inhibitor SB203580,¹⁶¹ thus strongly pointing to ERK-induced phosphorylation during cell proliferation. With the use of mass spectrometry it was shown that phosphorylation at Ser759 and Ser789 of mammalian smooth muscle CaD (or Ser497 and Ser527 in nonmuscle CaD; Fig. 1) indeed dissociates one of the actin-binding sites of CaD and weakens its inhibitory effect on the actomyosin ATPase activity.¹⁰⁹ The regulatory role of CaD phosphorylation by MAPK in smooth muscle cells still remains controversial, because inhibition of MAPK failed to produce detectable differences in contractility of smooth muscle tissues;¹⁶² however, there is little doubt that phosphorylation of CaD is involved in nonmuscle cell division and locomotion. As shown by Yamboliev and Gerthoffer,¹⁶³ inhibition of ERK phosphorylation slowed down the PDGF-induced migration of cultured smooth muscle cells by 50%.

For nonmuscle CaD a number of pioneering discoveries on phosphorylation were made by Matsumura's group. Of particular interest are the findings that CaD transiently dissociates from actin filaments during mitosis¹⁶⁴ and that p34^{cdc2} is responsible for regulating this process.¹⁶⁵ In contrast to ERK, which phosphorylates CaD primarily at only 2 sites near one of the actin-binding sites, there are a total of 7 residues in the C-terminal region of CaD that can be phosphorylated by cdc2 kinase.^{166,167} These residues encompass both actin-binding sites, such that when all are phosphorylated CaD dissociates from actin completely.¹⁶⁸ Immunofluorescence images indicate that, in contrast to other actin-binding proteins such as myosin, α -actinin and Tm, CaD is not concentrated at the cleavage furrow until the later stages of cytokinesis.¹⁶⁹ Based on this finding, it was postulated that CaD inhibits activation of the contractile ring at early stages of assembly, presumably by blocking either the actomyosin interaction, or the severing activities of gelsolin, both being required for cytokinesis. Upon phosphorylation by cdc2 kinase, CaD dissociates from actin filaments, thus removing the inhibitory effects and allowing activation of the contractile ring. Prevention of CaD phosphorylation at all potential cdc2 sites by mutagenesis indeed slows down progression through the cell cycle.^{156,170} More direct evidence for CaD phosphorylation in individual cells at various cell cycle stages was obtained by immuno-staining studies. Anti-phosphopeptide antibodies revealed a dynamic change of CaD phosphorylation throughout the cell cycle progression, in a manner that is reciprocal to the change of actin stress fibers.¹⁷¹ These results suggest that phosphorylation of CaD has a broad functional significance. It is more likely to be involved in the change of cell shape during cell division as well as in postmitotic spreading. Interestingly, the same residues of the major phosphorylation sites during mitosis¹⁷² are also phosphorylated when cultured smooth muscle cells are stimulated to migrate.^{173,174} Since both ERK and cdc2 kinase are simultaneously activated under a wide range of conditions,^{175–177} CaD phosphorylation may thus provide a common mechanism to link cell proliferation and cell migration, both requiring cell shape changes.

When the cdc2-sites or the ERK-sites were blocked by mutagenesis, cell division and detachment were slowed down, but not stopped,^{171,178} indicating that there must be alternative pathways that cells can adopt to circumvent the impediment. Indeed, CaD is also known to be phosphorylated by the p21-activated protein kinase (PAK).¹⁷⁹ The major PAK-phosphorylated sites (Ser672 and Ser702, in the chicken smooth muscle sequence)¹⁸⁰ are also in the C-terminal region, but different from the ERK sites. It was reported¹⁸¹ that constitutively active PAK causes Ca²⁺-independent contractility in smooth muscle fibers accompanied by an increase in the level of CaD phosphorylation at the same position as Ser672 in the chicken sequence. When these residues were mutated to Ala, CaD appeared to accumulate at cell leading edges, suggesting a potential role for phosphorylation in cell motility. Since PAK is a downstream effector in the Rac1/Cdc42 signaling pathways¹⁸² under a variety of agonist stimulations, it is not at all surprising that PAK-induced phosphorylation affects migratory behaviors. Whether CaD is indeed subject to PAK regulation and the structural and functional consequences of this are of great interest. These issues have been addressed in parallel with the investigation of ERK-phosphorylation. An interesting cellular feature is the formation of podosomes, which exist in many cell types including smooth muscle cells.^{183,184} In A7r5 cells podosomes are formed upon stimulation with phorbol dibutyrate (PDBu), which activates PKC. Interestingly, l-CaD is present in such podosomes¹⁸⁵ and is phosphorylated at ERK sites.¹⁸⁶ By over-expressing a phosphorylation-deficient CaD-GFP mutant it was found that, although both the size and the lifetime of the podosomes are modulated by ERK-mediated CaD phosphorylation, the modes of ERK and CaD in their modulation of the formation and dynamics of podosomes are different.¹⁸⁶ This suggests involvement of other players in addition to ERK. It turned out that the formation of podosomes was more tightly correlated with PAK-mediated phosphorylation of CaD.¹⁸⁷ It was suggested that PAK phosphorylation renders CaD more effective in displacing Arp2/3¹¹⁵ and thereby increases its ability to inhibit podosome formation. Such an effect should be readily testable by biochemical experiments. Another

notable finding in these studies is that ERK-phosphorylated CaD (although not so clear about PAK-phosphorylated CaD) is localized at the ring of podosomes¹⁸⁶ where Tm5a/5b was found in the osteoclasts.⁵⁸ Whether such colocalization suggests interactions or common targets is worthy of further investigation.

Nonmuscle CaD and Its Roles During Cell Proliferation and Migration

Over-expression of CaD in nonmuscle cells has led to a number of conflicting phenotypes including stabilized or disrupted stress fibers, increases or decreases of focal adhesions and podosome structures, hampered or unaffected cell division process and enhanced or compromised cell motility.^{155,156,171,185,188–192} These interesting yet confusing results may have stemmed from (i) the difference of cell types used, (ii) different stages of cells and (iii) different levels of over-expression. Nevertheless, several observations are particularly noteworthy. Antisense oligonucleotide targeted to CaD dramatically inhibited hormone-induced stress fiber formation.¹⁹³ Transfection of adenovirus l-CaD suppressed the growth and survival in vascular smooth muscle cells and inhibited neointimal formation after angioplasty.¹⁹⁴ Most recently it has been shown that ectopic expression of l-CaD suppresses the invasive activity of cancer cells.¹⁹⁵

Based on the biochemical properties of CaD, it may be assumed that its primary function is to stabilize the actin filaments. Depending on the phosphorylation states and differential affinities toward HMW and LMW Tm isoforms, CaD may target different pools of actin filaments. Over-expression of CaD would be expected to reinforce the actin cytoskeleton. However, excess CaD may displace crosslinked actomyosin bundles and thereby disrupt stress fibers. Similarly, properly regulated CaD should enhance the actin meshwork and promote cell movement, but too much unphosphorylated CaD, as seen in the case of over-expression of the nonphosphorylatable mutant,¹⁷¹ cell adhesion could be stifled and both cell migration and cell proliferation could be slowed down. This is consistent with the finding that during cell division l-CaD undergoes phosphorylation, which weakens actin-binding. Since Rho activation induces cell adhesion, it had been suggested that focal adhesion involves actomyosin-based contractility.¹⁹² What was not addressed is how much CaD in the cell was phosphorylated. It was observed that phosphorylated, but not unphosphorylated, CaD colocalizes with vinculin.¹⁷¹ It is thus possible that phospho-CaD recruits essential partners to the newly formed focal adhesions and this process may be blocked by unphosphorylated CaD when expressed in large quantities. By suppressing the expression of CaD in cells and monitoring the adhesion and migration properties, one may be able to assess its overall functions. Interestingly, hampered cytokinesis was also observed upon over-expression of a HMW-and-LMW chimeric nonmuscle Tm that exhibited a much higher affinity toward actin than the wild-type Tm,¹⁹⁶ indicating that disassembly of microfilament organization is an essential step during cell division. Evidently, both CaD and Tm are part of stabilizing factors for the actin cytoskeleton.

In addition to cell division and migration, a number of other functions for l-CaD have been previously suggested. It was reported¹⁹⁷ that a monoclonal antibody against the C-terminal region of CaD inhibited granular movement in fibroblast cells, similar to the effect of anti-Tm antibodies,¹⁹⁸ once again indicating that both CaD and Tm are involved in the organization of the cytoskeleton and trafficking. Furthermore, CaD is associated with Grb2, Shc and Sos; in this complex CaD was found to be tyrosine-phosphorylated.^{199,200} More recently, it has also been suggested that the interaction between phospho-CaD and Tm is further influenced by Hsp27.²⁰¹ The significance of all these findings awaits further investigation.

Future Directions

Cytoskeleton remodeling involves numerous actin-binding proteins. The fact that all of them including CaD contain multiple target-specific domains suggests that cytoskeleton proteins

work together as partners. CaD most likely works with other protein components in addition to Tm for synergistic maintenance and regulation of actin filament function. Many of these binding partners and structural/functional consequences of such interactions remain to be identified. Phosphorylation is also likely to have an effect on the interactions. One example is cortactin, which is an ABP mainly found in the cell cortex.²⁰² Cortactin is known to bind Arp2/3²⁰³ and also interacts with CaD.²⁰⁴ Not only was binding of CaD to cortactin demonstrated in vitro, but the two proteins were also shown to colocalize in cells at the cortex, where CaD was found to be phosphorylated.¹⁷¹ The functional significance of this interaction needs further elucidation. Another prominent candidate is Arp2/3, which competes with CaD for actin binding.¹¹⁵ Since cortactin promotes Arp2/3-mediated actin polymerization, it would be of interest to see how the interactions among CaD, cortactin and Arp2/3 play out in this process and what ERK-or PAK-phosphorylation would do to them. Myosin may also serve as a partner. Nonmuscle myosin II, like smooth muscle myosin, is activated through light chain phosphorylation.²⁰⁵ It is possible that the N-terminal region of CaD binds the S2 region and stabilizes the elongated configuration of unphosphorylated myosin II in nonmuscle cells as well. Whether and how this interaction affects the behaviors of nonmuscle cells is not known and should also be tested.

All in all, the role of CaD in the regulation of actin cytoskeleton is still a fertile field. With recent advancement in cell biology, it is timely to perform more detailed biochemical and biophysical studies on cytoskeleton proteins. In light of the fact that both CaD and Tm are involved in cell growth and cell motility, new information should enhance our understanding of cell proliferation and metastasis and lead to development of novel therapeutic agents for the treatment of cancers.

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References

1. Pollard TD, Borisy GG. Cellular motility driven by assembly and disassembly of actin filaments. *Cell* 2003;112:453–465. [PubMed: 12600310]
2. Winder SJ. Structural insights into actin-binding, branching and bundling proteins. *Current Opinion in Cell Biology* 2003;15:14–22. [PubMed: 12517699]
3. Huber PA. Caldesmon. *Int J Biochem Cell Biol* 1997;29:1047–1051. [PubMed: 9415999]
4. Marston, SB.; Huber, PAJ. Caldesmon. In: Bárány, M., editor. *Biochemistry of Smooth Muscle Contraction*. San Diego, CA: Academic Press, Inc; 1996. p. 77-90.
5. Matsumura F, Yamashiro S. Caldesmon. *Curr Opin Cell Biol* 1993;5:70–76. [PubMed: 8448032]
6. Wang C-LA. Caldesmon and smooth-muscle regulation. *Cell Biochem Biophys* 2001;35:275–288. [PubMed: 11894847]
7. Hai CM, Gu Z. Caldesmon phosphorylation in actin cytoskeletal remodeling. *Eur J Cell Biol* 2006;85:305–309. [PubMed: 16546574]
8. Humphrey MB, Herrera-Sosa H, Gonzalez G, et al. Cloning of cDNAs encoding human caldesmons. *Gene* 1992;112:197–204. [PubMed: 1555769]
9. Ueki N, Sobue K, Kanda K, et al. Expression of high and low molecular weight caldesmons during phenotypic modulation of smooth muscle cells. *Proc Natl Acad Sci USA* 1987;84:9049–9053. [PubMed: 3321066]
10. Paul ER, Ngai PK, Walsh MP, et al. Embryonic chicken gizzard: expression of the smooth muscle regulatory proteins caldesmon and myosin light chain kinase. *Cell Tissue Res* 1995;279:331–337. [PubMed: 7895272]

11. Bretscher A, Lynch W. Identification and localization of immunoreactive forms of caldesmon in smooth and nonmuscle cells: a comparison with the distributions of tropomyosin and alpha-actinin. *J Cell Biol* 1985;100:1656–1663. [PubMed: 2985624]
12. Novy RE, Lin JL, Lin JJ. Characterization of cDNA clones encoding a human fibroblast caldesmon isoform and analysis of caldesmon expression in normal and transformed cells. *J Biol Chem* 1991;266:16917–16924. [PubMed: 1885618]
13. Hayashi K, Yano H, Hashida T, et al. Genomic structure of the human caldesmon gene. *Proc Natl Acad Sci USA* 1992;89:12122–12126. [PubMed: 1465449]
14. Duband JL, Gimona M, Scatena M, et al. Calponin and SM 22 as differentiation markers of smooth muscle: spatiotemporal distribution during avian embryonic development. *Differentiation* 1993;55:1–11. [PubMed: 8299876]
15. Vrhovski B, McKay K, Schevzov G, et al. Smooth Muscle-specific {alpha} Tropomyosin Is a Marker of Fully Differentiated Smooth Muscle in Lung. *J Histochem Cytochem* 2005;53:875–883. [PubMed: 15995146]
16. Matsumura F, Yamashiro S. Caldesmon. *Curr Opin Cell Biol* 1993;5:70–76. [PubMed: 8448032]
17. Wang C-LA. Caldesmon and smooth-muscle regulation. *Cell Biochem Biophys* 2001;35:275–288. [PubMed: 11894847]
18. Wang C-LA, Chalovich JM, Graceffa P, et al. A long helix from the central region of smooth muscle caldesmon. *J Biol Chem* 1991;266:13958–13963. [PubMed: 1856225]
19. Guo H, Wang C-LA. Specific disruption of smooth muscle caldesmon expression in mice. *Biochem Biophys Res Commun* 2005;330:1132–1137. [PubMed: 15823561]
20. Owada MK, Hakura A, Iida K, et al. Occurrence of caldesmon (a calmodulin-binding protein) in cultured cells: comparison of normal and transformed cells. *Proc Natl Acad Sci USA* 1984;81:3133–3137. [PubMed: 6328499]
21. Dingus J, Hwo S, Bryan J. Identification by monoclonal antibodies and characterization of human platelet caldesmon. *J Cell Biol* 1986;102:1748–1757. [PubMed: 3517005]
22. Owens GK, Loeb A, Gordon D, et al. Expression of smooth muscle-specific alpha-isoactin in cultured vascular smooth muscle cells: relationship between growth and cytodifferentiation. *J Cell Biol* 1986;102:343–352. [PubMed: 3944187]
23. Rovner AS, Murphy RA, Owens GK. Expression of smooth muscle and nonmuscle myosin heavy chains in cultured vascular smooth muscle cells. *J Biol Chem* 1986;261:14740–14745. [PubMed: 3533925]
24. Shanahan CM, Weissberg PL, Metcalfe JC. Isolation of gene markers of differentiated and proliferating vascular smooth muscle cells. *Circ Res* 1993;73:193–204. [PubMed: 8508530]
25. Volberg T, Sabanay H, Geiger B. Spatial and temporal relationships between vinculin and talin in the developing chicken gizzard smooth muscle. *Differentiation* 1986;32:34–43. [PubMed: 3096800]
26. Kashiwada K, Nishida W, Hayashi K, et al. Coordinate expression of alpha-tropomyosin and caldesmon isoforms in association with phenotypic modulation of smooth muscle cells. *J Biol Chem* 1997;272:15396–15404. [PubMed: 9182570]
27. Gunning PW, Schevzov G, Kee AJ, et al. Tropomyosin isoforms: divining rods for actin cytoskeleton function. *Trends Cell Biol* 2005;15:333–341. [PubMed: 15953552]
28. Sobue K, Hayashi K, Nishida W. Expressional regulation of smooth muscle cell-specific genes in association with phenotypic modulation. *Mol Cell Biochem* 1999;190:105–118. [PubMed: 10098977]
29. Shah V, Bharadwaj S, Kaibuchi K, et al. Cytoskeletal organization in tropomyosin-mediated reversion of ras-transformation: Evidence for Rho kinase pathway. *Oncogene* 2001;20:2112–2121. [PubMed: 11360195]
30. Momiyama T, Hayashi K, Obata H, et al. Functional involvement of serum response factor in the transcriptional regulation of caldesmon gene. *Biochem Biophys Res Commun* 1998;242:429–435. [PubMed: 9446812]
31. Cerda-Nicolas M, Lopez-Gines C, Gil-Benso R, et al. Solitary fibrous tumor of the orbit: morphological, cytogenetic and molecular features. *Neuropathology* 2006;26:557–563. [PubMed: 17203593]

32. Leonardi CL, Warren RH, Rubin RW. Lack of tropomyosin correlates with the absence of stress fibers in transformed rat kidney cells. *Biochim Biophys Acta* 1982;720:154–162. [PubMed: 6282338]
33. Ryan MP, Higgins PJ. Cytoarchitecture of Kirsten sarcoma virus-transformed rat kidney fibroblasts: butyrate-induced reorganization within the actin microfilament network. *J Cell Physiol* 1988;137:25–34. [PubMed: 2844835]
34. Watanabe K, Kusakabe T, Hoshi N, et al. h-Caldesmon in leiomyosarcoma and tumors with smooth muscle cell-like differentiation: its specific expression in the smooth muscle cell tumor. *Hum Pathol* 1999;30:392–396. [PubMed: 10208459]
35. Comin CE, Dini S, Novelli L, et al. h-Caldesmon, a Useful Positive Marker in the Diagnosis of Pleural Malignant Mesothelioma, Epithelioid Type. *Am J Surg Pathol* 2006;30:463–469. [PubMed: 16625092]
36. Zheng PP, van der Weiden M, Kros JM. Differential expression of Hela-type caldesmon in tumour neovascularization: a new marker of angiogenic endothelial cells. *J Pathol* 2005;205:408–414. [PubMed: 15682433]
37. Zheng PP, Hop WC, Sillevius Smitt PA, et al. Low-molecular weight caldesmon as a potential serum marker for glioma. *Clin Cancer Res* 2005;11:4388–4392. [PubMed: 15958622]
38. Zheng PP, Luidier TM, Pieters R, et al. Identification of tumor-related proteins by proteomic analysis of cerebrospinal fluid from patients with primary brain tumors. *J Neuropathol Exp Neurol* 2003;62:855–862. [PubMed: 14503641]
39. Haeberle JR, Hathaway DR, Smith CL. Caldesmon content of mammalian smooth muscles [see comments]. *J Muscle Res Cell Motil* 1992;13:81–89. [PubMed: 1556173]
40. Haeberle JR, Hathaway DR. Correspondence. *J Muscle Res Cell Motility* 1992;13:584–585.
41. Lehman W, Denault D. Correspondence. *J Muscle Res Cell Motility* 1992;13:582–583.
42. Lehman W, Denault D, Marston S. The caldesmon content of vertebrate smooth muscle. *Biochim Biophys Acta* 1993;1203:53–59. [PubMed: 8218392]
43. Szymanski PT, Chacko TK, Rovner AS, et al. Differences in contractile protein content and isoforms in phasic and tonic smooth muscles. *Am J Physiol* 1998;275:C684–692. [PubMed: 9730952]
44. Glukhova MA, Kabakov AE, Frid MG, et al. Modulation of human aorta smooth muscle cell phenotype: a study of muscle-specific variants of vinculin, caldesmon and actin expression. *Proc Natl Acad Sci USA* 1988;85:9542–9546. [PubMed: 3143999]
45. Lehman W, Craig R, Lui J, et al. Caldesmon and the structure of smooth muscle thin filaments: immunolocalization of caldesmon on thin filaments. *J Muscle Res Cell Motil* 1989;10:101–112. [PubMed: 2760189]
46. Mabuchi K, Li Y, Carlos A, et al. Caldesmon exhibits a clustered distribution along individual chicken gizzard native thin filaments. *J Muscle Res Cell Motil* 2001;22:77–90. [PubMed: 11563552]
47. Katayama E, Ikebe M. Mode of caldesmon binding to smooth muscle thin filament: possible projection of the amino-terminal of caldesmon from native thin filament. *Biophys J* 1995;68:2419–2428. [PubMed: 7647246]
48. Craig R, Megerman J. Assembly of smooth muscle myosin into side-polar filaments. *J Cell Biol* 1977;75:990–996. [PubMed: 562890]
49. Mabuchi K, Li Y, Tao T, et al. Immunocytochemical localization of caldesmon and calponin in chicken gizzard smooth muscle. *J Muscle Res Cell Motil* 1996;17:243–260. [PubMed: 8793726]
50. Cooke PH, Fay FS, Craig R. Myosin filaments isolated from skinned amphibian smooth muscle cells are side-polar. *J Muscle Res Cell Motil* 1989;10:206–220. [PubMed: 2503537]
51. Yamashiro-Matsumura S, Matsumura F. Characterization of 83-kilodalton nonmuscle caldesmon from cultured rat cells: stimulation of actin binding of nonmuscle tropomyosin and periodic localization along microfilaments like tropomyosin. *J Cell Biol* 1988;106:1973–1983. [PubMed: 3384851]
52. Abd-el-Basset EM, Ahmed I, Fedoroff S. Actin and actin-binding proteins in differentiating astroglia in tissue culture. *J Neurosci Res* 1991;30:1–17. [PubMed: 1795393]
53. Tanaka J, Watanabe T, Nakamura N, et al. Morphological and biochemical analyses of contractile proteins (actin, myosin, caldesmon and tropomyosin) in normal and transformed cells. *J Cell Sci* 1993;104:595–606. [PubMed: 8505382]

54. Kira M, Tanaka J, Sobue K. Caldesmon and low Mr isoform of tropomyosin are localized in neuronal growth cones. *J Neurosci Res* 1995;40:294–305. [PubMed: 7745623]
55. Bretscher A, Lynch W. Identification and localization of immunoreactive forms of caldesmon in smooth and nonmuscle cells: a comparison with the distributions of tropomyosin and alpha-actinin. *J Cell Biol* 1985;100:1656–1663. [PubMed: 2985624]
56. Lin JJ, Hegmann TE, Lin JL. Differential localization of tropomyosin isoforms in cultured nonmuscle cells. *J Cell Biol* 1988;107:563–572. [PubMed: 3047141]
57. Fukui Y, Lynch TJ, Brzeska H, et al. Myosin I is located at the leading edges of locomoting *Dictyostelium amoebae*. *Nature* 1989;341:328–331. [PubMed: 2797149]
58. McMichael BK, Kotadiya P, Singh T, et al. Tropomyosin isoforms localize to distinct microfilament populations in osteoclasts. *Bone* 2006;39:694–705. [PubMed: 16765662]
59. Warren KS, Lin JL, Wamboldt DD, et al. Overexpression of human fibroblast caldesmon fragment containing actin-, Ca^{++} /calmodulin- and tropomyosin-binding domains stabilizes endogenous tropomyosin and microfilaments. *J Cell Biol* 1994;125:359–368. [PubMed: 8163552]
60. Zheng PP, Weiden MV, Sillevs Smitt PA, et al. Hela/-CaD Undergoes a DNA Replication-Associated Switch in Localization from the Cytoplasm to the Nuclei of Endothelial Cells/Endothelial Progenitor Cells in Human Tumor Vasculature. *Cancer Biol Ther* 2007;6.
61. Smith CW, Pritchard K, Marston SB. The mechanism of Ca^{2+} regulation of vascular smooth muscle thin filaments by caldesmon and calmodulin. *J Biol Chem* 1987;262:116–122. [PubMed: 2947901]
62. Graceffa P. Evidence for interaction between smooth muscle tropomyosin and caldesmon. *FEBS Lett* 1987;218:139–142. [PubMed: 3595858]
63. Watson MH, Kuhn AE, Mak AS. Caldesmon, calmodulin and tropomyosin interactions. *Biochim Biophys Acta* 1990;1054:103–113. [PubMed: 2383599]
64. Czurlyo EA, Emelyanenko VI, Permyakov EA, et al. Spectrofluorimetric studies on C-terminal 34 kDa fragment of caldesmon. *Biophys Chem* 1991;40:181–188. [PubMed: 1883949]
65. Nomura M, Yoshikawa K, Tanaka T, et al. The role of tropomyosin in the interactions of F-actin with caldesmon and actin-binding protein (or filamin). *Eur J Biochem* 1987;163:467–471. [PubMed: 3830166]
66. Fujii T, Ozawa J, Ogoma Y, et al. Interaction between chicken gizzard caldesmon and tropomyosin. *J Biochem (Tokyo)* 1988;104:734–737. [PubMed: 3235448]
67. Huber PA, Fraser ID, Marston SB. Location of smooth-muscle myosin and tropomyosin binding sites in the C-terminal 288 residues of human caldesmon. *Biochem J* 1995;312:617–625. [PubMed: 8526878]
68. Wang Z, Horiuchi KY, Chacko S. Characterization of the functional domains on the C-terminal region of caldesmon using full-length and mutant caldesmon molecules. *J Biol Chem* 1996;271:2234–2242. [PubMed: 8567684]
69. Lamb NJ, Fernandez A, Mezgueldi M, et al. Disruption of the actin cytoskeleton in living nonmuscle cells by microinjection of antibodies to domain-3 of caldesmon. *Eur J Cell Biol* 1996;69:36–44. [PubMed: 8825022]
70. Hnath EJ, Wang CL, Huber PA, et al. Affinity and structure of complexes of tropomyosin and caldesmon domains. *Biophys J* 1996;71:1920–1933. [PubMed: 8889167]
71. Hayashi K, Yamada S, Kanda K, et al. 35 kDa fragment of h-caldesmon conserves two consensus sequences of the tropomyosin-binding domain in troponin T. *Biochem Biophys Res Commun* 1989;161:38–45. [PubMed: 2730665]
72. El-Mezgueldi M, Copeland O, Fraser ID, et al. Characterization of the functional properties of smooth muscle caldesmon domain 4a: evidence for an independent inhibitory actin-tropomyosin binding domain. *Biochem J* 1998;332:395–401. [PubMed: 9601068]
73. Horiuchi KY, Chacko S. Interaction between caldesmon and tropomyosin in the presence and absence of smooth muscle actin. *Biochemistry* 1988;27:8388–8393. [PubMed: 3242591]
74. Watson MH, Kuhn AE, Novy RE, et al. Caldesmon-binding sites on tropomyosin. *J Biol Chem* 1990;265:18860–18866. [PubMed: 2229046]
75. Childs TJ, Watson MH, Novy RE, et al. Calponin and tropomyosin interactions. *Biochim Biophys Acta* 1992;1121:41–46. [PubMed: 1599949]

76. Ishikawa R, Yamashiro S, Kohama K, et al. Regulation of actin binding and actin bundling activities of fascin by caldesmon coupled with tropomyosin. *J Biol Chem* 1998;273:26991–26997. [PubMed: 9756949]
77. Graceffa P, Wang C-LA, Stafford WF. Caldesmon. Molecular weight and subunit composition by analytical ultracentrifugation. *J Biol Chem* 1988;263:14196–14202. [PubMed: 3170543]
78. Wang C-LA, Chalovich JM, Graceffa P, et al. A long helix from the central region of smooth muscle caldesmon. *J Biol Chem* 1991;266:13958–13963. [PubMed: 1856225]
79. Lupas A, Van Dyke M, Stock J. Predicting coiled coils from protein sequences. *Science* 1991;252:1162–1164.
80. Berger B, Wilson DB, Wolf E, et al. Predicting coiled coils by use of pairwise residue correlations. *Proc Natl Acad Sci USA* 1995;92:8259–8263. [PubMed: 7667278]
81. Graceffa P, Lehrer SS. Dynamic equilibrium between the two conformational states of spin-labeled tropomyosin. *Biochemistry* 1984;23:2606–2612. [PubMed: 6087878]
82. Wang E, Wang C-LA. (i, i + 4) Ion pairs stabilize helical peptides derived from smooth muscle caldesmon. *Arch Biochem Biophys* 1996;329:156–162. [PubMed: 8638947]
83. Mabuchi K, Wang C-LA. Electron microscopic studies of chicken gizzard caldesmon and its complex with calmodulin. *J Muscle Res Cell Motil* 1991;12:145–151. [PubMed: 2061408]
84. Gao Y, Patchell VB, Huber PA, et al. The interface between caldesmon domain 4b and subdomain 1 of actin studied by nuclear magnetic resonance spectroscopy. *Biochemistry* 1999;38:15459–15469. [PubMed: 10569928]
85. Bartegi A, Fattoum A, Derancourt J, et al. Characterization of the carboxyl-terminal 10-kDa cyanogen bromide fragment of caldesmon as an actin-calmodulin-binding region. *J Biol Chem* 1990;265:15231–15238. [PubMed: 2394719]
86. Fujii T, Imai M, Rosenfeld GC, et al. Domain mapping of chicken gizzard caldesmon. *J Biol Chem* 1987;262:2757–2763. [PubMed: 2434491]
87. Riseman VM, Lynch WP, Nefsky B, et al. The calmodulin and F-actin binding sites of smooth muscle caldesmon lie in the carboxyl-terminal domain whereas the molecular weight heterogeneity lies in the middle of the molecule. *J Biol Chem* 1989;264:2869–2875. [PubMed: 2914935]
88. Szpacenko A, Dabrowska R. Functional domain of caldesmon. *FEBS Lett* 1986;202:182–186. [PubMed: 2941315]
89. Wang C-LA, Wang L-WC, Xu SA, et al. Localization of the calmodulin-and the actin-binding sites of caldesmon. *J Biol Chem* 1991;266:9166–9172. [PubMed: 2026616]
90. Velaz L, Ingraham RH, Chalovich JM. Dissociation of the effect of caldesmon on the ATPase activity and on the binding of smooth heavy meromyosin to actin by partial digestion of caldesmon. *J Biol Chem* 1990;265:2929–2934. [PubMed: 2137453]
91. Wang Z, Jiang H, Yang ZQ, et al. Both N-terminal myosin-binding and C-terminal actin-binding sites on smooth muscle caldesmon are required for caldesmon-mediated inhibition of actin filament velocity. *Proc Natl Acad Sci USA* 1997;94:11899–11904. [PubMed: 9342334]
92. Li Y, Zhuang S, Guo H, et al. The major myosin-binding site of caldesmon resides near its N-terminal extreme. *J Biol Chem* 2000;275:10989–10994. [PubMed: 10753900]
93. Wang C-LA. Photocrosslinking of calmodulin and/or actin to chicken gizzard caldesmon. *Biochem Biophys Res Commun* 1988;156:1033–1038. [PubMed: 3190671]
94. Wang C-LA, Wang L-WC, Lu RC. Caldesmon has two calmodulin-binding domains. *Biochem Biophys Res Commun* 1989;162:746–752. [PubMed: 2757638]
95. Szczesna D, Graceffa P, Wang C-LA, et al. Myosin S1 changes the orientation of caldesmon on actin. *Biochemistry* 1994;33:6716–6720. [PubMed: 8204606]
96. Zhuang S, Mabuchi K, Wang C-LA. Heat treatment could affect the biochemical properties of caldesmon. *J Biol Chem* 1996;271:30242–30248. [PubMed: 8939977]
97. Mabuchi K, Lin JJ, Wang CL. Electron microscopic images suggest both ends of caldesmon interact with actin filaments. *J Muscle Res Cell Motil* 1993;14:54–64. [PubMed: 8478429]
98. Chen YD, Chalovich JM. A mosaic multiple-binding model for the binding of caldesmon and myosin subfragment-1 to actin. *Biophys J* 1992;63:1063–1070. [PubMed: 1420925]

99. Fredricksen S, Cai A, Gafurov B, et al. Influence of ionic strength, actin state and caldesmon construct size on the number of actin monomers in a caldesmon binding site. *Biochemistry* 2003;42:6136–6148. [PubMed: 12755616]
100. Moody CJ, Marston SB, Smith CW. Bundling of actin filaments by aorta caldesmon is not related to its regulatory function. *FEBS Lett* 1985;191:107–112. [PubMed: 2932345]
101. Dabrowska R, Goch A, Galazkiewicz B, et al. The influence of caldesmon on ATPase activity of the skeletal muscle actomyosin and bundling of actin filaments. *Biochim Biophys Acta* 1985;842:70–75. [PubMed: 2931121]
102. Cuneo P, Magri E, Verzola A, et al. 'Macromolecular crowding' is a primary factor in the organization of the cytoskeleton. *Biochem J* 1992;281:507–512. [PubMed: 1736897]
103. Isambert H, Venier P, Maggs AC, et al. Flexibility of actin filaments derived from thermal fluctuations. Effect of bound nucleotide, phalloidin and muscle regulatory proteins. *J Biol Chem* 1995;270:11437–11444. [PubMed: 7744781]
104. Ishikawa R, Yamashiro S, Matsumura F. Annealing of gelsolin-severed actin fragments by tropomyosin in the presence of Ca^{2+} . Potentiation of the annealing process by caldesmon. *J Biol Chem* 1989;264:16764–16770. [PubMed: 2550459]
105. Ishikawa R, Yamashiro S, Matsumura F. Differential modulation of actin-severing activity of gelsolin by multiple isoforms of cultured rat cell tropomyosin. Potentiation of protective ability of tropomyosins by 83-kDa nonmuscle caldesmon. *J Biol Chem* 1989;264:7490–7497. [PubMed: 2540194]
106. Chalovich JM, Chen YD, Dudek R, et al. Kinetics of binding of caldesmon to actin. *J Biol Chem* 1995;270:9911–9916. [PubMed: 7730374]
107. Lehman W, Vibert P, Craig R. Visualization of caldesmon on smooth muscle thin filaments. *J Mol Biol* 1997;274:310–317. [PubMed: 9405141]
108. Foster DB, Huang R, Hatch V, et al. Modes of caldesmon binding to actin: sites of caldesmon contact and modulation of interactions by phosphorylation. *J Biol Chem* 2004;279:53387–53394. [PubMed: 15456752]
109. Huang R, Li L, Guo H, et al. Caldesmon binding to actin is regulated by calmodulin and phosphorylation via different mechanisms. *Biochemistry* 2003;42:2513–2523. [PubMed: 12614145]
110. Mornet D, Harricane MC, Audemard E. A 35-kilodalton fragment from gizzard smooth muscle caldesmon that induces F-actin bundles. *Biochem Biophys Res Commun* 1988;155:808–815. [PubMed: 3421967]
111. Takiguchi K, Matsumura F. Role of the Basic C-Terminal Half of Caldesmon in Its Regulation of F-Actin: Comparison between Caldesmon and Calponin. *J Biochem (Tokyo)* 2005;138:805–813. [PubMed: 16428310]
112. Tang JX, Janmey PA. The polyelectrolyte nature of F-actin and the mechanism of actin bundle formation. *J Biol Chem* 1996;271:8556–8563. [PubMed: 8621482]
113. Galazkiewicz B, Mossakowska M, Osinska H, et al. Polymerization of G-actin by caldesmon. *FEBS Lett* 1985;184:144–149. [PubMed: 2985442]
114. Makuch R, Kulikova N, Graziewicz MA, et al. Polymerization of actin induced by actin-binding fragments of caldesmon. *Biochim Biophys Acta* 1994;1206:49–54. [PubMed: 8186249]
115. Yamakita Y, Oosawa F, Yamashiro S, et al. Caldesmon inhibits Arp2/3-mediated actin nucleation. *Journal of Biological Chemistry* 2003;278:17937–17944. [PubMed: 12637566]
116. Ngai PK, Walsh MP. Inhibition of smooth muscle actin-activated myosin Mg^{2+} -ATPase activity by caldesmon. *J Biol Chem* 1984;259:13656–13659. [PubMed: 6150036]
117. Onji T, Takagi M, Shibata N. Caldesmon specifically inhibits the effect of tropomyosin on actomyosin system in platelet. *Biochem Biophys Res Commun* 1987;143:475–481. [PubMed: 2952114]
118. Sobue K, Takahashi K, Wakabayashi I. Caldesmon150 regulates the tropomyosin-enhanced actin-myosin interaction in gizzard smooth muscle. *Biochem Biophys Res Commun* 1985;132:645–651. [PubMed: 4062943]
119. Chacko S, Conti MA, Adelstein RS. Effect of phosphorylation of smooth muscle myosin on actin activation and Ca^{2+} regulation. *Proc Natl Acad Sci USA* 1977;74:129–133. [PubMed: 189302]

120. Chacko S, Eisenberg E. Cooperativity of actin-activated ATPase of gizzard heavy meromyosin in the presence of gizzard tropomyosin. *J Biol Chem* 1990;265:2105–2110. [PubMed: 2137124]
121. Dabrowska R, Hinssen H, Galazkiewicz B, et al. Modulation of gelsolin-induced actin-filament severing by caldesmon and tropomyosin and the effect of these proteins on the actin activation of myosin Mg(2+)-ATPase activity. *Biochem J* 1996;315:753–759. [PubMed: 8645154]
122. Horiuchi KY, Samuel M, Chacko S. Mechanism for the inhibition of acto-heavy meromyosin ATPase by the actin/calmodulin binding domain of caldesmon. *Biochemistry* 1991;30:712–717. [PubMed: 1824822]
123. Hemric ME, Freedman MV, Chalovich JM. Inhibition of actin stimulation of skeletal muscle (A1) S-1 ATPase activity by caldesmon. *Arch Biochem Biophys* 1993;306:39–43. [PubMed: 8215419]
124. Chalovich JM, Cornelius P, Benson CE. Caldesmon inhibits skeletal actomyosin subfragment-1 ATPase activity and the binding of myosin subfragment-1 to actin. *J Biol Chem* 1987;262:5711–5716. [PubMed: 2952642]
125. Yan B, Sen A, Chalovich JM, et al. Theoretical studies on competitive binding of caldesmon and myosin S1 to actin: Prediction of apparent cooperativity in equilibrium and slow-down in kinetics of S1 binding by caldesmon. *Biochemistry* 2003;42:4208–4216. [PubMed: 12680775]
126. Sen A, Chen YD, Yan B, et al. Caldesmon reduces the apparent rate of binding of myosin S1 to actin-tropomyosin. *Biochemistry* 2001;40:5757–5764. [PubMed: 11341841]
127. Marston SB, Redwood CS. The essential role of tropomyosin in cooperative regulation of smooth muscle thin filament activity by caldesmon. *J Biol Chem* 1993;268:12317–12320. [PubMed: 8509369]
128. Alahyan M, Webb MR, Marston SB, et al. The mechanism of smooth muscle caldesmon-tropomyosin inhibition of the elementary steps of the actomyosin ATPase. *J Biol Chem* 2006;281:19433–19448. [PubMed: 16540476]
129. Rembold CM, Wardle RL, Wingard CJ, et al. Cooperative attachment of cross bridges predicts regulation of smooth muscle force by myosin phosphorylation. *Am J Physiol Cell Physiol* 2004;287:C594–602. [PubMed: 15151901]
130. Marston S, Burton D, Copeland O, et al. Structural interactions between actin, tropomyosin, caldesmon and calcium binding protein and the regulation of smooth muscle thin filaments. *Acta Physiol Scand* 1998;164:401–414. [PubMed: 9887964]
131. Marston SB, Fraser ID, Huber PA. Smooth muscle caldesmon controls the strong binding interaction between actin-tropomyosin and myosin. *J Biol Chem* 1994;269:32104–32109. [PubMed: 7798205]
132. Fraser ID, Marston SB. In vitro motility analysis of smooth muscle caldesmon control of actin-tropomyosin filament movement. *J Biol Chem* 1995;270:19688–19693. [PubMed: 7649978]
133. Rosenfeld SS, Taylor EW. The dissociation of 1-N6-ethenoadenosine diphosphate from regulated actomyosin subfragment 1. *J Biol Chem* 1987;262:9994–9999. [PubMed: 3611074]
134. McKillop DF, Geeves MA. Regulation of the acto.myosin subfragment 1 interaction by troponin/tropomyosin. Evidence for control of a specific isomerization between two acto. myosin subfragment 1 states. *Biochem J* 1991;279(Pt 3):711–718. [PubMed: 1953663]
135. Horiuchi KY, Miyata H, Chacko S. Modulation of smooth muscle actomyosin ATPase by thin filament associated proteins. *Biochem Biophys Res Commun* 1986;136:962–968. [PubMed: 2941015]
136. Vibert P, Craig R, Lehman W. Three-dimensional reconstruction of caldesmon-containing smooth muscle thin filaments. *J Cell Biol* 1993;123:313–321. [PubMed: 8408215]
137. Hodgkinson JL, Marston SB, Craig R, et al. Three-dimensional image reconstruction of reconstituted smooth muscle thin filaments: effects of caldesmon. *Biophys J* 1997;72:2398–2404. [PubMed: 9168017]
138. Lehman W, Vibert P, Uman P, et al. Steric-Blocking By Tropomyosin Visualized In Relaxed Vertebrate Muscle Thin Filaments. *Journal of Molecular Biology* 1995;251:191–196. [PubMed: 7643394]
139. Lehrer SS, Geeves MA. The muscle thin filament as a classical cooperative/allosteric regulatory system. *J Mol Biol* 1998;277:1081–1089. [PubMed: 9571024]

140. Lehrer SS, Golitsina NL, Geeves MA. Actin-tropomyosin activation of myosin subfragment 1 ATPase and thin filament cooperativity. The role of tropomyosin flexibility and end-to-end interactions. *Biochemistry* 1997;36:13449–13454. [PubMed: 9354612]
141. Word RA, Stull JT, Casey ML, et al. Contractile elements and myosin light chain phosphorylation in myometrial tissue from nonpregnant and pregnant women. *J Clin Invest* 1993;92:29–37. [PubMed: 8392087]
142. Ikebe M, Reardon S. Binding of caldesmon to smooth muscle myosin. *J Biol Chem* 1988;263:3055–3058. [PubMed: 3257755]
143. Hemric ME, Chalovich JM. Effect of caldesmon on the ATPase activity and the binding of smooth and skeletal myosin subfragments to actin. *J Biol Chem* 1988;263:1878–1885. [PubMed: 2962997]
144. Huber PA, Redwood CS, Arent ND, et al. Identification of functioning regulatory sites and a new myosin binding site in the C-terminal 288 amino acids of caldesmon expressed from a human clone. *J Muscle Res Cell Motil* 1993;14:385–391. [PubMed: 8227296]
145. Onishi H, Wakabayashi T. Electron microscopic studies of myosin molecules from chicken gizzard muscle I: the formation of the intramolecular loop in the myosin tail. *J Biochem (Tokyo)* 1982;92:871–879. [PubMed: 7142124]
146. Suzuki H, Onishi H, Takahashi K, et al. Structure and function of chicken gizzard myosin. *J Biochem (Tokyo)* 1978;84:1529–1542. [PubMed: 153905]
147. Suzuki H, Stafford WF 3rd, Slayter HS, et al. A conformational transition in gizzard heavy meromyosin involving the head-tail junction, resulting in changes in sedimentation coefficient, ATPase activity and orientation of heads. *J Biol Chem* 1985;260:14810–14817. [PubMed: 2932450]
148. Woodhead JL, Zhao FQ, Craig R, et al. Atomic model of a myosin filament in the relaxed state. *Nature* 2005;436:1195–1199. [PubMed: 16121187]
149. Katayama E, Scott-Woo G, Ikebe M. Effect of caldesmon on the assembly of smooth muscle myosin. *J Biol Chem* 1995;270:3919–3925. [PubMed: 7876138]
150. Marston S, Pinter K, Bennett P. Caldesmon binds to smooth muscle myosin and myosin rod and crosslinks thick filaments to actin filaments. *J Muscle Res Cell Motil* 1992;13:206–218. [PubMed: 1534566]
151. Haeberle JR, Trybus KM, Hemric ME, et al. The effects of smooth muscle caldesmon on actin filament motility. *J Biol Chem* 1992;267:23001–23006. [PubMed: 1429647]
152. Marston SB, Latchbridges J. *Muscle Res Cell Motil* 1989;10:97–100.
153. Horiuchi KY, Chacko S. Effect of unphosphorylated smooth muscle myosin on caldesmon-mediated regulation of actin filament velocity. *J Muscle Res Cell Motil* 1995;16:11–19. [PubMed: 7751401]
154. Earley JJ, Su X, Moreland RS. Caldesmon inhibits active crossbridges in unstimulated vascular smooth muscle: an antisense oligodeoxynucleotide approach. *Circ Res* 1998;83:661–667. [PubMed: 9742062]
155. Numaguchi Y, Huang S, Polte TR, et al. Caldesmon-dependent switching between capillary endothelial cell growth and apoptosis through modulation of cell shape and contractility. *Angiogenesis* 2003;6:55–64. [PubMed: 14517405]
156. Li Y, Wessels D, Wang T, et al. Regulation of caldesmon activity by Cdc2 kinase plays an important role in maintaining membrane cortex integrity during cell division. *Cell Mol Life Sci* 2003;60:198–211. [PubMed: 12613668]
157. Ngai PK, Walsh MP. The effects of phosphorylation of smooth-muscle caldesmon. *Biochem J* 1987;244:417–425. [PubMed: 2822003]
158. Adam LP, Haeberle JR, Hathaway DR. Phosphorylation of caldesmon in arterial smooth muscle. *J Biol Chem* 1989;264:7698–7703. [PubMed: 2708386]
159. Adam LP, Gapinski CJ, Hathaway DR. Phosphorylation sequences in h-caldesmon from phorbol ester-stimulated canine aortas. *FEBS Lett* 1992;302:223–226. [PubMed: 1601129]
160. Childs TJ, Watson MH, Sanghera JS, et al. Phosphorylation of smooth muscle caldesmon by mitogen-activated protein (MAP) kinase and expression of MAP kinase in differentiated smooth muscle cells. *J Biol Chem* 1992;267:22853–22859. [PubMed: 1331069]
161. D'Angelo G, Graceffa P, Wang C-LA, et al. Mammal-specific, ERK-dependent, caldesmon phosphorylation in smooth muscle. Quantitation using novel anti-phosphopeptide antibodies. *J Biol Chem* 1999;274:30115–30121. [PubMed: 10514499]

162. Nixon GF, Iizuka K, Haystead CM, et al. Phosphorylation of caldesmon by mitogen-activated protein kinase with no effect on Ca^{2+} sensitivity in rabbit smooth muscle. *J Physiol (Lond)* 1995;487:283–289. [PubMed: 8558463]
163. Yamboliev IA, Gerthoffer WT. Modulatory role of ERK MAPK-caldesmon pathway in PDGF-stimulated migration of cultured pulmonary artery SMCs. *American Journal of Physiology—Cell Physiology* 2001;280:C1680–C1688. [PubMed: 11350764]
164. Yamashiro S, Yamakita Y, Ishikawa R, et al. Mitosis-specific phosphorylation causes 83K nonmuscle caldesmon to dissociate from microfilaments. *Nature* 1990;344:675–678. [PubMed: 2157986]
165. Yamashiro S, Yamakita Y, Hosoya H, et al. Phosphorylation of nonmuscle caldesmon by p34cdc2 kinase during mitosis. *Nature* 1991;349:169–172. [PubMed: 1986309]
166. Mak AS, Carpenter M, Smillie LB, et al. Phosphorylation of caldesmon by p34cdc2 kinase. Identification of phosphorylation sites. *J Biol Chem* 1991;266:19971–19975. [PubMed: 1939059]
167. Yamashiro S, Yamakita Y, Yoshida K, et al. Characterization of the COOH terminus of nonmuscle caldesmon mutants lacking mitosis-specific phosphorylation sites. *J Biol Chem* 1995;270:4023–4030. [PubMed: 7876150]
168. Yamakita Y, Yamashiro S, Matsumura F. Characterization of mitotically phosphorylated caldesmon. *J Biol Chem* 1992;267:12022–12029. [PubMed: 1534804]
169. Hosoya N, Hosoya H, Yamashiro S, et al. Localization of caldesmon and its dephosphorylation during cell division. *J Cell Biol* 1993;121:1075–1082. [PubMed: 8388877]
170. Yamashiro S, Chern H, Yamakita Y, et al. Mutant Caldesmon lacking cdc2 phosphorylation sites delays M-phase entry and inhibits cytokinesis. *Mol Biol Cell* 2001;12:239–250. [PubMed: 11160835]
171. Kordowska J, Hetrick T, Adam LP, et al. Phosphorylated l-caldesmon is involved in disassembly of actin stress fibers and postmitotic spreading. *Exp Cell Res* 2006;312:95–110. [PubMed: 16289153]
172. Yamashiro S, Yamakita Y, Yoshida K, et al. Characterization of the COOH terminus of nonmuscle caldesmon mutants lacking mitosis-specific phosphorylation sites. *J Biol Chem* 1995;270:4023–4030. [PubMed: 7876150]
173. Yamboliev IA, Gerthoffer WT. Modulatory role of ERK MAPK-caldesmon pathway in PDGF-stimulated migration of cultured pulmonary artery SMCs. *Am J Physiol Cell Physiol* 2001;280:C1680–1688. [PubMed: 11350764]
174. Goncharova EA, Vorotnikov AV, Gracheva EO, et al. Activation of p38 MAP-kinase and caldesmon phosphorylation are essential for urokinase-induced human smooth muscle cell migration. *Biol Chem* 2002;383:115–126. [PubMed: 11930938]
175. Manes T, Zheng DQ, Tognin S, et al. Alpha(v)beta3 integrin expression up-regulates cdc2, which modulates cell migration. *J Cell Biol* 2003;161:817–826. [PubMed: 12771130]
176. Juliano R. Movin' on through with Cdc2. *Nat Cell Biol* 2003;5:589–590. [PubMed: 12833057]
177. Liu X, Yan S, Zhou T, et al. The MAP kinase pathway is required for entry into mitosis and cell survival. *Oncogene* 2004;23:763–776. [PubMed: 14737111]
178. Yamashiro S, Chern H, Yamakita Y, et al. Mutant caldesmon lacking cdc2 phosphorylation sites delays M-phase entry and inhibits cytokinesis. *Molecular Biology of the Cell* 2001;12:239–250. [PubMed: 11160835]
179. Van Eyk JE, Arrell DK, Foster DB, et al. Different molecular mechanisms for Rho family GTPase-dependent, Ca^{2+} -independent contraction of smooth muscle. *J Biol Chem* 1998;273:23433–23439. [PubMed: 9722579]
180. Guo H, Bryan J, Wang C-LA. A note on the caldesmon sequence. *J Muscle Res Cell Motil* 1999;20:725–726. [PubMed: 10672521]
181. McFawn PK, Shen L, Vincent SG, et al. Calcium-independent contraction and sensitization of airway smooth muscle by p21-activated protein kinase. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L863–870. [PubMed: 12513968]
182. Vidal C, Geny B, Melle J, et al. Cdc42/Rac1-dependent activation of the p21-activated kinase (PAK) regulates human platelet lamellipodia spreading: implication of the cortical-actin binding protein cortactin. *Blood* 2002;100:4462–4469. [PubMed: 12453877]

183. Linder S, Kopp P. Podosomes at a glance. *J Cell Sci* 2005;118:2079–2082. [PubMed: 15890982]
184. Hai CM, Hahne P, Harrington EO, et al. Conventional protein kinase C mediates phorbol-dibutyrate-induced cytoskeletal remodeling in a7r5 smooth muscle cells. *Exp Cell Res* 2002;280:64–74. [PubMed: 12372340]
185. Eves R, Webb BA, Zhou S, et al. Caldesmon is an integral component of podosomes in smooth muscle cells. *J Cell Sci* 2006;119:1691–1702. [PubMed: 16595550]
186. Gu Z, Kordowska J, Williams GL, et al. Erk1/2 MAPK and caldesmon differentially regulate podosome dynamics in A7r5 vascular smooth muscle cells. *Exp Cell Res* 2007;313:849–866. [PubMed: 17239373]
187. Morita T, Mayanagi T, Yoshio T, et al. Changes in the balance between caldesmon regulated by p21-activated kinases and the Arp2/3 complex govern podosome formation. *J Biol Chem* 2007;282:8454–8463. [PubMed: 17224451]
188. Warren KS, Shutt DC, McDermott JP, et al. Overexpression of microfilament-stabilizing human caldesmon fragment, CaD39, affects cell attachment, spreading and cytokinesis. *Cell Motil Cytoskeleton* 1996;34:215–229. [PubMed: 8816288]
189. Surgucheva I, Bryan J. Over-expression of smooth muscle caldesmon in mouse fibroblasts. *Cell Motil Cytoskeleton* 1995;32:233–243. [PubMed: 8581978]
190. Mirzapoiarzova T, Kolosova IA, Romer L, et al. The role of caldesmon in the regulation of endothelial cytoskeleton and migration. *J Cell Physiol* 2005;203:520–528. [PubMed: 15521070]
191. Helfman DM, Levy ET, Berthier C, et al. Caldesmon inhibits nonmuscle cell contractility and interferes with the formation of focal adhesions. *Mol Biol Cell* 1999;10:3097–3112. [PubMed: 10512853]
192. Grosheva I, Vittitow JL, Goichberg P, et al. Caldesmon effects on the actin cytoskeleton and cell adhesion in cultured HTM cells. *Exp Eye Res* 2006;82:945–958. [PubMed: 16679125]
193. Castellino F, Ono S, Matsumura F, et al. Essential role of caldesmon in the actin filament reorganization induced by glucocorticoids. *J Cell Biol* 1995;131:1223–1230. [PubMed: 8522585]
194. Yokouchi K, Numaguchi Y, Kubota R, et al. l-Caldesmon regulates proliferation and migration of vascular smooth muscle cells and inhibits neointimal formation after angioplasty. *Arterioscler Thromb Vasc Biol* 2006;26:2231–2237. [PubMed: 16888241]
195. Yoshio T, Morita T, Kimura Y, et al. Caldesmon suppresses cancer cell invasion by regulating podosome/invadopodium formation. *FEBS Lett* 2007;581:3777–3782. [PubMed: 17631293]
196. Warren KS, Lin JL, McDermott JP, et al. Forced expression of chimeric human fibroblast tropomyosin mutants affects cytokinesis. *J Cell Biol* 1995;129:697–708. [PubMed: 7730405]
197. Hegmann TE, Schulte DL, Lin JL, et al. Inhibition of intracellular granule movement by microinjection of monoclonal antibodies against caldesmon. *Cell Motil Cytoskeleton* 1991;20:109–120. [PubMed: 1751965]
198. Hegmann TE, Lin JL, Lin JJ. Probing the role of nonmuscle tropomyosin isoforms in intracellular granule movement by microinjection of monoclonal antibodies. *J Cell Biol* 1989;109:1141–1152. [PubMed: 2670955]
199. Boerner JL, McManus MJ, Martin GS, et al. Ras-independent oncogenic transformation by an EGF-receptor mutant. *J Cell Sci* 2000;113:935–942. [PubMed: 10683142]
200. Boerner JL, Danielsen AJ, Lovejoy CA, et al. Grb2 regulation of the actin-based cytoskeleton is required for ligand-independent EGF receptor-mediated oncogenesis. *Oncogene* 2003;22:6679–6689. [PubMed: 14555981]
201. Somara S, Bitar KN. Phosphorylated HSP27 modulates the association of phosphorylated caldesmon with tropomyosin in colonic smooth muscle. *Am J Physiol Gastrointest Liver Physiol*. 2006
202. Weed SA, Parsons JT. Cortactin: coupling, membrane dynamics to cortical actin assembly. *Oncogene* 2001;20:6418–6434. [PubMed: 11607842]
203. Daly RJ. Cortactin signalling and dynamic actin networks. *Biochem J* 2004;382:13–25. [PubMed: 15186216]
204. Huang R, Cao G-J, Guo H, et al. Direct interaction between caldesmon and cortactin. *Arch Biochem Biophys* 2006;456:175–182. [PubMed: 16962992]

205. Totsukawa G, Yamakita Y, Yamashiro S, et al. Activation of myosin phosphatase targeting subunit by mitosis-specific phosphorylation. *J Cell Biol* 1999;144:735–744. [PubMed: 10037794]
206. Mabuchi K, Li YH, Carlos A, et al. Caldesmon exhibits a clustered distribution along individual chicken gizzard native thin filaments. *J Muscle Res Cell Motil* 2001;22:77–90. [PubMed: 11563552]

Figure 1.

CaD has two isoforms resulting from alternative splicing. The domain structures of mammalian h-CaD (upper bar) and l-CaD (lower bar) indicate the common functional regions for myosin-binding (light green), CaM-binding (red) and actin-binding (blue and turquoise). All functional domains are shared between both isoforms, except that the central 'spacer' (yellow) is missing in l-CaD. Also shown are the two phosphorylation sites common for ERK and cdc2 kinase. A color version of this figure is available at www.Eurekah.com.

Figure 2.

The compensatory isoform switchover depends on the tissue type. Western analysis by Odyssey imaging of tissue extracts from bladder and aorta of wild-type (+/+) and CaD-deficient (-/-) mice. Note that the up-regulation of nonmuscle CaD is more robust in bladder than in aorta. There appeared to be more severe proteolysis in bladder than in aorta. β -actin (green) was used as a reference. A color version of this figure is available at www.Eurekah.com.

Figure 3.

CaD binds actin longitudinally with the N-terminus appearing on one side of the actin filament. A model illustrates the position of h-CaD on the two-stranded actin filament. CaD molecules on one strand are shown in solid lines and those on the opposite strand in broken lines. Note that the ends of all CaD molecules appear on the same side of an actin filament. Such an arrangement is only possible with h-CaD. Taken from Mabuchi et al.²⁰⁶

Figure 4.

A model with obliquely arranged contractile units allows clustered distribution of h-CaD along both actin and myosin filaments. Actin filaments (thin lines attached to the dense body) and myosin filaments (thick lines with a helical twist to represent the side-polar filaments) intersect each other at an angle. Both the intersecting angles and the twist of myosin filaments are exaggerated in this drawing. The myosin heads on opposite sides of the myosin filament interact with two bundles of actin filaments in reverse orientation, allowing muscle shortening. As suggested by this model, because of the nonparallel alignment, a given myosin filament is bound to interact with multiple actin filaments in the same bundle. Assuming that h-CaD (red dots) is only present at the junctions of these filaments, it naturally has a clustered distribution along either the actin or the myosin filament. Taken from Wang.⁶ A color version of this figure is available at www.Eurekah.com.

Figure 5.

3-D reconstruction shows an unusual binding mode of CaD on the actin filament. Surface views (a–c) of thin filament reconstructions showing the position of the C-terminal CaD fragment, H32K and phospho-H32K on F-actin and transverse sections (d–f) through maps of 3D reconstructions. The extra density (open bold arrows in b and c) contributed by H32K is associated with subdomains 1 and 2 of actin. In b the density that spans like a staple from the back of subdomain 1 to subdomain 3 of the neighboring actin monomer of the genetic helix (red ellipse). This inter-strand connectivity is present in b (red arrow), but is absent in a and c (green arrows). Open bold arrows in e indicate regions of significant H32K density and the red arrow points to the inter-strand density. Taken from Foster et al.¹⁰⁸ A color version of this figure is available at www.Eurekah.com.

Figure 6.

CaD is able to crosslink neighboring actin subunits. Disulfide crosslinking occurs between a C-terminal CaD fragment that contains two cysteine residues (at positions 595 and 766; H32Kqc), or its variant that contains only one cysteine residue (at position 766; H32Kqc/ca) and actin, as shown by the SDS-PAGE with Coomassie staining in the absence of reducing agent. Note that ERK-induced phosphorylation resulted in less H32Kqc/ca•actin crosslinking and almost no H32Kqc•actin₂ species.

Figure 7.

Actin filaments form bundles in the presence of CaD. CaD is known to bundle actin filaments owing to its multiple actin-binding sites. However, full-length h-CaD forms loose bundles (top panel), whereas the C-terminal fragment forms tight and straight bundles (bottom panel). Both samples were processed for rotary shadowing and viewed by electron microscopy. Scale bar: 1 μ m.(Mabuchi and Wang, unpublished results).

Figure 8.

A model depicting how CaD could change the configuration of myosin. Smooth muscle myosin heavy chains (orange) undergo a conformational change upon phosphorylation (red dots) at the light chains (yellow) from an elongated (6S) to a compact (10S) configuration. In this model binding of the N-terminal domain of CaD (blue) to the S-2 region of myosin is thought to interfere with the head-rod interaction and shift the myosin conformation to the 6S form even in the absence of phosphorylation. A color version of this figure is available at www.Eurekah.com.