Diversification of caldesmon-linked actin cytoskeleton in cell motility

Taira Mayanagi and Kenji Sobue*

Department of Neuroscience; Osaka University Graduate School of Medicine; Yamadaoka Suita, Osaka Japan;
Department of Neuroscience; Iwate Medical University; Nishikotuda Yahaba-Cho, Shiwa-gun, Iwate Japan

Key words: caldesmon, actin cytoskeleton, cell migration, cell invasion, cytokinesis, transcription, smooth muscle contraction, cytokinesis

Abbreviations: CaD, caldesmon; Ca²⁺, calcium; CaM, calmodulin; TM, tropomyosin; F-actin, filamentous actin; N-terminal, amino-terminal; C-terminal, carboxy-terminal; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; SMC, smooth muscle cell; SRF, serum response factor; MRTF, myocardin-related transcription factor; GC, glucocorticoid; GR, glucocorticoid receptor; GRE, glucocorticoid responsive element; VSMC, vascular smooth muscle cell; IGF, insulin-like growth factor; PI3K, phosphatidylinositol-3 kinase; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; ERK, extracellular signal regulated protein kinase; LPA, lysophosphatidic acid; PAK, p21-activated protein kinase; CaMKII, Ca²⁺/CaM-dependent protein kinase II; NPC, neural progenitor cell; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition

The actin cytoskeleton plays a key role in regulating cell motility. Caldesmon (CaD) is an actin-linked regulatory protein found in smooth muscle and non-muscle cells that is conserved among a variety of vertebrates. It binds and stabilizes actin filaments, as well as regulating actin-myosin interaction in a calcium (Ca²⁺)-calmodulin (CaM)- and/or phosphorylation-dependent manner. CaD function is regulated qualitatively by Ca²⁺/CaM and by its phosphorylation state and quantitatively at the mRNA level, by three different transcriptional regulation of the CALD1 gene. CaD has numerous functions in cell motility, such as migration, invasion and proliferation, exerted via the reorganization of the actin cytoskeleton. Here we will outline recent findings regarding CaD's structural features and functions.

Introduction

Regulation of cell adhesion and migration is essential, not only for development, immune response and wound healing, but also for various pathological events such as cancer progression. During organogenesis and cancer metastasis, cells dissociate from their origin and penetrate into distant target tissues. Cell adhesion and migration are controlled by complex changes in the cytoskeleton, particularly the actin cytoskeleton, which plays a pivotal role in controlling cell motility, such as cell morphology, adhesion and migration.

During a keen dispute over the Ca²⁺-dependent regulation of smooth muscle and non-muscle contraction, CaD appeared on the cytoskeletal stage as a CaM- and actin-binding protein. Subsequent studies on Ca²⁺-dependent regulation in both smooth muscle and non-muscle cells showed CaD to be an actin-linked regulatory protein that functions in the actomyosin contractile system. CaD binds filamentous (F-) actin and inhibits actin-myosin interactions in a Ca²⁺/CaM-dependent manner, as determined by superprecipitation assays and actin-activated myosin ATPase activity. CaD is an actin-, myosin-, CaM- and tropomyosin(TM)-binding protein. In vitro, Ca²⁺/CaM's binding to CaD reduces its affinity for actin, which reverses CaD's inhibitory effect on the actomyosin system. Thus, CaD is a major regulatory component of smooth muscle thin filaments. CaD also enhances the actin binding of TM and stabilizes actin filaments directly by binding along the side of F-actin. In vitro reconstitution experiments revealed that CaD competes with fascin, filamin, Arp2/3 complex, gelsolin and coflin for binding to F-actin. CaD's vital roles in various actin cytoskeleton-related cellular processes have been demonstrated in many different cell types, as described below.

Structural and Functional Relationships of CaD Isoforms

CaD is expressed by many vertebrates. There are two different molecular weight (Mr) isoforms of CaD: high-Mr CaD (h-CaD, 120–150 kDa) and low-Mr CaD (l-CaD, 70–80 kDa). The two isoforms have common amino(N)- and carboxy(C)-terminal domains, but h-CaD has a specific insertion of the central repeating domain containing a lysine-, arginine-, glutamate- and alanine-rich unit (Fig. 1A). The human CaD gene (CALD1), which maps to 7q33 (Fig. 1B), generates at least eight species of CaD mRNA from its 13 exons. CaD's isoforms are mainly generated by the selective splicing together of exon 1a or 1b, exon 3a or 3ab and/or exon 4. Exons 1a and 1b encode the fibroblast- (fibro-) and HeLa-type N-terminal sequences, respectively. Exon 3b encodes the unique central repeating domain, consisting of the 229 amino acids specific to h-CaD. mRNA transcripts in which exon 3 is represented only
Both CaD proteins are composed of approximately 50% charged amino acids, such as glutamate, lysine and arginine, which form a particularly prominent cluster in the h-CaD central repeating domain. Owing to its peculiar amino acid composition, h-CaD exhibits slower mobility on SDS-PAGE than predicted simply by deducing the molecular mass from the amino acid sequence.\textsuperscript{26} The actin-binding site, one CaM-binding site and the myosin ATPase inhibition site are located within CaD's C-terminal region,\textsuperscript{26-28} whereas the myosin-binding and second CaM-binding sites are in the N-terminal region.\textsuperscript{29-32} Both CaD isoforms have an elongated shape when viewed by electron microscopy.\textsuperscript{30,33}

How has CaD changed evolutionally? Diligent genomic analyses reveal that CaD is ubiquitously distributed among vertebrates (Fig. 2A). The N-terminal myosin- and CaM-binding regions and the C-terminal actin-, CaM- and TM-binding regions are highly conserved among vertebrate species, whereas several spacers are inserted in the internal region in some species (Fig. 2A). CaD has a partial homology to troponin T,\textsuperscript{26} which is a critical regulator of Ca\textsuperscript{2+}-dependent actin-myosin contraction. These observations may provide a new insight into the evolutionary diversity of CaD isoforms, human CALD1 gene and the transcripts. (A) The domain structures of h- and l-CaD.\textsuperscript{7,115} The myosin-binding site and the potential CaM-binding site are located in N-terminal (light gray). The actin (black), TM (horizontal line)-CaM (diagonal line)-binding sites are located in C-terminus. The central repeating domain (dark gray) is inserted in h-CaD. (B) The exon/intron organization of the human CALD1 gene. The CALD1 locus is located in 7q33 (chromosome 7). Boxes indicate the exons. The fibro- and HeLa-type promoters are located in the flanking regions of exon 1a-1 and 1b, respectively (black arrows). A potential exon (1a-1') and the promoter region were identified by a database search of Ensembl (http://uswest.ensembl.org/index.html). (C) The five major mRNA species are shown. CaD isoform diversity is primarily generated by alternative transcriptional initiation from exon 1a or 1b, and by alternative splicing of exon 3a or 3ab and/or exon 4. The h-CaD mRNA containing exon 3ab is specifically generated in differentiated SMCs.
skeletal, smooth and cardiac muscle, as well as non-muscle contractile systems.

Although h- and l-CaD display the same biochemical character in vitro, their distribution is mutually exclusive. h-CaD is abundantly and specifically expressed in smooth muscle cells (SMCs), whereas l-CaD is ubiquitously expressed in non-muscle cells. h-CaD is localized to the actomyosin contractile structure in SMCs as an integral component of the thin filaments. In

Figure 2. The vertebral orthologs of CaD. (A) The domain structures of CaD orthologs among vertebrates. The homology/similarity values to N- or C-terminal domain of human-l-CaD are shown, respectively. Human (Homo sapiens) h-CaD [NP_149129.2], fibro l-CaD I [NP_004333.1], rat (Rattus norvegicus) l-CaD [AA68521.1], mouse (Mus musculus) I-CaD [AAH19435.1], chicken (Gallus gallus) h-CaD [BAA04539.1], I-CaD [BAA04540.1], lizard (Anolis carolinensis) CaD [Ensemble ENSACAP00000011703], frog (Xenopus tropicalis) CaD [XP_002932341.1], fish (Danio rerio) CaD [XP_692347.4]. (B) A result of phylogenetic analysis using MAGA4 software based on ClustalW program from the homologies with the exception of the central inserted domain. The branch lengths are proportional to the inferred evolutionary change. The scale bar corresponds to 0.1 estimated amino acid substitutions per site. The bootstrap values are shown at nodes.

152 Cell Adhesion & Migration Volume 5 Issue 2
migrating cells, l-CaD is localized to stress fibers, membrane ruffles and lamellipodial extensions. Stress fibers provide the structural and contractile cytoarchitecture for cell morphology and motility. Both CaD and myosin II are localized to stress fibers, but are not in the focal adhesions that provide attachment sites to the substratum at the stress-fiber termini. It is not clear whether CaD isoforms function differently in vivo. Guo and Wang have generated transgenic h-CaD-specific knockout mice without affecting l-CaD expression. The homozygotes are viable, though some newborns die 5–7 h after birth with symptoms similar to an umbilical hernia. It has recently been reported that knockdown of CaD ortholog in zebrafish causes defects in vasculogenesis and angiogenesis, as well as in cardiac organogenesis. These organotypic abnormalities may cause serious functional defects in cardiac contractility and blood circulation. Together, these reports suggest that CaD is essential for normal vertebrate development, in particular for development of organs composed of SMCs.

Transcriptional Regulation of the CaD (CALD1) Gene

Three different mechanisms for CALD1 gene transactivation have been demonstrated. In SMCs and non-muscle cells, transcription of the CALD1 gene primarily depends on serum response factor (SRF), which binds to the CC(A/T)GG motif (known as a CArG box) within the CALD1 promoter region and activates its transcription. There is a conserved CArG element in the proximal site of the fibro-type 1st exon (exon 1a-1), and SMG-specific CALD1 gene transcription regulated by the coordinated function of a triad of transcription factors: SRF, Nkx homeobox (Nkx 3.2) factor and GATA6. Recently, myocardin was identified as a potent co-factor for SRF-mediated transcription in cardiac and SMC gene expression, as it is restricted to cardiac muscle and SMCs. In non-muscle cells, myocardin-related transcription factors (MRTFs) activate actin cytoskeletal gene transcription, including the CALD1 gene, via the Rho-MRTF-SRF pathway. Glucocorticoid receptor (GR) mediates a second pathway for activating the CALD1 gene transcription. In lung adenocarcinoma A549 cells and neural progenitor cells (NPCs), GR binds directly to the two glucocorticoid (GC)-response elements (GREs) located near the transcriptional start site of the fibro-type CALD1 promoter, and enhances CALD1 transcription. The MRTF/SRF-dependent and GR-dependent CALD1 transcription pathways are mutually independent, and GC-dependent CaD upregulation is prominent in cells expressing low levels of CaD before stimulation. A third regulatory pathway, in which CaD expression is upregulated by p53 was recently reported by Mukhopadhyay et al. The activation of p53 correlates with increased CaD expression. Although a chromatin-immunoprecipitation (ChIP) genome-wide analysis show that p53 binds directly to the CALD1 gene flanking region, the precise mechanism of this novel transactivation pathway remains to be elucidated.

h-CaD is specifically expressed at high levels in differentiated SMCs, and serves as a marker for these cells. In developing chicken gizzards, a CaD isoform conversion from the l- to the h-form, accompanied by an increase to high expression levels, correlates with SMC differentiation. Conversely, CaD expression decreases and converts from the h- to the l-form in association with vascular SMC (VSMC) dedifferentiation in primary culture. Thus, the change in the CaD isoforms expressed precisely reflects the change in SMC phenotypes.

Differentiated VSMCs are spindle-shaped, show ligand-induced contractility, and are positive for SMC molecular markers, whereas dedifferentiated VSMCs lose these properties. Earlier culture methods produced only dedifferentiated VSMCs. By using the sensitive, phenotype-related CaD isoform conversion and other SMC molecular markers to monitor SMC phenotypes, our research group attempted to establish a primary culture system for VSMCs that preserved the differentiated phenotype (Fig. 3A and B). VSMCs cultured on laminin under IGF-I-stimulated conditions are spindle-shaped, have carbachol-induced contractility and express high levels of h-CaD and other SMC markers; i.e., they display a fully differentiated phenotype. Using this culture system, Hayashi et al. elucidated the signaling pathways regulating the VSMC phenotypes. IGF-I, IGF-II and insulin can maintain a differentiated phenotype via the PI3K-PKB/Akt pathway, whereas PDGF, EGF, bFGF, and angiotensin II and serum induce VSMC dedifferentiation via the coordinated activation of the ERK and p38MAPK pathways. Thus, the VSMC phenotype is determined by the balance of strength between the PI3K-PKB/Akt pathway and the ERK and p38MAPK pathways (Fig. 3C).

The phenotypic modulation of VSMCs from differentiated to dedifferentiated is critically involved in the development and progression of atherosclerosis. In particular, early events in atherosclerosis are initiated by the formation of neointima, which is primarily composed of dedifferentiated, proliferating VSMCs. Using this VSMC culture system, we further identified bioactive phospholipids, lysophosphatidic acids (LPAs), which are abundant in human serum, but not in plasma, as potent VSMC dedifferentiation factors. In vivo, unsaturated LPAs induce VSMC dedifferentiation by activating both ERK and p38MAPK, resulting in neo-intimal formation. Epiregulin, a member of the EGF growth factor family, was further identified as a paracrine factor that is involved in the dedifferentiation of normal VSMCs near neointima. Unsaturated LPAs actually also induce epiregulin expression. Thus, the expression of epiregulin triggered by unsaturated LPAs may be a progression factor during the early onset of atherosclerosis. These findings shed light on molecular mechanisms involved in the early stages of atherosclerosis.
CaD’s Role in Cellular Functions

CaD regulates cell morphology and motility. A number of reports have shown that increased CaD expression in non-muscle cells enhances stress fiber formation and reduces cell motility. In contrast, depleting CaD by gene silencing impairs or eliminates stress fibers. The formation of straight, thick stress fibers is thought to be mediated by CaD’s F-actin stabilizing and bundling/cross-linking activities. CaD’s binding to F-actin protects the filaments from actin-severing factors such as gelsolin and coflin, in vitro. Furthermore, in vivo, thick stress fibers stabilized by CaD are more resistant to the actin-depolymerizing drug cytochalasin B. Myosin ATPase inhibition by blebbistatin, a selective myosin II inhibitor, disrupts stress fibers. These results suggest that the formation of thick stress fibers requires CaD, both to stabilize F-actin and to properly regulate myosin activity.

CaD is phosphorylated by several protein kinases. PAK phosphorylation sites are located close to CaD’s CaM-binding sites. CaD phosphorylation by ERK1/2 and/or p38MAPK is involved in cell migration. When CaD is phosphorylated by PKC, both its binding to actin and Ca2+/CaM and its inhibitory effect on myosin ATPase activity are weakened in proportion to the degree of phosphorylation.

CaD phosphorylation sites for various kinases, including PKC, ERK and p38MAPK, are very near the C-terminal region that contains actin-, CaM- and TM-binding sites and inhibits myosin ATPase activity. CaD is also phosphorylated by other kinases, such as Ca2+/CaM-dependent protein kinase II (CaMKII), cdc2 protein kinase, myosin light chain kinase (MLCK), protein kinase A (PKA) and casein kinase II. In addition to serine/threonine phosphorylation, CaD is phosphorylated on tyrosine residues. Highly phosphorylated CaD tyrosine residues are located in the N-terminal region, which possesses myosin- and secondary CaM-binding sites. CaD is phosphorylated on tyrosine during the cellular response to viral-type EGFR (v-erbB) activation, which promotes CaD binding to myosin II, Grb2, Shc, Nck, MLCK and PAK. Although the role of CaD’s interactions with these scaffold proteins has not been elucidated, they are thought to contribute to actin cytoskeletal reorganization and to anchorage-independent growth in EGFR-activated tumor cells. High levels of tyrosine phosphorylation in CaD are observed in zyxin-deficient cells, in which focal adhesion assembly and cell motility are enhanced, further implicating CaD in actin cytoskeletal remodeling and motility.
Upregulated CaD mediates glucocorticoid-induced inhibition of cell migration. In addition to the qualitative regulation of CaD by Ca<sup>2+</sup>/CaM and phosphorylation, quantitative regulation through transcription regulation has been documented. GCs are major stress-response mediators that regulate numerous cellular functions. In the human lung adenocarcinoma A549 cell line, GC-induced CaD upregulation enhances the formation of thick stress fibers and focal adhesions, which suppress cell migration.45,46 This GC-dependent CaD upregulation also delays neuronal migration during neocortical development. CaD is upregulated by activated GR binding to two GREs in the fibrotype CALD1 promoter.

It is well documented that stress-triggered GCs, as well as acute or chronic GC treatment, impair the structural and functional plasticity of the brain. The exposure of perinatal animals and humans to excess stress/GCs can affect brain development, leading to altered behaviors in adult offspring in animals, and an increased risk of psychiatric disorders in humans (reviewed in ref. 84). Fukumoto et al. recently showed that excessive GC exposure retards NPC migration by dysregulating actin-myosin interactions via CaD upregulation.47,48 Migrating NPCs are normally bipolar, with a characteristic long leading process and rear retracting process. GC-treated NPCs become multipolar and CaD, F-actin and myosin IIA are found in the soma and concentrated in the tips of the multipolar processes. As with GC-treated NPCs, CaD-overexpressing cells exhibit a bipolar-to-multipolar transition, with dynamic growth endings at the tips of their multipolar processes, and they migrate randomly. These data indicate that an appropriate level of CaD expression is critical for the actin-myosin II interactions in NPCs that maintain their bipolar shape and linear migration. Elucidating the molecular mechanisms underlying the detrimental effect of GCs on neocortical development will expand our understanding of how stress/GCs alter neural network formation and affect behaviors later in life.

CaD regulates cell morphology and motility in the Rho-dependent MRTF/SRF transactivation pathway. The Rho-MRTF/SRF pathway plays important roles in numerous physiological processes by upregulating actin cytoskeletal/focal adhesion genes, including CaD. RhoA is a crucial regulator of stress fiber formation, focal adhesion assembly and cell morphology and motility, mediated by the reorganization of the actin cytoskeleton.45 It is well-documented that the RhoA-ROCK-LIMK pathway increases actin polymerization by inactivating its downstream target, ADF/cofilin, which severs F-actin.86 Rho’s interactions with diaphanous-related formin (DRF) family proteins leads to the nucleation and elongation of non-branched F-actin.87 Rho activation triggers the translocation of MRTFs to the nucleus, and enhances the MRTF/SRF-dependent transcription of actin cytoskeletal/focal adhesion genes such as CaD, TM, vinculin and zyxin.88 MRTF activation enhances stress fiber formation and focal adhesion assembly, in parallel with actin cytoskeletal/focal adhesion protein upregulation. On the other hand, MRTF inhibition leads to morphological changes, including decreased cell spreading and adherence and fewer stress fibers and focal adhesions.45 The transcriptional regulation of actin cytoskeletal/focal adhesion genes via the MRTF/SRF-dependent pathway is essential to maintaining and changing cell morphology in response to stimulation and to changes in the extracellular environment.

MRTF/SRF-dependent transcriptional regulation also contributes to epithelial-mesenchymal transition (EMT). EMT is a critical process that occurs during embryonic development, fibrosis and tumor progression. It is defined as a reduction in cell-cell adhesion accompanying decreased epithelial gene expression,
acquisition of motility, cell-shape alteration and increased mesenchymal gene expression.99-101 The remodeling of the actin cytoskeleton is a major EMT event, and it is part of the alteration from cell-adhesion to cell motility.

Transforming growth factor β (TGFβ) is one of the main EMT inducers; it triggers the dissociation of cell-cell contacts and actin cytoskeletal remodeling, permitting adherent epithelial cells to scatter and migrate directionally through the extracellular matrix.22 In human, mouse and canine epithelial cells, TGFβ1 stimulation causes a series of actin cytoskeletal genes, including CaD, to be upregulated during EMT via the Rho-MRTF/SRF pathway.23 TGFβ stimulation also increases Snail expression via MRTF/Smad activation, in which Snail is a transcription factor that induces cell-cell detachment by repressing E-cadherin,94 which leads to cells being released from cell-cell attachments simultaneously with the remodeling of their actin cytoskeleton.95 In kidney tubule cells as well, the Rho-MRTF/SRF-dependent pathway mediates the disruption of cell-cell contact, inducing EMT.95 These regulatory mechanisms maybe involved in various pathological events, including cancer metastasis and fibrosis, as well as in development.96,97

CaD is a suppressor protein in podosome/invadopodium formation. The podosome and invadopodium are highly dynamic cell adhesion structures that degrade the extracellular matrix (ECM) and promote cell invasion.98-101 While CaD is not localized at focal adhesions, it is a component of the podosome.102-105 CaD suppresses podosome/invadopodium formation in smooth muscle cells and in transformed or cancer cells.102-103 CaD depletion results in the increased formation of numerous small podosomes/invadopodia, which have a significantly shorter lifetime and more mobility than control cells.103 Forced CaD expression, on the other hand, suppresses the formation of podosomes/invadopodia, ECM degradation and cell invasion.104 CaD suppresses podosome/invadopodium formation by competing with the Arp2/3 complex, which is activated by N-WASP and is required for podosome formation.105 In addition, CaD's role in podosome formation is regulated by PAK phosphorylation and Ca2+/CaM binding.102,103 These results are consistent with CaD being a potent suppressor of cancer cell invasion.

CaD expression is decreased in certain transformed and cancer cells.22,37,104 A decrease in the expression of CaD and TM correlates with suppressed MRTF nuclear translocation in Ras- or Src-transformed intestinal epithelial cells, suggesting that MRTF/SRF-dependent transcription is also downregulated.105 Furthermore, the ectopic expression of active-form MRTFs reverses the inhibition of CaD and TM expression and the associated morphological phenotypes, resulting in less invasive, anchorage-independent growth. In vivo, cancer cells that express active MRTFs form fewer subcutaneous cancer foci and show fewer liver metastases from the spleen.105 In another metastasis models in which breast carcinoma or melanoma cells were injected into the mouse tail vein, inactivating the MRTF/SRF pathway reduces cell motility and metastasis to the lung, whereas forced expression of active-MRTF-A increased colonization of the lung.106 This discrepancy may be due to differences in the metastasis model. A recent report shows that p53 activation upregulates CaD expression and suppresses tumorigenic phenotypes;107 p53 suppresses podosome/invadopodium formation, promotes stress fiber stability and attenuates cell migration/invasion mediated by upregulation of CaD.48,107 These results suggest that CaD is a critical target of p53 in preventing invasion and metastasis.

CaD is a regulatory protein and cytokinesis component. During mitosis, CaD is phosphorylated by cdc2 kinase, which greatly reduces CaD's binding affinity for actin filaments.79,76,108 During cytokinesis, CaD is not detected in cleavage furrows mainly composed of contractile elements, such as myosin and actin.109 A CaD mutation containing alanine substitutions in all seven cdc2 phosphorylation sites inhibits cell division and delays M-phase entry in Xenopus embryos and CHO cells.110 This mutated CaD inhibits microfilament disassembly during mitosis. These results suggest that the regulation of actin filament assembly/disassembly in cleavage furrows by CaD via cdc2-dependent phosphorylation is important for cell cycle progression.

Manes et al. reported that cdc2 activity increases in cell migration through its specific association with cyclinB2 in the αvβ3 integrin signaling pathway.111 CaD and cdc2 are co-localized in membrane ruffles in motile cells. Their finding suggests that CaD phosphorylation by cdc2 is required to activate cell motility via the αvβ3 integrin-cdc2-dependent pathway, since a CaD mutation affecting all seven cdc2 phosphorylation sites blocks cdc2-induced motility. Cdc2-dependent CaD regulation may therefore be significant in tumorigenesis, which involves successive cell proliferation and configuration.

CaD regulates secretion. Stimulation of adrenal chromaffin cells increases the concentration of intracellular free Ca2+3, which initiates catecholamine secretion by exocytosis. CaD acts to regulate the organization of actin filaments beneath the cell periphery during the secretory process.112 CaD also plays a role in GnRH (gonadotropin releasing hormone)-stimulated gonadotropin release from the pituitary.113 Similarly, GC induces negative feedback regulation of ACTH (adrenocorticotropic hormone) secretion by upregulating CaD to stabilize actin filaments.113 Further, it is likely that TM and CaD affect intracellular granule movement by regulating the contractile system in response to Ca2+-changes inside non-muscle cells.114 These data indicate that CaD regulates secretion and intracellular granule trafficking in a Ca2+-dependent manner.

Conclusion

It has become clear that CaD is an important regulator of cell motility, such as cell morphology, migration, cytokinesis and secretion as well as of smooth muscle contraction (Fig. 4). CaD regulates the organization of the actin cytoskeleton and actin/myosin-dependent contractility. Because these mechanisms are fundamental for cell structure and force generation, they link to numerous cellular processes, such as intracellular trafficking, cell morphology maintenance and changes, cytokinesis, secretion, cell adhesion and migration. CaD’s functions are controlled by Ca2+/CaM binding and/or phosphorylation,
which provide spatio-temporal control of directed motility. Further, CaD expression levels are regulated by at least three different signaling pathways—the GR-, Rho-MRTF/SRF- and p53-dependent pathways, all of which are all deeply involved in development, stress response and various pathological changes. Although we have made remarkable progress in understanding the physiological roles of CaD, this may be only a partial list of its functions. CaD’s ubiquitous distribution and fundamental functions suggest that it is likely to play significant roles in other, as yet unknown, cellular processes.

Acknowledgements

This work was supported by Grant-in-aid for Scientific Research 20240038 (to K.S.) and Grant-in-aid for Young Scientist 21700352 (to T.M.) from the Japan Society for the Promotion of Science.

References

1. Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, et al. Cell migration: integrating signals from front to back. Science 2003; 302:1784-9.
2. Condeelis J, Segall JE. Intravital imaging of cell movement in tumours. Nat Rev Cancer 2003; 3:921-30.
3. Wang W, Goswami S, Sahai E, Byskov JB, Segall JE, Condeelis JS. Tumor cells caught in the act of invading: their strategy for enhanced cell motility. Trends Cell Biol 2005; 15:338-45.
4. Pantaloni D, Le Clinchae C, Carlier MF. Mechanism of actin-based motility. Science 2001; 292:1502-6.
5. Chihbara ES, Higgi HN. Themyary faces of actin: matching assembly factors with cellular structures. Nat Cell Biol 2007; 9:1110-21.
6. Sobue K, Muramoto Y, Fujita M, Kakiuchi S. Purification of a calmodulin-binding protein from chicken gizzard that interacts with F-actin. Proc Natl Acad Sci USA 1981; 78:5652-5.
7. Sobue K, Seller JR. Caldesmon, a novel regulatory protein in smooth muscle and nonmuscle actin-myosin systems. J Biol Chem 1991; 266:2415-8.
8. Sobue K, Morimoto K, Inui M, Kanda K, Kakiuchi S. Control of actin-myosin interaction of gizzard smooth muscle by calmodulin- and caldesmon-linked flip-flop mechanism. Biomed Res 1982; 3:188-96.
9. Ngai PK, Walsh MP. Inhibition of smooth muscle actin-activated myosin Mg2+-ATPase activity by caldesmon. J Biol Chem 1984; 259:13656-9.
10. Marston SB, Lehman W. Caldesmon is a Ca2+-regulated component of native smooth-muscle thin filaments. Biochem J 1985; 231:517-22.
11. Brezcher A, Lynch W. Identification and localization of immune reactive forms of caldesmon in smooth and nonmuscle cells: a comparison with the distributions of tropomyosin and alpha-actinin. J Cell Biol 1985; 100:1656-63.
12. Furst DO, Cross RA, De Mey J, Small JV. Caldesmon is an elongated, flexible molecule localized in the acto-myosin domains of smooth muscle. EMBO J 1986; 5:251-7.
13. Yamazaki K, Iroh K, Sobue K, Mori T, Shibata N. Purification of caldesmon and myosin light chain (MLC) kinase from gizzard smooth muscle: comparisons with gizzard caldesmon and MLC kinase. J Biochem 1987; 100:1-9.
14. Dings J, Hw0 S, Bryan J. Identification by monoclonal antibodies and characterization of human platelet caldesmon. J Cell Biol 1986; 102:1748-57.
15. Ishikawa R, Yamashiro S, Matsumura F. Differential modulation of actin-severing activity of gelolin by multiple isoforms of cultured rat cell cytoplasm. Potentiation of protective ability of tropomyosins by 83 kDa nonmuscle caldesmon. J Cell Biol 1989; 108:507-16.
16. Warren KS, Shutt DC, McDermott JP, Lin JL, Soll DR, Lin JJ. Overexpression of microfilament-stabilizing human caldesmon fragment, CaD39, affects cell attachment, spreading and cytoskeleton. J Cell Biol 1994; 125:359-68.
17. Ishikawa R, Yamashiro S, Matsumura F. Regulation of actin binding and actin bundling activities of fascin by caldesmon coupled with tropomyosin. J Biol Chem 1998; 273:26901-7.
18. Nomura M, Yoshikawa K, Tanaka T, Sobue K, Matsumura F. The role of tropomyosin in the interaction of F-actin with caldesmon and actin-binding protein (or filamin). Eur J Biochem 1987; 163:467-71.
19. Yamakita Y, Oosawa F, Yamashiro S, Matsumura F. Caldesmon inhibits Arp2/3-mediated actin nucleation. J Biol Chem 2003; 278:17975-44.
20. Yonezawa NO, Deshpande R, Sakai H. Studies on the interaction between actin and collin purified by a new method. Biochem J 1988; 251:121-7.
21. Owada MK, Hakura A, IidaK, Yahara I, Sobue K, Kakiuchi S. Occurrence of caldesmon (a calmodulin-binding protein) in cultured cells: comparison of normal and transformed cells. Proc Natl Acad Sci USA 1984; 81:3133-7.
22. Sobue K, Tanaka T, Kanda K, Ashino N, Kakiuchi S. Purification and characterization of caldesmon77: a calmodulin-binding protein that interacts with actin filaments from bovine adrenal medulla. Proc Natl Acad Sci USA 1985; 82:9205-9.
23. Hayashi K, Yano H, Hashida T, Takeuchi R, Takeda O, Asada K, et al. Genomic structure of the human caldesmon gene. Proc Natl Acad Sci USA 1992; 89:12412-6.
24. Yano H, Hayashi K, Harana H, Ito J, Kobayashi M, Identification of two distinct promoters in the chicken caldesmon gene. Biochem Biophys Res Commun 1994; 201:618-26.
25. Hayashi K, Fujino Y, Kato I, Sobue K. Structural and functional relationships between h- and l-caldesmons. J Biol Chem 1991; 266:255-61.
26. S installed A, Dohuwawska R, Sakai H, Modulated actin microfilament dynamics of F-actin with caldesmon and actin-binding proteins (actin, myosin, caldesmon and tropomyosin) in normal and transformed cells. J Cell Sci 1993; 104:595-606.
27. Guo H, Wang CL. Specific disruption of smooth muscle caldesmon expression in mice. Biochem Biophys Res Commun 2003; 300:1132-7.
28. Zheng PP, Severijnen LA, van der Weiden M, Willemsen R, Kros JM. A crucial role of caldesmon in vascular development in vivo. Cardiovasc Res 2009; 81:562-9.
29. Zheng PP, Severijnen LA, Willemsen R, Kros JM. Caldesmon is essential for cardiac morphogenesis and function: in vivo study using a zebrafish model. Biochem Biophys Res Commun 2009; 378:37-40.
30. Yan H, Hayashi K, Momiyama T, Saga H, Haruna M, Sobue K. Transcriptional regulation of the chicken caldesmon gene. Activation of gizzard-type caldesmon promoter requires a CAAG box-like motif. J Biol Chem 1999; 274:2661L6.
31. Momiyama T, Hayashi K, Obata H, Chimiyo Y, Nishida T, Ito T, et al. Functional involvement of serum response factor in the transcriptional regulation of caldesmon gene. Biochem Biophys Res Commun 1996; 242:429-35.
32. Nishida W, Nakamura M, Mori S, Takahashi M, Ohkawa Y, Tadakad S, et al. A triad of serum response factor and the GATA and NK families governs the transcription of smooth and cardiac muscle genes. J Biol Chem 2002; 277:7308-17.
33. Wang D, Chang PS, Wang Z, Sutherland L, Richardson JA, Small E, et al. Activation of cardiac gene expression by myocardin, a transcriptional cofactor for serum response factor. Cell 2001; 105:851-62.
34. Morita T, Mayanagi T, Sobue K. Reorganization of the actin cytoskeleton via transcriptional regulation of cytoskeletal/focal adhesion genes by myocardin-related transcription factors (MRTFs/MAL/MKLs). Exp Cell Res 2007; 313:3432-45.
35. Mayanagi T, Morita T, Hayashi K, Fukumoto K, Sobue K. Glucocorticoid receptor-mediated expression of caldesmon regulates cell migration via the reorganization of the actin cytoskeleton. J Biol Chem 2008; 283:31183-96.
36. Fukumoto K, Morita T, Mayanagi T, Tanokashira D, Yoshida T, Sakai A, et al. Dermal effects of glucocorticoids on actin cytoskeletons during skin development. Mol Psychiatry 2009; 14:1119-31.
37. Mukhopadhyay UK, Eves R, Jia L, Mooney P, Mak AS. p53 suppresses Src-induced podosome and rosette formation and cellular invasiveness through the upregulation of caldesmon. Mol Cell Biol 2009; 29:3088-98.
38. Wei CL, Wu Q, Vega VB, Chi K, Ng P, Zhang T, et al. A global map of p53 transcription-factor binding sites in the human genome. Cell 2006; 124:20719.
39. Ueki N, Sobue K, Kanda K, Hada T, Higashino K. Expression of high and low molecular weight caldesmon during phenotypic modulation of smooth muscle cells. Proc Natl Acad Sci USA 1987; 84:9049-53.
51. Hayashi K, Saga H, Chimori Y, Kimura K, Yamazaki Y, Sobe K. Differentially phosphoryte of smooth muscle cells depends on signaling pathways through insulin-like growth factors and phosphatidylinositol 3-kinase. J Biol Chem 1998; 273:28860-7.
52. Yamazaki Y, Saga H, Hayashi K, Ohkawa Y, Ogara A, Aoki J, et al. Vascular remodeling induced by naturally occurring unsaturated lysophosphatidic acid in vivo. Circulation 2003; 108:1746-52.
53. Takahashi M, Hayashi K, Yoshida K, Ohkawa Y, Komurasaki T, Kiribatake A, et al. Epiregulin as a mammalian paracrine factor released from ERK and p38MAP-activated vascular smooth muscle cells. Circulation 2003; 108:2524-9.
54. Sobe K. Expression regulation of smooth muscle cell-specific genes in association with phenotypic modula-
06; 63:54-62.
55. Hayashi K, Takahashi M, Kimura K, Nishida W, Saga H, Sobe K. Changes in the balance of phosphoinositol-3-kinase/protein kinase B (Akt) and the mitogen-activated protein kinase pathways in human platelets. J Biol Chem 2001; 276:78-80.
56. Ross R. The pathogenesis of atherosclerosis: a perspec-
tive for the 1990s. Nature 1993; 362:801-9.
57. Glukhova MA, Frid MG, Kotelsiansky VE. Phenotypic changes of human aortic smooth muscle cells during development in the adult vessel. Am J Physiol 1991; 261:715-80.
58. Hayashi K, Takahashi M, Nishida W, Yoshida K, Ohkawa Y, Kiribatake A, et al. Phenotypic modulation of vascular smooth muscle cells induced by unsaturated lysophosphatic acids. Circ Res 2001; 89:251-8.
59. Castellino F, Heuser J, Michetti S, Bruino B, Luini A. Glucocorticoid stabilization of actin filaments: a possible mechanism for inhibition of corticotropin release. Proc Natl Acad Sci USA 1992; 89:3775-9.
60. Castellino F, Ono S, Matsumura F, Luini A. Essential role of caldesmon in the actin filament reorganiza-
tion induced by glucocorticoids. J Cell Biol 1995; 131:1223-29.
61. Li Y, Lin JL, Reiter RS, Daniels K, Soll DR, Lin JJ. Caldesmon mutant defective in Ca2+-calmodulin bind-
ing interferes with assembly of stress fibers and affects cell morphology, growth and motility. J Cell Sci 2004; 117:5939-604.
62. Mirzapouzoa T, Kolosova IA, Romen L, Garcia JG, Verin AD. The role of caldesmon in the regulation of epithelial cytoskeleton and migration. J Cell Physiol 2005; 203:520-6.
63. Eppinga RD, Li Y, Lin JL, Mak AS, Lin JJ. Requirement of reversible caldesmon phosphorylation at P21-
activated kinase-responsive sites for lamellipodia extension during cell migration. Cell Motil Cytoskeleton 2004; 58:181-91.
64. Lynch WP, Riseman VM, Brencher A. Smooth muscle caldesmon is an extended flexible monomeric protein in solution that can readily undergo reversible intra-
and intermolecular sulphydryl cross-linking. A mecha-
nism for caldesmon's F-actin bundling activity. J Biol Chem 1987; 262:7429-37.
65. Van Eyk JE, Arrell DK, Foster DB, Strauss JD, Heimonen YT, Furmaniak-Kazmczak E, et al. Different molecular mechanisms for Rho family GDP-dependent GTP-convertases in motile cells in culture. J Biol Chem 1998; 273:34343-9.
66. Foster DB, Shen LH, Kelly J, Thibault JE, Elyk V, Mak AS. Phosphorylation of caldesmon by p21-activated kinase. Implications for the Ca2+ sensitivity of smooth muscle contraction. J Biol Chem 2000; 275:17959-65.
67. Jiang Q, Huang R, Cai S, Wang CLA. Caldesmon regu-
lates the motility of vascular smooth muscle cells by modulating the actin cytoskeleton stability. J Biomed Sci 2010; 17:6.
68. Umekawa H, Hidaka H. Phosphorylation of calde-
smon by protein kinase C. Biochem Biophys Res Commun 1985; 132:56-62.
69. Lichtfield DW, Ball EH. Phosphorylation of calde-
smon77 by protein kinase C in vitro and in intact human platelets. J Biol Chem 1987; 262:8056-60.
70. Tanaka T, Ohra H, Kanda K, Hidaka H, Sobe K. Phosphorylation of high-Mr caldesmon by protein kinase C modulates the regulatory function of this protein on the interaction between actin and myosin. Eur J Biochem 1990; 188:495-500.
71. Childs TJ, Watson MH, Sanghera JS, Campbell DL, Peiche SL, Mak AS. 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; 267:22853-9.
72. Adam LP, Hataway DR. Identification of mitogen-
activated protein kinase phosphorylation sequences in mammalian b-caldesmon. FEBS Lett 1993; 322:36-60.
73. Patchell VB, Vorontsova AV, Gao Y, Low DG, Evans JS, Fattoum A, et al. Phosphorylation of the minimal inhibitor region at the C-terminus of caldesmon alters its structural and actin binding properties. Biochem Biophys Acta 2002; 1596:121-30.
74. Goncharova EA, Vorontsova AV, Gracheva EO, Wang CL, Paneri RA, Stefansson YY, et al. Activation of p38 MAP-kinase and caldesmon phosphorylation are essential for urokinase-induced human smooth muscle cell migration. Biochem Biophys Acta 2002; 1585-116.
75. Yamashita S, Yamakita Y, Hosoya H, Matsumura F. Phosphorylation of caldesmon by p34cdc2 during mitosis. Nature 1991; 349:169-72.
76. Mak AS, Watson MH, Litwin CM, Wang JH. Phosphorylation of caldesmon by cd2 kinase. J Biol Chem 1991; 266:6678-81.
77. Sobieczk A, Sarg B, Lindner H, Seow CY. Phosphorylation of myosin by myotonic light chain kinase increases its binding affinity for phosphorylated myosin filaments. J Biol Chem 2010; 391:109-114.
78. Hettasch JM, Sellers JR. Caldesmon phosphorylation in intact human platelets by cAMP-dependent pro-
tein kinase and protein kinase C. J Biol Chem 1991; 266:1876-81.
79. Vorontsova AV, Shirinsky VP, Gusev NB, Manus MJ, Lingle WL, Salisbury JL, Maihle NJ. A splice-
variant of calmodulin binding to the Shc Grb2 complex. J Biol Chem 1999; 274:33807-13.
80. McManus MJ, Li J, Edjkane S, Perez-Sanchez C, Gaggioli C, Sahai E, Rotstein OD, et al. Cell contact-dependent regulation of cell movement and survival: implications for domain mapping. FEBS Lett 2007; 581:777-82.
81. McManus MJ, Borrer JL, Daniels AJ, Wang Z, Matsumura F, Maile NH. An oncogenic epidermal growthfactor receptor signals via a p21-activated kinase-caldesmon-phospho-myosin phosphorysensitive complex. J Biol Chem 2000; 275:35328-34.
82. Hofmann LM, Jensen CC, Kloekers S, Wang CL, Yousagi M, Beckerle MC. Genetic alteration of zyxin causes Menke's syndrome, increased motil-
ity and deficits in actin remodeling. J Cell Biol 2006; 172:771-82.
83. Sobe K, Fukumoto K, Caldesmon, an actin-linked regulatory protein, comes across glucocorticoids. Cell Adh Migr 2010; 4:185-93.
84. Hall A. Rho GTPases and the actin cytoskeleton. Science 1998; 279:509-14.
85. Rafopoulos M, Hall A. Cell migration: Rho GTPases lead the way. Dev Bio 2004; 265:23-28.
86. Wallar BJ, Alberts AS. The formins: active scaffold binding that remodels the cytoskeleton. Trends Cell Biol 2003; 13:435-46.
87. Miralles F, Posern G, Zanorymuidou AI, Treisman R. Actin dynamics control SRF activity by regulation of its coactivator MAL. Cell 2003; 113:329-42.
109. Hosoya N, Hosoya H, Yamashiro S, Mohri H, Matsumura F. Localization of caldesmon and its dephosphorylation during cell division. J Cell Biol 1993; 121:1075-82.
110. Yamashiro S, Chern H, Yamakita Y, Matsumura F. Mutant caldesmon lacking cdk2 phosphorylation sites delays M-phase entry and inhibits cytokinesis. Mol Biol Cell 2001; 12:239-50.
111. Manes T, Zheng DQ, Togain S, Woodard AS, Marchisio PC, Languino LR. αvβ3 integrin expression upregulates cdk2, which modulates cell migration. J Cell Biol 2003; 161:817-26.
112. Burgoyne RD, Cheek TR, Norman KM. Identification of a secretory granule-binding protein as caldesmon. Nature 1986; 319:68-70.
113. Janovick JA, Natarajan K, Longo F, Conn PM. Caldesmon: a bifunctional (calmodulin and actin) binding protein which regulates stimulated gonadotrophin release. Endocrinology 1991; 129:68-74.
114. Hegemann TE, Schulte DL, Lin JL, Lin JJ. Inhibition of intracellular granule movement by microinjection of monoclonal antibodies against caldesmon. Cell Motil Cytoskeleton 1991; 20:109-20.
115. Helfman DM, Levy ET, Berthier C, Shrutman M, Riveline D, Grosheve I, et al. Caldesmon inhibits non-muscle cell contractility and interferes with formation of focal adhesions. Mol Biol Cell 1999; 10:3097-117.
116. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 2007; 24:1596-9.
117. Thompson JD, Higgins DG, Gibson TJ. Clustal W—improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994; 22:4673-80.