Sangdun Choi
Editor

Encyclopedia of Signaling Molecules

Second Edition

With 1893 Figures and 247 Tables

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CalDAG-GEFII

▶ RasGRP1

CALM1

▶ Calmodulin (CALM1)

CALM2

▶ Calmodulin (CALM1)

CALM3

▶ Calmodulin (CALM1)

Calmodulin (CALM1)

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Synonyms

CALM1: CaM; caM; CAMI; PHKD; CPVT4; DD132; LQT14; CALML2
CALM2: PHKD; CAMII; LQT15; PHKD2
CALM3: CaM; PHKD; PHKD3; CaMIII; HEL-S-72

Historical Background

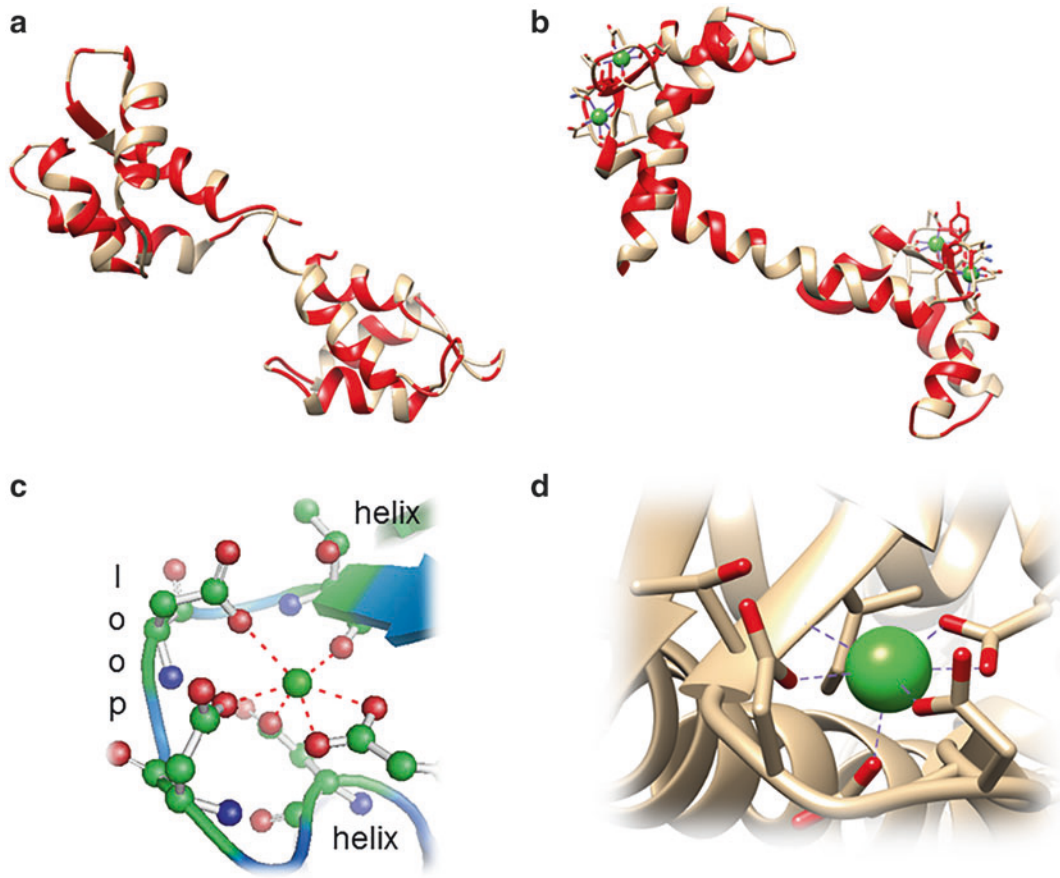
Calmodulin (CaM) was first discovered as an unidentified activator of cyclic 3′,5′-nucleotide phosphodiesterase (Cheung 1970). Human CaM is encoded by three distinct genes (CALM1, CALM2, and CALM3), which exhibit minor variations at the nucleotide level, yet express the same amino acid sequence. CaM is a predominantly helical intracellular protein (Fig. 1a) that mediates numerous Ca^{2+}-signaling activities in response to changes in cytosolic Ca^{2+} levels. CaM can be divided into two globular domains, each containing two cooperatively paired EF-hand motifs, allowing it to bind up to four Ca^{2+} ions (Kretsinger and Nockolds 1973) (Fig. 1b).

Each canonical EF-hand site consists of a highly-conserved 29-residue helix-loop-helix structure. The Ca^{2+} binding residues comprise twelve residues in the sequence, where residues in relative positions 1, 3, 5, 7, 9, and 12 typically coordinate binding of Ca^{2+} (Fig. 1c). The two structurally similar domains are separated by a transdomain linker region (residues 76–84) variously described as either helical in the Ca^{2+}-bound state (Fig. 1b), or as a flexible loop (Fig. 1a). The apparent flexibility of this region likely results in an equilibrium between conformational states and allows the protein to surround target biomolecules during Ca^{2+}-mediated signalling events. Subsequent to binding with intracellular calcium, the Ca^{2+}-CaM complex mediates numerous biological processes associated with cell division, cell death, signal transduction, inflammation, muscle contraction, memory, nerve growth, and the immune response (Fig. 2).

Thus, activation of CaM by Ca^{2+} allows the protein to adopt conformations necessary for interaction with a wide variety of biomolecules, including protein kinases, calcineurin, PDE, MLCK, NMDAR, ryanodine receptors, and DAPK, among others (Ikura and Ames 2006) (Fig. 3).

Activation of CaM

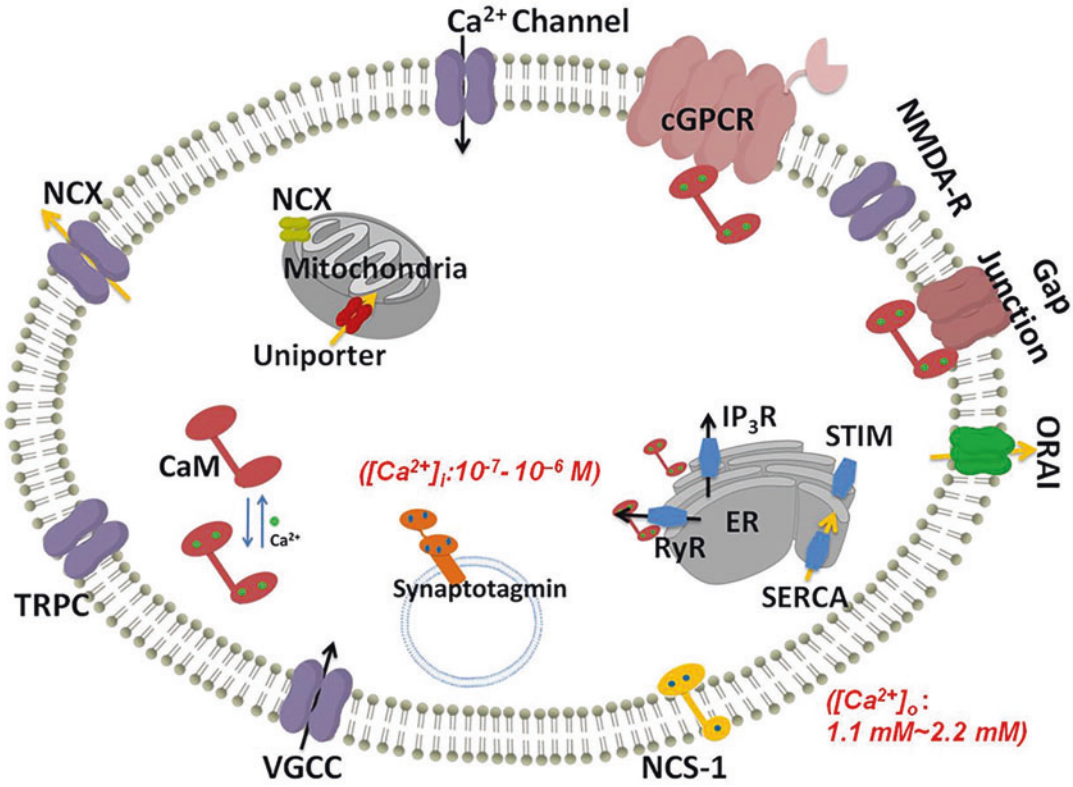
Cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_{CYT}$) in resting cells are maintained at levels ranging from 1000 to 10,000 times lower than extracellular concentrations. This gradient across the plasma membrane required to maintain



Calmodulin (CALM1), Fig. 1 (**a**) Calcium-free (apo) CaM, and (**b**) calcium-loaded (holo) CaM. (**c**) The canonical EF-Hand loop is comprised of a helix-loop-helix structure, with ligands forming a pentagonal bipyramidal structure coordinating the Ca^{2+} ion. Residues in the loop are assigned relative position numbers 1–12. Binding ligands are usually residues in positions 1, 3, 5, 7, 9, and 12. (**d**) Ca^{2+} is bound by oxygen ligands, including bidentate carboxyl groups Glu and Asp, mainchain oxygen, and oxygen from water molecules

intracellular Ca^{2+} homeostasis and regulate Ca^{2+} signalling involves influx of Ca^{2+} from the extracellular medium via Ca^{2+} channels (triggered by different stimuli including membrane depolarization, mechanical stretch, external agonists, depletion of internal stores, and intracellular messengers). The release or sequestration of internal Ca^{2+} levels within internal Ca^{2+} stores (ER or SR) is mediated by the IP_3 receptors (IP_3R) and the ryanodine receptors (RyR), and cytosolic Ca^{2+} homeostasis is further maintained by the actions of proteins, including the plasma membrane Ca^{2+}-ATPase (PMCA), the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), the secretory pathway Ca^{2+}-ATPase (SPCA), and the Na^{+}/Ca^{2+} exchanger (NCX), among others (Fig. 2).

CaM is activated by fluctuations in $[Ca^{2+}]_{CYT}$ that result in binding of Ca^{2+} ions in the four EF-hand binding sites (two paired sites in both the N- and C-domains) of the protein. Binding appears to occur in two steps, and several studies have established a relative order of occupancy for Ca^{2+} by NMR chemical shifts (Kirberger et al. 2013) indicating that ions are bound first in the C-domain EF-hand sites EF-III and EF-IV, followed by the N-domain sites EF-I and EF-II. Formation of the Ca^{2+}/CaM complex alters the conformation

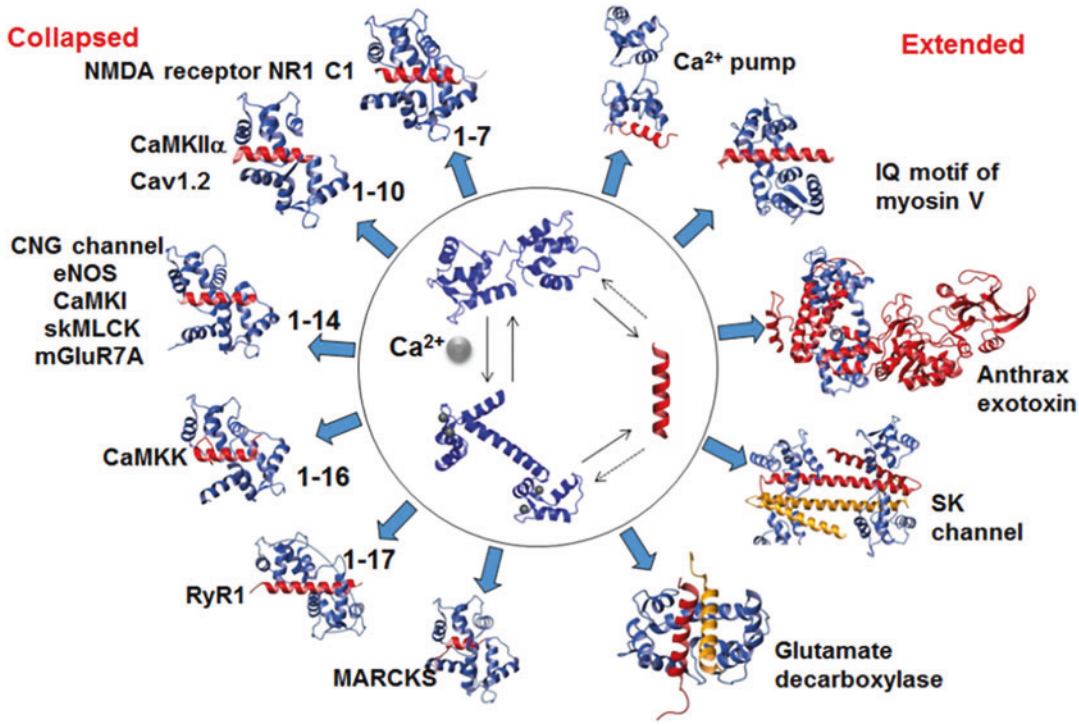


Calmodulin (CALM1), Fig. 2 Overview of Ca^{2+}-signaling. Intracellular Ca^{2+} concentration is maintained and regulated by ingress of extracellular Ca^{2+} through various Ca^{2+} channels, and release or uptake of Ca^{2+} by internal Ca^{2+} stores in the SR and ER. Fluctuations in intracellular Ca^{2+} activate CaM and other intracellular proteins, which allow Ca^{2+} to interact indirectly with a variety of other proteins and enzymes

of CaM, producing hydrophobic methionine-rich grooves that, coupled with the flexibility of the trans-domain helix, allow it to surround and anchor the basic amphiphilic helices with hydrophobic sidechains found in the CaM-binding domains (CBDs) of target proteins and peptides (Ikura and Ames 2006).

Binding Affinity and Cooperativity

In CaM, the paired EF-hand sites in the respective N- and C-domains reportedly bind Ca^{2+} ions cooperatively (Linse and Forsen 1995), with formation of a short β-strand connecting the two sites through hydrogen bonding interactions. This cooperativity between paired sites has made it difficult to accurately determine binding affinity values for individual sites. Methods used to determine individual affinity values have included the introduction of mutations to deactivate one of the paired sites, grafting sites into scaffold proteins, or development of thermodynamic models, all of which fail to adequately address the effects of intradomain cooperativity. Alternatively, various studies have reported domain-specific affinity values by monitoring changes in intrinsic fluorescence of Phe and Tyr residues (VanScyoc and Shea 2001; Jiang et al. 2010). Results of these studies report that the C-domain of CaM appears to bind Ca^{2+} with slightly higher affinity ($K_d \approx 2.0$) than the N-domain ($K_d \approx 10.0$) (Jiang et al. 2010; Kirberger et al. 2013), which is consistent with the reported order of site occupancy. Binding of Ca^{2+} to the C-domain is the rate-limiting step in Ca^{2+}/CaM complex



Calmodulin (CALM1), Fig. 3 CaM interacts with various target proteins through two binding modes, collapsed or extended, based on the distance between N- and C-domains of CaM. While CaM binds predominantly in the Ca^{2+}-loaded state (holo-CaM), it may also bind in a Ca^{2+}-free state (e.g., apo-CaM, with the IQ motif) or in a semiloaded state (Ca^{2+}-binding in only the higher affinity C-terminal domain). Identified binding modes include 1–10, 1–14, 1–11, 1–16 (Osawa et al. 1999), 1–17 (Maximciuc et al. 2006), and 1–10–14 (Gifford et al. 2007)

formation, as the on/off-rates of Ca^{2+} can be 30- to 150-fold slower for the C-domain than for the N-domain; however, binding of CaM with the PEP-19 enzyme appears to increase the on-rate for Ca^{2+} up to 40-fold, so the Ca^{2+}/CaM complex formation isn't solely regulated by temporal and spatial changes in cytosolic Ca^{2+} and CaM concentrations (Wang and Putkey 2016).

Evidence also indicates that the domains bind cooperatively, as well, so that Ca^{2+} occupies both EF-hand sites in the higher affinity C-terminal domain sites first, resulting in conformational changes that facilitate Ca^{2+}-binding in the N-domain sites (Evans and Shea 2009), which is also consistent with the reported rate-limiting effects. These conformational changes, as noted above, are responsible for formation of the hydrophobic groove regions necessary for CaM to interact with other proteins and peptides along the Ca^{2+}-signalling pathway, through different molecular interactions. CaM also binds various toxic metals, particularly Pb^{2+}, with higher affinity than Ca^{2+}. At low concentrations, Pb^{2+} can mimic Ca^{2+} by occupying the EF-hand sites, thus activating CaM. Our research also indicates that when Pb^{2+} occupies an EF-hand site, due to its slightly larger ionic radius, it produces small conformational changes in the site that are insufficient to alter protein function, but may disrupt cooperativity between sites (Kirberger et al. 2013), thus resulting in similar binding affinity values between paired sites. However, at higher concentrations of Pb^{2+}, the protein conformation is altered, and the protein activity is inhibited. This biphasic effect may be due to binding of Pb^{2+} in the flexible central helix joining the N- and C-domains (Kirberger et al. 2013), a region that may include Zn^{2+} and Mg^{2+} auxilliary binding sites capable of allosterically regulating the function of CaM.

CaM Binding Modes for Molecular Interactions

CaM is known to reversibly or irreversibly bind its targets which possess regions of net positive charge, moderate hydrophilicity, and moderate-to-high helical hydrophobic moments. Targets for holo-CaM contain amphipathic α-helices with two suitably spaced and oriented hydrophobic anchors which can insert into the methionine rich pockets (Maximciuc et al. 2006) and utilizes both N- and C-domains. Many target sequences are intrinsically disordered and undergo a disorder-to-order conformational transition (Ikura and Ames 2006). CaM interacts with its targets in two general binding modes: an extended or collapsed form (Fig. 3). In extended mode, the two domains of CaM interact with different sites of targets, which typically doesn't significantly alter the distance between the two domains. Conversely, in the collapsed or canonical wrap-around mode, when the two CaM domains bind to the hydrophobic anchor residues in the binding motif and surround the helical target, the distance between two domains is reduced, via flexibility of the central helix, from 50 Å to less than 10 Å. Based on separation of hydrophobic anchor residues in target sequences, 1–10, 1–14, 1–11, 1–16, 1–17, and 1–10–14 modes have been identified in crystal structures (Fig. 3). An interesting example of the extended mode of interaction is observed with calcineurin A, where two CaM units combine with two peptides (2:2 arrangement), which resembles the unliganded holo-CaM. In the collapsed binding form, several studies uisng peptide models have shown that Holo CaM binds to sites located in the second half of the intracellular loop of α-subfamily connexins ($Cx50p_{141–166}$, $Cx44p_{132–153}$, and $Cx43p_{136–158}$) which falls into the 1–5–10 subclass binding mode, where each number represents the presence of a hydrophobic residue (Zou et al. 2014).

While Ca^{2+}-loaded (holo) CaM is widely known as a mediator of Ca^{2+} signaling, Ca^{2+}-free (apo) CaM can also interact with proteins. In the extended binding style, CaM can bind to different target proteins such as the IQ motif of myosin V Ca^{2+} channels, anthrax toxin, SK channels (small conductance Ca^{2+}-activated potassium channels), Ca^{2+} pump, and glutamate decarboxylase (Fig. 3) (Zou et al. 2014). The IQ motif can also interact through the C lobe in a collapsed form, and most structures involve at least partially Ca^{2+}-loaded CaM. However, some IQ motifs may bind with Ca^{2+}-loaded CaM, as well (Feldkamp et al. 2011). Generally, residues Ile and Gln initiate the consensus sequence (IQXXXRGXXXR) of IQ motifs, but other hydrophobic residues such as Leu and Val can replace the Ile. Gly at position 7 is not conserved in some CaM-binding proteins like PEP19 and some myosin proteins (Bahler and Rhoads 2002), and Arg at position 11 can be replaced by Lys. The pattern [I, L, V]QxxxRxxx[R, K] is a generalized version of an IQ motif. Furthermore, IQ-like motifs can be represented as ([FILV]Qxxx [RK]xxxxxxxx) for those sequences that do not strictly follow the generalized IQ motif sequence. The IQ motif binds relatively tightly to CaM at basal levels of intracellular calcium and these interactions change with changes in calcium levels. For example, this interaction can be viewed in two phases where, in phase 1, Ca^{2+} bound to the C-domain EF hand pair in CaM increases the K_d for the neuromodulin IQ complex from the Ca^{2+} free value of 2.3 ± 0 l1 μM to a value of 14.4 ± 1.3 μM. In Phase 2, the Ca^{2+} bound to the N-domain EF hand pair reduces the K_d for the IQ motif complex to a value of 2.5 ± 0.1 μM.

CaM Signaling Pathways

CaM plays on important role in the calcium-signaling network, the extent of which remains to be fully mapped and understood. Within cells, CaM may function as either an activator or inhibitor in the mediation of Ca^{2+} signaling and intracellular Ca^{2+} concentrations that regulate cellular activity, synaptic plasticity, and signal transduction required for long-term potentiation (LTP). Here, we will review some recent work highlighting the more prominent roles of CaM in regulating biological functions in various cellular environments.

CaM-Mediated GPCR Signaling

N-methyl-D-aspartate (NMDA) is a member of the ionotropic glutamate receptor family (iGluR) and is a fundamental receptor involved in synaptic plasticity, LTP, learning, and memory. An influx of Ca^{2+} through NMDAR activates CaM, which then binds CaMKII. However, the Ca^{2+}/CaM complex acts within a negative feedback loop as well, by inactivating the NMDAR receptor through displacement of α-actinin from the C0 domain of the NR1 subunit of the receptor (Merrill et al. 2007). As seen in Fig. 3, the metabotropic glutamate receptors (mGluR's) are G protein-coupled receptors that bind the neurotransmitter L-glutamate. In the CNS, mGluR7 (Fig. 3), which plays an important role in synaptic plasticity and neurotransmission, is regulated by PICK1 and the Ca^{2+}/CaM complex. PICK1 recruits PKCα for phosphorylation of mGluR7 at Ser-862 (Suh et al. 2013) and competes with Ca^{2+}/CaM for the same binding site on mGluR7, where binding of the Ca^{2+}/CaM complex releases G protein C-tail G_β and G_γ units, thus mediating glutamatergic autoinhibition. Additionally, CaM may regulate the activity of calcium sensing receptor (CaSR), which belongs to the family C of GPCRs along with mGluR.

CaM Regulates Calcium Dynamics via Interaction with Intracellular Calcium Channels

CaM functions as a biphasic regulator of skeletal muscle ryanodine receptor Ca^{2+} release channel (RyR1) to regulate muscle contraction. At low cytosolic Ca^{2+} concentrations, CaM interacts with RyR1 to rapidly increase the release of Ca^{2+} from intracellular stores in the SR into the cytosol (Fig. 2). However, as the concentration of Ca^{2+} increases, binding of Ca^{2+} with CaM appears to produce an inhibitory effect on RyR1 to restrict further influx of Ca^{2+} into the cell (Jiang et al. 2010). When binding to RyR1, CaM recognizes two hydrophobic anchor residues that pull the CaM N- and C-domains closer to one another, without making contact. According to NMR residual dipolar couplings and relaxation data, this conformation protects the structure of the lobes in solution, while still allowing their motions within the complex (Maximciuc et al. 2006). Moreover, this process may be additionally regulated by Ca^{2+}-S100A1, which competes with Ca^{2+}-CaM for the same binding site on RyR1 (Wright et al. 2008). Inositol 1,4,5-trisphosphate receptors (IP_3R) share significant structural homology with RyR proteins, as well as functional properties, as they are both initialized by low levels of cytosolic Ca^{2+}, then subsequently inhibited by increasing concentrations of Ca^{2+} to restrict Ca^{2+} release from intracellular ER stores. For IP_3R1, this is mediated in part, via CaM, although it is not clear whether CaM is critically important to this process, as other molecules may participate in this regulation.

CaM Regulation of Intracellular Biological Processes

CaM activates CaM-dependent kinase II (CaMKII) (Fig. 3), a serine/threonine kinase with homologous α, β, and δ isoforms found in various tissue types. The CaMKII regulatory domain includes an autophosphorylation site (Thr286 and Thr287 in CaMKIIα and CaMKIIβ, respectively), required for activation by the Ca^{2+}/CaM complex. Once activated, CaMKII in the brain plays an important role in synaptic plasticity and long-term potentiation (LTP) of neural signaling. It is also critical to both normal activity and pathologies within cardiac tissues. Following the initial interaction with the Ca^{2+}/CaM complex, CaMKII remains active even in the absence of the Ca^{2+}/CaM complex.

In addition, calcium-loaded CaM binds to and activates phosphodiesterase 1A (PDE1A), hydrolyzing cGMP to 5′GMP, which regulates cyclic nucleotide signaling in vascular smooth muscle cells. CaM also interacts with calcineurin (CaN), a serine/threonine protein phosphatase with three regulatory domains for calcineurin B, calmodulin-binding, and an auto-inhibitory domain that binds the active site in the absence of the Ca^{2+}/CaM complex. An increase in intracellular Ca^{2+} concentration leads to formation of Ca^{2+}-calmodulin which then binds CaN, exposing the active cleft, hence regulating CaN

activity. CaN subsequently activates T cells of the immune system and may play an important role in cognitive function.

Another protein that interacts with CaM is Myosin V, an actin-based, dimeric motor protein that performs a variety of different functions including mRNA transport, membrane trafficking, and the establishment of cell polarity. Each myosin V head includes an actin-binding motor domain that hydrolyzes ATP, and a long neck region (lever arms) consisting of six IQ motifs that bind apo-CaM. At low concentrations of intracellular Ca^{2+}, apo-CaM remains bound to the IQ motif of the lever arms, which maintains the motility of Myosin V. However, at higher Ca^{2+} concentrations, Ca^{2+}-loaded CaM apparently dissociates, which inhibits mobility of the protein and prevents communication between the two heads of the dimer (Ikura and Ames 2006).

Role of CaM in Disease

CaM Mutations – Species Differences

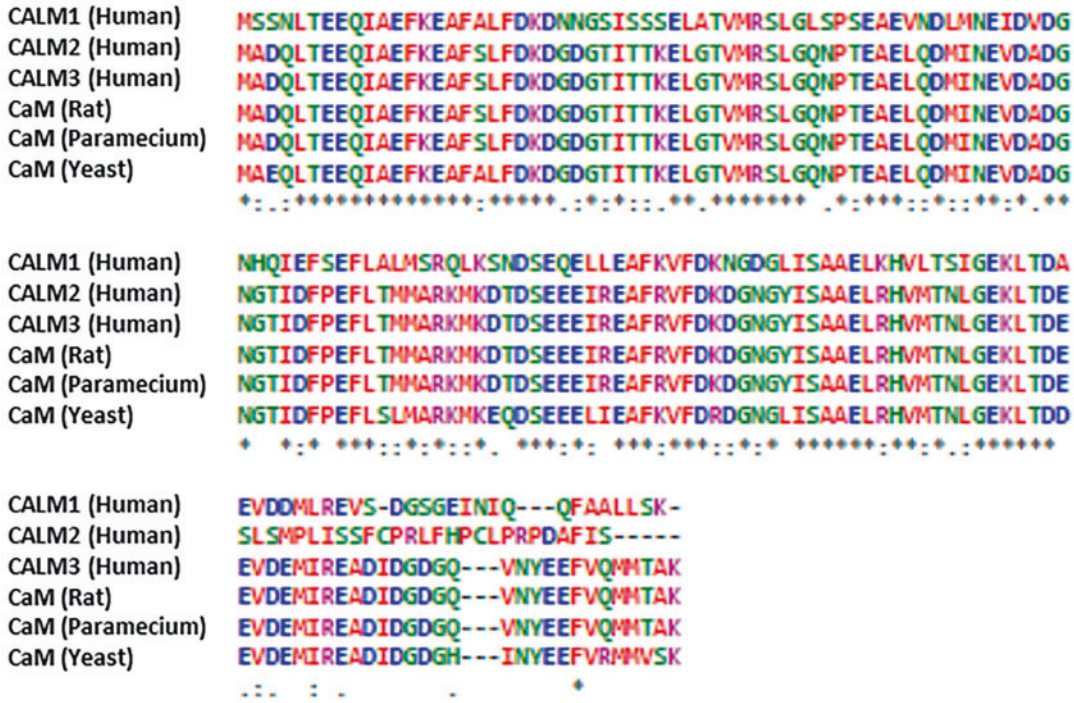
The amino acid sequence of CaM is highly conserved across isoforms and species (Fig. 4), indicating minimal evolutionary divergence and the importance of CaM. In higher vertebrates, the three CaM isoforms, expressed by independent genes (CALM1–3), and situated on different chromosomes (14q24-q31, 2p21.1-p21.3, and 19q13.2-q13.3, respectively), exhibit ~80% identity (Fischer et al. 1988). These are transcribed into at least eight mRNAs that encode identical CaM proteins.

CaM Mutations Associated with Heart Diseases

Mutations associated with CALM1–3 have been associated with heart arrhythmias, where CaM signaling is disrupted, altering calcium signaling processes through ion channels in the heart. Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a syndrome and condition characterized by abnormal heart rhythm (arrhythmia), caused by emotional stress or exercise-induced ventricular tachycardia. Studies have shown that CaM mutations in the CALM1 gene (N54I and N98S) (Fig. 5) are associated with CPVT which can cause severe ventricular arrhythmia as well as sudden cardiac death. Among these mutations, N98S did not show any effect on RyR2, while N54I increased the activity of RyR2 in sheep. Since these CaM mutants have higher affinity compared to wild-type CaM, they still bind to RyR2 even in the presence of excess wild-type CaM, therefore, mutations in only one out of three CaM genes can result in the CPVT phenotype (Crotti et al. 2013).

Long QT syndrome (LQTS) is a heart rhythm condition that may produce rapid, chaotic heartbeats. LQTS results from delayed repolarization of the ventricular action potential. Three CaM mutations (D96V, D130G, and F142 L) in either CALM1 or CALM2 genes have been linked to LQTS. It is notable that all LQTS-associated CaM mutations occur in the higher affinity EF-hand motifs in the CaM C-domain (Crotti et al. 2013) which prevents Ca^{2+} binding to CaM and suppresses Ca^{2+}-dependent inactivation of L-type Ca^{2+} channels. In addition, it has been shown that CaM binding to RyR2 during heart failure is reduced by 50% (Walweel et al. 2017) due to a decrease in CaM binding affinity causing severe cardiac hypertrophy and early death in mutant mice.

In another study, three heterozygous de novo mutations were identified in either CALM1 or CALM2, which were presumably linked to life-threatening ventricular arrhythmias combined variably with epilepsy and delayed neurodevelopment in infants (Crotti et al. 2013) (Fig. 5). A recent study reported that the interaction between Ca^{2+}-loaded CaM with the IQ-motif of p68 RNA helicase plays a significant role in cancer metastasis and cell migration (Wang et al. 2013). Specifically, a peptide fragment that spans the IQ motif of p68 strongly inhibits cancer metastasis in two different animal models. The peptide interrupts p68 and Ca^{2+}/CaM interaction and inhibits cell migration. These results demonstrate that the p68-Ca-calmodulin interaction is essential for the formation of lamellipodia and filopodia in migrating cells.



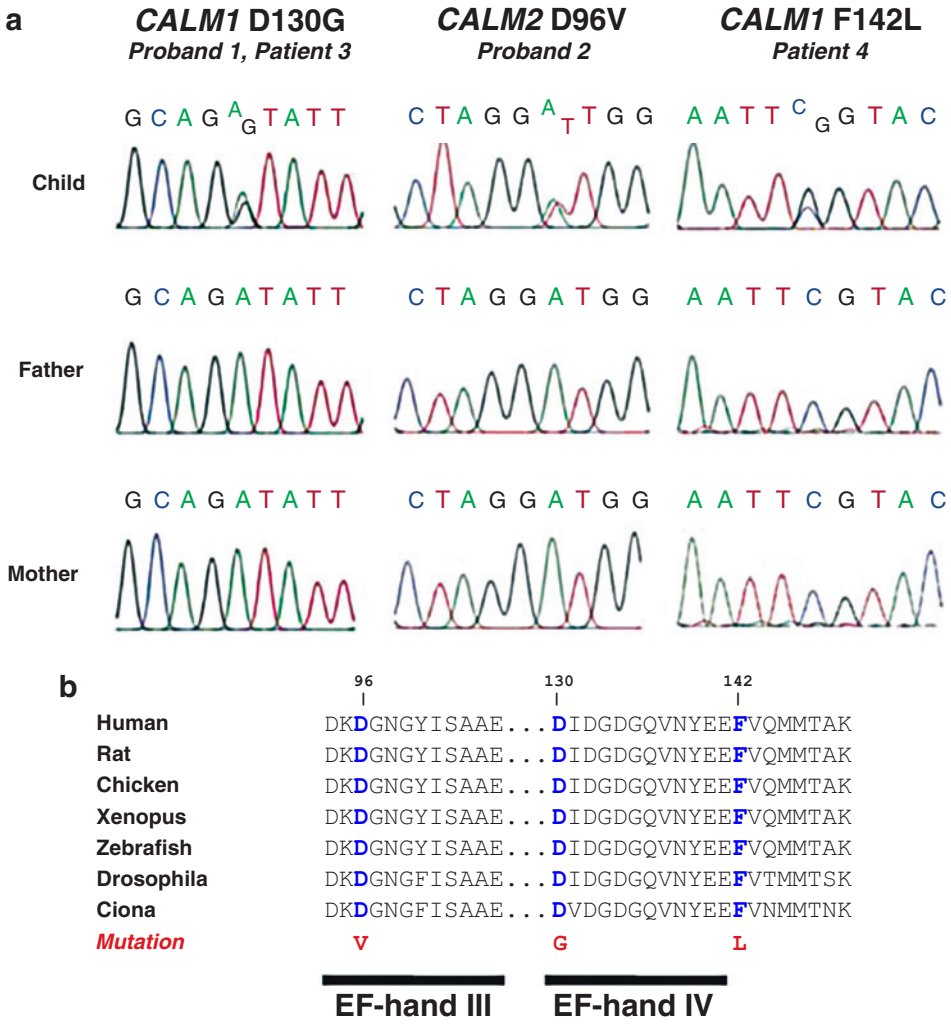
Calmodulin (CALM1), Fig. 4 Amino acid MSA using Clustal Omega reveals high sequence identity shared by human CaM (1, 2, and 3) and other diverse species, indicating conservation of the sequence over time, and limited evolutionary divergence

CaM – Metal Toxicity

As previously noted, Lead (Pb^{2+}) ions, which appear to bind promiscuously in proteins with multiple coordination geometries, can replace Ca^{2+} ions in CaM, and research suggests that the interaction between Pb^{2+} and CaM represents one potential avenue of Pb^{2+} neurotoxicity. CaM may be activated by low concentrations of Pb^{2+}, which would result in improper signaling and/or binding with downstream targets. Studies have shown that both PDE and MLCK, in the presence of CaM, are initially activated and then deactivated with increasing Pb^{2+} concentration. While the mechanism associated with this biphasic response remains unclear, evidence suggests that Pb^{2+} may bind to allosteric sites on CaM, in addition to, or as an alternative to, binding in the EF-hand sites (Kirberger et al. 2013). Hence, the combination of ionic displacement and opportunistic binding may activate and/or inhibit CaM, which would have profound effects on CaM-dependent signaling activities.

Summary and Perspectives

CaM is a 148 amino acid protein with a highly-conserved sequence that has diverged little over time and across species (Fig. 4), and its importance is evident by the redundance of three separate genes encoding the protein. CaM cooperatively binds four Ca^{2+} ions in canonical EF-hand motifs in response to cellular calcium concentration changes. As a result of calcium-dependent conformational changes, cooperativity, flexibility, and different interaction modes, CaM regulates numerous biological processes through interactions with targeted proteins in different cellular environments. An increased understanding of calcium binding, dynamic effects of cellular concentration changes, metal selectivity, cooperativity, and conformational changes of CaM and its target complexes will no doubt further illuminate this amazing protein as a dominant regulator of cellular activity, synaptic plasticity, cognitive function, muscle contraction,



Calmodulin (CALM1), Fig. 5 De novo calmodulin gene mutations in infants with severe cardiac arrhythmias. (**a**) Nucleotide sequence showing heterozygous calmodulin gene mutations. (**b**) Amino acid sequence alignments for calmodulins from different species with location of missense mutations

and immunity, and as an integral component in the molecular basis of diseases, including Pb^{2+}-induced neurotoxicity.

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Calmodulin-Dependent Cyclic Nucleotide Phosphodiesterase

▶ Phosphodiesterase 1

Calpain

Volker Nimmrich, Anton Bespalov and Achim Möller
Neuroscience Research, GPRD, Abbott, Ludwigshafen, Germany

Synonyms

Calcium-activated neutral protease; EC 3.4.22.52; EC 3.4.22.53; Nonlysosomal cysteine protease

Historical Background

Calpains are a family of non-lysosomal cysteine proteases that are activated by calcium. Calcium-dependent protease activity has already been detected in 1964 in brain tissue from rats, and this activity was later related to calpain. Yet, the identification of the protein started off with structural analysis of muscle tissue and its alteration by *post mortem* degradation (history broadly reviewed by Goll et al. 1990, 2003): In the late 1960s, Wayne Busch and Darrel Goll studied the physiological effects of Ca^{2+} in tissue specimen of rabbit muscle. When muscle strips were left overnight in a 1 mM Ca^{2+} buffer solution, it became apparent that the Z-line (a structural element separating sarcomers in skeletal muscle) had completely disappeared. Busch and Goll subsequently incubated the stripes in EGTA, thereby preventing degradation of the Z-line. With the help of Marvin Stromer, they performed electron