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Review Article

Calmodulin: The switch button of calcium signaling

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Abstract

Calmodulin (CaM), a calcium sensor, decodes the critical calcium-dependent signals and converts them into the driving force to control various important cellular functions, such as ion transport. This small protein has a short central linker to connect two globular lobes and each unit is composed of a pair of homologous domains (HD) which are responsible for calcium binding. The conformation of each HD is sensitive to the levels of the intracellular Ca^{2+} concentrations while the flexible structure of the central domain enables its interactions with hundreds of cellular proteins. Apart from calcium binding, posttranslational modifications (PTMs) also contribute to the modulations of CaM functions by affecting its protein-protein interaction networks and hence drawing out the various downstream signaling cascades. In this mini-review, we first aim to elucidate the structural features of CaM and then overview the recent studies on the engagements of calcium binding and PTMs in Ca^{2+}/CaM-mediated conformational alterations and signaling events. The mechanistic understanding of CaM working models is expected to be a key to decipher the precise role of CaM in cardiac physiology and disease pathology.

Keywords: *Calcium, Calmodulin, Conformational change, Posttranslational modification*

Introduction

Calcium impacts nearly every aspect of cell life mainly through participating in the activation processes of many intracellular signaling pathways [1]. To carry out these tremendously important tasks, calcium has to work in coordination with various cellular proteins. There are two groups of calcium-responding proteins, including calcium buffers/or transporters and calcium sensors. Calcium sensors undergo conformational changes once they expose to and bind to intracellular Ca^{2}+, known as a process that enables the conversion of calcium dependent signals into biological functions [2]. Calmodulin (CaM), a well-defined calcium sensor, is central to the activation of calcium-dependent signaling pathways and certain types of mutations in CaM's calcium-binding domain has been implicated in cardiac arrhythmias [3].

CaM has been investigated extensively for more than four decades. It was first discovered as an activator of cyclic nucleotide phosphodiesterase in the early 1970s [4,5]. At that time [4,5], the changes of phosphodiesterase enzymatic activity were observed during a process of protein purification and subsequent studies have led to identify CaM as a phosphodiesterase activator [6]. CaM was named for CALcium MODULating protein (CALMODULIN) according to its pleotropic functions have been widely characterized in many calcium-dependent cellular processes.

CaM is highly conserved and expressed ubiquitously in eukaryotic cells. In higher vertebrates, CaM is encoded by three distinct genes (CaM I, II, and III) which are transcribed into several different mRNAs while they are translated into an identical CaM protein ultimately [7]. Apart from it appears an abundant protein and constitutes around 0.1% of all cellular proteins [2], CaM is widely distributed in cellular compartments where it mediates interactions with cellular proteins [2]. Calcium is a critical second messenger that participates in various important cellular events. Ca^{2+}-dependent signalings driven by CaM act as an activator for phosphodiesterase and ensure CaM to mediate interactions with hundreds of cellular proteins [6]. To this end, CaM possesses a highly flexible structure to facilitate its interactions with various proteins while its structural conformation is finely adjusted by calcium binding and a number of posttranslational modifications (PTMs). The

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presence of multi-faced states of CaM leads to produce its multiplexed bioactivities.

In this mini-review, we first address the structural features of CaM and then have an in-depth overview on how calcium binding and PTMs are linked to its conformational alterations and functions. Furthermore, we centralize the mechanistic picture of CaM-driven cellular signalings by briefly revisiting current highlights on the PTMs-mediated CaM's protein-protein-interacting networks. Details for the major concerns and highlights are described in the following three sections:

The protein structural profiles of calmodulin

CaM is a small, acidic, and relatively stable protein with a molecular weight of 16.7 kDa [8], comprising of 148 amino acid residues and none of them is cysteine nor hydroxyproline [9]. CaM contains four homologous domains which were initially characterized as a helix-loop-helix structure [10], and later were defined as EF-hands because their three-dimensional arrangements resemble a thumb and index finger [2]. Calcium ion binding can occur independently in all four EF domains [11]. The first two EF-hands are connected by a short antiparallel beta-sheet to make up the CaM's N-terminal lobe [12], and the same linking method is employed to hold the third and fourth EF-hands to build up its C-terminus. The two EF-hands in each lobe coordinate the regulators needed for calcium binding and the N-and C-terminal lobes are further bridged by a central linker to be incorporated into the CaM protein context. CaM's four calcium-binding motifs exhibit structural similarities. The first EF-hand is more similar to the third one while the second EF-hand is more similar to the fourth one [9]. The calcium-binding domain of CaM is similar to that of other calcium-binding proteins, such as troponin C and parvalbumins [8]. Although the four EF-hands in CaM are structurally similar their binding abilities to calcium are different from each other. The C-terminal domain has a higher affinity to calcium than the N-terminus [13], whereas the N-terminus binds faster to calcium than the C-terminus [13]. In the absence of target proteins, calcium binds first to the C-terminus and latter to the N-terminus. On the contrary, the four calcium binding sites will cooperate to dramatically increase the protein's affinity to calcium once target proteins are presented [14]. The above biochemical characteristics enable CaM to relay the complex calcium signalings to produce their downstream pleotropic effects.

CaM's central linker is short, flexible, highly conserved, and invariant in length [15], comprising of 25 amino residues to make up two alpha helices separated by a hinge domain [16]. The central linker is featured by its structural flexibility that enables CaM to adopt a wide range of conformations with a variety of orientations of the lobes, for instance, the extended and compact formats [15]. The central linker is critical for protein-protein interactions and the distance between its two ends affects the interactions between CaM and the target proteins [15]. In particular, each alpha helix of the central linker is sufficient to provide its specific affinity to substrate binding. One study employed nuclear magnetic resonance to demonstrate that ten residues in the linker region modulate the changes of the local conformation upon substrate binding [17]. Five out of the ten residues (Met76, Lys77, Thr79, Asp80 and Ser81) are highly flexible in the linker domain while the remaining five residues (Arg74, Lys75, Asp78, Glu82, and Glu83) are more stringent [17]. Although the central linker has been identified to be an alpha-helix under a context of crystal format or in solutions, it has been shown that this region bends when it interacts with some target proteins [18]. Current knowledge suggests that the linker is balanced by the reverse switch from helical to nonhelical modes.

The effects of calcium binding on calmodulin functions

In addition to acting as a calcium sensor, CaM can exhibit a variety of calcium independent functions mostly relying on its capability to mediate interactions with cellular targets at a calcium-free state [19]. Calcium-free CaM, known as apo-CaM, is typically characterized as a compact and closed structure [Figure 1a]. In the absence of Ca^{2+} and target peptides, the hydrophobic residues are buried and packed together in the interior of the protein core [12,20]. When the target proteins are present, the C-terminal lobe of apo-CaM adopts a semi-open conformation to hold its target peptide from the interacting proteins [21]. In the calcium-free environment, when CaM meets IQ motif (IQXXXRGXXXR), a consensus sequence capable of binding CaM in a Ca^{2+}-independent manner, the C-terminal lobe forms a shallow cleft and becomes more accessible to the target peptides. On the other hand, the closed conformation of the N-terminal lobe can only maintain a low binding affinity to the GXXXR motif, and consequently these weaker interactions collectively urge CaM to surround the central part of the IQ motif [22]. In addition to the IQ motif, apo-CaM is also noted to mediate interactions with other motifs from the target proteins, such as actin-binding proteins, cytoskeletal and membrane proteins, enzymes, receptors and ion channels [19]. Accordingly, studies have demonstrated that the presence of calcium is not

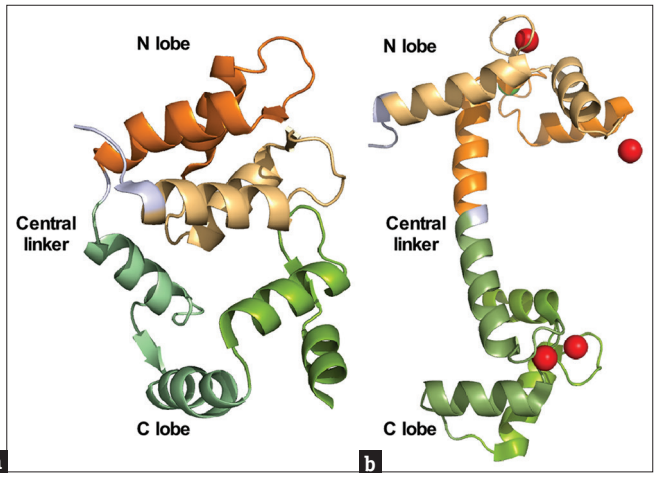

Figure 1: Crystal structure of Calmodulin in various conformations. (a) Apo Calmodulin (PDB: 1QX5); (b) Calcium-bound Calmodulin (PDB: 1EXR)

absolutely necessary for CaM to carry out some of its critical functions, such as mitosis [23] and endocytosis [24].

Calcium binding promotes CaM to change its conformation from a compact structure into a more open format [Figure 1b] and this transition is the key to support calcium-dependent interactions between CaM and its targets. Calcium binding promotes the changes of CaM conformation to expose the methionine-rich hydrophobic patches at each lobe. This structural plasticity ensures CaM is suitable to have interactions with a variety of targets, such as protein kinases, phosphatases, receptors, ion-channel proteins, phosphodiesterases, and nitric oxide synthases [2,25]. A contiguous stretch of methionine residues create a highly polarized environment to facilitate CaM's interactions with target proteins [14]. Moreover, the methionine's long flexible side chain makes the hydrophobic patches to become accessible to a broad spectrum of cellular proteins [14]. The alpha helix of the central linker unwinds and bends to various degrees in response to calcium binding [26]. Both the high flexibility of the hydrophobic patches and the unwinding potency of the central linker contribute to CaM's structural adjustability, leading to generate an ideal domain to carry out versatile protein-protein interactions [13]. Taking advantage of these features, Ca^{2+}-CaM can interact with different target proteins through a variety of binding domains which are not very similar in the primary sequence [25]. When encountering the target proteins, Ca^{2+}-CaM wraps around the binding sequences to enable the N-and C-terminal domains to interact with the hydrophobic anchor of the binding motifs from the targets [13]. The above binding patterns could be parallel or antiparallel, which are mainly determined by the charge density of the binding motif [14].

Aberrant behaviors of ion channels caused by mutations-induced alterations of CaM's Ca^{2+}-binding ability and structural conformations have been implicated in severe cardiac arrhythmia [27,28]. Among the amino acids that are required for Ca^{2+} coordination in the EF-hands, Asp95 and Asn97 are identified to be arrhythmogenic hot spots. The occurrence of mutations at different sites can lead to various influences on the functions of CaM. For instance, the D95V mutation completely disrupts calcium binding to the third EF-hand by inducing a drastic conformational change not only in the third EF-hand but also in the distant areas away from the mutated site [29]. Although this mutation does not prevent CaM for binding to other proteins completely, the mode of protein-protein interaction is largely altered [29]. In addition to CaM Asp95, Asn97 is also essential for calcium binding in the third EF-hand. Interestingly, the N97S mutation results in a distinct phenotype to that of D95V. The N97S mutation only modestly reduces the calcium-binding affinity[27] and it causes a relatively smaller conformation change at the local area [29]. Interestingly, a replacement of the Asn97 residue by another amino acid could bring different outcomes. For example, the conformational change caused by the N97I mutation is similar to the phenotype produced by the D95V mutation [29]. These studies suggest that both the positions and the types of amino acids contribute to the formation and the maintenance of CaM's structural characteristics and calcium binding ability. In summary, the biochemical properties of the amino acids in the EF-hands appear to be the key determinant that affects the interactions between CaM and its target proteins. CaM has evolved to act as a dynamic and sensitive calcium sensor as it adjusts its conformation upon encountering calcium signals to interact with a variety of proteins, thus eliciting multiple biological impacts.

Importantly, these mutations not only induce alterations on conformation and calcium binding affinity of CaM, they also result in cardiac problems on clinical setting. These mutations disrupt the homeostasis of multiple ion channels that are regulated by CaM, altering the cellular ion current and then lead to cardiac arrhythmia. The first study for mutant CaM-induced cardiac arrhythmia was reported on 2012 [30]. The N97S mutation was found to cause catecholaminergic polymorphic ventricular tachycardia and sudden cardiac death [30]. Following studies further demonstrated that D95V and N97I mutations lead to long QT syndrome [31,32]. Although CaM has been widely studied for many decades, the awareness and link of mutations on CaM to cardiac arrhythmia was relatively recent. The true incidence of mutant CaM-induced cardiac arrhythmia on clinical setting remains unclear so far.

The importance of posttranslational modifications to calmodulin functions

Eukaryotic cells employ PTMs as a strategy to modulate protein functions and enable them to exquisitely regulate signaling pathways to properly respond to the diverse stimuli [33]. CaM also undergoes many types of PTMs, including oxidation, phosphorylation, trimethylation, carboxylmethylation, acetylation, and proteolytic cleavage [7]. PTMs offer selective strategies for CaM to produce multifaceted biological functions. In this section, the current understanding in respect to the involvements of phosphorylation, oxidation, and tyrosine nitration (TN) in the modulations of CaM functions is discussed, and the effect of each PTM is summarized in Table 1.

Phosphorylation

Protein phosphorylation, a reversible PTM which is triggered by kinases and phosphatases, is one of the most common cellular events that have been used as an effective way to control the intrinsic activity of the target proteins. Phosphorylation occurs mostly on two types of amino acids: serine/threonine and tyrosine [34]. There are many candidate phosphorylation sites at each EF-hand of CaM were discovered, including Thr26, Thr29, Thr44, Tyr99, Tyr138, Ser101, and Thr117, as well as Thr79 and Ser81 of the flexible central linker [35]. Several serine/threonine kinases have been reported to mediate the phosphorylation of the above indicated residues. Among those known CaM kinases, Casein kinase II (CK II) is a serine/threonine protein kinase which has been studied widely [35]. CKII phosphorylates CaM at Thr79, Ser81, and Ser101 [36]. Phosphorylation of Thr79 reduces the sensitivity of small conductance Ca^{2+}-activated K^+ channels to calcium [37], whereas phosphorylation of Ser101 impairs the ability of CaM to activate endothelial

Table 1: PTMs and the related functional alterations of calmodulin

PTM	Residue	Proposed consequence	Ref.
Phosphorylation	Thr79	Reduce the sensitivity of small conductance Ca^{2+}-activated K^+ channels to calcium	37
	Ser101	Impair the ability of eNOS activation	38
		Alter CaM's affinity to calcium	39, 40
	Tyr99	Enhance EGFR activation	43
		Reduce the biological activity of CaM	44
		Activate nNOS	35, 45
		Promote phosphorylation and activation of c-Src	46
	Tyr138	Enhance EGFR activation	43
		Reduce the biological activity of CaM	44
		Promote phosphorylation and activation of c-Src	46
Sulfoxidation	Met51	Promote CaM degradation	64
	Met71	Promote CaM degradation	64
	Met72	Promote CaM degradation	64
	Met109	Perturb Ca^{2+}-induced structural shift	60
	Met124	Perturb Ca^{2+}-induced structural shift	60
	Met144	Induce structural changes in the C-terminus	58
		Block activation of plasma membrane Ca^{2+}-ATPase	51
		Disrupt the interaction with CAMKII	52,61
	Met145	Induce structural changes in the C-terminus	58
		Block activation of plasma membrane Ca^{2+}-ATPase	51
		Disrupt the interaction with CAMKII	52,61
		Promote CaM degradation	63
Nitration	Tyr99	Reduce affinity for calcium	68
		More eNOS decoupling	68
	Tyr138	Reduce affinity for calcium	68
		Less eNOS decoupling	68
Carboxylmethylation		Decrease activation of cAMP phosphodiesterase	70
Acetylation	Lys75	Reduce the affinity to MLC kinase	72
Trimethylation	Lys115	Dysfunction of mitochondrial respiratory chain	75

NOS (eNOS), an enzyme responsible for generating nitric oxide in the vascular endothelium [38]. Because Ser101 resides in the calcium-binding pocket of the third EF-hand, the phosphorylation at this residue also affects CaM's binding to calcium. According to its promising capability to control the phosphorylation of Ser101, CKII can drive the phosphorylation dependent PTM to refine CaM's affinity to calcium and to shape the protein-protein interacting networks induced by CaM [39,40].

CaM contains two tyrosine residues, Tyr99 and Tyr138, and phosphorylation of these residues have been implicated in CaM-mediated protein-protein interactions. Epidermal growth factor receptor (EGFR) and insulin receptor are two receptor tyrosine kinases known to trigger the phosphorylation of CaM although their contributions are biologically distinct [41]. The tyrosine-defective mutants have been previously characterized and were found to be biologically active [42]. Once Tyr99/Tyr138-CaM is phosphorylated by EGFR this PTM leads to enhance EGFR's ligand-dependent activation [43]. Thus, phospho-Tyr-CaM can be considered an intracellular co-activator of EGFR. On the other hand, insulin receptor-induced phosphorylation reduces the biological activity of CaM [44]. It should be noted that there are a number of nonreceptor tyrosine kinases, including c-Src, c-Fyn and c-Fgr, have been shown to mediate CaM phosphorylation of Tyr99/Tyr138. Although the Src kinase induced phosphorylation of Tyr99 has been well-characterized the same enzyme is also reported to participate in the phosphorylation dependent PTM of Tyr138 [41]. In the mouse brains, hypoxia activates c-Src to phosphorylate Tyr99 of CaM and this PTM leads to neuronal NOS activation, subsequently inducing neuronal cell death [35,45]. The interaction of apo-CaM or in low degree of Ca^{2+}/CaM with c-Src was shown to promotes its activation [46,47] while phospho-Tyr138-CaM is not Src associated [59]. A study reported that the phospho-mimetic mutant of Tyr99/Tyr138 on CaM facilitates the autophosphorylation and activation of c-Src irrespective of the presence of Ca^{2+} [46]. To sum up, the way that CaM undergoes phosphorylation dependent PTMs to reversely control its functions and activation of cellular kinases makes CaM a versatile regulator with capability to translate different messages into pleiotropic signalings, hence resulting in diverse biological outcomes.

Sulfoxidation

Proteins oxidation can be driven by either a reversible or irreversible mode. CaM is subjected to an oxidation dependent PTM by reactive oxygen species (ROS) or reactive nitrogen species (RNS). Among the 20 amino acids, cysteine and methionine are the only two candidates that contain a sulfur atom and both are eligible to carry out ROS-mediated oxidation [49,50]. CaM contains nine methionine residues but

none of cysteine. Oxidation of nine methionine residues in CaM has been verified *in vitro*[51] while only two of them were found to be oxidized *in vivo* [51]. CaM's susceptibility to oxidation highly depends on its conformational state [52]. The potency of the individual methionine residues to mediate the oxidative modification is enhanced when they are exposed to solvents [53,54]. For example, upon Ca^{2+} binding to CaM, Ca^{2+}-CaM exposes methionine residues from the hydrophobic patches to the solvents, thereby facilitating the oxidation dependent PTM to occur. Two residues, Met72 and Met145, were shown to have elevated susceptibility to perform oxidative reaction in Ca^{2+}-CaM [52]. Ca^{2+}/CaM-dependent protein kinase II (CAMKII) is an important protein kinase regulated by Ca^{2+}/CaM complex. Binding of CAMKII to the C lobe of Ca^{2+}-CaM blocks methionine residues from accessing the environmental solvents and ultimately prevents oxidation dependent PTM to take place [52]. Oxidation is also known to promote conformational changes in many proteins, including CaM [55-57]. For example, oxidation of Met144 and/or Met145 causes changes in the tertiary structure of the CaM C-terminus, and subsequently impairs its affinity to calcium [58-60]. Oxidation mimic mutations at methionine residues (M109Q and M124Q) decrease the calcium-induced close-to-open conformational change of CaM [60]. The oxidation dependent PTM is expected to influences the interactions between CaM and its target proteins according to oxidized CaM has an altered conformation. Oxidation can elicit different effects depending on the sites of oxidized methionine and the number of the oxidized methionine residues. Ca^{2+}-ATPase is a transport protein responsible for maintaining intracellular Ca^{2+} homeostasis and ensuring proper cellular signaling. Oxidation at Met144 and Met145 of CaM blocks its activation of Ca^{2+}-ATPase [51]. It also disrupts the interaction of CaM with CAMKII [61,67]. Ryanodine receptor 1 (RyR1), a protein responsible for calcium release from sarcoplasmic reticulum, is also regulated by CaM. The ability of CaM to modulate RyR1 is reduced when only few methionine residues are oxidized while oxidation occurring at nine methionine residues will cause a loss-of-the function phenotype [62]. Methionine residues of CaM can be defined as redox sensors because they not only contribute a critical role in calcium homeostasis but also produce calcium-dependent signalings to sense an oxidative stress condition. In addition to interfering the interactions of CaM with its target proteins, oxidation accelerates the degradation of CaM. Two studies have proposed that oxidation of Met51, Met71, Met72 and Met145 play a part in proteasome-mediated degradation of CaM [63,64]. Ca^{2+}-CaM becomes more susceptible to proteasome dependent degradation when it exposes to oxidative stress.

Tyrosine nitration

Nitration of tyrosine (Y) residues of proteins is a low abundant PTM and this event is typically induced by RNS [65,66]. TN occurs at the same sites as the phosphotyrosine residues and this type of PTMs results in the alterations of chemical and physical properties of CaM, such as pKa and electron-density factors [67]. These features are tightly linked to the CaM functions and its extensive biological activities. TN occurs at two tyrosine residues of CaM, Tyr99 and Tyr138, and this PTM only causes a slightly lower affinity for calcium binding compared to apo-CaM [71]. On the other hand, nitrotyrosine-CaM can bind to eNOS in a calcium free environment while CaM with no nitrotyrosine losses its binding capability to eNOS. When nitroTyr99-CaM is presented, eNOS-decoupling becomes dominant and a decline of NO production is observed, whereas nitration at Tyr138 results in a poor efficacy of eNOS decoupling but a dominant route for NO biosynthesis [68]. These evidences suggest that TN affect the function of CaM depending on which PTM sites is selected.

Other posttranslational modifications

CaM is also subjected to some other modifications, through which the function of this protein could be modulated. For example, CaM is a good substrate for protein carboxylmethyltransferase [69], and carboxylmethylation of CaM leads to the decrease activation of cAMP phosphodiesterase in the brain [70]. Acetylation of specific residues on CaM can change the interaction between CaM and other proteins. For example, CaM interacts with myosin light chain (MLC) kinase, a serine/threonine kinase that phosphorylates MLC, at the region of residue Lys75 [71]. Acetylation on Lys75 reducing the affinity of CaM to MLC kinase markedly [72]. Trimethylation on Lys115, which is catalyzed by CaM methyltransferase (KMT) [73], is also a common PTM on CaM. Although this modification did not affect its activation of cyclic nucleotide phosphodiesterase [74], deletion of CaM KMT cause dysfunction of mitochondrial respiratory chain, especially in the brain [75].

Current evidences about mutations on posttranslational modification sites of calmodulin and human diseases

Although the functional regulation by PTMs has been studied widely, the exploration of consequence of mutations on those residues where PTM occurs is still in a very limited extent. Several mutations on CaM have been reported to be involved in cardiac arrhythmias on human subjects (detailed in section 2), however, none of these mutations occurs on the PTM residues mentioned above [76]. Since PTMs on specific residues are critical for modulating the CaM-dependent signaling and regulating the subsequent physiological functions, mutations occurring on those residues could disrupt these precise regulations and result in various diseases. Some evidences show that PTMs of CaM regulate its interaction with EGFR, Src and eNOS [35], suggesting CaM-dependent signaling may be involved in the pathogenesis of cancer and brain hypoxia. However, currently there are no data about the presence and incidence of CaM mutation-induced cancers and other diseases on human subjects.

Conclusions

CaM is critical for the maintenance of normal cellular functions as it decodes the important signals via a calcium

dependent manner and translates them into a number of various biological messages. Several types of PTMs are required to modulate the conformation of CaM as they create unique hallmarks in the calcium binding domains, known to be a prerequisite for most CaM dependent functions. Some missense mutations in CaM, even in one allele, can result in a significant pathologic condition, such as life-threatening arrhythmia [76,77]. Although this area of research is particularly interesting and important the further insight regarding the detail linkages of CaM to the development of diseases remains to be explored. Many studies have investigated the possible mechanisms underlying CaM mutations-induced arrhythmia and the resulting deregulation of ion channel has been suggested as the major cause to this type of cardiac disorder [13,29,76]. Nevertheless, given the diversity of CaM functions has been widely reported, there may be other mechanisms could also contribute to the pathogenesis of these arrhythmias. For example, it is expected that the involvements of other CaM-dependent proteins in cardiac disorders should be identified in the near future. Herein, we attempt to have a brief review on recent advances of CaM research from the aspects of structural and cellular biology. By doing so, we have built up a clearer picture to delineate how CaM is being regulated in cellular environments. With this knowledge in hand, researchers may make a wiser step to explore the precise role of CaM in the development of cardiac diseases and maybe other diseases.

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Conflicts of interest

There are no conflicts of interest.

References

1. Clapham DE. Calcium signaling. Cell 2007;131:1047-58.
2. Chin D, Means AR. Calmodulin: A prototypical calcium sensor. Trends Cell Biol 2000;10:322-8.
3. Jensen HH, Brohus M, Nyegaard M, Overgaard MT. Human calmodulin mutations. Front Mol Neurosci 2018;11:396.
4. Cheung WY. Cyclic 3',5'-nucleotide phosphodiesterase. Demonstration of an activator. Biochem Biophys Res Commun 1970;38:533-8.
5. Kakiuchi S, Yamazaki R. Calcium dependent phosphodiesterase activity and its activating factor (Paf) from brain – Studies on Cyclic 3', 5'-nucleotide phosphodiesterase (Iii). Biochem Bioph Res Co 1970;41:1104-10.
6. Cheung WY. Calmodulin plays a pivotal role in cellular regulation. Science 1980;207:19-27.
7. Kortvely E, Gulya K. Calmodulin, and various ways to regulate its activity. Life Sci 2004;74:1065-70.
8. Stevens FC. Calmodulin: An introduction. Can J Biochem Cell Biol 1983;61:906-10.
9. Watterson DM, Sharief F, Vanaman TC. The complete amino acid sequence of the Ca^{2+}-dependent modulator protein (calmodulin) of bovine brain. J Biol Chem 1980;255:962-75.
10. Strynadka NC, James MN. Crystal structures of the helix-loop-helix calcium-binding proteins. Annu Rev Biochem 1989;58:951-98.
11. Lakowski TM, Lee GM, Okon M, Reid RE, McIntosh LP. Calcium-induced folding of a fragment of calmodulin composed of EF-hands 2 and 3. Protein Sci 2007;16:1119-32.
12. Kuboniwa H, Tjandra N, Grzesiek S, Ren H, Klee CB, Bax A. Solution structure of calcium-free calmodulin. Nat Struct Biol 1995;2:768-76.
13. Sorensen AB, Sondergaard MT, Overgaard MT. Calmodulin in a heartbeat. Febs J 2013;280:5511-32.
14. Yamniuk AP, Vogel HJ. Calmodulin's flexibility allows for promiscuity in its interactions with target proteins and peptides. Mol Biotechnol 2004;27:33-57.
15. Anthis NJ, Clore GM. The length of the calmodulin linker determines the extent of transient interdomain association and target affinity. J Am Chem Soc 2013;135:9648-51.
16. Bradley LH, Bricken ML, Randle C. Expression, purification, and characterization of proteins from high-quality combinatorial libraries of the mammalian calmodulin central linker. Protein Expr Purif 2011;75:186-91.
17. Kukic P, Camilloni C, Cavalli A, Vendruscolo M. Determination of the individual roles of the linker residues in the interdomain motions of calmodulin using NMR chemical shifts. J Mol Biol 2014;426:1826-38.
18. Kretsinger RH. The linker of calmodulin – To helix or not to helix. Cell Calcium 1992;13:363-76.
19. Jurado LA, Chockalingam PS, Jarrett HW. Apocalmodulin. Physiol Rev 1999;79:661-82.
20. Houdusse A, Silver M, Cohen C. A model of Ca^{2+}-free calmodulin binding to unconventional myosins reveals how calmodulin acts as a regulatory switch. Structure 1996;4:1475-90.
21. Swindells MB, Ikura M. Pre-formation of the semi-open conformation by the apo-calmodulin C-terminal domain and implications binding IQ-motifs. Nat Struct Biol 1996;3:501-4.
22. Houdusse A, Gaucher JF, Krementsova E, Mui S, Trybus KM, Cohen C. Crystal structure of apo-calmodulin bound to the first two IQ motifs of myosin V reveals essential recognition features. Proc Natl Acad Sci U S A 2006;103:19326-31.
23. Sweet SC, Rogers CM, Welsh MJ. Calmodulin stabilization of kinetochore microtubule structure to the effect of nocodazole. J Cell Biol 1988;107:2243-51.
24. Kübler E, Schimmöller F, Riezman H. Calcium-independent calmodulin requirement for endocytosis in yeast. EMBO J 1994;13:5539-46.
25. Vetter SW, Leclerc E. Novel aspects of calmodulin target recognition and activation. Eur J Biochem 2003;270:404-14.
26. Zhang M, Abrams C, Wang L, Gizzi A, He L, Lin R, et al. Structural basis for calmodulin as a dynamic calcium sensor. Structure 2012;20:911-23.
27. Hwang HS, Nitu FR, Yang Y, Walweel K, Pereira L, Johnson CN, et al. Divergent regulation of ryanodine receptor 2 calcium release channels by arrhythmogenic human calmodulin missense mutants. Circ Res 2014;114:1114-24.
28. Søndergaard MT, Tian X, Liu Y, Wang R, Chazin WJ, Chen SR, et al. Arrhythmogenic calmodulin mutations affect the activation and termination of cardiac ryanodine receptor-mediated Ca2+release. J Biol Chem 2015;290:26151-62.
29. Wang K, Holt C, Lu J, Brohus M, Larsen KT, Overgaard MT, et al. Arrhythmia mutations in calmodulin cause conformational changes that affect interactions with the cardiac voltage-gated calcium channel. Proc Natl Acad Sci U S A 2018;115:E10556-65.
30. Nyegaard M, Overgaard MT, Søndergaard MT, Vranas M, Behr ER, Hildebrandt LL, et al. Mutations in calmodulin cause ventricular tachycardia and sudden cardiac death. Am J Hum Genet 2012;91:703-12.
31. Crotti L, Johnson CN, Graf E, De Ferrari GM, Cuneo BF, Ovadia M, et al. Calmodulin mutations associated with recurrent cardiac arrest in infants. Circulation 2013;127:1009-17.
32. Makita N, Yagihara N, Crotti L, Johnson CN, Beckmann BM, Roh MS, et al. Novel calmodulin mutations associated with congenital arrhythmia susceptibility. Circ Cardiovasc Genet 2014;7:466-74.
33. Wang YC, Peterson SE, Loring JF. Protein post-translational modifications and regulation of pluripotency in human stem cells. Cell

Res 2014;24:143-60.
34. Ubersax JA, Ferrell JE Jr. Mechanisms of specificity in protein phosphorylation. Nat Rev Mol Cell Biol 2007;8:530-41.
35. Villalobo A. The multifunctional role of phospho-calmodulin in pathophysiological processes. Biochem J 2018;475:4011-23.
36. Sacks DB, Davis HW, Crimmins DL, McDonald JM. Insulin-stimulated phosphorylation of calmodulin. Biochem J 1992;286:211-6.
37. Allen D, Fakler B, Maylie J, Adelman JP. Organization and regulation of small conductance Ca^{2+}-activated K^{+}channel multiprotein complexes. J Neurosci 2007;27:2369-76.
38. Greif DM, Sacks DB, Michel T. Calmodulin phosphorylation and modulation of endothelial nitric oxide synthase catalysis. Proc Natl Acad Sci U S A 2004;101:1165-70.
39. Aiuchi T, Hagiwara T, Omata K, Nakaya K, Nakamura Y. Effect of phosphorylation of calmodulin on calcium binding affinity as estimated by terbium fluorescence. Biochem Int 1991;23:145-9.
40. Quadroni M, L'Hostis EL, Corti C, Myagkikh I, Durussel I, Cox J, et al. Phosphorylation of calmodulin alters its potency as an activator of target enzymes. Biochemistry 1998;37:6523-32.
41. Benaim G, Villalobo A. Phosphorylation of calmodulin. Functional implications. Eur J Biochem 2002;269:3619-31.
42. Salas V, Sánchez-Torres J, Cusidó-Hita DM, García-Marchan Y, Sojo F, Benaim G, et al. Characterisation of tyrosine-phosphorylation-defective calmodulin mutants. Protein Expr Purif 2005;41:384-92.
43. Stateva SR, Salas V, Benguría A, Cossío I, Anguita E, Martín-Nieto J, et al. The activating role of phospho-(Tyr)-calmodulin on the epidermal growth factor receptor. Biochem J 2015;472:195-204.
44. Williams JP, Jo H, Sacks DB, Crimmins DL, Thoma RS, Hunnicutt RE, et al. Tyrosine-phosphorylated calmodulin has reduced biological activity. Arch Biochem Biophys 1994;315:119-26.
45. Mishra OP, Ashraf QM, Delivoria-Papadopoulos M. Mechanism of increased tyrosine (Tyr (99)) phosphorylation of calmodulin during hypoxia in the cerebral cortex of newborn piglets: the role of nNOS-derived nitric oxide. Neurochem Res 2010;35:67-75.
46. Stateva SR, Salas V, Anguita E, Benaim G, Villalobo A. Ca2+/calmodulin and apo-calmodulin both bind to and enhance the tyrosine kinase activity of c-Src. PLoS One 2015;10:e0128783.
47. Anguita E, Villalobo A. Src-family tyrosine kinases and the Ca^{2+}signal. Biochim Biophys Acta Mol Cell Res 2017;1864:915-32.
48. Wu J, Masci PP, Chen C, Chen J, Lavin MF, Zhao KN. β-Adducin siRNA disruption of the spectrin-based cytoskeleton in differentiating keratinocytes prevented by calcium acting through calmodulin/epidermal growth factor receptor/cadherin pathway. Cell Signal 2015;27:15-25.
49. Shacter E. Quantification and significance of protein oxidation in biological samples. Drug Metab Rev 2000;32:307-26.
50. Lee BC, Gladyshev VN. The biological significance of methionine sulfoxide stereochemistry. Free Radic Biol Med 2011;50:221-7.
51. Bartlett RK, Urbauer RJ, Anbanandam A, Smallwood HS, Urbauer JL, Squier TC. Oxidation of Met (144) and Met (145) in calmodulin blocks calmodulin dependent activation of the plasma membrane Ca-ATPase. Biochemistry 2003;42:3231-8.
52. Snijder J, Rose RJ, Raijmakers R, Heck AJ. Site-specific methionine oxidation in calmodulin affects structural integrity and interaction with Ca^{2+}/calmodulin dependent protein kinase II. J Struct Biol 2011;174:187-95.
53. Gao J, Yin DH, Yao Y, Sun H, Qin Z, Schöneich C, et al. Loss of conformational stability in calmodulin upon methionine oxidation. Biophys J 1998;74:1115-34.
54. Yin D, Kuczera K, Squier TC. The sensitivity of carboxyl-terminal methionines in calmodulin isoforms to oxidation by H_2O_2 modulates the ability to activate the plasma membrane Ca-ATPase. Chem Res Toxicol 2000;13:103-10.
55. Sharp JS, Tomer KB. Analysis of the oxidative damage-induced conformational changes of apo- and holocalmodulin by dose-dependent protein oxidative surface mapping. Biophys J 2007;92:1682-92.
56. Ortiz de Orué Lucana D, Roscher M, Honigmann A, Schwarz J. Iron-mediated oxidation induces conformational changes within the redox-sensing protein HbpS. J Biol Chem 2010;285:28086-96.
57. Song IK, Lee JJ, Cho JH, Jeong J, Shin DH, Lee KJ. Degradation of redox-sensitive proteins including peroxiredoxins and DJ-1 is promoted by oxidation-induced conformational changes and ubiquitination. Sci Rep 2016;6:34432.
58. Anbanandam A, Bieber Urbauer RJ, Bartlett RK, Smallwood HS, Squier TC, Urbauer JL. Mediating molecular recognition by methionine oxidation: Conformational switching by oxidation of methionine in the carboxyl-terminal domain of calmodulin. Biochemistry 2005;44:9486-96.
59. Schallreuter KU, Gibbons NC, Zothner C, Abou Elloof MM, Wood JM. Hydrogen peroxide-mediated oxidative stress disrupts calcium binding on calmodulin: More evidence for oxidative stress in vitiligo. Biochem Biophys Res Commun 2007;360:70-5.
60. McCarthy MR, Thompson AR, Nitu F, Moen RJ, Olenek MJ, Klein JC, et al. Impact of methionine oxidation on calmodulin structural dynamics. Biochem Biophys Res Commun 2015;456:567-72.
61. Robison AJ, Winder DG, Colbran RJ, Bartlett RK. Oxidation of calmodulin alters activation and regulation of CaMKII. Biochem Biophys Res Commun 2007;356:97-101.
62. Balog EM, Norton LE, Bloomquist RA, Cornea RL, Black DJ, Louis CF, et al. Calmodulin oxidation and methionine to glutamine substitutions reveal methionine residues critical for functional interaction with ryanodine receptor-1. J Biol Chem 2003;278:15615-21.
63. Sacksteder CA, Whittier JE, Xiong Y, Li J, Galeva NA, Jacoby ME, et al. Tertiary structural rearrangements upon oxidation of methionine (145) in calmodulin promotes targeted proteasomal degradation. Biophys J 2006;91:1480-93.
64. Balog EM, Lockamy EL, Thomas DD, Ferrington DA. Site-specific methionine oxidation initiates calmodulin degradation by the 20S proteasome. Biochemistry 2009;48:3005-16.
65. Ng JY, Boelen L, Wong JW. Bioinformatics analysis reveals biophysical and evolutionary insights into the 3-nitrotyrosine post-translational modification in the human proteome. Open Biol 2013;3:120148.
66. Radi R. Protein tyrosine nitration: Biochemical mechanisms and structural basis of functional effects. Acc Chem Res 2013;46:550-9.
67. Zhan X, Wang X, Desiderio DM. Mass spectrometry analysis of nitrotyrosine-containing proteins. Mass Spectrom Rev 2015;34:423-48.
68. Porter JJ, Jang HS, Haque MM, Stuehr DJ, Mehl RA. Tyrosine nitration on calmodulin enhances calcium-dependent association and activation of nitric-oxide synthase. J Biol Chem 2020;295:2203-11.
69. Gagnon C, Kelly S, Manganiello V, Vaughan M, Odya C, Strittmatter W, et al. Modification of calmodulin function by enzymatic carboxyl methylation. Nature 1981;291:515-6.
70. Billingsley M, Kuhn D, Velletri PA, Kincaid R, Lovenberg W. Carboxylmethylation of phosphodiesterase attenuates its activation by Ca^{2+}-calmodulin. J Biol Chem 1984;259:6630-5.
71. Jackson AE, Carraway KL 3rd, Puett D, Brew K. Effects of the binding of myosin light chain kinase on the reactivities of calmodulin lysines. J Biol Chem 1986;261:12226-32.
72. Jackson AE, Carraway KL 3rd, Payne ME, Means AR, Puett D, Brew K. Association of calmodulin and smooth muscle myosin light chain kinase: Application of a label selection technique with trace acetylated calmodulin. Proteins 1987;2:202-9.
73. Magnani R, Dirk LM, Trievel RC, Houtz RL. Calmodulin methyltransferase is an evolutionarily conserved enzyme that trimethylates Lys-115 in calmodulin. Nat Commun 2010;1:43.
74. Rowe PM, Wright LS, Siegel FL. Calmodulin N-methyltransferase. Partial purification and characterization. J Biol Chem 1986;261:7060-9.
75. Haziza S, Magnani R, Lan D, Keinan O, Saada A, Hershkovitz E,

et al. Calmodulin methyltransferase is required for growth, muscle strength, somatosensory development and brain function. PLoS Genet 2015;11:e1005388.

76. Chazin WJ, Johnson CN. Calmodulin mutations associated with heart arrhythmia: A status report. Int J Mol Sci 2020;21:1418.

77. Jiménez-Jáimez J, Palomino Doza J, Ortega Á, Macías-Ruiz R, Perin F, Rodríguez-Vázquez del Rey MM, et al. Calmodulin 2 mutation N98S is associated with unexplained cardiac arrest in infants due to low clinical penetrance electrical disorders. PLoS One 2016;11:e0153851.