Calmodulin regulation (calmodulation) of voltage-gated calcium channels

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Calmodulin regulation (calmodulation) of the family of voltage-gated Ca_V1-2 channels comprises a prominent prototype for ion channel regulation, remarkable for its powerful Ca^{2+} sensing capabilities, deep in elegant mechanistic lessons, and rich in biological and therapeutic implications. This field thereby resides squarely at the epicenter of Ca^{2+} signaling biology, ion channel biophysics, and therapeutic advance. This review summarizes the historical development of ideas in this field, the scope and richly patterned organization of Ca^{2+} feedback behaviors encompassed by this system, and the long-standing challenges and recent developments in discerning a molecular basis for calmodulation. We conclude by highlighting the considerable synergy between mechanism, biological insight, and promising therapeutics.

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The ancient Ca^{2+} sensor protein calmodulin (CaM) has emerged as a pervasive modulator of ion channels (Saimi and Kung, 2002), manifesting remarkable Ca^{2+} sensing capabilities in this context (Dunlap, 2007; Tadross et al., 2008) and furnishing essential Ca^{2+} feedback in numerous biological settings (Alseikhan et al., 2002; Xu and Wu, 2005; Mahajan et al., 2008; Adams et al., 2010; Morotti et al., 2012). Such modulation was discovered via mutations in CaM that altered the motile behavior of *Paramecium*, stemming from blunted activation of Ca^{2+}-dependent Na^+ current or K^+ current (Kink et al., 1990). Since then, numerous ion channels have been found to be modulated by CaM, as extensively reviewed (Budde et al., 2002; Saimi and Kung, 2002; Wei et al., 2003; Halling et al., 2006; Tan et al., 2011; Nyegaard et al., 2012). This paper focuses on the CaM regulation (calmodulation) of voltage-gated Ca^{2+} channels, which are extensively regulated by intracellular Ca^{2+} binding to CaM (Ca^{2+}/CaM; Lee et al., 1999; Peterson et al., 1999; Zühlke et al., 1999; Haeseleer et al., 2000; DeMaria et al., 2001; Budde et al., 2002; Halling et al., 2006). Such feedback regulation by Ca^{2+}/CaM demonstrates versatile functional capabilities, represents a prominent prototype for ion-channel modulation, and holds far-reaching biological consequences. These attributes contribute much to the standing of voltage-gated Ca^{2+} channels as the queen of ion channels, molecules that not only sculpt membrane electrical waveforms but also serve as gatekeepers of the ubiquitous second messenger Ca^{2+} (Yue, 2004).

Classic history of discovery

The earliest signs of Ca^{2+}-dependent regulation of Ca^{2+} channels came from data showing that increased Ca^{2+} could accelerate the inactivation of Ca^{2+} currents in *Paramecium* (Brehm and Eckert, 1978), invertebrate neurons (Tillotson, 1979), and insect muscle (Ashcroft and Stanfield, 1981). These results gave birth to the concept of Ca^{2+}-dependent inactivation (CDI), the then counterintuitive notion that entities other than transmembrane voltage could modulate the rapid gating of ion channels (Eckert and Tillotson, 1981).

Methodological advances permitting routine isolation and recording of Ca^{2+} currents within vertebrate preparations clearly revealed the existence of CDI of cardiac L-type Ca^{2+} currents (carried by $Ca_V1.2$ channels) in both multicellular preparations (Kass and Sanguinetti, 1984; Mentrard et al., 1984) and isolated myocytes (Lee et al., 1985). Classic data illustrating CDI of cardiac L-type currents is shown in Fig. 1 (A–C). Fig. 1 A (Ca) shows Ca^{2+} current from frog cardiocytes, elicited by a test voltage pulse to +80 mV (Mentrard et al., 1984). CDI becomes apparent upon Ca^{2+} entry during a voltage prepulse to +80 mV (Fig. 1 B), which causes sharply attenuated Ca^{2+} current in a subsequent test pulse (diminished area of red shading). To exclude voltage depolarization itself as the cause of inactivation, a prepulse to +120 mV (Fig. 1 C) can be demonstrated to produce far less inactivation within a subsequent test-pulse current. Because this prepulse imposed strong depolarization, but diminished Ca^{2+} entry via decreased driving force, the reemergence of test Ca^{2+} current suggests that the

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Abbreviations used in this paper: CaM, calmodulin; CaM regulation, calmodulation; CaBP, Ca^{2+}-binding protein; CDF, Ca^{2+}-dependent facilitation; CDI, Ca^{2+}-dependent inactivation; NSCaTE, N-terminal spatial Ca^{2+}-transforming element.

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strong inactivation evident in Fig. 1 B could be attributed to CDI. Data of this type specifies an inactivation mechanism that depends on voltage as a U-shaped function, now considered a hallmark of CDI (Eckert and Tillotson, 1981).

Still, the actual nature of effects of Ca^{2+} upon gating remained unclear. Fig. 1 (D–F) reproduces the profile of CDI at the single-molecule level (Yue et al., 1990; Imredy and Yue, 1992, 1994). Resolving data such as these is technically challenging, owing to the diminutive size of unitary Ca^{2+} currents (∼0.3 pA) and the sub-millisecond gating timescale involved. These data demonstrate the existence of U-shaped inactivation in the gating of a single cardiac L-type Ca^{2+} channel fluxing Ca^{2+}, as present in an adult rat ventricular myocyte (Imredy and Yue, 1994). These results established that Ca^{2+} influx of a single channel suffices to trigger CDI, and demonstrated the clear correspondence of single-channel and multicellular behavior (Fig. 1). Thus, CDI emerged as a legitimate molecular-level modulatory process.

Advent of Ca^{2+} channel calmodulation

The Ca^{2+} sensor mediating Ca^{2+} regulation of Ca^{2+} channels has long been a matter of debate, with proposals of direct binding of Ca^{2+} to the channel complex (Standen and Stanfield, 1982; Plant et al., 1983; Eckert and Chad, 1984), and of Ca^{2+}-dependent phosphorylation and/or dephosphorylation of channels (Chad and Eckert, 1986; Armstrong, D., C. Erxleben, D. Kalman, Y. Lai, A. Nairn, and P. Greengard. 1988. Abstracts of papers presented at the forty-second annual meeting of the Society of General Physiologists. Abstr. 20). The road toward identification of the actual Ca^{2+} sensor began with the cloning and expression of recombinant Ca^{2+} channels, thereby enabling structure–function studies (Snutch and Reiner, 1992). This phase of discovery was initiated by a bioinformatic observation (Babitch,

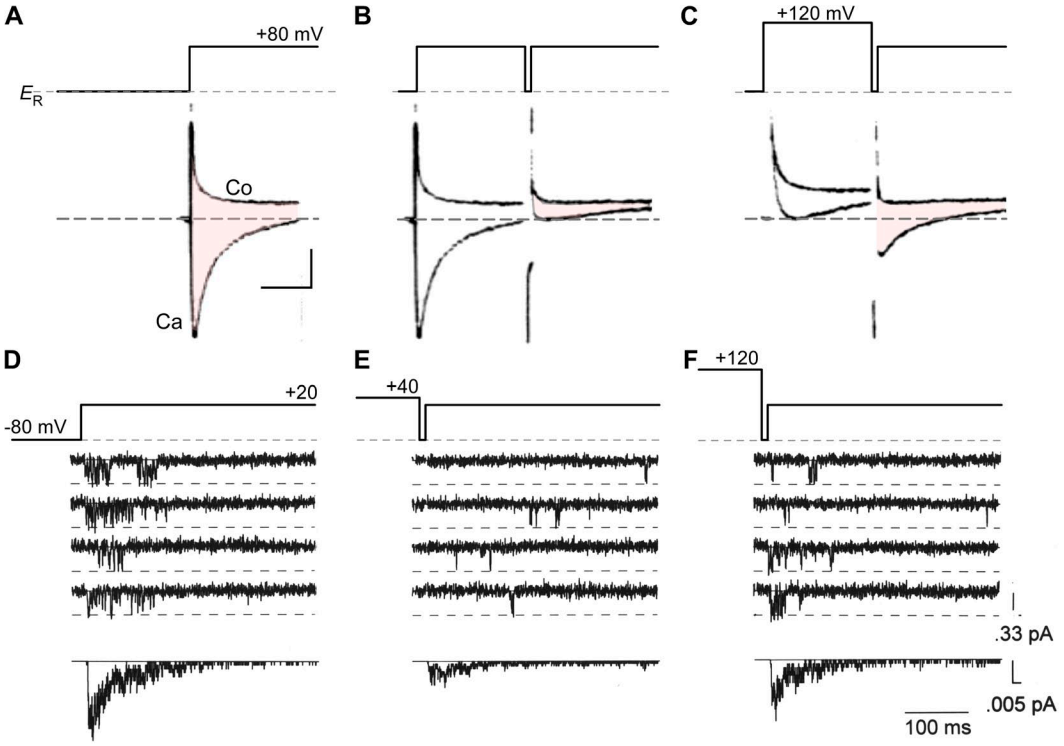
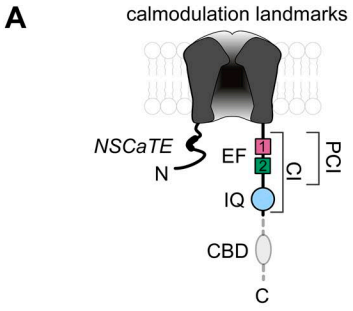


Figure 1. Classic U-shaped signature of CDI. (A–C) Early voltage-clamp recordings from a multicellular preparation of frog atrial trabeculae cells demonstrates CDI of Ca^{2+} currents. All listed voltages are relative to resting potential E_R (∼−80 mV) Adapted from Mentrard et al. (1984). (A) Ca^{2+} currents (shaded pink) evoked in response to an +80-mV depolarizing test pulse show robust inactivation. Fast capacitive transient was estimated by blocking these Ca^{2+} currents with 3 mM Co^{2+} solution (Co). Vertical bar corresponds to 0.5 μA of current; horizontal bar, 100 ms. (B) The Ca^{2+} current in response to the test pulse is sharply attenuated when preceded by an +80 mV prepulse. This reduction in current amplitude reflects the inactivation of Ca^{2+} channels. Format is as in A. (C) Further increase in the prepulse potential to +120 mV restores Ca^{2+} current amplitude (compare with B). Here, the diminished Ca^{2+} entry during the prepulse was insufficient to trigger CDI. The format is as in A. (D–F) Single channel recordings of L-type Ca^{2+} channels from adult rat ventricular myocytes also exhibit U-shaped dependence of Ca^{2+} channel inactivation. Adapted from Imredy and Yue (1994) with permission from Elsevier. (D) Depolarizing voltage pulse to +20 mV evokes representative elementary Ca^{2+} currents. Ensemble average currents are shown on the bottom. (E) When depolarizing pulse was preceded by +40 mV prepulse, the elementary Ca^{2+} currents are sparser and the first opening is delayed. This reduction in open probability parallels the sharp attenuation of macroscopic Ca^{2+} currents seen in B. The ensemble average is shown on the bottom. (F) Further increase in prepulse voltage to +120 mV led to a reversal of this gating pattern, once again highlighting the U-shaped dependence of CDI. Ensemble average is shown on the bottom.

1990), pointing out that the carboxy terminus of voltage-gated Ca^{2+} channels contains a region with weak homology to an EF hand Ca^{2+}-binding motif (Fig. 2 A, EF1). After the recognition of EF1, the existence of a second EF hand–like motif became apparent (Fig. 2 A, EF2). This led to chimeric channel analysis (de Leon et al., 1995), wherein segments of the carboxy termini of L-type ($Ca_V1.2$) and R-type ($Ca_V2.3$) Ca^{2+} channels were swapped. These two types of channels exhibit strong and weak CDI, respectively, under conditions of strong intracellular Ca^{2+} buffering (Liang et al., 2003), making them advantageous for chimeric analysis. Results from these experiments revealed that the proximal third of the carboxy terminus (Fig. 2 A, CI region) was important for CDI, and that EF1 of $Ca_V1.2$ channels was essential for the strong CDI in these channels (de Leon et al., 1995). One possible explanation was that EF1 might be the Ca^{2+} binding site for CDI postulated previously (Standen and Stanfield, 1982; Plant et al., 1983; Eckert and Chad, 1984). However, mutations within the Ca^{2+} binding loop of EF1 failed to eliminate CDI (Zhou et al., 1997; Peterson et al., 2000), focusing the search for the Ca^{2+} sensor elsewhere.

Targets of CaM binding themselves often resemble CaM, a bilobed molecule with each lobe composed of two EF hands (Jarrett and Madhavan, 1991). The dual vestigial EF hands in the carboxy terminus of channels (Fig. 2 A) may be seen as resembling a lobe of CaM, which suggests that CaM itself might be the Ca^{2+} sensor for CDI. Initial studies exploring this notion, however, showed that pharmacological inhibition of CaM did not eliminate CDI of L-type Ca^{2+} channels (Imredy and Yue, 1994; Zühlke and Reuter, 1998). Nonetheless, deletions within the $Ca_V1.2$ channel CI region identified an IQ motif (Fig. 2 A) as a critical element in CDI (Zühlke and Reuter, 1998), and mutagenesis of this IQ domain weakens CDI (Qin et al., 1999). As IQ motifs often bind Ca^{2+}-free CaM (apoCaM; Jurado et al., 1999), the possibility that CaM acts as a CDI sensor reemerged. Definitive evidence came with studies involving a Ca^{2+}-insensitive mutant form of CaM (CaM_{1234}), in which point mutations within all four EF hands eliminate Ca^{2+} binding (Xia et al., 1998). If "preassociation" of apoCaM with target molecules were a prerequisite for subsequent regulation by Ca^{2+} binding to this apoCaM, then CaM_{1234} could act as a dominant negative to silence regulation, as seen for small-conductance Ca^{2+}-activated K channels (Xia et al., 1998). Indeed, when $Ca_V1.2$ channels were coexpressed with CaM_{1234} or its analogues, CDI was ablated or strongly suppressed (Peterson et al., 1999; Zühlke et al., 1999). Additionally, apoCaM was found to bind to the $Ca_V1.2$ CI region, with critical dependence upon the IQ segment (Erickson et al., 2001; Pitt et al., 2001; Erickson et al., 2003). In retrospect, apoCaM preassociation with $Ca_V1.2$ channels would sterically protect CaM from pharmacological effects (Dasgupta et al., 1989), rationalizing the prior insensitivity of CDI to such small-molecule perturbation. In all, these data firmly substantiated CaM as the sensor for $Ca_V1.2$ channel Ca^{2+} regulation.



	N-lobe triggered modulation	C-lobe triggered modulation
$Ca_V1.2$ L-type channel	CDI[1] (local*)	CDI[2-3] (local)
$Ca_V1.3$ L-type channel	CDI[4] (local)	CDI[4] (local)
$Ca_V1.4$ L-type channel	CDI[5,6] (global)	CDI[5,6] (local)
$Ca_V2.1$ P/Q-type channel	CDI[7,8] (global)	CDF[7,8] (local)
$Ca_V2.2$ N-type channel	CDI[9] (global)	(not found)[9]
$Ca_V2.3$ R-type channel	CDI[9] (global)	(not found)[9]

* intermediate local / global (Tadross *et al*, 2008)

[1](Dick *et al*, 2008)
[2](Peterson *et al*, 1999)
[3](Zuhlke *et al*, 1999)
[4](Yang *et al*, 2006)
[5](Singh *et al*, 2006)
[6](Wahl-Schott *et al*, 2006)
[7](DeMaria *et al*, 2001)
[8](Lee *et al*, 2003)
[9](Liang *et al*, 2003)

Figure 2. Calmodulation: the ingredients and the flavors. (A) Channel diagram illustrates overall arrangement of structural landmarks critical for CDI. The Ca^{2+}-inactivation (CI) region, spanning ~160 residues of the channel carboxy terminus, is highly conserved across $Ca_V1/2$ channel families and is elemental for CDI. The proximal segment (PCI) of the CI region includes the dual vestigial EF hand (EF) segments (shaded purple and blue-green). The IQ domain, a canonical CaM binding motif critical for CDI, is located just downstream of the PCI segment. The NSCaTE element in the amino terminus of $Ca_V1.2/1.3$ channels is an N-lobe Ca^{2+}/CaM effector site. The CBD element (gray) in the carboxy terminus of Ca_V2 channels is a CaM binding segment thought to be critical for CDI. (B) The table outlines functional bipartition of CaM in Ca_V channels and the corresponding spatial Ca^{2+} selectivities. In $Ca_V1.2$ and $Ca_V1.3$ channels, both the C-lobe and N-lobe of CaM enable fast CDI with local Ca^{2+} selectivity. Latent CDI of $Ca_V1.4$ channels is revealed upon deletion of an autoinhibitory domain in the distal carboxy terminus. Throughout the Ca_V2 channel family, the N-lobe of CaM evokes slow CDI with global Ca^{2+} spatial selectivity. In $Ca_V2.1$ channels, the C-lobe of CaM supports ultra-fast facilitation (CDF) with local Ca^{2+} selectivity. No C-lobe triggered modulation has been reported for $Ca_V2.2$ and $Ca_V2.3$ channels.

Initially, it was believed that only $Ca_V1.2$ L-type channels (Fig. 2 B) were subject to CaM-mediated Ca^{2+} regulation (Zamponi, 2003). However, this system of calmodulation was gradually recognized to pertain to most of the Ca_V1 and Ca_V2 (but not Ca_V3) branches of the Ca_V channel superfamily (Liang et al., 2003; Fig. 2 B). P-type ($Ca_V2.1$) channels were found to be Ca^{2+} regulated (Lee et al., 1999), and a Ca^{2+}/CaM binding site downstream of the IQ element (CBD; Fig. 2 A) was argued to be important. In this initial study, no role for CaM preassociation was recognized, and no role for the IQ domain was found. Moreover, Ca^{2+} regulation of $Ca_V2.1$ channels manifests as a facilitation of current (Ca^{2+}-dependent facilitation; CDF), followed by a slowly developing CDI. Thus, it appeared possible that the Ca^{2+} regulation of $Ca_V2.1$ channels might diverge mechanistically from that of $Ca_V1.2$ channels. However, apoCaM was later found to preassociate with $Ca_V2.1$ channels (Erickson et al., 2001), Ca^{2+}-insensitive mutant CaM molecules were found to eliminate Ca^{2+} regulation in $Ca_V2.1$ channels (DeMaria et al., 2001; Lee et al., 2003), and the IQ domain was determined to be structurally essential for this regulation (DeMaria et al., 2001; Lee et al., 2003; Kim et al., 2008; Mori et al., 2008). That said, the role of the CBD segment remains contentious, with some studies still arguing for this segment's importance in CDI (Lee et al., 2003). In contrast, deletion of the entire carboxy terminus after the IQ domain (including the CBD element) completely spared CDF and CDI of $Ca_V2.1$ channels (DeMaria et al., 2001; Chaudhuri et al., 2005). Overall, $Ca_V2.1$ and $Ca_V1.2$ channels appear to be Ca^{2+} regulated by a largely conserved scheme.

Soon thereafter, calmodulation was established for the remaining members of the Ca_V2 class of channels (Fig. 2 B, $Ca_V2.2$ and $Ca_V2.3$), which indicates that this modulatory system pertains throughout the Ca_V1-2 channel superfamily (Liang et al., 2003). Strong CaM-mediated CDI was found in $Ca_V1.3$ channels (Xu and Lipscombe, 2001; Shen et al., 2006; Yang et al., 2006); a latent capacity for CaM-mediated CDI was observed in $Ca_V1.4$ channels (Singh et al., 2006; Wahl-Schott et al., 2006); and potential indications of CaM-mediated regulation of $Ca_V1.1$ channels have been described (Stroffekova, 2008, 2011).

A few more nuanced points nonetheless merit attention. First, some types of calmodulation are insensitive to strong intracellular Ca^{2+} buffering (e.g., $Ca_V1.2$ CDI), whereas others are not ($Ca_V2.3$ CDI; Fig. 2 B). This contrasting sensitivity enables the use of chimeric channels under increased Ca^{2+} buffering to identify key structural determinants (de Leon et al., 1995), despite the existence of calmodulation across the channel superfamily. Second, the mechanisms underlying CDF in $Ca_V1.2$ channels remain mysterious, as the CDF of native channels of the heart is weak to start, and attenuated by blockade of Ca^{2+} release from neighboring ryanodine receptor channels (RYRs; Wu et al., 2001). Additionally, CDF of $Ca_V1.2$ channels is strongly manifest only in recombinant channels containing point mutations within the IQ domain, and only when they are expressed in frog oocytes (Zühlke et al., 2000). Curiously, CDF is not observed in recombinant $Ca_V1.2$ channels expressed in mammalian cell lines (Peterson et al., 1999). In contrast, CDI of $Ca_V1.2$ channels is strong and universally observed across experimental platforms. Third, Ca^{2+}/CaM-dependent dephosphorylation by calcineurin was initially suggested as a mechanism for CDI of Ca^{2+} channels (Chad and Eckert, 1986); however, this proposition has remained controversial as previously reviewed (Budde et al., 2002). Recently, calcineurin was found to preassemble with the $Ca_V1.2$ channel complex through the scaffolding protein AKAP79/150 bound to the distal carboxy terminus of the channel (Oliveria et al., 2007). Furthermore, two additional maneuvers were reported to impede CDI of these channels (Oliveria et al., 2012): the overexpression of a mutant AKAP79/150 incapable of binding calcineurin, and inhibition of calcineurin activity. However, others have found that direct inhibition of calcineurin has no effect on the CDI of L-type channels within multiple neuronal preparations (Branchaw et al., 1997; Victor et al., 1997; Zeilhofer et al., 1999). Moreover, deletion of the entire distal carboxy terminus (including the AKAP79/150 binding segment) of $Ca_V1.2$ channels preserves strong CDI (de Leon et al., 1995; Erickson et al., 2001; Crump et al., 2013). Similarly, short variants of $Ca_V1.3$ channels lacking the AKAP harboring distal carboxy termini (Marshall et al., 2011) also fully support CDI (Xu and Lipscombe, 2001; Shen et al., 2006; Yang et al., 2006; Bock et al., 2011). Altogether, it may be that calcineurin and AKAP79/150 are indirect and context-dependent modulators of CDI, rather than direct effector molecules (Budde et al., 2002).

Functional bipartition of CaM and selectivity for local/global Ca^{2+} sources

The calmodulatory mechanism for Ca^{2+} channels supports remarkable forms of Ca^{2+} decoding. This feature echoes earlier discoveries of "functional bipartition" of CaM in *Paramecium* (Kink et al., 1990; Saimi and Kung, 2002). Here, "under-excitable" behavioral strains had mutations only in the N-lobe of CaM, whereas "over-excitable" strains had mutations only in the C-lobe. Thus, one lobe of CaM can mediate signaling to one set of functions, whereas the other lobe signals to an alternative set of operations. It was unclear, however, whether this bipartition extended to mammals. The requirement that Ca^{2+} regulation of mammalian Ca^{2+} channels requires apoCaM preassociation permitted direct exploration of this question. Coexpressing channels with "half mutant" CaM molecules (CaM_{12} and CaM_{34}, where only C- or N-terminal lobes, respectively, bind Ca^{2+}) revealed

that the individual lobes of CaM can evoke distinct components of channel regulation (Fig. 2 B). In most Ca_V1 channels, the C-lobe induces a kinetically rapid phase of CDI, whereas the N-lobe yields a slower component (Peterson et al., 1999; Yang et al., 2006; Dick et al., 2008). For $Ca_V1.2$ channels, early studies that utilized high intracellular Ca^{2+} buffering found only minimal N-lobe–mediated CDI (Peterson et al., 1999). However, when interrogated under low intracellular Ca^{2+} buffering, slow but recognizable N-lobe–mediated CDI of $Ca_V1.2$ channels emerges (Dick et al., 2008; Simms et al., 2013). Most strikingly, in $Ca_V2.1$ channels, the lobes of CaM produce opposing polarities of regulation: the C-lobe of CaM triggers a kinetically rapid CDF, whereas the N-lobe evokes a slower CDI (DeMaria et al., 2001; Lee et al., 2003). $Ca_V2.2$ and $Ca_V2.3$ channels manifest CDI triggered mainly by the N-lobe of CaM (Liang et al., 2003). Whether this bipartition orchestrates divergent classes of behavior in higher-order animals remains an intriguing and open question (Wei et al., 2003).

Beyond simply splitting the Ca^{2+} signal, however, the calmodulation of mammalian Ca^{2+} channels revealed that the lobes of CaM can selectively decode Ca^{2+} in different ways. Hints as to this capability came from the differential sensitivity of calmodulation in various channels to Ca^{2+} buffering. Processes evoked by the C-lobe of CaM are invariably insensitive to introduction of strong Ca^{2+} buffering, whereas N-lobe–mediated processes in Ca_V2 channels can be eliminated by the same maneuver. Strong buffering would only spare Ca^{2+} signals near the cytoplasmic mouth of channels where strong point-source Ca^{2+} influx would overwhelm buffer capacity (Neher, 1986; Stern, 1992). This argues that local Ca^{2+} influx through individual channels triggers C-lobe signaling; in other words, the C-lobe of CaM exhibits a "local Ca^{2+} selectivity." In contrast, a spatially global elevation of Ca^{2+} (present in the absence of strong Ca^{2+} buffering) is required for N-lobe signaling of Ca_V2 channels (DeMaria et al., 2001; Lee et al., 2003; Liang et al., 2003); thus, the N-lobe in this context exhibits a "global selectivity." The existence of global selectivity is notable, given that local Ca^{2+} influx yields far larger Ca^{2+} increases near a channel than does globally sourced Ca^{2+} (Tay et al., 2012). One exception to this pattern is the local Ca^{2+} selectivity of the N-lobe component of CDI in $Ca_V1.2/1.3$ channels (see two paragraphs below). Corroboration of spatial Ca^{2+} selectivity is provided by the ability of single $Ca_V1.2$ channels to undergo CDI (Fig. 1, D–F). By definition, only a local Ca^{2+} source is present in the single-channel configuration; thus, the presence of CDI in this context indicates local Ca^{2+} selectivity. Additionally, single-channel records of $Ca_V2.1$ channels exhibit CDF (driven by C-lobe), but not CDI (triggered by N-lobe; Chaudhuri et al., 2007), which is consistent with the proposed differential selectivities for this channel. Fig. 2 B summarizes the arrangement of spatial Ca^{2+} selectivities according to the lobes of CaM.

The mechanisms underlying these contrasting spatial Ca^{2+} selectivities can be interpreted as emergent behaviors of a system in which a lobe of apoCaM must transiently detach from a preassociation site before binding Ca^{2+}, and where a Ca^{2+}-bound lobe of CaM must associate with a channel effector site to mediate regulation (Tadross et al., 2008). When the slow Ca^{2+}-unbinding kinetics of the C-lobe of CaM are imposed on this scenario, local Ca^{2+} sensitivity invariably results. In contrast, when the rapid Ca^{2+}-unbinding kinetics of the N-lobe are interfaced with this architecture, global Ca^{2+} selectivity arises if the channel preferentially binds apoCaM versus Ca^{2+}/CaM, as in the case of Ca_V2 channels (Fig. 2 B). When the channel preferentially interacts with Ca^{2+}/CaM, local Ca^{2+} selectivity emerges, as in the context of Ca_V1 channels (Fig. 2 B). The relative roles of the affinities and kinetics of Ca^{2+} binding to the two lobes of CaM in mediating local and global Ca^{2+} selectivities have been considered at length elsewhere (Tadross et al., 2008).

As an explicit example of how spatial Ca^{2+} selectivity of N-lobe regulation is specified, we consider the effects of an N-terminal spatial Ca^{2+}-transforming element (NSCaTE) present only on the amino terminus of $Ca_V1.2/1.3$ channels (Fig. 2 A). NSCaTE is a Ca^{2+}/CaM binding site whose presence enhances the aggregate channel affinity for Ca^{2+}/CaM over apoCaM (Ivanina et al., 2000; Dick et al., 2008), endowing the N-lobe component of CDI of these channels with a largely local Ca^{2+} selectivity of N-lobe CDI in these channels. Elimination of the NSCaTE site in these channels, which tilts channels toward a preference for apoCaM, then switches their N-lobe CDI to a global profile. Conversely, donation of NSCaTE to Ca_V2 channels, which causes channels to favor Ca^{2+}/CaM binding, then endows their N-lobe CDI with local Ca^{2+} selectivity (Dick et al., 2008). In this manner, the presence of NSCaTE can tune the spatial Ca^{2+} selectivity of N-lobe–mediated channel regulation. This overall arrangement of CaM interactions with a target molecule could endow numerous other regulatory systems with spatial Ca^{2+} selectivity.

Molecular basis of calmodulation

Elucidating the arrangement of apoCaM and Ca^{2+}/CaM on Ca^{2+} channels is fundamental for the field, given the biological influence of calmodulation, and the importance of this system as an ion-channel regulatory prototype (Dunlap, 2007). This critical task, however, remains an ongoing challenge, given the >2,000 amino acids comprising the main pore-forming α_1 subunit alone, the ability of multiple peptide segments of the channel to bind CaM in vitro, and the formidable nature of obtaining atomic structures for these channels.

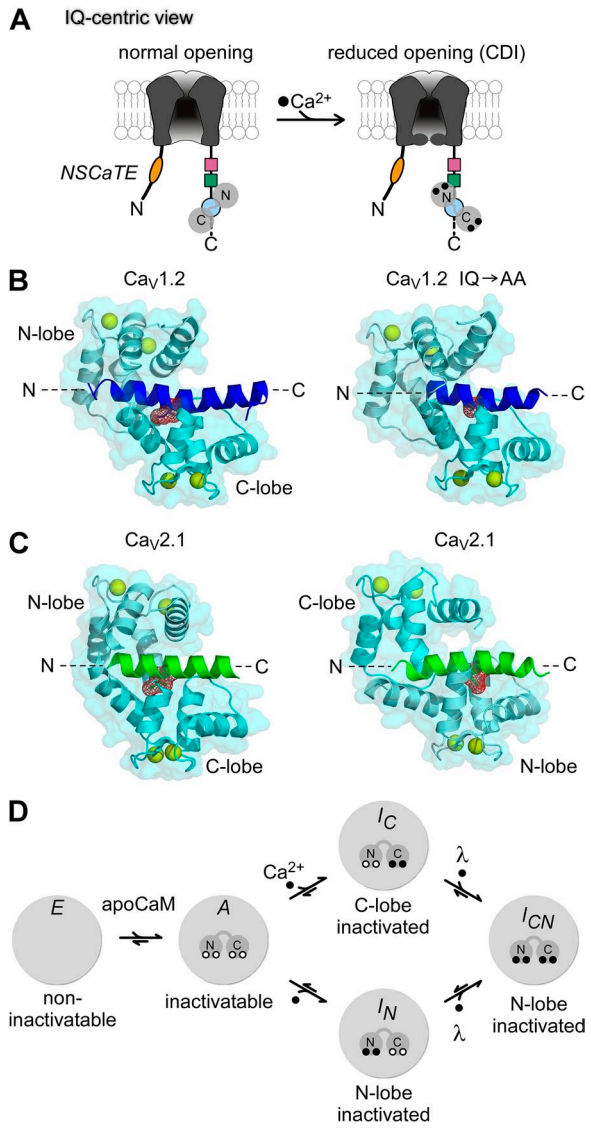


Figure 3. Toward an atomic-level understanding of CDI. (A) IQ-centric view of calmodulation of Ca_V channels. In this mechanistic scheme, ApoCaM is preassociated with the IQ domain (blue). Upon Ca^{2+} binding, CaM rebinds the same IQ domain with a higher affinity, and subtle conformational rearrangements are presumed to trigger CaM-mediated channel regulation. (B, left) Crystal structure of $Ca_V1.2$ IQ domain peptide in complex with Ca^{2+}/CaM (Protein Data Bank accession no. 2BE6). The IQ domain is colored blue. CaM is show in cyan (N-lobe, pale cyan; C-lobe, cyan). Ca^{2+} ions are depicted as yellow spheres. The key isoleucine residue (red) serves as a hydrophobic anchor for CaM. Ca^{2+}/CaM adopts a parallel arrangement with the IQ domain in which the N-lobe binds closer to the amino terminus of the IQ domain and the C-lobe binds further downstream. (B, right) Crystal structure of Ca^{2+}/CaM bound to mutant $Ca_V1.2$ IQ domain with alanines substituted for the key isoleucine-glutamine residues (accession no. 2F3Z). This double mutation abolishes CDI. Structurally, however, Ca^{2+}/CaM hugs the mutant IQ domain in a similar conformation as to its interaction with the wild-type (left). (C) Crystal structure of Ca^{2+}/CaM bound to $Ca_V2.1$ IQ domain (left, accession no. 3BXK; right, accession no. 3DVM). The IQ domain is shaded green, CaM in cyan. Ca^{2+}/CaM was reported to bind to the $Ca_V2.1$ IQ domain in both parallel (left) and antiparallel (right) arrangements. The antiparallel arrangement in which the C-lobe of CaM binds upstream of IQ domain has led to speculation that CDF may result from this inverted polarity of Ca^{2+}/CaM association. (D) Simplified configurations of the CaM/channel complex relevant for calmodulation (*E*, *A*, I_C, I_N, and I_{CN}). In configuration *E*, channels lack preassociated CaM and therefore cannot undergo CDI. Configuration *A* corresponds to channels bound to apoCaM, and thus capable of undergoing robust CDI. Ca^{2+} binding to the C-lobe and N-lobe of CaM leads to the inactivated configurations I_C and I_N, respectively. Ca^{2+} binding to both lobes of CaM then yields configuration I_{CN}, with both C and N lobes of CaM engaged toward CDI. This final transition may exhibit positive cooperativity as specified by the constant $\lambda >> 1$. Under endogenous levels of CaM, channels may reside in any of the five configurations. Strong coexpression of wild-type or mutant CaM restricts accessibility to various states. Reproduced with permission from Ben Johny et al., 2013.

The stoichiometry of CaM interaction with channels has been debated. Crystal structures of Ca^{2+}/CaM complexed with portions of the carboxy tail CI region of $Ca_V1.2$ channels (Fallon et al., 2009; Kim et al., 2010) suggest that multiple CaM molecules may interact to produce the full spectrum of Ca^{2+} regulatory functions. Moreover, CaM can interact with multiple peptide segments of the channel (Ivanina et al., 2000; Tang et al., 2003; Zhou et al., 2005; Dick et al., 2008; Ben Johny et al., 2013). In contrast, covalent fusion of single CaM molecules to $Ca_V1.2$ channels suggests that only one CaM per channel suffices to elicit CDI (Mori et al., 2004). Moreover, live-cell FRET studies of the interactions between CaM and holochannels ($Ca_V1.2$) also point to a 1:1 CaM/channel ratio (Ben Johny et al., 2012). In the holochannel, the various CaM binding segments may be arranged in close proximity so as to allow the two lobes of CaM to preferentially interact with the distinct channel segments during Ca^{2+} regulation (Dick et al., 2008; Ben Johny et al., 2013). Recent biochemical studies of the NSCaTE element show that this segment preferentially interacts with a single lobe of CaM (N-lobe) at a time (Liu and Vogel, 2012). Furthermore, the NSCaTE element can interact also with Ca^{2+}/CaM prebound to an IQ domain peptide (Taiakina et al., 2013). Thus, we favor the interpretation that only one CaM interacts within holochannels, although peptide fragments of Ca^{2+} channels may bind to multiple CaM molecules.

The IQ domain (Fig. 3 A, blue circle) is critical for calmodulation. Mutations within this domain markedly modulate the Ca^{2+} regulation of $Ca_V1.2$ channels (Zühlke and Reuter, 1998; Qin et al., 1999; Zühlke et al., 1999, 2000; Erickson et al., 2003), $Ca_V1.3$ channels (Yang et al., 2006; Bazzazi et al., 2013; Ben Johny et al., 2013), $Ca_V2.1$ channels (DeMaria et al., 2001; Lee et al., 2003; Kim et al., 2008; Mori et al., 2008), and $Ca_V2.2$ and $Ca_V2.3$ channels (Liang et al., 2003). Furthermore, apoCaM preassociation with $Ca_V1.2/1.3$ channels requires the IQ domain (Erickson et al., 2001; Pitt et al.,

2001; Erickson et al., 2003; Bazzazi et al., 2013; Ben Johny et al., 2013). Finally, Ca^{2+}/CaM binds well to IQ-domain peptides of many Ca_V channels (Peterson et al., 1999; Zühlke et al., 1999; DeMaria et al., 2001; Pitt et al., 2001; Liang et al., 2003; Bazzazi et al., 2013; Ben Johny et al., 2013). All these results gave rise to the IQ-centric hypothesis shown in Fig. 3 A. Here, the IQ domain is important for both apoCaM preassociation (left) and Ca^{2+}/CaM binding (effector configuration shown on the right). This IQ-centric paradigm has motivated efforts to resolve crystal structures of Ca^{2+}/CaM complexed with IQ-domain peptides of various Ca_V1-2 channels (Fallon et al., 2005; Van Petegem et al., 2005; Kim et al., 2008; Mori et al., 2008), as shown in Fig. 3 (B and C). Throughout, the IQ peptide segment appears as an α-helical entity with N and C termini as labeled.

However, this viewpoint remains problematic in three regards. (1) The atomic structures of Ca^{2+}/CaM bound to wild-type and mutant IQ peptides of $Ca_V1.2$ (Fig. 3 B) show that a central isoleucine (side chains explicitly shown) in the IQ element is deeply buried within the C-lobe of Ca^{2+}/CaM (Van Petegem et al., 2005), and that alanine substitution at this site hardly changes structure (Fallon et al., 2005). Additionally, Ca^{2+}/CaM dissociation constants for corresponding wild-type and mutant IQ peptides are nearly the same (Zühlke et al., 2000; Bazzazi et al., 2013). Why then would alanine substitution at this well-ensconced site influence the rest of the channel to blunt regulation (Fallon et al., 2005)? (2) For $Ca_V1.2/1.3$ channels, the N-lobe of Ca^{2+}/CaM effector site appears to be an NSCaTE element (Fig. 3 A, oval) of the channel amino terminus (Dick et al., 2008; Tadross et al., 2008; Liu and Vogel, 2012), separate from the IQ element. (3) Crystal structures of Ca^{2+}/CaM in complex with the IQ peptide of $Ca_V2.1$ channels show that CaM can adopt both a parallel (Mori et al., 2008) and an antiparallel configuration (Kim et al., 2008; Fig. 3 C, left and right, respectively). The apparent inversion in configuration of CaM binding to IQ domain between $Ca_V1.2$ and $Ca_V2.1$ has been proposed as a mechanism for the opposing polarity of Ca^{2+} regulation observed in the two channels (Kim et al., 2008; Minor and Findeisen, 2010). However, detailed structural analysis along with functional systematic alanine scanning mutagenesis and chimeric channel analysis argues that the C-lobe effector site resides at a site beyond the IQ module (Mori et al., 2008).

A major concern with older IQ domain analyses is that the regulatory system was not considered conceptually as a whole, as shown in Fig. 3 D (drawn with specific reference to $Ca_V1.3$ channels; Ben Johny et al., 2013). Configuration *E* portrays channels lacking apoCaM. Such channels can open normally, but do not manifest CDI because Ca^{2+}/CaM from bulk solution cannot efficiently access a channel in configuration *E* to induce CDI (Mori et al., 2004; Yang, P.S., M.X. Mori, E.A. Antony, M.R. Tadross, and D.T. Yue. 2007. *Biophysical Journal* abstracts issue. 1669-Plat; Liu et al., 2010; Findeisen et al., 2011). ApoCaM binding with configuration *E* gives rise to configuration *A*, where opening can also occur normally, but CDI can now take place. For CDI, Ca^{2+} binding to both lobes of CaM yields configuration I_{CN}, which underlies a fully inactivated channel with strongly reduced opening. For intermediate configurations, Ca^{2+} binding only to the C-lobe brings about configuration I_C, equivalent to a C-lobe inactivated channel; Ca^{2+} binding only to the N-lobe yields the N-lobe–inactivated arrangement (I_N). Of particular importance, ensuing entry into I_{CN} likely involves positively cooperative interactions specified by cooperativity factor $\lambda >> 1$.

This scheme emphasizes several challenges for older analyses of the IQ domain, where CDI was mostly assessed with only endogenous CaM present. Results thus obtained would be ambiguous, because IQ-domain mutations could alter calmodulation via changes at multiple steps depicted in Fig. 3 D, whereas other interpretations largely attributed the effects to altered Ca^{2+}/CaM binding with an IQ effector site. In contrast, IQ mutations could well weaken apoCaM preassociation and reduce CDI by favoring configuration *E*. Moreover, functional deficits caused by mutations that do attenuate interaction with one lobe of Ca^{2+}/CaM may be masked by positively cooperative steps (λ in Fig. 3 D).

To minimize these issues, CDI of $Ca_V1.3$ channels was recently characterized during strong coexpression with various mutant CaM molecules (Bazzazi et al., 2013; Ben Johny et al., 2013) as shown in Fig. 4 (A–C, top). For orientation, CDI of channels expressed with only endogenous CaM present is shown in the upper portion of Fig. 4 A. Strong CDI produces a rapid decay of whole-cell Ca^{2+} current (red trace), compared with the negligible decline of Ba^{2+} current (black trace). Because Ba^{2+} binds CaM poorly (Chao et al., 1984), the decline of Ca^{2+} versus Ba^{2+} current after 300 ms of depolarization quantifies CDI (*CDI* parameter plotted below in bar graphs). One can isolate the diamond-shaped subsystem lacking configuration *E* (Fig. 4 B, top) by leveraging mass action with strong coexpression of wild-type CaM (CaM_{WT}). Further simplification of CDI was obtained by strongly coexpressing channels with CaM_{12} (Fig. 4 C, top), which empties configuration *E* by mass action, and forbids configurations I_N and I_{CN}. Thus, the isolated C-lobe component of CDI can be studied, with the signature rapid time course of current decay shown near the top of Fig. 4 C. Critically, this layout avoids interplay with cooperative λ steps in Fig. 3 D. Likewise, strongly coexpressing CaM_{34} focuses on the slower N-lobe form of CDI, with attendant simplifications.

Accordingly, a new framework of CaM/channel configurations that underlie calmodulation could be deduced, as diagrammed in Fig. 5 (Ben Johny et al., 2013). For convenience, Fig. 4 compiles results from a systematic

alanine scan covering the entire CI domain of the carboxyl tail of $Ca_V1.3$ channels (Bazzazi et al., 2013; Ben Johny et al., 2013), where substitution positions are denoted to the left of the bar graph in Fig. 4 A. The N-terminal end of the CI segment corresponds to the top of the graph, purple and blue-green regions indicate EF1 and EF2, and the lavender zone denotes the IQ domain. Electrophysiological characterization for each of the substitutions was performed for all the various subsystems (Fig. 4, A–D, top), and bar graphs plot the strength of *CDI* for the corresponding subsystems in each of the panels. The blue-green vertical lines reference the profile for wild-type channels. The results thus obtained were combined with CaM binding assays (not depicted) to identify meaningful structure–function correlations.

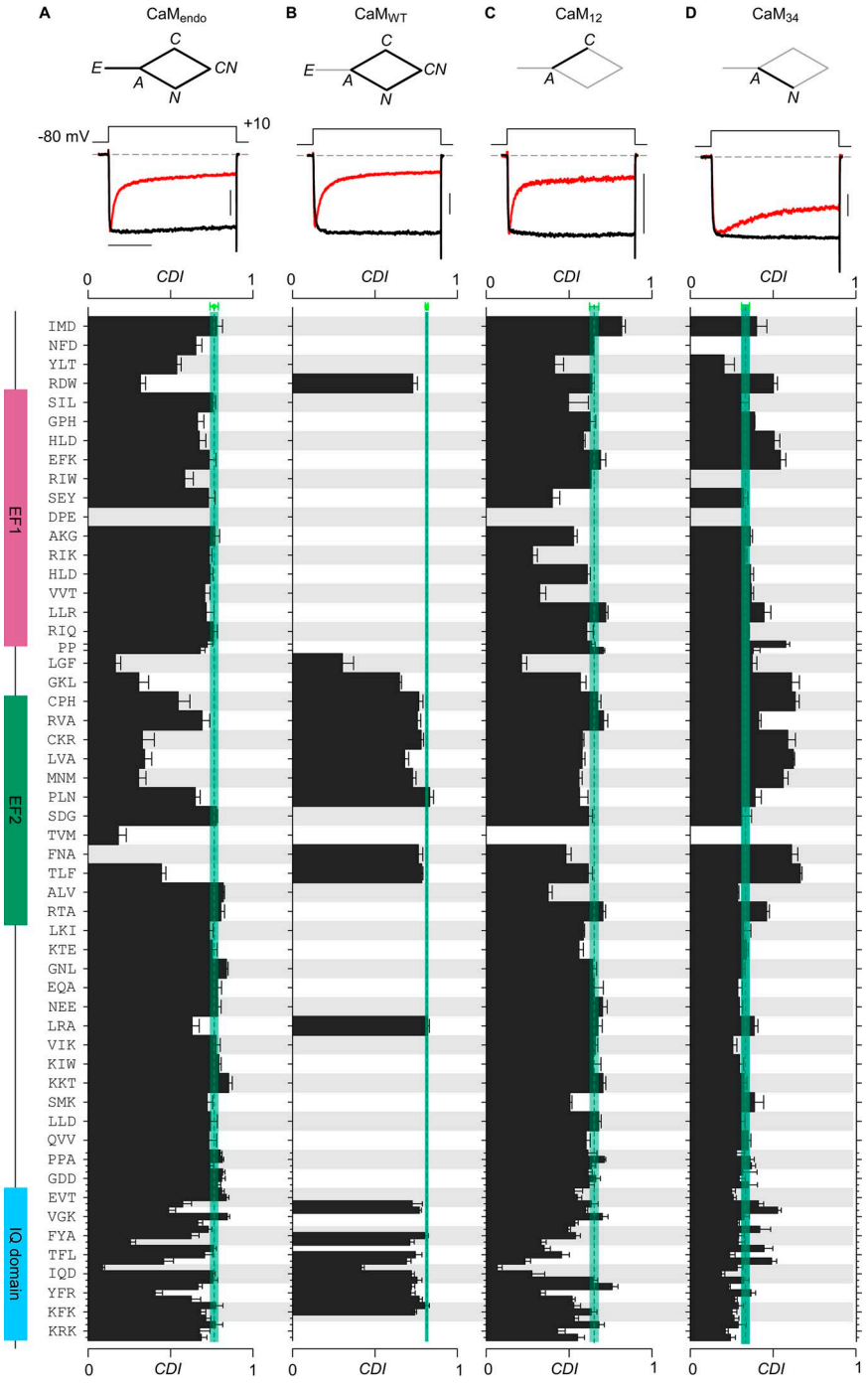


Figure 4. Residue-level roadmap for calmodulation of $Ca_V1.3$ channels. Systematic alanine scanning mutagenesis of the entire CI domain of $Ca_V1.3$ channels. (A) CDI of wild-type and mutant $Ca_V1.3$ channels recombinantly expressed in HEK293 cells was measured with only endogenous CaM present. CDI observed here reflects properties of the entire system (stick figure) diagrammed in Fig. 3 D. Exemplar whole-cell current shows robust CDI for wild-type (WT) $Ca_V1.3$, reflecting their high affinity for apoCaM. Here and throughout, the vertical bar pertains to 0.2 nA of Ca^{2+} current (black); the Ba^{2+} current (gray) has been scaled ∼3-fold downward to aid comparison of decay kinetics. Horizontal bar, 100 ms. *CDI* is measured as the fractional reduction of Ca^{2+} current in comparison to Ba^{2+} at 300 ms (Ben Johny et al., 2013). Bottom, bar graph summary of *CDI* for alanine substitutions for indicated residues. *CDI* for WT channels, blue-green vertical line. Alanine substitutions in both EF hand regions and IQ domain resulted in diminished CDI. (B) Overexpression of CaM_{WT} isolates the behavior of the diamond-shaped subsystem. Such overexpression of CaM_{WT} should rescue CDI for mutations that weakened apoCaM preassociation. For WT $Ca_V1.3$, exemplar current shows that CDI is unaltered, as expected for a channel with high affinity for apoCaM. Bottom, bar graph summary of CDI for various mutant channels with CaM_{WT} overexpressed. Format is as in A. CDI is rescued for several mutations in the EF hand region (EF2) and the IQ domain, reflecting the preassociation of N-lobe and C-lobe of apoCaM. (C) Overexpression of CaM_{12} isolates C-lobe CDI. Exemplar currents for WT channels show the fast C-lobe form of CDI. Bottom, bar graph summary shows the footprint of C-lobe CDI. Mutants in both the first EF hand and the IQ domain disrupted the C-lobe CDI (see the LGF loci in the EF hand regions and TFL and IQD loci in the IQ domain). The format is as in A. (D) Overexpression of CaM_{34} isolates N-lobe CDI. N-lobe CDI was largely preserved by mutations in the CI region. Mutations that weakened N-lobe apoCaM preassociation exhibited enhanced N-lobe CDI (see EF2 segment). Reassuringly, alanine scanning mutagenesis of NSCaTE element resulted in specific disruption of N-lobe CDI of $Ca_V1.3$ channels (Tadross et al., 2008). Adapted with permission from Ben Johny et al. (2013) and Bazzazi et al. (2013).

Under the regimen in Fig. 4 A, where all configurations are potentially accessible, diminished CDI by alanine substitutions occurred not only in the IQ domain, but upstream in the EF hand regions (between LGF and TLF residues). The latter effects strongly suggested that something outside the IQ domain was essential. Some of these deficits in CDI could be attributed to weakening of apoCaM preassociation with channels, because overexpressing wild-type CaM to depopulate configuration *E* (in Fig. 4 B) substantially recovered many of these CDI deficits. Binding assays of apoCaM with various CI segments confirmed this interpretation (Bazzazi et al., 2013; Ben Johny et al., 2013).

Turning to potential deficits relating to diminished Ca^{2+}/CaM action through channel effector sites, Fig. 4 (C and D) separately interrogates the C-lobe and N-lobe components of CDI. Importantly, isolating the C- and N-lobe components minimizes masking of mutational effects by positive cooperativity, allowing CDI deficits to be more sensitively observed in this regimen. That said, Fig. 4 C displays C-lobe CDI deficits in the EF hand region (strongest for LGF substitution), as well as in the IQ domain (with the strongest effect upon substitution at the central isoleucine). These outcomes support a hypothesis where the Ca^{2+}/CaM effector configuration for the C-lobe component of CDI involves both of these functional hotspots, as developed two paragraphs below. For the N-lobe component of CDI (Fig. 4 D), no appreciable deficits were observed, as would be expected if interaction with the channel NSCaTE element (in the channel amino terminus) serves as the effector element.

Fig. 5 A displays a proposed configuration *A* for apoCaM interaction with the channel. This includes a homology model of the apoCaM C-lobe complexed with the IQ domain (blue), based on analogous Na_V structures (Chagot and Chazin, 2011; Feldkamp et al., 2011). The portrayal of the apoCaM N-lobe incorporates ab initio structural prediction of the CI domain, with two vestigial EF hands (EF), and a protruding helix (preIQ subelement). The EF hand module (EF) resembles the structure of a homologous Na_V segment (Chagot et al., 2009; Wang et al., 2012), and the preIQ helix resembles a helical segment observed in crystal structures of analogous $Ca_V1.2$ peptides (Fallon et al., 2009; Kim et al., 2010). The atomic structure of the apoCaM N-lobe (1CFD) was interfaced with shape-complementarity docking algorithms. Regarding the proposed interaction interface of the N-lobe with the channel EF domain, we note that alanine substitution therein produced a curious enhancement of N-lobe CDI seen with certain alanine substitutions, especially in the EF region (Fig. 4 D). This feature is interesting because weakening of channel interaction with the N-lobe of apoCaM would be predicted to strengthen CDI produced by the N-lobe of Ca^{2+}/CaM (Tadross et al., 2008).

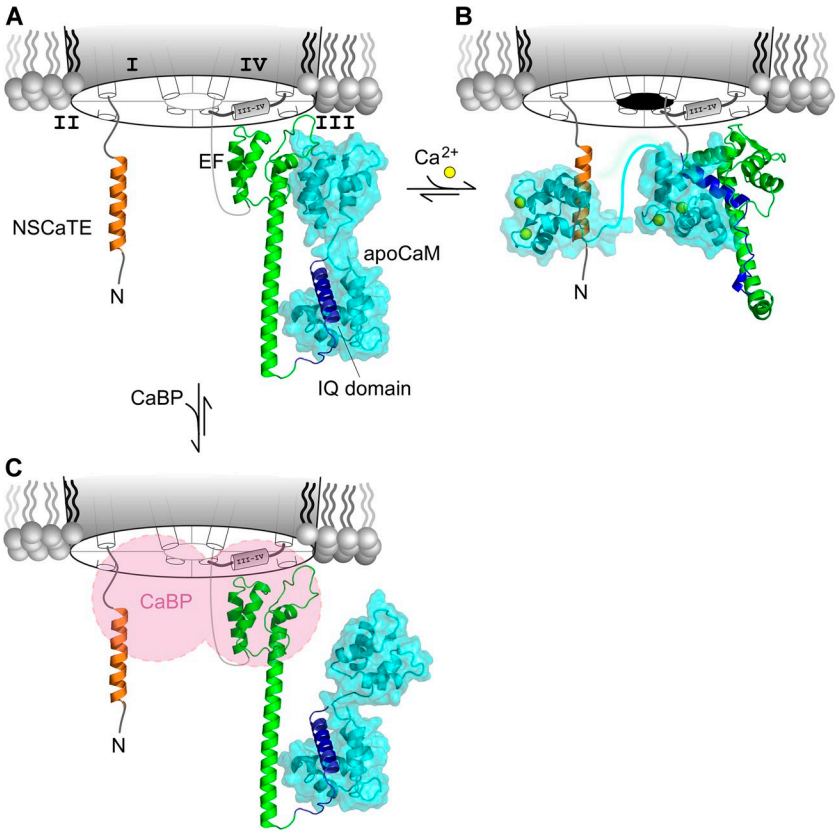


Figure 5. Next-generation blueprint for CaM/CaBP regulation of Ca_V channels. (A) De novo molecular model of $Ca_V1.3$ CI region docked to apoCaM. The N-lobe of apoCaM is thought to preassociate with the PCI region (green), whereas the C-lobe binds to the IQ domain (blue). The NSCaTE segment (tan) is unoccupied. This model corresponds to configuration *A* in Fig. 3 D, where the channels are charged with an apoCaM and ready to undergo CDI. (B) Proposed model for the Ca^{2+} inactivated state of the channel. The N-lobe of Ca^{2+}/CaM is shown binding to the NSCaTE segment based on a recent NMR structure (Protein Data Bank accession no. 2LQC). This configuration is believed to result in N-lobe CDI. The de novo molecular model shows C-lobe of Ca^{2+}/CaM, the EF hand segment, and the IQ domain forming a tripartite complex resulting in C-lobe CDI. Overall, this model corresponds to the inactive configuration I_{CN} in Fig. 3 D. (C) Proposed allosteric mechanism of CaBP action. CaBP and apoCaM may dually bind the Ca_V1 channel. However, CaBP may allosterically inhibit CDI of Ca_V1 channels by interacting with the NT, III-IV loop, or PCI segment, thereby preventing Ca^{2+}/CaM from reaching its effector configuration. (A–C) Adapted with permission from Yang et al. (2014).

Fig. 5 B displays a proposed arrangement for the Ca^{2+}/CaM-bound configuration I_{CN}. The N-lobe complex with NSCaTE is an NMR structure (Liu and Vogel, 2012). An alternative ab initio model of the PCI is computationally docked with the C-lobe of Ca^{2+}/CaM (Protein Data Bank accession no. 3BXL) and IQ module, which together form a ternary complex. This ternary arrangement is consistent with the importance of both IQ and EF domains for C-lobe CDI (Fig. 4 C). Intriguingly the C-lobe configuration resembles a rather canonical CaM–peptide complex, where the channel EF module contributes a surrogate lobe of CaM. Importantly, assays of Ca^{2+}/CaM binding to the IQ segment alone would correlate poorly with functional effects relating to the ternary complex, as experimental studies observe (Bazzazi et al., 2013; Ben Johny et al., 2013). Overall, the proposed framework in Fig. 5 (A and B) may aid future structural biology and structure–function work. In addition, this scheme of CaM exchange within its target molecule may generalize beyond the Ca^{2+} channel family.

Biological consequences and prospects for new disease therapies

The biological consequences of Ca^{2+} regulation by CaM promise to be wide ranging and immense. In the heart, elimination of $Ca_V1.2$ CDI by means of dominant-negative CaM elicits marked prolongation of ventricular action potential duration (APD), implicating CDI as a dominant control factor in specifying APD (Alseikhan et al., 2002; Mahajan et al., 2008). As APD is one of the main determinants of electrical stability and arrhythmias in the heart, pharmacological manipulation of such regulation looms as a future antiarrhythmic strategy (Mahajan et al., 2008; Anderson and Mohler, 2009). Most recently, genome-wide linkage analysis in humans has uncovered heritable and de novo CaM mutations as the probable cause of several cases of catecholaminergic polymorphic ventricular tachycardia (CPVT), with altered CaM-ryanodine receptor function implicated as a major contributing factor (Nyegaard et al., 2012). Whole-exome sequencing has revealed an association between three de novo missense CaM mutations and severe long-QT (LQT) syndrome with recurrent cardiac arrest (Crotti et al., 2013), quite plausibly acting via disruption of $Ca_V1.2/1.3$ channel CDI (Limpitikul et al., 2014).

In brain, state-of-the-art analysis of genome-wide single-nucleotide polymorphisms (SNPs) has identified Ca_V channels as a major risk factor for several psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). More specifically to calmodulation, $Ca_V1.3$ channels constitute a prominent Ca^{2+} entry portal into pacemaking and oscillatory neurons (Bean, 2007), owing to the more negative voltages required to open these ion channels. These channels are subject to extensive alternative splicing (Hui et al., 1991; Xu and Lipscombe, 2001; Shen et al., 2006; Bock et al., 2011; Tan et al., 2011) and RNA editing (Huang et al., 2012) in the carboxy tail, in ways that strongly modulate the strength of CDI (Shen et al., 2006; Liu et al., 2010; Huang et al., 2012). This fine tuning of CDI appears to be important for circadian rhythms (Huang et al., 2012). From the specific perspective of disease, $Ca_V1.3$ channels contribute a substantial portion of Ca^{2+} entry into substantia nigral neurons (Bean, 2007; Chan et al., 2007; Puopolo et al., 2007; Guzman et al., 2009), which exhibit high-frequency pacemaking (Chan et al., 2007) that drives dopamine release important for movement control. Notably, loss of these neurons is intimately related to Parkinson's disease, and Ca^{2+} disturbances and overload are critical to this neurodegeneration (Bezprozvanny, 2009; Surmeier and Sulzer, 2013). Accordingly, an attractive therapeutic possibility for Parkinson's disease involves discovery of small molecules that selectively down-regulate the opening of $Ca_V1.3$ versus other closely related Ca^{2+} channels (Kang et al., 2012). Thus, understanding the mechanisms underlying the modulation of $Ca_V1.3$ CDI is crucial, particularly to furnish specific molecular interfaces as targets of rational screens for small-molecule modulators.

The fundamental mechanism of action of a natural modulator of $Ca_V1.3$ CDI may be especially relevant. In particular, recent discoveries identify Ca^{2+}-binding proteins (CaBPs), a family of CaM-like brain molecules (Haeseleer et al., 2000) that may also bind Ca_V channels and other targets (Yang et al., 2002; Kasri et al., 2004). Like CaM, CaBPs are bilobed, with each lobe containing two EF hand Ca^{2+} binding motifs (Haeseleer et al., 2000), and a recent crystal structure shows overall similarity to CaM (Findeisen and Minor, 2010). However, whereas all four EF hands bind Ca^{2+} in CaM, one lobe is nonfunctional in CaBPs. Coexpressing CaBPs with Ca_V1 channels eliminates their CDI, thereby potentially influencing diverse biological processes (Zhou et al., 2004; Yang et al., 2006). Some have argued that CaBPs may simply compete for apoCaM on channels (Lee et al., 2002; Findeisen et al., 2013; Oz et al., 2013). More recent data argue for a different mechanism (Fig. 5 C), in which CaBP and apoCaM can both preassociate with the channel (Yang et al., 2014). In particular, by binding to channel regions that overlap Ca^{2+}/CaM effector loci, CaBPs may preemptively retard the ability of Ca^{2+}/CaM to reach its effector configuration (Fig. 5 B), allowing low concentrations of CaBP molecules in the CNS to still exert functional effects in the presence of much higher CaM levels (Yang et al., 2014). If this scheme is correct, the basic mechanisms of CaBP action may have implications for drug discovery, in that small molecules that target channel interaction surfaces for CaBP and/or Ca^{2+}/CaM may exert potent modulatory actions.

In conclusion, this overview of the calmodulation of voltage-gated Ca^{2+} channels highlights an impressive

synergy among elegant molecular regulatory mechanisms, vital biological functions, and pathogenesis and potential therapy. As such, this field promises considerable mystery, enrichment, and enlightenment in the years ahead.

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