Calcium Channels Are Models of Self-Control

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When Ca^{2+} ions flow through the pore of an individual voltage-gated Ca^{2+} channel, they act back on the channel they've passed through and alter subsequent Ca^{2+} flow. Such local, almost instantaneous regulation involves both positive and negative feedback mechanisms: Ca^{2+}-dependent facilitation (CDF) and Ca^{2+}-dependent inactivation (CDI), respectively. Indeed, some types of Ca^{2+} channel are capable of undergoing both CDF and CDI, with each form of modulation following a different time course and having a different dependency on the rate, extent, and spatial localization of Ca^{2+} entry. These channels are equipped with a special Ca^{2+}-sensitive toolkit, which they use to exquisitely manipulate their own Ca^{2+} influx, and thereby adjust the many effector responses that lie downstream of the Ca^{2+} entry. Although such self-regulation has been recognized at the cellular level for decades (Brehm and Eckert, 1978; Marban and Tsien, 1982), recent work has begun to illuminate the underlying molecular mechanisms and their inherent complexity. Chaudhuri et al. (see p. 385 of this issue) have added several essential links to this chain of discovery.

CDF and CDI are present in most, but not all, members of the high voltage–activated Ca_v1 and Ca_v2 channels, but are entirely absent in the low voltage–activated Ca_v3 channels. Curiously, despite their diametrically opposite effects on channel gating and consequent Ca^{2+} influx, both CDF and CDI are mediated by calmodulin (CaM). Site-directed mutagenesis of recombinant Ca_v1 and Ca_v2 channels demonstrated that CaM interacts with a canonical "IQ" domain (a highly conserved region in the cytoplasmic C-terminal tail of all Ca_v1 and Ca_v2 pore-forming subunits). Removal of the IQ domain and mutations in the IQ domain that prevent CaM binding eliminate both CDF and CDI (Zühlke and Reuter, 1998; Qin et al., 1999; Zühlke et al., 1999). The high selectivity of CaM for Ca^{2+} over Ba^{2+} (Chao et al., 1984) explains the long-recognized inability of Ba^{2+} to evoke CDF and CDI. Although at first glance this might seem like a limited toolkit, CaM operates a bit like a fully loaded Swiss Army knife (Fig. 1).

How Can CaM Produce Two Opposing Actions on the Same Channel?

This dumbbell-shaped molecule coordinates two calcium ions (via a pair of EF hands) in both its N- and C-terminal lobes (Babu et al., 1988; see Fig. 2). In previous work, the Yue lab introduced selective mutations into CaM to obliterate Ca^{2+} binding by the N lobe (CaM_{12}), the C lobe (CaM_{34}), or both (CaM_{1234}), and these recombinant probes have identified key features of CaM-Ca_v channel signaling. Overexpressed CaM_{1234} acts in a dominant-negative fashion to eliminate both CDF and CDI in $Ca_v2.1$ channels, effectively competing with endogenous wild-type CaM (DeMaria et al., 2001). This result argues (a) that CaM can bind directly to the channel even in its Ca^{2+}-free form and (b) that Ca^{2+} binding to CaM is essential for both CDF and CDI. In fact, the Yue lab has further demonstrated that preventing N lobe Ca^{2+} binding (with CaM_{12}) selectively eliminates CDI in $Ca_v2.1$ channels, whereas preventing C lobe Ca^{2+} binding (with CaM_{34}) selectively eliminates CDF (DeMaria et al., 2001).

CDF and CDI operate in distinctly different time domains as well. During a train of action potentials, for example, CDF develops over the first few milliseconds of train initiation, while CDI requires many tens or hundreds of milliseconds (which is nicely illustrated in Fig. 1 A of Chaudhuri et al., 2007). To explain this kinetic difference, investigators have speculated that CDF mediated by the C lobe may be most effectively activated by rapid spikes of calcium (such as those that occur locally at the inner mouth of an open calcium channel), whereas CDI mediated by the N lobe may result from more slowly developing Ca^{2+} elevations (such as those produced globally through contributions from many channels opening in concert) (DeMaria et al., 2001). In other words, CaM relies on spatiotemporal aspects of the Ca^{2+} signal in choosing which knife blade (or set of blades) to use on the channel. Support for such speculation has been indirect, based primarily on two experiments that have analyzed macroscopic currents through $Ca_v2.1$. First, Ca^{2+} chelators, capable of buffering Ca^{2+} globally in the cytoplasm but not locally near the channel's mouth, eliminate CDI without altering the development of CDF in $Ca_v2.1$ (Liang et al., 2003). Second, CDI (but not CDF) depends upon the amplitude of the macroscopic current, which affects global more than local Ca^{2+} (Soong et al., 2002).

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Abbreviations used in this paper: CaM, calmodulin; CDF, Ca^{2+}-dependent facilitation; CDI, Ca^{2+}-dependent inactivation.

Direct Test of the Local–Global Hypothesis

In the current article, Chaudhuri et al. provide a more direct test of the local–global hypothesis for CaM signaling by studying CDF and CDI at the level of single $Ca_v2.1$ channels in cell-attached patches. Because Ca^{2+} ions flowing through a single open channel will change the Ca^{2+} concentration in the immediate vicinity of the channel pore without significantly affecting the global Ca^{2+} concentration in the cytoplasm at large, Chaudhuri et al. reasoned that a single active $Ca_v2.1$ channel should undergo CDF but not CDI. Channel activity was recorded using 100 mM Ca^{2+} (or 90 mM Ba^{2+}) as charge carrier and depolarizing test pulses of sufficient amplitude (20 mV) and duration (250 ms) for both CDF (rapid) and CDI (slow) to be observed, if present. Immediately following the onset of depolarization, Chaudhuri et al. found that the single channel open probabilities (p_o) were comparable in Ca^{2+} and Ba^{2+}. Over the ensuing 50 ms, however, p_o in Ca^{2+} rose monotonically to a new level and stayed there throughout the remainder of the pulse (indicating CDF had occurred), while Ba^{2+} currents remained constant at the initial p_o for the entire length of the depolarization (consistent with the observation that CDF, and CaM, are not activated by Ba^{2+}). In contrast, CDI is all but absent from the single channel records; at the end of the 250-ms-long test pulse, a period over which macroscopic $Ca_v2.1$ currents decline as much as 30% (Lee et al., 2000; DeMaria et al., 2001), the single channel p_o decreased no more than ~5% in Ca^{2+}. These data uphold the authors' prediction that CDF, but not CDI, would be manifest at the single channel level.

Additional experiments performed by Chaudhuri et al. (2007) further strengthen the linkage between CDF measured at the single channel and macroscopic levels. First, when a conditioning depolarization was delivered immediately before the test pulse, the channel opened directly to the higher probability facilitated state, mirroring previous results on macroscopic currents (Lee et al., 2000; DeMaria et al., 2001). Second, a splice variant of $Ca_v2.1$ that was shown previously to be incapable of undergoing CaM-induced CDF, measured macroscopically (Chaudhuri et al., 2004), also lacked CDF measured on single channels. The new publication from the Yue lab thus provides strong support for the "local calcium preference" of C lobe–induced CDF and the "global Ca^{2+} preference" of N lobe–induced CDI in $Ca_v2.1$ channels.

What Is the Mechanistic Basis for the Local/Global Preference?

CaM is a small molecule; in its most extended form, the N and C lobes are separated by no more than ~65 Å (Babu et al., 1988). By contrast, the rapid Ca^{2+} transients that activate local C lobe–mediated responses of CaM extend over at least tens of nanometers (Naraghi
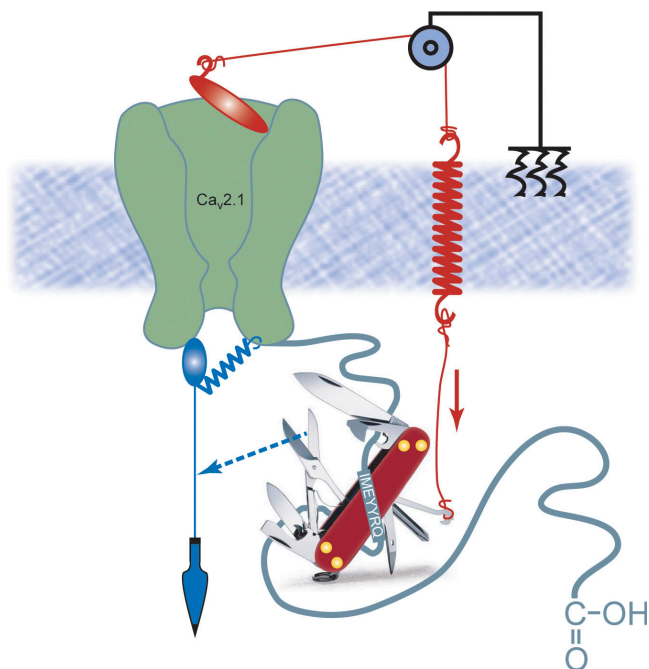

Figure 1. Calcium/calmodulin-mediated regulation of $Ca_v2.1$ calcium channel. Calmodulin (Swiss Army knife) is shown interacting with the IQ domain in the carboxy-terminal tail of a $Ca_v2.1$, voltage-gated calcium channel (light green). When calmodulin binds calcium ions (yellow circles), its two functional lobes differentially activate a rapid mechanism (red) that facilitates current flow and a slower mechanism (blue) that inhibits current flow by changing the probability of channel opening. With apologies to Reuben Garrett L. Goldberg.

and Neher, 1997), making it unlikely (based solely on a spatial argument) that the two ends of CaM are surrounded by different concentrations of Ca^{2+}. Thus, the apparent local/global preference of CaM's lobes must result from the differential kinetics with which the two lobes of CaM bind Ca^{2+} under physiological conditions (Pitt et al., 2001; Tadross, M., H. Liang, and D.T. Yue. 2005. *Biophys. Soc.* Abstr. 439). That is, the local C lobe Ca^{2+} sensor may respond better to the rapid, local Ca^{2+} signal because it binds Ca^{2+} more quickly and/or loses it more slowly than does the N lobe, perhaps involving differential allosteric effects of the channel on the two lobes of CaM.

Proving that differences in the kinetics of Ca^{2+} binding to the two lobes of CaM actually underlie their local/global preferences is a formidable challenge, as methods have yet to be developed that will allow quantification of Ca^{2+} binding kinetics in the native cellular environment. As a first step, however, Hamilton and colleagues have edged closer to the in vivo situation by using stopped-flow fluorescence methods to study the interaction dynamics between Ca^{2+}, CaM, and Ca_v channel IQ peptides in vitro (Black et al., 2005). They find that IQ peptides differentially alter the rates of Ca^{2+} binding to (and dissociation from) the N and
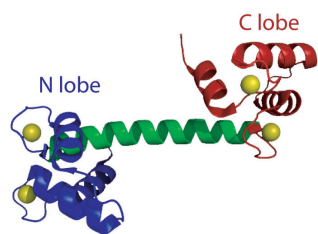
	N lobe	C lobe
Ca$_V$1.2	CDF*	CDI
Ca$_V$1.3	?	CDI§
Ca$_V$2.1	CDI	CDF
Ca$_V$2.2	CDI	- - -
Ca$_V$2.3	CDI	- - -

* CDF only present in Ca$_V$1.2 channels with I1624A mutation.
§ lobe specificity not yet tested for CDI in Cav1.3.

Figure 2. Lobe specificity of calmodulin. Summary of the effects of the N and C lobes of calmodulin on the voltage-gated calcium channel types noted in the leftmost column. CDI (calcium-dependent inactivation); CDF (calcium-dependent facilitation); - - - (no effect). Crystal structure of chicken calmodulin complexed with four calcium ions (yellow spheres) shown at top (from Rupp et al., 1996; Protein Data Bank entry 1UP5). Adapted from Liang et al., 2003.

C lobes of CaM. On average, the channel peptides slow Ca^{2+} dissociation from the C lobe more than from the N lobe, while speeding Ca^{2+} association to the N lobe more than to the C lobe. Furthermore, some Ca$_V$ IQ domain peptides are more effective than others at regulating Ca^{2+} binding to CaM. Although it is too early to quantitatively extrapolate the in vitro results of the Hamilton lab to the native channel (in which multiple regions outside of the IQ domain are also known to alter CaM's effects on the channel), it is clear that variation in the two-way conversation between CaM and Ca$_V$ channels offers abundant opportunities for fine tuning Ca^{2+} influx.

What Is the Biophysical Mechanism Underlying CDF?

In addition to providing direct support for the differential detection of Ca^{2+} transients by the two lobes of CaM, Chaudhuri et al. also used their single channel approach to discriminate between two separate mechanisms that could, in theory, give rise to the CDF detected macroscopically. They reason that facilitation might result from a Ca^{2+}-CaM–mediated transition to a unique gating state with a p$_o$ higher than that of the normal open state (the "enhanced opening model"). Alternatively, facilitation might encourage an otherwise sluggish channel to open more quickly, speeding the transition to the normal open state (the "accelerated activation model"). Chaudhuri et al. clearly summarize how these two models predict very different outcomes

of experiments to quantify the latency to first channel opening, conditional open probability, and voltage dependence of p$_o$. Experiments on both single channel and macroscopic currents explore each of these in turn and lead the reader to the unequivocal conclusion that a unique open state (with a p$_o$ significantly higher than that of the normal open state) is responsible for CDF in Ca$_V$2.1 channels.

Variable Actions of CaM on Ca$_V$1 and Ca$_V$2 Channels

Previous work has demonstrated that Ca$_V$1 and Ca$_V$2 channels are regulated unequally by CaM (Liang et al., 2003); in fact, given the high level of conservation of IQ domains in Ca$_V$ channels, there is an amazing variation in CaM action (Fig. 2). CDF appears to be the more variable of the two Ca^{2+}-dependent regulatory mechanisms. The C lobe of CaM promotes CDF in Ca$_V$2.1, for example, but not in Ca$_V$2.2 and Ca$_V$2.3, the two other members of the Ca$_V$2 family; in fact, the C lobe mutant CaM$_{34}$ appears functionally silent on Ca$_V$2.2 and Ca$_V$2.3 (Liang et al., 2003). Even more surprising, alternative splicing of Ca$_V$2.1 in regions outside the IQ domain is capable of abrogating CDF (Chaudhuri et al., 2004), leading to the conclusion that although the IQ domain is necessary for CDF, it is not sufficient. Studies on Ca$_V$1.2 channels have further underscored the range of variation; CDF is absent from wild-type Ca$_V$1.2 channels but emerges following mutations in single hydrophobic residues in the IQ domain (Zühlke et al., 1999; Van Petegem et al., 2005).

Unlike CDF, CDI is found in all Ca$_V$1 and Ca$_V$2 channels (Budde et al., 2002; Liang et al., 2003), but the lobe of CaM responsible for CDI varies. For example, the C lobe of CaM mediates CDI in Ca$_V$1.2, not CDF as it does in Ca$_V$2.1, described above (Peterson et al., 1999). Interestingly, whether the C lobe is mediating CDF or CDI, its effects are refractory to cytoplasmic Ca^{2+} buffers (Soong et al., 2002; Liang et al., 2003), indicating that the local Ca^{2+}-sensing properties of the C lobe are conserved across the Ca$_V$1/Ca$_V$2 family. Similarly, in all situations where the N lobe is active (e.g., across all Ca$_V$2 family members, where it mediates CDI; Fig. 2) its effects are reduced or eliminated by cytoplasmic Ca^{2+} chelators (Zühlke et al., 1999; Liang et al., 2003), underscoring the conserved global Ca^{2+}-sensing properties of the N lobe.

What these examples illustrate is that the mechanistic underpinnings of CDF and CDI are complex and channel specific, with seemingly subtle structural differences having dramatic biochemical and biophysical consequences. There is still much to learn from time resolved measurements of channel gating (such as employed in Chaudhuri et al., 2007) and Ca^{2+}-CaM-channel binding (Black et al., 2005) applied to engineered channels carrying unique subsets of the known domains important for CaM's action on Ca$_V$ channels. Such functional

studies will provide the necessary framework for building models that can then be validated by crystallographic snapshots of the interacting pairs.

The first structural pictures of Ca^{2+}-CaM in complex with the $Ca_v1.2$ IQ domain have recently been obtained (Fallon et al., 2005; Van Petegem et al., 2005). This work demonstrates that several hydrophobic residues in the IQ domain are involved in the Ca^{2+}-dependent interaction of CaM with $Ca_v1.2$. The Ca^{2+}-bound C lobe associates with the IQ domain more tightly than does the N lobe and provides the primary means of anchoring CaM to the channel (Van Petegem et al., 2005). The N lobe, by contrast, appears free to assume different conformations, two of which have been captured thus far. Although, as mentioned above, the N lobe appears to lack functional consequences on wild-type $Ca_v1.2$, the Minor lab demonstrated that mutating one aromatic C lobe anchor point (I1624) in the IQ domain of the full-length $Ca_v1.2$ channel allowed the normally silent N lobe to evoke CDF (Van Petegem et al., 2005), confirming earlier mutagenesis experiments highlighting the ability of this residue to normally suppress CDF in wild-type $Ca_v1.2$ channels (Zühlke et al., 1999).

These structural studies provide a robust predictive framework for evaluating differences in CaM's lobe specificity for Ca_v1 and Ca_v2 channels. The dominant interaction demonstrated between the C lobe and the IQ domain is consistent with the dominance of the C lobe in $Ca_v1.2$ channel function. Given the predominance of N lobe–mediated CDI in Ca_v2 channels, however, one wonders whether the N lobe might bind more tightly than does the C lobe in these channels. Consistent with this idea, Van Petegem et al. (2005) note that several of the aromatic anchors for C lobe binding in $Ca_v1.2$ are not well conserved in the IQ domains of Ca_v2 channels. It will be interesting to determine whether mutations in aromatic residues predicted to be necessary for N lobe binding to $Ca_v2.2$ and/or $Ca_v2.3$ (which normally lack CDF) might breathe new life into the otherwise ineffective CaM C lobe to evoke CDF in these channels. Other key questions remain as well: (a) can mutating aromatic anchoring residues in $Ca_v2.1$ further strengthen CDF; (b) does splice variation in $Ca_v1.2$, $Ca_v2.2$, and $Ca_v2.3$ contribute to heterogeneity in these channels' responses to CaM as it does for $Ca_v2.1$ and $Ca_v1.3$ (Soong et al., 2002; Chaudhuri et al., 2004; Shen et al., 2006); and (c) what are the additional endogenous mechanisms (see, for example, Yang et al. 2006) that naturally regulate CaM-Ca_v interactions in vivo?

Final Thoughts on the Potential Physiological Impact of Ca_v Channel Self-Regulation

Calcium's job description as a cellular regulator is formidable: to initiate synaptic vesicle fusion, transcriptional activation, muscle contraction, and membrane permeability changes. Each of these effector responses is optimally activated by a uniquely tailored Ca^{2+} signal. Furthermore, some types of Ca^{2+} signals are clearly out of bounds, as an overabundance of Ca^{2+} is toxic. Not surprisingly, therefore, mechanisms that regulate Ca^{2+} are assumed to be of great physiological consequence, although definitive tests of this assumption have only begun to emerge.

CDF and CDI offer two opposing, activity-dependent means of altering Ca^{2+} influx and the many effector responses it triggers. Several aspects of this dual regulatory system are intriguing. CDF is one of nature's rare positive feedback mechanisms. The enhanced opening mechanism proposed by Chaudhuri et al. predicts that both the amplitude and the duration of Ca^{2+} influx will increase during CDF. This prediction has been confirmed in experiments that directly measure Ca^{2+} influx during action potential trains. The suggestion is that CDF provides an effective means of bringing about use-dependent enhancement of cellular responses that are mediated by Ca^{2+}, particularly those effector responses (e.g., exocytosis at synapses mediated by $Ca_v2.1$ and $Ca_v2.2$) that vary as a nonlinear function of Ca^{2+} influx (Cuttle et al., 1998).

When left unchecked, however, CDF is a potentially dangerous form of regulation, given the toxic nature of sustained elevations in cytoplasmic Ca^{2+} concentration. Might this explain why CDF appears to be absent from most Ca_v channel types in vivo (Liang et al., 2003)? Given the sparse expression of CDF amongst the Ca_v channels, it is also interesting to speculate that the presence of CDF might signal a unique functional role for those rare variants capable of experiencing it. The hazards of runaway CDF may also provide a rationale for CDI as a coexisting countermeasure to terminate Ca^{2+} influx. Conveniently, CDI develops with some delay relative to CDF, which would allow short-term physiological potentiation of Ca^{2+} influx, while avoiding long-term pathophysiological consequences.

Unlike CDF, CDI is ubiquitous amongst the Ca_v1 and Ca_v2 channels (Liang et al., 2003) and, as such, clearly also has functions outside the context of CDF (Budde et al., 2002). In cardiac myocytes, for example, C lobe–mediated CDI of $Ca_v1.2$ channels appears to be an essential regulator of action potential duration (Alseikhan et al., 2002); in nerve terminals, N lobe–mediated CDI appears to underlie use-dependent short-term plasticity of synaptic transmitter release via $Ca_v2.1$ and $Ca_v2.2$ (Forsythe et al., 1998; Xu and Wu, 2005). Such studies, in combination with the impressive contributions of Chaudhuri et al. in this issue, move us inexorably closer to the era of CaM-Ca_v channel enlightenment in which we will understand not only how many blades CaM's knife contains but how each is shaped and, thereby, carves out a unique physiological niche.

REFERENCES

Alseikhan, B.A., C.D. DeMaria, H.M. Colecraft, and D.T. Yue. 2002. Engineered calmodulins reveal the unexpected eminence of

Ca^{2+} channel inactivation in controlling heart excitation. *Proc. Natl. Acad. Sci. USA.* 99:17185–17190.

Babu, Y.S., C.E. Bugg, and W.J. Cook. 1988. Structure of calmodulin refined at 2.2 Å resolution. *J. Mol. Biol.* 204:191–204.

Black, D.J., D.B. Halling, D.V. Mandich, S.E. Pedersen, R.A. Altschuld, and S.L. Hamilton. 2005. Calmodulin interactions with IQ peptides from voltage-dependent calcium channels. *Am. J. Physiol. Cell Physiol.* 288:C669–C676.

Brehm, P., and R. Eckert. 1978. Calcium entry leads to inactivation of calcium channels in *Paramecium. Science.* 202:1203–1206.

Budde, T., S. Meuth, and H.C. Pape. 2002. Calcium-dependent inactivation of neuronal calcium channels. *Nat. Rev. Neurosci.* 3:873–883.

Chao, S.H., Y. Suzuki, J.R. Zysk, and W.Y. Cheung. 1984. Activation of calmodulin by various metal cations as a function of ionic radius. *Mol. Pharmacol.* 26:75–82.

Chaudhuri, D., S.Y. Chang, C.D. DeMaria, R.S. Alvania, T.W. Soong, and D.T. Yue. 2004. Alternative splicing as a molecular switch for Ca^{2+}/calmodulin-dependent facilitation of P/Q-type Ca^{2+} channels. *J. Neurosci.* 24:6334–6342.

Chaudhuri, D., J.B. Issa, and D.T. Yue. 2007. Elementary mechanisms producing facilitation of Ca$_v$2.1 (P/Q-type) channels. *J. Gen. Physiol.* 129:385–401.

Cuttle, M.F., T. Tsujimoto, I.D. Forsythe, and T. Takahashi. 1998. Facilitation of the presynaptic calcium current at an auditory synapse in rat brainstem. *J. Physiol.* 512:723–729.

DeMaria, C.D., T.W. Soong, B.A. Alseikhan, R.S. Alvania, and D.T. Yue. 2001. Calmodulin bifurcates the local Ca^{2+} signal that modulates P/Q-type Ca^{2+} channels. *Nature.* 411:484–489.

Fallon, J.L., D.B. Halling, S.L. Hamilton, and F.A. Quiocho. 2005. Structure of calmodulin bound to the hydrophobic IQ domain of the cardiac Ca$_v$1.2 calcium channel. *Structure.* 13:1881–1886.

Forsythe, I.D., T. Tsujimoto, M. Barnes-Davies, M.F. Cuttle, and T. Takahashi. 1998. Inactivation of presynaptic calcium current contributes to synaptic depression at a fast central synapse. *Neuron.* 20:797–807.

Lee, A., T. Scheuer, and W.A. Catterall. 2000. Ca^{2+}/calmodulin-dependent facilitation and inactivation of P/Q-type Ca^{2+} channels. *J. Neurosci.* 20:6830–6838.

Liang, H., C.D. DeMaria, M.G. Erickson, M.X. Mori, B.A. Alseikhan, and D.T. Yue. 2003. Unified mechanisms of Ca^{2+} regulation across the Ca^{2+} channel family. *Neuron.* 39:951–960.

Marban, E., and R.W. Tsien. 1982. Enhancement of calcium current during digitalis inotropy in mammalian heart: positive feed-back regulation by intracellular calcium? *J. Physiol.* 329:589–614.

Naraghi, M., and E. Neher. 1997. Linearized buffered Ca^{2+} diffusion in microdomains and its implications for calculation of [Ca^{2+}] at the mouth of a calcium channel. *J. Neurosci.* 17:6961–6973.

Peterson, B.Z., C.D. DeMaria, J.P. Adelman, and D.T. Yue. 1999. Calmodulin is the Ca^{2+} sensor for Ca^{2+}-dependent inactivation of L-type calcium channels. *Neuron.* 22:549–558.

Pitt, G.S., R.D. Zühlke, A. Hudmon, H. Schulman, H. Reuter, and R.W. Tsien. 2001. Molecular basis of calmodulin tethering and Ca^{2+}-dependent inactivation of L-type Ca^{2+} channels. *J. Biol. Chem.* 276:30794–30802.

Qin, N., R. Olcese, M. Bransby, T. Lin, and L. Birnbaumer. 1999. Ca^{2+}-induced inhibition of the cardiac Ca^{2+} channel depends on calmodulin. *Proc. Natl. Acad. Sci. USA.* 96:2435–2438.

Rupp, B., D.R. Marshak, and S. Parkin. 1996. Crystallization and preliminary X-ray analysis of two new crystal forms of calmodulin. *Acta Crystallogr. D Biol. Crystallogr.* 52:411–413.

Shen, Y., D. Yu, H. Hiel, P. Liao, D.T. Yue, P.A. Fuchs, and T.W. Soong. 2006. Alternative splicing of the Cav1.3 channel IQ domain, a moleculear switch of Ca^{2+}-dependent inactivation within auditory hair cells. *J. Neurosci.* 26:10690–10699.

Soong, T.W., C.D. DeMaria, R.S. Alvania, L.S. Zweifel, M.C. Liang, S. Mittman, W.S. Agnew, and D.T. Yue. 2002. Systematic identification of splice variants in human P/Q-type channel $\alpha_1$2.1 subunits: implications for current density and Ca^{2+}-dependent inactivation. *J. Neurosci.* 22:10142–10152.

Van Petegem, F., F.C. Chatelain, and D.L. Minor. 2005. Insights into voltage-gated calcium channel regulation from the structure of the Ca$_v$1.2 IQ domain-Ca^{2+}/calmodulin complex. *Nat. Struct. Mol. Biol.* 12:1108–1115.

Xu, J., and L.G. Wu. 2005. The decrease in the presynaptic calcium current is a major cause of short-term depression at a calyx-type synapse. *Neuron.* 46:633–645.

Yang, P.S., B.A. Alseikhan, H. Hiel, L. Grant, M.X. Mori, W. Yang, P.A. Fuchs, and D.T. Yue. 2006. Switching of Ca^{2+}-dependent inactivation of Ca$_v$1.3 channels by calcium binding proteins of auditory hair cells. *J. Neurosci.* 26:10677–10689.

Zühlke, R.D., and H. Reuter. 1998. Ca^{2+}-sensitive inactivation of L-type Ca^{2+} channels depends on multiple cytoplasmic amino acid sequences of the α_{1C} subunit. *Proc. Natl. Acad. Sci. USA.* 95:3287–3294.

Zühlke, R.D., G.S. Pitt, K. Deisseroth, R.W. Tsien, and H. Reuter. 1999. Calmodulin supports both inactivation and facilitation of L-type calcium channels. *Nature.* 399:159–162.