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Mechanism of Local and Global Ca^{2+} Sensing by Calmodulin in Complex with a Ca^{2+} Channel

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SUMMARY

Calmodulin (CaM) in complex with Ca^{2+} channels constitutes a prototype for Ca^{2+} sensors that are intimately colocalized with Ca^{2+} sources. The C-lobe of CaM senses local, large Ca^{2+} oscillations due to Ca^{2+} influx from the host channel, and the N-lobe senses global, albeit diminutive Ca^{2+} changes arising from distant sources. Though biologically essential, the mechanism underlying global Ca^{2+} sensing has remained unknown. Here, we advance a theory of how global selectivity arises, and we experimentally validate this proposal with methodologies enabling millisecond control of Ca^{2+} oscillations seen by the CaM/channel complex. We find that global selectivity arises from rapid Ca^{2+} release from CaM combined with greater affinity of the channel for Ca^{2+}-free versus Ca^{2+}-bound CaM. The emergence of complex decoding properties from the juxtaposition of common elements, and the techniques developed herein, promise generalization to numerous molecules residing near Ca^{2+} sources.

INTRODUCTION

Ca^{2+} constitutes a ubiquitous signal with wide-ranging biological impact (Berridge et al., 2000). Despite the pervasive nature of Ca^{2+}, its detection can be highly selective in space and time, as required for specificity in signaling to appropriate targets (Bootman et al., 2001; Cullen, 2006; De Koninck and Schulman, 1998; Dolmetsch et al., 1998; Gu and Spitzer, 1995; Li et al., 1998; Oancea and Meyer, 1998; Winslow and Crabtree, 2005). Among the most critical of these detection modes are those relating to Ca^{2+} sensors positioned in close proximity, i.e., within nanometers, of Ca^{2+} sources. This placement of sensors within such a "nanodomain" promotes rapid and privileged Ca^{2+} signaling (Augustine et al., 2003; Bootman et al., 2001; Catterall, 1999). However, such proximity to a Ca^{2+} source challenges a sensor's ability to integrate Ca^{2+} signals from distant sources, which is essential for coordinated signaling at the whole-cell level.

A prototype for coupled sensors and sources is the Ca^{2+} sensor calmodulin (CaM), in its regulation of the Ca_V1-2 family of Ca^{2+} channels (Dunlap, 2007). CaM is continuously complexed with channels as a resident Ca^{2+} sensor (Erickson et al., 2001; Pitt et al., 2001), and Ca^{2+} binding to the C- and N-terminal lobes of CaM can each induce a separate form of regulation on the same channel (DeMaria et al., 2001; Yang et al., 2006). Given the approximate diameter of Ca^{2+} channels (Wang et al., 2002), the resident CaM would be ~10 nm from the channel pore, well within the channel nanodomain. Despite this proximity, each lobe responds selectively to distinct Ca^{2+} signals (cartooned in Figure 1A), which differ in both their spatial distribution (top row) and temporal characteristics (bottom row). Under physiological conditions, the composite Ca^{2+} signal (Figure 1A, left column) is the sum of two distinct components. First, Ca^{2+} inflow during channel openings produces a "local signal" component (Figure 1A, middle column) comprising brief yet intense local spikes of amplitude Ca_{spike} ~100 μM (bottom row). These spikes are tightly synchronized with openings of the host channel, and localized to the nanodomain (top row, green hemisphere) (Neher, 1998; Sherman et al., 1990) (see Supplemental Data [3], available online). Second, accumulation of Ca^{2+} from distant sources (e.g., other Ca^{2+} channels) generates a "global signal" component (Figure 1A, right column) consisting of a far smaller (~5 μM) global pedestal (bottom row), which is spatially widespread (top row, green shading). In the Ca_V1-2 family of Ca^{2+} channels, regulation triggered by the C-lobe of CaM exploits channel proximity and responds almost maximally to the local Ca^{2+} signal alone (Liang et al., 2003). This "local selectivity" is schematized for a Ca^{2+}-dependent inactivation process (CDI) triggered by the C-lobe (Figure 1B). Such CDI produces a strong decay of Ca^{2+} current during sustained voltage activation whether Ca^{2+} is buffered at physiological levels, or much more strongly (Figure 1B). Since high Ca^{2+} buffering eliminates the global pedestal while hardly affecting local spikes (Figure 1A, middle column; Supplemental Data [3]) (Neher, 1998), the sparing of CDI under this condition indicates that the local signal alone is sufficient. By contrast, N-lobe mediated regulation of all Ca_V2 channels somehow prefers the diminutive global pedestal over the far larger local spikes. The hallmark of this "global selectivity" is the presence of strong CDI in physiological buffering (Figure 1C, left), and its near absence in high buffering (Figure 1C, middle) (DeMaria et al., 2001; Liang et al., 2003).

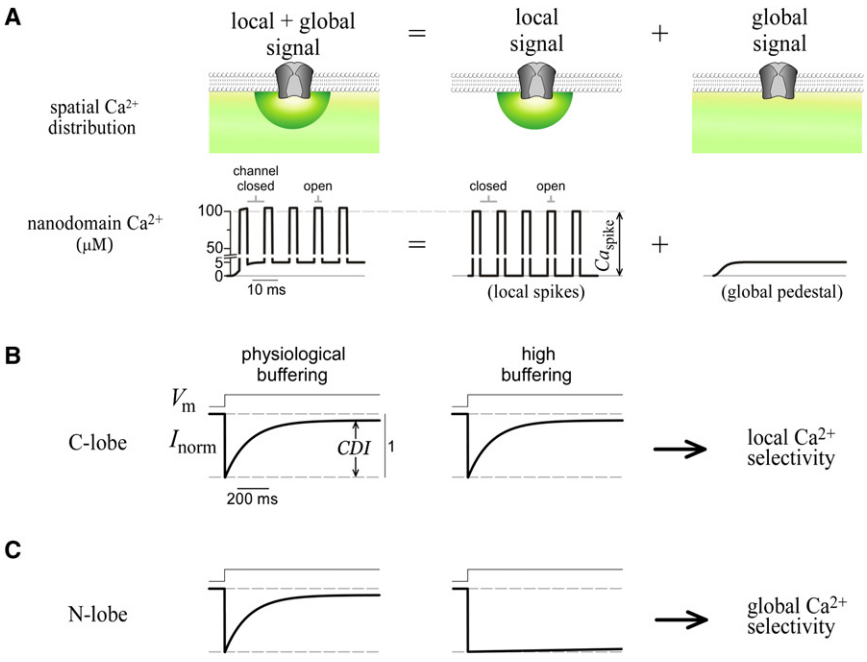

Figure 1. Definition of Spatial Selectivity

(A) Cartoon of Ca^{2+} signals near a channel. Spatial (top row) and temporal (bottom row) profiles are shown. Note axis break in nanodomain Ca^{2+}. Composite signal (left column) is the sum of local (middle column) and global (right column) components.

(B) Schematic of C-lobe CDI, which exemplifies local selectivity. Intracellular buffering conditions cause the Ca^{2+} input signals to correspond to those shown directly above in (A).

(C) Schematic of N-lobe CDI, which exemplifies global selectivity, buffering as in (B).

Without this detection mode, Ca^{2+} feedback would be restricted to isolated complexes, and lack coordination over larger regions. Global selectivity is thus critical to the signaling repertoire of Ca^{2+} sensors positioned near Ca^{2+} sources.

What are the mechanisms for the contrast in spatial Ca^{2+} selectivity of the lobes of CaM? The simplest explanation would presume that while the C-lobe resides within the nanodomain, the N-lobe lies outside this zone, where the local signal would be smaller than the global pedestal (Figure 1A, top row). However, each channel is constitutively complexed with a single CaM (Mori et al., 2004; Yang et al., 2007), and the lobes of CaM are very close to one another (<6 nm), indicating that both lobes are likely within the nanodomain (Dunlap, 2007; Stern, 1992). Hence, the N-lobe must be insensitive to Ca^{2+} intensity, and instead may respond to certain temporal features of nanodomain Ca^{2+} (Figure 1A, bottom row). Though some Ca^{2+}-dependent mechanisms that favor specific temporal patterns of Ca^{2+} have been characterized (De Koninck and Schulman, 1998; Oancea and Meyer, 1998), none can respond to signals of low amplitude and frequency (global pedestal), while ignoring signals of high amplitude and frequency (local spikes). Hence, global selectivity must employ a thus far unknown mechanism.

Here, theoretical and experimental advances explain how this unusual selectivity for global Ca^{2+} signals arises from the combination of two common elements: rapid Ca^{2+} release from CaM, together with greater channel affinity for Ca^{2+}-free (apoCaM) versus Ca^{2+}-bound CaM (Ca^{2+}/CaM). Since our proposed mechanism requires CaM/channel interactions as present within intact channels, we develop the means to probe Ca^{2+} dynamics within this integrated setting, using channels engineered for enhanced opening, with a "voltage block" electrophysiological technique to precisely control nanodomain Ca^{2+}. These tools resolve Ca^{2+} response characteristics clearly distinctive of the proposed mechanism. Combining this approach with manipulation of a recently identified CaM regulatory site (Dick et al., 2008) enables quantitative confirmation of a key prediction—selectivity can be incrementally changed from global to local by adjusting the ratio of channel affinity for apoCaM versus Ca^{2+}/CaM. Our findings generalize across Ca_V1–Ca_V2 channels, and likely extend to diverse Ca^{2+} sensors situated near Ca^{2+} sources.

RESULTS

Dominant Conformations of the CaM/Channel Complex Involved in Ca^{2+} Sensing

To explore how the CaM/channel complex could produce both local and global Ca^{2+} selectivity, we outlined a basic system comprised of the dominant conformations of this molecular assembly (Figure 2A). Several established features were considered. First, a 1:1 CaM/channel stoichiometry has been demonstrated (Mori et al., 2004; Yang et al., 2007). Second, two distinct types of CaM/channel interactions are known to exist: apoCaM binding, which anchors CaM to the channel as a resident sensor; and Ca^{2+}/CaM binding, which here produces CDI. Finally, an appropriate first-order approximation was to separately consider the operation of the C- and N-lobes of CaM, each with simultaneous (un)binding of two Ca^{2+} ions (Linse et al., 1991; Martin et al., 1985). This single-lobe approximation was reinforced in our experiments by the use of mutant CaM molecules that restrict Ca^{2+} binding to one lobe or the other (DeMaria et al., 2001; Peterson et al., 1999). Based upon these features, four main conformations result (Figure 2A, valid for either the C- or N-lobe). State 1 represents apoCaM (yellow circle) bound to the channel preassociation site (round pocket). Direct Ca^{2+} binding to CaM in state 1 is not considered, as such interaction is unlikely according to an analogous apoCaM/peptide structure (Houdusse et al., 2006). State 2 portrays apoCaM after it releases from the preassociation site, at which point it can bind Ca^{2+} to produce Ca^{2+}/CaM (square) in state 3. We reason that a transiently dissociated lobe of CaM (state 2 or 3) does not diffuse away (retained within a channel alcove), because of the slow