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Minireview

Ion channel regulation by calmodulin binding

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Abstract

While many ion channels are modulated by phosphorylation, there is growing evidence that they can also be regulated by Ca^{2+}-calmodulin, apparently through direct binding. In some cases, this binding activates channels; in others, it modulates channel activities. These phenomena have been documented in *Paramecium*, in *Drosophila*, in vertebrate photoreceptors and olfactory receptors, as well as in ryanodine receptor Ca^{2+}-release channels. Furthermore, studies on calmodulin mutants in *Paramecium* have shown a clear bipartite distribution of two groups of mutations in the calmodulin gene that lead to opposite behavioral and electrophysiological phenotypes. These results indicate that the N-lobe of calmodulin specifically interacts with one class of ion-channel proteins and the C-lobe with another.

Key words: Ion channel; Calmodulin; Ca^{2+}; Signal transduction; Channel regulation

1. Channels as 'permeases'

'Ion channels', a term with a strong electric connotation, is often taken to mean 'some special entities only of electrophysiological interest'. This misunderstanding is unfortunate, because it impedes understanding and hampers communication concerning an important class of effector molecules. In reality, ion channels are quite similar to many highly regulated enzymes. Channels are integral membrane proteins, some 10's to 100's kDa in molecular weight. Many of their corresponding genes have been cloned and sequenced. As expected of proteins, channels are made of α-helices, β-sheets, turns, binding sites, regulatory domains etc. Like enzymes each with a certain turnover number, open channels turn over some 10^5 to 10^7 ions per second from one to the other side of membrane. Thus, channels are ion 'permeases'. Ion fluxes are passive, driven by the electrochemical gradients for charged solutes (ions). This property distinguishes channels from pumps that use energy to transfer ions in the reverse direction to erect the gradients. Just like enzymes with their substrate specificities, some channels are very selective, allowing the passage of only one type of ions; others discriminate ions only by their + or − charges. There are also channels which pass uncharged molecules including water [1]. Many enzymes and channels are regulated between their active and inactive conformations. Key enzymes in signal-transduction pathways, such as protein kinases, phosphatases, cyclases, proteases, etc., are controlled directly through their bindings to second messengers and their mediators: cyclic nucleotides, IP_3, G-proteins, or Ca^{2+}-calmodulin. Similarly, there, too, are G-protein activated channels, nucleotide-activated channels, IP_3-activated channels, as well as Ca^{2+}-calmodulin regulated channels. The last type of channels is reviewed here. See [2] and [3] for general introductions to the topic of ion channels.

2. Ca^{2+}-calmodulin activates ion channels in paramecium

Ca^{2+}-calmodulin activated enzymes, such as certain types of protein kinases, calcineurins, cyclases, phosphodiesterases, Ca^{2+}-ATPases, etc., are well known [4]. This activation results from a direct protein–protein interaction between Ca^{2+}-calmodulin and the enzyme protein or a subunit of the enzyme complex. An exactly parallel mechanism of channel activation has been proposed (Fig. 1). Here, one envisions the channel protein to have a binding site(s) for Ca^{2+}-calmodulin. When this site is not occupied, the channel is in its inactivated (closed) conformation. Ca^{2+} that emerges in the cytoplasm through influx or internal release calcifies calmodulin in the vicinity of the channel and the Ca^{2+}-calmodulin complex binds to the channel, which then leads to a conformational change in the channel protein to the activated (openable) form.

Saimi and Ling [5] provided a direct demonstration of this type of activation with *Paramecium* channels. Using the patch-clamp technique [6], a *patch* of membrane (~1 μm^2) adhering to the mouth of a micropipet upon slight suction was excised from *Paramecium* and then subjected to voltage control (*clamped*). The solutions in the micropipet (the electrode) and in the perfusable bath, which the cytoplasmic side of the patch membrane was facing, and also the transmembrane voltage were all arranged, so that a current carried by Na^+ flowed from the

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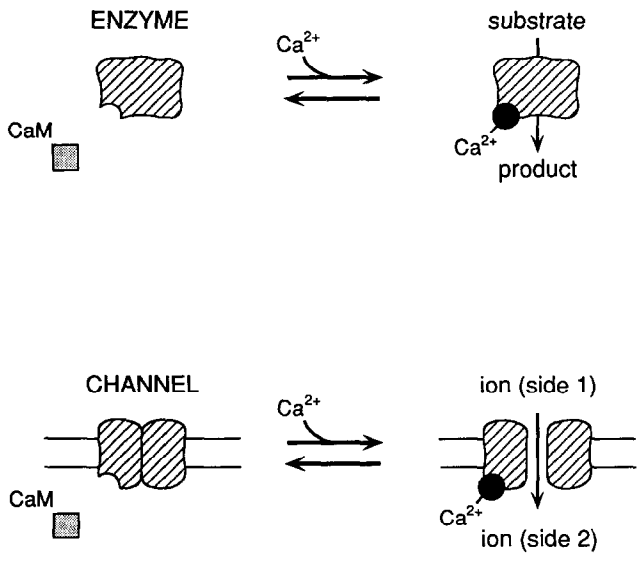

Fig. 1. Parallelism of Ca^{2+}-calmodulin regulation of enzymes (upper diagram) and ion channels ('ion permeases', lower diagram). While some are up-regulated as depicted here, others are down-regulated by Ca^{2+}-calmodulin binding (see text).

bath through the open channel into the pipet. The minute current, in the order of 10^{-12} A, through individual channel proteins was then amplified and registered. In their experiments, Saimi and Ling first captured the channels in their 'activated' state in the presence of high Ca^{2+} (10^{-5} M in the bath) as evidenced by the steps of current they passed. (Note that activation means making the channel openable. Whether the activated channel is in the open (O) or closed (C) conformation probabilistically depends on the thermal energy available. Channel activation and enzyme activation are directly comparable. We can study activities of individual channels as well as populations of channels, but we can only examine activities of populations of enzyme molecules because of lower turnover numbers.) This quantal nature of the current (Fig. 2, upper right) is consistent with the notion that we are observing the behavior of individual molecules. After a brief exposure of the patch to low Ca^{2+} (10^{-8} M) through perfusion of the bath, the channel was no longer active (B) and remained inactive even when the bath was returned to 10^{-5} M Ca^{2+} (C). This observation is consistent with the loss of a component other than Ca^{2+}. The channel could be reactivated by the addition of exogenous calmodulin to the bath (returning to A). These channels could be repeatedly inactivated and reactivated in this manner, consistent with reversible channel interaction with Ca^{2+}-calmodulin. Because the bath solutions were simple pH- and pCa-buffered salt solutions, containing no enzymes or energy source, it is not possible to invoke covalent modifications, such as phosphorylation, as the mechanism of channel activation. The simplest interpretation of these observation is a direct protein–protein interaction between calmodulin and the channel protein directly, paralleling the interactions between calmodulin and enzymes (Fig. 1). However, because the channel protein has not been purified and examined biochemically, the Ca^{2+}-calmodulin binding subunit may or may not be the pore-forming subunit, though all subunits must interact and are all delimited to the membrane. Calmodulin-activated enzymes can often become activated permanently after limited proteolytic digestion, leading to the view that the calmodulin activation is in fact a disinhib-

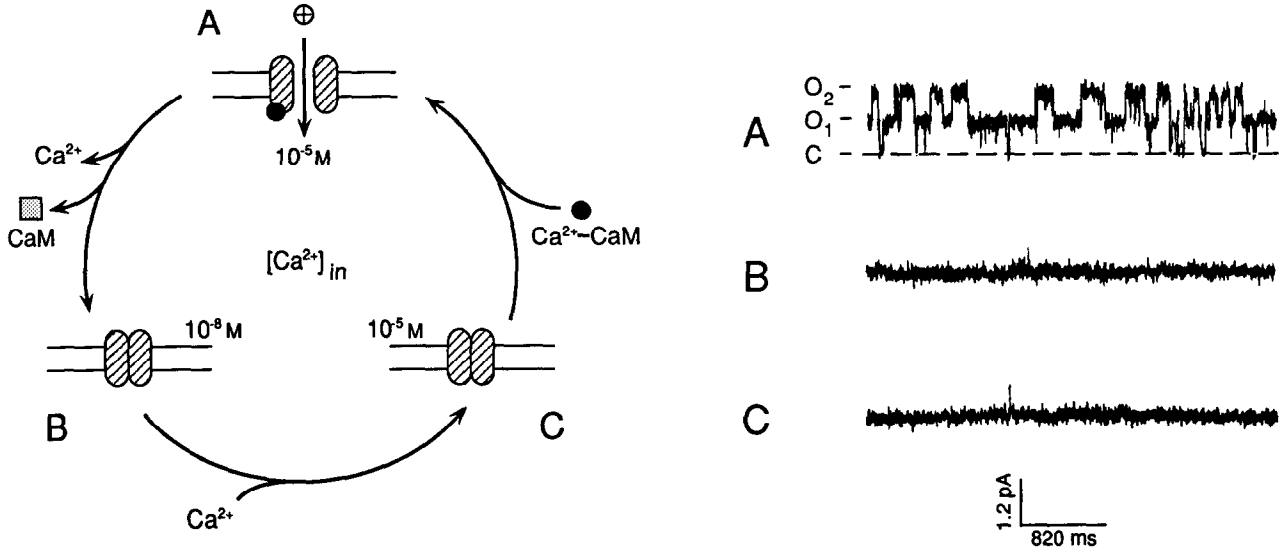

Fig. 2. Direct activation of *Paramecium* Na^+ channels by Ca^{2+}-calmodulin. (Left) A diagram of the proposed channel activation and inactivation cycle. (Right) Observed channel activities (Na^+ current through individual channel proteins in a patch of membrane, on which a +50 mV voltage drop was imposed). The channels had all components and were active (A in both left and right: O_1 and O_2 for open states, C for closed state). Release of Ca^{2+} and calmodulin inactivated the channels (B). Restoring Ca^{2+} alone did not reactivate them (C), but restoring Ca^{2+}-calmodulin did (A). Modified from [5].

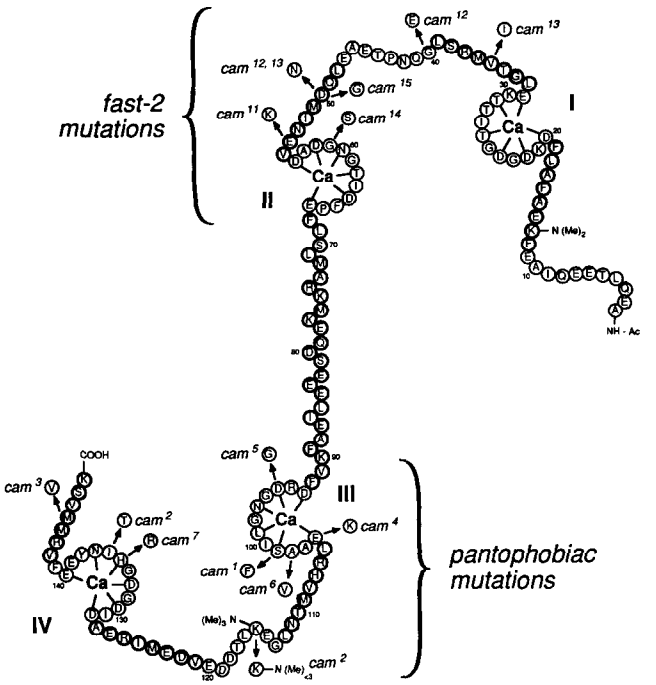

Fig. 3. Bipartite distribution of calmodulin mutations discovered in vivo. Ca^{2+}-calmodulin activated Na^+ channels and K^+ channels participate in membrane excitation in *Paramecium*. Mutants were selected on the basis of prolonged or abbreviated backward swimming, the excitation behavior. All 'pantophobiac' mutants (*cam*^{1} through *cam*^{7}) that show prolonged excitation are found to have mutations located in the C-terminal lobe of calmodulin. All 'fast-2' mutants (*cam*^{11} through *cam*^{15}) that show abbreviated excitation have mutations in the N-terminal lobe. No mutations have found in the central helix. These results indicate a functional bipartition, in terms of channel regulation, that reflects the structural bipartition of calmodulin [8,9].

ition [4]. Interestingly, we also found a Ca^{2+}-calmodulin activated K^+ channel in *Paramecium* that is permanently activated by such proteolytic digestions [7].

3. Mutants and functional bipartition of calmodulin in vivo

As in many neurons and muscles, a Ca^{2+}-based action potential can be triggered in *Paramecium*. Each action potential induces a reversal of the ciliary motion and causes a bout of backward swimming (excitation) in this unicellular animal. The Na^+ current is among those inward currents that sustain the action potential and lengthen the backward swimming. *Paramecium* is also equipped with several K^+ channels. Outward K^+ currents tend to terminate the action potential and shorten the backward swim time. Behavioral mutants have been isolated that show either prolonged or abbreviated behavioral responses to certain stimuli. Among these mutants are those mutated in the one and only structural gene for calmodulin. Curiously, all those calmodulin mutations that shorten the excitation, the 'fast-2' subtype, mapped to the N-terminal lobe of calmodulin, while those that lengthen the excitation, the 'pantophobiac' subtype, mapped to the C-terminal lobe of calmodulin. The former mutants have little or no Ca^{2+}-calmodulin activated Na^+ currents, the latter mutants have reduced Ca^{2+}-calmodulin activated K^+ currents. Of the 12 mutations scrutinized, none were found in the central helix [8,9] (Fig. 3). Although each mutant uniquely exhibits additional subtle pleiotropic phenotypes, this distinct phenotypic grouping of these mutations into the two lobes clearly indicated a functional bipartition of the calmodulin molecule. The simplest interpretation is that there are crucial sites of interaction in the N-lobe with the Na^+ channel and crucial sites in the C-lobe with the K^+ channel. Note that these are not site-directed mutations generated in vitro but random mutations selected through phenotypes in vivo. Recent studies showed that such a bipartition holds true when mutant calmodulins were examined for their ability to stimulate a mammalian enzyme [10].

4. Modulation of ryanodine receptor Ca^{2+}-release channels (RyR/CaCh)

RyR/CaCh's, which release Ca^{2+} from SR during the excitation-contraction coupling of muscles, have now been found in mammalian brains and in invertebrates [11]. The RyR/CaCh of skeletal muscle, the best studied, passes all cations and is strongly regulated by many factors, one of which is calmodulin. Although calmodulin dependent kinases can phosphorylate the channel protein, Ca^{2+}-calmodulin interacts directly with the channel in the absence of ATP and inhibits ^{45}Ca uptake into SR vesicles by >1,000-fold [12] and reduces reconstituted channel activity by 2-fold [13]. Based on the deduced amino-acid sequence of RyR/CaCh, 2–3 putative calmodulin binding sites have been proposed [14,15]. The role of this calmodulin inhibition in the physiological function of RyR/CaCh remains unclear, however.

5. Vertebrate olfactory receptor and photoreceptor

Recently Chen and Yau [16] showed that Ca^{2+}-calmodulin strongly modulates a channel situated at the end of the cascade of olfactory sensory transduction: odorant → receptor → G-protein → cyclase → cAMP/cGMP → channel. By using rat receptor channels cloned and expressed in a cell line, these authors showed that Ca^{2+}-calmodulin reduces the apparent affinity of the channel for cAMP by up to 20-fold. These findings help to explain the Ca^{2+}-mediated channel adaptation to the odorant stimuli. Because the channels were examined in excised patches bathed in a simple buffer, as in the case of the *Paramecium* work described above, the effects of

calmodulin addition and perfusion cannot be attributed to indirect effects through a covalent modification, but participation of a third protein or subunit in the patch cannot be ruled out.

In the vertebrate rod outer segment, the signal transducing cation channel is activated by cGMP in the dark. Hsu and Molday [17] showed that Ca^{2+}-calmodulin reduces the affinity between the channel and cGMP. This reduced affinity lowers the probability of channel opening, thus decreasing the cation influx at low cGMP concentrations. This also has an effect of increasing the sensitivity of the channel to small increments in the cGMP concentration during phototransduction, since the cGMP-channel activity relationship is sigmoidal. Thus, Ca^{2+} modulate the sensitivity of the cGMP-activated channel through calmodulin. While this channel and the olfactory channel described above are closely related, these authors suggested that Ca^{2+}-calmodulin binds to a 240-kDa protein that is tightly associated with the pore-forming channel subunit.

6. trp and trpl of Drosophila

Phillips et al. [18] used ^{125}I-labelled calmodulin to probe an expression library of *Drosophila* head cDNA. By selecting clones that express calmodulin-binding fragments, they retrieved a full length gene that corresponded to a 128-kDa protein. This gene, called *trpl* for *trp-l*ike, was found to be a homologue of a previously cloned gene called *trp* [19]. Sequence analysis showed that the proteins corresponding to *trp* and *trpl* have putative membrane-crossing segments similar to those of voltage-gated ion channels. The *trp*-product has one and the *trpl*-product has two putative calmodulin binding domains which, like those of other calmodulin targets, contain aromatic or long chain hydrophobic residues, basic amino acids and few, if any, acidic residues. Message hybridization in situ showed that these two genes are expressed almost exclusively in the retina of the compound eye. Whole-fly electroretinography as well as voltage-clamp experiments with individual photoreceptors using both wild-type and mutants strongly suggested that these gene products are key cation (Ca^{2+}?) channels in the generation of reception potential during phototransduction in the fly [20,21].

7. Conclusion

Direct interactions between Ca^{2+}-calmodulin and channel proteins described here parallel those of the well known Ca^{2+}-calmodulin activated enzymes. This mode of channel regulation is apparently employed by many eucaryotic organisms in different sensory transduction cascades.

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