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Review

Mechanisms for localising calcineurin and CaMKII in dendritic spines

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ABSTRACT

Calcineurin and calmodulin-dependent protein kinase II (CaMKII) are both highly abundant in neurons, and both are activated by calmodulin at similar Ca^{2+} concentrations in the test tube. However, they fulfill opposite functions in dendritic spines, with CaMKII activity driving long-term synaptic potentiation following large influxes of Ca^{2+} through NMDA-type glutamate receptors (NMDARs), and calcineurin responding to smaller influxes of Ca^{2+} through the same receptors to induce long-term depression. In this review, we explore the notion that precise dynamic localisation of the two enzymes at different sites within dendritic spines is fundamental to this behaviour. We describe the structural basis of calcineurin and CaMKII localisation by their interaction with proteins including AKAP79, densin-180, α-actinin, and NMDARs. We then consider how interactions with these proteins likely position calcineurin and CaMKII at different distances from Ca^{2+} microdomains emanating from the mouths of NMDARs in order to drive the divergent responses. We also highlight shortcomings in our current understanding of synaptic localisation of these two important signalling enzymes.

1. Introduction

Ca^{2+} entry into dendritic spines is essential for triggering changes in both spine morphology and the strength of synaptic connections. Ca^{2+} signalling mechanisms have been most intensively researched in spines that lie postsynaptic to hippocampal CA3-CA1 synapses. These synapses are the leading model for understanding the molecular basis of synaptic plasticity, and their properties are thought to be characteristic of excitatory synapses in general. Ca^{2+} entry through NMDA-type glutamate receptors (NMDARs) in the postsynaptic membrane is the trigger for long-term changes in the strength of CA3-CA1 synapses [1], with the degree of Ca^{2+} influx determining the direction of plasticity [2]. Both low-frequency (*e.g.*, 1 Hz) homo-synaptic stimulation, and hetero-synaptic 'post' before 'pre' spike timing [3], trigger relatively small Ca^{2+} influxes into dendritic spines that induce long-term depression (LTD) [1]. Larger Ca^{2+} influxes that induce long-term potentiation (LTP) may be triggered by high-frequency homosynaptic stimulations (evenly-spaced or tetanic), and 'pre' before 'post' spike timing [3]. The molecular basis of Ca^{2+}-driven synaptic plasticity has been intensively researched as the process is thought to underlie learning and memory. Two highly abundant [4,5] Ca^{2+}/Calmodulin (CaM)-sensitive enzymes have emerged as the key inducers of LTP and LTD of excitatory glutamatergic synapses following Ca^{2+} influx: the phosphatase calcineurin and CaM-dependent protein kinase II (CaMKII). The two enzymes control the phosphorylation state of synaptic proteins including AMPA-type glutamate receptors (AMPARs), thereby determining the conductance and numbers of these receptors in the postsynaptic density (PSD) [6]. This is an effective mechanism for altering synaptic strength, as AMPARs are the major mediator of fast excitatory synaptic transmission. Calcineurin dephosphorylation of AMPARs at residue Ser845, following smaller influxes of Ca^{2+}, is an important component of LTD induction [7,8]. Larger influxes of Ca^{2+} activate Ca^{2+}/CaM-dependent protein kinase II (CaMKII) resulting in phosphorylation of proteins, including AMPARs at residue Ser831 to induce LTP [9–11]. Activated CaMKII is also thought to play a key structural role in LTP that supports enduring modifications in spine morphology [12]. Despite its status as the foremost mechanism for driving bidirectional synaptic plasticity [13], Ca^{2+} activation of calcineurin and CaMKII presents a paradox: how do enzymes that are activated with similar half-maximal Ca^{2+} and CaM concentrations in the test tube [14,15] respond to different modes of Ca^{2+} influx through NMDARs in dendritic spines?

Elevations in second messengers including Ca^{2+} are localised within neurons [16]. Two-photon fluorescence lifetime imaging microscopy shows that CaMKII activation can be insulated within single spines [17]. Experiments with fast and slow Ca^{2+} chelators indicate that Ca^{2+} signalling may be further localised at the sub-spinal level [18,19]. Ca^{2+} 'microdomains' are known to occur in the vicinity of L-type Ca^{2+} channels, and enable spatial decoding of Ca^{2+} signals by these channels [20]. Highly localised Ca^{2+} signalling is emerging as an important feature of cellular signalling in further contexts, including coupling of Ca^{2+} release from acidic organelles and the endoplasmic reticulum [21]. It is likely that highly localised elevation of Ca^{2+} in the vicinity of

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NMDARs is also important in dendritic spines. For example, localised activation of calcineurin in restricted Ca^{2+} microdomains could explain why only the phosphatase is activated during LTD. Such a model is consistent with the patterns of electrical sitmulation that induce LTP or LTD [1]. Investigation of localised cAMP signalling has revealed that cAMP cyclases, receptors, and phosphodiesterases are precisely co-ordinated within cAMP microdomains [22]. Protein-protein interactions involving anchoring proteins are fundamental to establishing this sub-cellular structure. In an analogous way, the internal structure of dendritic spines – in particular mechanisms for positioning CaMKII and calcineurin – will direct responses to localised Ca^{2+} signals. On this basis, the aim of this review is to consider how CaMKII and calcineurin are positioned in dendritic spines, and how this relates to their roles in responding to Ca^{2+} signals. We begin by discussing high-resolution structural information for calcineurin (Part 2) and CaMKII (Part 3), with a focus on describing sites on each enzyme that are known to mediate protein-protein interactions. After summarising the overall architecture of dendritic spines (Part 4), we consider how interaction sites on CaMKII and calcineurin are likely to determine the localisation of the enzymes in idealised 'naïve' spines (Part 5), and how this positioning may underlie their functions. Both calcineurin and CaMKII relocate in spines upon larger influxes of Ca^{2+} that drive LTP. In Part 6, we discuss the implications of this structural reorganisation.

2. Calcineurin: structural overview & key interaction sites

Calcineurin is a heterodimer consisting of a catalytic subunit (A) of ~60 kD that presents a helix following the catalytic domain that interacts with a myristylated B subunit of ~19 kD [23] (Fig. 1a). Calcineurin is highly expressed throughout the brain, accounting for 1% of total brain protein in hippocampus [4], and it was first identified as a major neuronal CaM-binding protein ('calci*neur*in') prior to its subsequent characterisation as the Ca^{2+}/CaM-dependent phosphatase known as protein phosphatase 2B (PP2B) [24]. The B subunit resembles CaM with four EF hands that can each coordinate Ca^{2+}. At resting cellular Ca^{2+} concentrations, high affinity sites 3 and 4 are fully Ca^{2+} occupied while sites 1 and 2 act as Ca^{2+} sensors. Ca^{2+} binding to sites 1 and 2 serves as a gateway for Ca^{2+}/CaM binding, which releases an autoinhibitory element to bring about a further ~10-fold increase in phosphatase activity [15]. *In vitro* enzymatic assays suggest that calcineurin is activated with a half-maximal Ca^{2+} concentration in the range of a few hundred nM to a few μM, depending on factors including CaM and Mg^{2+} concentration [25]. The half-maximal CaM concentration for activation *in vitro* is ~15–30 nM [26,27], much lower than the concentration of CaM present in dendritic spines with CaM accounting for ~1.5% of total protein in hippocampus [28]. Two interaction sites have been identified on calcineurin that enable interaction with both substrates and anchoring proteins that present short linear motifs encoded by the amino acid sequence 'PxIxIT' and/or 'LxVP'.

2.1. PxIxIT-type interactions

The PxIxIT motif was first identified in the calcineurin substrate NFAT [29], which enters the nucleus following dephosphorylation to regulate transcription. NFAT dephosphorylation by calcineurin can be antagonised using a high affinity peptide with the consensus sequence PVIVIT with comparable efficacy to the calcineurin inhibitor cyclosporine [30]. PxIxIT motifs bind at a site equivalent to the RVxF site on protein phosphatase 1 (PP1) [31], with the PxIxIT sequence adopting a β-strand conformation (blue, Fig. 1b) that packs against and extends a

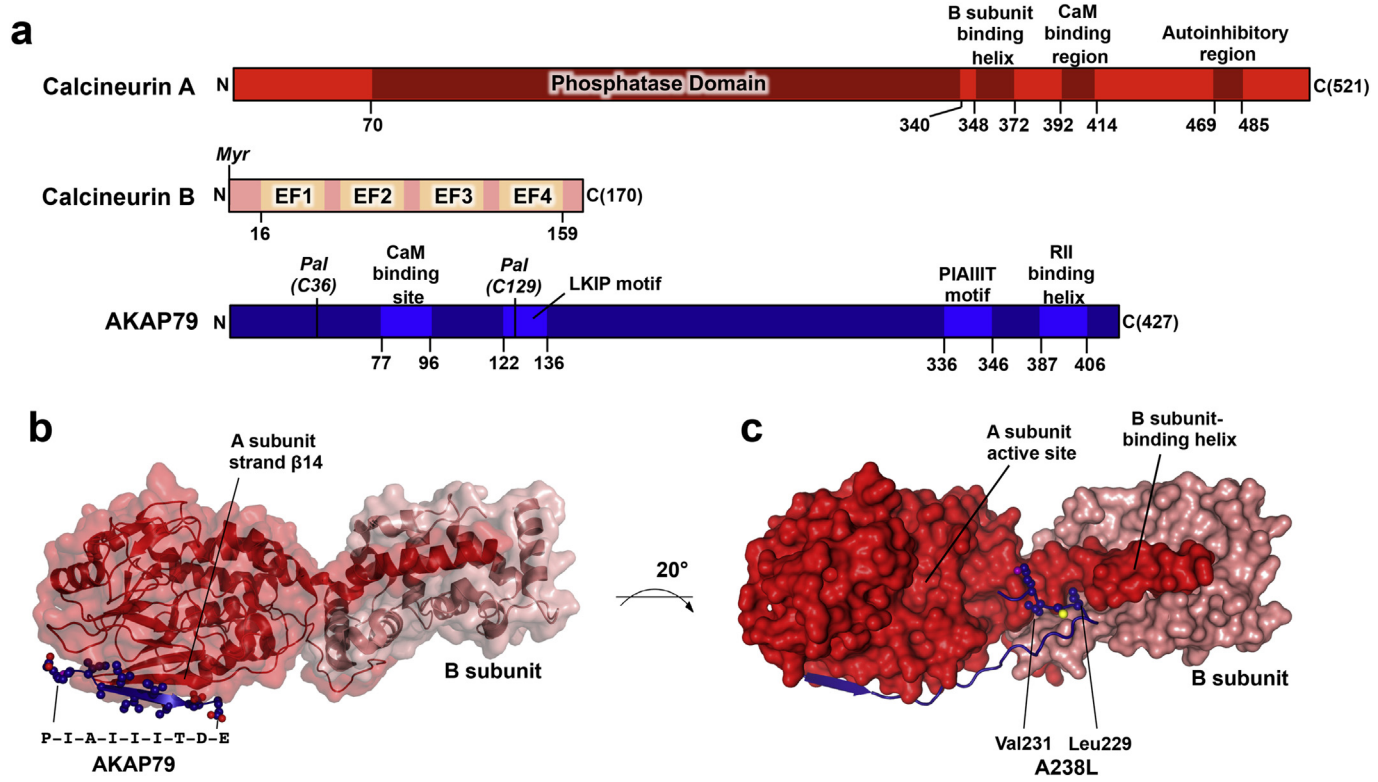

Fig. 1. Calcineurin interaction sites. (a) Topology diagrams for mouse calcineurin A and B subunits, and human AKAP79. Lipid attachment sites for myristyl ('Myr') and palmitoyl ('Pal') groups are indicated. (b) Cartoon representation of crystal structure (PDB ID 3LL8) of calcineurin (red) in complex with a peptide encompassing the constitutive PIAIIIT anchoring motif of AKAP79 (blue). (c) The location of the LxVP motif binding site on calcineurin is demonstrated by the structure of calcineurin in complex with the viral inhibitor A238L (blue), which contains the sequence 'LCVK' (PDB ID 4F0Z). A238L also contains a PxIxIT-type motif that binds in the conventional way to strand β-14. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)