REVIEW ARTICLE

Targeting of calcium/calmodulin-dependent protein kinase II

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Calcium/calmodulin-dependent protein kinase II (CaMKII) has diverse roles in virtually all cell types and it is regulated by a plethora of mechanisms. Local changes in Ca^{2+} concentration drive calmodulin binding and CaMKII activation. Activity is controlled further by autophosphorylation at multiple sites, which can generate an autonomously active form of the kinase (Thr^{286}) or can block Ca^{2+}/calmodulin binding ($Thr^{305/306}$). The regulated actions of protein phosphatases at these sites also modulate downstream signalling from CaMKII. In addition, CaMKII targeting to specific subcellular microdomains appears to be necessary to account for the known signalling specificity, and targeting is regulated by Ca^{2+}/calmodulin and autophosphorylation. The present review focuses on recent studies revealing the diversity of CaMKII interactions with proteins localized to neuronal dendrites. Interactions with various subunits of the NMDA (*N*-methyl-D-aspartate) subtype of glutamate receptor have attracted the most attention, but binding of CaMKII to cytoskeletal and several other regulatory proteins has also been reported. Recent reports describing the molecular basis of each interaction and their potential role in the normal regulation of synaptic transmission and in pathological situations are discussed. These studies have revealed fundamental regulatory mechanisms that are probably important for controlling CaMKII functions in many cell types.

Key words: α-actinin, calcium/calmodulin-dependent protein kinase II (CaMKII), densin-180, *N*-methyl-D-aspartate receptor (NMDA receptor), postsynaptic density, protein phosphatase.

INTRODUCTION

The ubiquitously expressed calcium/calmodulin-dependent protein kinase II (CaMKII) mediates diverse physiological responses to increases of intracellular Ca^{2+} concentrations by virtue of its activation by Ca^{2+}/calmodulin and autophosphorylation. CaMKII is most highly expressed in neurons, where it modulates most aspects of neuronal function, including gene expression, neurotransmitter synthesis and exocytosis, neurotransmitter receptor and ion-channel functions, cytoskeletal interactions and morphology, and various signalling pathways. Thus targeting of CaMKII to appropriate subcellular compartments is critical for directing efficient and specific responses to diverse signals that may elevate calcium in discrete neuronal subdomains. The regulation of postsynaptic responses at excitatory glutamatergic synapses in the hippocampus is perhaps the most widely investigated physiological role of CaMKII. The AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)-type glutamate receptor is a major locus of its actions: CaMKII drives synaptic insertion of new receptor subunits and phosphorylates receptor subunits directly to enhance channel conductance. These processes are widely believed to play critical roles in the long-term regulation of synaptic transmission (long-term potentiation and depression) at the cellular level, and in complex animal behaviour, such as learning and memory (reviewed in [1]). The synaptic specificity of many of these changes indicates that localization of CaMKII actions to specific synapses is critical to its function *in vivo*. The present review briefly summarizes necessary information about the structure and regulation of CaMKII, but focuses on the available, sometimes contradictory, data suggesting various protein–protein interactions that may contribute to the localization of CaMKII actions at excitatory postsynaptic elements in neurons. The potential impact of these interactions on CaMKII functions is also discussed. Several more comprehensive discussions of the regulatory properties and other cellular roles of CaMKII are also available [1–5].

STRUCTURE, FUNCTION AND REGULATION OF CaMKII

Gene/protein structure

CaMKIIs are encoded by four genes in mammals (α, β, γ and δ), each of which is transcribed and processed into a variety of alternatively spliced mRNA products. The translated protein products are generally 50–60 kDa in size, and contain a highly conserved N-terminal catalytic domain (approx. 280 amino acids) followed by a regulatory domain (approx. 40 amino acids), and a 150–220 amino acid C-terminal domain (Figure 1A). The regulatory domain contains the autoinhibitory domain, a calmodulin-binding domain and several regulatory autophosphorylation sites (Thr^{286} and $Thr^{305/306}$). The variable size of the C-terminal domain results from extensive alternative splicing of the mRNAs (Figure 1A). An additional CaMKIIα variant, termed αKAP, lacks catalytic and regulatory domains, containing instead a short, novel, hydrophobic N-terminal domain fused to the association domain [6]. All CaMKII isoforms studied to date are capable of

Abbreviations used: AKAP, A-kinase anchoring protein; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CaMKAP, calcium/calmodulin-dependent protein kinase II-anchoring protein; CaMKII, calcium/calmodulin-dependent protein kinase II; CaM-KIIN, CaMKII inhibitor; cdk5, cyclin-dependent protein kinase 5; GAP, GTPase-activating protein; GFP, green fluorescent protein; GST, glutathione S-transferase; MAP2, microtubule-associated protein 2; MAPK, mitogen-activated protein kinase; NMDA, *N*-methyl-D-aspartate; PDZ domain, protein domain sharing homology with PSD95/Dlg-A/ZO-1; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PP, protein phosphatase; PSD, postsynaptic density; SAP97, synapse-associated protein of 97 kDa; SR, sarcoplasmic reticulum.

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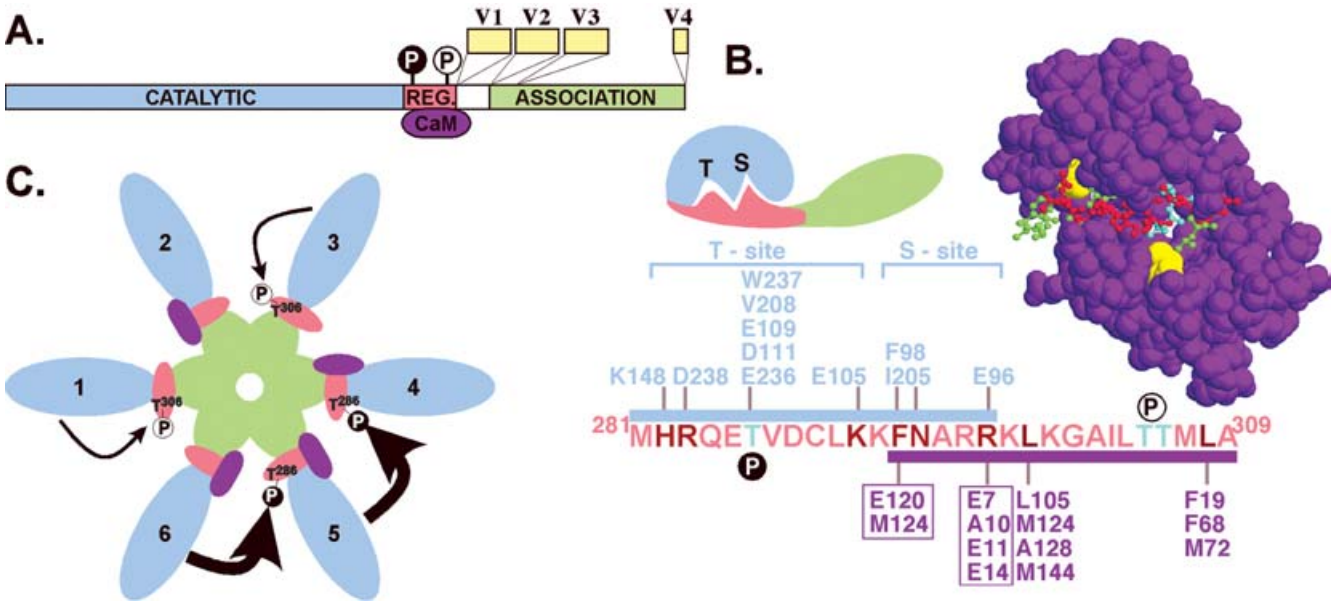

Figure 1 Structure and regulation of CaMKII

(**A**) CaMKII isoforms contain an N-terminal catalytic domain (blue), a central regulatory (REG) domain (pink) and a C-terminal association domain (green). The regulatory domain contains autoinhibitory and calmodulin (CaM; purple)-binding sequences, as well as Ca^{2+}/calmodulin-dependent (white P in black circle) and Ca^{2+}-independent (black P in white circle) autophosphorylation sites. Four genes encoding CaMKII isoforms share approx. 90 % amino acid sequence identity in the catalytic and regulatory domains, and approx. 75 % amino acid sequence identity in the association domain. In addition, each gene product is subject to alternative mRNA splicing, with the variable insertion of up to four cassettes (V1–V4), generating approx. 30 distinct known mRNAs encoding CaMKII variants. (**B**) The amino acid sequence of the regulatory domain (residues 281–309; pink) contains several residues (maroon or cyan) that are critical for interactions with residues in the catalytic domain (above in blue) and/or in Ca^{2+}/calmodulin (below in purple). The C-terminal end of the autoinhibitory domain occupies the catalytic site (S-site) of the kinase by acting as a pseudosubstrate, whereas residues surrounding Thr^{286} occupy a distinct hydrophobic pocket in the catalytic domain, termed the T-site (inset above). A space-filled structure of Ca^{2+}/calmodulin (purple) bound to a 'ball and stick' structure of an α-helical calmodulin-binding peptide from CaMKII (residues 293–310) is also shown (prepared with RasMac from the Protein Data Base structure 1CDM [13]). The peptide structure is oriented with the N-terminus on the left, with residues in green making critical interactions with Ca^{2+}/calmodulin. These include Leu^{299} and Leu^{308}, which make initial low-affinity interactions with several amino acids in calmodulin, including Met^{72} and Met^{124} (yellow in space-filled structure). Ca^{2+}/calmodulin-dependent autophosphorylation at Thr^{286} (white P in black circle) blocks interactions of the autoinhibitory domain with the T-site, generating an autonomously active form of CaMKII, and also enhances interactions of Phe^{293}, Asn^{294} and Arg^{287} with calmodulin, resulting in a substantial increase of the binding affinity. Ca^{2+}-independent autophosphorylation (black P in white circle) at Thr^{305} or Thr^{306} blocks Ca^{2+}/calmodulin binding to CaMKII. (**C**) CaMKII holoenzymes appear as a stacked pair of hexameric rings by electron microscopy, with association domains forming the hub and catalytic domains projecting out (see text); a single ring is shown in the diagram for clarity. Autophosphorylation at Thr^{286} requires simultaneous binding of Ca^{2+}/calmodulin to adjacent subunits in the holoenzyme, one of which serves as the catalytic unit and the other as the substrate; thus, in this example, Ca^{2+}/calmodulin-bound subunits 4 and 5 will be autophosphorylated (wide arrows), whereas subunits 2 and 6 will not. In contrast, autophosphorylation at $Thr^{305/306}$ is an intrasubunit reaction that occurs only in the absence of Ca^{2+}/calmodulin (e.g. in subunits 1 and 3; narrow arrow). Thr^{306} is the preferred Ca^{2+}/calmodulin-independent site in the absence of prior Thr^{286} autophosphorylation (as shown), but is only slowly phosphorylated; CaMKII is inactivated because binding of Ca^{2+}/calmodulin is blocked. In contrast, removal of Ca^{2+}/calmodulin from Thr^{286}-autophosphorylated kinase results in rapid autophosphorylation at Thr^{305} or Thr^{306} (not shown), which also blocks Ca^{2+}/calmodulin binding, but the kinase remains active due to the Thr^{286} autophosphorylation. Thus PPs can potently regulate CaMKII activity.

homo- and hetero-multimerization via their C-terminal domains to form 300–700 kDa holoenzymes, with the subunit composition being dictated in a stochastic manner by the relative isoform expression levels (reviewed in [2,3]).

Regulation: Ca^{2+} binding and autophosphorylation

The catalytic domain shares substantial amino acid sequence similarity with the catalytic domains of other kinases. This domain forms a bi-lobed structure with an N-terminal ATP-binding lobe formed primarily of β-sheets and a C-terminal substrate-binding lobe containing mostly α-helices. The catalytic site is formed in a cleft at the interface between the two lobes. The combination of experimental data obtained using synthetic peptides [7–9] and site-directed mutagenesis [10–12] with molecular modelling [11] suggests that the autoinhibitory subdomain (amino acids 282–302) makes multiple interactions with the catalytic domain (Figure 1B). Residues 297–300 of the autoinhibitory domain occupy the substrate-binding site (S-site) of the kinase, whereas more N-terminal portions (between residues 282 and 294) make additional interactions with a hydrophobic pocket on the C-terminal lobe of the catalytic domain (T-site). Interactions at the T-site are thought to induce secondary conformational changes that interfere with ATP binding to the kinase (reviewed in [2,3]). Thus the kinase is maintained in an inactive conformation in the absence of Ca^{2+}/calmodulin.

Binding of Ca^{2+}/calmodulin involves pivotal interactions between residues in the C- and N-terminal lobes of calmodulin, and Leu^{299} and Leu^{308} respectively in CaMKII [13], inducing an α-helical structure. Binding of Ca^{2+}/calmodulin is presumed to disrupt autoinhibitory interactions, because certain amino acids in the regulatory domain (e.g. Phe^{293} and Arg^{297}) are critical for both Ca^{2+}/calmodulin binding and autoinhibition [9,11–14] (Figure 1B). Thus substrates and ATP gain access to the catalytic site. Simultaneous Ca^{2+}/calmodulin binding to adjacent subunits in a single holoenzyme results in the efficient *trans*-autophosphorylation of Thr^{286} in the autoinhibitory domain (Figure 1C) [15–17]. This mechanism allows for ultrasensitivity of the Thr^{286}-autophosphorylation reaction to increasing concentrations of Ca^{2+} [18]. The Thr^{286}-autophosphorylated kinase displays a 1000-fold enhanced affinity for Ca^{2+}/calmodulin [19], perhaps because calmodulin interactions with Phe^{293}, Asn^{294} and Arg^{297} are promoted [14,20,21]. In addition, Thr^{286}-autophosphorylated kinase retains autonomous activity when calmodulin dissociates

[22–25], apparently because interaction of the autoinhibitory domain with the T-site is abrogated [11]. Interactions with other proteins are also modulated by Thr^{286}-autophosphorylation (see below). In an *in vitro* model system, Thr^{286}-autophosphorylation is sensitive to the duration, magnitude and frequency of imposed calcium transients [26], providing strong evidence that Thr^{286}-autophosphorylation of CaMKII serves as an integrator of repetitive calcium signals *in vivo*. However, these studies lacked counteracting phosphatases that may also be regulated directly or indirectly by calcium, and clearly play a role in the physiological regulation of CaMKII autophosphorylation (see below). Thus the relationship of CaMKII activity to calcium transient frequency, magnitude and duration is probably more complex in intact cells. However, studies using 'knock-in' mice, in which Thr^{286} of the endogenous CaMKIIα gene was mutated to alanine to abrogate this autophosphorylation, clearly revealed its importance in the regulation of synaptic plasticity and behaviour [27,28].

CaMKII also undergoes Ca^{2+}-independent/autonomous autophosphorylation that can occur via two mechanisms. First, slow intra-subunit autophosphorylation at Thr^{306} in the basal state blocks subsequent binding of Ca^{2+}/calmodulin [29,30] (Figure 1C). Thus CaMKII may be 'locked' in an inactive form until these residues are dephosphorylated [29]. Secondly, following initial autophosphorylation at Thr^{286}, removal of Ca^{2+}/calmodulin induces a rapid burst of autonomous autophosphorylation at Thr^{305} or Thr^{306}, as well as Ser^{314} [30–32]. Once again, autophosphorylation at Thr^{305} or Thr^{306} blocks Ca^{2+}/calmodulin binding, but CaMKII remains active toward exogenous substrates due to prior autophosphosphorylation at Thr^{286} [30–32]. Confirmation of the physiological phosphorylation of Thr^{305} or Thr^{306} was problematic for some time, perhaps because of strongly opposing actions of cellular phosphatases. However, recent studies using phospho-$Thr^{305/306}$-specific antibodies have indicated that CaMKII is indeed phosphorylated at these residues *in vivo* [33,34]. Knock-in mutations of the endogenous mouse CaMKIIα gene that change Thr^{305} and Thr^{306} to alanine or aspartic acid implicate a role for these phosphorylation sites in the regulation of synaptic plasticity and learning [33]. Moreover, phosphorylation of Thr^{305} or Thr^{306} is elevated in a mouse model of Angelman's mental retardation syndrome, and this is associated with a reduction of total CaMKII activity in hippocampal homogenates [34]. These studies also suggest that $Thr^{305/306}$-autophosphorylation may regulate the subcellular localization of CaMKII in addition to its activity (see below).

Regulation: the role of protein phosphatases (PPs)

The actions of cellular serine/threonine PPs can play a significant role in regulating CaMKII by opposing the effects of autophosphorylation, as well as phosphorylation of its substrates. PP1, PP2A and PP2C, but not PP2B, exhibit activity towards Thr^{286} *in vitro* [35–38]. Targeting of the kinase to specific subcellular compartments may also modulate its availability to different phosphatases. CaMKII associated with postsynaptic densities (PSDs) is primarily dephosphorylated by PP1 [35–37,39,40], whereas soluble CaMKII appears to be targeted selectively by PP2A [37]. Although differential dephosphorylation of distinct cellular pools of CaMKII has not been demonstrated *in situ*, it presents an attractive mechanism to permit selective modulation of CaMKII functions in those compartments.

It appears that PP1 acts as a 'gate' for CaMKII autophosphorylation at Thr^{286} in the hippocampus and that this gate modulates synaptic transmission (induction of long term potentiation) and behaviour. Recent experiments demonstrated that the presence of counteracting PP1 enhances the ultrasensitivity of Thr^{286}-autophosphorylation to stimulation by Ca^{2+} *in vitro* [18]. Although PP2A and/or PP2C may regulate CaMKII autophosphorylation under other conditions and/or in different cells, high frequency or theta-burst electrical-stimulation-induced CaMKII autophosphorylation in hippocampal slices was only observed when PP1 was inhibited, presumably by its endogenous regulator, inhibitor-1, following its phosphorylation by cAMP-dependent protein kinase (PKA) [41,42]. Moreover, transgenic overexpression of constitutively active inhibitor-1 in mice resulted in reduced PP1 activity, enhanced Thr^{286}-autophosphorylation and enhanced phosphorylation of a downstream substrate (the GluR1 subunit of the AMPA-type glutamate receptor), in addition to alterations in learning and memory [43]. Although specific phosphatases acting on the $Thr^{305/306}$-autophosphorylation sites have not been identified, elevated phosphorylation at these sites in the mouse model of Angelman's mental retardation syndrome was associated with decreased PP1 or PP2A phosphatase activity in hippocampal extracts [34]. In combination, these data confirm the importance of PP1 as a regulator of CaMKII implicated in the regulation of hippocampal synaptic transmission.

Holoenzyme structure

The holoenzyme structure is critical for efficient Thr^{286}-autophosphorylation between adjacent CaMKII subunits (see above), as well as for association with other cellular proteins (see below). Initial studies of the hydrodynamic properties of CaMKII holoenzymes containing mixtures of various isoforms purified from brain, muscle and liver tissues predicted holoenzymes ranging in size from 300 to 700 kDa (reviewed in [2,5]). Based on subunit sizes of 50–60 kDa and low-resolution electron micrographs, the holoenzymes were predicted to contain 6–12 subunits [44,45]. Deletion mutagenesis studies have suggested the importance of portions of the C-terminal domain for oligomerization *in vitro* and in intact cells [46,47].

More recently, computer-assisted image averaging techniques have been applied to analyse images captured by electron microscopy, resulting in higher-resolution structures [48,49]. CaMKIIα expressed in insect cells consists of a hub formed by a stacked pair of hexameric rings surrounded by 12 projections, a structure that is also observed in the purified rat forebrain enzyme and recombinant CaMKIIβ. A CaMKIIα C-terminal domain construct that lacks the catalytic and regulatory domains forms the double hexameric ring hub, but lacks projecting domains. Thus the holoenzyme appears to be a dodecameric hub formed from the C-terminal association domains surrounded by projecting regulatory/catalytic domains [48,49]. Although secondary-structural features in the holoenzymes could not be inferred, the two studies differed in how the catalytic domains were distributed around the holoenzyme core. In the first study, catalytic domains of adjacent subunits were positioned too far apart to allow for intersubunit autophosphorylation at Thr^{286} [48], whereas the authors of the second study positioned the catalytic domains in a single plane and in close proximity such that intersubunit Thr^{286}-autophosphorylation can occur [49].

The recent derivation of a high-resolution X-ray crystal structure of the CaMKIIα C-terminal association domain has called into question the accuracy of interpretations from the electron microscopy images [50]. In this case, the bacterially expressed protein formed a stacked pair of heptameric rings, suggesting a 14-subunit holoenzyme core. Careful measurement of the hydrodynamic properties of this protein supported further a tetradecameric structure. Modelling studies suggested that

the tetradecameric structure would allow the catalytic domains to be sufficiently close to permit efficient Thr^{286} autophosphorylation [50]. However, it is difficult to reconcile the tetradecameric X-ray crystal structure of the holoenzyme core [50] with the dodecameric full-length enzymes previously observed in images obtained by electron microscopy [48,49]. Additional studies are needed to demonstrate that the intact CaMKII holoenzyme can indeed form the tetradecameric structure seen for bacterially expressed C-terminal domains. This is especially important because bacterially expressed full-length CaMKIIα has been reported to have subtle defects in catalytic properties that may result from abnormal intersubunit contacts [51,52].

POTENTIAL IMPACT OF SUBCELLULAR LOCATION ON CaMKII FUNCTION

It is well established that appropriate targeting of signalling molecules plays an important role in establishing the fidelity and efficacy of cellular responses to extracellular stimuli. In considering the impact that targeting may have on CaMKII function, it is apparent that three interrelated factors may come into play. (i) The subcellular location of CaMKII must be matched to the localization of the calcium signals to which the kinase is intended to respond. For example, synaptic activation stimulates different magnitudes and dynamics of calcium changes in dendritic shafts and dendritic spines [53]. (ii) CaMKII may be exposed to different levels of phosphatase activity, or even to distinct phosphatases, in discrete subcellular locations. This may endow the subcellular pools of CaMKII with different activation characteristics because phosphatases antagonize autophosphorylation (see above). (iii) CaMKII must be co-localized with the correct substrate to provide an appropriate cellular response. As a simple example, restricted activation of CaMKII in dendritic spines (for example, see [54]) would not allow for the phosphorylation of substrates in the dendritic shaft {such as MAP2 (microtubule-associated protein 2); see [55]} in the absence of other factors. Although CaMKII also plays important regulatory roles in presynaptic terminals and probably in the soma, the remainder of this article will focus on the localization of CaMKII functions in dendrites and the protein–protein interactions that may contribute to this localization.

LOCALIZATION OF CaMKII TO PSDs

Electron-microscopy studies of neuronal morphology have revealed that mature excitatory synapses contain a specialized electron-dense cytoskeletal structure in the dendritic spine, termed the PSD. PSDs isolated by differential centrifugation are enriched in cytoskeletal proteins and neurotransmitter receptors as well as scaffolding and signalling proteins, which include protein kinases and phosphatases. A series of elegant studies identified a plethora of protein–protein interactions that form the core PSD structure (reviewed in [56–59]). Early studies reported that CaMKII constitutes 20–50 % of the total protein in isolated PSDs [60–62], perhaps exceeding the amounts needed for purely catalytic functions and suggesting that the enzyme may play some sort of structural role. However, examination of slices from brains fixed *in situ* using immuno-electron microscopy indicated that the amount of CaMKII in PSDs is highly variable [63,64]. Subsequently, it was reported there is a post-mortem accumulation of CaMKII in the PSD, and that CaMKII is only 2–10 % of the total protein in PSDs isolated from tissue that is rapidly dissected and homogenized [40,65]. In addition, new particulate forms of self-associated CaMKII that appear to be formed under pathological conditions have been identified in PSD preparations and in neurons. As detailed below, it is now apparent that the association of CaMKII with PSDs is dynamically regulated by multiple physiological processes, and that some of these processes appear to be mis-regulated under certain pathological conditions.

Pathological association of CaMKII with PSDs and formation of CaMKII clusters

The rapid depletion of cellular ATP levels after even brief ischaemic episodes induces membrane depolarization, cellular Ca^{2+} influx and neurotransmitter release. Among the many intracellular consequences of prolonged ischaemic episodes is a persistent down-regulation of CaMKII levels (reviewed in [66]). However, this appears to be preceded by the activation of soluble CaMKII, Thr^{286}-autophosphorylation and subsequent translocation to PSDs [67–70]. Recent work suggests that NMDA (*N*-methyl-D-aspartate) receptor blockade or CaMKII inhibition by KN62 attenuates ischaemia-induced autophosphorylation of CaMKII and partially attenuates the accumulation of CaMKII in PSD fractions [71]. Alternatively, or perhaps in addition, the translocated CaMKII may be associated with co-fractionating particulate structures, and a novel particulate form of CaMKII that may be involved has been identified. These 'CaMKII clusters' can be separated from PSDs and exhibit a quite distinct morphology, as seen using electron microscopy [72]. Their formation is stimulated *in vivo* following killing, and in cultured neurons and/or brain slices by excitotoxic stimulation, metabolic poisoning or sustained Ca^{2+} elevation. CaMKII clusters formed in cells appear to be composed almost entirely of CaMKII protein [72–74] and they may be related to self-associated CaMKII holoenzymes that can be formed *in vitro* following autophosphorylation at low pH [75,76]. Thus both bona fide association of CaMKII with PSDs and the apparently independent formation of CaMKII clusters can be driven by excitotoxic insults.

Physiological regulation of CaMKII association with PSDs

Since pathological responses often arise by mis-regulation of normal pathways, it was natural to wonder whether or not physiological stimuli also might regulate CaMKII association with PSDs. Initially, isolated PSDs were shown to possess the capacity to bind additional CaMKII in a saturable, Thr^{286}-autophosphorylation-dependent manner [40,77]. Additional studies revealed that incubation of hippocampal slices with the broad-spectrum potassium-channel blocker tetraethylammonium, or other pharmacological agents that enhance CaMKII activity/autophosphorylation, resulted in up to a 2.2-fold increase in the amount of both CaMKIIα and CaMKIIβ associated with a PSD-enriched cytoskeletal fraction [40]. Tetraethylammonium treatment was confirmed to induce a long-lasting enhancement of synaptic transmission similar to classical long-term potentiation. Subsequent electron-microscopy studies using immuno-gold techniques demonstrated that brief (1.5–3 min) depolarization of cultured neurons and/or brain slices induces a <2-fold thickening of the PSD and a 5-fold enhancement of CaMKII labelling associated with PSDs [78]. Moreover, glutamate induced similar changes in cultured neurons, and these changes were reversed within 5 min of agonist removal [78]. Thus CaMKII association with PSDs is dynamically regulated in an activation-dependent manner under physiological conditions.

More details about the dynamics of CaMKII localization *in situ* were revealed by fluorescent microscopy of cultured hippocampal neurons transiently transfected to express CaMKII

isoforms fused to the green fluorescent protein (GFP–CaMKII) [79]. GFP–CaMKIIα is uniformly cytosolic under basal conditions, but becomes punctate and co-localizes with PSD markers within 20 s of the addition of glutamate. In contrast, GFP–CaMKIIβ associates with F-actin under basal conditions and glutamate addition induces a slow dissociation from F-actin followed by accumulation in PSDs. These changes in localization required activation of NMDA receptors and calmodulin binding to GFP–CaMKII [79]. Subsequent work demonstrated that the localization of overexpressed GFP–CaMKIIα is also regulated by synaptic transmission or by localized application of glutamate 'puffs' [80]. Following removal of agonist, GFP–CaMKIIα dissociates from synapses over a time course of a few minutes; dissociation is accelerated by mutation of Thr^{286} to alanine and slowed by mutation of Thr^{286} to aspartic acid, which mimics the autophosphorylated enzyme, or by PP inhibition [79,80]. In combination, these data suggest that Thr^{286}-autophosphorylation stabilizes the association of CaMKII with PSDs and that PP1 activity promotes dissociation by dephosphorylating Thr^{286}.

Besides being regulated by Thr^{286}-autophosphorylation, autophosphorylation at Thr^{305} and/or Thr^{306} also regulates the interaction of CaMKII with PSDs. Mutation of Thr^{305} and Thr^{306} to alanine resulted in slower dissociation of GFP–CaMKIIα from PSDs in transfected neurons [80], suggesting that CaMKII dissociation from PSDs is promoted by phosphorylation at these sites. Consistent with this hypothesis, PSDs isolated from transgenic knock-in mice harbouring a mutation of Thr^{305} and Thr^{306} to alanine contain higher levels of CaMKIIα [33]. In addition, PSDs isolated from a mouse model of Angelman's mental retardation syndrome contain less total CaMKII, which is associated with enhanced levels of phospho-$Thr^{305/306}$ in these animals [34]. Thus it appears that a major function of $Thr^{305/306}$ phosphorylation is to promote the dissociation of CaMKII from PSDs. The mechanism for this effect is unclear. Since Ca^{2+}/calmodulin binding is blocked by autophosphorylation at $Thr^{305/306}$ (see above) and is necessary for CaMKII interactions with some PSD proteins (see below), dissociation of calmodulin may be necessary to promote CaMKII dissociation from PSDs. Alternatively, phosphorylation at $Thr^{305/306}$ may directly interfere with such interactions (see below). Whichever mechanism underlies these effects, it may provide an additional pathway by which information about the magnitude and duration of calcium transients can be sensed by CaMKII and translated into a meaningful biological response.

In addition to the complex and interrelated roles of glutamate receptors, calcium, calmodulin binding, and autophosphorylation at Thr^{286}, Thr^{305} and Thr^{306} in regulating the localization of CaMKII to PSDs, it is apparent that other signalling pathways also play a role in directing CaMKII localization. For example, synaptic localization of CaMKII endogenous to cultured neurons is induced by the addition of phorbol esters [81]. However, in this case, synaptic translocation is independent of CaMKII activation, but rather appears to be dependent on protein kinase C (PKC) activity. Thus it seems likely that activation of G_q-coupled metabotropic heptahelical receptors and/or other pathways that stimulate PKC may also modulate CaMKII association with PSDs.

MECHANISMS OF DENDRITIC CaMKII TARGETING

Subcellular targeting of protein kinases, PPs and other signalling molecules is often mediated by specific protein–protein interactions [82]. Perhaps the best-studied example is the targeting of PKA by AKAPs (A-kinase anchoring proteins) [83]. Based on this precedent, much attention has focused on identifying CaMKAPs (CaMKII anchoring proteins): hypothetical proteins that may play a key role in CaMKII-dependent signalling. Initial screening to identify CaMKII-binding activities using a gel overlay assay detected multiple proteins in tissue extracts and subcellular fractions, representing putative CaMKAPs [84]. The strongest signal was a PSD-enriched protein of 180–190 kDa, termed p190, which interacted with CaMKIIα or CaMKIIβ autophosphorylated at Thr^{286}, but not non-phosphorylated enzyme or enzyme that is only autophosphorylated at $Thr^{305/306}$. This observation suggested that association of CaMKII with PSDs is regulated by Thr^{286}-autophosphorylation [84]. Subsequent studies demonstrated that this is in fact the case both *in vitro* and in intact cells (see above). However, it now appears that at least two proteins contribute to the p190 CaMKII-binding activity (see below), and several additional dendritic proteins have been shown to interact with the kinase. Whether and how these CaMKII-binding proteins function as CaMKAPs in neuronal dendrites remains unclear.

This section reviews information about dendritic CaMKII-binding proteins and their potential contributions to CaMKII localization and/or function. Despite some apparent inconsistencies in the literature, it is clear that CaMKII is targeted dynamically to synapses by diverse mechanisms.

NMDA receptor subunits

Identification of interacting subunits

The NMDA subtype of glutamate receptor is a Ca^{2+}-permeable cation channel that is opened by coincident binding of glutamate to the extracellular domain and plasma membrane depolarization (reviewed in [85]). It is formed from a tetrameric (or pentameric) assembly of the essential NR1 subunit with NR2 subunit family members (A–D) and/or NR3 subunits, whose predicted transmembrane topology indicates that the variable C-terminus is the only significant cytosolic domain. Interactions of diverse proteins with these C-terminal domains (Figure 2) are thought to provide a structural framework for the PSD [56–59]. For example, NR2A and NR2B are highly enriched in PSDs and their C-termini interact with PDZ (after PSD95/Dlg-A/ZO-1) domains in proteins from the PSD95 family. It is well established that neuronal CaMKII is regulated by Ca^{2+} influx via NMDA receptors [86,87]. Association of CaMKII with the NMDA receptor complex would provide an attractive means for tight regulation of kinase activity.

The NR2A and NR2B subunits have a molecular mass similar to that of the abundant PSD-enriched p190 CaMKII-binding protein [84]. Moreover, CaMKII partially co-localizes with NMDA receptor subunits and/or other components of the NMDA receptor complex in cultured neurons [79,88], and CaMKII co-immunoprecipitates with NMDA receptor subunits from brain extracts [88–91]. Although roles for other components of NMDA receptor complexes cannot be excluded, these data could be explained by a direct interaction of CaMKII with NMDA receptor subunit(s), a possibility that has been investigated using a variety of approaches.

Strack and Colbran [88] expressed the entire cytosolic C-terminal domains of NR1, NR2A and NR2B in bacteria and examined CaMKII binding by gel overlay assay. Thr^{286}-autophosphorylated CaMKII bound to the NR2B cytosolic domain more than 10-fold better than that to NR2A or NR1, and kinase autophosphorylated only at $Thr^{305/306}$ did not bind significantly to any of the proteins. The primary-interaction domain in NR2B was narrowed to within amino acids 1260–1309 using gel overlay and glutathione–agarose co-sedimentation assays. Thr^{286}-autophosphorylation appears to be essential for interaction

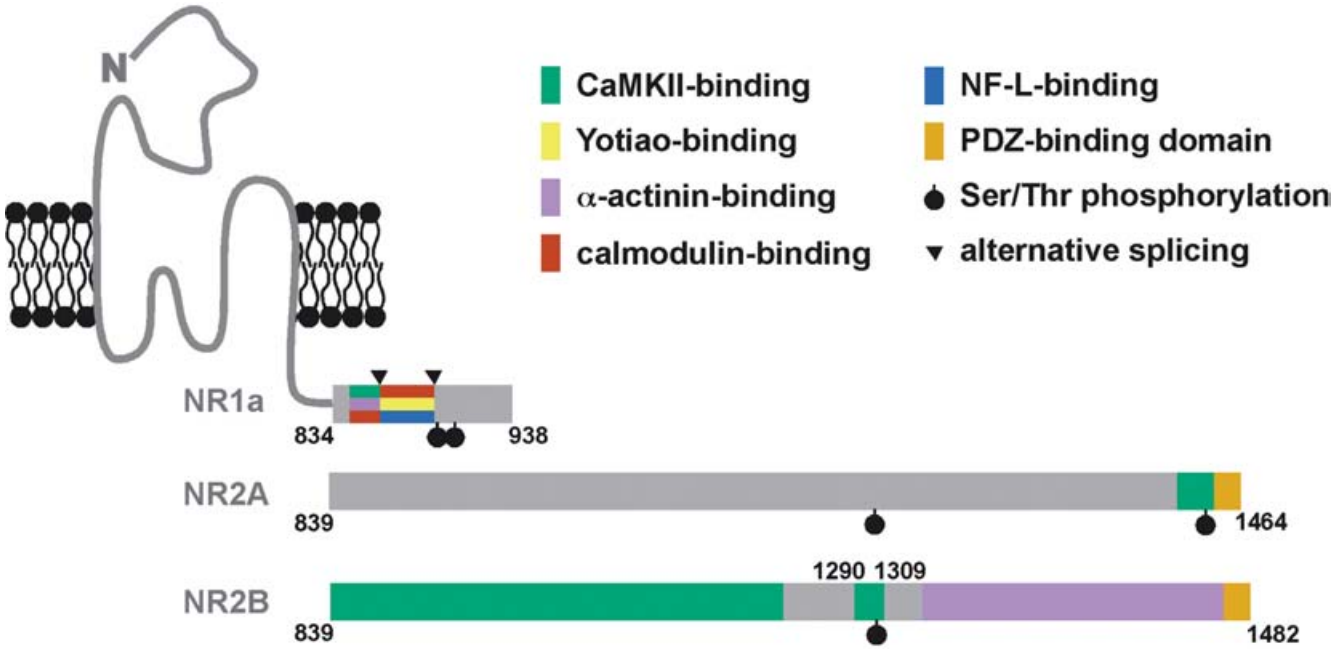

Figure 2 Multiple CaMKII interaction sites on NMDA receptor subunits

The NMDA receptor is thought to be a heterotetrameric assembly of NR1, NR2 and NR3 subunits that form a ligand- and voltage-gated Ca^{2+} channel. The diagram emphasizes the more divergent C-terminal cytoplasmic domains of the NR1A, NR2A and NR2B subunits, which have been shown to bind CaMKII by dissimilar sequences (green) and mechanisms. Additional protein–protein interaction sites and known Ser/Thr phosphorylation sites in the C-terminal domains are indicated as in the key. The PDZ-binding domain interacts with PSD95 family proteins, an interaction that is believed to be critical for normal assembly of PSDs. NF-L, neurofilament-L.

with this domain, since kinase activation by Ca^{2+}/calmodulin binding failed to support detectable binding. CaMKII forms a stoichiometric complex with NR2B(1260–1309) *in vitro* with an apparent binding affinity of 140 nM (subunit concentration), approx. 100-fold lower than the average concentration of CaMKII subunits in forebrain [88]. Subsequent work identified several amino acids within residues 1290–1309 of NR2B that are important for the interaction *in vitro* [92]. The functionally significant residues are not conserved in the corresponding NR2A domain, probably accounting for the observed binding selectivity. Thus these studies found that high-affinity and stoichiometric binding of CaMKII is specific to NR2B, is mediated primarily by residues 1290–1309 of NR2B, and is supported by Thr^{286} autophosphorylation, but not Ca^{2+}/calmodulin binding alone.

At about the same time, Gardoni et al. [89] identified an interaction of CaMKII with intact NR2A and/or NR2B subunits using co-immunoprecipitation and gel overlay assays. Later studies showed that CaMKII (solubilized from a PSD fraction using 0.1% SDS) bound to the extreme C-terminus of NR2A (residues 1349–1464) in a glutathione–agarose co-sedimentation assay, but longer NR2A proteins (e.g. residues 1244–1464) exhibited weaker binding of CaMKII [93]. PSD95 also was associated with the GST (glutathione S-transferase)–NR2A–CaMKII complex, as expected, since PDZ domains in PSD95 interact with the extreme C-terminus of NR2A [93]. The potential presence of NR2B in these isolated complexes (due to its own interaction with PSD95) was not investigated, although this might account for some of the CaMKII co-purifying with the complex. Binding of CaMKII to NR2A was potentiated by autophosphorylation at Thr^{286}, but not by the presence of Ca^{2+}/calmodulin [93]. Subsequent studies identified residues 1412–1419, close to the C-terminal PSD95-interaction site, as critical for CaMKII interaction with NR2A; this domain exhibits no obvious similarity to CaMKII-binding domains identified in other proteins (Figure 3). Binding of CaMKII to the NR2A C-terminus was reduced by PKC-mediated phosphorylation at Ser^{1416} [91], and appears to be competitive with PSD95 binding [94]. However, it is difficult to reconcile the competitive interactions of PSD95 and CaMKII with NR2A with the initial report that PSD95 co-purifies with a NR2A–CaMKII complex [93]. Moreover, interpretation of the apparent competition is complicated by the fact that both the NR2A ligand for CaMKII and the PSD95 competitor were presented as GST-fusion proteins in this experiment and complexes were isolated using glutathione–agarose [94]. Thus some of the apparent competition may be due to displacement of the entire complex from glutathione–agarose, rather than displacement of CaMKII from immobilized GST–NR2A. Nevertheless, it seems likely that CaMKII binds to residues 1412–1419 of NR2A. However, CaMKII has not been shown to form stoichiometric complexes with NR2A and the binding affinity has not been estimated. Thus, since other laboratories

CaMKII(281-302)	MHRQETVDCLKKFNARRKLK
NR2B(1290-1309)	AQKKNRNKLRRQHSYDTFVDL
NR2A(1413-1419)	SRDSRGH
NR1a(845-861)	RKQMQLAFAAVNVWRKN
Densin-180 (1353-1380)	PYEGNINKVTIQQFQSPLPIQIPSSQAT
synGAPβ(1146-1166)	SPSLQADAGGGGAAPGGPRHG
CaM-KIIN(43-68)	RPPKLCQICRAKRVVIEDDRIDDVLK

Figure 3 CaMKII-interacting sequences

Amino acid sequences of small (< 30 amino acid) domains that have been shown to interact with CaMKII are shown. Specific residues implicated as critical for CaMKII-binding are underlined where known. There is no apparent sequence identity among the CaMKII-binding domains other than the similarity between residues 1290–1309 of NR2B and the autoinhibitory domain of CaMKII itself. The available biochemical data suggest that there are multiple mechanisms for CaMKII binding to these interacting proteins (see text).

Table 1 Summary of isoform-selectivity and regulation of CaMKII interactions with binding proteins

The table lists CaMKII-binding proteins with the smallest identified CaMKII-binding domains. The CaMKII-isoform-selectivity and domains in the kinase responsible for interaction with each binding protein are listed to the extent that this has been established. Relative strengths of interactions with different forms of the kinase are indicated, and should be compared between different forms for each binding protein (i.e. horizontally), not for each form of the kinase between binding proteins (i.e. not vertically). NonP, non-autophosphorylated without bound Ca^{2+}/calmodulin; Ca^{2+}/CaM, non-autophosphorylated with bound Ca^{2+}/calmodulin; P-T^{286}, Thr^{286} autophosphorylated; P-T^{306}, Thr^{306} autophosphorylated; P-T^{286}/P-$T^{305/306}$, CaMKII phosphorylated at both Thr^{286} and $Thr^{305/306}$; N.D., not determined. Note that not all forms of the kinase have been tested with each binding protein and in only two cases were apparent affinities quantified. Variability in detection of the apparently weaker interactions may reflect differences in the assay protocols (see text).

Binding protein		CaMKII		Relative binding of CaMKII forms					
Identity	Domain	Isoform	Domain	NonP	Ca^{2+}/CaM	P-T^{286}	P-T^{306}	P-T^{286}/P-$T^{305/306}$	Reference(s)
NR1	845–861	α	N.D.	–	–	++++	N.D.	++	[90,95]
NR2A	1412–1419	α	Catalytic	++	++	++++	–	–	[91,93]
NR2B	839–1120	α	N.D.	–	*	++++	N.D.	++	[90,95,96]
	1290–1309	α	Catalytic (Ile^{205})	–	†	++++	–	++	[92,96]
F-Actin	N.D.	β (not α)	Variable	++++	–	N.D.	N.D.	N.D.	[109,118]
α-Actinin	806–871	α, β	Catalytic	++++	N.D.	N.D.	N.D.	N.D.	[110,111]
Densin-180	1353–1380	α (not β)	Association	‡	N.D.	++++	N.D.	N.D.	[110,122]
SynGAPβ	1146–1166	α	N.D.	++++	N.D.	–	N.D.	N.D.	[128]
Cdk5: p39	114–369	α	Catalytic + regulatory	+	++++	N.D.	N.D.	N.D.	[111]
p35	99–307	α	Catalytic + regulatory	+	++++	N.D.	N.D.	N.D.	[111]
CaM-KIIN	43–69	α, β	Catalytic	–	++++	++++	N.D.	N.D.	[133]

* Non-phosphorylated CaMKII was initially reported to bind NR2B(839–1120) [90], but subsequent studies found that Thr^{286} autophosphorylation was necessary and sufficient [95].

† Binding of CaMKII to NR2B(1290–1309) was reported to require Thr^{286} autophosphorylation [88,92], but variable interactions in the presence of only Ca^{2+}/CaM have also been demonstrated [95,96].

‡ The relative binding of non-phosphorylated and Thr^{286}-autophophorylated CaMKII to densin-180 varies between reports and assays [110,122].

failed to detect direct interactions of CaMKII with NR2A [90,95] or detected only weak interactions [88], it appears that CaMKII may bind only weakly to the NR2A subunit of NMDA receptors.

A third laboratory examined the interactions of CaMKII with individual full-length NMDA receptor subunits immuno-isolated from SDS-solubilized PSDs and then partially renatured by dilution of the detergent [90]. Stable, saturable interactions of autophosphorylated CaMKII with NR1 and NR2B, but not NR2A, were detected [90]. However, it is difficult to judge the relative strengths of these interactions because of probable differences in the amounts of immunoprecipitated protein available in the assay and probable variability in the efficiency of the renaturation step. Studies using recombinant fragments of the C-terminal tail of NR2B mapped two CaMKII-binding domains, one within residues 839–1120 and a second within residues 1120–1482 (Figure 2) [90], presumably corresponding to the domain defined by Strack and Colbran [88]. The relative importance of these two domains for CaMKII binding to the entire C-terminal domain of NR2B was not determined. CaMKII interaction with the membrane-proximal region (residues 839–1120) was initially reported to be independent of kinase activation [90], but a later study found that this interaction was dependent on Thr^{286}-autophosphorylation [96]. Specific amino acids that are critical for CaMKII binding to this membrane-proximal domain have not yet been identified. Bayer et al. [96] also found that CaMKII interaction with residues 1120–1482 of NR2B could be supported by Ca^{2+}/calmodulin-binding alone and that autophosphorylation at Thr^{286} potentiated the interaction, in contrast with other work suggesting that Thr^{286}-autophosphorylation was required for the interaction [88,92]. Possible explanations for these apparently contradictory results may lie in differences in the binding assays. For example, faster washing protocols used by Leonard and colleagues [90,96] may permit the detection of weaker Ca^{2+}/calmodulin-dependent interactions with residues 1120–1482, or weaker autophosphorylation-dependent interactions with residues 839–1120. In combination, these data suggest that CaMKII activation by Ca^{2+}/calmodulin binding may be sufficient for NR2B binding, but that autophosphorylation strongly potentiates the interaction.

CaMKII binding to the NR1 subunit also was shown to require autophosphorylation [95]. The interaction with NR1 appears to be mediated via residues within the so-called C0 region of the NR1 subunit [95], but the affinity and stoichiometry of CaMKII binding were not evaluated. Peptides corresponding to residues 845–861 of NR1 were shown to be capable of CaMKII-binding, but this domain shares no sequence identity with other CaMKII binding domains (Figure 3). Ca^{2+}/calmodulin and α-actinin also interact competitively and directly with NR1 subunits via the C0 region, and peptide mapping experiments suggest that these interaction domains overlap the CaMKII-binding domain (Figure 2) [97–99]. Thus it would seem that CaMKII and Ca^{2+}/calmodulin must either compete for interaction with NR1 or interact simultaneously with NR1; these possibilities have not yet been tested experimentally.

In summary, the current biochemical data indicate that CaMKII may make multiple interactions with NMDA receptor subunits that may only require binding of Ca^{2+}/calmodulin, but are at least strongly enhanced by Thr^{286}-autophosphorylation. CaMKII appears to interact most strongly with residues 1290–1309 of NR2B *in vitro*, and this presumably represents the primary interaction. However, CaMKII anchored to residues 1290–1309 may make secondary interactions with other NMDA receptor subunits/domains in the context of native heterotetrameric receptors. Careful characterization of the affinities, dynamics and regulation of these diverse interactions may provide more insight into their relative importance in cells.

Mechanism of CaMKII interaction with NMDA receptors

The precise mechanisms by which CaMKII binds to NR1, NR2A and NR2B are unclear. All of these interactions appear to require CaMKII activation, whether by Ca^{2+}/calmodulin binding alone or by additional phosphorylation at Thr^{286} (see Table 1). The interaction of CaMKII with residues 1290–1309 of NR2B has been examined the most intensively. Although this domain contains a high-affinity (K_m approx. 50 nM) substrate for CaMKII (Ser^{1303}) [100], a classical substrate-binding mechanism does not appear to be involved, because syntide-2 (a model peptide

substrate for CaMKII) failed to compete for the interaction [92]. However, residues 1290–1309 of NR2B have considerable sequence similarity to the autoinhibitory domain of CaMKII (Figure 3) and peptide inhibitors modelled after the autoinhibitory domain were found to be very effective competitors for binding [90,92]. These data suggested that the autoinhibitory domain itself or the catalytic domain contributes to CaMKII binding. Monomeric truncation mutants of CaMKII lacking a functional association domain were able to bind residues 1260–1309 from NR2B, albeit weaker than the holoenzyme [92]. Thus the holoenzyme structure may serve to increase the avidity of an interaction between the CaMKII catalytic/regulatory domain and residues 1290–1309 of NR2B, perhaps by effectively enhancing the local concentration of catalytic domains.

The similarity of residues 1290–1309 from NR2B with the autoinhibitory domain of CaMKII (Figure 3) suggested that they both interact with the catalytic domain by a similar mechanism. A model for NR2B interactions with the CaMKII catalytic domain was proposed [96], based on a model of the autoinhibitory domain interaction with the catalytic domain (see above; Figure 1B) and biochemical analyses of wild-type and mutated proteins. A key observation was that peptides containing residues 1290–1309 of NR2B were able to 'trap' an active Ca^{2+}-independent form of CaMKII following pre-incubation of the kinase with Ca^{2+}/calmodulin in the absence of ATP [96]. The authors of that study suggested that the NR2B peptide mimics the portion of the autoinhibitory domain surrounding Thr^{286}, but not the pseudosubstrate portion of the autoinhibitory domain. Ca^{2+}/calmodulin binding removes the autoinhibitory domain from the catalytic domain, allowing binding of the NR2B peptide to the T-site, which is occupied by Thr^{286} in the autoinhibitory domain in the inactive kinase (Figure 1B). Thus the NR2B peptide prevents the autoinhibitory domain from interacting with the T-site when Ca^{2+}/calmodulin is removed from the kinase. However, the NR2B peptide lacks inhibitory pseudosubstrate sequences present in the autoinhibitory domain, and the S-site remains available for substrate binding and catalysis in the absence of Ca^{2+}/calmodulin and autophosphorylation [96]. Consistent with an important role of the catalytic domain in the interaction, an $Ile^{205} \rightarrow$ Lys mutation within the catalytic domain of CaMKII blocked NR2B binding *in vitro* [96]. In contrast, this mutation has only modest effects on the interaction of the autoinhibitory domain with the catalytic domain, as reflected by the very modest elevation of autonomous activity [11]. Thus, although the T-site in the CaMKII catalytic domain can bind to NR2B in addition to the autoinhibitory domain, there may be some differences in the specific interactions involved.

Little is known about the domains of CaMKII that are important for interactions with NR1 and the membrane-proximal domain in NR2B. The interaction of wild-type CaMKII with NR2A is competed by a catalytic/regulatory domain fragment of CaMKII (residues 1–316) [93], suggesting the involvement of this domain in binding NR2A. However, the role of the T-site in CaMKII binding to NR2A, NR1 or the membrane-proximal domain of NR2B has not been investigated. It seems likely that CaMKII interacts with the other NMDA receptor subunits by different mechanisms because CaMKII-binding domains in these proteins share no significant amino acid sequence identity (Figure 3).

Role of NMDA receptor subunits in CaMKII localization in neurons

A variety of data are consistent with a CaMKII interaction with NMDA receptors in neurons. CaMKII translocates to PSDs under a variety of physiological and pathological conditions that promote Thr^{286}-autophosphorylation (see above), a reaction that stabilizes or is required for binding to NR1, NR2A and NR2B *in vitro*. In addition, the amount of CaMKII that co-immunoprecipitates with NMDA receptors from hippocampal slice homogenates is enhanced under conditions that activate CaMKII and increase Thr^{286}-autophosphorylation, including high-frequency tetanic stimulations that induce long-term potentiation [90,94]. Similarly, co-immunoprecipitation of CaMKII with NR2B is enhanced following ischaemia, and this enhancement is attenuated by NMDA receptor blockade or by a CaMKII inhibitor [70,71]. However, the identity of NMDA receptor subunits and domains that interact with CaMKII and how these interactions affect CaMKIIα localization and function in neurons is unclear.

One way to determine the impact of specific NMDA receptor subunits on CaMKII localization is to assess changes in localization following manipulation of their expression levels in a controlled manner, such as by overexpression or knock-down/out strategies [gene knockout, small interfering RNA (siRNA) or antisense]. However, interpretation of any effects might be confounded by the presence of additional CaMKII-binding proteins (see below) and by the complex effects of genetic disruption of NMDA receptor subunits (e.g. [101–103]). One simpler alternative approach is to co-express either wild-type or mutated CaMKIIα with functional NMDA receptors in heterologous cells. In cells expressing NR1, NR2B and CaMKIIα, the kinase is not significantly co-localized with the receptor under basal conditions (i.e. in the presence of a NMDA receptor antagonist). Addition of NMDA for 15 min results in a significant increase in the proportion of cells containing clusters of CaMKIIα immunofluorescence that co-localize with NR2B in a manner that depends on Ca^{2+} influx, CaMKII activity and Thr^{286}-autophosphorylation [88,92,96]. Moreover, co-localization of CaMKII with NR2B is sensitive to mutations within residues 1290–1309 of NR2B that interfere with CaMKII binding to NMDA fragments *in vitro* [92]. NMDA-induced redistribution of CaMKIIα is not observed when NR2A is substituted for NR2B in the transfections [88], suggesting that NR1 and NR2A alone are insufficient to interact with CaMKIIα under these conditions. In addition, residues 1260–1316 from NR2B were shown to be sufficient to modulate CaMKIIα localization when fused to a heterologous protein; treatment with Ca^{2+} ionophore transiently enhanced CaMKII co-localization with the fusion protein [92]. In combination, these data indicate that CaMKII co-localization with NMDA receptors in HEK-293 cells is mediated primarily by Thr^{286}-autophosphorylation-enhanced interaction with residues 1290–1309 in NR2B, the domain that appears to exhibit strongest binding to CaMKII *in vitro*. However, it is possible that these studies missed a transient co-localization with NR2B that required the binding of Ca^{2+}/calmodulin to CaMKII, but was not stable in the absence of Thr^{286}-autophosphorylation. In addition, the impact of interactions with other sites in NMDA receptor subunits, and of NMDA receptor association with other PSD proteins, on CaMKII localization was not assessed.

The evidence available suggests that NR2B plays a dominant role in the association of CaMKII with PSDs. First, GST–NR2B(1260–1309), but not a GST-fusion protein containing the analogous domain from NR2A, competes for approx. 70% of exogenous CaMKII binding to isolated PSDs *in vitro* [92]. These data suggest that NR2B, or other proteins that interact with CaMKII via a similar mechanism, make a major contribution to this binding activity. Since NR2B(1260–1309) shares no identity with other identified CaMKII-binding domains, these data are consistent with a dominant role for NR2B in the interaction. Secondly, the localization dynamics of overexpressed GFP–CaMKIIα in cultured neurons [79,80] are consistent with the dynamic nature of CaMKII–NR2B (and CaMKII–NR1)

interactions *in vitro* [95]. Moreover, the translocation of soluble GFP–CaMKIIα to synaptic sites is abrogated by a single point mutation of CaMKIIα that interferes with binding to NR2B(1290–1309) [96]. Although these observations are consistent with a major role for NR2B residues 1290–1309 in the interaction of CaMKII with NMDA receptors in cells, autophosphorylation-enhanced interactions of CaMKII with additional NMDA receptor domains or subunits, and also other synaptic proteins (see below), prevents definitive conclusions from being drawn. Furthermore, it is clear that localization of CaMKII is not always determined by NMDA receptor subunits because phorbol ester treatment of cultured hippocampal neurons results in reciprocal translocation of CaMKII to synapses and NMDA receptors away from synapses [81].

Besides directing CaMKII localization, interaction with NMDA receptors may have additional impact on kinase function(s). For example, as mentioned above, peptides corresponding to residues 1289–1310 of NR2B trap an autonomous form of CaMKII *in vitro* [96], suggesting that downstream CaMKII signalling events may be enhanced in intact cells. However, like the similar autoinhibitory domain peptides, NR2B peptides also can function as potent inhibitors of CaMKII activity under other conditions (S. Strack, A. J. Robison, M. A. Bass and R. J. Colbran, unpublished work). Significantly, these divergent effects of NR2B on kinase activity have only been demonstrated *in vitro* using relatively short synthetic peptides or recombinant proteins. It is not clear how many subunits in a CaMKII holoenzyme may be able to interact simultaneously with NR2B in the context of the intact heterotetrameric NMDA receptor associated with other PSD proteins in its native membrane environment. Thus, if only a small proportion of the subunits in a CaMKII holoenzyme can interact simultaneously with NMDA receptors due to such steric constraints, the impact of NR2B binding on downstream signalling may not be as great as effects observed *in vitro*. In addition, it is unclear whether or not interactions with NR1 or NR2A have the potential to modulate CaMKII activity further. Therefore it will be important to assess experimentally the impact of NMDA receptor binding on signalling downstream of CaMKII in intact cells.

Effects of CaMKII on NMDA receptor function

Interaction of CaMKII with NMDA receptors may also modulate NMDA receptor properties. For example, the balance of competition between CaMKII and PSD95 or SAP97 (synapse-associated protein of 97 kDa) for binding to the NR2A C-terminal domain appears to be modulated by NMDA receptor activation, as well as the activities of CaMKII and PKC, and by the induction of long-term potentiation [91,94,104]. Displacement of NR2A from PSD95 or SAP97 might be expected to enhance trafficking of these NMDA receptor subunits.

The CaMKII-binding domain in the NR1 subunit overlaps with domains that also bind α-actinin and calmodulin, and these interactions are competitive [95]. An intact actin cytoskeleton enhances overall ion movement through NMDA receptors [105], and displacement of α-actinin from NMDA receptors by competitive binding of Ca^{2+}/calmodulin is important for Ca^{2+}-dependent inactivation of the channel [99]. Thus it might be expected that CaMKII binding to NR1 would modulate NMDA receptors, but this has not yet been investigated.

Ser^{1303} in the NR2B subunit of the NMDA receptor is a high-affinity *in vitro* substrate for CaMKII and is phosphorylated under physiological conditions [100]. Whereas this phosphorylation regulates CaMKII binding to NMDA receptors *in vitro* and in HEK-293 cells [92], its role in regulating neuronal NMDA receptors has not been explored specifically. In a recent study, co-expression of CaMKII enhanced the desensitization of recombinant NMDA receptors containing the NR2B subunit, but did not affect the properties of NMDA receptors containing NR2A in place of NR2B; moreover, CaMKII autophosphorylation at Thr^{286} was required for enhanced receptor desensitization (S. S. Sikes, D. M. Lovinger and R. J. Colbran, unpublished work). Thus the requirements for modulation of NMDA receptor desensitization by CaMKII appear to be similar to those for co-localization of CaMKII with NR2B [88,92]. Additional studies are needed to determine the roles of CaMKII interactions with, and phosphorylation of, NR2B and other subunits in NMDA receptor desensitization.

F-actin and α-actinin

Rat brain CaMKII was shown to bind actin filaments *in vitro* and Ca^{2+}/calmodulin promotes dissociation of the kinase from actin [106]. CaMKII also associates with microtubules in brain extracts [107] and phosphorylation of MAP2 by CaMKII blocks its actin-filament-bundling activity [108]. CaMKIIβ has been shown to be responsible for F-actin binding *in vitro* [109]. Moreover, overexpressed GFP–CaMKIIβ associates with F-actin in multiple cell types under basal conditions, whereas GFP–CaMKIIα is localized diffusely in the cytoplasm. Interestingly, co-expression of CaMKIIβ with GFP–CaMKIIα results in F-actin-localized fluorescence, suggesting that the presence of a limited number of CaMKIIβ subunits in each holoenzyme is sufficient for F-actin targeting [109]. Treatment of cells expressing GFP–CaMKIIβ under conditions that activate the kinase results in dissociation of GFP–CaMKIIβ from F-actin due to Ca^{2+}/calmodulin binding [79]. In cells co-expressing CaMKIIα and GFP–CaMKIIβ, the time course of dissociation is controlled by the ratio of expressed isoforms. Moreover, transfer of alternatively spliced inserts from CaMKIIβ into CaMKIIα confers F-actin-binding ability [79]. Thus these data indicate that CaMKIIβ makes a specific and direct interaction with F-actin.

In addition to the direct binding of CaMKIIβ to F-actin, it has also been shown that CaMKII isoforms bind α-actinin [110,111]. α-Actinin contains two putative EF-hand Ca^{2+}-binding domains and was originally identified as an F-actin-binding protein, but it also interacts with NMDA receptor subunits [98] and densin-180 [110]. A domain close to the C-terminus of α-actinin (residues 638–871) interacts with the catalytic domains of CaMKIIα or CaMKIIβ, permitting the formation of a ternary complex containing CaMKIIα, α-actinin and densin-180 [110]. It would appear that these interactions represent a mechanism for targeting of CaMKIIα to portions of the F-actin cytoskeleton that contain α-actinin. Interestingly, α-actinin binds directly to a variety of receptors and ion channels [95,98,99,112–114] and associates with F-actin in dendritic spines and other structures [115]. Thus the ternary complex of CaMKIIα with densin-180 and α-actinin may serve to target kinase activity to associated receptors.

The different mechanisms for association of CaMKIIα and CaMKIIβ with the actin cytoskeleton might confer different functions. Recent studies found that expression of these two isoforms is regulated in opposite directions by synaptic activity; enhanced synaptic activity drives increased CaMKIIα expression, whereas CaMKIIβ expression is increased when synaptic activity is reduced [116,117]. Hippocampal neurons transfected to overexpress CaMKIIα had enhanced unitary synaptic currents, but a decreased frequency of such events, whereas CaMKIIβ-transfected cells exhibited inverse changes [116]. Independent studies demonstrated that overexpression of CaMKIIβ, but not

CaMKIIα, regulates the movement, extension and branching of filopodia and fine dendrites, and the total number of synapses [118]. Since the actin cytoskeleton regulates neuronal morphology, as well as synaptic glutamate receptors, it is tempting to suggest that the specificity of these effects of CaMKIIα and CaMKIIβ overexpression arise from targeting to different portions of the F-actin cytoskeleton. Indeed, transfer of the alternatively spliced F-actin-targeting sequence from CaMKIIβ to CaMKIIα conferred the ability to modulate at least some of the morphological features [118]. However, specific substrates and mechanisms underlying the morphological and synaptic changes remain unknown.

Densin-180

Densin-180 was identified as a relatively abundant PSD-enriched $\approx$ 180 kDa protein containing leucine-rich repeats near the N-terminus and a PDZ domain at the C-terminus [119]. Based on the presence of a single predicted unconventional membrane-spanning domain and the sensitivity of the electrophoretic mobility to glycosidases and proteases specific to O-sialoglycoproteins, densin-180 was suggested to be a transmembrane protein. The large putative extracellular N-terminus was predicted to interact with presynaptic or other extracellular proteins, whereas the C-terminal 400 amino acids might interact with cytoplasmic proteins [119]. However, recent cell-surface biotinylation experiments found that densin-180 is not available for modification in cultured hippocampal neurons [120]. Although a failure to detect cell-surface biotinylation of densin-180 may result from blockade due to its association with other extracellular molecules, it may be that densin-180 is cytosolic, like other members of the LAP family of proteins. LAP family proteins, containing both leucine-rich repeats and a PDZ domain, are localized to cell–cell contact sites, such as basolateral membranes in polarized epithelia, where they may play important roles in cell polarity and epithelial homoeostasis [121]. Thus the question as to whether densin-180 is in fact a transmembrane protein or whether it is cytosolic like the other family members remains unresolved.

Interactions of CaMKII with densin-180 were identified in two laboratories at about the same time [110,122]. As part of a search for additional $\approx$ 180 kDa CaMKII-binding proteins localized to PSDs [84], cDNAs corresponding to splice variants of the putative intracellular domain of densin-180 were isolated [122]. Proteins encoded by three of the four variants interacted with CaMKIIα in overlay and co-sedimentation assays. In addition, the CaMKII-binding domain of densin-180 was capable of directing CaMKII localization when transfected into HEK-293 cells [122]. CaMKIIα interactions with densin-180 were also identified using a yeast two-hybrid strategy to screen for densin-180-interacting proteins; CaMKIIα and densin-180 co-localized in cultured neurons and co-immunoprecipitated from brain extracts [110]. Thus it appears that CaMKII interacts with densin-180 under physiological conditions.

The CaMKII-binding domain of densin-180 lies within an exon present in three of four putative C-terminal splice variants [122]. Additional deletion mutagenesis restricted further the CaMKII-binding domain to 27 amino acids within this exon (residues 1354–1381 in the originally isolated densin-180 sequence [119]). However, there is no apparent sequence similarity between the CaMKII binding domain in densin-180 and CaMKII binding domains in NR2B, NR2A, NR1 or α-actinin (Figure 3). Thus it is not surprising that the interactions of densin-180 and residues 1290–1309 of NR2B with CaMKII differ in several ways. First, NR2B cannot compete with densin-180 for binding to CaMKII [122]. Secondly, binding to densin-180 is partially independent of CaMKII activation [110,122], whereas interaction with NR2B requires Ca^{2+}/calmodulin binding and/or autophosphorylation [88,90,92,96]. Thirdly, densin-180 appears to bind CaMKIIα specifically [110], whereas NR2B binds all CaMKII isoforms (A. J. Robison and R. J. Colbran, unpublished work). These data implicate more variable regions of the C-terminal domain of CaMKIIα as important for densin-180 binding, as originally suggested by yeast two-hybrid analysis [110], whereas the catalytic domain is important in binding NR2B [90,92,96]. Fourthly, densin-180 has no significant effect on CaMKII activity *in vitro* (M. A. Bass, S. Strack, A. J. Robison and R. J. Colbran, unpublished work), whereas NR2B either inhibits CaMKII or 'traps' the autonomous form (see above). Taken together, these data indicate that CaMKIIα holoenzymes are capable of binding both densin-180 and NR2B simultaneously, at least *in vitro* (see Figure 4); whether or not this ternary complex occurs in intact cells remains to be determined.

Overall, the available data indicate that densin-180 is a compelling candidate to play a significant role in determining CaMKII localization *in situ*. In addition, the PDZ domain of densin-180 is involved in several other interesting interactions. For example, association of densin-180 with the δ-catenin/NPRAP (neural plakophilin-related armadillo repeat protein)–N-cadherin complex via the PDZ domain may contribute to the organization of synaptic junctions [120]. In addition, the densin-180 PDZ domain interacts with MAGUIN-1, a PSD95-interacting protein, providing a potential structural link between densin-180 and NMDA receptors in brain [123]. Most significantly to this article, the C-terminus of α-actinin also binds to the PDZ domain in densin-180 [110]. Since α-actinin also makes independent interactions with CaMKII, it appears that a ternary complex can be formed containing α-actinin, densin-180 and CaMKIIα [110] (see above; and Figure 4). Additional studies are needed to elucidate the specific impact of densin-180 on CaMKII targeting and function.

SynGAPβ

SynGAP is a Ras GTPase-activating protein (GAP) that was identified as a PSD95-interacting protein, serves as a negative regulator of MAPK (mitogen-activated protein kinase) signalling [124–126] and is present in the NMDA receptor multiprotein complex [124,127]. CaMKII phosphorylates synGAP at unidentified site(s) and inhibits its GAP activity, suggesting one mechanism by which NMDA receptor activation may signal to activate the MAPK signalling pathway [125]. Multiple synGAP variants presumed to arise by alternative splicing were recently identified and one (synGAPβ) possesses a unique C-terminal sequence that lacks the PSD95-binding motif [128]. Nevertheless, synGAPβ is targeted to PSDs, perhaps more avidly than its PSD95-binding counterparts. This finding may be explained by the fact that non-phosphorylated CaMKIIα interacts with synGAPβ, but not other synGAP variants; the available evidence suggests that CaMKII binding to synGAPβ is inhibited by Thr^{286}-autophosphorylation [128]. The synGAPβ C-terminal domain is sufficient for CaMKII binding, but bears no striking identity with CaMKII-binding domains identified in other PSD proteins (Figure 3); the molecular mechanism of the interaction remains unclear. It will be interesting to compare the impact of CaMKII on the regulation of different synGAP isoforms. One might predict that synGAPβ is the preferred target of CaMKII signalling and that other synGAP isoforms are modulated by different mechanisms. Selective coupling of synGAP isoforms to different regulatory mechanisms may contribute to the complexity

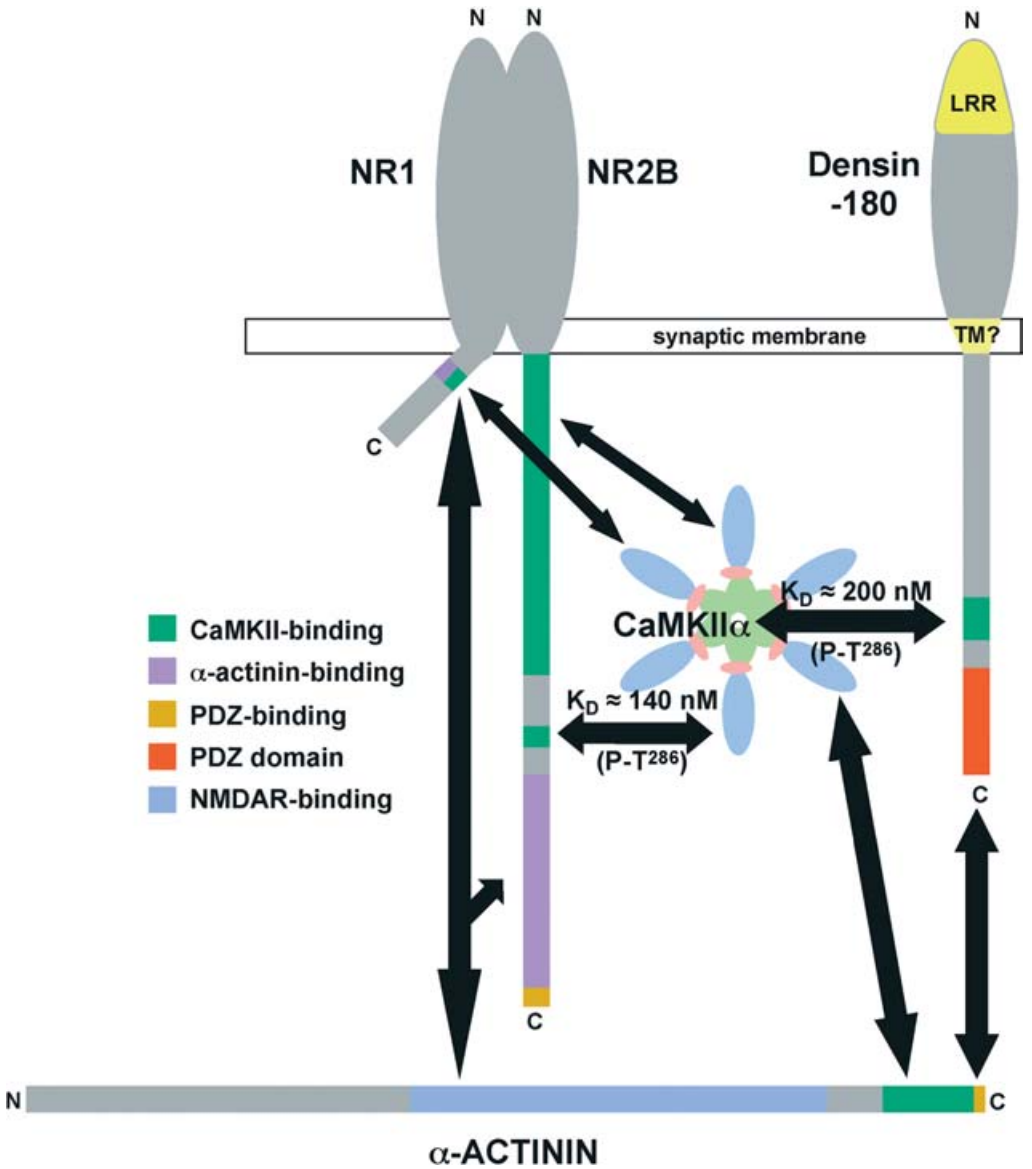

Figure 4 Model for multivalent CaMKII interactions with NMDA receptors, densin-180 and α-actinin

CaMKII possesses multiple domains that can bind to NMDA receptor (NMDAR) subunits, densin-180 and α-actinin. Domains in CaMKII are coloured as in Figure 1 and protein–protein interaction domains in other proteins are coloured as in the key. Apparent interaction affinities are given where known. Additional interactions of CaMKII with NR2A, F-actin, synGAPβ, cdk5 and CaM-KIIN are not depicted. In densin-180, 'LRR' and 'TM?' refer to the leucine-rich repeat domain and putative transmembrane domain respectively. P-T^{286} depicts CaMKII autophosphorylated on Thr^{286}.

of NMDA receptor coupling to Ras and MAPK pathways, as has been revealed in heterozygous synGAP knockout mice [126].

cdk5 (cyclin-dependent protein kinase 5)

Unlike most cyclin-dependent protein kinases, cdk5 is expressed at high levels in post-mitotic cells including neurons, where it is associated with cytoskeletal elements (reviewed in [129]). The NMDA receptor NR2A subunit is phosphorylated by cdk5 at Ser^{1232} *in vitro* and in cells, and inhibitors of cdk5 block long-term potentiation in the hippocampus [130]. In addition, cdk5 has been shown to modulate the PP1-inhibitor proteins DARPP-32 (dopamine- and cAMP-regulated phosphoprotein) and inhibitor-1 [131,132]. Modulation of PP1 activity by inhibitor-1 plays an important role in regulating CaMKII autophosphorylation and postsynaptic responses [41–43,80]. Recently, it was shown that the p35 and p39 activator subunits of cdk5 associate with CaMKIIα, as well as α-actinin-1 [111]. Yeast two-hybrid analyses indicate that the C-terminal 200–250 amino acids of p35/p39 interact with the catalytic/regulatory domain of CaMKIIα. The association of CaMKII and α-actinin with p35/p39 is potentiated by Ca^{2+} *in vitro*, and is enhanced by NMDA receptor activation in intact neurons in a CaMKII-activity-dependent manner [111]. It was suggested that the interaction may target cdk5 activity to the PSD, since the total cdk5 activity in cell extracts is unaffected by glutamate stimulation of neurons. However, verification of this finding and the elucidation of functional roles for the interaction will require additional studies.

CaM-KIIN

A yeast two-hybrid screen for CaMKII-interacting proteins identified two isoforms of an 8 kDa protein that stably binds to

and potently inhibits CaMKIIα and CaMKIIβ activity [133,134]. This naturally occurring CaMKII-inhibitor protein was named CaM-KIIN, and interaction of CaM-KIIN with CaMKII *in vitro* required Ca^{2+}/calmodulin binding to the kinase. A 28-amino-acid domain near the C-terminus of CaM-KIIN contains the inhibitory determinants, and CaMKII inhibition was shown to be non-competitive with a conventional peptide substrate [133,134]. Interestingly, this domain is not similar to the CaMKII auto-inhibitory domain, or to other known CaMKII-binding proteins (Figure 3). When overexpressed in COS cells, CaM-KIIN is distributed between nuclear and cytoplasmic compartments; co-transfection of a constitutively active mutant of CaMKIIα, but not the wild-type protein, appears to sequester CaM-KIIN in the cytoplasm [133]. In cultured neurons, CaM-KIIN is not nuclear, but co-localizes with CaMKII in the somatic cytoplasm and dendrites, but not with CaMKII in dendritic spines [134]. Thus CaM-KIIN may function to negatively regulate CaMKII signalling in dendrites, localizing further CaMKII actions to spines and PSDs after synaptic activation.

CaMKII TARGETING IN OTHER SYSTEMS

Although most attention has focused on targeting of CaMKII in neuronal dendrites, information is emerging that localization of CaMKII by specific interactions with other cellular proteins may be important in other systems.

Presynaptic targeting and function

A form of CaMKIIα modified by an unknown mechanism is tightly associated with presynaptic vesicles, but has identical catalytic properties with soluble CaMKIIα [135]. The vesicle-associated CaMKII binds to and phosphorylates synapsin I [136], promoting dissociation of synapsin I from synaptic vesicles, thereby making more vesicles available at the active zone for neurotransmitter release (reviewed in [137]). In addition, CaMKII activity is enhanced by its binding to myosin V, a Ca^{2+}-regulated F-actin-based motor protein implicated in modulation of vesicle trafficking [138,139]. Thus interactions of CaMKII with other proteins may regulate the availability of synaptic vesicles at the active zone of presynaptic terminals by diverse mechanisms.

CaMKII has also been implicated in a more direct regulation of the exocytotic machinery. Autophosphorylated CaMKII interacts with and phosphorylates syntaxin 1A, and the interaction appears to promote synaptic vesicle exocytosis [140,141]. Syntaxin interacts with the so-called 'synprint' domain of N-type Ca^{2+} channels, and phosphorylation in the synprint domain by CaMKII (or PKC) strongly inhibits binding of syntaxin to the channel [142]. Thus Ca^{2+} influx and CaMKII autophosphorylation may promote formation of a syntaxin–CaMKII complex associated with the N-type Ca^{2+} channel, thereby enhancing phosphorylation of the Ca^{2+} channel and subsequent dissociation of syntaxin. Enhanced dissociation of syntaxin from the synprint site can be envisaged as a positive or negative modulation of exocytosis (see discussion in [142]).

In combination, these data suggest that CaMKII interaction with multiple presynaptic proteins enhances phosphorylation of substrates and promotes neurotransmitter release. However, the molecular basis of these interactions remains poorly defined.

Nuclear targeting and function

CaMKII has been implicated in the regulation of gene transcription. For example, CaMKII phosphorylates the positive regulatory site (Ser^{133}) in CREB (cAMP-response-element-binding protein) as well as a dominant-negative regulatory site (Ser^{142}) [143]. Although most CaMKII holoenzymes are excluded from the nucleus because of their size, a CaMKIIδ splice variant (δ_B) was shown to contain a functional nuclear localization signal [144] that permits regulation of cardiac gene expression by CaMKII [145]. The nuclear localization sequence can be inactivated by phosphorylation catalysed by CaMKI or CaMKIV [146]. A similar alternatively spliced cassette is found in variants of CaMKIIα (α_B) and CaMKIIγ; α_B (also known as α-33) has a restricted expression pattern in brain [147,148]. Interestingly, expression of CaMKIIα in the nucleus was recently shown to inhibit the neuron-like differentiation of PC12 cells [149]. In summary, certain CaMKII splice variants are targeted specifically to the nucleus where they can participate in the regulation of gene transcription.

Targeting of CaMKII in cardiac muscle

CaMKII has been physically and functionally linked to regulation of cardiac Ca^{2+} homoeostasis. CaMKII co-localizes with and facilitates L-type Ca^{2+} channels in cardiomyocytes [150]. Moreover, unidentified isoforms of CaMKII appear to be tightly associated with cardiac L-type Ca^{2+} channels in excised membrane patches, and this association is sensitive to cytoskeleton-disrupting drugs [151]. Beside regulating L-type Ca^{2+} channels in the sarcolemma, cardiac CaMKII isoforms also regulate the Ca^{2+}-ATPase in longitudinal sarcoplasmic reticulum (SR) by phosphorylating phospholamban [152], ryanodine receptors in junctional SR [153], as well as gene transcription in the nucleus (see above). Targeting to longitudinal SR may be achieved by association of conventional CaMKII isoforms with αKAP [154], an alternative product of the CaMKIIα gene that lacks a catalytic domain, but contains a unique hydrophobic N-terminal domain that tightly associates with membranes [6]. Inhibition of CaMKII on the longitudinal SR by targeted expression of a CaMKII inhibitor peptide suppressed phosphorylation of phospholamban without affecting phosphorylation of the ryanodine receptor in junctional SR [152]. However, specific mechanisms targeting CaMKII to the junctional SR and the sarcolemma remain unknown. In combination, it appears that specific association of CaMKII isoforms with different subcellular structures dictates their signalling specificity in the heart.

SUMMARY, PERSPECTIVES AND FUTURE DIRECTIONS

Over the last 5 years, CaMKII has been shown to bind to several proteins that are localized to diverse subcellular compartments in multiple tissues. Many of these studies focused on interactions of CaMKIIα and CaMKIIβ with neuronal proteins, but it seems likely that investigation of other CaMKII gene products and splice variants in different tissues will reveal additional interacting proteins. The available data show that interactions of CaMKII with some of the neuronal proteins are non-competitive, and sometimes co-operative, indicating that different kinase domains are involved. Consistent with this model, it has not yet been possible to identify a 'consensus' CaMKII-binding sequence. However, as more CaMKII-interacting proteins are characterized, it may be possible to categorize them into subclasses based on their interaction mechanisms.

The apparent diversity in CaMKII targeting mechanisms is in marked contrast with the mechanisms targeting other signalling enzymes. For example, PKA is targeted by interaction of the N-terminal dimerization domain of its regulatory subunits with amphipathic α-helices found in almost all of the approx. 50 known AKAPs [83]. Similarly, PP1 catalytic subunits are targeted in

large part by interaction with consensus [Arg/Lys]-[Val/Ile]-Xaa-Phe motifs in a diverse family of PP1-targeting proteins [155]. It will be very interesting to establish how the CaMKII holoenzyme is able to accommodate the diversity of these interactions at a structural level.

It remains to be determined whether or not any of the known CaMKII-binding proteins function as the original CaMKAP hypothesis predicts: i.e. to provide stable anchoring of CaMKII in specific subcellular locations. Most of the interactions characterized to date are dynamically regulated by the activation state of the kinase. Whether the CaMKAP hypothesis is proven or not, CaMKII-interacting proteins may be downstream effector substrates for CaMKII, or they may be associated with a distinct downstream effector substrate, thereby bringing kinase and substrate in close proximity. In addition, there is some evidence to suggest that these interactions may modify the kinase activity. Although CaMKIIα and CaMKIIβ possess similar catalytic properties, information is emerging that they can have significantly different physiological roles in neuronal dendrites. Part of the explanation for this selectivity of function may lie in the fact that they interact differentially with neuronal CaMKII-binding proteins (Table 1). However, formal proof that the specific cellular functions of CaMKIIα and CaMKIIβ are linked to distinct protein–protein interactions is currently lacking. Studies to obtain such proof will require exquisite subcellular/spatial and temporal resolution to circumvent likely synapse-to-synapse variability in the nature of CaMKII complexes, even within a single neuron. This variability may determine the unique properties of each synapse, or subset of synapses, in a neuron. Despite the lack of formal proof, it seems likely that CaMKII-binding proteins will confer specific physiological functions to different kinase holoenzymes.

Apart from dictating different physiological functions, the ability of CaMKII to interact simultaneously with multiple proteins suggests that, under some circumstances, the kinase may nucleate large multiprotein complexes and thereby serve a structural role in addition to its catalytic function. Such a structural role for CaMKII was previously suggested based on its abundance in neurons (reviewed in [156]). However, catalytic function appears to be essential for the putative structural role, because intra-holoenzyme CaMKII autophosphorylation at Thr^{286} is necessary for, or at least potentiates, several of the protein–protein interactions that are required for appropriate creation of these complexes. This regulated multivalent property has been suggested to create new docking sites for AMPA-type glutamate receptors in PSDs at neuronal synapses that already contain the NMDA receptor [157]. Recent findings suggest that Thr^{305} and/or Thr^{306} autophosphorylation negatively regulates CaMKII association with the PSD [33,34,80], although the mechanism by which this occurs is unclear. More detailed biochemical analyses will be necessary to develop a deeper understanding of the dynamics of formation of these complexes, the relative stoichiometries of the various CaMKII-binding proteins in intact cells and their specific contributions to CaMKII targeting.

In summary, while much has recently been learned about the participation of neuronal CaMKII isoforms in diverse protein complexes, additional interacting proteins surely remain to be discovered, especially for the divergent CaMKIIγ and CaMKIIδ splice variants. It seems likely that continued characterization of dynamic relationships of the interactions of CaMKII with its binding proteins will provide new insights into the physiological roles of this fascinating enzyme in many tissues.

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