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Review

CaMKII: Claiming Center Stage in Postsynaptic Function and Organization

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While CaMKII has long been known to be essential for synaptic plasticity and learning, recent work points to new dimensions of CaMKII function in the nervous system, revealing that CaMKII also plays an important role in synaptic organization. Ca^{2+}-triggered autophosphorylation of CaMKII not only provides molecular memory by prolonging CaMKII activity during long-term plasticity (LTP) and learning but also represents a mechanism for autoactivation of CaMKII's multifaceted protein-docking functions. New details are also emerging about the distinct roles of CaMKIIα and CaMKIIβ in synaptic homeostasis, further illustrating the multilayered and complex nature of CaMKII's involvement in synaptic regulation. Here, I review novel molecular and functional insight into how CaMKII supports synaptic function.

Introduction

CaMKII is a highly unusual kinase. Accounting for 1%–2% of total brain protein, its abundance is only rivaled by a few other, mostly cytoskeletal, proteins (Lisman et al., 2002). By autophosphorylating itself upon activation by Ca^{2+} and calmodulin (CaM), it retains its catalytic activity beyond the initial stimulation, constituting a molecular memory device, and has long been considered to be important for long-term potentiation (LTP) and learning. CaMKII activation by Ca^{2+} influx via NMDA receptors (NMDARs) is essential for standard hippocampal LTP and hippocampus-based learning (Kerchner and Nicoll, 2008; Lisman et al., 2012; Malenka and Bear, 2004; Morris, 2013). The pivotal role of CaMKII in LTP cannot be overemphasized. This Review will focus on recent work that has unearthed novel functions of CaMKII in spines, focusing on the hippocampal CA1 region.

CaMKII Structure and Regulation

CaMKII is formed by 12 catalytically active subunits (Figure 1) (Chao et al., 2011; Colbran and Brown, 2004). Four different genes (*CAMK2A*, *CAMK2B*, *CAMK2G*, and *CAMK2D*) encode CaMKIIα–CaMKIIδ, respectively, with α and β being highly prevalent in brain. CaMKII accounts for 2%–6% of total protein in the PSD (~80 dodecameric complexes per 0.1 μm^2 of PSD [Chen et al., 2005]); larger PSDs in mushroom-shaped spines will have up to ~240 dodecamers [Feng et al., 2011]), trumping the abundance of the prototypal postsynaptic scaffold protein PSD-95 (~250 per 0.1 μm^2 of PSD [Chen et al., 2008]) in total mass (Dosemeci et al., 2007). Forebrain CaMKII consists mostly of nine α and three β subunits, whereas this ratio is inverted for cerebellar CaMKII (Miller and Kennedy, 1985).

CaMKII is inactive under resting conditions as substrate access to its binding site in the catalytic domain is blocked by the autoinhibitory pseudosubstrate segment of the protein (Figures 1A and 1B) (Braun and Schulman, 1995; Colbran and Brown, 2004; Coultrap and Bayer, 2012). Upon Ca^{2+} influx, Ca^{2+}/calmodulin binding to the pseudosusbtrate segment of CaMKII relieves it from this autoinhibition. When Ca^{2+}/CaM binds to two neighboring subunits, autophosphorylation at T286 (T287 in CaMKIIβ) can occur, which results in the persistence of kinase activity even beyond removal of Ca^{2+}/CaM (Braun and Schulman, 1995; Colbran and Brown, 2004; Coultrap and Bayer, 2012) (Figure 1C). However, this so-called autonomous activity of CaMKII is significantly below the maximal activity (~40%–80% at physiological ATP concentrations, i.e., >1 mM) (Coultrap et al., 2010).

Autophosphorylation of the 12 subunits within a holoenzyme allows for graded translation of Ca^{2+} spike frequency into kinase activity in vitro (De Koninck and Schulman, 1998) and in intact neurons (Fujii et al., 2013). Furthermore, as part of this molecular memory mechanism, T286 residues that lose their phosphoryl moieties during periods of suboptimal Ca^{2+} influx can be rephosphorylated. Rephosphorylation of T286 is greatly enhanced when the neighboring subunit is still T286 phosphorylated because T286 phosphorylation dramatically enhances Ca^{2+}/CaM binding, a phenomenon called CaM trapping. Notably, autonomous CaMKII activity due to T286 phosphorylation, which is lower than Ca^{2+}/CaM-stimulated CaMKII activity and varies between substrates, is especially high with respect to T286 autophosphorylation of neighboring subunits (Coultrap et al., 2010).

CaMKII Localization and Interactions in Spines

Under basal conditions, endogenous CaMKII appears to be enriched by a factor of ~2 in spines compared to dendritic shafts (Feng et al., 2011; Merrill et al., 2005; Strack and Hell, 2008). Under basal conditions, ~80% of CaMKII molecules exit spines and exchange with dendritic shaft CaMKII with a time constant between 1 and 5 min (~1 s for free GFP), with ~15% remaining firmly anchored in spines after 30 min (Lee et al., 2009; Sharma et al., 2006; Sturgill et al., 2009). Protein-protein interactions play a critical role in retaining CaMKII in spines, and F-actin, α-actinin, NMDARs, and to some degree densin-180, are emerging as major CaMKII binding partners (Figure 2) (Strack and Hell, 2008).

CaMKII Binding to F-Actin

Most of CaMKII and of F-actin within spines are in the spine interior (Ding et al., 2013; Feng et al., 2011; Tao-Cheng et al., 2007)

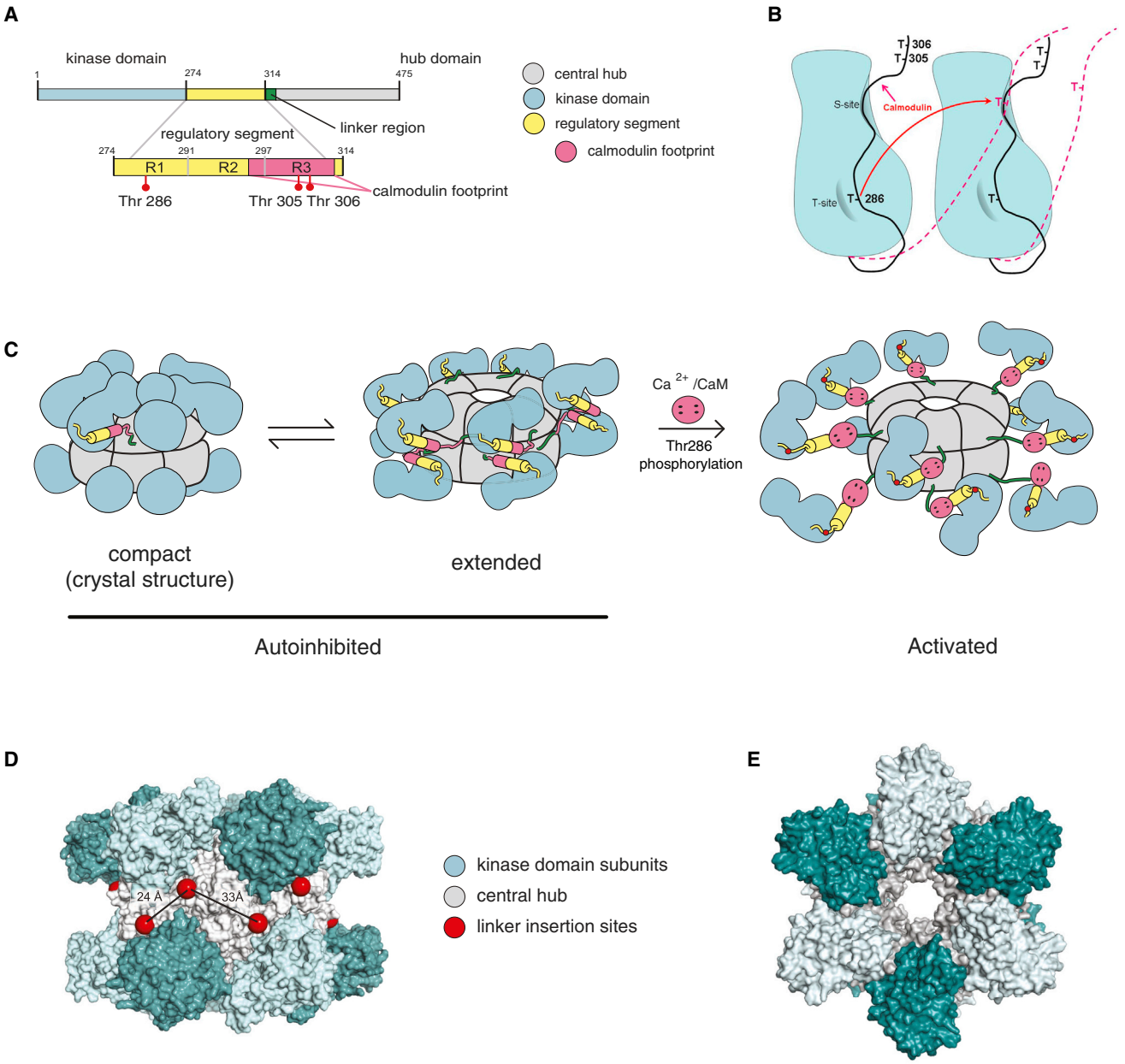

Figure 1. CaMKII Structure

(A) Linear depiction of one CaMKII subunit. It shows kinase domain (blue; numbering according to mouse CaMKIIα), autoinhibitory segment (yellow; formed by R1, which includes T286, R2, which binds to S site, and R3, which includes most of the CaM binding site; red), linker region (green), and association domain (gray).

(B) Autoinhibition and autophosphorylation of CaMKII subunits. The diagram illustrates schematically two neighboring subunits with the inhibitory segment in black. The T site (gray half-moon) accommodates T286 under resting conditions, fostering the interaction of the pseudosubstrate region immediately downstream of T286 with the catalytic site (S site; gray surface on right side of each subunit). The S site is formed by the cleft between N and C domains and is in close proximity to the T site. Upon binding of Ca^{2+}/CaM to the region defined by T305/306, the inhibitory segments are displaced from the S and T sites (red dashed lines). If two neighboring subunits simultaneously bind Ca^{2+}/CaM, T286 from one subunit can reach the catalytic site of the other (red arrow) and becomes phosphorylated.

(C–E) CaMKII dodecamer. Each model depicts the two stacked hexameric rings.

(C) Schematic of a structural model of CaMKII dodecamer. According to the model, the CaMKII dodecamer can exist in three main conformations: (1) a closed inhibited/inactive conformation with the linker folded into the association domain, rendering it inaccessible for Ca^{2+}/CaM binding and activation; (2) an open inhibited/inactive conformation with the linker extended outward; and (3) a fully extended active conformation with Ca^{2+}/CaM bound to the regulatory segment. The kidney-shaped segments represent the catalytic domains. They consist of a smaller globular N and a larger globular C domain. The catalytic cleft is nested in the cleft between the two domains. Adopted with permission from Stratton et al. (2013).

(D and E) Space-filling atomic model of the crystal structure of the CaMKII dodecamer in the closed, inhibited conformation. Shown are views from side (D) and top (E). The individual catalytic domains of each subunit are alternating light and dark blue for clarity. The association domains, which form the central hub, are gray. The positions of the linkers are depicted by red spheres. Adopted with permission from Chao et al. (2011).

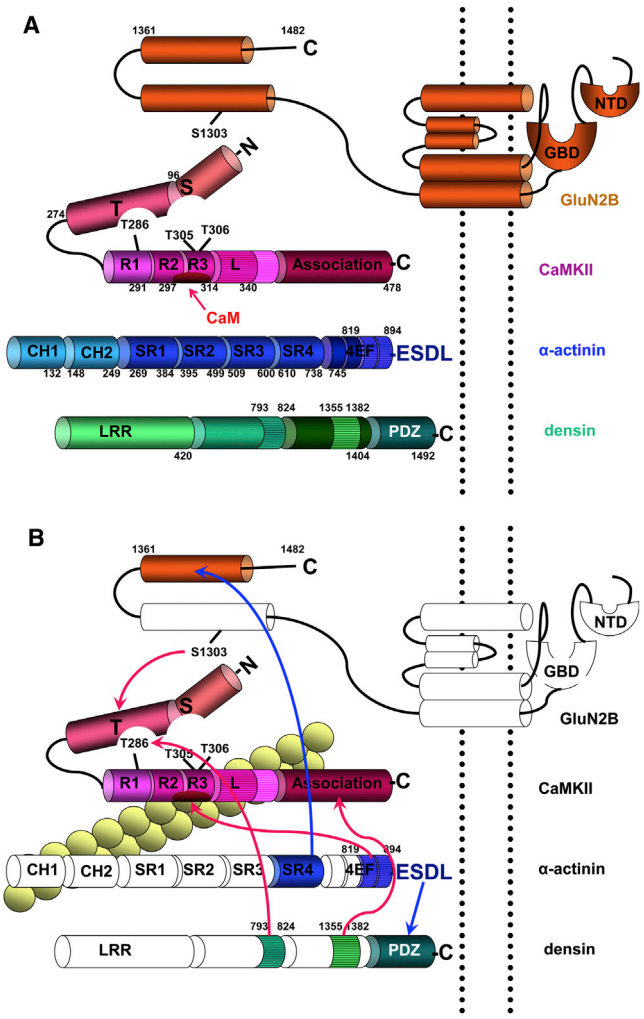

Figure 2. Domains that Mediate Interactions between CaMKII, α-Actinin, F-Actin, Densin, and NMDARs

(A) Linear structures of CaMKII and its most prevalent and functionally important binding proteins in spines. The CaMKII diagram (red) shows the N domain (aa 1–96; numbering according to mammalian CaMKIIα), C domain (aa 97–274), catalytic site nested between N and C domains (includes substrate binding site [S site]), autoinhibitory segment (aa 275–340) consisting of R1 (aa 275–291; contains T286 for interaction with the T site under resting conditions), R2 (aa 291–297; binds to the S site under resting conditions), and R3 (aa 297–314; includes the Ca^{2+}/CaM binding site, which overlaps with R2; dark red oval; T305/T306 inhibit Ca^{2+}/CaM binding if phosphorylated), linker segment L (aa 314–340), and association domain (aa 341–478). α-Actinin (blue) consists of two calponin homology domains (CH1: aa 1–132; CH2: aa 148–249; numbering according to mammalian α-Actinin-1), four spectrin repeat domains (SR1: aa 269–384; SR2: aa 395–499; SR3: aa 509–600; SR4: 610–738), and four EF hands (aa 745–894). The linker between CH2 and SR1 (aa 250–268) is an important attachment site for the EF hands in the antiparallel dimer (second protomer is not depicted). Densin (green) is formed by a leucine-rich repeat domain (LRR; consists of 16 leucine-rich repeats; aa 1–420), a central domain of less certain structural identity (aa 421–1,404), and a C-terminal PDZ domain (aa 1,405–1,492). The NMDAR GluN2B subunit (orange) consists of an extracellular N-terminal domain (NTD) and glutamate binding domain (GBD), which is formed by the N terminus and the extracellular loop between transmembrane segments 2 and 3, three transmembrane segments, which form, together with a membrane-reentry loop, the pore, and an intracellular C terminus (aa 838–1,482).

(B) Depiction of CaMKII interaction sites. The linker of CaMKIIβ (horizontal stripes), which is of variable length and different from the linker of CaMKIIα, is important for binding to F-actin (yellow beaded double string crossing underneath the linker). The exact binding site on CaMKIIβ for F-actin is unclear. F-actin also binds to CH1 of α-actinin (crossing underneath CH1). Other connections of CaMKII are depicted by red arrows. EF3 and EF4 hands of α-actinin (aa 819–194; horizontal stripes) and Ca^{2+}/CaM compete for binding to R2/R3 on CaMKII (dark red oval). The association domain of CaMKII (aa 341–478) binds to aa 1,335–1,382 of densin (horizontal stripes) independent of CaMKII activation. The T site of CaMKII binds to aa 1,290–1,309 of GluN2B (including S1303, which is involved in CaMKII binding and phosphorylated by CaMKII) and aa 793–824 of densin (horizontal stripes) upon addition of Ca^{2+}/CaM or phosphorylation of T286. α-actinin binds with its C-terminal ESDL motif to the PDZ domain of densin and with its C-terminal portion of SR4 to the C-terminal portion of GluN2B (blue arrows).

and turn over with comparable rates (~1 min; [Feng et al., 2011; Frost et al., 2010; Honkura et al., 2008; Lee et al., 2009; Sharma et al., 2006; Sturgill et al., 2009]). CaMKIIβ binds to and crosslinks F-actin filaments (Fink et al., 2003; Lin and Redmond, 2008; Okamoto et al., 2007; Shen and Meyer, 1999) via the variable linker between the regulatory and association domains of CaMKIIβ, which is different from CaMKIIα (Figure 2) (Fink et al., 2003; O'Leary et al., 2006; Lin and Redmond, 2008; Shen and Meyer, 1999). F-actin binding targets CaMKII dodecamers to spines, where F-actin is concentrated (Figure 3). Upon Ca^{2+} influx, Ca^{2+}/CaM and T287 autophosphorylation displace CaMKIIβ from F-actin (Lin and Redmond, 2008; Shen and Meyer, 1999) (Figure 3). Notably, CaMKIIβ has an ~9-fold higher affinity for Ca^{2+}/CaM (EC_{50} for autophosphorylation: 15 nM) than CaMKIIα (Brocke et al., 1999), suggesting that CaMKIIβ-containing dodecamers are readily released from F-actin before CaMKIIα subunits bind Ca^{2+}/CaM and are activated. However, Ca^{2+}/CaM binding to CaMKIIα is necessary for T287 phosphorylation of CaMKIIβ in native forebrain dodecamers as CaMKIIα subunits phosphorylate CaMKIIβ subunits (Brocke et al., 1999).

CaMKII Binding to α-Actinin

α-actinin is an F-actin binding protein concentrated at cell adhesion points and in spines (Otey and Carpen, 2004; Wyszynski et al., 1998). Four genes (*ACTN1–ACTN4*) encode the highly homologous α-actinin-1 through α-actinin-4 with α-actinin-1, α-actinin-2, and α-actinin-4, but not α-actinin-3, being expressed in forebrain neurons (Schnizler et al., 2009; Wyszynski et al., 1998). α-actinins contain two calponin homology domains (CH1 and CH2), followed by four spectrin homology repeats (SR1–SR4), four EF hand motifs (EF region), and a C-terminal PDZ binding motif (ESDL; Figure 2A). The same segment in the CaMKII autoinhibitory domain that binds to Ca^{2+}/CaM binds to the EF hand motifs of α-actinin-2 and α-actinin-4 (Figure 2B) (Jalan-Sakrikar et al., 2012; Robison et al., 2005b; Walikonis et al., 2001). Indeed, Ca^{2+}/CaM outcompetes α-actinin for CaMKII binding (Meyer et al., 1992; Robison et al., 2005a), thus Ca^{2+} influx dislodges CaMKII from α-actinin and enables CaMKII to redistribute within spines (Figure 3). As α-actinin supports CaMKII association with F-actin under basal conditions (Jalan-Sakrikar et al., 2012), disruption of α-actinin binding to one or more subunits of a CaMKII dodecamer by Ca^{2+}/CaM might act in parallel with disruption of the direct binding of CaMKIIβ subunits to F-actin. Furthermore, the two N-terminal EF hands in α-actinin-1 and α-actinin-4 can bind Ca^{2+}, which inhibits their binding to F-actin (Burridge and Feramisco, 1981; Sjöblom

et al., 2008; Witke et al., 1993). Multiple interactions of single CaMKIIα/CaMKIIβ dodecamers with F-actin via β subunits and with F-actin-associated α-actinin dimers are likely to be critical for keeping a defined amount of CaMKII anchored within each spine. Ca^{2+} influx has the potential to liberate CaMKIIα/CaMKIIβ dodecamers from F-actin and α-actinin for redistribution within individual spines to the PSD within seconds.

T286 autophosphorylation does not affect binding of the EF hands of α-actinin, suggesting that α-actinin binds to a portion of the inhibitory region that is accessible in the resting conformation of CaMKII (conformation b in Figure 1C). Slow autophosphorylation in the Ca^{2+}/CaM binding site of CaMKII abrogates binding of Ca^{2+}/CaM only if on T305, but of both Ca^{2+}/CaM and α-actinin if on T306 (Jalan-Sakrikar et al., 2012), consistent with a structural model that proposes that T306 but not T305 is involved in binding to the EF region of α-actinin, which only engages one face of the α-helical structure of this CaMKII region (Jalan-Sakrikar et al., 2012). Thus, T305 autophosphorylation potentially serves to protect the α-actinin-CaMKII interaction from being disrupted by Ca^{2+}/CaM. The impact of CaMKIIβ phosphorylation on T306/T307 has not been studied. Like Ca^{2+}/CaM, α-actinin binding stimulates CaMKII activity but to a lesser extent than Ca^{2+}/CaM and only for certain substrates (Jalan-Sakrikar et al., 2012). This mechanism could act to ensure a certain but low level of kinase activity of α-actinin-associated CaMKII.

CaMKII Binding to Densin

Densin consists of multiple leucine-rich repeats (LRRs), a middle region of less clear homologies, and a PDZ domain located at its C terminus (Figure 2A). Densin binds to the T site of CaMKIIα and CaMKIIβ (Jiao et al., 2011) and to the C-terminal oligomerization domain of CaMKIIα but not CaMKIIβ (Figure 2B) (Strack et al., 2000b). Whereas binding of densin to the CaMKII association domain is independent of CaMKII activation, binding of densin to the T site of CaMKII requires either Ca^{2+}/CaM or T286 autophosphorylation to provide access to the site (Figure 2B) (Jiao et al., 2011). At present, the relevance of the two densin interaction sites is not clear. α-actinin can bind to the PDZ domain of densin (Robison et al., 2005b; Walikonis et al., 2001), which synergistically promotes the CaMKII-α-actinin interaction probably by forming a ternary complex (Robison et al., 2005b).

Densin knockout (KO) mice do not show loss of CaMKII from spines or PSDs under basal conditions, suggesting that densin's role as a CaMKII-anchoring protein is redundant or auxiliary (Carlisle et al., 2011). Nevertheless, GluN1/densin double KO neurons, but not GluN1 single KO neurons, show a strongly reduced spine accumulation of CaMKII (Carlisle et al., 2011). Apparently in GluN1, KO neurons in which NMDARs and hence GluN2B are absent, densin is needed for compensating loss of CaMKII anchoring under basal conditions, possibly by fostering CaMKII binding to α-actinin. In fact, α-actinin is reduced in the PSD in densin KO mice supporting the notion that densin helps localize α-actinin at postsynaptic sites and spines (Carlisle et al., 2011). Thus, densin might augment CaMKII interactions with F-actin via α-actinin.

Despite the contribution of densin to basal CaMKII targeting to spines and the activity-driven densin-T site interaction, a role of densin in Ca^{2+}/CaM-induced postsynaptic CaMKII clustering appears to be of low prevalence, as abrogating CaMKII binding to GluN2B is sufficient to completely abolish activity-driven spine accumulation of CaMKII (see below) (Halt et al., 2012). Accordingly, the activity-triggered recruitment of CaMKII from shaft to spines is mainly GluN2B, and not densin, dependent.

Paradigm Shifting Structural Roles for CaMKII: F-Actin Bundling and Branching

CaMKIIβ binding to F-actin not only anchors CaMKII in spines but also stabilizes and bundles F-actin to augment spine size (Lin and Redmond, 2008; Okamoto et al., 2007). Knockdown of CaMKIIβ (but not CaMKIIα) causes a reduction in spine head size and loss of mature spines, which is fully rescued by ectopic expression of kinase dead CaMKIIβ K43R or of a fragment comprised of the association domain and the preceding linker region that mediates F-actin bundling (Okamoto et al., 2007). Accordingly, F-actin bundling by CaMKIIβ enhances spine size in a kinase activity-independent manner. Also, CaMKIIβ overexpression stabilizes F-actin-rich structures in cultured cortical neurons and decreases F-actin motility in spines (Okamoto et al., 2007). Furthermore, in CaMKIIβ A303R knockin (KI) mice, CaMKIIβ cannot be activated (as Ca^{2+}/CaM binding is abrogated), yet spine targeting of CaMKIIα as well as hippocampal LTP and learning are all normal. This lack of effect is in contrast to CaMKIIβ KO mice in which CaMKIIα spine targeting, LTP, and learning are impaired (Borgesius et al., 2011). The modest phenotypes of CaMKIIβ A303R KI mice probably reflect the recurring theme of redundancy and the engagement of compensatory mechanisms in postsynaptic CaMKII targeting.

When neuronal network activity is decreased, postsynaptic AMPA receptor (AMPAR) content and responses and probably spine size increase over the synapse population to maintain homeostasis of overall excitatory inputs into this neuron (Murthy et al., 2001; Turrigiano, 2008). In parallel, expression and spine content of CaMKIIβ are increased and CaMKIIα expression is decreased (Thiagarajan et al., 2002). With the emerging role of CaMKIIβ in stabilizing F-actin, it appears likely that the homeostatic increase in synaptic strength is at least in part due to the CaMKIIβ-mediated increase in F-actin content, which in turn leads to larger spine size and thereby higher postsynaptic strength. Indeed, knockdown of CaMKIIβ prevents the increase in postsynaptic GluA1 that is otherwise observed upon chronic inhibition of neuronal activity by TTX (Groth et al., 2011) and overexpression of CaMKIIβ increases miniature excitatory postsynaptic current (mEPSC) frequency probably by increasing synapse density (Thiagarajan et al., 2002).

As discussed above, α-actinin fosters the interaction of CaMKII with F-actin (Jalan-Sakrikar et al., 2012). The interplay among α-actinin, CaMKIIα/β dodecamers, and F-actin probably helps to organize the F-actin network in spines (Burette et al., 2012; Korobova and Svitkina, 2010). α-actinin by itself mediates formation of parallel F-actin filaments or F-actin bundling (Meyer and Aebi, 1990; Pavalko and Burridge, 1991; Wachsstock et al., 1993). In neurons, overexpression of α-actinin-2 induces long filopodia-like structure on dendrites (Hoe et al., 2009; Nakagawa et al., 2004), which mainly contain parallel F-actin bundles (Korobova and Svitkina, 2010). Furthermore, CaMKIIβ knockdown

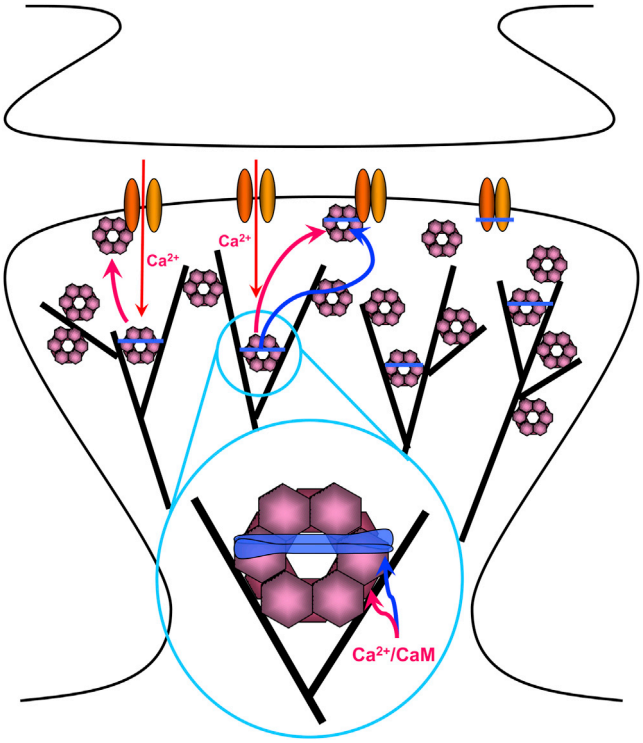

Figure 3. Interactions of CaMKII with F-Actin, α-Actinin, and NMDARs in Spines

Under basal conditions, CaMKII (pink) is mostly associated with F-actin (black lines). This interaction might also localize CaMKII to an area 50–100 nm underneath the center of the PSD with high CaMKII concentrations also present at the lateral edges of the PSD (Ding et al., 2013). The figure envisions that this association with F-actin occurs in conjunction with α-actinin (blue) as CaMKIIβ subunits within the dodecameric CaMKII complexes as well as α-actinin directly bind to F-actin and to each other. The resulting "triades" (magnified area) are predicted to foster a highly branched F-actin cytoskeleton rather than the parallel fiber arrangement induced by α-actinin alone in the absence of CaMKII. Ca^{2+} influx via NMDARs, which consist of GluN1 (yellow) and GluN2 (orange) subunits, triggers release of CaMKII from F-actin as Ca^{2+}/CaM will displace CaMKIIβ from F-actin (red arrow in insert) and CaMKIIα and CaMKIIβ from α-actinin (blue arrow in insert). CaMKII will then bind to the NMDAR subunit GluN2B (top left area of PSD), which requires either Ca^{2+}/CaM or the more lasting T286/287 autophosphorylation. After removal of Ca^{2+}/CaM, α-actinin will reassociate with CaMKII, possibly forming a trimeric complex with GluN2B (top middle area; right area depicts an α-actinin-NMDAR complex without CaMKII).

not only reduces the number and size of spines but also increases the number of such filopodia-like dendritic protrusions (Okamoto et al., 2007). This outcome of CaMKIIβ knockdown is consistent with and best explained by α-actinin being the prevailing determinant of the parallel F-actin fibers in those protrusions, contrasting the CaMKIIβ-supported branched F-actin in spines (Korobova and Svitkina, 2010). Thus, in conjunction with CaMKIIβ, α-actinin may support the branched F-actin cytoskeleton rather than a parallel arrangement of F-actin fibers in spines (Figure 3). Binding of α-actinin to F-actin opens up access to the EF hands near α-actinin's C terminus (Figure 4) (Travers et al., 2013), which in turn bind to the regulator domain of CaMKII (Figure 2). In fact, the density of F-actin branching points appear to be highest ~20 nm interior to the PSD, with dense accumulation of branching points at the lateral edges of the PSD (Burette et al., 2012). This distribution of branched F-actin matches quite well the distribution of CaMKII in spines (Ding et al., 2013). Other F-actin regulators and especially the Arp2/3 complex, which promotes F-actin branching, are likely to assist in induction of branched fiber formation (Korobova and Svitkina, 2010; Rácz and Weinberg, 2008).

Role of CaMKII Anchoring at the PSD

At first glance, CaMKII's abundance suggests that it might not need to be precisely targeted within PSDs to fulfill its role in postsynaptic signaling. However, anchoring by densin and α-actinin can refine its substrate selectivity and, as I will discuss below, activity-driven binding to GluN2B is important for LTP and spine stabilization, indicating that its accurate anchoring within the PSD does matter. Anchoring of CaMKII makes phosphorylation faster, more efficient, and much more selective. The relative slow kinetics of CaMKII (~10/s [Coultrap and Bayer, 2012]) renders kinase anchoring all the more important for effective phosphorylation of key targets and probably reflects that it mainly phosphorylates substrates within its immediate vicinity rather than mediating high throughput phosphorylation of many proteins within a larger area. In parallel, notably, the upstream regulator of CaMKII, CaM, is itself anchored at the PSD by neurogranin, which recruits apo-CaM to postsynaptic sites and releases Ca^{2+}/CaM upon Ca^{2+} influx. This mechanism ensures that sufficient CaM is present in spines to allow for effective signaling (Zhabotinsky et al., 2006; Zhong et al., 2009). The precise spatial and consequent functional arrangements of CaM and neurogranin with respect to CaMKII remain to be defined.

Activity-Dependent CaMKII Binding to NMDAR

The bulk of CaMKII is in the interior of a spine (Ding et al., 2013; Feng et al., 2011; Tao-Cheng et al., 2007), as this space accounts for a much larger fraction of the spine volume than the area immediately beneath the PSD. Interestingly, in quickly perfused rat brain (1.5 min; to prevent postmortem CaMKII clustering at PSDs), the concentration of CaMKII shows a distinct peak about 40 nm away from PSD along the axodendritic position but falls off toward the spine center to about one-third of the peak concentration. It declines to even lower values (approximately one-tenth of peak at 40 nm) at the PSD center near the plasma membrane along the axodendritic axis, although it is larger at the periphery than in the center of the PSD (Ding et al., 2013). Ca^{2+} influx via NMDAR upon LTP induction induces relocation of CaMKII from spine center to PSD within <2 min (Ding et al., 2013; Dosemeci et al., 2002; Otmakhov et al., 2004; Tao-Cheng et al., 2007), possibly much faster (Figure 3). These findings and the above biochemical data showing that CaMKIIα/β dodecamers are linked to F-actin directly via CaMKIIβ and indirectly via α-actinin and that Ca^{2+}/CaM severs these interactions, suggest the following scenario. Under basal conditions, CaMKII is largely anchored to F-actin in the interior of spines. Within seconds, perhaps milliseconds, of Ca^{2+} influx, Ca^{2+}/CaM will release CaMKII from F-actin and α-actinin for rapid relocation to the PSD. Within 1–2 min of Ca^{2+} influx, redistribution of bulk CaMKII from shaft to spines also becomes obvious (Otmakhov et al., 2004; Shen and Meyer, 1999; Shen et al., 2000) (for endogenous CaMKII, see Ding et al., 2013;

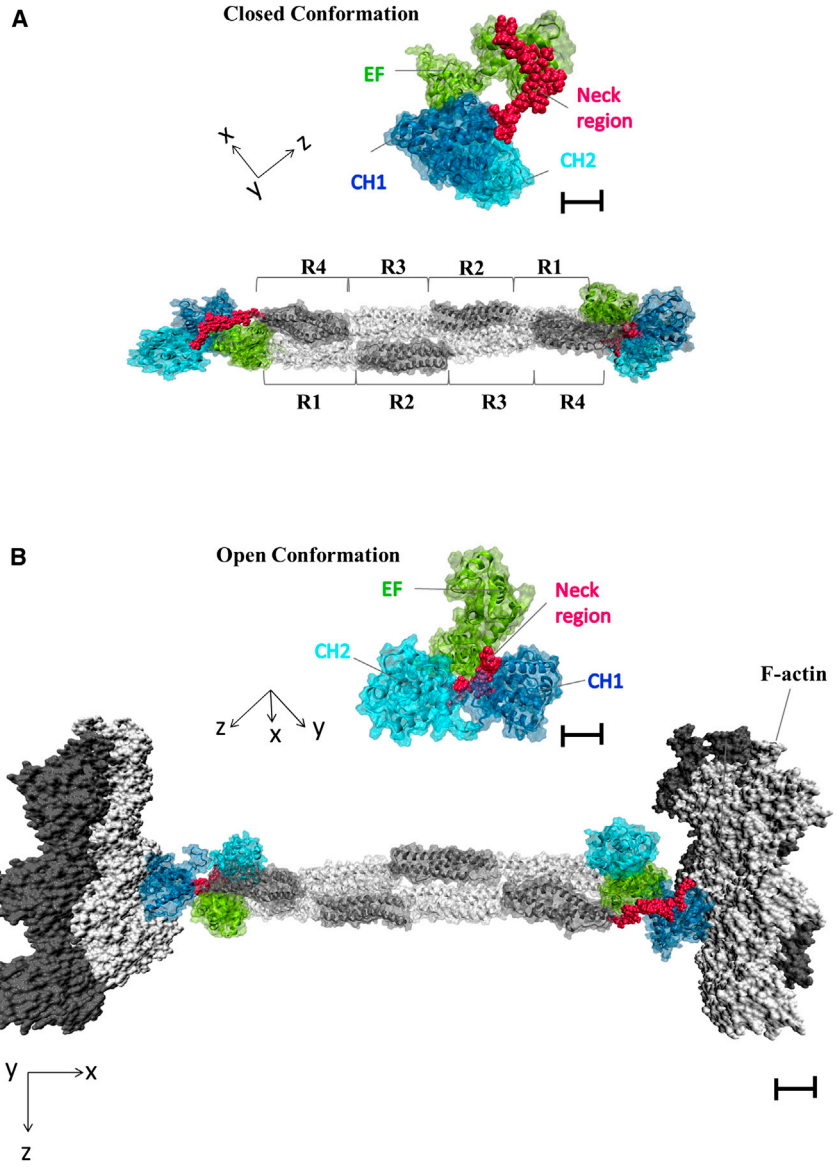

Figure 4. Binding of α-Actinin to F-Actin Makes EF3 and EF4 Accessible for CaMKII Binding

(A) Native structure of α-actinin dimer (PDB ID: 1SJJ) with both actin binding domains (CH1 and CH2) in a closed conformation (insert on top of the full-length α-actinin structural model). In this structure, EF3 and EF4 from the C terminus of one protomer interacts with the neck region, which connects CH2 with SR1 in the other protomer (Travers et al., 2013). CH1 is quasifolded back like a hook toward the SR region.

(B) Actin dimers as present in F-actin (PDB ID: 3G37) are docked onto the α-actinin structure depicting the F-actin crosslinking activity of α-actinin. CH1 binds to F-actin (Travers et al., 2013). The CH1 and CH2 domains are in an open conformation in this F-actin-bound state (insert on top of the full-length α-actinin-F-actin structural model) as observed (Galkin et al., 2010). EF3 and EF4 of the second antiparallel α-actinin protomer are displaced from the neck region as predicted rendering them accessible for CaMKII binding. Scale bars represent 20 Å for main figure and 10 Å for enlarged inserts.

Merrill et al., 2005; Strack and Hell, 2008). A two-step process is likely in place in which CaMKII will relocate to postsynaptic sites rather quickly from the spine interior and more slowly from shafts.

The Ca^{2+}-triggered increases in CaMKII content of spines and isolated PSDs depend on the activity-driven binding of CaMKII to the C-terminal tail of GluN2B (aa 1,290–1,309); both effects are abrogated in $GluN2B_{L1298A/R1300Q}$ KI mice in which CaMKII binding to GluN2B is eliminated (Halt et al., 2012). Hence, GluN2B association is a critical requirement for recruitment of CaMKII to postsynaptic sites. The dependence of activity-triggered CaMKII accumulation in spines on GluN2B binding is especially remarkable as the space in the spine interior is much larger than underneath the PSD. Even though it has been estimated that ~5% of the total CaMKII content within a spine is concentrated at its PSD (Feng et al., 2011), it appears that a clearly distinguishable change in total spine CaMKII would have to involve a change in the spine cytosol and not just at the PSD. CaMKII binding to GluN2B might have effects that reach throughout the whole spine. Newly recruited CaMKII might bind to CaMKII that is already anchored within the spine, including CaMKII associated with GluN2B (Hudmon et al., 2005a) (see also tower-like CaMKII structures in Petersen et al., 2003) and with F-actin/α-actinin due to Ca^{2+}/CaM-induced CaMKII self-aggregation. Perhaps association of CaMKII with GluN2B at the PSD somehow fosters CaMKII autophosphorylation and thereby aggregation in the spine interior potentially by saturating the phosphatases that otherwise dephosphorylate T286 (Lisman et al., 2002).

The stoichiometry of CaMKII and GluN2B in PSD also deserves further consideration. Given that a typical PSD has ~80 CaMKII dodecamers/0.1 μm^2 (Chen et al., 2005), which translates into up to ~240 dodecamers in larger PSDs (Feng et al., 2011), but has only at most 10–20 GluN2B-containing NMDARs (Feng et al., 2011), it appears likely that CaMKII is anchored not only via GluN2B but also other binding sites. Additional sites that require CaMKII activation via Ca^{2+}/CaM or T286 autophosphorylation for binding are present on GluN1 (aa 845–861) and another site within the membrane proximal half of the C terminus of GluN2B (aa 839–1,120) (Leonard et al., 2002; Merrill et al., 2005). Densin is also part of the PSD (Walikonis et al., 2000) and α-actinin can associate with the PSD via binding to densin and NMDAR subunits (Walikonis et al., 2001; Wyszynski et al., 1997), constituting additional CaMKII attachment sites. It is unclear whether densin and α-actinin form CaMKII anchor sites

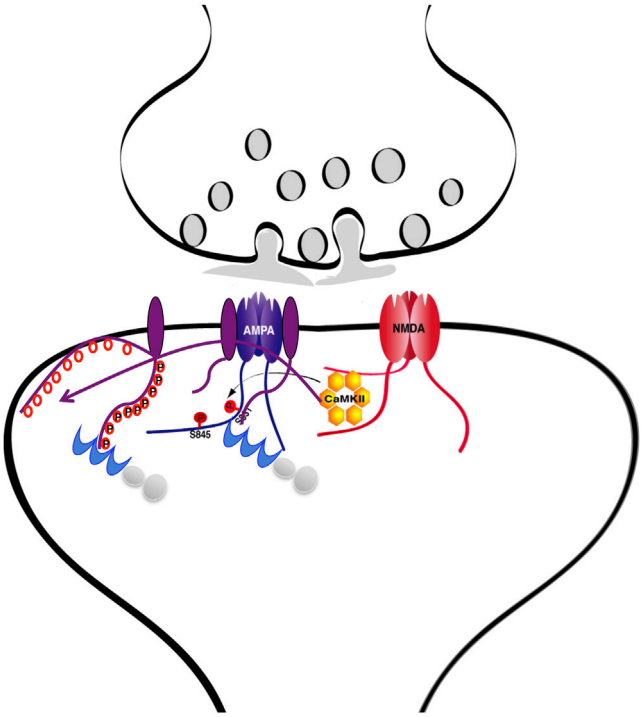

Figure 5. Role of CaMKII Binding to GluN2B in AMPAR Phosphorylation

Ca^{2+} influx during LTP will induce association of CaMKII with the NMDAR. From there, CaMKII can reach and phosphorylate neighboring AMPARs. Phosphorylation of GluA1 on S831 will immediately increase conductance through AMPARs (dark purple). The Ca^{2+} influx will also facilitate detachment of the cytosolic C termini of TARPs (dark magenta), which have multiple positively charged Arg and Lys residues. The detachment will make nine phosphorylation sites on Stg (red circles) available for CaMKII. The ensuing phosphorylations will reduce the net positive charge and thereby reassociation with the plasma membrane. As a result, the number of TARPs whose C termini are available for binding to PDZ domains of PSD-95 (blue) will be increased for enhanced trapping of AMPAR-TARP complexes at postsynaptic sites (Opazo et al., 2010).

separate from GluN2B and from each other or if they are part of the same complex. Formation of such a complex is conceivable as the dodecameric CaMKII can simultaneously interact with all three proteins (Robison et al., 2005b), which can also interact with each other. If densin, α-actinin, and GluN2B together form CaMKII anchoring sites, their number would be 10–20 but with multiple attachment sites for CaMKII.

CaMKII binding to $GluN2B_{1290-1309}$ needs either Ca^{2+}/CaM or T286 phosphorylation because binding occurs at the T site, similar to T286 (Figure 2) (Bayer et al., 2001; Leonard et al., 1999, 2002). As long as a CaMKII subunit is bound to $GluN2B_{1290-1309}$, T286 and with it the downstream inhibitory segment will not be able to rebind to the T and S site, respectively, keeping CaMKII constitutively active (Bayer et al., 2001). This autonomous activity is lower than that induced by T286 phosphorylation; nevertheless, this displacement is analogous to T286 phosphorylation in that it can promote T286 rephosphorylation upon suboptimal Ca^{2+} influx of a neighboring subunit that lost its T286 phosphate due to suboptimal Ca^{2+} influx. This effect is in place because only that neighboring subunit and not the $GluN2B_{1290-1309}$-bound subunit requires new Ca^{2+}/CaM binding in contrast to unbound and completely unphosphorylated CaMKII. This mechanism has the potential to further perpetuate CaMKII activation beyond T286 autophosphorylation while being impervious to dephosphorylation (Lisman et al., 2002). Furthermore, analogous to autophosphorylation (Singla et al., 2001), GluN2B binding could increase the affinity of CaMKII for Ca^{2+}/CaM resulting in Ca^{2+}/CaM trapping. Ca^{2+}/CaM trapping by GluN2B-bound CaMKII would also promote rephosphorylation of a neighboring subunit by making it easier for the GluN2B-associated subunit to capture Ca^{2+}/CaM upon submaximal Ca^{2+} influx, thereby fostering simultaneous binding of Ca^{2+}/CaM to this and the neighboring subunit.

Role of CaMKII Binding to NMDAR in LTP

Ca^{2+} influx via NMDARs induces CaMKII binding to GluN2B (Leonard et al., 1999, 2002; Strack and Colbran, 1998; Strack et al., 2000a). This interaction is important for LTP. Ectopic expression of GluN2B with two point mutations that eliminate CaMKII binding ($GluN2B_{R1300Q/S1303D}$) in cultured hippocampal slices abrogates LTP induced by pairing postsynaptic depolarization with a 3 Hz/90 s stimulus train (Barria and Malinow, 2005). Furthermore, field LTP induced by two 100 Hz/1 s stimulus trains or by theta burst stimulation is reduced by 50% in $GluN2B_{L1298A/R1300Q}$ knockin mice (Halt et al., 2012). Why overexpression of $GluN2B_{R1300Q/S1303D}$ would completely prevent LTP induced by a rather strong pairing protocol when similar point mutations in KI mice only partially affect two forms of LTP induced by milder induction protocols is unclear.

GluN2B binding recruits CaMKII to strategically ideal locations, placing CaMKII at the source of Ca^{2+} influx and near AMPAR (Figure 5) (Leonard et al., 1999). CaMKII can reach substrates that are 20 nm, if not farther, away from its anchoring sites, as the dodecameric cylinder of CaMKII is ~20 nm long and the 12 kinase domains point to multiple directions (Chao et al., 2011). If we assume that a typical PSD harbors 10–20 GluN2B-containing NMDARs (Feng et al., 2011) and that they are relatively evenly spaced across a PSD and interspersed with 40 AMPARs, their distance is well within this range. Functional studies in $GluN2B_{L1298A/R1300Q}$ knockin mice clearly show that CaMKII binding to GluN2B is important for NMDA-induced phosphorylation of GluA1 on S831 and chemical LTP-induced augmentation of the AMPAR auxiliary subunit γ8 in the PSD beyond the initial stimulation period of 5 min (Halt et al., 2012).

As α-actinin also directly binds to GluN1 and GluN2B (Figure 2) (Wyszynski et al., 1997), it stabilizes CaMKII binding to NMDARs and augments GluN2B phosphorylation on S1303. However, at the same time, α-actinin inhibits GluA1 phosphorylation on S831 as it antagonizes Ca^{2+}/CaM binding to CaMKII when ectopically expressed in HEK293 cells (Jalan-Sakrikar et al., 2012). S831 in the C-terminal tail of GluA1 is an important CaMKII site for upregulation of AMPAR channel conductance (Kristensen et al., 2011; Oh and Derkach, 2005). Thus α-actinin might stabilize the postsynaptic structure by linking the NMDAR-CaMKII complexes via multiple interactions to F-actin. It might in parallel curb in such stabilized structures of upregulation of AMPAR activity by CaMKII-mediated S831 phosphorylation to

preserve the status quo of the postsynaptic site with a rather modest level of S831 phosphorylation. To caution, however, it is also quite possible that α-actinin binding to one or two CaMKII subunits in a dodecameric complex recruits CaMKII to the neighborhood of postsynaptic AMPAR, with the other subunits being freely available for enhanced GluA1 S831 phosphorylation in vivo upon such α-actinin-mediated CaMKII anchoring.

Activity-Driven Accumulation of CaMKII in Spines and a Role for L-type Ca^{2+} Channels

In single spines, potentiation by high-frequency glutamate uncaging doubles the amount of total as well as anchored CaMKII within 30–40 min, largely paralleling the lasting increase in spine size (Lee et al., 2009; Zhang et al., 2008). However, it should be noted that CaMKII clustering trailed spine enlargement by ~10 min in one study (Zhang et al., 2008) but not in another (Lee et al., 2009). The former timing of CaMKII clustering is similar to the delayed accumulation of GluA1 at the spine surface in other work (Kopec et al., 2006), which hints at a potentially causal relationship. The correlated increases in the spine size and bulk CaMKII content upon potentiation lead to the suggestion that the increase in bulk CaMKII accumulation during later phases of potentiation is to a good degree due to binding of CaMKII to F-actin (Lisman et al., 2012), which is consistent with a structural role for CaMKII in maintaining F-actin in an interdependent relationship as discussed in detail above.

Chemically induced LTP, which activates all functional synapses, results in postsynaptic CaMKII clustering that persists for >2 hr (Otmakhov et al., 2004). However, it is unclear whether this lasting increase would apply to individually stimulated spines as puffing glutamate onto a small dendritic region results in postsynaptic CaMKII accumulation throughout the dendritic arbor, indicating that activation of multiple synapses utilizes mechanisms for CaMKII accumulation that might differ from those for individual spine stimulation (Rose et al., 2009) (for lack of this widespread CaMKII clustering in a different hippocampal culture system without astrocyte feeder layers and without AP5 used during culturing, see Lemieux et al., 2012). Similarly, electrophysiologically induced LTP, which probably potentiates much less than 1% of all synapses, results in CaMKII T286 autophosphorylation throughout the dendritic area, suggesting widespread CaMKII activation (Ouyang et al., 1997, 1999). The widespread CaMKII clustering in spines that is induced by a local glutamate puff (but not global glutamate application) depends not only on NMDAR but also L-type Ca^{2+} channels (Rose et al., 2009). The L-type channel $Ca_v1.2$ itself also functions as a CaMKII anchor protein and allows CaMKII stimulation within the Ca^{2+} nanodomain at the pore (Abiria and Colbran, 2010; Grueter et al., 2006; Hudmon et al., 2005b). This interaction is complex as CaMKII can bind to the IQ motif region (Hudmon et al., 2005b) and the auxiliary β subunits β_1 and β_2 (Abiria and Colbran, 2010; Grueter et al., 2006). The IQ region in the C terminus of the central pore-forming $\alpha_1 1.2$ subunit has been implicated in both Ca^{2+}-dependent inactivation (CDI) and Ca^{2+}-dependent facilitation (CDF) of $Ca_v1.2$ currents (Zühlke et al., 1999). CDI is mediated by CaM, which also interacts directly with this region in a rather complex manner (Johny et al., 2013). CDF can be observed upon repeated stimulation (Dzhura et al., 2000), requires CaMKII binding to the IQ region (Hudmon et al., 2005b) and β_1 or β_2 (Abiria and Colbran, 2010; Grueter et al., 2006), and phosphorylation of β_2 by CaMKII in the $Ca_v1.2$ complex (Abiria and Colbran, 2010; Grueter et al., 2006).

L-type channels are enriched at postsynaptic sites (Davare et al., 2001; Hell et al., 1993, 1996), but it is unclear whether CaMKII binding to L-type channels per se is required for the widespread CaMKII clustering in spines throughout dendrites upon their localized stimulation. However, CaMKII binding to the $Ca_v1.2$-related $Ca_v1.3$ L-type channel is required for activation of CaMKII-dependent CREB-mediated gene transcription upon weak but not strong depolarization of cultured superior cervical ganglion (SCG) neurons (Wheeler et al., 2012). Activation of CREB upon L-type channel activation is blocked by the fast Ca^{2+}-chelator BAPTA but not the slower chelator EGTA (Wheeler et al., 2012), suggesting that influx of Ca^{2+} activates CaMKII associated with L-type channels in a spatially restricted manner, potentially limited to nanodomains surrounding individual channels (Neher, 1998). Interestingly, clustering of CaMKII at $Ca_v1.3$ puncta (the main L-type channel in SCG) can be induced by both weak depolarizations that selectively activate $Ca_v1.3$ but also stronger depolarizations that selectively activate N-type currents in the presence of L-type blockers. CaMKII does not cluster at N-type Ca^{2+} channel puncta (the main non-L-type high threshold Ca^{2+} channel in SCG) (Wheeler et al., 2012). Accordingly, L-type channels can serve as hubs for CaMKII signaling, probably bringing together various signaling components even if they have to ultimately reach far away sites such as the nucleus.

Monitoring CaMKII Dynamics in Spines by FLIM

Measuring fluorescence lifetime (FLIM) to monitor activation of CaMKII doubly tagged with mEGFP and REACh, Lee et al. found that induction of LTP by repetitive glutamate uncaging resulted in a surprisingly transient change in the FLIM signal (Lee et al., 2009). Earlier evidence had indicated that CaMKII activity undergoes a prolonged increase in CaMKII activity upon LTP induction at potentiated spines (Lisman et al., 2002). While Lee et al.'s data suggest that activation of bulk CaMKII may be only short lived in spines, a more cautious interpretation is warranted. However, CaMKII can exist in different activated states with Ca^{2+}/CaM, resulting in maximal activation without T286 autophosphorylation (Braun and Schulman, 1995; Coultrap et al., 2010). T286 phosphorylation keeps the kinase active beyond Ca^{2+} and CaM dissociation but at a significantly lower level than Ca^{2+}/CaM (Braun and Schulman, 1995; Coultrap et al., 2010). Yet, the FLIM signals are actually several fold more strongly influenced by T286 autophosphorylation than by Ca^{2+}/CaM binding (Lee et al., 2009). For instance, T286A mutant CaMKII shows far fewer changes in FLIM upon Ca^{2+}/CaM addition than wild-type (WT) CaMKII even though it can be fully activated by Ca^{2+}/CaM (Lee et al., 2009). Accordingly, the FLIM signals faithfully reflect only conformational changes of CaMKII and are not a direct measure of its catalytic activity. The relaxation of FLIM signals shortly after LTP might thus indicate a conformational change to a discrete, yet still catalytically active, conformation. Also, it is unknown how binding of CaMKII to its anchoring sites affects the FLIM signals. It is possible that

binding to GluN2B, densin, or α-actinin substantially reduces fluorescence lifetime without strongly affecting CaMKII activity.

The above FLIM studies also suggest that CaMKII activation in spines upon depolarization (in the absence of glutamate receptor activation) depends on Ca^{2+} influx via L-type channels even though this influx only contributes a small amount of total Ca^{2+} influx into spines in this scenario (Lee et al., 2009). Furthermore, 20 mM of the fast Ca^{2+} chelator BAPTA, but not 20 mM of the slow Ca^{2+} chelator EGTA, blocked this CaMKII activation in spines. As BAPTA but not EGTA is fast enough to intercept Ca^{2+} close to the channel mouth to interfere with its signaling to nearby Ca^{2+} target sites, these results suggest once more that depolarization-induced Ca^{2+} influx activates CaMKII molecules that reside within nanodomains near L-type channels (Neher, 1998) and are likely tethered to $Ca_v1.2$ (Hudmon et al., 2005b). Such a highly localized effect of Ca^{2+} influx via L-type channels is also in agreement with most recent findings that stimulating hippocampal slices with glutamate leads to displacement of α-actinin from the IQ motif of $Ca_v1.2$, which otherwise anchors $Ca_v1.2$ at postsynaptic sites (Hall et al., 2013). This effect is blocked by inhibition of L-type channels, but not NMDARs, reflecting a highly localized Ca^{2+}-mediated effect in spines within the immediate environment of $Ca_v1.2$ that cannot be mediated by robust Ca^{2+} influx via NMDARs within the same spines.

What is, however, not immediately compatible with the finding that depolarization-induced activation of CaMKII is mediated by highly localized Ca^{2+} influx via L-type channels is that CaMKII activation in spines upon robust Ca^{2+} influx through NMDAR was fully sensitive not only to 5 mM BAPTA but also to 5 mM EGTA (Lee et al., 2009). In other words, these observations suggest that EGTA-sensitive global rather than local Ca^{2+} signaling is important for CaMKII activation, although this latter finding is consistent with the model that the bulk of CaMKII is associated with F-actin in the spine interior under resting conditions and activated upon delocalized Ca^{2+} influx (Figure 3). Clearly more work is needed to reconcile these findings. Also, it is quite possible that a subpopulation of CaMKII that is too small to be detected by the above FLIM studies would relocate to the PSD and stay active for much longer than the bulk of CaMKII in spines (Lisman et al., 2012).

Dissociation of Spine Size and Postsynaptic Strength by T305/T306 Phosphorylation

Expression of the phosphomimetic CaMKIIα mutation T286D induces synaptic weakening (Pi et al., 2010b). This finding is surprising because strengthening rather than weakening would have been predicted, as CaMKIIα T286D is constitutively active like truncated $CaMKII\alpha_{1\text{-}290}$, which increases EPSC amplitude (Pi et al., 2010b). As it turns out, the CaMKIIα T286D mutant becomes phosphorylated on T305 or T306. Preventing T305/T306 phosphorylation in the CaMKIIα T286D/T305A/T306A triple mutant leads to the expected potentiation rather than depression of synaptic transmission (Pi et al., 2010b). The phosphomimetic CaMKIIα T286D/T305D/T306D mutant, however, induces depression. At the same time, all of the CaMKIIα mutants tested that include T286D increased spine size in organotypic cultures (Pi et al., 2010a), potentially by inducing T site interaction with GluN2B or another T site binding protein. Accordingly, T286 phosphorylation is necessary and sufficient for CaMKIIα to augment spine size independent of T305/T306 phosphorylation status. CaMKIIα activation is neither sufficient nor necessary for the increase in spine size or the decrease in EPSC amplitude, as the kinase dead mutation K42R did not prevent spine enlargement or EPSC reduction by CaMKIIαK42R/T286D expression. At the same time, CaMKIIα activity appears to be critical for the increase in EPSC amplitude seen with $CaMKII\alpha_{1\text{-}290}$, as overexpression of WT CaMKIIα had no effect on AMPAR EPSC amplitude (Pi et al., 2010b). Further puzzling, CaMKIIα T286D/T305D/T306D as well as CaMKIIα T286D/T305A/T306A prevented LTP, the latter potentially by occlusion as it induces potentiation upon its ectopic expression, whereas single mutant CaMKIIα T286D showed nearly normal LTP (Pi et al., 2010b).

Given that the T306D mutation and thus likely T306 phosphorylation block α-actinin binding (Jalan-Sakrikar et al., 2012), it is conceivable that T305/T306-phosphorylated CaMKIIαT286D and CaMKIIαT286D/T305D/T306D show reduced EPSCs due to loss of α-actinin binding. Binding to α-actinin may play a hitherto unappreciated role in CaMKII anchoring at postsynaptic sites, possibly via formation of a complex between CaMKII, α-actinin, densin, and the NMDAR (Figure 2), which could be important for synaptic strength independent of spine size.

The fact that T305/T306-phosphorylated CaMKIIαT286D and CaMKIIαT286D/T305D/T306D increases spine size when decreasing postsynaptic response strength is remarkable because it shows that the two parameters do not have to always be correlated (see also Wang et al., 2007). This loss of correlation could indicate that T305/T306 phosphorylation affects the coupling of size and AMPAR content of spines. Many molecular mechanisms could be invoked. As T286 phosphorylated or T286D mutated CaMKII binds to GluN2B and also the central densin domain (Figure 2), these interactions could support CaMKII functions that are not engaged under basal conditions and are only modestly affected by T305/T306 phosphorylation (Leonard et al., 2002) but can augment F-actin in spines and thereby spine size and postsynaptic AMPAR number or activity upon stimulation. One candidate mechanism is CaMKII-mediated phosphorylation of Kalirin 7, which promotes F-actin formation via Rac (Xie et al., 2007), although there is no evidence that its phosphorylation by CaMKII requires CaMKII binding to GluN2B or densin. On the other hand, T305/T306 phosphorylation may engage a second mechanism that acts to reduce AMPAR strength by recruiting proteins that negatively regulate availability of functional AMPARs at postsynaptic sites such as the kinase Cdk5 (Morabito et al., 2004; Seeburg et al., 2008). Cdk5 binds via its activator p35 to CaMKII (Dhavan et al., 2002) and this interaction is augmented by CaMKII activation by Ca^{2+}/CaM. It is possible, but highly speculative, that T305/T306 autophosphorylation subsequent to T286 phosphorylation is responsible for this increase in CaMKII-Cdk5 binding, thereby recruiting Cdk5 to postsynaptic sites for downregulation of AMPARs. Finally, T305/T306 phosphorylation impairs retention of CaMKII that accumulates upon Ca^{2+} influx in spines (Shen et al., 2000) and modestly reduces binding of CaMKII to GluN1 and GluN2B by ~50% (Leonard et al., 2002), which could negatively affect its actions at the PSD. Clearly, we are missing

important details in our understanding of how CaMKII regulates spine size and especially postsynaptic strength.

CaMKII as Docking Protein for Arc

Further underscoring its structural functions, CaMKII is emerging as a docking protein for several other proteins in spines, including Arc/Arg3.1. Arc binds to the endocytic proteins dynamin and endophilin and is important for postsynaptic removal of AMPAR especially upon homeostatic down scaling of postsynaptic strength (Chowdhury et al., 2006; Shepherd et al., 2006). Although Arc expression requires synaptic activity, it is preferably recruited by CaMKIIβ to spines of low activity (Okuno et al., 2012). CaMKIIβ binds Arc much more tightly in the absence of Ca^{2+}/CaM and T287 autophosphorylation (Arc binding to CaMKIIα is weak under all conditions) (Okuno et al., 2012). Thus, CaMKIIβ acts to curb an increase in synaptic strength under basal conditions and can in fact counteract spine size expansion by recruiting Arc to less active synapses in vivo and in culture (Okuno et al., 2012). This "inverse tagging" of inactive spines might contribute to the synapse specificity of LTP as it will mainly affect nonpotentiated synapses. In this way, it might cause a modest homeostatic synaptic down scaling of the large majority of nonpotentiated synapses as required to keep the overall synaptic input within a defined dynamic range and to prevent overexcitability after potentiation of a subpopulation of synapses.

CaMKII as Docking Protein for Proteasomes

CaMKII recruits proteasomes to spines (Bingol et al., 2010). Notably, proteasome activity is not only needed for long-term depression (LTD) but also LTP, perhaps because negative regulators of postsynaptic strength have to be removed, including rigid scaffolds formed by structural proteins (Bingol and Sheng, 2011), Arc (Chowdhury et al., 2006; Shepherd et al., 2006), the small G proteins Rap1 and Rap2 and their upstream activators EPAC2 (Woolfrey et al., 2009; Zhu et al., 2002, 2005), the Rho family of small G proteins and its upstream activators Ephexin 1 and 5 (Margolis et al., 2010), the cyclin-dependent kinase Cdk5 (Morabito et al., 2004; Seeburg et al., 2008), and the polo-like kinase Plk2 (Morabito et al., 2004; Seeburg et al., 2008). Ca^{2+} influx via NMDARs augments CaMKII accumulation in stimulated spines just before proteasome accumulation. The capability of CaMKII to bind and thereby recruit proteasomes depends on its activation by Ca^{2+}/CaM and on T286 autophosphorylation and on its binding to GluN2B (Hamilton et al., 2012). This dual requirement for CaMKII activation for binding to its own docking site on GluN2B on one hand and to proteasome on the other will assure that proteasome accumulation mainly occurs in spines that are experiencing high activity. Overexpression of CaMKII with the phosphomimetic T286D mutation to allow GluN2B and proteasome binding plus the K42R mutation in the catalytic site to inactivate the kinase activity (and thereby perhaps T305/T306 phosphorylation) also promotes postsynaptic proteasome accumulation (Bingol et al., 2010). These results once more indicate that CaMKII can play a structural role by demonstrating its ability to function as an activity-dependent, autoregulated postsynaptic proteasome scaffold. This mechanism is not only important for LTP but also for activity-induced formation (Hamilton et al., 2012) and stabilization (Hill and Zito, 2013) of new spines.

CaMKII as Docking Protein for Casein Kinase 2

CaMKII also acts as a scaffold to recruit casein kinase 2 (CK2) to GluN1/GluN2B complexes. CK2 phosphorylates S1480 in the GluN2B SXV motif (Chung et al., 2004; Sanz-Clemente et al., 2010), which mediates binding of the receptor to PSD-95 or its homologs PSD-93 or SAP102 and regulates its postsynaptic targeting (Elias et al., 2006, 2008; Prybylowski et al., 2005). Activation of CaMKII by Ca^{2+}/CaM is required for phosphorylation of GluN2B S1480 by CK2, which blocks PSD-95 binding and impairs postsynaptic NMDAR targeting (Sanz-Clemente et al., 2010, 2013). As for proteasomes, CaMKII autophosphorylation probably enhances the recruitment of CK2 to GluN2B synergistically with changes in Ca^{2+} concentration such that the effect becomes rapidly stronger with further Ca^{2+} influx above a certain Ca^{2+} threshold.

The activation-dependent recruitment of proteasomes and CK2 to GluN2B by CaMKII illuminates once again CaMKII's role as a multivalent adaptor protein whose protein interactions at various sites are regulated by the kinase activity intrinsic to each subunit of the dodecamer (see also Robison et al., 2005b).

Role of CaMKII and Its Anchoring by GluN2B in LTP and Synapse Selectivity of LTP

CaMKII (and PKC) can phosphorylate GluA1 on S831 to increase AMPAR conductivity (Kristensen et al., 2011; Oh and Derkach, 2005) and EPSCs during LTP (Benke et al., 1998). S831 phosphorylation depends on CaMKII binding to $GluN2B_{1290-1309}$ (Halt et al., 2012). CaMKII (and PKC) can also phosphorylate stargazin (Stg/γ2) on as many as nine serine residues (Figure 5). Stg/γ2 is a member of the TARP family of AMPA receptor auxiliary subunits that mediate postsynaptic AMPAR recruitment by PSD-95 and PSD-93 (Chen et al., 2000; Elias et al., 2006, 2008; Schnell et al., 2002). Ectopic expression of phosphomimetic and -preventive Asp and Ala mutants of all nine residues occludes or prevents, respectively, pairing-induced LTP (Tomita et al., 2005) and postsynaptic AMPAR trapping by CaMKII activity (Opazo et al., 2010). As Ca^{2+} potently disrupts electrostatic interactions between proteins and the plasma membrane (Zilly et al., 2011), Ca^{2+} influx through the NMDAR probably promotes this Stg/γ2 phosphorylation by decreasing the association of the Stg/γ2 C terminus with the plasma membrane, thereby rendering it more accessible for CaMKII (Figure 5). The consequent phosphorylation prevents the Stg/γ2 C terminus from reassociating with the plasma membrane, thereby fostering PSD-95 binding (Sumioka et al., 2010). In this manner, CaMKII can elevate the number of postsynaptic AMPAR anchoring sites or "slots" that might be formed by TARPs in conjunction with PSD-95. The interaction between TARPs and PSD-95 will enhance their mutual accumulation at postsynaptic sites, thereby recruiting more AMPARs, which is thought to underlie LTP (Kerchner and Nicoll, 2008; Lisman and Hell, 2008). The nine serine residues that are phosphorylated by CaMKII in Stg/γ2 are conserved in other TARPs including γ8, the prevalent hippocampal TARP (Rouach et al., 2005). ChemLTP augments the content of γ8 and other TARPs in PSD preparations (Halt

et al., 2012). The correlation between loss of NMDAR-mediated CaMKII anchoring and loss of persistent γ8 postsynaptic accumulation following chemLTP in GluN2B KI mice (Halt et al., 2012) provides evidence for the model that phosphorylation of TARPs by CaMKII enhances clustering of AMPAR-TARP complexes at the PSD upon LTP (Hayashi et al., 2000; Sumioka et al., 2010; Tomita et al., 2005). However, it should be noted that postsynaptic localization of AMPARs by Stg/γ2 and γ8 can to some, though much limited, degree be accomplished independent of the whole C terminus of Stg/γ2 and of γ8 including their very C-terminal PDZ binding segment for PSD-95 (Milstein and Nicoll, 2009).

Given that phosphorylation of GluA1 on S831 and a lasting accumulation of γ8 in PSDs following chemLTP depend on the activity-driven CaMKII binding to $GluN2B_{1290-1309}$, it is quite conceivable that phosphorylation of Stg/γ2 and potentially of γ8 also requires CaMKII binding to GluN2B. This might also be true for CaMKII-mediated phosphorylation of the Rac GTP exchange factor kalirin-7, which augments Rac activity, F-actin formation, spine enlargement, and postsynaptic AMPAR accumulation (Xie et al., 2007). In this context, it is tempting to speculate that T305/T306 phosphorylation prevents GluN2B-associated CaMKII from phosphorylating some targets (e.g., TARPs, which directly mediate postsynaptic AMPAR targeting) but not other targets (e.g., Rac, which augments F-actin and spine size). Such a mechanism would explain why CaMKII when phosphorylated on T286 and T305 or T306 reduces postsynaptic response strength when it increases spine size. Interestingly, the structural protein CASK fosters T306/T307 phosphorylation in *Drosophila* CaMKII, circumventing T287 phosphorylation, constituting an endogenous mechanism that downregulates CaMKII activity at synapses of low activity (Hodge et al., 2006).

Further evidence for the importance of CaMKII binding to GluN2B in the maintenance phase of LTP comes from the following observations. The membrane-permeant tatCN21 peptide derived from the endogenous CaMKII inhibitory protein CaMKIIN can directly inhibit CaMKII and displace CaMKII from GluN2B (Buard et al., 2010; Sanhueza et al., 2011). Other catalytic site binding peptides (e.g., syntide) cannot disrupt the CaMKII-NMDAR interaction probably because they cannot bind with sufficient affinity to the T site. Whereas 5 μM tatCN21 is sufficient to fully block CaMKII activation in acute hippocampal slices (Buard et al., 2010), 20 μM tatCN21 is required for CaMKII displacement from GluN2B (Sanhueza et al., 2011). Although 5 μM tatCN21 is sufficient to block LTP induction when applied before the tetanus reflecting the requirement of CaMKII activity during the initiation of LTP (Buard et al., 2010), 20 μM tatCN21 concentration is necessary when applied after the tetanus to reverse LTP and prevent its maintenance (Sanhueza et al., 2011). Accordingly, it is the displacement of CaMKII from GluN2B and not its inactivation that interferes with LTP maintenance. As other inhibitors of CaMKII activity did not affect LTP maintenance, it is quite possible that CaMKII's role when bound to GluN2B is structural rather than catalytic. Additional activation-dependent binding sites for CaMKII in the C termini of GluN1, GluN2A, and a second, membrane-proximal site in the long C terminus of GluN2B that is upstream of $GluN2B_{1290-1309}$ (Leonard et al., 1999, 2002) and $densin_{793-824}$ (Carlisle et al., 2011) appear to be much less relevant (Halt et al., 2012). It should be noted, however, that peptides similar to tatCN21 can also affect CaMKII binding to densin (Jiao et al., 2011).

CaMKII activation and accumulation is limited to individual spines when those undergo potentiation by repetitive glutamate uncaging (Lee et al., 2009; Zhang et al., 2008). Given that CaMKII is necessary for standard LTP in CA1 (Lisman and Hell, 2008; Lisman et al., 2012), that abrogating postsynaptic CaMKII accumulation in GluN2B KI mice inhibits LTP (Halt et al., 2012), and that CaMKII constitutes 2%–6% of total protein in PSDs (Chen et al., 2005), it appears that activity-dependent CaMKII binding to GluN2B is a central part of the mechanism that accounts for the synapse specificity of LTP, a prerequisite for LTP's role in information storage, by recruiting CaMKII to those synapses that experience heightened Ca^{2+} influx.

Role of CaMKII in Synaptic Homeostasis

Prolonged decreases in neuronal network activity trigger increases in postsynaptic AMPAR content and responses and spine size increase over most synapses of a neuron to maintain the set point for total excitatory input (Murthy et al., 2001; Turrigiano, 2008). In parallel, levels of CaMKIIα decrease and CaMKIIβ increase (Thiagarajan et al., 2002). The opposite is true upon chronic increase of network activity, i.e., AMPAR-mediated synaptic transmission and CaMKIIβ levels decrease and CaMKIIα levels increase (Thiagarajan et al., 2002). CaMKIIα overexpression in dissociated hippocampal cultures drastically decreases mEPSC frequency (but increases mEPSC amplitude) (Thiagarajan et al., 2002). CaMKIIβ overexpression increases GluA1 protein levels (Groth et al., 2011), the number of PSD-95 positive puncta (Fink et al., 2003), and mEPSC frequency (but not amplitude) (Thiagarajan et al., 2002). Overexpression of CaMKIIα might impair spine stability and thereby synapse number by reducing the interaction of the enzyme with F-actin due to decreased CaMKIIβ content in the dodecamer, while overexpression of CaMKIIβ might have the opposite effect. The increase in mEPSC amplitude by CaMKIIα overexpression could be via GluA1 S831 and Stg phosphorylation, which would increase AMPAR conductance and abundance, respectively. Of note, EPSC amplitude was unaltered upon overexpression of CaMKIIα in CA1 pyramidal neurons in organotypic slice cultures (Pi et al., 2010b), in contrast to its effect in dissociated hippocampal cultures (Thiagarajan et al., 2002).

Four related proteins known as GKAPs (or SAPAPs) bind to the GK domain of PSD-95 to foster its postsynaptic localization. GKAPs connect PSD-95 to Shank, another important postsynaptic structural protein, which is linked to F-actin. GKAP is surfacing as an important target for CaMKII under conditions of chronically decreased as well as increased neuronal activity. Decreasing network activity in hippocampal cultures with TTX augments the number of GKAP- and PSD-95-positive synapses as well as the postsynaptic content of GKAP and PSD-95 at individual synapses (Shin et al., 2012). These increases are prevented by blocking L-type Ca^{2+} channels and by knockdown of CaMKIIβ. Knockdown of CaMKIIβ by itself reduces GKAP and PSD-95 cluster density (Fink et al., 2003; Shin et al., 2012) and spine size and number (Okamoto et al., 2007) and prevents homeostatic upregulation of GluA1 upon chronic block of AMPAR

activity (Groth et al., 2011). The observation that Ca^{2+} channel inhibition counteracts the effect of decreased network activity on GKAP and PSD-95 is surprising, as is the increase in CaMKIIβ T287 autophosphorylation upon TTX treatment, as Ca^{2+} influx via L-type channels will be reduced. Also, the finding that L-type block alone did not affect GKAP or PSD-95 clustering (Shin et al., 2012) contrasts earlier observations that such a block mimics the TTX-induced elevation of mEPSC frequency (Thiagarajan et al., 2005), the latter predicting increased synapse density and thereby PSD-95 cluster density upon chronic L-type block. Thus, more work is required to define how L-type Ca^{2+} channels mediate inactivity-triggered upregulation of postsynaptic GKAP and PSD-95 and AMPAR function.

A chimeric CaMKIIα construct carrying the F-actin binding domain of CaMKIIβ was able to rescue the loss of TTX-induced upregulation of postsynaptic GKAP clustering upon CaMKIIβ knockdown (Shin et al., 2012). This finding indicates that F-actin recruits native CaMKIIβ-containing CaMKII dodecamers to the postsynaptic site for homeostatic upregulation of postsynaptic size and strength and provides further support for the above model that CaMKIIβ is important for postsynaptic F-actin function.

How does CaMKII regulate GKAP and PSD-95 in spines? GKAP binds to dynein light chain (DLC), which links GKAP to myosin 5A (Naisbitt et al., 2000). Disrupting the DLC-GKAP interaction or knockdown of myosin 5A impairs postsynaptic GKAP localization under basal conditions (Shin et al., 2012). Further evidence suggests that CaMKII phosphorylates GKAP in its DLC binding domain on S340 and S384, which disrupts DLC binding and synaptic targeting of GKAP under resting conditions. Ectopic expression of GKAP with either phosphodeficient S340A,S384A or phosphomimetic S340D,S384D double mutations prevents the inactivity-induced postsynaptic accumulation of GKAP. These findings suggest that GKAP requires myosin 5A-dependent transport and that GKAP has to undergo a phosphorylation-dephosphorylation cycle for its postsynaptic accumulation. CaMKII-mediated phosphorylation might release GKAP from DLC after it arrives in spines. During chronic inactivity, an increase in F-actin-anchored CaMKII might augment accumulation of GKAP and thereby of PSD-95 in spines. In general agreement with these findings, expression of dominant-negative myosin 5A and myosin 5A knockdown reduces postsynaptic AMPAR content and activity in organotypic hippocampal slice cultures, especially under conditions of basal synaptic activity and blocked LTP as well as CaMKII-driven postsynaptic targeting of GluA1 (Correia et al., 2008).

Increasing network activity with a $GABA_A$ receptor antagonist bicuculline decreases the density and intensity of GKAP and PSD-95 immunofluorescent puncta (Shin et al., 2012). This effect is inhibited by NMDAR blockade and knockdown of CaMKIIα but not of CaMKIIβ. Bicuculline treatment induces ubiquitination and proteasomal degradation of GKAP, which is prevented by KN93 implicating CaMKII as one of its targets although KN93 inhibits also other CaMKs and various ion channels. In support for a role of CaMKII in regulating GKAP degradation, CaMKII disrupts binding of GKAP to PSD-95 by phosphorylating GKAP on S54 and S201 (Shin et al., 2012). Furthermore, S54 phosphorylation is required for GKAP polyubiquitination and removal from the synapse (Shin et al., 2012). Accordingly, increased Ca^{2+} influx during enhanced network activity stimulates CaMKIIα. The ensuing phosphorylation of GKAP displaces it from PSD-95, thereby leading to its ubiquitination and degradation. The loss of GKAP then translates into loss of postsynaptic AMPARs with GKAP mutation or knockdown preventing the homeostatic scaling (Shin et al., 2012).

CaMKII in Brain Diseases

Synapse dysfunction is implicated in many, perhaps the majority, of brain diseases. In disorders in which Ca^{2+} influx is dysregulated, CaMKII function is likely to be affected. While CaMKII's role in disease is far from being fully understood, we have evidence for CaMKII involvement in multiple neurological disorders. Here, I will briefly discuss emerging evidence that has begun to provide insight into how dysregulation of CaMKII activity contributes to disease.

During and subsequent to spontaneous seizures, Ca^{2+} influx through NMDARs and Ca^{2+}-permeable AMPARs can contribute to the etiology of epilepsy in part by dysregulation of CaMKII (McNamara et al., 2006). During ischemic conditions that lead to excitotoxic insult, the Ca^{2+} influx through NMDARs causes postsynaptic CaMKII activation (Westgate et al., 1994) and CaMKII inhibitors can alleviate the insult at least in the early phases (Hajimohammadreza et al., 1995; Vest et al., 2010). However, longer treatments with CaMKII inhibitors have the potential to exacerbate the damage (but see Vest et al., 2010) in part by inhibiting glutamate reuptake by astrocytes (Ashpole et al., 2013).

Accumulation of amyloid beta peptide (Aβ) is one of the two main hallmarks of Alzheimer's disease. Aβ can induce synaptic loss by chronically increasing NMDAR activity (Hu et al., 2009; Wei et al., 2010), which in turn would usually affect CaMKII activation status, postsynaptic localization, and function. However, it appears that at least in organotypic hippocampal slices, viral expression of Aβ acts through a novel metabotropic rather than ionotropic NMDAR function (Kessels et al., 2013). An analogous metabotropic NMDAR function seems to underlie NMDAR-dependent LTD. These metabotropically induced forms of LTD are independent of postsynaptic Ca^{2+} rises and thereby likely of CaMKII activation, although they could quite well involve structural changes of GluN2B that could affect CaMKII binding as GluN2B mediates the metabotropic Aβ effect (Hu et al., 2009; Kessels et al., 2013).

In a rat model of Parkinson's disease, CaMKIIα T286 phosphorylation and its association with the NMDAR is increased (Picconi et al., 2004). Importantly, the dysfunctions in motor performance and LTP at corticostriatal synapses were rectified by injection of CaMKII inhibitors, causally linking upregulation of CaMKII activity to this disease model.

Mutations in the chromatin remodeling protein ATRX have been implicated in mental retardation. In ATRX KO mice, spines were enlarged in the medial prefrontal cortex but not in the hippocampal CA1 area (Shioda et al., 2011). This enlargement was paralleled by increased CaMKII autophosphorylation. This increase was correlated with increased phosphorylation of Kalirin-7 and of Tiam 1, a guanine nucleotide exchange factor for Rac, both of which would be expected to elevate spine size.

Increased T305/T306 phosphorylation of CaMKII has been demonstrated in Angelman's syndrome, which is characterized

by motor dysfunction, epilepsy, and mental retardation (van Woerden et al., 2007; Weeber et al., 2003). As T305/T306 phosphorylation decreases basal synaptic strength (Pi et al., 2010b), it is now important to define the precise functions of this phosphorylation to further define the molecular mechanism of Angelman's syndrome.

Dissecting the precise role of CaMKII in these and other brain diseases has proven to be a challenge, probably because CaMKII fulfills many different functions. Some of these functions might contribute to neuronal damage upon CaMKII dysregulation and others might actually counteract it. Defining the function of CaMKII in these diseases will not only advance our understanding of CaMKII-regulated mechanisms but also pave the way for developing innovative treatments. Disentangling the precise mechanisms of postsynaptic regulation must be a central focus in our quest to comprehend brain function and molecular basis of disease.

Conclusions

CaMKII commanded early attention in the synaptic signaling field based on its high expression levels, its size, and its intricate and lasting autoregulation. Recent discoveries have shed new light onto the function of CaMKII at the postsynapse, revealing a major autoregulated structural role for CaMKII in addition to its kinase function. It is now apparent that CaMKII is a central organizer of the postsynaptic F-actin network and acts as an autotuning machine that regulates its own PSD localization and simultaneously recruiting key effector proteins including the proteasome and CK2. A full understanding of the molecular details of synaptic plasticity and learning awaits a rigorous biochemical analysis of the precise structural and mechanistic properties of CaMKII and its interactions. I predict CaMKII's versatile and multifaceted functions will keep us in suspense for years to come.

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