



HHS Public Access

Author manuscript

Neuron. Author manuscript; available in PMC 2015 September 15.

Published in final edited form as:

Neuron. 2014 January 22; 81(2): 249–265. doi:10.1016/j.neuron.2013.12.024.

CaMKII: Claiming Center Stage in Postsynaptic Function and Organization

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SUMMARY

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. Ca²⁺-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 CaMKII α and CaMKII β 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 few other, mostly cytoskeletal, proteins (Lisman et al., 2002). By autophosphorylating itself upon activation by Ca²⁺ 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 Ca²⁺ influx via 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 over-emphasized. This review, will focus on recent work that has unearthed novel functions of CaMKII in spines, focus 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*, *2B*, *2G*, *2D*) encode CaMKII α - δ , respectively, with α and β being highly prevalent in brain. CaMKII accounts for 2–6% of

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total protein in the PSD (~80 dodecameric complexes per $0.1 \mu\text{m}^2$ 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 $0.1 \mu\text{m}^2$ of PSD (Chen et al., 2008)) in total mass (Dosemeci et al., 2007). Forebrain CaMKII consists mostly of 9 α and 3 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 (Figure 1A,B) (Braun and Schulman, 1995; Colbran and Brown, 2004; Coultrap and Bayer, 2012). Upon Ca^{2+} influx, Ca^{2+} /calmodulin binding to the pseudosubstrate segment of CaMKII relieves it from this autoinhibition. When Ca^{2+} /CaM binds to two neighboring subunits, autophosphorylation at T286 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 re-phosphorylated. Re-phosphorylation 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 (~1s 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) 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)). CaMKII β binds to and cross links 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 CaMKII β , which is different from CaMKII α (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 Ca²⁺ influx, Ca²⁺/CaM and T287 autophosphorylation displace CaMKII β from F-actin (Lin and Redmond, 2008; Shen and Meyer, 1999) (Figure 3). Notably, CaMKII β has a ~9 fold higher affinity for Ca²⁺/CaM (EC₅₀ for autophosphorylation: 15 nM) than CaMKII α (Brocke et al., 1999) suggesting that CaMKII β -containing dodecamers are readily released from F-actin before CaMKII α subunits bind Ca²⁺/CaM and are activated. However, Ca²⁺/CaM binding to CaMKII α is necessary for T287 phosphorylation of CaMKII β in native forebrain dodecamers as CaMKII α subunits phosphorylate CaMKII β 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 (*ACTN1-4*) encode the highly homologous α -actinin-1 through -4 with α -actinin-1, -2, and -4 but not -3 being expressed in forebrain neurons (Schnizler et al., 2009; Wyszynski et al., 1998). α -actinins contain two calponin homology domains (CH1, CH2), followed by four spectrin homology repeats (SR1-4), 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 Ca²⁺/CaM binds to the EF hand motifs of α -actinin-2 and -4 (Figure 2B) (Jalan-Sakrikar et al., 2012; Robison et al., 2005b; Walikonis et al., 2001). Indeed, Ca²⁺/CaM out-competes α -actinin for CaMKII binding (Meyer et al., 1992; Robison et al., 2005a), thus Ca²⁺ 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 Ca²⁺/CaM might act in parallel with disruption of the direct binding of CaMKII β subunits to F-actin. Furthermore, the two N-terminal EF hands in α -actinin-1 and -4 can bind Ca²⁺, which inhibits their binding to F-actin (BurrIDGE and Feramisco, 1981; Sjoblom et al., 2008; Witke et al., 1993). Multiple interactions of single CaMKII α/β 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. Ca²⁺ influx has the potential to liberate CaMKII α/β 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 Ca²⁺/CaM binding site of CaMKII abrogates binding of Ca²⁺/CaM only if on T305 but of both, Ca²⁺/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 Ca²⁺/CaM. The impact of CaMKII β phosphorylation on T306/T307 has not been studied. Like Ca²⁺/CaM, α -actinin

binding stimulates CaMKII activity but to a lesser extent than $\text{Ca}^{2+}/\text{CaM}$ 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 CaMKII α and β (Jiao et al., 2011) and to the C-terminal oligomerization domain of CaMKII α but not β (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 $\text{Ca}^{2+}/\text{CaM}$ 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 also bind to the PDZ domain of densin (Robison et al., 2005b; Walikonis et al., 2001), which synergistically promotes the CaMKII - α -actinin interaction likely by forming a ternary complex (Robison et al., 2005b).

Densin 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 $\text{Ca}^{2+}/\text{CaM}$ – 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

CaMKII β 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 CaMKII β (but not CaMKII α) causes a reduction in spine head size and loss of mature spines, which is fully rescued by ectopic expression of kinase dead CaMKII β 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 CaMKII β enhances spine size in a kinase activity – independent manner. Also, CaMKII β overexpression stabilizes F-actin-rich structures in cultured cortical neurons and decreases F-actin motility in spines (Okamoto et al., 2007). Furthermore, in CaMKII β A303R KI mice CaMKII β cannot be activated (as $\text{Ca}^{2+}/\text{CaM}$ binding is abrogated) yet spine targeting of

CaMKII α as well as hippocampal LTP and learning are all normal. This lack of effect is in contrast to CaMKII β KO mice in which CaMKII α spine targeting, LTP, and learning are impaired (Borgesius et al., 2011). The modest phenotypes of CaMKII β A303R KI mice likely reflects the recurring theme of redundancy and the engagement of compensatory mechanisms in postsynaptic CaMKII targeting

When neuronal network activity is decreased, postsynaptic AMPAR content and responses and likely spine size increase over the synapse population to maintain homeostasis of overall excitatory inputs into this neuron (Murthy et al., 2001; Turrigiano, 2008b). In parallel, expression and spine content of CaMKII β are increased and CaMKII α expression is decreased (Thiagarajan et al., 2002). With the emerging role of CaMKII β in stabilizing F-actin, it appears likely that the homeostatic increase in synaptic strength is at least in part due to the CaMKII β -mediated increase in F-actin content, which in turn leads to larger spine size and thereby higher postsynaptic strength. Indeed, knock down of CaMKII β 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 CaMKII β increases mEPSC frequency likely 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 between α -actinin, CaMKII α/β dodecamers, and F-actin likely 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 Burrridge, 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, CaMKII β knock down 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 CaMKII β knock down is consistent with and best explained by α -actinin being the prevailing determinant of the parallel F-actin fibers in those protrusions contrasting the CaMKII β -supported branched F-actin in spines (Korobova and Svitkina, 2010). Thus, in conjunction with CaMKII β , 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; Racz 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 likely 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 Ca²⁺/CaM upon Ca²⁺ 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 post mortem CaMKII clustering at PSDs) the concentration of CaMKII shows a distinct peak about 40 nm away from PSD along the axo-dendritic position but falls off towards the spine center to about 1/3 of the peak concentration. It declines to even lower values (~1/10 of peak at 40 nm) at the PSD center near the plasma membrane along the axo-dendritic axis although it is larger at the periphery than in the center of the PSD (Ding et al., 2013). Ca²⁺ 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 CaMKII α/β dodecamers are linked to F-actin directly via CaMKII β and indirectly via α -actinin and that Ca²⁺/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 Ca²⁺ influx, Ca²⁺/CaM will release CaMKII from F-actin and α -actinin for rapid relocation to the PSD. Within 1–2 min of Ca²⁺ 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; 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 Ca²⁺-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 1290–1309); 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 / $0.1 \mu\text{m}^2$ (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 839–1120) (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 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 3 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., 2002; Leonard et al., 1999). 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 re-bind 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 re-phosphorylation 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 re-phosphorylation 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., 2002; Leonard et al., 1999; 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 sec stimulus train (Barria and Malinow, 2005). Furthermore, field LTP induced by two 100 Hz/1 sec stimulus trains or by theta burst stimulation is reduced by 50% in GluN2B_{L1298A/R1300Q} knock-in 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 twelve 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} knock-in 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 $\gamma 8$ 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 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 Ca²⁺ 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 (Zhang et al., 2008) but not the other study (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 >2h (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 potentiates likely much less than 1% of all synapses, results in CaMKII T286 autophosphorylation throughout the dendritic area suggesting widespread CaMKII activation (Ouyang et al., 1997; Ouyang et al., 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 Ca²⁺ 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 Ca²⁺ 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 α_1 1.2 subunit has been implicated in both, Ca²⁺-dependent inactivation (CDI) and Ca²⁺-dependent facilitation (CDF) of Ca_v1.2 currents (Zuhlke 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., 1996; Hell et al., 1993) but it is unclear if 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 $\text{Ca}_v1.3$ puncta (the main L-type channel in SCG) can be induced by both weak depolarizations that selectively activate $\text{Ca}_v1.3$ 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 likely 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 life time (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, as. However, CaMKII can exist in different activated states with $\text{Ca}^{2+}/\text{CaM}$ 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 $\text{Ca}^{2+}/\text{CaM}$ (Braun and Schulman, 1995; Coultrap et al., 2010). Yet, the FLIM signals are actually several fold more strongly influenced by T286 autophosphorylation than by $\text{Ca}^{2+}/\text{CaM}$ binding (Lee et al., 2009). For instance, T286A mutant CaMKII shows much less changes in FLIM upon $\text{Ca}^{2+}/\text{CaM}$ addition than wild type CaMKII even though it can fully be activated by $\text{Ca}^{2+}/\text{CaM}$ (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 life time 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 Ca²⁺ 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 Ca²⁺-mediated effect in spines within the immediate environment of Ca_v1.2 that cannot be mediated by robust Ca²⁺ 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 Ca²⁺ influx via L-type channels is that CaMKII activation in spines upon robust Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ 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 CaMKII α mutation T286D induces synaptic weakening (Pi et al., 2010b). This finding is surprising because strengthening rather than weakening would have been predicted as CaMKII α T286D is constitutively active like truncated CaMKII α ₁₋₂₉₀, which increases EPSC amplitude (Pi et al., 2010b). As it turns out the CaMKII α T286D mutant becomes phosphorylated on T305 or T306. Preventing T305/306 phosphorylation in the CaMKII α T286D/T305A/T306A triple mutant leads to the expected potentiation rather than depression of synaptic transmission (Pi et al., 2010b). The phosphomimetic CaMKII α T286D/T305D/T306D mutant, however, induces depression. At the same time, all of the CaMKII α mutants tested that include T286D increased spine size in organotypic cultures (Pi et al., 2010a), potentially by inducing T-site interaction with GluN2B or an. Accordingly, T286 phosphorylation is necessary and sufficient for CaMKII α to augment spine size independent of T305/T306 phosphorylation status. CaMKII α 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 CaMKII α K42R/T286D expression. At the same time CaMKII α activity appears to be critical for the increase in EPSC amplitude seen with CaMKII α ₁₋₂₉₀ as overexpression of WT CaMKII α had no effect on AMPAR EPSC amplitude (Pi et al., 2010b). Further puzzling, CaMKII α T286D/T305D/T306D as well as CaMKII α T286D/T305A/T306A prevented LTP, the latter potentially by occlusion as it induces potentiation upon its ectopic expression, whereas single mutant CaMKII α 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 CaMKII α T286D and CaMKII α T286D/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 CaMKII α T286D and CaMKII α T286D/T305D/T306D increases spine size when decreasing postsynaptic response strength is remarkable because it shows that the two parameters do not have always to 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 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 CaMKII β to spines of low activity (Okuno et al., 2012). CaMKII β binds Arc much more tightly in the absence of Ca^{2+} /CaM and T287 autophosphorylation (Arc binding to CaMKII α is weak under all conditions) (Okuno et al., 2012). Thus, CaMKII β 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 non-potentiated synapses. In this way, it might cause a modest homeostatic synaptic down scaling of the large majority of non-potentiated synapses as required to keep the overall synaptic input within a defined dynamic range and to prevent overexcitability following 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 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 2 and their upstream activators EPAC2 (Woolfrey et al., 2009; Zhu et al., 2002; Zhu et al., 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 homologues PSD-93 or SAP102 and regulates its postsynaptic targeting (Elias et al., 2008; Elias et al., 2006; 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., 2013; Sanz-Clemente et al., 2010). 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/ γ 2) on as many as nine serine residues (Figure 5). Stg/ γ 2 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., 2008; Elias et al., 2006; Schnell et al., 2002). Ectopic expression of phospho-mimetic 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 Ca²⁺ potentially disrupts electrostatic interactions between proteins and the plasma membrane (Zilly et al., 2011) Ca²⁺ influx through the NMDAR likely promotes this Stg/ γ 2 phosphorylation by decreasing the association of the Stg/ γ 2 C-terminus with the plasma membrane, thereby rendering it more accessible for CaMKII (Figure 5). The consequent phosphorylation prevents the Stg/ γ 2 C-terminus from re-associating 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’, which 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/ γ 2 are conserved in other TARPs including γ 8, the prevalent hippocampal TARP (Rouach et al., 2005). ChemLTP augments the content of γ 8 and other TARPs in PSD preparations (Halt et al., 2012). The correlation between loss of NMDAR-mediated CaMKII anchoring and loss of persistent γ 8 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/ γ 2 and γ 8 can to some, though much limited, degree be accomplished independent of the whole C-terminus of Stg/ γ 2 and of γ 8 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 γ 8 in PSDs following chemLTP depend on the activity-driven CaMKII binding to GluN2B_{1290–1309}, it is quite conceivable that phosphorylation of Stg/ γ 2 and potentially of γ 8 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 T286 phosphorylation, constituting an endogenous mechanism that down regulates 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 likely because they cannot bind with sufficient affinity to the T-site. Whereas 5 μ M tatCN21 is sufficient to fully block CaMKII activation in acute hippocampal slices (Buard et al., 2010), 20 μ M tatCN21 is required for CaMKII displacement from GluN2B (Sanhueza et al., 2011). Although 5 μ M 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 μ M 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., 2002; Leonard et al., 1999) 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, 2008a). In parallel, levels of CaMKII α decrease and CaMKII β increase (Thiagarajan et al., 2002). The opposite is true upon chronic increase of network activity, i.e., AMPAR-mediated synaptic transmission and CaMKII β levels decrease and CaMKII α levels increase (Thiagarajan et al.,

2002). CaMKII α overexpression in dissociated hippocampal cultures drastically decreases mEPSC frequency (but increases mEPSC amplitude) (Thiagarajan et al., 2002). CaMKII β 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 CaMKII α might impair spine stability and thereby synapse number by reducing the interaction of the enzyme with F-actin due to decreased CaMKII β content in the dodecamer, while overexpression of CaMKII β might have the opposite effect. The increase in mEPSC amplitude by CaMKII α 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 CaMKII α 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 Ca²⁺ channels and by knock down of CaMKII β . Knock down of CaMKII β 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 up regulation of GluA1 upon chronic block of AMPAR activity (Groth et al., 2011). The observation that Ca²⁺ channel inhibition counteracts the effect of decreased network activity on GKAP and PSD-95 is surprising, as is the increase in CaMKII β T287 autophosphorylation upon TTX treatment, as Ca²⁺ 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 Ca²⁺ channels mediate inactivity-triggered upregulation of postsynaptic GKAP and PSD-95 and AMPAR function.

A chimeric CaMKII α construct carrying the F-actin binding domain of CaMKII β was able to rescue the loss of TTX-induced upregulation of postsynaptic GKAP clustering upon CaMKII β knock down (Shin et al., 2012). This finding indicates that F-actin recruits native CaMKII β -containing CaMKII dodecamers to the postsynaptic site for homeostatic upregulation of postsynaptic size and strength and provides further support for the above model that CaMKII β 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 knock down 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 phospho-deficient S340A,S384A or phospho-mimetic 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 knock down 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 CaMKII α but not of CaMKII β . 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 poly-ubiquitination and removal from the synapse (Shin et al., 2012). Accordingly, increased Ca²⁺ influx during enhanced network activity stimulates CaMKII α . 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 knock down 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 Ca²⁺ 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, Ca²⁺ influx through NMDARs and Ca²⁺-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 Ca²⁺ 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 (A β) is one of the two main hallmarks of Alzheimer's disease. A β 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 A β acts through a novel metabotropic rather than ionotropic NMDAR function (Kessels et al., 2013). An analogous metabotropic NMDAR function seems to underlie NMDAR-dependent long-term depression (LTD). These metabotropically-induced forms of LTD are independent of postsynaptic Ca²⁺ 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 A β effect (Hu et al., 2009; Kessels et al., 2013)..

In a rat model of Parkinson's disease, CaMKII α T286 phosphorylation and its association with the NMDAR is increased (Picconi et al., 2004). Importantly, the dysfunctions in motor performance and LTP at cortico-striatal 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 T305/306 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, likely 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 structural, autoregulated 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 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 multi-faceted functions will keep us in suspense for years to come.

Acknowledgments

I wish to thank Dr. H Schulman (Allosteros Therapeutics) for discussions, Dr. M. M. Stratton and Dr. J. Kuriyan (UC Berkeley) for discussions and for putting Figure 1A,C,D,E together; Ms. H. Shams, Dr. M.R.K. Mofrad (UC Berkeley) for discussions and for providing Figure 4; Mr. Pang-Yen Tseng (UC Davis) for help with Figure 1B, 2, and 3. Work by the author was supported by NIH grants R01NS035563, R01AG017502, and R01NS078792.

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Highlights

1. CaMKII - F-actin interaction controls CaMKII docking and release within spines
2. CaMKII β collaborates with α -actinin to stabilize branched F-actin in spines
3. CaMKII is an auto-regulated postsynaptic docking hub protein
4. Emerging roles for CaMKII in LTP, synaptic homeostasis, and brain diseases

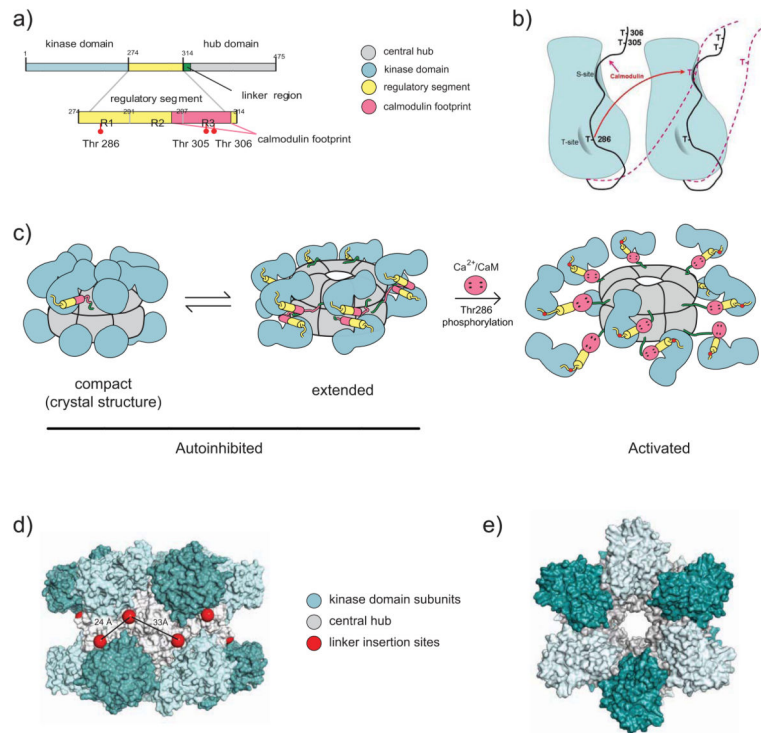


Figure 1. CaMKII Structure

(A) Linear depiction of one CaMKII subunit. It shows kinase domain (blue; numbering according to mouse CaMKII α), 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 (grey).

(B) Autoinhibition and autophosphorylation of CaMKII subunits. The diagram illustrates schematically two neighboring subunits with the inhibitory segment in black. The T-site (grey half moon) accommodates T286 under resting conditions, fostering the interaction of the pseudosubstrate region immediately downstream of T286 with the catalytic site (S site; grey 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 Ca²⁺/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 Ca²⁺/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: a) a closed inhibited/inactive conformation with the linker folded into the association domain, rendering it inaccessible for Ca²⁺/CaM binding and activation; b) an open inhibited/inactive conformation with the linker extended outward; c) a fully extended active conformation with Ca²⁺/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,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 grey. The positions of the linkers are depicted by red spheres. Adopted with permission from (Chao et al., 2011).

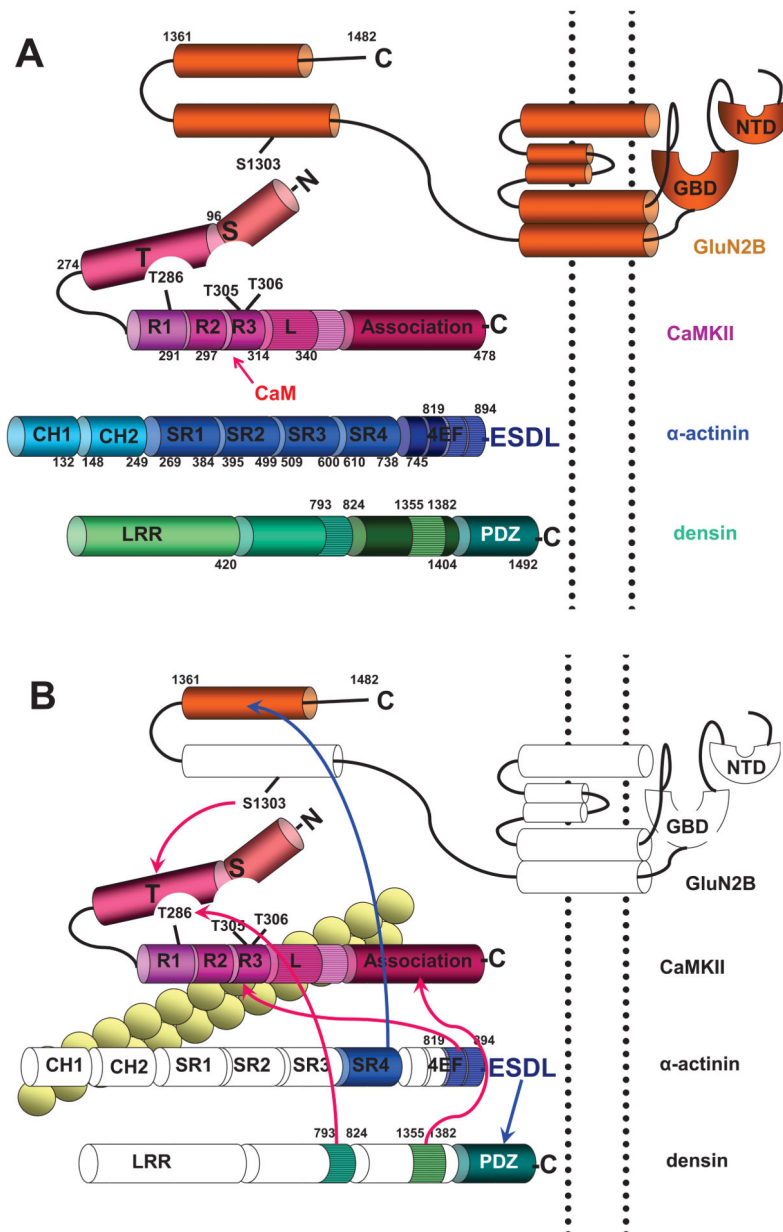


Figure 2. Domains that Mediate Interactions between CaMKII, α -actinin, F-actin, Densin, and NMDARs

(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 CaMKII α), 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 interact 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 Ca²⁺/CaM binding site, which overlaps with R2; dark red oval; T305/T306 inhibit Ca²⁺/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 421–1404) and a C-terminal PDZ domain (aa 1405–1492).

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 838–1482).

(B) Depiction of CaMKII interaction sites. The linker of CaMKII β (horizontal stripes), which is of variable length and different from the linker of CaMKII α , is important for binding to F-actin (yellow beaded double string crossing underneath the linker). The exact binding site on CaMKII β 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 Ca²⁺/CaM compete for binding to R2/R3 on CaMKII (dark red oval). The association domain of CaMKII (aa 341–478) binds to aa 1335–1382 of densin (horizontal stripes) independent of CaMKII activation. The T site of CaMKII binds to aa 1290–1309 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 Ca²⁺/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).

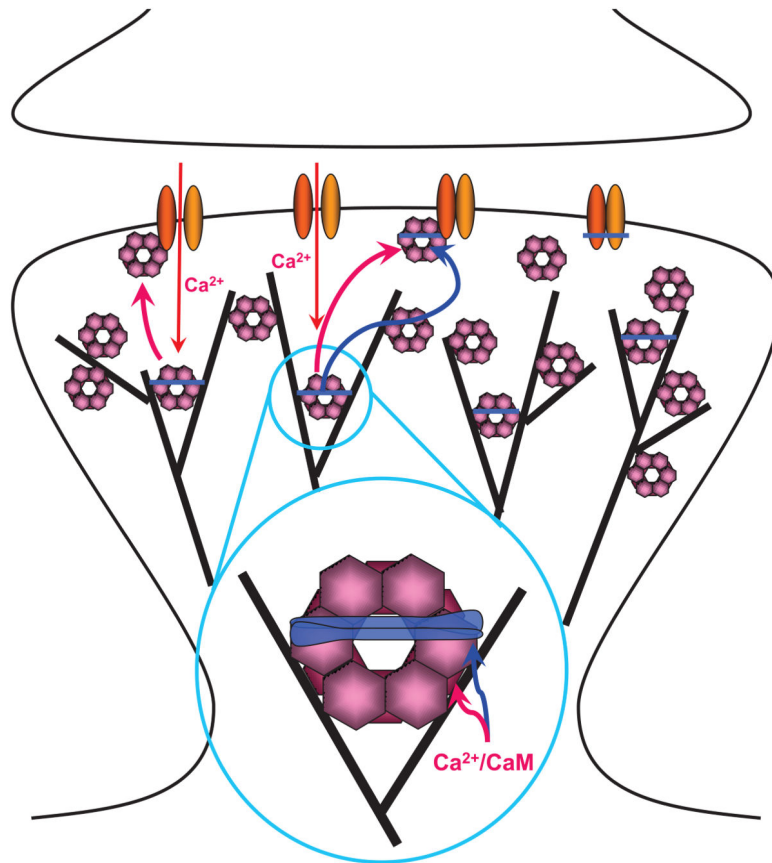


Figure 3. Interactions of CaMKII with F-actin, α -actinin, and NMDARs in Spines

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 CaMKII β 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 subunits (orange), triggers release of CaMKII from F-actin as $\text{Ca}^{2+}/\text{CaM}$ will displace CaMKII β from F-actin (red arrow in insert) and CaMKII α and β from α -actinin (blue arrow in insert). CaMKII will then bind to the NMDAR subunit GluN2B (top left area of PSD), which requires either $\text{Ca}^{2+}/\text{CaM}$ or the more lasting T286/287 autophosphorylation. After removal of $\text{Ca}^{2+}/\text{CaM}$, α -actinin will re-associate with CaMKII possibly forming a trimeric complex with GluN2B (top middle area; right area depicts an α -actinin – NMDAR complex without CaMKII).

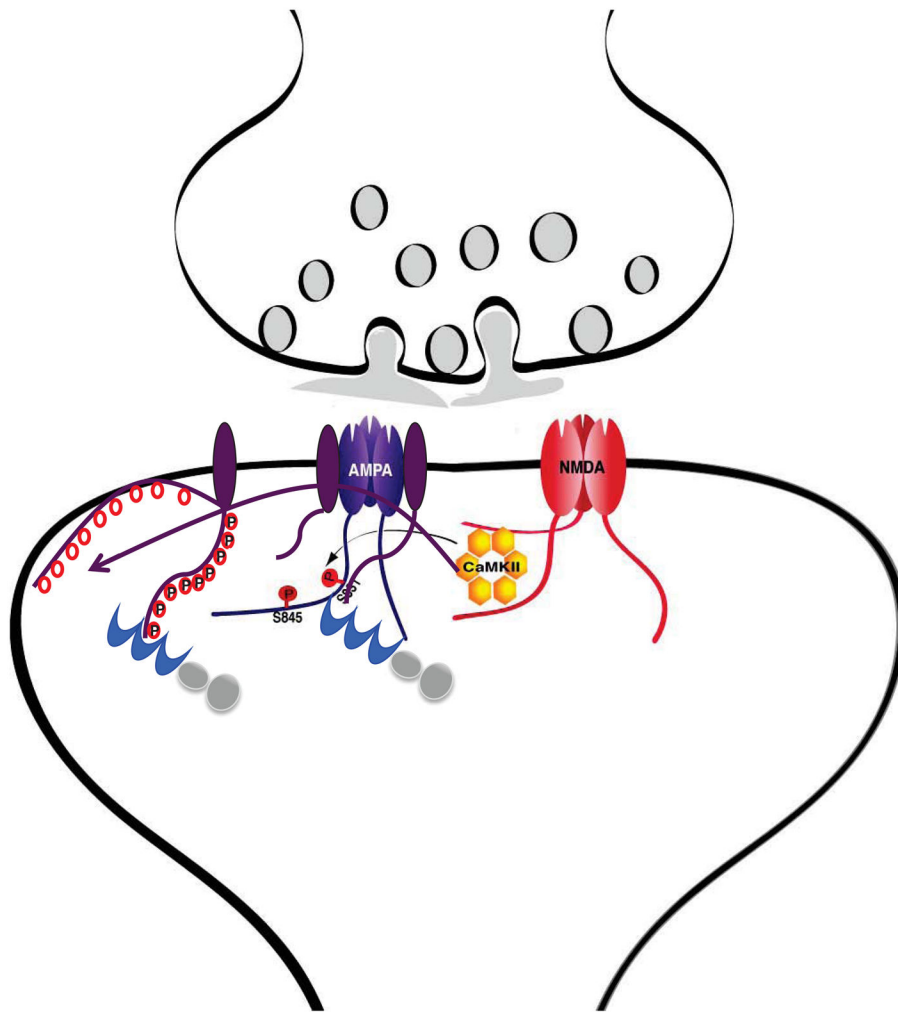


Figure 4. Binding of α -actinin to F-actin Makes EF3 and EF4 Accessible for CaMKII Binding
 (A) Native structure of α -actinin dimer (PDB ID: 1SJJ) with both actin binding domains (CH1, CH2) in a closed conformation (insert on top of the full length α -actinin structural model). In this structure EF3 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 quasi folded back like a hook towards 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 cross-linking 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 are 20 Angstrom for main figure and 10 Angstroms for enlarged inserts.

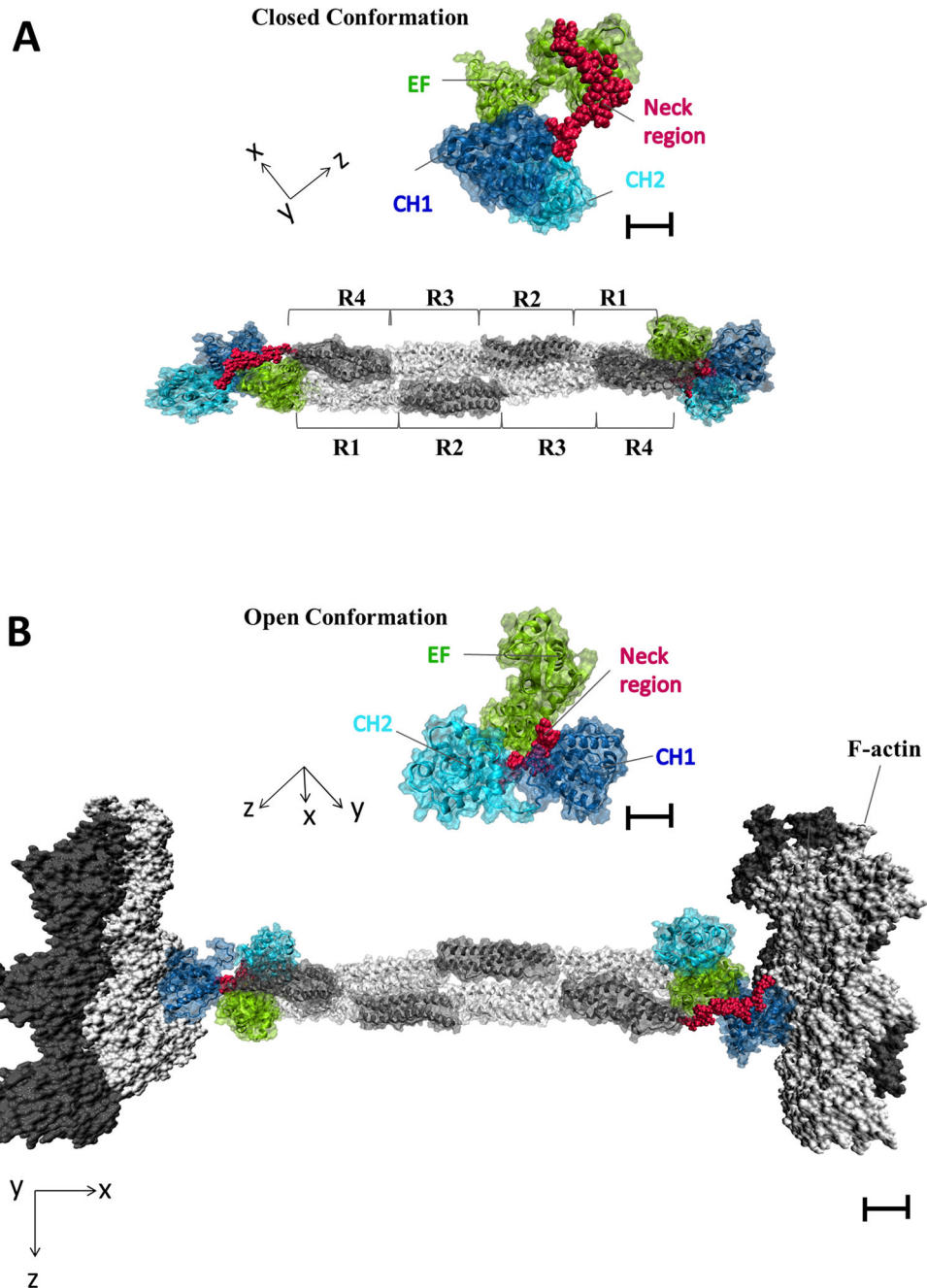


Figure 5. Role of CaMKII Binding to GluN2B in AMPAR Phosphorylation

Ca²⁺ 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 Ca²⁺ 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 9 phosphorylation sites (red circles) available for CaMKII. The ensuing phosphorylations will reduce the net positive charge and thereby re-association 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).

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