

## CaMKII substrates in synaptic plasticity

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### Introduction

Excitatory synaptic transmission in brain occurs at synapses that use glutamate as their neurotransmitter. This signaling is primarily mediated by two classes of glutamate gated ion channels, NMDA and AMPA receptors. Activity-dependent changes in signaling by AMPA receptors represent a key mechanism for brain plasticity and underlie aspects of learning and memory. Long-term potentiation (LTP) in the hippocampal pyramidal cells is the best-established model for this plasticity. Although the molecular mechanism for LTP is not entirely defined; it is clear that  $\text{Ca}^{2+}$  influx through NMDA receptors initiates the changes seen during LTP (Collingridge et al., 2004; Lisman, 2003; Malenka and Bear, 2004; Malenka and Nicoll, 1999). It has been also shown that activation of calcium/calmodulin dependent protein kinase II (CaMKII) can be necessary and sufficient for the expression of LTP (Hayashi et al., 2000; Lledo et al., 1995; Silva et al., 1992). The relevant targets for CaMKII and the downstream mechanism that

enhances synaptic transmission however remain unclear.

## **Is the AMPA receptor a substrate of CaMKII in LTP?**

LTP increases both the number of synaptic AMPA receptors and their single-channel unitary conductance (Andrasfalvy and Magee, 2004; Benke et al., 1998; Luthi et al., 2004; Manabe et al., 1992; Muller et al., 1988). AMPA receptors are heterotetramers composed of the four subunits GluR1-4 (Dingledine et al., 1999; Hollmann and Heinemann, 1994). In the adult rat hippocampus, GluR1-3 are highly expressed (Monyer et al., 1991), and in pyramidal cells each heterotetramer contains at least one GluR2 subunit (Verdoorn et al., 1991). GluR1 knockout mice show impaired LTP (Zamanillo et al., 1999), whereas GluR2 knockout mice show enhanced LTP (Jia et al., 1996). These studies suggest that GluR1 plays a dominant role in LTP.

CaMKII can directly phosphorylate GluR1 on serine residue 831 (S831, Barria et al., 1997a; Mammen et al., 1997); and in brain slices, S831 is phosphorylated after LTP induction (Barria et al., 1997b; Lee et al., 2000). Furthermore, single-channel analysis showed that this phosphorylated GluR1 exhibits a larger unitary conductance as compared to non-phosphorylated GluR1 (Derkach et al., 1999). These studies suggest a model in which CaMKII enhances channel conductance of AMPA receptors during LTP. Indeed, mutant mice lacking serines 831 and 845 (a protein kinase A phosphorylation site) have impaired LTP (Lee et al., 2003).

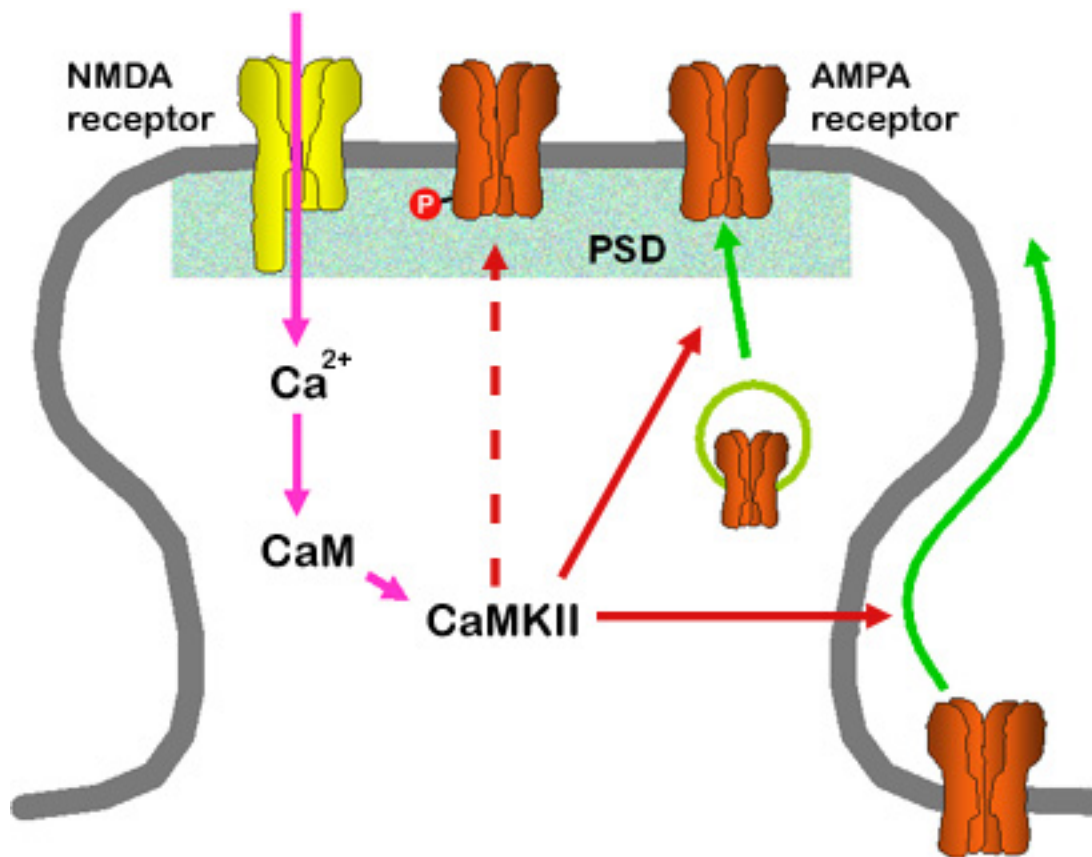
Complicating this simple model however, a recent study by Oh and Derkach (2005) suggests that CaMKII increases the single channel conductance of homomeric GluR1 channel, but not that of the heteromeric GluR1/GluR2 channel. The authors did confirm that GluR1 was indeed phosphorylated by CaMKII in heteromeric channels with GluR2. Oh et al. concluded that GluR2 determines the single-channel conductance of heteromeric channels independently of

the phosphorylation-state of GluR1. These findings raise two important questions. How do GluR1 phosphorylation sites function during LTP? More importantly, what other CaMKII substrates play a role in LTP?

## Are there other CaMKII substrates?

The lack of robust and specific inhibitors of CaMKII makes it difficult to identify its substrates during LTP. The best current CaMKII inhibitors, KN-62 and KN-93, do not inhibit a constitutively active, autophosphorylated CaMKII that is likely important in LTP (Colbran and Brown, 2004; Hudmon and Schulman, 2002; Lisman et al., 2002).

Both



systematic proteomic and candidate protein approaches have been used to identify the crucial CaMKII substrates involved in LTP. More than 100 proteins in the genomic database contain the CaMKII consensus sequence, Arg-X-X-Ser/Thr (Pearson et al., 1985). Proteomic analysis identified more than 30 synaptic proteins as

potential targets for phosphorylation by CaMKII (Yamauchi, 2002). The role for these substrates in the regulation of AMPA receptor function in LTP remains unclear.

The restricted search for CaMKII substrates of relevance to LTP has focused on AMPA receptor interacting proteins. The best characterized associated protein partner for AMPA receptors is the tetraspanning membrane protein, stargazin, which is essential for the clustering of AMPA receptors at synapses of cerebellar granule cells (Chen et al., 2000; Hashimoto et al., 1999). Stargazin and three related transmembrane AMPA receptor regulatory proteins (TARPs) show distinct expression patterns in brain (Tomita et al., 2003). TARPs modulate not only the trafficking of AMPA receptors, but also control the gating and pharmacology of the channel (Tomita et al., 2005a; Tomita et al., 2004).

Interestingly, stargazin is quantitatively phosphorylated in brain, and stargazin phosphorylation promotes the synaptic trafficking of AMPA receptors (Tomita et al., 2005b). NMDA receptor activity can induce stargazin phosphorylation via activation of CaMKII and PKC, which both directly phosphorylate stargazin in vitro (Tomita et al., 2005b). At hippocampal synapses LTP requires stargazin phosphorylation (Tomita et al., 2005b). These results establish stargazin as a critical substrate in LTP.

## **Conclusions**

It is unclear what CaMKII substrates modulate AMPA receptor function during LTP. CaMKII substrates relevant to LTP should meet the following criteria: 1) the substrate should be phosphorylated by CaMKII during LTP; 2) Substrate phosphorylation should promote synaptic AMPA receptor function; 3) Disrupting substrate phosphorylation should prevent LTP. No protein has yet convincingly met all three of these criteria. Thirty years after the description of LTP,

substantial progress has been made. Yet we still lack a molecular mechanism for LTP.

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