Synaptic plasticity: **A molecular memory switch**

John E. Lisman and Charmian C. McIntyre

Recent work shows that two molecules with major roles in synaptic plasticity – CaMKII and the NMDA receptor – bind to each other. This binding activates CaMKII and triggers its autophosphorylation. In this state, it may act as a memory switch and strengthen synapses through enzymatic and structural processes.

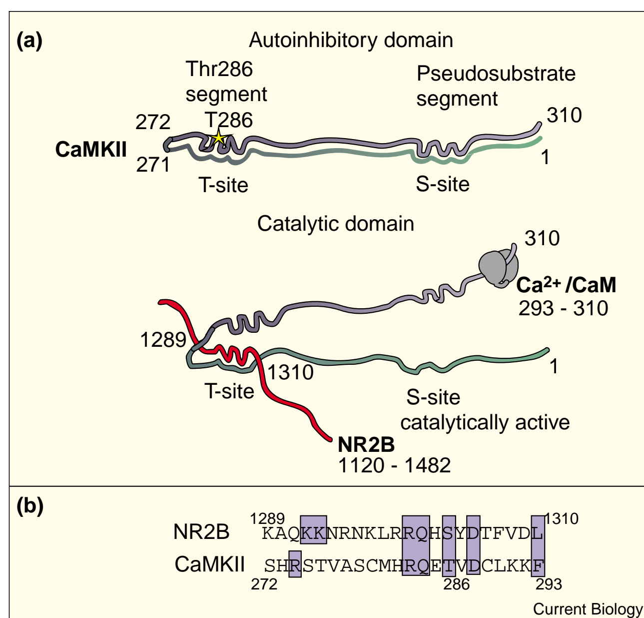
Address: Department of Biology and Volen Center for Complex Systems, Brandeis University, Waltham, Massachusetts 02453, USA.

Current Biology 2001, 11:R788–R791

0960-9822/01/$ – see front matter
© 2001 Elsevier Science Ltd. All rights reserved.

There is widespread agreement that memory can be encoded by activity-dependent changes in the strength of synapses. The molecular mechanisms which control synaptic strength are now being elucidated at a breathtaking pace. A particularly important recent development is the finding that two of the primary molecules involved — Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) and the NMDA subtype of glutamate receptor — form a tight complex with each other at the synapse [1]. A new study [2] has shown that this binding has more effects than previously suspected; the binding enhances both the autophosphorylation of the kinase and the ability of the entire holoenzyme, which has twelve subunits, to become hyperphosphorylated.

Figure 1



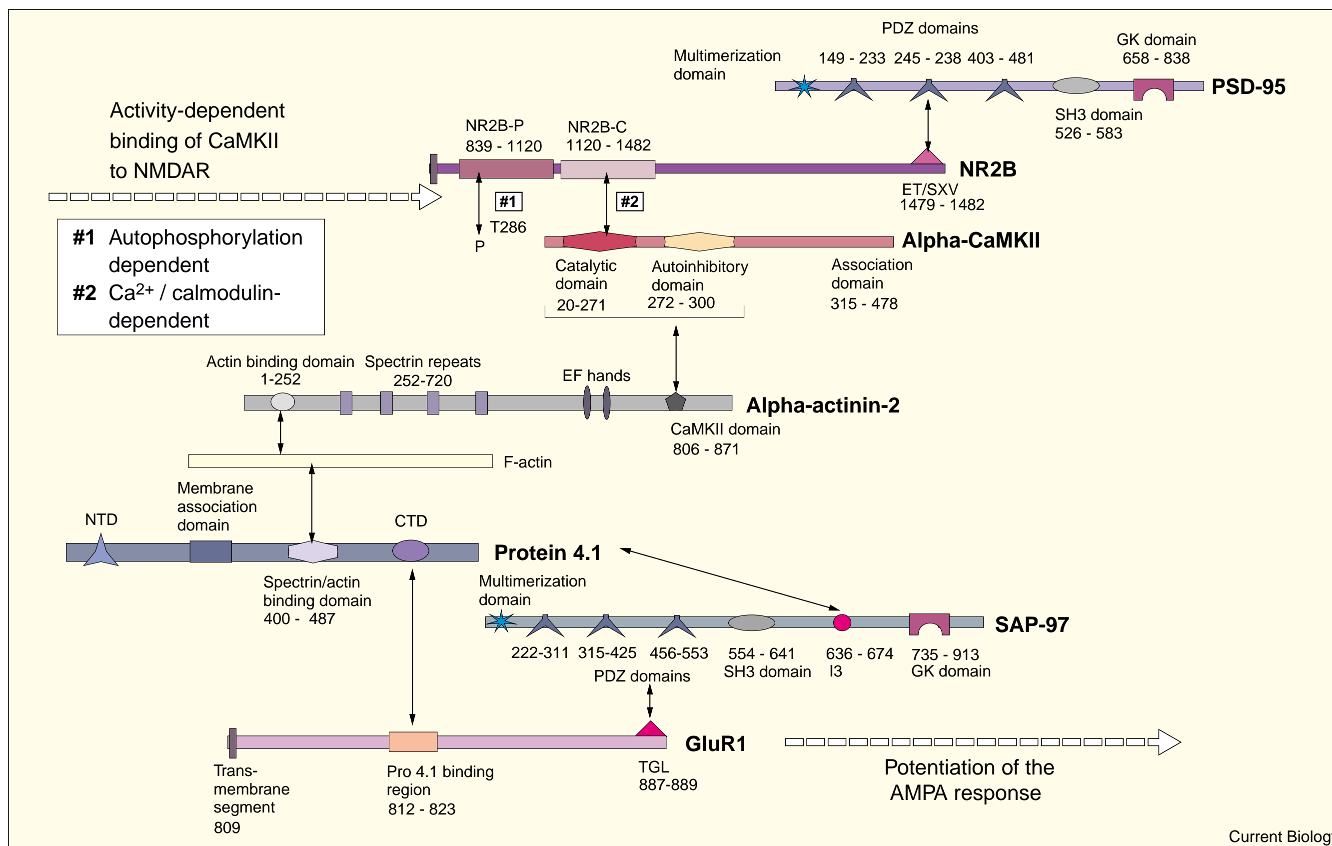
Binding of the cytoplasmic tail of NR2B to the catalytic domain of CaMKII renders CaMKII activity Ca^{2+}-independent. **(a)** A schematic of how NR2B (red) interacts with the T-site of the catalytic domain of CaMKII (green). Binding of Ca^{2+}/calmodulin displaces the autoinhibitory domain from the catalytic domain; this exposes the T-site, allowing NR2B to bind. NR2B prevents rebinding of the pseudosubstrate segment with the S-site after Ca^{2+}/calmodulin unbinding, thereby keeping the catalytic S-site exposed and maintaining catalytic activity. **(b)** A segment of NR2B is homologous to the Thr286 segment of CaMKII. Adapted from [2].

Other recent work has extended our understanding of how CaMKII may function as a memory switch [3]. This work suggests that the phosphorylated ‘on’ state of the kinase is stable because the kinase autophosphorylates faster than it is dephosphorylated by the local phosphatase pool; hyperphosphorylation augments this difference by saturating the phosphatase. In its ‘on’ state, the ‘memory switch’ can lead to long-term strengthening of the synapse by multiple mechanisms. One involves direct phosphorylation of the glutamate-activated AMPA receptors, which increases their conductance [4]. A second [3] appears to be a structurally mediated process: CaMKII, once bound to the NMDA receptor, may organize additional anchoring sites for AMPA receptors at the synapse. We are thus beginning to understand how structural and enzymatic processes can lead to information storage and retrieval at synapses.

The idea that synaptic modification underlies memory storage was developed by the psychologist Donald Hebb. He argued that a set of synaptically connected cells in a neural network could store associative memories if synapses obeyed a simple, synapse-specific modification rule: strengthening occurs if there is both presynaptic activity (input to the synapse) and sufficient convergent excitatory input to fire the postsynaptic cell. This is now termed the ‘Hebb rule’. In 1973, Bliss and Lomo [5] provided the first evidence that brief periods of high-frequency synaptic activity could, in fact, lead to long-lasting strengthening of synapses. Synaptic strengthening is defined as a sustained increase in the size of the graded synaptic response generated by an action potential in the presynaptic axon. This process is termed long-term potentiation (LTP) and has been most extensively studied in the CA1 region of the hippocampus, where synaptic modification obeys the Hebb rule.

The mechanism by which the Hebb rule is implemented is now known (for a general review of LTP, see [6]). Transmission at glutamatergic synapses is mediated by two types of receptor: the AMPA receptors, which are responsible for basal synaptic transmission, and the

Figure 2



Autophosphorylated CaMKII may enhance synaptic transmission by organizing anchoring sites for AMPA receptors at the synapse. The NMDA receptor complex binds, via subunit NR2B, to autophosphorylated CaMKII, which binds to alpha-actinin-2, providing a link to the F-actin cytoskeleton. F-actin binds to SAP-97 and Protein 4.1, which bind to AMPA receptors via subunit GluR1. This hypothetical linkage [3] suggests how CaMKII phosphorylation could lead to AMPA insertion at sites already containing NMDA receptors. [2,15–21].

NMDA receptors, which have a special role during LTP induction. NMDA receptors require glutamate to open, but glutamate alone is not sufficient; there must also be a substantial positive shift in the membrane potential of the postsynaptic cell. Thus, NMDA receptors are activated only under the conditions specified by the Hebb rule. Pharmacologically blocking NMDA receptors, or genetically removing them, prevents LTP induction. NMDA receptor channels have a high Ca^{2+} permeability, and it is now generally agreed that this NMDA-receptor-mediated increase in postsynaptic $[Ca^{2+}]_i$ triggers the biochemical processes responsible for LTP.

An important Ca^{2+} ‘detector’ in the postsynaptic neuron is CaMKII. This enzyme is highly concentrated in the postsynaptic density, a structure directly attached to the cytoplasmic face of the postsynaptic membrane. Several lines of evidence indicate that activation of CaMKII is a critical step in LTP induction. For instance, LTP induction can be blocked by pharmacologically inhibiting CaMKII or genetically removing it. Other experiments have shown that introducing an active form of CaMKII into the postsynaptic neuron induces a potentiation which prevents subsequent LTP induction. CaMKII is thus both necessary and sufficient for LTP induction.

The properties of CaMKII relevant to memory function have been extensively investigated. The kinase has twelve nearly identical subunits, which are arranged in two hexameric rings [7]. Each subunit can be activated by the binding of a single Ca^{2+}/calmodulin to that subunit. This simple form of activation lasts only as long as the increase in $[Ca^{2+}]_i$. Under certain conditions, however, CaMKII activation can outlast the increase in $[Ca^{2+}]_i$ [8]. This occurs when the increase in $[Ca^{2+}]_i$ is sufficient to cause Ca^{2+}/calmodulin to bind simultaneously to two neighboring subunits [9]. When this happens, one subunit phosphorylates the other at residue threonine 286. This causes the phosphorylated subunit to remain active even after $[Ca^{2+}]_i$ levels return to baseline. Previously it was thought

that this two-calmodulin reaction was the only way to persistently activate CaMKII, but the new results reported by Bayer *et al.* [2] indicate that when CaMKII binds to the NMDA receptor, persistent activity can be triggered by a sequence of more probable one-calmodulin reactions.

The new work [2] has revealed fascinating new details about the CaMKII–NMDA receptor interaction. In the 'off' state, the kinase is inactive because an autoinhibitory domain binds to the catalytic domain, which contains both the T-site and the S-site (Figure 1). Ca^{2+}/calmodulin binding causes the autoinhibitory domain to move away from the S- and T-sites, thereby activating the kinase. The principal finding of Bayer *et al.* [2] is that a 22 amino acid region of the NMDA receptor subunit NR2B can bind to the T-site of CaMKII after it is exposed by Ca^{2+}/calmodulin. This prevents the return of the autoinhibitory domain to the S-site even after dissociation of Ca^{2+}/calmodulin, and thus causes the kinase to remain active. Importantly, this could facilitate further autophosphorylation by a one-calmodulin reaction. When a neighboring CaMKII subunit binds Ca^{2+}/calmodulin, it will be phosphorylated at threonine 286 by the active NR2B-bound subunit. The reaction could continue around the ring, leading to hyperphosphorylation of the holoenzyme and enhancement of its Ca^{2+}-independent activity. Thus, the following picture emerges: during neuronal activity, CaMKII translocates to postsynaptic regions [10] and binds directly to the NR2B subunits [1,11]. This then leads to hyperphosphorylation of CaMKII entirely through one-calmodulin reactions.

There are at least four consequences of this hyperphosphorylation. First, CaMKII will bind more tightly to NR2B, as Bayer *et al.* [2] also show that a second region on NR2B, called the P-region, binds only phosphorylated CaMKII. Second, phosphorylated subunits of CaMKII will phosphorylate existing AMPA receptors, enhancing their conductance [4]. This has been shown to contribute to LTP [12]. Third, hyperphosphorylation may saturate local phosphatase molecules, thereby preventing dephosphorylation of CaMKII. This could result in CaMKII remaining active for very long periods [3]. Fourth, hyperphosphorylation of CaMKII may trigger the assembly of a molecular linkage [3] joining NMDA receptors to AMPA receptors (Figure 2). The formation of this linkage may be the process by which AMPA receptors are inserted into synaptic sites that already contain NMDA receptors. Like AMPA receptor phosphorylation, AMPA receptor insertion may contribute to LTP [13].

This is an exciting time in the field of synaptic plasticity. As details of the interactions among synaptic molecules are revealed, the roles of the molecules in synaptic mechanisms of information storage and retrieval are starting to emerge. Still other important pieces of the puzzle are tantalizing. For instance, the binding of CaMKII to the NMDA receptor displaces a scaffolding protein called PSD-95 from the NMDA receptor [14]. This scaffolding protein organizes a complex of important second messenger systems, but the functional consequences of separating CaMKII from PSD-95 are not yet known. Similarly, presynaptic and postsynaptic components of the synapse interact structurally, but the functional implications of these interactions are also unknown.

References

1. Gardoni F, Caputi A, Cimino M, Pastorino L, Cattabeni F, Di Luca M: **Calcium/calmodulin-dependent protein kinase II is associated with NR2A/B subunits of NMDA receptor in postsynaptic densities.** *J Neurochem* 1998, **71**:1733-1741.
2. Bayer K-U, De Koninck P, Leonard AS, Hell JW, Schulman H: **Interaction with the NMDA receptor locks CaMKII in an active conformation.** *Nature* 2001, **411**:801-805.
3. Lisman JE, Zhabotinsky AM: **A model of synaptic memory: a CaMKII/PP1 switch that potentiates transmission by organizing an AMPA-receptor anchoring assembly.** *Neuron* 2001, **31**:191-201.
4. Derkach V, Barria A, Soderling TR: **Ca^{2+}/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors.** *Proc Natl Acad Sci USA* 1999, **96**:3269-3274.
5. Bliss TV, Lomo T: **Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path.** *J Physiol* 1973, **232**:331-356.
6. Malenka RC, Nicoll RA: **Long-term potentiation — a decade of progress?** *Science* 1999, **285**:1870-1874.
7. Kolodziej SJ, Hudmon A, Waxham MN, Stoops JK: **Three-dimensional reconstructions of calcium/calmodulin-dependent (CaM) kinase IIalpha and truncated CaM kinase IIalpha reveal a unique organization for its structural core and functional domains.** *J Biol Chem* 2000, **275**:14354-14359.
8. Miller SG, Kennedy MB: **Regulation of brain type II Ca^{2+}/calmodulin-dependent protein kinase by autophosphorylation: a Ca^{2+}-triggered molecular switch.** *Cell* 1986, **44**:861-870.
9. Hanson PI, Meyer T, Stryer L, Schulman H: **Dual role of calmodulin in autophosphorylation of multifunctional CaM kinase may underlie decoding of calcium signals.** *Neuron* 1994, **12**:943-956.
10. Shen K, Meyer T: **Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation.** *Science* 1999, **284**:162-166.
11. Strack S, Colbran RJ: **Autophosphorylation-dependent targeting of calcium/calmodulin-dependent protein kinase II by the NR2B subunit of the N-methyl-D-aspartate receptor.** *J Biol Chem* 1998, **273**:20689-20692.
12. Benke TA, Luthi A, Isaac JT, Collingridge GL: **Modulation of AMPA receptor unitary conductance by synaptic activity.** *Nature* 1998, **393**:793-797.
13. Hayachi Y, SHi HH, Esteban JA, Piccini A, Poncer JC, Malinow R: **Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction.** *Science* 2000, **287**:2262-2267.
14. Gardoni F, Schrama LH, Kamal A, Gispen WH, Cattabeni F, Di Luca M: **Hippocampal synaptic plasticity involves competition between Ca^{2+}/calmodulin-dependent protein kinase II and postsynaptic density 95 for binding to the NR2A subunit of the NMDA receptor.** *J Neurosci* 2001, **21**:1501-1509.
15. Kornau HC, Schenker LT, Kennedy MB, Seeburg PH: **Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95.** *Science* 1995, **269**:1737-1740.
16. Shen L, Liang F, Walensky LD, Huganir RL: **Regulation of AMPA receptor GluR1 subunit surface expression by a 4. 1N-linked actin cytoskeletal association.** *J Neurosci* 2000, **20**:7932-7940.
17. Leonard AS, Davare MA, Horne MC, Garner CC, Hell JW: **SAP97 is associated with the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit.** *J Biol Chem* 1998, **273**:19518-19524.

18. Wu H, Reuver SM, Kuhlendahl S, Chung WJ, Garner CC: **Subcellular targeting and cytoskeletal attachment of SAP97 to the epithelial lateral membrane.** *J Cell Sci* 1998, **111:**2365-2376.
19. Galliano MF, Huet C, Frygelius J, Polgren A, Wewer UM, Engvall E: **Binding of ADAM12, a marker of skeletal muscle regeneration, to the muscle-specific actin-binding protein, alpha-actinin-2, is required for myoblast fusion.** *J Biol Chem* 2000, **275:**13933-13939.
20. Walikonis RS, Oguni A, Khorosheva EM, Jeng CJ, Asuncion FJ, Kennedy MB: **Densin-180 forms a ternary complex with the (alpha)-subunit of Ca^{2+}/calmodulin-dependent protein kinase II and (alpha)-actinin.** *J Neurosci* 2001, **21:**423-433.
21. Schischmanoff PO, Winardi R, Discher DE, Parra MK, Bicknese SE, Witkowska HE, Conboy JG, Mohandas N: **Defining of the minimal domain of protein 4.1 involved in spectrin-actin binding.** *J Biol Chem* 1995, **270:**21243-21250.