Calcium-calmodulin Dependent Protein Kinase II: *An Unforgettable Story*

Cedarburg SMART Team: Laura Tiffany, Alex Bothe, Sarah Dyke, Theresa Eggleston, Savannah Kenny, Erin Kuhn, Alex Satchie, Kathryn Tiffany and Emily Zietlow
Teacher: Mrs. Karen Tiffany

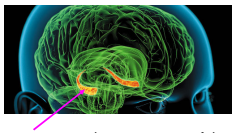
Mentors: Audra Kramer, Kanwardeep Kaleka, and Nashaat Gerges, Ph.D.
Department of Cell Biology, Neurobiology, and Anatomy Medical College of Wisconsin

Abstract

According to the National Institutes of Health, 5.1 million Americans have Alzheimer's disease (AD), which affects memory and the ability to learn. In long-term potentiation (LTP), a correlate of learning and memory, the number of receptors at the synapse between neurons, increases. Calcium/calmodulin dependent protein kinase II (CaMKII), a large dodecameric enzyme comprising 1-2% of all proteins in the brain, is part of a signaling pathway implicated in LTP. In this pathway, Ca^{+2} binds calmodulin (CaM) and the Ca^{+2}/CaM complex activates CaMKII, which then phosphorylates other proteins in the cell, like α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. To investigate the role of CaMKII, the Cedarburg SMART (Students Modeling A Research Topic) Team used 3D printing technology to design a CaMKII model, highlighting the catalytic, self-association, and autoinhibitory domains. The Ca^{+2}/CaM complex activates CaMKII by displacing a portion of the autoinhibitory domain that blocks the active site of the enzyme, exposing both the catalytic base and Thr286, the residue involved in autophosphorylation. When CaMKII phosphorylates AMPA receptors, their numbers increase in the post synaptic neuron and they are more sensitive to glutamate. Impaired LTP may lead to the cognitive decline seen in AD.

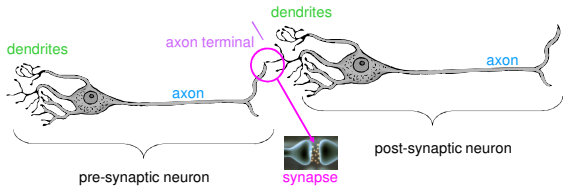
Learning results in changes in cellular structure in the brain.

The brain is the center of the nervous system and controls other organs in the body. It is also the location of learning and memory.



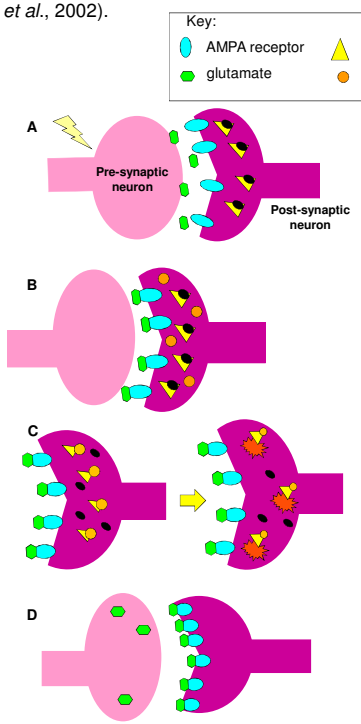
The **hippocampus**, a major component of the vertebrate brain, functions in memory and is one of the first regions of the brain to show damage in Alzheimer's disease (AD). AD is a progressive neurologic disorder that interferes with memory and learning.

The functional unit of the brain is the nerve cell, or **neuron**. There are an estimated 15 - 100 billion neurons in the brain and as many as 100 - 500 trillion synapses, junctions between adjacent neurons. Neurotransmitters, such as glutamate, are released from axon terminals, diffuse across synapses, and bind to dendritic receptors in the adjacent neuron, enabling neurons to communicate with one another.



Learning is a result of signals being passed along neurons and across the synapse connecting adjacent neurons. It is believed that information is stored when neuronal signaling pathways connect, resulting in memory (Lynch, 2004). Through long-term potentiation (LTP), the number of receptors and sensitivity of receptors in the post-synaptic membrane are increased. This allows for more neurotransmitters to bind to the receptors and, therefore, results in stronger neural connections and improved memory and learning ability (Cooke and Bliss, 2006). It is hypothesized that misprocessing of a brain protein results in the accumulation of protein fragments that impairs hippocampal LTP and may lead to the cognitive decline observed in AD (Rowan, *et al.*, 2003).

Calcium/calmodulin dependent protein kinase II (CaMKII) is part of a signaling pathway that modulates the number and sensitivity of glutamate receptors into the post-synaptic membrane and is necessary for long term potentiation (LTP) (Lisman, *et al.*, 2002).



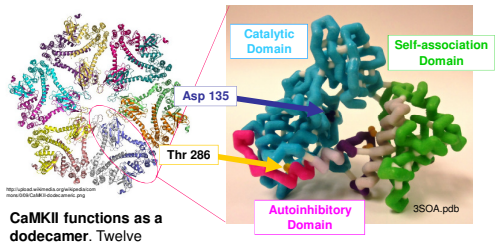
A. A signal passes along a pre-synaptic neuron.
A nerve impulse (lightning symbol) reaches the axon terminal of a pre-synaptic neuron. Glutamate, a neurotransmitter, is released into the synapse.

B: The pre-synaptic neuron signals the post-synaptic neuron.
Glutamate diffuses across the synapse and binds to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors on the post-synaptic neuron. As a result of signaling initiated by glutamate binding AMPA receptors, Ca^{+2} levels increase in the post-synaptic neuron. Neurogranin localizes inactive calmodulin (CaM) in the postsynaptic density, increasing the probability that Ca^{+2} will find and bind to CaM.

C: A signaling pathway results in activation of CaMKII.
Ca^{+2} binds to CaM, and neurogranin releases the active Ca^{+2}/CaM complex. The Ca^{+2}/CaM complex then binds CaMKII and induces a conformational change in CaMKII that exposes its active site.

D: Active CaMKII is an integral part of LTP.
Active CamKII phosphorylates AMPA receptors, increasing both receptor numbers and their sensitivity to neurotransmitters. The up-regulation of receptors in the postsynaptic neuron correlates with long-term potentiation (LTP).

The structure of CaMKII is important for its function in LTP.



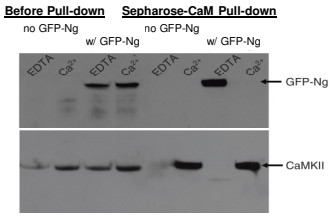
CaMKII functions as a dodecamer. Twelve identical monomers associate to form the functional holoenzyme.

A backbone model of a CaMKII monomer, a kinase involved in LTP.

Each monomer is comprised of a catalytic domain (cyan), a self-association domain (lime), and an autoinhibitory domain (portions in purple, lavender and pink). The autoinhibitory domain blocks the active site when Ca^{+2}/CaM is not bound. Binding of Ca^{+2}/CaM to the autoinhibitory domain (region shown in purple), causes the regulatory segment to move away from the active site, exposing the catalytic Asp135 (blue) as well as Thr286 (yellow). Autophosphorylation of Thr286 prevents the regulatory segment from blocking the active site, even when Ca^{+2}/CaM dissociates. This prolonged activation of CaMKII facilitates LTP.

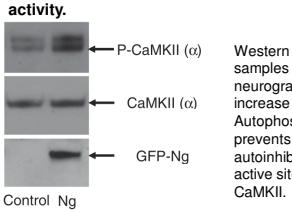
Increased calmodulin (CaM) availability increases CaMKII phosphorylation, and thus modulates CaMKII activity.

A. CaMKII binds to calmodulin (CaM) in the presence of Ca^{+2}.



Western blot analysis of a CaM "pull-down" assay shows that CaMKII binds to CaM only in the presence of Ca^{+2}. Conversely, GFP-labeled neurogranin (Ng) binds to CaM only in the absence of Ca^{+2}. GFP-labeled neurogranin (GFP-Ng) was expressed in brain cells. Brain tissue samples were homogenized and incubated with CaM-sepharose beads in the presence of 2 mM EDTA or 2 mM Ca^{+2}.

B. Neurogranin (Ng) binds CaM, increasing its availability in the post-synaptic neuron. Greater CaM availbility results in greater CaMKII activity.



Western blot analysis of hippocampal tissue samples indicates that the presence of neurogranin (GFP-Ng) corresponds to an increase in active CaMKII (P-CaMKII). Autophosphorylation of T286 in CaMKII prevents the regulatory segment of the autoinhibitory domain from blocking the active site, resulting in constitutively active CaMKII.

Concluding Remarks

- CaMKII is an important protein in neurons, comprising 1 - 2 % of neuronal proteins.
- As part of a signal pathway, CaMKII is activated when a Ca^{+2}/CaM complex binds to CaMKII and induces a conformational change.
- Active CaMKII phosphorylates AMPA receptors, resulting in increased numbers of receptors in the post-synaptic neuron and a greater sensitivity of the AMPA receptors to glutamate.
- Prolonged activation of CaMKII facilitates LTP, a cellular mechanism that underlies learning and memory.
- Alzheimer's disease (AD) is a progressive neurodegenerative disorder that results in obvious cognitive decline and dementia. The deterioration of the hippocampus that occurs early in AD may result in impaired LTP.

References

Alzheimer's Disease, PubMed Health (http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001767/).
Cooke, S., and Bliss, T. (2006). Plasticity in the human central nervous system. *Brain* **129**: 1659–1673.
Lisman, J., Schulman, H., Cline, H. (2002). The Molecular Basis of CaMKII Function in Synaptic and Behavioural Memory. *Nat Rev Neurosci* **3**: 175-90.
Lynch, M. (2004). Long-term potentiation and memory. *Physiol Rev* **84(1)**: 87-136.
Rowan, M., Klyubin, I., Cullen, W., Anwyl, R. (2003). Synaptic plasticity in animal models of early Alzheimer's disease. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **358 (1432)**: 821–8.
Zhong, L., Cherry, T., Bies, C., Florence, M., Gerges, N. (2009). Neurogranin enhances synaptic strength through its interaction with calmodulin. *The EMBO Journal* **28**: 3027-3039.

The SMART Team Program (Students Modeling A Research Topic) is funded by a grant from NIH-SEPA 1R25OD010505-01 from NIH-CTSA UL1RR031973.