

Calcium/Calmodulin-Dependent Protein Kinases

Key References

- Braun, A.P., Schulman, H. "The multifunctional calcium/calmodulin-dependent protein kinase: From form to function." *Annu. Rev. Physiol.* **57**, 417-445 (1995).
- De Koninck, P., Schulman, H. "Sensitivity of CaM kinase II to the frequency of Ca²⁺ oscillations." *Science* **279**, 227-230 (1998).
- Gallager, P.J. et al. "Myosin light chain kinases." *J. Muscle Res. Cell Motil.* **18**, 1-6 (1997).
- Goldberg, J. et al. "Structural basis for the auto-inhibition of calcium calmodulin-dependent protein kinase I." *Cell* **84**, 875-887 (1996).
- Hanson, P.I. et al. "Dual role of calmodulin in autophosphorylation of multifunctional CaM kinase may underlie decoding of calcium signals." *Neuron* **12**, 943-956 (1994).
- Marin, P. et al. "Glutamate-dependent phosphorylation of elongation factor-2 and inhibition of protein synthesis in neurons." *J. Neurosci.* **17**, 3445-3454 (1997).
- Morris, E.P., Torok, K. "Oligomeric structure of α -calmodulin-dependent protein kinase II." *J. Mol. Biol.* **308**, 1-8 (2001).
- Palfrey, H.C., Nairn, A.C. "Calcium-dependent regulation of protein synthesis." *Adv. Second Messenger Phosphoprotein Res.* **30**, 191-223 (1995).
- Ryazanov, A.G. et al. "Alpha-kinases: A new class of protein kinases with a novel catalytic domain." *Curr. Biol.* **9**, R43-R45 (1999).
- Shen, K., Meyer, T. "Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation." *Science* **284**, 162-166 (1999).
- Soderling, T.R. "The Ca²⁺-calmodulin-dependent protein kinase cascade." *Trends Biochem. Sci.* **24**, 232-236. (1999).
- Soderling, T.R. "CaM-kinases: Modulators of synaptic plasticity." *Curr. Opin. Neurobiol.* **10**, 375-380 (2000).

Overview

Free calcium is a major second messenger in all cell types. One mechanism by which calcium ions exert their effects is by binding to a 17 kDa protein, calmodulin (CaM). The binding of four calcium ions to calmodulin changes its conformation and promotes its interaction with a number of other proteins, including several classes of protein kinases that are activated by the calcium/CaM complex. A practical way of classifying the calcium/CaM-dependent protein kinases is based on their substrate specificity: some of these enzymes have only one substrate, and are designated as 'dedicated' calcium/CaM-dependent protein kinases, while others have broad substrate specificity and are termed 'multifunctional' kinases.

The dedicated calcium/CaM-dependent protein kinases comprise three enzymes; phosphorylase kinase, myosin light chain kinase and eEF2-kinase. Phosphorylase kinase, the first protein kinase to be identified, phosphorylates and activates glycogen phosphorylase, the enzyme that degrades glycogen. Phosphorylase kinase is activated either by phosphorylation by cAMP-dependent protein kinase or by binding of calcium/CaM. This mechanism of regulation is especially important in muscle where glycogen breakdown and muscle contraction are coordinated by the transient increase in cytosolic calcium levels. Myosin light chain kinases (MLCK) are a group of enzymes that phosphorylate the regulatory light chain of myosin. Smooth muscle MLCK induces smooth muscle contraction by increasing actin-activated myosin ATPase activity. In contrast, striated muscle MLCK plays only a modulatory role in contraction by potentiating the effects of troponin-bound calcium on actin/myosin. In non-muscle cells, MLCKs are key factors in the numerous processes which involve actin/myosin-based organelle movement or cell motility. eEF2-kinase (also known

as CaM-kinase III) phosphorylates eukaryotic elongation factor 2 (eEF2), a GTPase necessary for the elongation step in protein translation. eEF2-kinase belongs to a separate class of protein kinases that also includes myosin heavy chain kinases, and is distinct from the main family of protein kinases with which they have no sequence similarity. Phosphorylation of eEF2 by eEF2-kinase accounts for a calcium-dependent interruption of protein synthesis that may be responsible for a rapid change in the nature of the mRNA being translated.

Multifunctional calcium/CaM-dependent protein kinases comprise three enzymes referred to as CaM-kinases I, II and IV. CaM-kinase II (CaMKII) is an oligomer of probably 12 subunits which has unique properties and is also the most extensively studied. It is a ubiquitously distributed enzyme that is very highly enriched in neurons, especially in post-synaptic densities. As is the case of other CaM-kinases, the activity of CaMKII is inhibited by an autoinhibitory domain. This inhibition is alleviated by binding of calcium/CaM which allows autophosphorylation of the autoinhibitory domain. Once autophosphorylation has occurred, the presence of calcium/CaM is no longer necessary and the enzyme becomes calcium/CaM-independent. Interestingly, the oligomeric structure of CaMKII and the fact that autophosphorylation is a 'trans' reaction between different subunits of the oligomer has important consequences. Autophosphorylation promotes calcium/CaM trapping and occurs only when two adjacent subunits are bound to calcium/CaM. Thus, CaMKII is sensitive to the duration and frequency of calcium transients, and therefore is capable of decoding the frequency of calcium spikes. CaMKII may also remain active for some time while calcium levels return to normal, thereby maintaining a transient 'memory' of

neuronal activation. Its unusual properties, in particular its abundance in synaptic regions and its actions on many proteins including ion channels, make CaMKII a very important contributor to the processes of synaptic plasticity and LTP induction. The precise intracellular localization of CaMKII also appears to be regulated and recent work has demonstrated the ability of the ionotropic glutamate receptor agonist NMDA (N-Methyl-D-Aspartate) to induce translocation of CaMKII from actin filaments to post-synaptic densities.

CaMKI and CaMKIV are monomeric enzymes that share the common property of being activated by calcium/CaM binding and by phosphorylation by a CaM-kinase-kinase (CaMKK). Thus, together these kinases are organized as a calcium/CaM-dependent protein kinase cascade. CaMKI is a ubiquitously expressed cytosolic enzyme which phosphorylates many substrates, including synapsin I. In contrast, CaMKIV (also known as CaMK-Gr because of its abundance in cerebellar granule cells) is located in the nucleus and is predominantly expressed in neurons, testis and T-cells. CaMKIV phosphorylates transcription factors, including cAMP responsive element binding protein (CREB) and the associated CREB-binding protein (CBP), and thus plays a major role in calcium-regulated gene transcription. CaMKK controls the activity of both CaMKI and CaMKIV. There are two isoforms of CaMKK, α and β , enriched in the cytoplasm and the nucleus, respectively. CaMKK is also able to phosphorylate and activate PKB, and thus exert anti-apoptotic effects. Recently, a family of pro-apoptotic serine/threonine protein kinases has been identified and termed Death Associated Protein Kinases (DAP-kinases). Two of these DAP-kinases possess a CaM-binding domain and are activated by calcium-CaM.

Calcium/Calmodulin-Dependent Protein Kinases

| | Dedicated CaM-Kinases | | | Multifunctional CaM-Kinases | | | CaMKK |
|---|---|--|--------------|--|--|--|------------------------|
| | Phosphorylase Kinase (P 2014) | MLCK | eEF2-Kinase | CaMKI | CaMKII (C 7331) | CaMKIV | |
| MOLECULAR WEIGHT CATALYTIC SUBUNIT (kDa) | γ: 45 | 70-150 | ~100 | ~42 | 50-60 | 65-67 | 60-70 |
| SUBUNIT COMPOSITION | 16 subunits (α ₄ , β ₄ , γ ₄ , δ ₄) | Monomer | Monomer | Monomer | Oligomer (12) | Monomer | Monomer |
| GENES, ISOFORMS | — | Several | — | — | α, β, γ, δ | — | α, β |
| SUBSTRATES | Glycogen phosphorylase (79700) | Regulatory myosin light chain | eEF-2 | Multiple | Multiple | Multiple | CaMKI CaMKIV PKB |
| INHIBITORS | — | ML-7 (I 2764) ML-9 (C 1172) | TS-2 TS-4 | KN-62 (I 2142) KN-93 (K 1385) | KN-62 (I 2142) KN-93 (K 1385) | KN-62 (I 2142) KN-93 (K 1385) | — |

ABBREVIATIONS

CaMK: Calcium/Calmodulin-Dependent Protein Kinase

CaMKK: Calcium/Calmodulin-Dependent Protein Kinase Kinase

eEF2: eukaryotic Elongation Factor 2

MLCK: Myosin Light Chain Kinase

PKB: Protein Kinase B (also known as Akt)

ML-7: 1-(5-Iodonaphthalene-1-sulfonyl)-1H-hexahydro-1,4-diazepine

ML-9: 1-(5-Chloronaphthalene-1-sulfonyl)-1H-hexahydro-1,4-diazepine

KN-62: 2-[N-(4'-Methoxybenzenesulfonyl)]amino-N-(4'-chlorophenyl)-2-propenyl-N-methylbenzylamine phosphate

KN-93: N-(2-[N-[4-Chlorocinnamyl]-N-methylaminomethyl]phenyl)-N-(2-hydroxyethyl)-4-methoxybenzenesulphonamide

TS-2: 4-Ethyl-4-hydroxy-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine

TS-4: 4-Hydroxy-6-isopropyl-4-methyl-2-p-tolyl-5,6-dihydro-4H-1,3-selenazine