

## Ca/CaMKs

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### 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. 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 (previously 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, the  $\alpha$  kinases, with no sequence similarity with the main family of protein kinases. 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 12 subunits which has unique properties and is also the most extensively studied. It is a ubiquitously distributed enzyme 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. CaMKII is a very important contributor to the processes of synaptic plasticity and LTP induction.

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 largely 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. 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,  $\alpha$  and  $\beta$ , 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 kinases possess a CaM-binding domain and are activated by calcium/CaM.

## Ca/CaMKs

<b>FAMILY MEMBERS</b>	CaMKK	DAP-K	Phosphorylase Kinase ( <b>P2014</b> )
<b>OTHER NAMES</b>	Calcium/calmodulin-dependent protein kinase kinase	Death-associated protein kinase	PHK, glycogen phosphorylase kinase, GPK ( <b>P6635</b> )
<b>MOLECULAR WEIGHT/ STRUCTURAL DATA</b>	60-70 kDa Monomer	165 kDa Monomer	$\gamma$ : 45 kDa 16 subunits ( $\alpha 4$ , $\beta 4$ , $\gamma 4$ , $\delta 4$ )
<b>ISOFORMS</b>	$\alpha$ , $\beta$	One, several shorter related kinases	Two catalytic (M muscle, L/T liver/testis)
<b>SPECIES</b>	Mammals, birds	Vertebrates	Vertebrates, <i>Drosophila</i>
<b>DOMAIN ORGANIZATION</b>	Catalytic, CaM-R	Catalytic, CaM-R, ankyrin repeats, cytoskeleton binding, death domain	$\gamma$ , Catalytic, CaM-R
<b>PHOSPHORYLATION SITES</b>	Not characterized	Ser <sup>308</sup> (autophos., inhibition)	Ser <sup>27</sup> in $\beta$ subunit (PKA, activation)
<b>TISSUE DISTRIBUTION</b>	Ubiquitous, brain, testis, spleen	Ubiquitous	Ubiquitous, liver, muscle
<b>SUBCELLULAR LOCALIZATION</b>	Cytoplasm, nucleus	Cytoplasm	Cytoplasm
<b>BINDING PARTNERS/ ASSOCIATED PROTEINS</b>	Not known	Actin filaments	Not known
<b>UPSTREAM ACTIVATORS</b>	Ca <sup>2+</sup> /calmodulin	Ca <sup>2+</sup> /calmodulin, dephosphorylation	Ca <sup>2+</sup> /calmodulin, PKA ( <b>P2645</b> ), ADP ( <b>A2754</b> )
<b>DOWNSTREAM ACTIVATION</b>	CaMKI, CaMKIV, PKB ( <b>A8729</b> ), AMP-kinase ( <b>P6998</b> )	Myosin light chain ( <b>M9891</b> , <b>M4064</b> )	Glycogen phosphorylase ( <b>79700</b> )
<b>ACTIVATORS</b>	Not known	Not known	Not known
<b>INHIBITORS</b>	STO-609 ( <b>S1318</b> )	K252A ( <b>K1639</b> ), MW01-026Z, 3-amino-6-phenyl-pyridazine	Not known
<b>SELECTIVE ACTIVATORS</b>	Not known	Not known	Not known
<b>PHYSIOLOGICAL FUNCTION</b>	Activates CaMKI, IV, cell survival	Membrane blebbing	Promotes glycogen degradation
<b>DISEASE RELEVANCE</b>	Not known	Apoptosis, neuronal death	Mutations in glycogenesis

## FOOTNOTES

## Ca/CaMKs

<b>FAMILY MEMBERS</b>	MLCK	eEF2-Kinase	CaMKI
<b>OTHER NAMES</b>	Myosin light chain kinase ( <b>K1639</b> )	Eukaryotic elongation factor-2 kinase; Cam kinase III	Calcium/calmodulin-dependent protein kinase 1
<b>MOLECULAR WEIGHT/ STRUCTURAL DATA</b>	70-150 kDa Monomer	~100 kDa Monomer Unrelated to classical kinases	~42 kDa Monomer
<b>ISOFORMS</b>	Several	One	$\alpha$ , $\beta$ , $\gamma$
<b>SPECIES</b>	Vertebrates, <i>Drosophila</i>	Vertebrates	Vertebrates, <i>Drosophila</i>
<b>DOMAIN ORGANIZATION</b>	Actin binding, PEVK repeats, Ig, FN Catalytic, CaM-R	N-terminal $\alpha$ -kinase d	Catalytic, CaM-R
<b>PHOSPHORYLATION SITES</b>	Thr <sup>803</sup> (autophos., Ca <sup>2+</sup> /CaM-independence) Ser <sup>815</sup> and Ser <sup>823</sup> (autophos., inhibition)	Ser <sup>359</sup> (p38-MAPK), Ser <sup>366</sup> (p90RSK), Ser <sup>377</sup> (MAPKAP-K2), Ser <sup>500</sup> (PKA)	Thr <sup>177</sup> (CaMKK, activation)
<b>TISSUE DISTRIBUTION</b>	Ubiquitous	Ubiquitous	Ubiquitous
<b>SUBCELLULAR LOCALIZATION</b>	Cytoplasm	Cytoplasm	Cytoplasm
<b>BINDING PARTNERS/ ASSOCIATED PROTEINS</b>	Actin ( <b>A9718</b> )	Not known	Not known
<b>UPSTREAM ACTIVATORS</b>	Ca <sup>2+</sup> /calmodulin, ERK ( <b>E9402</b> )	Ca <sup>2+</sup> /calmodulin	Ca <sup>2+</sup> /calmodulin, CaMKK
<b>DOWNSTREAM ACTIVATION</b>	Myosin light chain ( <b>K1639</b> )	eEF-2	Multiple (e.g. synapsin I, CREB)
<b>ACTIVATORS</b>	Not known	Not known	Not known
<b>INHIBITORS</b>	ML-7 ( <b>I2764</b> ), ML-9 ( <b>C1172</b> )	TS-2, TS-4	KN-62 ( <b>I2142</b> ), KN-93 ( <b>K1385</b> )
<b>SELECTIVE ACTIVATORS</b>	Not known	Not known	Not known
<b>PHYSIOLOGICAL FUNCTION</b>	Smooth muscle contraction	Protein synthesis interruption	Many (e.g. transcription regulation)
<b>DISEASE RELEVANCE</b>	Vasospasm, asthma	Not known	Not known

## FOOTNOTES

## Ca/CaMKs

<b>FAMILY MEMBERS</b>	CaMKII ( <b>C7331</b> )	CaMKIV
<b>OTHER NAMES</b>	Calcium/calmodulin-dependent protein kinase 2	Calcium/calmodulin-dependent protein kinase 4 ( <b>C0843</b> )
<b>MOLECULAR WEIGHT/ STRUCTURAL DATA</b>	50-60 kDa Oligomer (12)	65-67 kDa Monomer
<b>ISOFORMS</b>	$\alpha$ , $\beta$ , $\gamma$ , $\delta$	Not known
<b>SPECIES</b>	Vertebrates, <i>Drosophila</i>	Mammals
<b>DOMAIN ORGANIZATION</b>	Oligomerization domain, catalytic, CaM-R	Catalytic, CaM-R
<b>PHOSPHORYLATION SITES</b>	Autophosphorylation (CaM-R), others	CaMKK (Thr <sup>196</sup> , activation loop)
<b>TISSUE DISTRIBUTION</b>	Ubiquitous	Neurons, testis, T cells
<b>SUBCELLULAR LOCALIZATION</b>	Cytoplasm, nucleus	Cytoplasm, nucleus
<b>BINDING PARTNERS/ ASSOCIATED PROTEINS</b>	Not known	Not known
<b>UPSTREAM ACTIVATORS</b>	Ca <sup>2+</sup> /calmodulin, autophosphorylation	Ca <sup>2+</sup> /calmodulin, CaMKK
<b>DOWNSTREAM ACTIVATION</b>	Multiple (e.g. NMDA and AMPA receptors)	Multiple (e.g. MEF2)
<b>ACTIVATORS</b>	Not known	Not known
<b>INHIBITORS</b>	KN-62 ( <b>I2142</b> ), KN-93 ( <b>K1385</b> )	KN-62 ( <b>I2142</b> ), KN-93 ( <b>K1385</b> )
<b>SELECTIVE ACTIVATORS</b>	Not known	Not known
<b>PHYSIOLOGICAL FUNCTION</b>	Many (e.g. synaptic plasticity)	Many (e.g. transcription regulation)
<b>DISEASE RELEVANCE</b>	Not known	Not known

### Abbreviations

**CaMK:** Calcium/Calmodulin-Dependent Protein Kinase

**CaMKK:** Calcium/Calmodulin-Dependent Protein Kinase Kinase

**CaM-R:** Calmodulin regulatory domain: binds calmodulin and regulates kinase activity

**CREB:** cAMP-response element-binding protein

**DAP-kinase:** Death-Associated Protein kinase

**eEF2:** Eukaryotic Elongation Factor 2

**FN:** Fibronectin domain

**Ig:** Immunoglobulin domain

**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

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

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

**MAPK:** Mitogen-activated protein kinase

**MAPKAP-K:** MAPK-activated protein kinase

**MEF2:** Myocyte Enhancer Factor-2

**MLCK:** Myosin Light Chain Kinase

**PKA:** cAMP-dependent protein kinase

**PKB:** Protein Kinase B (also known as Akt)

**RSK:** Ribosome subunit S6 kinase

**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

## FOOTNOTES