

Ca/CaMKs

Key References

- Bain, J., et al., The specificities of protein kinase inhibitors: an update., *Biochem J.*, **371**, 199-204 (2003).
- Colbran, R.J., Targeting of calcium/calmodulin-dependent protein kinase II., *Biochem. J.*, **378**, 1-16 (2004).
- Gallager, P.J., et al., Myosin light chain kinases., *J. Muscle Res. Cell Motil.*, **18**, 1-6 (1997).
- Hoelz, A., et al., Crystal structure of a tetradecameric assembly of the association domain of Ca²⁺/calmodulin-dependent kinase II., *Mol. Cell*, **11**, 1241-1251 (2003).
- Hudmon, A. and Schulman, H., Neuronal Ca²⁺/calmodulin-dependent protein kinase II: the role of structure and autoregulation in cellular function., *Annu. Rev. Biochem.*, **71**, 473-510 (2002).
- Lisman, J., et al., The molecular basis of CaMKII function in synaptic and behavioural memory., *Nat. Rev. Neurosci.*, **3**, 175-190 (2002).
- Rosenberg, O.S., et al., Structure of the autoinhibited kinase domain of CaMKII and SAXS analysis of the holoenzyme. *Cell*, **123**, 849-860 (2005).
- Ryazanov, A.G., Elongation factor-2 kinase and its newly discovered relatives., *FEBS Lett.*, **514**, 26-29 (2002).
- Shohat, G., et al., The DAP-kinase family of proteins: study of a novel group of calcium-regulated death-promoting kinases., *Biochim. Biophys. Acta.*, **1600**, 45-50 (2002).
- Soderling, T.R., CaM-kinases: Modulators of synaptic plasticity., *Curr. Opin. Neurobiol.*, **10**, 375-380 (2000).
- Soderling, T.R., The Ca²⁺-calmodulin-dependent protein kinase cascade., *Trends Biochem. Sci.*, **24**, 232-236. (1999).
- Yamaguchi, H., et al., Crystal structure of the atypical protein kinase domain of a TRP channel with phosphotransferase activity., *Mol. Cell.*, **7**, 1047-1057 (2001).

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 α 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, 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 kinases possess a CaM-binding domain and are activated by calcium/CaM.

Ca/CaMKs

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

FOOTNOTES

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

FOOTNOTES

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