Minireview

Cellular Signaling through Multifunctional Ca\textsuperscript{2+}/Calmodulin-dependent Protein Kinase II*

Published, JBC Papers in Press, November 28, 2000, DOI 10.1074/jbc.R000013200

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Ca\textsuperscript{2+}/CaM-dependent protein kinase II (CaM-KII)\textsuperscript{1} is a ubiquitously expressed protein kinase that transduces elevated Ca\textsuperscript{2+} signals in cells to a number of target proteins ranging from ion channels to transcriptional activators. CaM-KII has a unique holoenzyme structure and autoregulatory properties that allow it to give a prolonged response to transient Ca\textsuperscript{2+} signals and to sense cellular Ca\textsuperscript{2+} oscillations. In neurons CaM-KII is highly expressed and localized with certain subcellular structures. Upon activation it can translocate to excitatory synapses where it regulates a number of proteins involved in synaptic transmission and its downstream signaling pathways.

Elevated intracellular free calcium (Ca\textsuperscript{2+}), in response to agonist stimulation or cell depolarization, is highly regulated and involves influx through voltage- and ligand-gated Ca\textsuperscript{2+}-permeable ion channels, release from intracellular stores through ryanodine- and inositol 1,4,5-trisphosphate-sensitive channels, sequestration by Ca\textsuperscript{2+} pumps and exchangers, and signaling through specific Ca\textsuperscript{2+} transducer proteins (1). Changes in intracellular calcium can display variable responses ranging from highly localized, transient elevations within subcellular structures (e.g. a dendritic spine of a neuron) to Ca\textsuperscript{2+} waves that spread throughout the cell including the nucleus. The most ubiquitous calcium-sensing protein is calmodulin (CaM), which contains four “EF” hand motifs with high specificity for binding Ca\textsuperscript{2+}. The Ca\textsuperscript{2+}/CaM complex interacts with and modulates the functionality of a large number of proteins (2) including several Ser/Thr protein kinases (CaM-Ks). This review, which is part of a series on Ca\textsuperscript{2+}/CaM-dependent protein kinases and phosphatase, will consider one member of this family, the multifunctional CaM-KII. The CaM-KII family is encoded by four genes (\(\alpha, \beta, \gamma, \) and \(\delta\)) that also exhibit alternative splicing. The \(\gamma\) and \(\delta\) isoforms are expressed in most tissues, whereas the \(\alpha\) and \(\beta\) isoforms are most prominent in neural tissues and comprise up to 2\% of total protein in hippocampus. This review will focus on selected advances over the past 5 years, especially for neuronal CaM-KII, and readers are referred to an earlier comprehensive review for general background information (3).

*This minireview will be reprinted in the 2001 Minireview Compendium, which will be available in December, 2001. This is the third article of four in the “Ca\textsuperscript{2+}-dependent Cell Signaling through Calmodulin-activated Protein Phosphatase and Protein Kinases Minireview Series.”

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The abbreviations used are: CaM-KII, Ca\textsuperscript{2+}/CaM-dependent protein kinase; AID, autoinhibitory domain; AMPA-R, \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolopropionic receptor; CaM, calmodulin; CPE, cytoplasmic polyadenylation element; CPEB, cytoplasmic polyadenylation element-binding protein; CREB, cAMP response element-binding protein; DG, disc large protein; GluR1, glutamate receptor subunit 1 of AMPA-R; cAK, CaM-KII association protein; LTP, long term potentiation; MDMA-R, N-methyl d-aspartate receptor; nNOS, neuronal nitric oxide synthase; PSD, postsynaptic density; PKA, protein kinase A; PDZ, Pse-Dlg-Zo-1; SAP, synapse-associated protein; SynGAP, synaptic GTPase-activating protein; UTR, untranslated region.

Structure and Regulation

The various CaM kinase II subunits are comprised of an N-terminal catalytic region, a central regulatory domain containing an autoinhibitory domain (AID) and Ca\textsuperscript{2+}/CaM binding motif, a variable sequence, and the C-terminal subunit association domain (4) (Fig. 1A). The holoenzyme is an oligomeric protein comprised of twelve 50–60-kDa subunits arranged as two stacked hexameric rings (5, 6). The C-terminal association domains form the central core of each ring with the N-terminal catalytic domains projecting outward (Fig. 1B). In the absence of bound Ca\textsuperscript{2+}/CaM, the CaM-KII is maintained in an inactive configuration because of the AID with the catalytic domain of its own subunit. Three distinct molecular models, based on structure/function studies, have been presented for how the AID might suppress catalytic activity (7–9), but definitive proof from a crystal structure has yet to be obtained. The Ca\textsuperscript{2+}/CaM complex binds to a sequence that partially overlaps the AID (Fig. 1A), presumably causing a conformational change and thereby disrupting interaction of the AID with the catalytic domain and producing kinase activation. Interaction of the AID with the sensitivity to activation by Ca\textsuperscript{2+}/CaM depends on the subunit composition of the holoenzyme (10).

Upon activation by Ca\textsuperscript{2+}/CaM binding, the kinase undergoes an immediate autophosphorylation on Thr-286 (numbering will be based on the \(\alpha\) isoform) (3). This autophosphorylation occurs within the oligomeric complex (i.e. intramolecular) but between adjacent subunits (intersubunit) that have bound Ca\textsuperscript{2+}/CaM (11, 12).

An interesting consequence of the oligomeric structure of CaM-KII discussed above is that it would restrict intramolecular autophosphorylation of Thr-286 within each of the two hexameric rings. This rapid autophosphorylation on Thr-286 has two important regulatory consequences. 1) The subsequent dissociation rate for Ca\textsuperscript{2+}/CaM upon removal of Ca\textsuperscript{2+} is decreased by several orders of magnitude (13, and 2) even after full dissociation of Ca\textsuperscript{2+}/CaM, the kinase retains partial activity (i.e. Ca\textsuperscript{2+}/CaM-independent or constitutive activity). Presumably the complex holoenzyme structure of CaM-KII (see Fig. 1B) endows the kinase with this unique regulatory property. Thus, transient elevation of intracellular Ca\textsuperscript{2+} can give a prolonged response through the constitutive activity of autophosphorylated CaM-KII, and this property appears to be critical for certain physiological functions of CaM-KII as discussed later.

Sensor of Cellular Ca\textsuperscript{2+} Oscillations

The efficiency of synaptic transmission between neurons can be modulated, a process known as synaptic plasticity. At excitatory synapses using glutamate as neurotransmitter, synaptic plasticity such as long term potentiation (LTP) is triggered by increased Ca\textsuperscript{2+} in postsynaptic spines (14) and is dependent on the frequency of afferent stimulation (15). How is the frequency of Ca\textsuperscript{2+} oscillations in the postsynaptic spine decoded? CaM-KII has been touted as a decoding mechanism (Fig. 2) because of its unique activation properties as discussed above and its localization in dendritic spines in an organelle called the postsynaptic density (PSD) (16). The magnitude of constitutive CaM-KII activity because of autophosphorylation of Thr-286 on adjacent subunits in the oligomeric holoenzyme should depend on the duration, amplitude, and frequency of elevated Ca\textsuperscript{2+}, and a recent in vitro study (17) shows this to be the case. The abilities of CaM-KII to decode the frequency of synaptic stimulation and to give a prolonged readout beyond the initial stimulus are two characteristics required for a molecule involved in generation of synaptic plasticity.

Subcellular Localization and Translocation

In many cells CaM-KII is largely soluble and widely distributed throughout the cell, but discrete subcellular pools of CaM-KII have recently become recognized. Localization of signaling enzymes close to their substrates has, in general, important regulatory consequences (18), especially for broad specificity enzymes such as...
CaM-KII. Thus, characterization of mechanisms for subcellular localization of CaM-KII and its physiological roles are intense areas of investigation.

Alternative splice variants of α, δ, and γ isoforms contain a nuclear localization signal (19, 20), and nuclear CaM-KII is likely to play a role in Ca$^{2+}$-mediated transcriptional regulation of genes such as brain-derived neurotrophic factor (21) and atrial natriuretic factor (22) through phosphorylation of transcription factors including CCAAT/enhancer-binding protein (C/EBP)(22, 24). It is intriguing that CaM kinases I and IV can phosphorylate a Ser adjacent to the nuclear localization signal and prevent nuclear localization of the CaM-KII, but whether this occurs physiologically is uncertain (25). CaM-KII exhibits broad cellular distribution and is largely cytoplasmic, whereas CaM-KIV has a rather restricted tissue distribution and exists as both cytosolic and nuclear isoforms (26). CaM-KII may also exert negative effects on transcription through phosphorylation of the transcription factor CREB. Surprisingly, although the activation site (Ser-133) in CREB can be efficiently phosphorylated by CaM-KII, it simultaneously phosphorylates another site (Ser-142) that exerts a dominant negative role (27). Thus, it is possible that nuclear CaM-KII can inhibit CREB-dependent transcription mediated by kinases such as PKA, but this needs to be verified under physiological conditions. Ca$^{2+}$-stimulated gene transcription through CREB is mediated in part by CaM-KIV (28, 29).

Anchoring proteins that localize PKA (18) and protein kinase C (30) close to physiological substrates have been well characterized, and recent studies indicate a similar regulatory scheme for CaM-KII. An anchoring protein, αKAP, has recently been identified that localizes skeletal muscle CaM-KII to the sarcoplasmic reticulum. This unique protein contains a hydrophobic N terminus that associates with the C-terminal association domain of CaM-KII (31). The C-terminal association domain of αKAP can form heteromers with the full-length CaM-KII subunit, and the hydrophobic N terminus of αKAP directs the resulting kinase complex to the sarcoplasmic reticulum membrane (32). Likely substrates for CaM-KII in the sarcoplasmic reticulum include the ryanodine receptor (33), phospholamban (34), and the Ca$^{2+}$-ATPase pump (35).

In brain, there is evidence for colocalization of the CaM-KII β isoform with the cytoskeleton. Upon stimulation of the Ca$^{2+}$-permeable NMDA-R channel in hippocampal neurons, CaM-KII appears to dissociate from F-actin and undergo translocation to membranous fractions including the PSD (36). The PSD, a complex of postsynaptic membrane proteins involved in mediating and modulating synaptic transmission, is held together and to the cytoskeleton through anchoring proteins of the PDZ/SAP family (16, 37). CaM-KII is a major constituent of the PSD where it is anchored in part through the protein densin-180 (38). This interaction of CaM-KII with densin-180 does not appear to depend on the activation state of the kinase. In contrast, additional CaM-KII can associate with the PSD through interaction with the NMDA-type glutamate-gated ion channel, but this translocation appears to require activation of the kinase and its autophosphorylation on Thr-286 (39, 40). Translocation to the PSD occurs in hippocampal slices upon treatments that activate CaM-KII, and it promotes the phosphorylation of CaM-KII substrates in the PSD such as the AMPA-R (39, 41). The anchoring interaction appears to occur between the catalytic domain of CaM-KII and residues 1290–1309 in the cytosolic tail of the NR2B subunit of the NMDA-R (42). Translocation would localize CaM-KII at a critical site of Ca$^{2+}$ influx into the dendritic spine because the NMDA channel has considerable Ca$^{2+}$ permeability, and its activation is required for several types of synaptic plasticity. In addition to localizing CaM-KII to a site of Ca$^{2+}$ influx, this translocation also situates the activated kinase in close proximity to several very important neuronal substrates (Fig. 3) as discussed below.

**Activation and Synthesis of CaM-KII in Neurons**

There is considerable evidence that activation of CaM-KII in pyramidal neurons of region CA1 in hippocampus is intimately involved in the phenomena of LTP (43, 44). LTP is touted as a cellular model of learning and memory because it represents a neuron-specific mechanism for increasing the efficacy of synaptic transmission (45). Induction of LTP, through activation of the NMDA-R, in hippocampus triggers CaM-KII autophosphorylation on Thr-286 and formation of its constitutively active form (46–48) (Fig. 3). Maintaining the constitutive activity of CaM-KII for at least 1 h during LTP requires inhibition of protein phosphatase 1, which can dephosphorylate Thr-286 (49), through PKA-mediated phosphorylation of the protein phosphatase 1 inhibitor (50). Induction of LTP by multiple tetanic stimuli results in global activation of CaM-KII throughout apical dendrites and the pyramidal cell somas (48). However, very mild NMDA-R stimulation of cultured neurons can result in very restricted activation of CaM-KII within individual dendritic spines (51).

Dendrites contain the machinery for localized protein synthesis, and one of the more abundant mRNAs in dendrites encodes the α subunit of CaM-KII. The 3'-untranslated region (UTR) of CaM-KII mRNA is responsible for its dendritic migration (52). Induction of LTP results in a selective increase in dendritic α CaM-KII protein, which is blocked by anisomycin and detected within 5 min, strongly suggestive of localized synthesis (53). How might dendritic synthesis of proteins such as CaM-KII be regulated (54)? The 3'-UTR in the mRNA encoding α CaM-KII contains two cytoplasmic polyadenylation elements (CPEs). These CPEs interact with a CPE-binding protein (CPEB), which is present in hippocampal dendrites and enriched in PSDs. Binding of CPEB to the 3'-UTR of CaM-KII mRNA promotes its polyadenylation, thereby enhancing translation (55). Certain forms of synaptic plasticity in the visual cortex result in changes in the 3'-UTR of CaM-KII mRNA with a 1.7-fold increase in CaM-KII protein isolated from synaptoneurosomes (55). The signaling pathway(s) between neural activity and enhanced CPEB-dependent polyadenylation is not known, but phosphorylation of CPEB may be involved (56).

**Neuronal Substrates of CaM-KII**

CaM-KII can phosphorylate a large number of proteins in vitro (3), and recently several substrates that may be involved in synaptic plasticity have been identified (Fig. 3). Numerous studies have documented that activated CaM-KII can phosphorylate the Glur1 subunit of the AMPA-R and enhance its current (43). A recent report documents AMPA-R phosphorylation by CaM-KII during LTP in region CA1 of hippocampus (57). Phosphorylation of Ser-831 in GluR1 by CaM-KII potentiates AMPA-R current by increasing single channel conductance (58). Indeed, about 60% of CA1 neurons that exhibit potentiation during LTP show an increase in unitary conductance (59). Experiments with transgenic mice also support a crucial role of CaM-KII phosphorylation of AMPA-R subunits in LTP (60). For example, mice lacking Glur1, the AMPA-R subunit phosphorylated by CaM-KII, show a specific deficit in LTP (61). Likewise, a single-site mutation in αCaM-KII (T286A) results in a mouse that is deficient in CA1 LTP (61). This subtle mutation does not effect activation of CaM-KII through binding of Ca$^{2+}$/CaM, but it precludes generation of constitutive activity by autophosphorylation. Thus, CaM-KII phosphorylation of AMPA-Rs with resultant potentiation of current is thought to contribute prominently to LTP at the CA1 synapse of hippocampus (43, 44).

Several other PSD proteins can also be phosphorylated by CaM-
Indeed, the NMDA-R that acts as an anchor for activated CaM-KII can be phosphorylated by CaM-KII (62). Although phosphorylation of Ser-1303 in the NR2B subunit has not been reported to directly regulate channel properties, it appears to decrease its binding affinity for CaM-KII (42). Another substrate is a novel Ras-GTPase-activating protein (SynGAP) localized at the PSD of hippocampal neurons through its interaction with PDZ domains of the scaffold proteins PSD-95 and SAP102 (63, 64). SynGAP is phosphorylated and potently inhibited by CaM-KII. This suggests that activation of the NMDA-R, which is also part of the PSD-95 complex, may result in activation of CaM-KII, which in turn phosphorylates and inhibits SynGAP, thereby potentiating activation of the mitogen-activated protein kinase pathway that appears to be important in some forms of synaptic plasticity. Regulation of mitogen-activated protein kinase through CaM-KII-mediated phosphorylation of SynGAP needs to be verified in intact neurons. Another CaM-KII substrate anchored to PSD-95 is neuronal nitric oxide synthase (nNOS). Phosphorylation of nNOS at Ser-847 results in partial inhibition of its activity (65). Nitric oxide, the product of NOS, appears to be an important signaling molecule in brain (66).
In Caenorhabditis elegans wild-type CaM-KII appears necessary for normal trafficking of glutamate receptors from the cell body and its clustering at neuromuscular synapses (67). In both C. elegans (67) and Drosophila (68), expression of constitutively active CaM-KII results in disruption of normal synaptic structure. In Drosophila this appears to be because of phosphorylation of the synaptic clustering protein DLG, a homologue of the mammalian SAP family involved in clustering of glutamate receptors. In mammals CaM-KII may also be involved in trafficking of glutamate receptors through interactions with SAP proteins. When GluR1 is overexpressed in CA1 hippocampal neurons, it translocates to the synapse in response to either activation of CaM-KII or LTP induction (69). This effect of CaM-KII was not because of phosphorylation of Ser-831 in GluR1, but insertion of GluR1 into the synapse was abolished by mutation of its C-terminal PDZ domain interaction site. GluR1 interacts with SAP97 (70), and SAP97 contains the CaM-KII phosphorylation site identified in Drosophila DLG. Thus, prolonged CaM-KII activity might promote recruitment of AMPA-Rs into synapses as predicted by the "silent synapse" hypothesis of LTP (44, 71).

Concluding Remarks

CaM-KII has a unique holoenzyme structure that endows it with unusual regulatory properties required for sensing and transducing various types of intracellular Ca\(^{2+}\) signals. Tremendous progress has occurred over the past 5 years in understanding the physiological substrates. The latter area has been hampered by the absence of highly specific CaM-KII inhibitors, but recently a naturally occurring CaM-KII inhibitor protein, CaM-KIIN, has been cloned (72). A 27-residue peptide derived from CaM-KIIN retains its high specificity and potency for inhibition of CaM-KII, and this naturally occurring CaM-KII inhibitor protein, CaM-KIIN, has been physiologically involved in clustering of glutamate receptors. In mammals it appears to be because of phosphorylation of the synaptic signals. Tremendous progress has occurred over the past 5 years in understanding the physiological substrates. The latter area has been hampered by the absence of highly specific CaM-KII inhibitors, but recently a naturally occurring CaM-KII inhibitor protein, CaM-KIIN, has been cloned (72). A 27-residue peptide derived from CaM-KIIN retains its high specificity and potency for inhibition of CaM-KII, and this naturally occurring CaM-KII inhibitor protein, CaM-KIIN, has been physiologically involved in clustering of glutamate receptors. In mammals it appears to be because of phosphorylation of the synaptic...
