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Calcium/Calmodulin-dependent Protein Kinase Kinase 2: Roles in Signaling and Pathophysiology*

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Many cellular Ca^{2+}-dependent signaling cascades utilize calmodulin (CaM) as the intracellular Ca^{2+} receptor. Ca^{2+}/CaM binds and activates a plethora of enzymes, including CaM kinases (CaMKs). CaMKK2 is one of the most versatile of the CaMKs and will phosphorylate and activate CaMKI, CaMKIV, and AMP-activated protein kinase. Cell expression of CaMKK2 is limited, yet CaMKK2 is involved in regulating many important physiological and pathophysiological processes, including energy balance, adiposity, glucose homeostasis, hematopoiesis, inflammation, and cancer. Here, we explore known functions of CaMKK2 and discuss its potential as a target for therapeutic intervention.

Ca^{2+} is pervasive second messenger that controls many cell functions by forming a complex with calmodulin (CaM)[3] (1), which serves as a ubiquitous intracellular Ca^{2+} receptor (2). Upon Ca^{2+} binding, CaM increases its affinity for a large number of CaM-binding proteins, including three multifunctional CaM kinases (CaMKI, CaMKII, and CaMKIV) (3). For full activation, CaMKI and CaMKIV require phosphorylation on an activation loop Thr by CaMKKα or CaMKKβ (CaMKK1 or CaMKK2, respectively). The requirement of two CaMKs in the same signaling pathway led to the concept of a CaMK cascade (4). In addition to its role in these enzymatic cascades, CaMKK2 is also a physiologically relevant upstream activator of the AMP-activated protein kinase (AMPK) (5–7), and one role for this CaMKK2-AMPK complex is the regulation of energy balance by acting in the hypothalamus (8).

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[3] The abbreviations used are: CaM, calmodulin; CaMK, CaM kinase; CaMKK, CaMK kinase; AMPK, AMP-activated protein kinase; CREB, cAMP response-element binding protein; CGC, cerebellar granule cell; GCP, granule cell precursor; EGL, external granule layer; ARC, hypothalamic arcuate nucleus; NPY, neuropeptide Y; AgRP, Agouti-related protein; WAT, white adipose tissue; HSC, hematopoietic stem cell; AR, androgen receptor; IP_3, inositol 1,4,5-trisphosphate.

CaMKK2: Structure and Function

CaMKK2 is a 66–68-kDa kinase in rat, mouse, and man (9–14). Analysis of *CaMKK2* cDNA clones revealed a >90% species similarity in the amino acid coding region but with heterogeneity in the 3′-noncoding termini (12–14). Similar to other CaMK family members, CaMKK2 consists of unique N- and C-terminal domains and a central Ser/Thr-directed kinase domain that is followed by a regulatory domain composed of overlapping autoinhibitory and CaM-binding regions (15). The deduced amino acid sequences of cloned *CaMKK2* cDNAs reveal 30–40% sequence identity of the kinase homology domain to other members of the CaMK family. However, CaMKK2 contains a unique 22-residue Pro/Arg/Gly-rich insert between the ATP-binding and protein substrate motifs (11).

The human *CaMKK2* locus spans over 40 kb pairs, maps to chromosome 12q24.2, and is organized into 18 exons and 17 introns (16). Two major transcripts are generated by use of polyadenylation sites present in the last two exons. Additionally, *CaMKK2* transcripts can be generated by alternative splicing of internal exons 14 and/or 16; this mechanism produces variants with different roles in neuronal differentiation (16, 17). PKA and CaMKIV have been reported to be involved in regulation of alternative splicing of the *CaMKK2* transcript (17).

Analysis of the sequences of the promoter and 5′-untranslated region of the human *CaMKK2* gene identified consensus DNA-binding sequences for several transcription factors, including Ikaros, RUNX1 (Runt-related transcription factor 1), and GATA1 (GATA-binding factor 1). The expression of these transcription factors is typically restricted to stem cell progenitors, in which their role is to regulate hematopoiesis and neuropoiesis (19–21). This may be relevant to the fact that CaMKK2 is expressed in a restricted number of cell types, as well as the involvement of CaMKK2 in processes regulating development of neurons and blood progenitors (22, 23).

The most well characterized substrates of CaMKK2 are CaMKI and CaMKIV. CaMKK2 phosphorylates CaMKIV and CaMKI on activation loop Thr residues (Thr-200 and Thr-177, respectively), which increases their kinase activities. Accordingly, mutation of the Thr residue abolishes both phosphorylation and activation of CaMKI/CaMKIV by CaMKK2 (12). More recently, AMPKα was shown to be an additional substrate of CaMKK2. Phosphorylation and activation of AMPK occur in response to an increase in intracellular Ca^{2+} in LKB1 (liver kinase B1)-deficient cells, and this effect is dependent on CaMKK2 (5). Down-regulation of CaMKK2 in mammalian cells using RNA interference almost completely abolishes AMPK activation (6). Finally, purified CaMKK2 will phosphorylate and activate AMPKα *in vitro* (6), and these two kinases form a stable multiprotein complex consisting of Ca^{2+}/CaM, CaMKK2, AMPKα, and AMPKβ (8), which is regulated by Ca^{2+}, but not by AMP, due to the absence of the AMP-binding subunit. Green *et al.* (24) demonstrated that CaMKK2 and AMPK associate through their kinase domains and found that CaMKK2 must be in an active conformation to bind AMPK, but not to associate with other substrates, such as CaMKIV. These

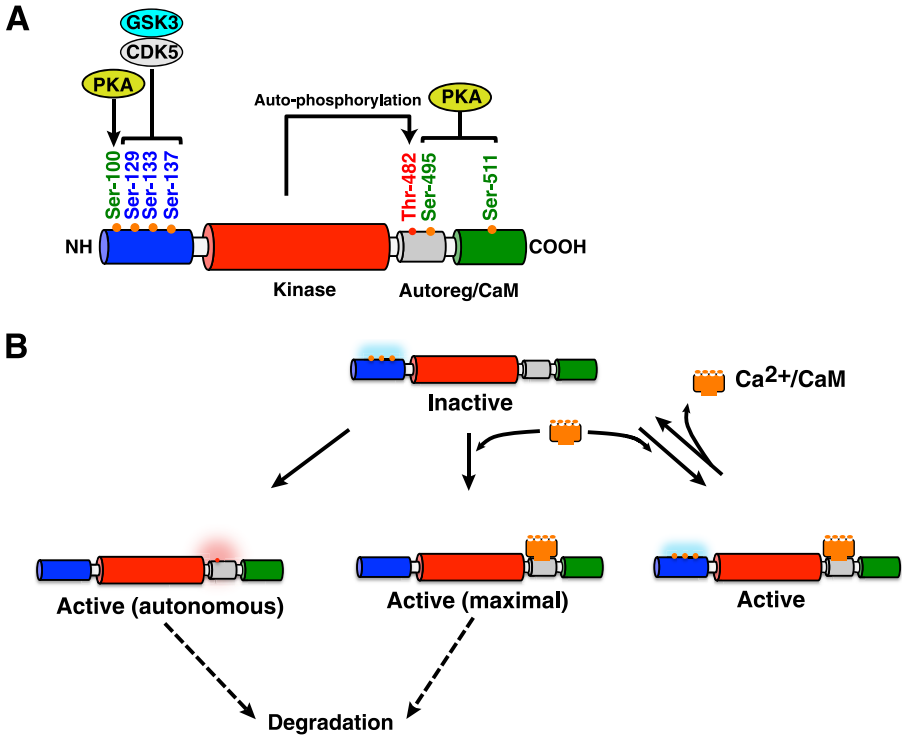

FIGURE 1. **Schematic representation of the structure-activity relationships of CaMKK2.** *A*, CaMKK2 consists of unique N- and C-terminal domains (*blue* and *green*, respectively) and a central Ser/Thr-directed kinase domain (*red*) that is followed by a regulatory domain composed of overlapping autoinhibitory and CaM-binding regions (*Autoreg/CaM*, *gray*). A region of 23 amino acids (residues 129–151) located at the N terminus of the catalytic domain has been identified as an important regulatory element. CDK5 and GSK3 phosphorylate Ser-129, Ser-133, and Ser-137. PKA phosphorylates Ser-100, Ser-495, and Ser-511. Thr-482 has been identified as an auto/transphosphorylation site. *B*, although CaMKK2 has autonomous activity for some substrates, binding to Ca^{2+}/CaM relieves autoinhibition, resulting in a fully active kinase. Mutation of Ser-129, Ser-133, and Ser-137 increases autonomous activity with little change in Ca^{2+}/CaM-dependent activity. Of note, mutation of Ser-129, Ser-133, and Ser-137 also decreases the stability of CaMKK2. This implies that the autonomously active CaMKK2 generated by dephosphorylation of these Ser residues would display a shorter half-life and be more rapidly degraded. Mutation of PKA residues does not affect CaMKK2 autonomous activity. Phosphorylation of Ser and Thr residues is depicted as *blue* and *red clouds*, respectively.

findings suggest the hypothesis that signals modifying the activation status of CaMKK2 may act as molecular switches to couple CaMKK2 with AMPK- and/or CaMK-dependent pathways.

The molecular mechanism regulating the enzymatic activity of CaMKK2 is still not completely defined (Fig. 1). CaMKKs are autoinhibited by a sequence located immediately C-terminal to their catalytic domain. This includes overlapping autoinhibitory and CaM-binding regions that are similar to those found in CaMKI and CaMKIV (11). However, the x-ray structure of Ca^{2+}/CaM bound to this region reveals that the structure of the bound CaMKK domain is markedly different from those of other CaMK CaM-binding domains whose structures have been solved (25). Clearly Ca^{2+}/CaM binding causes unique conformational changes in the CaMKKs relative to other CaMKs, although the mechanistic reason for this difference remains to be clarified.

CaMKK2 exhibits significant activity even in the absence of Ca^{2+}/CaM binding (autonomous activity) (12). By using truncation mutants of CaMKK2, a region of 23 amino acids (residues 129–151) located N-terminal to the catalytic domain was identified as an important regulatory element of autonomous activity (26). Mass spectrometry revealed three phosphorylation sites, Ser-129, Ser-133, and Ser-137 (26, 27), which are highly conserved in mouse, rat, and human isoforms of CaMKK2. CaMKK2 with mutations of these Ser residues exhibits increased autonomous activity but no change in Ca^{2+}/CaM-dependent activity (27). Notably, phosphorylation of Ser-129, Ser-133, and Ser-137 also increases the stability of CaMKK2. CDK5 (cyclin-dependent kinase 5) and GSK3 (glycogen synthase kinase 3) have been identified as upstream kinases responsible for phosphorylating these sites and, in turn, for the regulation of the autonomous activity of CaMKK2 (27). Furthermore, it has been proposed that a major consequence of relief from autoinhibition is autophosphorylation of Thr-482, a post-translational change that likely contributes to the increased autonomous activity of CaMKK2 (Fig. 1) (28). However, even though CaMKK2 has autonomous activity against CaMKI and CaMKIV, substrates that are not regulated by Ca^{2+}/CaM, such as AMPK, still require Ca^{2+}/CaM binding to CaMKK2 to become phosphorylated (7). This helps to explain the conundrum of why two enzymes in a cascade require the same allosteric activator. It also suggests that either a Ca^{2+} signal or other non-canonical signaling events could regulate CaMKK2 activity and serve as molecular switches to couple this kinase to several downstream effector pathways.

CaMMK2 Expression in Cells and Tissues

Although CaMKI and CaMKII are ubiquitously expressed, the expression of other CaMK family members is considerably more restricted. For example, CaMKIV is expressed at high levels in testis, as well as in nervous and immune systems (3, 29). CaMKK2 is present in many areas of the brain, including the

olfactory bulb, hippocampus, dentate gyrus, amygdala, hypothalamus, and cerebellum (Refs. 12 and 14; see the Gene Expression Nervous System Atlas (GENSAT) (30)). In addition to the nervous system, CaMKK2 is present at lower levels in testis, spleen, and lung (12, 31). In other tissues, such as kidney, intestine, and heart, the evidence for expression remains less clear (12, 31, 32).

CaMKK2 can be clearly detected in isolated murine preadipocytes, embryonic fibroblasts, and isolated hepatocytes and in human umbilical cord vein endothelial cells (32–34). Most recently, the presence of CaMKK2 in immune cells was examined and found exclusively in cells of the myeloid linage, including bone marrow-derived and freshly isolated peritoneal macrophages (35).

CaMKK2 and Brain Functions

Calcium controls many neuronal functions, such as neurotransmitter synthesis and secretion and dendritic spine morphology (36). Thus, it is not surprising that proteins involved in Ca^{2+}-dependent pathways, such as CaMKK2, play critical roles in the development of neurons and brain physiology (37, 38).

Hippocampal Memory—Germ-line ablation of CaMKK2 impairs long-term memory formation (39, 40). The absence of CaMKK2 is associated with selective loss of long-term potentiation at hippocampal CA1 synapses and with a decrease in spatial training-induced cAMP response-element binding protein (CREB) activation in the hippocampus (39). However, in contrast to CaMKK1$^{-/-}$ mice, loss of CaMKK2 does not correlate with deficits in fear conditioning (41).

Initiation and maintenance of synaptic plasticity in CA1 pyramidal neurons of the hippocampus require morphological changes in dendritic spines, which constitute the main structural basis for memory formation (42). Studies in cultured neurons revealed requirements for a CaMKK/CaMKI cascade in regulation of axonal growth cone morphology and outgrowth, dendritic arborization, and spine and synapse formation (37). CaMKK and CaMKI co-localize with βPIX (p21-activated kinase-interacting exchange factor) and GIT1 (G-protein-coupled receptor kinase-interacting protein 1) in dendritic spines as part of a multiprotein complex that regulates actin dynamics (43). Differential splicing and phosphorylation of critical Ser residues affect the ability of CaMKK2 to control dendrite/axon formation (17, 27). Thus, a CaMKK2/CaMKI cascade regulates learning-induced neuronal cytoskeleton remodeling associated with memory formation.

Cerebellar Development—Cerebellar granule cells (CGCs) are the most abundant neurons in the cerebellum. They develop from granule cell precursors (GCPs), which migrate from the rhombic lip to form a secondary proliferative zone in the external granule layer (EGL) (44, 45). During postnatal development, GCPs in the EGL cease proliferation and migrate again to form the internal granule layer, where they make synaptic connections with Purkinje cells. This complex process is fine-tuned by BDNF, which influences CGC development by promoting GCP exit from the cell cycle and acting as a chemokinetic factor to induce GCP migration. CaMKK2 is expressed in the cerebellum, as well as in isolated CGCs (14, 46). Studies with mouse models revealed that loss of CaMKK2 or its downstream target CaMKIV impairs the ability of GCPs to cease proliferation in the EGL and migrate to the internal granule layer (23). This phenotype is correlated with decreased CREB phosphorylation and reduced BDNF expression in GCPs. Thus, a CaMKK2/CaMKIV/CREB signaling cascade is required for regulation of BDNF production in the postnatal cerebellum and execution of the program that mediates CGC development (23).

Hypothalamus—The hypothalamus serves as a center for integration of hormonal and nutrient signals to modulate food intake, energy expenditure, and peripheral glucose metabolism (47, 48). Multiple neuronal populations residing in the hypothalamic arcuate nucleus (ARC) play a critical role in these regulatory circuits (47, 49). Due to their electrical activity and release of the orexigenic neuropeptide Y (NPY), NPY/Agouti-related protein (AgRP) neurons positively regulate feeding behavior. In contrast, pro-opiomelanocortin neurons inhibit feeding by releasing the α-melanocyte-stimulating hormone. AgRP neurons inhibit pro-opiomelanocortin neurons and thus serve a modulatory function to reduce satiety and promote food intake.

Ghrelin is a hormone produced in the intestine that exerts a potent central orexigenic effect by acting on hypothalamic NPY/AgRP neurons via activation of the growth hormone secretagogue receptor to promote release of NPY (50, 51). Ghrelin exerts its effects by binding to this G_q-coupled growth hormone secretagogue receptor, leading to an increase in intracellular Ca^{2+} that is required for transcriptional activation of the NPY gene, and AMPK has been identified as one relevant signaling component (52). Accordingly, genetic ablation of CaMKK2 impairs hypothalamic AMPK activity and down-regulates NPY and AgRP gene expression in NPY neurons, thus protecting mice from diet-induced obesity, hyperglycemia, and insulin resistance (8). Interestingly, because CaMKK2 forms a complex with AMPKα/β and Ca^{2+}/CaM, this has been proposed to function as the physiologically relevant signaling complex for mediating CaMKK2-mediated central effects on energy homeostasis (8).

CaMKK2 is present in the medial hypothalamus, especially in ventromedial nuclei (8, 14, 53, 54). In these neurons, cell-specific gene inactivation studies revealed that brain-derived serotonin uses a CaMK cascade involving CaMKK2 and CaMKIV to phosphorylate CREB in response to signaling through the Htr2c serotonin receptor. Thus, CaMKK2 regulates the expression of genes necessary for optimal sympathetic activity and, in turn, bone mass accrual, which is negatively correlated with sympathetic tone (54). Serotonin also acts via its Htr1a and Htr2b receptors in ARC neurons to favor appetite and decrease energy expenditure (55). However, to date, neither the nature of the molecular events elicited by serotonin nor the role of CaMKK2 in ARC neurons has been explored.

CaMKK2 in Adipose Tissue and Liver

Although CaMKK2$^{-/-}$ mice are protected from diet-induced obesity, glucose intolerance, and insulin resistance (8), they have more adipose tissue than WT mice when fed regular chow. Moreover, pair feeding of WT mice to match food consumption of CaMKK2 mice slows weight gain but fails to pro-

tect from diet-induced glucose intolerance (32). Taken together, these findings suggested that CaMKK2 may participate in circuits regulating the metabolic response to overnutrition in peripheral metabolic tissues. Indeed, this idea has been confirmed by recent studies revealing roles for this kinase in adipogenesis and hepatic glucose metabolism (32, 33).

Adipogenesis—White adipose tissue (WAT) is an organ whose major function is to regulate energy homeostasis (56). Adipocytes differentiate from mesenchymal stem cells in a complex process known as adipogenesis, which begins in late gestation and is largely regulated by nutrient availability. In the adult, there is a general consensus that the number of adipocytes cannot increase by >10% after puberty. Although CaMKK2 is barely detectable in adult mouse WAT or in isolated adipocytes, this kinase is expressed in preadipocytes (33). Indeed, genetic ablation of CaMKK2 led to an increase in WAT mass, which correlated with a decrease in the number of preadipocytes in this tissue. Interestingly, when exposed *in vitro* to adipogenic stimuli, primary CaMKK2$^{-/-}$ preadipocytes display a greater ability to differentiate into adipocytes than do WT cells. Moreover, during the differentiation process of WT adipocytes, the increase in molecular markers of the mature fat cell inversely correlates with the disappearance of CaMKK2. Interestingly, the silencing of AMPKα exerts effects comparable to genetic ablation of CAMKK2 by promoting terminal differentiation of preadipocytes. Finally, inhibition of the CaMKK2/AMPK signaling cascade in preadipocytes reduces Pref-1 (preadipocyte factor 1) and *Sox9* (SRY-related HMG box) mRNA, resulting in accelerated adipogenesis. Thus, CaMKK2/AMPKα functions in the signaling network that regulates adipocyte development (33).

Hepatic Glucose Metabolism—Although initial reports (11, 12, 31) failed to identify CaMKK2 in liver, this kinase is present in isolated hepatocytes (32). In fact, acute reduction of hepatic CaMKK2 reduces blood glucose in mice fed either a regular or high-fat diet (32). Notably, acute deletion of CaMKK2 in primary hepatocytes prevents the up-regulation of key enzymes of the gluconeogenesis pathway, such as glucose-6-phosphate dehydrogenase and phosphoenolpyruvate carboxykinase, in response to noradrenaline, an agonist of Ca^{2+} signaling. Moreover, freshly isolated hepatocytes from CaMKK2$^{-/-}$ mice also exhibit an increased rate of *de novo* lipogenesis relative to that of WT cells. Quiescent and noradrenaline-exposed CaMKK2-null hepatocytes express less mRNA encoding the PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1α) compared with WT hepatocytes, and this defect may be responsible for impaired glucose-6-phosphatase and phosphoenolpyruvate carboxykinase gene expression (32). The PGC-1α promoter can be activated by the CREB-CREB-binding protein-TORC2 complex in response to PKA signaling and repressed by HDAC5 (histone deacetylase 5), a putative target of CaMKs (57–59). In primary hepatocytes, loss of CaMKK2 prevents phosphorylation of HDAC5 on two residues that are critical for relief of repression (32). These data inspired the idea that a CaMKK2/CaMKI signaling cascade may control HDAC5 phosphorylation and, in turn, relief of repression of genes whose protein products are required for gluconeogenesis (32).

CaMKK2 and Myeloid Cell Physiology

CaMKK2 substrates control important functions in hematopoietic and immune cells (29, 60, 61). CaMKIV regulates survival of activated dendritic cells and the amplitude of the antibody response induced by vaccines (62, 63). CaMKI has been implicated in TLR4 (Toll-like receptor 4) signaling, as well as in the inflammatory response induced by sepsis (64), and AMPK plays an important role in the inflammatory response (65). Together, these findings suggest that CaMKK2 could be involved in the control of blood cell formation and inflammatory response. Indeed, recent reports identified functions for CaMKK2 in granulopoiesis and macrophage activation (22, 35).

Granulopoiesis—The formation of blood cells is a complex process in which hematopoietic stem cells (HSCs) self-renew or differentiate into more lineage-committed progenitors to ultimately generate all mature blood cells (66). The ability of HSCs to undergo self-renewal is partly regulated by external signals originating from the stem cell niche, and a high concentration of Ca^{2+} at the HSC-enriched endosteal surface provides a unique microenvironment for stem cells (67, 68). An implication of these data is that Ca^{2+}/CaM-dependent pathways may play critical roles in hematopoiesis. Supporting this idea, CaMKIV is involved in regulating HSC survival and maintenance (61).

Most recently, CaMKK2$^{-/-}$ mice were found to possess reduced numbers of HSCs and total bone marrow cells (22). However, unlike the case for loss of CaMKIV, depletion of CaMKK2 did not affect the proliferation, survival, or function of HSCs. Rather, loss of CaMKK2 resulted in marked defects in early myeloid progenitor populations. Surprisingly, engraftment of CaMKK2-null HSCs by bone marrow transplant led to an increased repopulation of myeloid cells, and an increased ability of CaMKK2-null myeloid progenitors to differentiate into colony-forming unit-granulocytes and Gr1^{+}Mac1^{-} granulocytes was confirmed *in vitro*. Intriguingly, similar to adipocytes, expression of CaMKK2 inversely correlates with progression of differentiation, and CaMKK2 mRNA is present in myeloid progenitors but is undetectable in mature granulocytes (22). Taken together, the findings on adipogenesis, granulopoiesis, and CGCs (22, 23, 33) suggest an intriguing scenario in which CaMKK2 plays a more general role in progenitor cell biology, operating as a critical component of the signaling pathway that regulates proliferation, terminal differentiation, and commitment of progenitor cells toward a specific lineage.

Macrophages—Macrophages are phagocytes that play an essential role in clearing the body of debris, apoptotic cells, and pathogens (69). In lean subjects, macrophages display an attenuated inflammatory phenotype and protect adipose tissue from metabolic stress. Contrariwise, in response to overnutrition, monocytes recruited to adipose tissue develop into macrophages with a proinflammatory phenotype that play a causative role in the glucose intolerance and metabolic syndrome associated with obesity (70). Because macrophages release mediators that regulate all aspects of host defense, inflammation, and homeostasis, they play a critical role in the pathogenesis of sepsis and many other conditions characterized by an abnormal response to pathogens and stressing agents (71, 72).

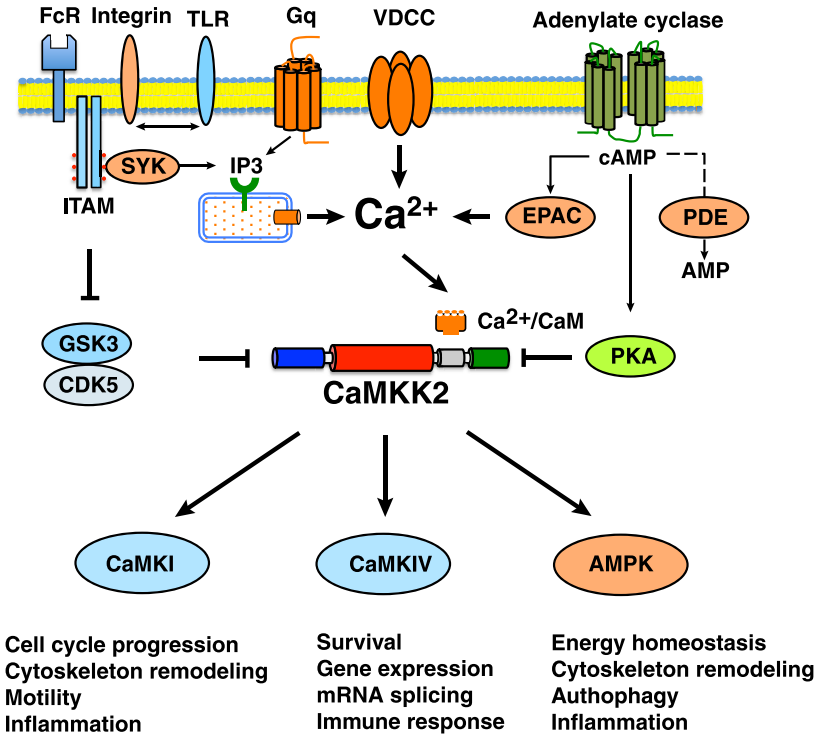

FIGURE 2. **CaMKK2 functions as a molecular hub to regulate critical cell functions.** CaMKK2 can be activated by signaling through G_q-coupled receptors, IP_3-mediated release of Ca^{2+} via activation of the IP_3 receptor, or Ca^{2+} entry into cells via plasma membrane ion channels (*i.e.* voltage-dependent calcium channel (*VDCC*)). Calcium signals from integrin and other immunoreceptor tyrosine-based activation motif (*ITAM*)-coupled immune receptors can activate IP_3-mediated release of Ca^{2+}, activate CaMKK2, and cross-regulate heterologous receptors, such as the Toll-like receptors (*TLR*). Signaling pathways modulating GSK3/CDK5 activities can modulate the stability and autonomous activity of CaMKK2. Signals regulating the intracellular level of cAMP can activate EPAC/PKA-dependent pathways and, in turn, regulate CaMKK2 activity. CaMKI, CaMKIV, and AMPK couple CaMKK2 to multiple downstream cascades, which conspire to regulate critical cell functions. *FcR*, Fc receptor; *PDE*, phosphodiesterase.

Genetic ablation of CaMKK2 protects mice from diet-induced obesity, insulin resistance, and glucose intolerance (8). The ability of CaMKK2 to control food intake in the hypothalamus and gluconeogenesis in the liver contributes to this phenotype (8, 32). However, obesity is associated with a chronic inflammatory response that, in turn, causes abnormalities in glucose metabolism (73, 74). Thus, loss of CaMKK2 could also exert its effects, at least in part, by mitigating the inflammatory response to overnutrition, as this would attenuate the detrimental effects of chronic inflammation on glucose metabolism. Indeed, genetic ablation of CaMKK2 protects mice from the effects of a high-fat diet by preventing accumulation of macrophages and inflammatory cytokines in the adipose tissue of obese mice (35). Interestingly, CaMKK2-null mice are also protected from endotoxin shock and fulminant hepatitis induced by bacterial LPS.

Among blood cell types, CaMKK2 is expressed selectively in macrophages, and its ablation impairs the ability of macrophages to spread, phagocytize bacteria, and release cytokines/chemokines in response to LPS. This might seem incongruous because AMPKα, a known downstream effector of CaMKK2 in macrophages, negatively regulates macrophage activation and polarization and contributes to protection against obesity, inflammation, and insulin resistance (75–77). However, analysis of events proximal to the TLR4 signaling cascade indicates that, at early time points, LPS stimulation induces a decrease in phospho-AMPKα, and an increase in phospho-AMPK can be observed only at later time points (77). Thus, AMPKα activation may not be a direct consequence of TLR4 engagement but rather mediated by the wave of cytokines released by activated macrophages, such as IL-10 (77). Taken together, these data are not compatible with AMPKα being a downstream effector of CaMKK2 in TLR4-mediated signaling.

Actually, loss of macrophage CaMKK2 uncouples TLR4 signaling from the phosphorylation of PYK2/PTK2B (protein tyrosine kinase 2) and from activation of PYK2 downstream effectors, such as ERK1/2, NFκB, c-Jun, and AKT (35). CaMKK2 may regulate the stability and/or endosome recycling of PYK2, thus tuning the cross-talk between integrin signaling and the TLR4-dependent cascade. Thus, in macrophages as well as in neurons, CaMKK2 participates in signaling pathways controlling cytoskeleton remodeling and morphological changes induced by external stimuli (17, 27, 37).

CaMKK2 and Prostate Cancer

The androgen receptor (AR) regulates prostate growth and is the principal target of therapy aimed at preventing growth and spreading of androgen-dependent prostate cancer (78). In an

attempt to determine androgen-responsive genes, two groups recently identified CaMKK2 to be associated with prostate cancer (79, 80). Frigo *et al.* (80) revealed that androgens stimulate the expression of CaMKK2 in androgen-dependent prostate cancer cells. These authors also identified AMPKα as the downstream effector of CaMKK2 and identified CaMKK2 and AMPK as components of the signaling pathway downstream of the AR that mediates prostate cancer cell migration and invasion. Subsequently, Massie *et al.* (79) used a different strategy to identify CaMKK2 as a direct AR target, reporting that it is consistently overexpressed in prostate cancer based on analysis of nine independent clinical gene expression studies. This study also revealed that the AR directly up-regulated expression of molecules required for glucose uptake and glycolysis. Pharmacological inhibition or siRNA-mediated down-regulation of CaMKK2 decreased phospho-AMPKα, glycolytic flux, glucose uptake, and lactate and citrate production. Thus, the authors proposed CaMKK2 to be an important regulator of anabolic pathways downstream of the AR in prostate cancer cells (79).

Concluding Remarks and Perspectives

CaMKK2 is regulated at many levels by many signaling pathways (Fig. 2). Generation and/or splicing of the primary transcript is a regulated event, and in prostate cancer cells, transcription can be regulated by androgens (16, 17, 79, 80). CaMKK2 protein is also subject to numerous post-translational modifications (Fig. 1) that affect protein stability and activity (27, 28). The cAMP/PKA pathway can inhibit CaMKK2 activity, and other inhibitory signals await discovery (81). As depicted in Fig. 2, CaMKK2 can be activated by signaling through G_q-coupled receptors, inositol 1,4,5-trisphosphate (IP_3)-mediated release of Ca^{2+} via activation of the IP_3 receptor, Ca^{2+} entry into cells via plasma membrane ion channels, and Toll-like receptors (5, 8, 34, 35). Intriguingly, CaMKK2 can also be activated by resveratrol, and because this field is still in its infancy, other stimulatory signals are likely to be discovered (82). Thus, it may be appropriate to consider CaMKK2 as a signaling hub that is capable of receiving and decoding signals transmitted via many diverse cellular regulatory pathways (Fig. 2). Considered in this light, CaMKK2 is one of the most versatile of the multifunctional CaMKs.

The information about CaMKK2 summarized herein suggests that it could be an attractive target for therapeutic intervention in liver and results in improved whole body glucose homeostasis, at least in part, by changing the primary hepatocyte fuel source from glucose to fat (32). In addition, depletion of CaMKK2 from preadipocytes accelerates their differentiation into adipocytes (33). Finally, among peripheral blood cells, CaMKK2 is expressed only in those of the myeloid lineage and controls the activation, cytokine production, phagocytosis, and motility of macrophages (35). Together, these actions conspire to render mice resistant to high-fat diet-induced glucose intolerance, insulin resistance, and diabetes. Mice deficient in CaMKK2 fail to accumulate fat when fed a high-fat diet probably due in part to depletion of the preadipocyte pool during development and to the inability of macrophages to move into adipose tissue and produce the proinflammatory cytokines/chemokines that accelerate diabetes and progression to metabolic syndrome (33, 35). Because, at present, only one small-molecule inhibitor of CaMKK2 has been reported (83), it might be prudent to renew the quest to identify potent, highly selective inhibitors of this versatile protein kinase.

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