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Calcium-regulated protein kinase cascades and their transcription factor targets

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Abstract. In the nervous system, calcium signals associated with electrical activation of neurons induce gene transcription that may be important for long-lasting adaptation. The type of transcriptional response is determined by the properties of the calcium signal that include subcellular localisation, amplitude, duration and the physical site of entry. Here we review calcium-regulated protein kinase cascades and discuss potential mechanisms through which they propagate calcium signals to and within the nucleus and control the activity of transcription factors and transcriptional co-activators.

Key words. Calcium signalling; gene expression; transcription factors; protein kinases.

Calcium and transcription

In eukaryotic cells, calcium ions not only regulate several basic cellular functions such as muscle contraction and neurotransmitter release but also act as key mediators of adaptive responses that involve changes in gene transcription. The latter role for calcium is particularly well documented in the nervous system, where transient increases in intracellular calcium concentration are responsible for electrical activity-dependent changes in gene expression [1–5; reviewed in 6–8]. Different types of electrical stimuli give rise to different transcriptional responses, most likely because the calcium signal associated with any given stimulus has distinct properties: calcium can flow into neurons from the extracellular space through neurotransmitter receptors or voltage-gated calcium channels or it can be released from intracellular stores; increases in calcium concentrations can be small or large, transient or sustained, and can be localised to the dendrites or invade the nucleus. Each of these parameters can influence the transcriptional response qualitatively as well as quantitatively. For example, in hippocampal neurons the physical site of calcium entry is an important determinant of the transcriptional response: calcium influx through L-type voltage-sensitive calcium channels very potently stimulates transcription mediated by the cAMP response element (CRE), whereas bath application of glutamate causing calcium to enter the neurons through the *N*-methyl-D-aspartate (NMDA) type of glutamate receptor poorly activates CRE-dependent gene expression [3, 9]. The importance of the spatial properties of calcium signals for transcription regulation was first demonstrated in the mouse pituitary cell line AtT20: nuclear and cytoplasmic calcium were shown to activate gene expression through distinct mechanisms [10, reviewed in 11]. Differential gene regulation by calcium signals may be explained by differences in the local availability of signal-processing molecules and their particular activation and inactivation properties. The predominant mechanism by which calcium signals are further processed within cytoplasm and the nucleus, ultimately leading to activation of components of the transcription-regulating machinery, involves protein kinase cascades. In this review we summarise current knowledge on calcium signal-regulated protein kinases and discuss possible mechanisms through which they control the activity of transcription factors and transcriptional coactivators.

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Calcium-regulated protein kinases

Calcium/calmodulin-dependent protein kinases

A consequence of increases in the intracellular calcium concentration is the association of calcium ions with the ubiquitous calcium receptor calmodulin (CaM). Calmodulin is a small (148 amino acids), acidic protein capable of binding four calcium ions. The calcium/CaM complex interacts and activates a variety of cellular effectors, which include protein kinases and phosphatases [12]. This activation process involves a conformational change of the target protein upon binding of calcium/CaM to a short region known as the CaM binding domain. Computer modelling based on the structures of the cAMP-dependent protein kinase (PKA) [13, 14] and analysis of the crystal structure of CaM kinase I [15] provided clues about theses structural changes. In the case of CaM-dependent kinases such as myosin-light chain kinase or the multifunctional CaM kinases I, II and IV, an autoinhibitory domain, interacts with the catalytic domain keeping the enzyme inactive at resting calcium levels. According to this 'intrasteric inhibition' model [13], calcium/CaM binding to a region adjacent to the autoinhibitory domain causes a change in conformation that permits access of peptide substrates and adenosine triphosphate (ATP) to the catalytic domain. In this manner, CaM-dependent kinases can rapidly sense elevations of intracellular calcium becoming active as calcium-bound CaM binds to them. In addition, some of these enzymes are also equipped with mechanisms that enable them to prolong their activity after calcium has returned to basal levels. This form of 'molecular memory' is best documented for CaM kinase II, a multimeric enzyme composed of at least 10 subunits [reviewed in 16]. Each one of the subunits is a 50–60-kDa polypeptide containing an N-terminal catalytic domain, a central autoinhibitory and CaM binding region and a C-terminal domain responsible for multimerisation and intracellular localisation. The unique multimeric structure of CaM kinase II allows very rapid trans-phosphorylation of subunits within the complex. As a consequence of trans-phosphorylation, a subunit acquires calcium/CaM-independent activity [17]. In addition, the affinity of the autophosphorylated subunits for CaM increases, making it more sensitive to further calcium elevations [18]. These properties of CaM kinase II allow the enzyme to remain active for some time after calcium levels have dropped below activation threshold, which explains why CaM kinase II activity is stimulated not only by sustained increases in calcium concentration but also by calcium oscillations [18].

The activity of CaM kinases I and IV, which act as monomeric enzymes, is also modulated by phosphorylation. However, in contrast to CaM kinase II, the regulatory phosphorylation events are catalysed by a distinct group of kinases termed CaM kinase kinases (CaMKK) [reviewed in 19]. Two CaMKK isoforms, α and β, have been cloned and were found to be expressed in many tissues (including the nervous system) [20–24]. CaMKKs themselves are calcium/CaM-dependent enzymes. They phosphorylate calcium/CaM-bound CaM kinase I and IV on a threonine residue situated within the activation loop (threonine residue 177 of the human CaM kinase I [22]; threonine residue 200 and 196 of the human and rat CaM kinase IV, respectively [23, 24]). These phosphorylation events cause the activity of CaM kinase I and IV to increase several fold. Similar to CaM kinase II, CaM kinase IV can autophosphorylate on serines 12 and 13, which is required for the enzyme to be active [25].

While CaM kinase IV is predominantly nuclear [26] and CaM kinase I appears to be a cytosolic enzyme [27], the subcellular distribution of CaM kinase II can vary [reviewed in 28]. Four types of subunits of CaM kinase II have been identified (α, β, γ, δ) that are encoded by different genes with differing tissue-specific expression. Alternative splicing within the C-terminal sequence of each gene produces further isoforms. Although the biochemical characteristics of CaM kinase II purified from many tissues are practically identical, the subunit composition, that is dependent on the source, seems to determine the subcellular localisation of the complex. The most studied forms of CaM kinase II (from forebrain or cerebellum) are rich in α- and β-subunits, respectively, are mainly cytoplasmic enzymes and are abundant in the postsynaptic densities [29]. Some splice variants of the α, γ and δ CaM kinase II genes contain a nuclear localisation signal (NLS) [30, 31], resulting in targeting of the kinase to the nucleus. Expression of the nuclear δ (B) isoform together with cytoplasmic isoforms of CaM kinase II can direct the heteromultimeric enzyme complex to the nucleus, suggesting that the relative abundance of cytoplasmic- or nuclear-targeted subunits may determine the subcellular localisation [31]. Nuclear localisation may also be subject to regulation by protein kinases, as phosphorylation of a serine residue adjacent to the NLS can prevent nuclear import [32].

CaM kinases are important regulators of gene expression. They can phosphorylate in vitro transcription factors such as CREB [33, 34], C/EBPβ [35] ATF-1 [36, 37] and SRF [34, 38] on important regulatory sites. In addition, pharmacological inhibition of CaM kinases greatly reduces the induction of, for example, c-*fos* expression and CREB-dependent transcription in response to calcium signals [3, 4, 39]. The evidence for nuclear CaM kinase IV being critical in this process is particularly good: (i) expression of catalytically inactive mutants of CaM kinase IV blocks calcium-activated,

CREB/CREB-binding protein (CBP)-mediated transcriptional activation in AtT20 cells and hippocampal neurons [9, 39]; (ii) thymic T cells derived from transgenic mice expressing the catalytically inactive mutants of CaM kinase IV under the control of the proximal promoter of the murine lck gene show a reduction in phorbolester/calcium ionophore-induced CREB phosphorylation [40], (iii) expression of a constitutively active form of CaM kinase IV is sufficient to activate CRE/CREB-dependent transcription [39, 41–43]. In contrast to CaM kinase IV, CaM kinase II does not activate CRE/CREB-mediated transcription [39, 41, 42]. However, a constitutively active form of CaM kinase II can stimulate the activity of the c-fos promoter [39, 42]. In addition, the nuclear δ (B) isoform of CaM kinase II can transactivate an atrial natriuretic factor (ANF) promoter-containing reporter gene in ventricular myocytes [44]. These transcriptional responses may be mediated by the c-fos serum response element (SRE) that is present in both the c-fos and ANF promoters. How signals generated by calcium flux across the plasma membrane are relayed to the nucleus to activate CaM kinases is not clear. It has been suggested that calcium-bound CaM translocates from submembranous regions of high calcium to the nucleus [45]. Alternatively, CaM kinase IV (or nuclear forms of CaM kinase II) may be directly activated by calcium transients propagated to the nucleus.

Extracellular signal-regulated kinases (ERKs)

ERK1 and ERK2 are the prototypical mitogen-activated protein (MAP) kinases. Their activity is controlled by a signalling cascade that is activated by environmental signals such as growth factor receptor stimulation [reviewed in 46, 47] and neurotransmitter-evoked activation of calcium entry into neurons [48, 49]. In the case of growth factors such nerve growth factor (NGF) or epidermal growth factor (EGF), the signalling pathways are very well worked out [reviewed in 50]. For example, upon binding of NGF, the high-affinity NGF receptor TrkA (a transmembrane receptor tyrosine kinase) autophosphorylates on tyrosine residues, allowing binding of the SH2 domain-containing adapter protein Grb-2. Grb-2 interacts and thereby activates the Ras GDP release factor SOS, resulting in binding of GTP to Ras. GTP-loaded Ras activates the serine/threonine kinase Raf, which in turn phosphorylates and activates a dual-specificity serine/threonine-tyrosine kinase MAP kinase/ERK kinases (MEK) 1 and 2. MEK 1/2 phosphorylate ERK1 and ERK2 at both a threonine and a tyrosine residue in the activation loop that stimulates their phosphotransferase activity. It appears that the section of the signalling cascade downstream of Ras is also used to link increase in the intracellular calcium concentration to the activation of ERKs. However, it is still unclear how calcium signals to Ras. A potential mechanism involves the calcium-regulated guanine-nucleotide releasing factor Ras-GRF [51]. Several other mechanisms have been described that involve, for example, activation of the nontransmembrane tyrosine kinases src [52] and Pyk2 [53], or inhibition by CaM kinase II of the Ras GTPase-activating protein p135synGap (an inactivator of Ras) [54].

One function of the Ras/ERK signalling cascade is to relay signals from the membrane to the nucleus. Upon activation, ERKs have been reported to translocate to the nucleus, where they phosphorylate and activate transcription factors [reviewed in 50]. The ternary complex factor (TCF) Elk-1 is one transcription factor target that mediates some of the effects of growth factors such as NGF, EGF and insulin on transcription [reviewed in 55]. CREB is another target of the Ras/ERK signalling pathway. However, phosphorylation of CREB on its activator site serine 133 is not mediated by ERKs but by the ERK-activated protein kinase ribosomal S6-kinase (RSK) 2 [56].

Whereas the Ras/ERK cascade is the prime mediator of growth factor signalling and controls the cells' physiological responses through the regulation of gene expression, the role of this signalling pathway for calcium regulation of gene expression is unclear. Several studies in which ERK activation was blocked by pharmacological means suggest that this signalling cascade may be responsible for neuronal activity-regulated CRE/CREB-dependent transcription [57, 58]. However, selective activation of the Ras/ERK pathway using the Ras mutant RasR12 is insufficient to stimulate CRE/CREB-mediated transcription [39], as is stimulation of cells with growth factors [56, 59–61]. In addition, at least in AtT20 and in the rat pheochromocytoma cell line PC12, activation of the Ras/ERK pathway is not essential for calcium-regulated transcription mediated by either CRE/CREB, TCF or SRF [60, 62]. Thus, the exact role of ERKs in the control of transcription factor and coactivator function remains to be investigated.

Stress-activated protein kinases

Stress-activated protein kinase (SAPK) is an umbrella name for a number of kinases that are at the bottom of a signalling cascade activated by various forms of stress, including ultraviolet (UV) irradiation, osmotic shock or the action of toxic compounds such as protein synthesis inhibitors [reviewed in 46]. SAPK1 was initially identified as a kinase that phosphorylates the N-terminal activation domain of c-Jun in response to stress signals and is also known as Jun N-terminal kinase (JNK) [63, 64]. Similar to ERKs, SAPKs are proline-directed protein kinases and recognise serine/proline and threonine/proline motifs.

First evidence for calcium regulation of SAPK came from experiments using Jurkat cells, a human T-cell line: activation of the T-cell receptor by treatment of Jurkat cells with phorbol esters (to stimulate PKC) and calcium ionophore strongly activates SAPK1/JNK [65]. One of the targets of SAPK1/JNK in T cells is c-Jun, which as part a complex with c-Fos and nuclear factor of activated T-cells (NF-AT) controls expression of the interleukin 2 gene [reviewed in 66]. In neurons, the profile of activation of SAPKs by calcium signals depends on the type of cells. For example, exposing cultured cerebellar granule cells to a high, excitotoxic dose of glutamate causes the activity of SAPK2/p38 MAP kinase to increase several fold, wherreas SAPK1/JNK is only poorly activated. This effect is dependent on calcium influx and is inhibited by an NMDA receptor antagonist [67]. In cultured striatal neurons, activation of calcium flux through NMDA receptors following bath application of 0.1 mM glutamate or NMDA induces a two- to three-fold activation of SAPK1/JNK [68]. A similar treatment of cultured hippocampal neurons failed to increase SAPK1/JNK activity [9]; however, it did stimulate (even at lower, nontoxic glutamate concentrations) SAPK2/p38 MAP kinase activity [G. E. Hardingham and H. Bading, unpublished]. Similarly, in AtT20 cells, calcium influx through L-type channels activates SAPK2/p38 MAP kinase but has no effect on SAPK1/JNK activity [69]. Whereas activation of SAPK2/p38 MAP kinase by low (nontoxic) concentrations in hippocampal neurons appears to regulate particular transcriptional events [G. E. Hardingham and H. Bading, unpublished], the correlation of glutamate-induced toxic damage to neurons and SAPK activation may indicate a causal relation between the two phenomena. This hypothesis seems to be supported by the observation that, compared with wild type, in mice lacking the JNK3 gene injection of kainate (a potent neurotoxin that induces seizures through stimulating particular types of ionotropic glutamate receptors) led to less cell death in the hippocampus [70]. However, kainate-induced seizures are much less severe in mice lacking the JNK3 gene, and thus a change in excitability rather than block of the cellular mechanism mediating cell death may explain the phenotype of the JNK3 knockout mouse.

Calcium-regulated transcription factor and coactivators

CREB/CREB-binding protein (CBP)

CREB is the best-studied calcium-regulated transcription factor. It binds as a dimer to the CRE, first identified in the promoter of the somatostatin gene as an element required for gene regulation by cAMP [71]. The c-fos CRE (at position -60 base pairs relative to the c-fos transcription start site) can also mediate transcriptional induction in response to elevated levels of cAMP, but in addition, it is a key regulatory element for calcium-activated gene expression in excitable cell lines and primary hippocampal neurons [reviewed in 6–8]. CREB-mediated transcription requires phosphorylation of CREB on its activator site serine 133 [72]. This phosphorylation can be catalysed by numerous signal-regulated protein kinases, including cAMP-dependent protein kinase (PKA) [72], CaM kinases I, II and IV [33, 41], the ERK-regulated kinase RSK2 [56] and the p38 MAP kinase-regulated MAPKAP kinase-2 [73]. However, CREB phosphorylation on serine 133 does not equal CREB-mediated transcription. For example, stimulation with growth factors such as nerve growth factor (NGF) very potently stimulates CREB phosphorylation (via the Ras/ERK/RSK2 pathway); however, growth factors do not efficiently activate CRE/CREB-mediated transcription [56, 59–61, 74]. Moreover, calcium-induced CREB phosphorylation can be uncoupled from CREB-mediated transcription by either blocking nuclear calcium transient or by inhibiting CaM kinase [39], indicating that nuclear calcium activating nuclear CaM kinase IV provides a second regulatory event required for calcium regulation of CREB-mediated transcription. This second event was recently identified and involves CBP [39]. CBP is a transcriptional coactivator that interacts with components of the basal transcription machinery. CBP may also affect transcription via its intrinsic or associated activity to acetylate histones, causes a relaxation of repressive chromatin structure and allows access to regions of DNA contained in nucleosomal complexes [reviewed in 75]. CBP does not bind to DNA directly but can be recruited to the promoter via several transcription factors, including CREB phosphorylated on serine 133. While the recruitment step of CBP to the promoter (i.e. CREB phosphorylation on serine 133) is controlled by many calcium-regulated kinase cascades (see above), activation of CBP function is controlled by nuclear calcium and CaM kinase IV [39]. Activation of the Ras/ERK/RSK2 pathway does not increase CBP activity [39]; however, CBP activity can also be stimulated by elevated levels of cAMP [39, 76, 77]. This explains why cAMP is the only other signal known to be sufficient to strongly stimulate CREB-mediated transcription. The mechanism through which CaM kinase IV activates CBP is unclear, although a recent study suggests that it may involve a calcium signal-regulated phosphorylation event on CBP [78].

c-Jun

One implication of the finding that CBP function is regulated by nuclear calcium and CaM kinase IV is that

CBP-interacting transcription factors other than CREB may function as nuclear calcium-regulated activators. Indeed the c-Jun transcription factor (which interacts with CBP [79]) can function as a calcium-regulated transcriptional activator in AtT20 cells [69] and primary hippocampal neurons [9]. Activation of c-Jun-dependent transcription by calcium signals is not accompanied by any increase in SAPK1/JNK kinase activity and does not require serine 63 and serine 73 [69], the two classical main regulatory SAPK1/JNK phosphorylation sites on c-Jun. Instead, similar to CREB, this transcriptional response is dependent on the activity of CaM kinases [69]. Given that CBP can interact with c-Jun independently of the phosphorylation of c-Jun on serine 63 and serine 73, and CBP activity is controlled by CaM kinase IV [39], these findings suggest that c-Jun-bound CBP is the site of regulation in c-Jun-mediated, calcium-activated transcription. This model is further supported by two additional observations: (i) expression of the adenovirus gene product E1A that can bind to CBP and disrupt its function inhibited calcium-activated, c-Jun-mediated transcription; and (ii) increasing levels of cAMP that can stimulate CBP activity also induce c-Jun-mediated transcription [69]. Thus, regulation of CBP activity appears to be the common mechanism through which nuclear calcium and CaM kinase IV (as well as cAMP) control CREB and c-Jun-dependent transcription and very likely also that of other CBP-recruiting transcription factors.

Ternary complex factors (TCFs)

The TCF family of transcriptional activators was initially identified as an activity in HeLa cells that formed a complex with DNA-bound serum response factor (SRF) on the c-fos promoter. The three known members of this family are Elk-1, SAP1 and SAP2/ERP/NET; their activity can be activated by ERKs [reviewed in 53]. In the case of Elk-1, several ERK phosphorylation sites have been identified that are important for Elk-1-dependent transcription upon serum or growth factor stimulation. Phosphorylation of Elk-1 can also occur in the nervous system: treatment of striatal brain slices with 0.1 mM glutamate increased the phosphorylation of Elk-1 on serine 383 [80], one of several regulatory phosphorylation sites in the C-terminal activation domain of Elk-1. While it is unclear whether calcium influx triggers this phosphorylation event, it is blocked by the MEK 1 inhibitor PD98059 and, thus, appears to be mediated by the Ras/ERK signalling pathway. However, stimulation of ERK activity upon activation of a transmembrane calcium flux does not necessarily lead to an increase in Elk-1 phosphorylation. For example, in PC12 cells, calcium flux through L-type calcium channels activates ERKs but does not increase the phosphorylation of Elk-1 on serine 383 [62]. In this study, calcium-induced Elk-1-dependent transcription was not analysed; however, experiments using cortical neurons showed that Elk-1 can mediate transcriptional activation upon calcium flux through NMDA receptor [81]. NMDA receptor regulation of Elk-1 in cortical neurons appears to be mediated by the Ras/ERK signalling pathway, as the transcriptional response was blocked by overexpression of the MAP kinase phosphatase MKP-1, and mutation of the two key regulatory phosphorylation sites serine 383 and serine 389 rendered Elk-1 functionally inactive [81] (although NMDA receptor-induced phosphorylation of Elk-1 on these sites was not analysed in this study). Evidence for an alternative mode of Elk-1 regulation comes from experiments in AtT20 cells. Calcium entry into AtT20 cells through L-type calcium channels activates Elk-1-mediated transcription in the absence of any detectable increase in Elk-1 phosphorylation on its activator sites [F. Cruzalegui, C. Lange and H. Bading, unpublished]. These findings indicate that calcium signals can stimulate Elk-1 function independently of the regulatory ERK phosphorylation sites. Given that Elk-1 (as well as SAP1a) can interact with CBP [82, 83], it is possible that CBP bound to Elk-1 is the target of a calcium-induced event that stimulates transcription. Thus, CREB, c-Jun and perhaps Elk-1 form a group of calcium-responsive transcriptional activators that function through recruitment of the nuclear calcium/CaM kinase IV-regulated coactivator CBP.

SRF

SRF binds as a dimer to the SRE found in the c-fos promoter and in the regulatory region of several other genes [reviewed in 84]. The c-fos SRE can also bind TCFs allowing for the formation of a ternary (i.e. SRE/SRF/TCF) complex; SREs without TCF binding sites exist in other genes. The introduction of specific mutations into the c-fos SRE that disrupt TCF binding made it possible to study transcriptional regulation by SRF only (as opposed to regulation by an SRF/TCF complex) [85–87]. SRF-dependent gene expression can be induced by calcium signals in a variety of cell types, including PC12 cells [60, 62], AtT20 cells [10, 60], hippocampal [3, 60] and cortical neurons [81]. In contrast to CREB/CBP, SRF-mediated gene expression is controlled by a mechanism that is triggered by cytoplasmic calcium signals [10]. The signalling pathway involved appears to function independently of the Ras/ERK cascade and may involve CaM kinases [60, 62]. However, phosphorylation of SRF residue serine 103, which is calcium-inducible and at least in vitro can be catalysed by CaM kinase II, does not appear to be critical for SRF to function as a calcium-regulated

transcription factor [38, 62]. Alternative regulatory mechanisms may involve small G proteins of the Rho family that regulate SRF-dependent transcription in fibroblast following serum stimulation [87], or changes in actin polymerisation [88].

Activating transcription factor-1 (ATF-1)

ATF-1 resembles CREB in many respects. It is a leucine zipper containing transcription factors, binds to the CRE and can mediate transcription induction in response to calcium signals and increased intracellular levels of cAMP [36, 37]. ATF-1 contains at least two regulatory phosphorylation sites (serine 63 and serine 72). Serine 63 of ATF-1 is critical for ATF-1 to function as an activator of gene transcription and thus appears to be the functional equivalent of the CREB phosphorylation site serine 133. Indeed, the amino acid sequence in the kinase-regulated domains of the two transcription factors' phosphorylation are so similar that the antibody to phospho-serine133-CREB also binds to ATF-1 phosphorylated on serine 63. Phosphorylation of serine 63 can be catalysed by several kinases, including CaM kinase I, II and IV [36, 37]. The function of serine 72, a target site for CaM kinase II, as the equivalent of the inhibitory CREB phosphorylation site serine 142, maybe be cell type-dependent. In the pituitary cell line GH3 cells, constitutively active forms of CaM kinase I and IV but not a constitutively active form of CaM kinase II (which phosphorylates serine 72) can activate wild-type ATF-1, whereas ATF-1 containing a serine-to-alanine mutation at position amino acid 72 is also activated by CaM kinase II [36]. These findings suggest an inhibitory function of serine 72. However, in F9 cells, expression of a constitutively active form of CaM kinase II did not significantly increase phosphorylation of ATF-1 on serine 72 and caused stimulation of wild-type ATF-1-dependent transcription [89].

Nuclear factor of activated T cells (NF-AT)

Calcium regulation of NF-AT is vital for the immune response. In resting cells, NF-AT is phosphorylated and resides in the cytoplasm. However, cytoplasmic calcium signals associated with, for example, antigen stimulation of B cells or T cells increases the activity of the calcium/CaM-dependent phosphatase calcineurin (CaN; also known as protein phosphatase 2B) that binds to and dephosphorylates NF-AT [90, 91]. The CaN/dephosphorylated NF-AT complex is subsequently imported into the cell nucleus and stimulates expression of target genes such as the interleukin 2 gene [reviewed in 66]. Nuclear translocation of NF-AT is a rapidly reversible process; once calcium levels in the nucleus have fallen below the activation threshold for CaN, the CaN/NF-AT complex falls apart, and NF-AT is rapidly exported from the nucleus by a Crm1-dependent mechanism [92]. This property of NF-AT may explain why its nuclear translocation and ability to activate transcription requires sustained (although low-amplitude) elevation of the calcium concentration [93]. In addition to the importance of nuclear calcium for the maintenance of a nuclear CaN/NF-AT complex, nuclear calcium transients may also be required for the trans-activating activity of NF-AT: NF-AT interacts with CBP [94], and nuclear calcium stimulating the activity of CBP may be a coregulator of NF-AT-mediated gene expression.

C/EBPβ

The CCAAT/Enhancer binding protein C/EBPβ (also termed NF-IL6/LAP) was one of the first transcription factors shown to be activated by a constitutively active form of CaM kinase II [35]. This transcriptional response requires phosphorylation of a serine residue (serine 276) within the leucine zipper of C/EBPβ that can be catalysed by CaM kinase II in vitro. More recent work also shows regulation of C/EBPβ (and C/EBPδ) by a constitutively active form of CaM kinase IV [95]. C/EBPβ is highly expressed in liver cell nuclei and is thought to be involved in inflammatory responses [96]. However, C/EBPβ itself is a signal-regulated immediate early gene in various cell types [97, 98]. In hippocampal neurons, expression of the C/EBPβ gene can be induced via a CaM kinase-dependent mechanism triggered by membrane depolarisation and calcium entry [95]. Thus C/EBPβ may be a transcription factor that is controlled by CaM kinases at the level of both expression and function.

Downstream regulatory element antagonist modulator (DREAM)

DREAM is an EF hand-containing calcium-binding protein that can function as a repressor of transcription [99]. DREAM-mediated repression required binding of DREAM to the downstream regulatory element (DRE), a process controlled by the concentration of intracellular calcium. Electrophoretic mobility shift assays show that at a calcium concentration of 0.5 μM, DREAM binds to the DRE, whereas at calcium concentrations above 10 μM the DREAM/DRE interaction is blocked. Functional DREs have been identified in the human dynorphin gene and in the c-fos gene. Their localisation in the transcribed region of the genes raises the possibility that DREAM functions by inhibiting transcription elongation. In addition to its regulation by calcium signals, increases in the intracellular concentration of cAMP can also relieve DREAM-mediated repression [99].

Conclusion

Several routes can link signals generated by calcium entry into neurons to the transcription-regulating machinery in the nucleus. Calcium can activate signalling cascades in the cytoplasm, but calcium transients propagated to the nucleus can also trigger intranuclear events. While gene regulation by cytoplasmic calcium signals is still poorly understood, a general mechanism for gene regulation by nuclear calcium is emerging. Central to this mechanism is the regulation of two aspects of CBP function, i.e. CBP recruitment by signal-regulated transcription factors and stimulation of the transcription-activating function of CBP by nuclear calcium signals. Given that CBP can interact with many transcription factors, control of CBP function by nuclear calcium may be a key mechanism underlying the numerous changes in gene expression associated with electrical activity-dependent neuronal plasticity.

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