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Regulation of gene expression by Ca^{2+} signals in neuronal cells

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Abstract

Calcium ions are ubiquitous second messengers that control diverse cellular functions. The versatility of Ca^{2+} arises both from the ability of cells to employ a range of mechanisms to generate stimulus-induced Ca^{2+} signals with defined characteristics and the existence of a large repertoire of Ca^{2+} receptive proteins that mediate the effects of Ca^{2+}. In neurons, the regulation of gene expression by electrical activity-induced increases in Ca^{2+} is critical for the long-term maintenance of neuronal adaptive responses. Different patterns of synaptic activity are able to generate Ca^{2+} signals varying in their amplitude, temporal profile, spatial properties and source or site of entry. The information embedded in Ca^{2+} signals is decoded by Ca^{2+}-responsive transcriptional regulators, including protein kinases, phosphatases and transcription factors, with differing Ca^{2+} sensitivities, kinetics of activation and deactivation, and subcellular localisation. The coordinated control of many transcriptional regulators by Ca^{2+} signals determines the qualitative and quantitative nature of the genomic response.

Keywords: Ca^{2+}; Transcription; cAMP response element binding protein (CREB); CREB binding protein (CBP); Serum response element (SRE)

1. Introduction

Neurons process information from the environment by the generation and propagation of electrical signals and release of neurotransmitter at synapses. Neuronal responses are adaptive or 'plastic', being molded by the nature and history of previous synaptic activity. The plasticity of the nervous system arises from the ability of neurons to undergo long-lasting morphological and functional changes in response to short bursts of synaptic activity and underlies learning and memory. Bursts of synaptic activity can have immediate short-term effects on the efficacy of synaptic transmission by the modification of pre-existing cellular proteins and also initiate the expression of new genes required for the long-term maintenance of plastic changes (reviewed in Goelet et al., 1986). These two temporal phases of synaptic plasticity have been demonstrated in both animal behavioral studies of memory and in synaptic plasticity models such as long-term potentiation in the mammalian hippocampus. Long-term potentiation is the persistent enhancement of synaptic transmission following brief high-frequency electrical stimulations (reviewed in Bliss and Collingridge, 1993), which can be induced in vitro in hippocampal slices or in animals where it may last for days to weeks. The early, protein synthesis-independent phase of long-term potentiation lasts for up to 3 h. For long-term potentiation to persist beyond this, a critical period of transcription (Nguyen et al., 1994) and protein synthesis (Frey et al., 1993) is required after its induction. Although many activity-induced genes have been identified, including transcription factors, protein kinases, synaptic vesicle proteins and neurotrophins (see, for example, Wisden et al., 1990; Nedivi et al., 1993; Lynch et al., 1994; Thomas et al., 1994), the molecular mechanisms by which the newly synthesized proteins alter synaptic function is not fully understood. This may involve changes in both presynaptic components such as an increase in neurotransmitter release and alterations in postsynaptic components resulting in enhanced responses to the neurotransmitter (reviewed in Kullmann and Siegelbaum, 1995).

Early experiments established that Ca^{2+} influx through either the *N*-methyl-D-aspartate (NMDA) type of glutamate receptor or L-type voltage-sensitive Ca^{2+} channels triggers long-term potentiation (Bliss and Collingridge, 1993; Kullmann and Siegelbaum, 1995). Ca^{2+} influx through either route is also the trigger for electrical activity-dependent gene expression and one of the earliest genomic consequences is the induction of immediate early genes (Cole et al.,

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1989; Wisden et al., 1990; Lerea et al., 1992; Bading et al., 1993, 1995). Many immediate early genes encode transcription factors such as c-*fos*, *fosB*, c-*jun*, *zif268* that in turn regulate the expression of late response genes the products of which contribute to the structural and functional changes underlying neuronal plasticity. Others, such as the neurotrophin brain-derived neurotrophic factor (BDNF), act directly. BDNF promotes neuronal survival (Schwartz et al., 1997), acts as a neurotransmitter eliciting postsynaptic action potentials (Kafitz et al., 1999) and modulates synaptic transmission by enhancing neurotransmitter release (reviewed in Poo, 2001).

It is now widely accepted that Ca^{2+} is able to specify distinct genomic responses by differential activation of transcriptional regulators that decipher the information contained in Ca^{2+} signals. Much of our understanding of Ca^{2+}-regulated transcription in neuronal cells comes from studying the activity-dependent induction of the c-*fos* gene, whose transcription is induced rapidly and robustly following stimulation, and without the need for new protein synthesis. Some of the recently unraveled molecular mechanisms that couple Ca^{2+} increase triggered at the synapse to c-*fos* transcription in the nucleus are reviewed here. The focus is on mechanisms controlling c-*fos* transcriptional initiation, although additional regulatory events, such as transcriptional elongation (Collart et al., 1991; Mechti et al., 1991; Thompson et al., 1995), control of mRNA stability and splicing (Treisman, 1985; Shyu et al., 1989), and mRNA translation (Takeuchi et al., 2001), may contribute to the regulation of gene expression by Ca^{2+}.

2. Ca^{2+}-responsive DNA regulatory elements

The c-*fos* promoter contains the two well-characterised Ca^{2+}-regulated DNA response elements, cAMP response element (CRE) and serum response element (SRE). The CRE, which was first identified as a promoter element conferring cAMP inducibility to the somatostatin gene (Montminy et al., 1986) and later as a Ca^{2+} and cAMP responsive element in the c-*fos* gene (Sheng et al., 1988; Bading et al., 1993), interacts with members of the basic motif/leucine zipper (bZIP) containing family of transcription factors that bind the CRE as dimers and either stimulate or repress transcription (reviewed in Sassone-Corsi, 1995). The cAMP response element binding protein (CREB), the first family member to by identified and the closely related family member activating transcription factor-1 (ATF-1), are activators of gene expression (Sheng et al., 1988). Alternate splicing of the cAMP response element modulator (CREM) gene, which encodes additional bZIP transcription factors, generates several CREM isoforms some of which like CREB bind the CRE but lack a particular domain required for transcriptional activation and, thus, function as repressors of CRE-regulated gene expression (Sassone-Corsi, 1995). The CRE/CREB system also mediates the effects of Ca^{2+} on induction of other activity-induced genes like BDNF (Tao et al., 1998).

The SRE, which mediates c-*fos* transcriptional induction associated with serum stimulation (Treisman, 1985; Greenberg et al., 1987), is also targeted by Ca^{2+} signalling pathways and was identified as the second Ca^{2+}-regulated DNA response element (Sheng et al., 1988; Bading et al., 1993; Misra et al., 1994; Johnson et al., 1997) in the c-*fos* gene. The c-*fos* SRE contains binding sites for at least two transcription factors. The serum response factor (SRF; Treisman, 1987; Norman et al., 1988; Schröter et al., 1987) which is the principal SRE-binding protein binds a core element referred to as the CArG box. Immediately, 5′ of the SRF binding site is an *ets* motif that interacts with members of the ternary complex factor (TCF) family of proteins (reviewed in Treisman, 1994). The best-characterised member of the TCF family is the transcription factor, Elk-1 (Hipskind et al., 1991; reviewed in Treisman, 1994).

Gene expression mediated by the transcription factor CREB is better characterised, having been the subject of many investigations, and is accordingly reviewed in greater detail here. CREB is a target for Ca^{2+}, cAMP and growth factor signalling pathways and has been implicated in physiological processes such as long-term memory (reviewed in Yin and Tully, 1996; Nguyen, 2001) and neuronal survival (Bonni et al., 1999). For example, Guzowski and McGaugh (1997) showed that injecting antisense oligos against CREB directly into the hippocampus of adult rats just hours before training affected the animals' long-term memory formation of a spatial task without affecting short-term memory. Similarly, induction of CREB repressor isoforms in transgenic *Drosophila* disrupted long-term memory without affecting early memory formation (Yin et al., 1995).

3. Signalling pathways targeting CREB

The ability of CREB to activate transcription requires its phosphorylation on serine 133 (Gonzalez and Montminy, 1989). The mutation of serine 133 to alanine renders CREB transcriptionally inactive. This phosphorylation event can be catalyzed by many stimulus-dependent protein kinases (Fig. 1) including cAMP-dependent protein kinase (PKA; Gonzalez and Montminy, 1989), Ca^{2+}/calmodulin-dependent kinase I, II and IV (Sheng et al., 1991; Sun et al., 1994; Matthews et al., 1994), ribosomal S6 kinase II (RSK2; Xing et al., 1996), MAP kinase-activated protein (MAPKAP) kinase-2 (Tan et al., 1996) and Akt/PKB (protein kinase B; Du and Montminy, 1998).

Ca^2/calmodulin-dependent kinases are activated by direct binding of Ca^{2+} and calmodulin and can be activated following Ca^{2+} entry into neurons (Ocorr and Schulman, 1991; Molloy and Kennedy, 1991; Bading et al., 1993), representing one route leading to CREB phosphorylation. Ca^{2+}/calmodulin-dependent kinases I and IV are also sub-

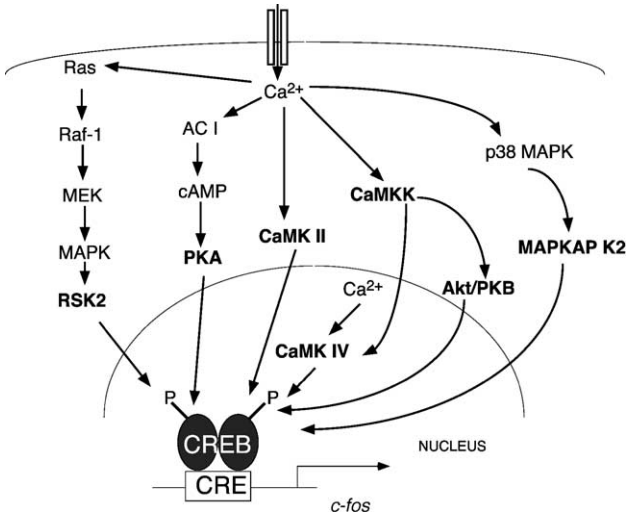

Fig. 1. Schematic representation of possible calcium-dependent routes to CREB phosphorylation. CREB phosphorylation on serine 133 can be catalyzed by RSK2, CaM kinases, PKA, MAPKAP kinase-2 and Akt/PKB.

ject to phosphorylation by upstream Ca^{2+}/calmodulin-dependent kinase kinases (reviewed in Corcoran and Means, 2001), which increases their activity although the physiological significance of this for Ca^{2+}/calmodulin-dependent kinase I- and IV-mediated transcription is unclear. The CREB kinase RSK2 is downstream of the Ras/MAP kinases (ERKs) signalling cascade (see Section 4.1), which is activated by growth factors and can also be triggered by Ca^{2+} entry into neurons (Bading and Greenberg, 1991; Rosen et al., 1994; Rusanescu et al., 1995). PKA-mediated phosphorylation of CREB is usually activated by cAMP generating stimuli, but represents a potential Ca^{2+}-activated route due to cross talk between Ca^{2+} and cAMP signalling mechanisms via the Ca^{2+}/calmodulin-dependent type I adenylate cyclase (reviewed in Cooper et al., 1995), an enzyme that catalyses the production of cAMP. A direct link between Ca^{2+} signals and stimulation of MAPKAP kinase-2 has not been demonstrated. However, the activity of p38 MAP kinase, the MAPKAP kinase-2 activator enzyme, is increased in cerebellar granule cells following glutamate-induced Ca^{2+} entry (Kawasaki et al., 1997) and in cortical neurons following membrane depolarization (Mao et al., 1999). CREB is also a target for the serine/threonine kinase Akt/PKB, which has been shown to block cellular apoptosis and to promote cell survival in response to growth factor induction. Yano et al. (1998) identified a Ca^{2+}-triggered signalling cascade in which Ca^{2+}/calmodulin-dependent kinase kinase activates Akt/PKB in the neuroblastoma NG108 cell line. In hippocampal neurons, Ca^{2+}/calmodulin-dependent kinase IV and RSK2 have emerged as the predominant CREB kinases in response to L-type Ca^{2+} channel and NMDA receptor activation (Bito et al., 1996; Hardingham et al., 1999, 2001a,b).

CREB phosphorylation, although essential for CREB-mediated gene expression, is not always sufficient for activation of transcription. An important determinant of CREB-mediated transcription is the duration for which CREB is phosphorylated: sustained and not transient CREB phosphorylation correlates with the ability of CREB to activate transcription in striatal (Liu and Graybiel, 1996) and hippocampal (Bito et al., 1996; Hardingham et al., 1999) neurons. The duration for which CREB is phosphorylated is controlled by the opposing actions of protein kinases and phosphatases on serine 133. The serine/threonine protein phosphatases PP-1 (Hagiwara et al., 1992; Alberts et al., 1994) and PP-2A (Wadzinski et al., 1993) can both dephosphorylate serine 133 resulting in CREB inactivation. PP1 has the potential to mediate CREB dephosphorylation in neurons because its phosphatase activity is regulated by synaptic transmission and it has been shown to play a role in long-term potentiation (Mulkey et al., 1994; Blitzer et al., 1998; Brown et al., 2000).

Not all intracellular signalling pathways that result in sustained CREB phosphorylation on serine 133 stimulate CREB-mediated transcription. For example, growth factors, such as epidermal growth factor (EGF) or nerve growth factor (NGF), do not or very poorly activate CRE/CREB-mediated transcription (Sheng et al., 1988; Ginty et al., 1994; Johnson et al., 1997) in spite of being potent activators of CREB phosphorylation via the Ras/MAP kinases (ERKs) signalling cascade (Ginty et al., 1994; Bonni et al., 1995; Xing et al., 1996). This indicates that additional regulatory events are required for CREB to activate transcription. Phosphorylated CREB recruits the transcriptional coactivator CREB binding protein (CBP) to the promoter (Chrivia et al., 1993; Kwok et al., 1994). CBP and its close relative, p300, (Lundblad et al., 1995) are important components of the transcriptional machinery and are thought to regulate gene expression by connecting sequence-specific transcriptional activators to components of the basal transcription machinery. In addition to acting as protein bridges, CBP/p300 also have the ability to influence gene expression by disrupting repressive chromatin structures through their intrinsic or associated histone acetyl transferase activity (for reviews, see Pazin and Kadonaga, 1997; Goodman and Smolik, 2000). Chawla et al. (1998) showed that in addition to CREB, its coactivator CBP is also a target for intracellular signalling pathways and that both a rise in Ca^{2+} and cAMP induce its ability to activate gene expression.

3.1. CRE/CREB activation by nuclear Ca^{2+} signals

Bursts of synaptic activity can increase the concentration of intracellular Ca^{2+} throughout the cell including the dendrites, cell body and nucleus. The localisation of protein kinases and phosphatases in different subcellular compartments raises the possibility that spatially distinct Ca^{2+} signals have different functions in the regulation of gene expression. The role of nuclear and cytoplasmic Ca^{2+} signals in the control of transcription was investigated by Hardingham et al. (1997) using a non-diffusible nuclear

Ca^{2+} chelator, 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) coupled to a 70-kDa dextran molecule, in the mouse pituitary cell line AtT20 cells. Hardingham et al. showed that an increase in nuclear Ca^{2+}, following L-type Ca^{2+} channel activation, controls gene expression through the CRE and CREB. Ca^{2+}-activated transcription via the SRE, on the other hand, is triggered in the cytoplasm and can function independently of increases in nuclear Ca^{2+} concentrations.

Given that a CREB kinase, Ca^{2+}/calmodulin-dependent kinase IV is found in the nucleus (Jensen et al., 1991) and, in its constitutively active form can stimulate CREB-dependent gene expression (Sun et al., 1994; Matthews et al., 1994), the mechanism by which nuclear Ca^{2+} activates CREB-mediated transcription likely involves phosphorylation of CREB on serine 133. However, chelation of nuclear Ca^{2+} did not block CREB phosphorylation (Chawla et al., 1998) indicating that nuclear Ca^{2+}-induced CREB phosphorylation on serine 133 is not the only regulatory step critical for transcriptional activation. Additional, nuclear Ca^{2+}-regulated events were found to impinge on the transcriptional coactivator CBP. CBP contains a signal-regulated transcriptional activation domain that is controlled by nuclear Ca^{2+} and Ca^{2+}/calmodulin-dependent kinase IV, and by cAMP (Chawla et al., 1998). Cytoplasmic Ca^{2+} signals that stimulate the Ras/MAP kinases (ERKs) signalling cascade, or expression of the activated form of Ras, induced CREB phosphorylation but did not increase CBP activity (Chawla et al., 1998). Thus, CBP recruitment to a promoter through interaction with, for example, CREB phosphorylated on serine 133 represents one of several regulatory steps needed for transcriptional activation (Fig. 2). This finding could explain why signalling mechanisms activated by Ca^{2+} but not those activated by growth factors efficiently induce CREB-mediated gene expression. Growth factors induce CREB phosphorylation on serine 133 and thus provide the CBP recruitment signal, but unlike Ca^{2+}, they are unable to stimulate CBP activity.

Transcriptional regulation by control of a coactivator or corepressor involved in chromatin remodeling has emerged as an important mechanism and is also seen during differentiation of skeletal muscle. The class II histone deactelyases HDAC4 and HDAC5 have been shown to directly bind to the transcription factor myocyte enhancer factor-2 (MEF2) and to repress its transcriptional activity and thereby inhibit skeletal myogenesis (reviewed in McKinsey et al., 2001). MEF-2 repression is relieved when HDAC4 and HDAC5 translocate to the cytoplasm during a complex process that includes phosphorylation of HDACs by a Ca^{2+}/calmodulin-dependent kinase in the nucleus (McKinsey et al., 2000; Miska et al., 2001). Nuclear Ca^{2+} may thus play a role in the nucleo-cytoplasmic shuttling of HDACs. Preliminary evidence suggests that HDAC4 and HDAC5 localisation is also controlled by electrical activity in hippocampal neurons (Chawla and Bading, unpublished observations).

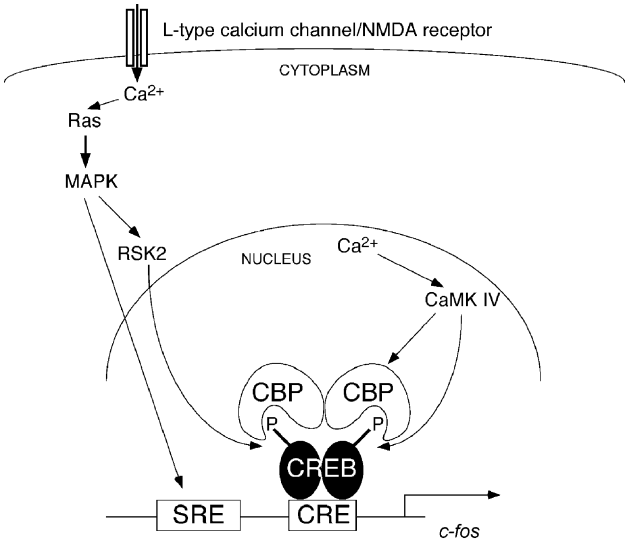

Fig. 2. Model for regulation of c-*fos* transcription by nuclear and cytoplasmic calcium signals. Calcium influx through the NMDA receptor or L-type calcium channel can activate the Ras/MAPK pathway in the cytoplasm leading to SRE-mediated transcription and CREB phosphorylation. Nuclear calcium activates CRE-mediated transcription, CREB phosphorylation and stimulates CBP activity.

Nuclear Ca^{2+} is necessary for CREB-mediated transcription but is it sufficient? Previous work (Deisseroth et al., 1996) suggested that CREB phosphorylation in response to synaptic stimulations of hippocampal neurons is mediated by a submembranous pool of Ca^{2+} and does not require increases in nuclear Ca^{2+}. The observation that a Ca^{2+}/calmodulin complex translocates to the nucleus following electrical activity (Deisseroth et al., 1998) led the authors to further conclude that calmodulin acts as the submembranous Ca^{2+} sensor and its translocation to the nucleus supports Ca^{2+}/calmodulin-dependent kinase IV-mediated CREB phosphorylation. However, Hardingham et al. (2001a), using wheat germ agglutinin to block the nuclear pore and inhibit nuclear import of calmodulin, recently showed that CREB phosphorylation in response to L-type Ca^{2+} channel activation can function in the absence of calmodulin translocation. Furthermore, they showed that isolated nuclei could support CREB phosphorylation, which is sensitive to Ca^{2+}/calmodulin-dependent kinase inhibitors. This suggests that the nucleus is an independent processing unit and nuclear Ca^{2+} alone can activate CREB. Their work also showed that a submembranous Ca^{2+} pool does in fact induce CREB phosphorylation in response to synaptic NMDA receptor activation, but this occurs by the Ras/MAPK pathway (Hardingham et al., 2001b).

Another example of nuclear Ca^{2+} as a direct mediator of transcriptional activation is provided by the transcription factor downstream regulatory element antagonist modulator (DREAM). DREAM was initially identified as an EF-hand containing protein in neurons that represses transcription from a DREAM response element (DRE) in the prodynorphin gene, which is also present in the c-*fos* gene. Binding

of Ca^{2+} to DREAM causes its dissociation from the DRE, thus relieving the repression (Carrion et al., 1999). Mice with a null mutation in DREAM show a reduction in pain behavior attributed to elevated levels of prodynorphin mRNA and dynorphin A peptides in the spinal cord (Cheng et al., 2002). In addition to its role in transcriptional repression, DREAM, also referred to as KChip3 and calsenilin, has been found to modulate A-type K^+ channels (An et al., 2000) and apoptosis (Jo et al., 2001).

3.2. Differential gene expression by spatially distinct Ca^{2+} signals in the nervous system

The activation of distinct signalling pathways by nuclear and cytoplasmic Ca^{2+} provides a mechanism for differential gene expression by electrical activity in the brain. However, are there physiological stimuli that cause differential increases in nuclear and cytoplasmic Ca^{2+} levels? It is conceivable that depending on the type and site of synaptic inputs, Ca^{2+} transients of different amplitudes occur in different cellular compartments. For example, brief bursts of synaptic activity at distal dendrites may not stimulate appreciable increases in nuclear Ca^{2+} levels, activating only cytoplasmic Ca^{2+} signalling mechanisms and, consequently, SRE-dependent transcription. One would predict that stronger or more prolonged stimuli, or synaptic inputs close to the cell body, would give rise to Ca^{2+} signals both in the cytoplasm and in the nucleus. This would activate transcription of CRE as well as SRE containing genes.

The possibility that different neuronal firing patterns may result in different cytoplasmic and nuclear Ca^{2+} levels is supported by recent work. Hardingham et al. (2001a) induced bursts of action potential firing in hippocampal neurons by inhibiting γ-aminobutyric acid ($GABA_A$) receptors with the antagonist bicuculline. This resulted in NMDA receptor-dependent Ca^{2+} oscillations that invaded the dendrites, cytoplasm and nucleus. Hardingham et al. observed that the nuclear Ca^{2+} transient outlasted the dendritic transient. Increasing the frequency of synaptic bursts also increased Ca^{2+} oscillation frequency and had an additive effect on the amplitude of the sustained nuclear Ca^{2+} signal. Higher burst frequencies not only resulted in an increase in amplitude of nuclear Ca^{2+} but also enhanced c-*fos* and CREB-mediated transcription. Thus, the nucleus can integrate the frequency of Ca^{2+} oscillations by converting them into a nuclear Ca^{2+} amplitude. The generation of different nuclear Ca^{2+} amplitudes by different synaptic stimuli offers an explanation for observations made by Impey et al. (1996). They reported that multiple trains of high-frequency electrical stimulations, but not a single high-frequency stimulus, could induce CRE-mediated transcription in hippocampal slices. The two stimulation paradigms may give rise to nuclear Ca^{2+} transients of different amplitudes, the former achieving levels sufficient to activate the CRE. Similarly, Worley et al. (1993) observed that expression of the immediate early genes c-*fos* and *zif268* was differentially induced, depending on the stimulus intensity during long-term potentiation induction. A stimulation pattern consisting of 50 repetitions of high-frequency pulses was needed to activate c-*fos* transcription, while 10 repetitions of high-frequency pulses induced expression of *zif268* but not of c-*fos*. Since the *zif268* gene contains four putative SREs in its promoter (Christy and Nathans, 1989; Changelian et al., 1989), it may be more sensitive to cytoplasmic Ca^{2+} signals. In contrast, expression of c-*fos* is more dependent on the CRE and, thus, may require stronger stimulations to elevate nuclear Ca^{2+} to levels that efficiently activate the nuclear Ca^{2+} signalling mechanism impinging on CREB/CBP.

3.3. Differential activation of CREB-dependent gene expression by the site of Ca^{2+} entry

Paradoxically, not all stimuli that efficiently increase nuclear Ca^{2+} levels activate CRE-dependent gene expression. In primary hippocampal neurons, activation of NMDA receptors by bath application of glutamate or L-type voltage-gated Ca^{2+} channel activation by bath depolarization gives rise to nuclear Ca^{2+} transients (Hardingham et al., 1999), induces CREB phosphorylation on serine 133 (Ginty et al., 1993), stimulates nuclear Ca^{2+}/calmodulin-dependent kinase IV (Bito et al., 1996) and CBP activity (Hardingham et al., 1999). While both types of Ca^{2+} channels can activate signalling pathways that stimulate transcription through the SRE, only Ca^{2+} influx through L-type Ca^{2+} channels efficiently activates CRE-mediated gene expression (Bading et al., 1993). The two channels differed in the duration for which CREB was phosphorylated (Hardingham et al., 1999). Activation of NMDA receptors by bath application of glutamate gave a transient increase in CREB phosphorylation, whereas L-type Ca^{2+} channel activation by bath depolarization resulted in sustained CREB phosphorylation. In contrast, synaptic activation of NMDA receptors resulted in prolonged CREB phosphorylation and efficiently induced CREB-mediated transcription (Hardingham et al., 2001a). This was in agreement with earlier work showing sustained CREB phosphorylation is required for CREB-mediated gene expression (Liu and Graybiel, 1996; Bito et al., 1996). Examining CREB phosphorylation in hippocampal neurons during synaptic stimulations, Bito et al. (1996) found that following brief stimulations CREB is only transiently phosphorylated on serine 133 while longer synaptic stimulation resulted in sustained CREB phosphorylation. Using pharmacological tools, Bito et al. (1996) showed that transient CREB phosphorylation by weak synaptic inputs could be converted into stimuli that give prolonged CREB phosphorylation by inhibition of PP-1 and not PP2A. Because stimulation of the serine/threonine phosphatase, calcineurin by Ca^{2+}/calmodulin indirectly activates PP1 by dephosphorylating and thereby inactivating the PP1 inhibitor, phospho-inhibitor 1, Bito et al.

(1996) tested the effect of calcineurin inhibition and showed that it delayed the rate of CREB dephosphorylation. They proposed that sustained CREB phosphorylation may be due to the production of superoxide during longer synaptic stimulations, resulting in inactivation of calcineurin (Bito et al., 1996). Liu and Graybiel (1996) described a similar role for calcineurin as a negative regulator of CREB function in striatal neurons.

How might Ca^{2+}, depending on the site of entry into neurons, control the duration of CREB phosphorylation? It is possible that calcineurin or PP-1 activity is differentially affected by Ca^{2+} channels explaining the differences in the kinetics of CREB phosphorylation on serine 133 by activation of L-type voltage-gated Ca^{2+} channels and synaptic and extrasynaptic activation of NMDA receptors. Differences in the amplitude or duration of Ca^{2+} signals and/or differences in the particular signalling mechanisms available at the site of Ca^{2+} entry could cause differential activation of CREB kinases and CREB phosphatases. Sala et al. (2000), using bath application of NMDA, showed that CREB is transiently phosphorylated in response to NMDA receptor activation in mature hippocampal neurons at 14 days in vitro. In contrast, NMDA receptor stimulation leads to prolonged CREB phosphorylation in immature hippocampal neurons at 7 days in vitro. The authors proposed that this could be due to a coupling of NMDA receptors of mature neurons to a CREB phosphatase, which they identified as PP1 on the basis of pharmacological inhibition using okadaic acid. Thus, NMDA receptors and not L-type Ca^{2+} channels may be coupled to a signalling mechanism that results in PP1-mediated dephosphorylation of CREB.

4. Gene regulation by cytoplasmic Ca^{2+} signals

4.1. Signalling pathways targeting the SRE

Although cytoplasmic Ca^{2+} signals are not sufficient to stimulate CRE-dependent transcription, they do control gene expression through the SRE (Hardingham et al., 1997). Two Ca^{2+} signalling pathways that impinge on the transcription factors SRF and TCF activate c-*fos* transcription from the SRE (Johnson et al., 1997). The signal transduction mechanisms controlling SRE-mediated transcription have been extensively studied in response to mitogenic stimuli, such as growth factors or serum. Some of the signalling pathways activated by mitogenic stimuli are also activated in response to Ca^{2+}, but none of them have so far been definitively shown to be responsible for Ca^{2+}-activated, SRE-dependent transcription.

Growth factors such as epidermal growth factor (EGF) or nerve growth factor (NGF) activate transcription via the classical Ras/MAP kinases (ERKs) signalling cascade which involves sequential activation of the small GTP binding protein Ras, the serine/threonine kinase Raf-1, MAP kinase/ERK kinases (MEKs) and MAP kinases (ERKs). Activated MAP kinase translocates to the nucleus where it phosphorylates and activates TCFs (Gille et al., 1992; reviewed in Marshall, 1994; Treisman, 1994). The TCF, Elk-1, is also reported to undergo phosphorylation in response to glutamate which activates NMDA receptors, in brain slices (Vanhoutte et al., 1999). The Ras/MAPK signalling cascade also impinges on SRF; the MAP kinases (ERKs) activated kinase, $pp90^{rsk}$, phosphorylates SRF on serine 103 following stimulation by growth factors, which accelerates the on-rate of the binding of SRF to DNA (Rivera et al., 1993). SRF phosphorylation on this residue also takes place in response to Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels in PC12 cells (Misra et al., 1994) but the functional significance of this event for SRF-mediated transcription remains unclear. SRF-dependent transcription following serum stimulation is also subject to regulation by a pathway involving activation of heterotrimeric G-proteins and members of the RhoA family of small GTPases (Hill et al., 1995; reviewed in Treisman et al., 1998).

In common with growth factors, Ca^{2+} is also a potent activator of the Ras/MAP kinases (ERKs) cascade. The initial observation by Bading and Greenberg (1991) that activation of NMDA receptors in primary hippocampal neurons induces tyrosine phosphorylation of ERK was followed by other studies showing Ca^{2+} activation of the Ras/MAP kinases (ERKs) signalling cascade in cultured cortical neurons and neuronal cell lines (Rosen et al., 1994; Rusanescu et al., 1995; Johnson et al., 1997). This suggested that Ca^{2+}, like growth factors, activates the SRE through the Ras/MAP kinases (ERKs) signalling cascade. In support of this, Xia et al. (1996) reported that in primary cortical neurons, overexpression of MAP kinase phosphatase-1 (MKP-1; Sun et al., 1993) that dephosphorylates and thereby inactivates MAP kinases (ERKs), blocked TCF-dependent transcriptional activation upon Ca^{2+} entry through NMDA receptors. In contrast, Johnson et al. (1997) found that inhibition of MAP kinases (ERKs) activation by pharmacological means had very little effect on Ca^{2+}-induced SRE-mediated transcription in PC12 and AtT20 cells, indicating that Ca^{2+} can stimulate gene expression via the SRE independently of the Ras/MAP kinases (ERKs) signalling cascade.

Several studies have also indicated a role for Ca^{2+}/calmodulin-dependent kinases in SRE-mediated transcription. Both the calmodulin antagonist calmidazolium and the Ca^{2+}/calmodulin-dependent kinase inhibitor, 1[*N*,*O*-bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl] 4-phenylpiperazine (KN-62), attenuate c-*fos* induction following L-type Ca^{2+} channel activation in PC12 cells and hippocampal neurons (Morgan and Curran, 1986; Bading et al., 1993; Bito et al., 1996). SRF may be a target for a Ca^{2+}/calmodulin-dependent kinase regulated mechanism since a Ca^{2+}-induced SRF-mediated transcriptional response is blocked by KN-62 (Miranti et al., 1995). Furthermore, the ability of SRF to activate transcription is enhanced in cells

overexpressing a constitutively active form of Ca^{2+}/calmodulin-dependent kinase IV (Miranti et al., 1995). In addition, overexpression of a constitutively active form of Ca^{2+}/calmodulin-dependent kinase II, although unable to activate a CRE-containing reporter, induced expression from a reporter containing the full-length c-*fos* promoter (Matthews et al., 1994). Since the SRE and the CRE are the two Ca^{2+}-responsive elements found in the c-*fos* promoter (Bading et al., 1993; Johnson et al., 1997), it is likely that the effect of constitutively active Ca^{2+}/calmodulin-dependent kinase II on c-*fos* transcriptional induction is mediated through the SRE.

Phospholipase A_2, a Ca^{2+}-regulated enzyme that catalyzes phospholipid metabolism leading to the formation of arachidonic acid, is another potential regulator of c-*fos* transcriptional regulation through the SRE. Arachidonic acid can be metabolised by cyclooxygenase and lipoxygenase to prostaglandins and leukotrienes, respectively. In hippocampal neurons, inhibition of phospholipase A_2 with quinacrine or aristolochic acid, or inhibition of the synthesis of prostaglandins attenuates c-*fos* induction following NMDA receptor activation (Lerea and McNamara, 1993; Lerea et al., 1997).

4.2. NF-AT activation in neurons and cells of the immune system

In addition to controlling neuronal gene expression, Ca^{2+}-activated transcription is also important for the growth, differentiation and function of immune cells. For example, antigen stimulation of B cells and T cells increases the intracellular Ca^{2+} concentration and activates the expression of a number of haematopoietic growth factor and cytokine genes through the transcription factors, nuclear factor of activated T cells (NF-AT) and NF-κB (for reviews, see Rao et al., 1997; Crabtree and Clipstone, 1994). The mechanism by which NF-AT activates transcription involves its dephosphorylation by calcineurin in the cytoplasm and subsequent translocation to the nucleus where it promotes transcription together with nuclear partners such as AP-1. The NF-AT isoform, NF-ATc4, is expressed in hippocampal neurons and translocates to the nucleus in response to electrical activity (Graef et al., 1999). While in immune cells only a continuous rise in intracellular Ca^{2+} concentration but not a brief Ca^{2+} spike induces persistent nuclear translocation of NF-AT (Dolmetsch et al., 1997) in neurons, even a brief depolarization for 3 min or physiological 5-Hz stimulation was sufficient for NF-ATc4 to persist in the nucleus for at least 15 min. Graef et al. (1999) also identified the inositol 1,4,5-triphosphate receptor type I (IP_3R1) gene, which encodes a endoplasmic reticulum Ca^{2+} channel as a downstream NF-AT target. Induction of the IP3R1 gene by NF-AT could play a role in plasticity by altering the amplitude and spatial properties of activity-induced Ca^{2+} transients.

5. Transcription factors are fast on–off switches: decoding temporal Ca^{2+} signals in neurons

In addition to the spatial properties, the duration of Ca^{2+} signals could also contribute to the specificity of transcription induction. In immune cells, Dolmetsch et al. (1997) reported differential activation of NF-AT and NF-κB by the duration of Ca^{2+} signals. A brief Ca^{2+} spike activated NF-κB, but not NF-AT, which required a sustained increase in intracellular Ca^{2+} for activation.

To assess the effect of the duration of elevated Ca^{2+} in neuronal cells, Chawla and Bading (2001) generated different-length Ca^{2+} transients by using an agonist and an antagonist of L-type Ca^{2+} channels. They found that in AtT20 cells and hippocampal neurons CREB, its coactivator CBP and SRE-interacting transcriptional regulators were induced by short-lasting Ca^{2+} signals, and they remained active for the duration of the Ca^{2+} signal. However, they were also shut-off rapidly after Ca^{2+} concentrations had returned to basal levels. Consequently, the magnitude of transcription activation was found to depend on the duration of increased Ca^{2+}. Chawla and Bading (2001) assessed the kinetics of CREB and ERK1/2 phosphorylation. CREB was activated by a fast, Ca^{2+}/calmodulin-dependent kinase-dependent mechanism that mediates CREB phosphorylation on serine 133 within 30 s of Ca^{2+} entry. The second Ca^{2+}-activated route to CREB, which involves the MAP kinase/ERK1/2 cascade, was triggered by brief, 30 to 60 s lasting Ca^{2+} transients. ERK1/2 activity peaked several min after Ca^{2+} entry and outlasted the Ca^{2+} transient. This was similar to earlier work (Wu et al., 2001) that assessed kinetic contributions of the Ca^{2+}/calmodulin-dependent kinase and MAP kinase pathways to CREB phosphorylation. The shut-off of CREB and ERK1/2 after termination of Ca^{2+} signals involved rapid dephosphorylation of their activator sites. The decoding of temporal features of Ca^{2+} transients by transcription factors and their regulating kinases and phosphatases adds another dimension to the mechanisms available for synaptic input-specific gene expression. The temporal profile of a Ca^{2+} transient induced by a burst of action potentials is likely to be different from that induced by a train of bursts of action potentials and will therefore differentially affect gene expression.

6. Concluding remarks

Neurons exploit the spatial and temporal diversity of Ca^{2+} transients to specify genomic responses. CRE and SRE-interacting proteins are fast on–off switches: their ability to activate transcription is induced rapidly following Ca^{2+} increases and is attenuated quickly after Ca^{2+} has returned to basal levels. In addition to the duration of the Ca^{2+} signal, the amplitude also affects transcription quantitatively. The spatial properties of Ca^{2+} signals and the site of Ca^{2+} entry specify qualitative differences in the tran-

scriptional response. Gene expression mediated by CREB and CBP requires nuclear Ca^{2+} while TCF/SRF and NF-AT-dependent transcription is controlled by Ca^{2+} transients in the cytoplasm. Ca^{2+} entry through the L-type Ca^{2+} channel activates CREB, CBP and SRE-interacting proteins, while influx through the NMDA receptor activates SRF/TCF. Thus, a neuronal Ca^{2+} code is emerging which forms the basis for activity-dependent gene expression. Activity-dependent changes in gene expression are not only important for neuronal plasticity but also play a crucial role in the shaping of synaptic connections during development and are important for neuronal survival.

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