Excitation-Transcription Coupling: Signaling by Ion Channels to the Nucleus

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Calcium (Ca^{2+}) is a highly pleiotropic second messenger that plays an important role in a wide variety of cellular events. One of the important functions of Ca^{2+} is to activate signaling pathways that lead to the expression of genes (*1*, *2*). Genome-wide screens have identified over 300 different genes (*3*, *4*) and approximately 30 transcription factors that are regulated by the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$). Studies over the past decade suggest that most Ca^{2+}-activated transcription factors require a rise in nuclear Ca^{2+} for activation. Recent evidence, however, indicates that Ca^{2+} elevations in the vicinity of plasma membrane Ca^{2+} channels also play a key role in regulating transcription. Thus, transcription factors integrate information about the electrical or chemical events affecting Ca^{2+} channels in distinct subcellular domains with nuclear signals that reflect events in the whole cell.

Cells respond to various types of stimulation with a remarkable diversity of $[Ca^{2+}]_i$ signals. Synaptic transmission, growth cone guidance, and action potential firing all trigger $[Ca^{2+}]_i$ elevations that are both temporally and spatially complex and are different from one another (*5*, *6*). A central question in Ca^{2+} signaling is how cells interpret these diverse Ca^{2+} signals and convert them into specific transcriptional responses. In cells with complex morphologies, such as neurons and muscle cells, the spatial localization of a rise in Ca^{2+} conveys important information about the type and intensity of the initiating stimulus. For example, in neurons, Ca^{2+} elevations that occur close to *N*-methyl-D-aspartate (NMDA) receptors reflect synaptic input (*7*-*9*), whereas Ca^{2+} elevations that occur close to N or P/Q type voltage-activated Ca^{2+} channels at synaptic terminals imply synaptic vesicle release (*10*, *11*). Information regarding the strength of an input is also encoded through the spatial distribution of a $[Ca^{2+}]_i$ elevation. Weak synaptic stimuli produce $[Ca^{2+}]_i$ elevations that are limited to a postsynaptic spine or to a limited region of the dendritic tree. Stronger or higher frequency stimulation produces $[Ca^{2+}]_i$ signals that spread to the cell body and nucleus of a cell (*12*-*14*). Thus, localized Ca^{2+} signals around ion channels provide information about the type of stimulus, whereas bulk $[Ca^{2+}]_i$ elevations provide information about the intensity of a stimulus.

Studies by a number of groups indicate that nuclear Ca^{2+} elevations activate enzymes that both regulate transcription factors directly and modulate chromatin structure. One of the best studied transcription factors in this regard is the cyclic adenosine 3′,5′-monophosphate-responsive element binding protein (CREB). CREB plays an important role in a wide variety of neuronal functions, including survival, neuronal morphology, and synaptic plasticity (*15*). CREB is activated by phosphorylation of several serines, including Ser^{133}, which recruits the coactivator CREB binding protein (CBP) (*16*). The requirement of nuclear and cytoplasmic Ca^{2+} for CREB-dependent transcription was investigated by injecting neuroblastoma cells with dextran-coupled BAPTA [1,2-bis(o-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid] a Ca^{2+} buffer that is too large to diffuse passively across the nuclear pores (*17*). Injection of dextran-BAPTA into the nucleus prevented the transcriptional activation of CREB, suggesting that either the kinetics or the amplitude of the nuclear Ca^{2+} rise is important for CREB activation. This conclusion agrees broadly with studies that suggest that weak synaptic stimulation fails to activate the transcription of CREB-activated genes such as c-*fos*, presumably because Ca^{2+} fails to invade the cell body or the nucleus of the neuron (*18*, *19*). Other results suggest that CREB phosphorylation on Ser^{133} can occur even in the presence of cytoplasmic EGTA [ethylene-glycol-bis-(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid], a Ca^{2+} buffer that prevents nuclear Ca^{2+} elevations but allows $[Ca^{2+}]_i$ elevation close to the plasma membrane (*20*, *21*). These results imply that a membrane-bound Ca^{2+} sensor can activate a signaling pathway that leads to phosphorylation of CREB Ser^{133}, but that CREB-dependent transcription requires additional Ca^{2+}-dependent steps that occur in the nucleus.

Other Ca^{2+}-activated transcription factors also require a rise in nuclear Ca^{2+} for activation. Elk-1 is an ETS family transcription factor that binds and regulates a second transcription factor called the serum response factor (SRF). SRF acts on the serum response element (SRE), a growth factor-regulated site found in many transcriptional enhancers (*22*). Elk-1 is activated by several Ca^{2+}-dependent signaling pathways, including the mitogen-activated protein kinase (MAPK) pathway and the Ca^{2+}-calmodulin (CaM)-activated kinase (CaMK) II and IV pathways (*23*). Initial studies using BAPTA-dextran injection in neuroblastoma cells suggested that a rise in cytoplasmic Ca^{2+} was sufficient to activate SRE-mediated transcription (*17*). However, a recent study using parvalbumen, a Ca^{2+}-binding protein, to buffer $[Ca^{2+}]_i$ elevations either in the nucleus or in the cytoplasm has found that stimulation of Elk-1 transcriptional activity in response to endothelial growth factor (EGF) requires a rise in nuclear Ca^{2+} (*24*). As is the case for CREB, Elk-1 phosphorylation and MAPK translocation into the nucleus, which are required for Elk-1 activation, are not affected by buffering the nuclear Ca^{2+} elevation. In contrast, Elk-1-mediated transcription is dramatically reduced by buffering nuclear Ca^{2+}, suggesting that both nuclear and cytoplasmic Ca^{2+} signals may be involved in regulating Elk-1-dependent transcription. The discrepancy of this recent study with the earlier study of SRE-dependent transcription has not been resolved, but may reflect the fact that the SRE is a target for multiple transcription factors other than Elk-1 (*25*).

Activation of the nuclear factor of activated T cells (NFAT), another well-characterized Ca^{2+}-dependent transcription factor, also requires a nuclear Ca^{2+} elevation (*26*). NFAT plays a cen-

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tral role in the activation of cells in the immune system and in the development of the cardiovascular and nervous systems (*27*). NFAT translocates from the cytoplasm into the nucleus in response to dephosphorylation of several of its serines by the Ca^{2+} CaM-activated phosphatase calcineurin (CaN) (*28*). CaN binds some isoforms of NFAT and translocates into the nucleus as a complex with NFAT, where it maintains NFAT in a dephosphorylated state as long as Ca^{2+} remains elevated (*29*). A drop in nuclear Ca^{2+} deactivates CaN and allows one of several NFAT kinases to rephosphorylate NFAT, causing it to leave the nucleus and thereby inactivating transcription (*30*, *31*). Thus, a sustained nuclear Ca^{2+} elevation is required for NFAT-dependent transcription.

Nuclear factor kappa B (NF-κB), another transcription factor that is regulated by translocation into the nucleus, also requires a nuclear Ca^{2+} elevation to sustain its activity. NF-κB translocates into the nucleus after the phosphorylation and degradation of an inhibitory subunit, IκB (*32*). Remarkably, a single spike of Ca^{2+} is sufficient to trigger IκB degradation and NF-κB translocation into the nucleus, where it persists for at least 30 min until IκB is replenished (*31*). Even in the nucleus, however, NF-κB requires additional $[Ca^{2+}]_i$ elevations for maximal transcription. Periodic $[Ca^{2+}]_i$ elevations every 15 or 30 min are not sufficient for maximal NF-κB transcription. Instead, the efficiency of NF-κB transcriptional activity increases as the time between Ca^{2+} elevations decreases and is maximal with a sustained $[Ca^{2+}]_i$ elevation (*33*). These results suggest that other Ca^{2+}-dependent events in addition to IκB degradation and NF-κB translocation are required for NF-κB transcriptional activity in response to elevations of $[Ca^{2+}]_i$. These additional Ca^{2+}-dependent events probably occur in the nucleus, because this is where the majority of NF-κB dimers are found after an initial Ca^{2+} elevation.

Finally, the transcriptional repressor Downstream regulatory element antagonistic modulator (DREAM), which is important in regulating the transcription of the prodynorphin gene in spinal cord neurons in response to elevations of $[Ca^{2+}]_i$, has an EF hand that can bind Ca^{2+} directly (*34*). At resting nuclear Ca^{2+} levels, DREAM is bound to DNA and suppresses transcription, possibly by preventing the interaction of transcription factors and coactivators such as CBP or p300 (*35*). Elevation of nuclear Ca^{2+} causes Ca^{2+} binding to the EF hand on DREAM, disrupting the interaction between DREAM and other proteins and relieving the transcriptional block. These events occur in the nucleus, suggesting that inactivation of DREAM requires a nuclear Ca^{2+} elevation.

The requirement for nuclear Ca^{2+} elevations to activate transcription but not phosphorylation of transcription factors such as CREB and Elk-1 suggests that there may be nuclear targets of Ca^{2+} other than the transcription factors themselves. The nucleus contains high concentrations of CaM, suggesting that it is rich in CaM targets (*36*). CaM also translocates to the nucleus in response to a $[Ca^{2+}]_i$ rise, which may further enhance the sensitivity of CaM-activated enzymes to changes in $[Ca^{2+}]_i$ (*37*, *38*). One prominent nuclear target for Ca^{2+} is the CaM-activated kinase CaMKIV (*39*). CaMKIV is found in the nucleus of a subpopulation of neurons and is known to phosphorylate CREB directly at Ser^{133} (*40*, *41*). Genetic disruption of the CaMKIV gene in mice reduces CREB phosphorylation in the cerebellum, and recent studies suggest that CaMKIV may mediate the early phase of CREB phosphorylation (*39*). CaMKIV also phosphorylates the histone deacetylases HDAC 4, 5, and 7, which are thought to repress gene expression by deacetylating histones and altering DNA structure (*42*). Phosphorylation of histone deacetylases (HDACs) by CaMKIV disrupts the association between HDACs and transcription factors such as the myocyte-enhancing factor-2 (MEF-2), triggering HDAC export from the nucleus (*43*, *44*). HDAC export allows MEF-2 to activate transcription by recruiting other Ca^{2+}-sensitive transcriptional factors such as NFAT and transcriptional coactivators such as p300 (*45-47*).

MEF-2 is also regulated by the CaN inhibitor protein CABIN1, which is another target of nuclear Ca^{2+}. CABIN1 binds and inhibits MEF-2 by recruiting a chromatin-remodeling complex composed of the proteins mSin-3 and HDACs 1 and 2, which modify histones and supress transcription. CABIN1 also prevents MEF-2 binding to p300, thus preventing MEF-2 from recruiting elements of the basal transcription machinery (*48*). Nuclear Ca^{2+} elevation causes dissociation of CABIN1 from MEF-2, possibly because CaM competes with MEF-2 for CABIN binding (*49*). Thus, nuclear Ca^{2+} elevations regulate CABIN binding to transcription factors such as MEF-2 and may thereby lead to general increases in transcription.

In addition to modulating transcriptional inhibitors such as HDACs 4, 5, and 7 and CABIN1, elevations in nuclear Ca^{2+} can also activate proteins that promote transcription. The coactivator protein CBP binds CREB as well as other transcription factors and recruits elements of the general transcription machinery to many promoters (*50*). CBP has histone acetylase (HAT) activity that can increase the activity of transcription factors by modifying the chromatin structure and can also recruit other HATs (*51*). Studies have shown that CBP can activate transcription in response to elevations in $[Ca^{2+}]_i$ when it is recruited to the DNA by fusion to a DNA binding domain from the yeast protein GAL-4 (*52*, *53*). CBP is phosphorylated at Ser^{301} by CamKIV, which appears to increase its transcriptional activity, although it is not clear whether this modification affects its binding to CREB and other transcription factors or whether it regulates the endogenous HAT activity of CBP (*54*). Other chromatin-remodeling enzymes such as methyl transferases and methylases may also be targets for calcium signaling in neurons, and this question is an active area of study in several laboratories. In summary, there is strong evidence that nuclear Ca^{2+} elevations are required for the activity of many transcription factors and that at least some of the targets of Ca^{2+} in the nucleus are not the transcription factors themselves but modulating enzymes that affect general aspects of transcription.

A requirement for nuclear Ca^{2+}, however, does not preclude an important role for cytoplasmic Ca^{2+} signals in activating nuclear signaling. A series of recent papers has highlighted the importance of localized channel-specific elevations of $[Ca^{2+}]_i$ in activating transcription. The fundamental idea underlying these papers is that local calcium-sensing proteins placed close to Ca^{2+}, channels may activate signaling pathways that regulate transcription. The Ca^{2+} concentration in the cloud of Ca^{2+} ions surrounding a Ca^{2+} channel is thought to be 100 to 1000 times higher than the Ca^{2+} concentration in the bulk of the cytoplasm (*55*). By placing Ca^{2+} sensors close to the intracellular mouth of a channel, it is possible to activate Ca^{2+}-dependent signaling pathways only when a particular Ca^{2+} channel admits Ca^{2+} and not when other types of Ca^{2+} channels are active. In this way, a cell can link the activation of a given signaling pathway to the specific electrical or chemical

stimuli that activate a particular type of Ca^{2+} channel.

One channel that has been implicated in signaling to the nucleus is the L-type voltage-activated Ca^{2+} channel. Early studies found that Ca^{2+} influx through L-type channels is particularly effective at activating the immediate-early gene c-*fos* in PC12 cells and cultured neurons (*56*). The effect of Ca influx through L-type channels on c-*fos* is mediated at least in part by CREB binding to regulatory sites on the c-*fos* promoter (*57*, *58*). In cultured hippocampal neurons (*59*) and hippocampal slices (*60*), Ca^{2+} influx through L-type channels leads to phosphorylation of CREB at Ser^{133} and activation of CREB-dependent transcription. Comparison of the Ca^{2+} influx mediated by L-type channels and by other types of Ca^{2+} channels suggests that the ability of L-type channels to activate CREB is not due to the ability of L-type channels to generate larger or more prolonged $[Ca^{2+}]_i$ rises than other channels (*37*). Instead, L-type channels appear to be associated with a complex of signaling proteins that can sense the activation of the channel and then signal to the nucleus. In a recent study using L-type Ca^{2+} channels that are insensitive to dihydropyridine inhibitors, we identified the calcium sensor CaM as a critical element of the signaling complex around the L-type calcium channel (*61*). Mutation of the CaM binding site on the COOH-terminus of L-type channels reduces their ability to activate CREB and also to activate the MAPK pathway. By activating the CREB kinases Rsk1 and 2 (*62*, *63*), the MAPK cascade leads to long-term CREB Ser^{133} phosphorylation and thus contributes substantially to CREB-dependent transcription (*64*). Thus, it appears that Ca^{2+} that is elevated in the immediate vicinity of L-type channels binds to CaM and leads to the activation of the MAPK enzymatic cascade, ultimately leading to the nucleus.

In addition to the L-type channel, another voltage-gated calcium channel, the P/Q-type channel encoded by the gene *CaV2.2*, also appears to be linked to the expression of genes (*65*). Introducing P/Q-type channels into 293T cells triggers expression of the synaptic protein syntaxin 1A. P/Q-type channel signaling to the syntaxin 1A gene appears to require communication between the channel and internal Ca^{2+} stores because it is blocked by thapsigargin, an inhibitor of the endoplasmic reticulum adenosine triphosphatase. The link between the P/Q type channel and syntaxin 1A expression is also observed in primary cerebellar granule cells, where it is blocked by P/Q channel inhibitors indicating that syntaxin 1A up-regulation requires Ca^{2+} flux through the P/Q-type channel. Syntaxin 1A binds to the P/Q-type channel and links it to the synaptic release machinery (*10*). Thus, the P/Q channel regulation of syntaxin 1A may be a mechanism by which Ca^{2+} channels regulate the expression of proteins that participate in synaptic vesicle release.

A final example of Ca^{2+} channels conveying information to the nucleus comes from recent studies of the NMDA receptor. In one set of studies, the authors examined the interaction of the ephrin family of tyrosine kinases with the NMDA receptor during synapse formation (*66*, *67*). Stimulation of neurons in culture with ephrin B triggers clustering of NMDA and ephrin receptors and association of the receptor complex with the Src family of tyrosine kinases. Src family kinases phosphorylate the NMDA receptor, increasing its ability to admit Ca^{2+} and increasing its ability to activate CREB-dependent gene expression. It is not clear from these studies whether the effects of ephrin B on NMDA receptor signaling are due to localized Ca^{2+} increases around the NMDA receptor or to a bulk Ca^{2+} increase in the nucleus.

A second set of studies has examined signaling to the nucleus by synaptic versus nonsynaptic NMDA receptors (*68*). The authors compared two methods of stimulating neurons in culture. They stimulated neurons with either bicuculline and 4-AP [an inhibitor of γ-aminobutyric acid (GABA) receptors and a potassium channel blocker, respectively], a combination that may activate neurons synaptically, or with glutamate in the bath, a treatment that activates all NMDA receptors indiscriminately. Treatment with bicuculline and 4-AP triggered a

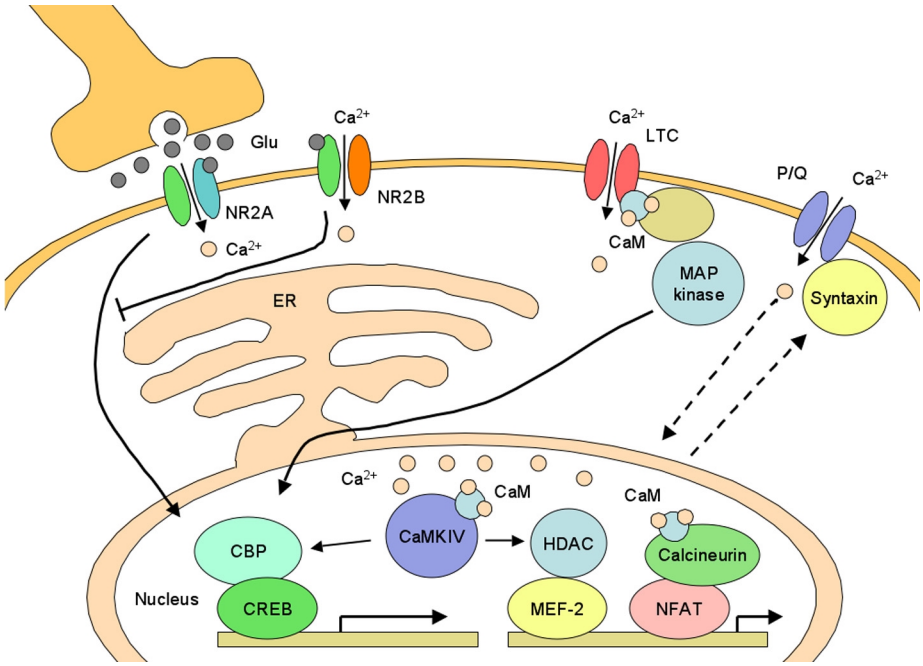

Fig. 1. Activation of Ca^{2+}-dependent transcription requires both cytoplasmic and nuclear Ca^{2+} signals. Ca^{2+} influx through synaptic NMDA receptors activates CREB, whereas influx through extrasynaptic NMDA receptors inhibits CREB. L-type channels use CaM to sense local Ca^{2+} elevations and activate the MAPK pathway. Activation of P/Q type channels leads to the expression of syntaxin 1A, which in turn binds to P/Q type channels. Some nuclear Ca^{2+} signals are mediated by CaM kinase IV phosphorylation of CBP and HDACs 4, 5, and 7. Nuclear Ca^{2+} also activates calcineurin, which dephosphorylates NFAT and promotes its transcriptional activity. Glu, glutamate; LTC, L-type channel; ER, endoplasmic reticulum.

robust activation of CREB-dependent transcription, whereas treatment with glutamate in the bath inhibited CREB activity and potentiated cell death. The authors argued that bicuculline and 4-AP activate NMDA receptors at the synapse that are linked to signaling pathways that activate CREB. In contrast, bath treatment with glutamate activates nonsynaptic NMDA receptors that inhibit CREB-dependent transcription and activate cell death pathways. The authors tested the idea that the difference between the two populations of NMDA receptors lies in their subunit composition. They found that a blocker of channels that contain the NR2B subunit prevented glutamate's inhibition of CREB activation, indicating that the NR2B subunit is located mainly outside of synapses. These results suggest that local events around the NR2A NMDA receptor subunit at synapses may underlie synapse-specific signaling to the nucleus.

The ability of the L-type, P/Q-type, and NMDA receptor channels to convey signals to the nucleus, and the wealth of evidence on Ca^{2+}-regulated enzymes that regulate transcription in the nucleus, provide a framework for understanding how Ca^{2+} channels regulate transcription. Ca^{2+}-dependent transcription requires both localized Ca^{2+} elevation around channels in the cytoplasm and Ca^{2+} elevations in the nucleus (Fig. 1). Ca^{2+} influx through L-type channels that do not bind CaM leads to elevated nuclear calcium but not to activation of CREB-dependent gene expression. Ca^{2+} influx through NMDA receptors in the synapse activates CREB, whereas Ca^{2+} influx through nonsynaptic NMDA receptors inhibits CREB, even though both lead to elevated nuclear $[Ca^{2+}]_i$. Thus, the dual regulation of signaling pathways by Ca^{2+} near channels and in the nucleus may be a general rule that allows neurons to tailor transcriptional activation to specific types of electrical or chemical stimulation and at the same time ensures that only robust stimuli that generate nuclear Ca^{2+} elevations are converted into long-term changes in gene expression.

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