

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

Ricardo Dolmetsch

(Published 21 January 2003)

Calcium ( $\text{Ca}^{2+}$ ) is a highly pleiotropic second messenger that plays an important role in a wide variety of cellular events. One of the important functions of  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ). Studies over the past decade suggest that most  $\text{Ca}^{2+}$ -activated transcription factors require a rise in nuclear  $\text{Ca}^{2+}$  for activation. Recent evidence, however, indicates that  $\text{Ca}^{2+}$  elevations in the vicinity of plasma membrane  $\text{Ca}^{2+}$  channels also play a key role in regulating transcription. Thus, transcription factors integrate information about the electrical or chemical events affecting  $\text{Ca}^{2+}$  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  $[\text{Ca}^{2+}]_i$  signals. Synaptic transmission, growth cone guidance, and action potential firing all trigger  $[\text{Ca}^{2+}]_i$  elevations that are both temporally and spatially complex and are different from one another (5, 6). A central question in  $\text{Ca}^{2+}$  signaling is how cells interpret these diverse  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  conveys important information about the type and intensity of the initiating stimulus. For example, in neurons,  $\text{Ca}^{2+}$  elevations that occur close to *N*-methyl-D-aspartate (NMDA) receptors reflect synaptic input (7-9), whereas  $\text{Ca}^{2+}$  elevations that occur close to *N* or *P/Q* type voltage-activated  $\text{Ca}^{2+}$  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  $[\text{Ca}^{2+}]_i$  elevation. Weak synaptic stimuli produce  $[\text{Ca}^{2+}]_i$  elevations that are limited to a postsynaptic spine or to a limited region of the dendritic tree. Stronger or higher frequency stimulation produces  $[\text{Ca}^{2+}]_i$  signals that spread to the cell body and nucleus of a cell (12-14). Thus, localized  $\text{Ca}^{2+}$  signals around ion channels provide information about the type of stimulus, whereas bulk  $[\text{Ca}^{2+}]_i$  elevations provide information about the intensity of a stimulus.

Studies by a number of groups indicate that nuclear  $\text{Ca}^{2+}$  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<sup>133</sup>, which recruits the coactivator

CREB binding protein (CBP) (16). The requirement of nuclear and cytoplasmic  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  fails to invade the cell body or the nucleus of the neuron (18, 19). Other results suggest that CREB phosphorylation on Ser<sup>133</sup> can occur even in the presence of cytoplasmic EGTA [ethylene-glycol-bis-( $\beta$ -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid], a  $\text{Ca}^{2+}$  buffer that prevents nuclear  $\text{Ca}^{2+}$  elevations but allows  $[\text{Ca}^{2+}]_i$  elevation close to the plasma membrane (20, 21). These results imply that a membrane-bound  $\text{Ca}^{2+}$  sensor can activate a signaling pathway that leads to phosphorylation of CREB Ser<sup>133</sup>, but that CREB-dependent transcription requires additional  $\text{Ca}^{2+}$ -dependent steps that occur in the nucleus.

Other  $\text{Ca}^{2+}$ -activated transcription factors also require a rise in nuclear  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ -dependent signaling pathways, including the mitogen-activated protein kinase (MAPK) pathway and the  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$  was sufficient to activate SRE-mediated transcription (17). However, a recent study using parvalbumin, a  $\text{Ca}^{2+}$ -binding protein, to buffer  $[\text{Ca}^{2+}]_i$  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  $\text{Ca}^{2+}$  (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  $\text{Ca}^{2+}$  elevation. In contrast, Elk-1-mediated transcription is dramatically reduced by buffering nuclear  $\text{Ca}^{2+}$ , suggesting that both nuclear and cytoplasmic  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ -dependent transcription factor, also requires a nuclear  $\text{Ca}^{2+}$  elevation (26). NFAT plays a cen-

Department of Molecular Pharmacology, Stanford University School of Medicine, Stanford, CA 94305, USA. E-mail: Dolmetsch@stanfordalumni.org

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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  remains elevated (29). A drop in nuclear  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  elevation is required for NFAT-dependent transcription.

Nuclear factor kappa B (NF- $\kappa$ B), another transcription factor that is regulated by translocation into the nucleus, also requires a nuclear  $\text{Ca}^{2+}$  elevation to sustain its activity. NF- $\kappa$ B translocates into the nucleus after the phosphorylation and degradation of an inhibitory subunit, I $\kappa$ B (32). Remarkably, a single spike of  $\text{Ca}^{2+}$  is sufficient to trigger I $\kappa$ B degradation and NF- $\kappa$ B translocation into the nucleus, where it persists for at least 30 min until I $\kappa$ B is replenished (31). Even in the nucleus, however, NF- $\kappa$ B requires additional  $[\text{Ca}^{2+}]_i$  elevations for maximal transcription. Periodic  $[\text{Ca}^{2+}]_i$  elevations every 15 or 30 min are not sufficient for maximal NF- $\kappa$ B transcription. Instead, the efficiency of NF- $\kappa$ B transcriptional activity increases as the time between  $\text{Ca}^{2+}$  elevations decreases and is maximal with a sustained  $[\text{Ca}^{2+}]_i$  elevation (33). These results suggest that other  $\text{Ca}^{2+}$ -dependent events in addition to I $\kappa$ B degradation and NF- $\kappa$ B translocation are required for NF- $\kappa$ B transcriptional activity in response to elevations of  $[\text{Ca}^{2+}]_i$ . These additional  $\text{Ca}^{2+}$ -dependent events probably occur in the nucleus, because this is where the majority of NF- $\kappa$ B dimers are found after an initial  $\text{Ca}^{2+}$  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  $[\text{Ca}^{2+}]_i$ , has an EF hand that can bind  $\text{Ca}^{2+}$  directly (34). At resting nuclear  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  causes  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  elevation.

The requirement for nuclear  $\text{Ca}^{2+}$  elevations to activate transcription but not phosphorylation of transcription factors such as CREB and Elk-1 suggests that there may be nuclear targets of  $\text{Ca}^{2+}$  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  $[\text{Ca}^{2+}]_i$  rise, which may further enhance the sensitivity of CaM-activated enzymes to changes in  $[\text{Ca}^{2+}]_i$  (37, 38). One prominent nuclear target for  $\text{Ca}^{2+}$  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<sup>133</sup> (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 phospho-

rylates 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  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$ . 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 suppress transcription. CABIN1 also prevents MEF-2 binding to p300, thus preventing MEF-2 from recruiting elements of the basal transcription machinery (48). Nuclear  $\text{Ca}^{2+}$  elevation causes dissociation of CABIN1 from MEF-2, possibly because CaM competes with MEF-2 for CABIN binding (49). Thus, nuclear  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $[\text{Ca}^{2+}]_i$  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<sup>301</sup> 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  $\text{Ca}^{2+}$  elevations are required for the activity of many transcription factors and that at least some of the targets of  $\text{Ca}^{2+}$  in the nucleus are not the transcription factors themselves but modulating enzymes that affect general aspects of transcription.

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

One channel that has been implicated in signaling to the nucleus is the L-type voltage-activated  $\text{Ca}^{2+}$  channel. Early studies found that  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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),  $\text{Ca}^{2+}$  influx through L-type channels leads to phosphorylation of CREB at Ser<sup>133</sup> and activation of CREB-dependent transcription. Comparison of the  $\text{Ca}^{2+}$  influx mediated by L-type channels and by other types of  $\text{Ca}^{2+}$  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  $[\text{Ca}^{2+}]_i$  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  $\text{Ca}^{2+}$  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<sup>133</sup>

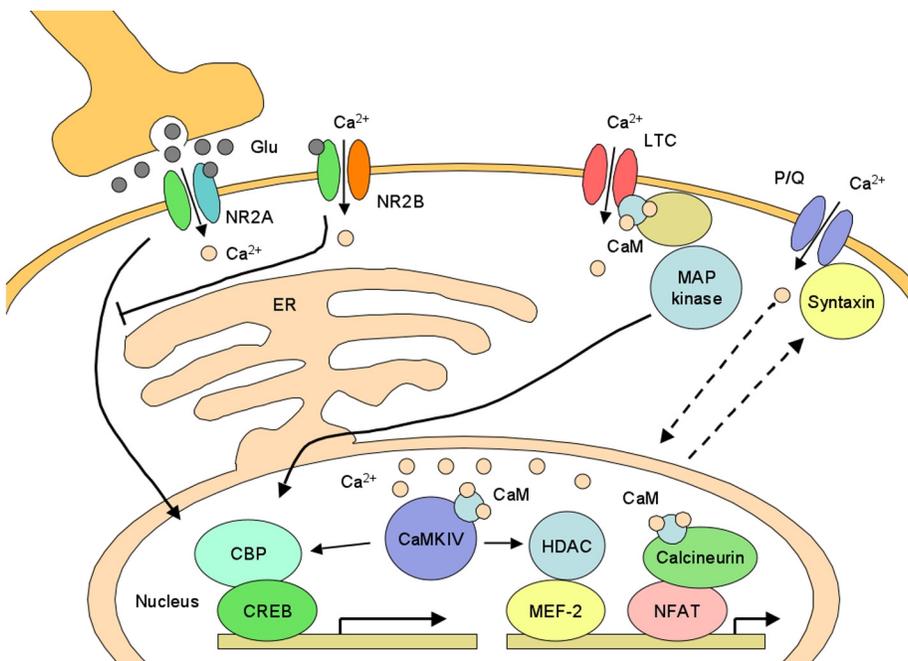
phosphorylation and thus contributes substantially to CREB-dependent transcription (64). Thus, it appears that  $\text{Ca}^{2+}$  that is elevated in the immediate vicinity of L-type channels binds to CaM and leads to the activation of the MAPK enzymatic cascade, ultimately leading to the nucleus.

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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  channels regulate the expression of proteins that participate in synaptic vesicle release.

A final example of  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  increases around the NMDA receptor or to a bulk  $\text{Ca}^{2+}$  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  $\gamma$ -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  $\text{Ca}^{2+}$ -dependent transcription requires both cytoplasmic and nuclear  $\text{Ca}^{2+}$  signals.  $\text{Ca}^{2+}$  influx through synaptic NMDA receptors activates CREB, whereas influx through extrasynaptic NMDA receptors inhibits CREB. L-type channels use CaM to sense local  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  signals are mediated by CaM kinase IV phosphorylation of CBP and HDACs 4, 5, and 7. Nuclear  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ -regulated enzymes that regulate transcription in the nucleus, provide a framework for understanding how  $\text{Ca}^{2+}$  channels regulate transcription.  $\text{Ca}^{2+}$ -dependent transcription requires both localized  $\text{Ca}^{2+}$  elevation around channels in the cytoplasm and  $\text{Ca}^{2+}$  elevations in the nucleus (Fig. 1).  $\text{Ca}^{2+}$  influx through L-type channels that do not bind CaM leads to elevated nuclear calcium but not to activation of CREB-dependent gene expression.  $\text{Ca}^{2+}$  influx through NMDA receptors in the synapse activates CREB, whereas  $\text{Ca}^{2+}$  influx through nonsynaptic NMDA receptors inhibits CREB, even though both lead to elevated nuclear  $[\text{Ca}^{2+}]_i$ . Thus, the dual regulation of signaling pathways by  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  elevations are converted into long-term changes in gene expression.

## References

1. A. E. West, W. G. Chen, M. B. Dalva, R. E. Dolmetsch, J. M. Kornhauser, A. J. Shaywitz, M. A. Takasu, X. Tao, M. E. Greenberg, Calcium regulation of neuronal gene expression. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 11024-11031 (2001).
2. A. E. West, E. C. Griffith, M. E. Greenberg, Regulation of transcription factors by neuronal activity. *Nat. Rev. Neurosci.* **3**, 921-931 (2002).
3. A. Lanahan, P. Worley, Immediate-early genes and synaptic function. *Neurobiol. Learn. Mem.* **70**, 37-43 (1998).
4. S. Feske, J. Giltman, R. Dolmetsch, L. M. Staudt, A. Rao, Gene regulation mediated by calcium signals in T lymphocytes. *Nat. Immunol.* **2**, 316-324 (2001).
5. M. D. Bootman, P. Lipp, M. J. Berridge, The organisation and functions of local  $\text{Ca}^{2+}$  signals. *J. Cell Sci.* **114**, 2213-2222 (2001).
6. M. J. Berridge, P. Lipp, M. D. Bootman, The versatility and universality of calcium signalling. *Nat. Rev. Mol. Cell Biol.* **1**, 11-21 (2000).
7. W. G. Regehr, D. W. Tank, Postsynaptic NMDA receptor-mediated calcium accumulation in hippocampal CA1 pyramidal cell dendrites. *Nature* **345**, 807-810 (1990).
8. R. Yuste, A. Majewska, K. Holthoff, From form to function: Calcium compartmentalization in dendritic spines. *Nat. Neurosci.* **3**, 653-659 (2000).
9. B. L. Sabatini, T. G. Oertner, K. Svoboda, The life cycle of  $\text{Ca}^{2+}$  ions in dendritic spines. *Neuron* **33**, 439-452 (2002).
10. S. E. Jarvis, G. W. Zamponi, Interactions between presynaptic  $\text{Ca}^{2+}$  channels, cytoplasmic messengers and proteins of the synaptic vesicle release complex. *Trends Pharmacol. Sci.* **22**, 519-525 (2001).
11. W. A. Catterall, Structure and function of neuronal  $\text{Ca}^{2+}$  channels and their role in neurotransmitter release. *Cell Calcium* **24**, 307-323 (1998).
12. J. Schiller, Y. Schiller, G. Stuart, B. Sakmann, Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *J. Physiol.* **505** (part 3), 605-616 (1997).
13. R. Yuste, W. Denk, Dendritic spines as basic functional units of neuronal integration. *Nature* **375**, 682-684 (1995).
14. F. Helmchen, K. Imoto, B. Sakmann,  $\text{Ca}^{2+}$  buffering and action potential-evoked  $\text{Ca}^{2+}$  signaling in dendrites of pyramidal neurons. *Biophys. J.* **70**, 1069-1081 (1996).
15. B. E. Lonze, D. D. Ginty, Function and regulation of CREB family transcription factors in the nervous system. *Neuron* **35**, 605-623 (2002).
16. A. J. Shaywitz, M. E. Greenberg, CREB: A stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu. Rev. Biochem.* **68**, 821-861 (1999).
17. G. E. Hardingham, S. Chawla, C. M. Johnson, H. Bading, Distinct functions of nuclear and cytoplasmic calcium in the control of gene expression. *Nature* **385**, 260-265 (1997).
18. M. Dragunow, W. C. Abraham, M. Goulding, S. E. Mason, H. A. Robertson, R. L. Faull, Long-term potentiation and the induction of *c-fos* mRNA and proteins in the dentate gyrus of unanesthetized rats. *Neurosci. Lett.* **101**, 274-280 (1989).
19. P. F. Worley, R. V. Bhat, J. M. Baraban, C. A. Erickson, B. L. McNaughton, C. A. Barnes, Thresholds for synaptic activation of transcription factors in hippocampus: Correlation with long-term enhancement. *J. Neurosci.* **13**, 4776-4786 (1993).
20. K. Deisseroth, H. Bito, R. W. Tsien, Signaling from synapse to nucleus: postsynaptic CREB phosphorylation during multiple forms of hippocampal synaptic plasticity. *Neuron* **16**, 89-101 (1996).
21. G. E. Hardingham, F. J. Arnold, H. Bading, Nuclear calcium signaling controls CREB-mediated gene expression triggered by synaptic activity. *Nat. Neurosci.* **4**, 261-267 (2001).
22. R. Treisman, Ternary complex factors: Growth factor regulated transcriptional activators. *Curr. Opin. Genet. Dev.* **4**, 96-101 (1994).
23. H. Enslin, H. Tokumitsu, P. J. Stork, R. J. Davis, T. R. Soderling, Regulation of mitogen-activated protein kinases by a calcium/calmodulin-dependent protein kinase cascade. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10803-10808 (1996).
24. T. Pusch, J. J. Wu, T. L. Zimmerman, L. Zhang, B. E. Ehrlich, M. W. Berchtold, J. B. Hoek, S. J. Karpen, M. H. Nathanson, A. M. Bennett, Epidermal growth factor-mediated activation of the ETS domain transcription factor Elk-1 requires nuclear calcium. *J. Biol. Chem.* **277**, 27517-27527 (2002).
25. Z. Xia, H. Dudek, C. K. Miranti, M. E. Greenberg, Calcium influx via the NMDA receptor induces immediate early gene transcription by a MAP kinase/ERK-dependent mechanism. *J. Neurosci.* **16**, 5425-5446 (1996).
26. H. Okamura, A. Rao, Transcriptional regulation in lymphocytes. *Curr. Opin. Cell Biol.* **13**, 239-243 (2001).
27. G. R. Crabtree, Calcium, calcineurin, and the control of transcription. *J. Biol. Chem.* **276**, 2313-2316 (2001).
28. N. A. Clipstone, G. R. Crabtree, Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. *Nature* **357**, 695-697 (1992).
29. F. Shibasaki, E. R. Price, D. Milian, F. McKeon, Role of kinases and the phosphatase calcineurin in the nuclear shuttling of transcription factor NF-AT4. *Nature* **382**, 370-373 (1996).
30. L. A. Timmerman, N. A. Clipstone, S. N. Ho, J. P. Northrop, G. R. Crabtree, Rapid shuttling of NF-AT in discrimination of  $\text{Ca}^{2+}$  signals and immunosuppression. *Nature* **383**, 837-840 (1996).
31. R. E. Dolmetsch, R. S. Lewis, C. C. Goodnow, J. I. Healy, Differential activation of transcription factors induced by  $\text{Ca}^{2+}$  response amplitude and duration. *Nature* **386**, 855-858 (1997).
32. I. Stancovski, D. Baltimore, NF- $\kappa$ B activation: The I  $\kappa$ B kinase revealed? *Cell* **91**, 299-302 (1997).
33. R. E. Dolmetsch, K. Xu, R. S. Lewis, Calcium oscillations increase the efficiency and specificity of gene expression. *Nature* **392**, 933-936 (1998).
34. A. M. Carrion, W. A. Link, F. Ledo, B. Mellstrom, J. R. Naranjo, DREAM is a  $\text{Ca}^{2+}$ -regulated transcriptional repressor. *Nature* **398**, 80-84 (1999).
35. F. Ledo, L. Kremer, B. Mellstrom, J. R. Naranjo,  $\text{Ca}^{2+}$ -dependent block of CREB-CBP transcription by repressor DREAM. *EMBO J.* **21**, 4583-4592 (2002).
36. O. Bachs, N. Agell, E. Carafoli, Calmodulin and calmodulin-binding proteins in the nucleus. *Cell Calcium* **16**, 289-296 (1994).
37. K. Deisseroth, E. K. Heist, R. W. Tsien, Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons. *Nature* **392**, 198-202 (1998).
38. P. G. Mermelstein, K. Deisseroth, N. Dasgupta, A. L. Isaksen, R. W. Tsien, Calmodulin priming: Nuclear translocation of a calmodulin complex and the memory of prior neuronal activity. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 15342-15347 (2001).
39. S. S. Hook, A. R. Means,  $\text{Ca}^{2+}$ /CaM-dependent kinases: from activation to function. *Annu. Rev. Pharmacol. Toxicol.* **41**, 471-505 (2001).
40. H. Enslin, P. Sun, D. Brickey, S. H. Soderling, E. Klamo, T. R. Soderling, Characterization of  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase IV. Role in transcriptional regulation. *J. Biol. Chem.* **269**, 15520-15527 (1994).
41. H. Bito, K. Deisseroth, R. W. Tsien, CREB phosphorylation and dephosphorylation: a  $\text{Ca}^{2+}$ - and stimulus duration-dependent switch for hippocampal gene expression. *Cell* **87**, 1203-1214 (1996).
42. J. Lu, T. A. McKinsey, C. L. Zhang, E. N. Olson, Regulation of skeletal

- myogenesis by association of the MEF2 transcription factor with class II histone deacetylases. *Mol. Cell* **6**, 233-244 (2000).
43. T. A. McKinsey, C. L. Zhang, J. Lu, E. N. Olson, Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature* **408**, 106-111 (2000).
  44. C. L. Zhang, T. A. McKinsey, S. Chang, C. L. Antos, J. A. Hill, E. N. Olson, Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. *Cell* **110**, 479-488 (2002).
  45. H. D. Youn, T. A. Chatila, J. O. Liu, Integration of calcineurin and MEF2 signals by the coactivator p300 during T-cell apoptosis. *EMBO J.* **19**, 4323-4331 (2000).
  46. H. Wu, F. J. Naya, T. A. McKinsey, B. Mercer, J. M. Shelton, E. R. Chin, A. R. Simard, R. N. Michel, R. Bassel-Duby, E. N. Olson, R. S. Williams, MEF2 responds to multiple calcium-regulated signals in the control of skeletal muscle fiber type. *EMBO J.* **19**, 1963-1973 (2000).
  47. F. Blaeser, N. Ho, R. Prywes, T. A. Chatila, Ca<sup>2+</sup>-dependent gene expression mediated by MEF2 transcription factors. *J. Biol. Chem.* **275**, 197-209 (2000).
  48. H. D. Youn, J. O. Liu, Cabin1 represses MEF2-dependent Nur77 expression and T cell apoptosis by controlling association of histone deacetylases and acetylases with MEF2. *Immunity* **13**, 85-94 (2000).
  49. H. D. Youn, L. Sun, R. Prywes, J. O. Liu, Apoptosis of T cells mediated by Ca<sup>2+</sup>-induced release of the transcription factor MEF2. *Science* **286**, 790-793 (1999).
  50. R. H. Goodman, S. Smolik, CBP/p300 in cell growth, transformation, and development. *Genes Dev.* **14**, 1553-1577 (2000).
  51. A. J. Bannister, T. Kouzarides, The CBP co-activator is a histone acetyltransferase. *Nature* **384**, 641-643 (1996).
  52. S. C. Hu, J. Chrivia, A. Ghosh, Regulation of CBP-mediated transcription by neuronal calcium signaling. *Neuron* **22**, 799-808 (1999).
  53. S. Chawla, G. E. Hardingham, D. R. Quinn, H. Bading, CBP: A signal-regulated transcriptional coactivator controlled by nuclear calcium and CaM kinase IV. *Science* **281**, 1505-1509 (1998).
  54. S. Impey, A. L. Fong, Y. Wang, J. R. Cardinaux, D. M. Fass, K. Obrietan, G. A. Wayman, D. R. Storm, T. R. Soderling, R. H. Goodman, Phosphorylation of CBP mediates transcriptional activation by neural activity and CaM kinase IV. *Neuron* **34**, 235-244 (2002).
  55. M. Naraghi, E. Neher, Linearized buffered Ca<sup>2+</sup> diffusion in microdomains and its implications for calculation of [Ca<sup>2+</sup>] at the mouth of a calcium channel. *J. Neurosci.* **17**, 6961-6973 (1997).
  56. T. H. Murphy, P. F. Worley, J. M. Baraban, L-type voltage-sensitive calcium channels mediate synaptic activation of immediate early genes. *Neuron* **7**, 625-635 (1991).
  57. M. Sheng, G. McFadden, M. E. Greenberg, Membrane depolarization and calcium induce c-fos transcription via phosphorylation of transcription factor CREB. *Neuron* **4**, 571-582 (1990).
  58. M. Sheng, M. A. Thompson, M. E. Greenberg, CREB: a Ca<sup>2+</sup>-regulated transcription factor phosphorylated by calmodulin-dependent kinases. *Science* **252**, 1427-1430 (1991).
  59. H. Bading, D. D. Ginty, M. E. Greenberg, Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. *Science* **260**, 181-186 (1993).
  60. S. M. Dudek, R. D. Fields, Mitogen-activated protein kinase/extracellular signal-regulated kinase activation in somatodendritic compartments: Roles of action potentials, frequency, and mode of calcium entry. *J. Neurosci.* **21**, RC122 (2001).
  61. R. E. Dolmetsch, U. Pajvani, K. Fife, J. M. Spotts, M. E. Greenberg, Signaling to the nucleus by an L-type calcium channel-calmodulin complex through the MAP kinase pathway. *Science* **294**, 333-339 (2001).
  62. L. B. Rosen, D. D. Ginty, M. J. Weber, M. E. Greenberg, Membrane depolarization and calcium influx stimulate MEK and MAP kinase via activation of Ras. *Neuron* **12**, 1207-1221 (1994).
  63. J. Xing, D. D. Ginty, M. E. Greenberg, Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. *Science* **273**, 959-963 (1996).
  64. G. Y. Wu, K. Deisseroth, R. W. Tsien, Activity-dependent CREB phosphorylation: Convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 2808-2813 (2001).
  65. K. G. Sutton, J. E. McRory, H. Guthrie, T. H. Murphy, T. P. Snutch, P/Q-type calcium channels mediate the activity-dependent feedback of syntaxin-1A. *Nature* **401**, 800-804 (1999).
  66. M. A. Takasu, M. B. Dalva, R. E. Zigmond, M. E. Greenberg, Modulation of NMDA receptor-dependent calcium influx and gene expression through EphB receptors. *Science* **295**, 491-495 (2002).
  67. M. B. Dalva, M. A. Takasu, M. Z. Lin, S. M. Shamah, L. Hu, N. W. Gale, M. E. Greenberg, EphB receptors interact with NMDA receptors and regulate excitatory synapse formation. *Cell* **103**, 945-956 (2000).
  68. G. E. Hardingham, Y. Fukunaga, H. Bading, Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat. Neurosci.* **5**, 405-414 (2002).

**Citation:** R. Dolmetsch, Excitation-transcription coupling: signaling by ion channels to the nucleus. *Sci. STKE* **2003**, pe4 (2003).

---

The following resources related to this article are available online at <http://stke.sciencemag.org>.  
This information is current as of January 1, 2017.

---

- Article Tools** Visit the online version of this article to access the personalization and article tools:  
<http://stke.sciencemag.org/content/2003/166/pe4>
- Related Content** The editors suggest related resources on *Science's* sites:  
<http://stke.sciencemag.org/content/sigtrans/5/211/ec50.abstract>  
<http://stke.sciencemag.org/content/sigtrans/2001/82/pe1.full>  
<http://stke.sciencemag.org/content/sigtrans/2006/360/tw376.abstract>  
<http://stke.sciencemag.org/content/sigtrans/2/60/ec83.abstract>  
<http://stke.sciencemag.org/content/sigtrans/5/237/pe34.full>  
<http://stke.sciencemag.org/content/sigtrans/3/109/ec50.abstract>  
<http://stke.sciencemag.org/content/sigtrans/7/348/ec293.abstract>
- References** This article cites 68 articles, 24 of which you can access for free at:  
<http://stke.sciencemag.org/content/2003/166/pe4#BIBL>
- Permissions** Obtain information about reproducing this article:  
<http://www.sciencemag.org/about/permissions.dtl>