Regulation of dendritic development by calcium signaling

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Abstract

Neuronal activity can have profound effects on dendrite morphology in the developing brain. The effects of neuronal activity on dendritic morphology are mediated by calcium signaling. While many effects of calcium on dendrite structure occur locally at the site of calcium entry into the cytoplasmic milieu, elevation of cytoplasmic calcium is also translated into changes in gene transcription. Decoding the calcium signal into specific changes in gene transcription involve coordinating the action of a number of kinases, phosphatases, transcription factors and transcriptional coactivators. This review focuses on the contribution of calcium-dependent transcription on the control of dendritic morphology.

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1. Introduction

An extensive literature supports a role for activity in controlling dendrite morphology (reviewed in [1]). For instance, the time of maximal dendrite growth and remodeling is concurrent with that of afferent innervation. In addition, during normal development of the somatosensory and visual systems dendrites dramatically reorganize in response to afferent input. Activity deprivation experiments also suggest that loss of normal activity during development leads to lasting deficits in dendritic development. For example, pharmacological blockade of synaptic activity in vitro and in vivo leads to deficits in neuronal dendritic growth [2,3]. Changes in synaptic input, as with monocular deprivation in mammals, alter dendritic development in both the lateral geniculate nucleus and the visual cortex [1]. In contrast, increasing activity by exposing animals to enriched environments enhanced dendritic branching and spine density of cortical pyramidal neurons and accelerated visual system development [4]. While dendrite development, the signaling mechanisms that link activity to morphology are not well understood.

As a first step in understanding how neuronal activity contributes to dendrite development and complexity, it is evident that calcium plays a key role. Afferent signaling predominately results in elevation of intracellular calcium levels in the postsynaptic neuron via influx of extracellular calcium or release from intracellular stores. This afferent activity-induced elevation in intracellular calcium leads to changes in dendrite morphology through two mechanisms. There is an immediate, local response mediated by calcium sensitive signaling proteins that results in alterations of the dendritic cytoskeleton. This fast local response to elevated calcium does not necessarily involve more than a portion of a dendrite. Local calcium signaling events have been shown to effect multiple aspects of dendrite elaboration including dendrite spine dynamics and formation, initiation of filopodia, and dendrite branch stability [1,5,6]. Local calcium signaling during dendrite development may also mediate local translational events in response to activity as reported in cultured hippocampal neurons [7].

In addition to the fast local signaling effects of calcium on dendrite structure, neuronal activity initiates a delayed, prolonged response on dendrite development. For example, a brief exposure to light that increased sensory neuron activ-
ity in the frog is sufficient to initiate an increase in the rate of dendrite growth that is sustained even in the absence of additional sensory activity [8]. Furthermore, in vitro studies have demonstrated that calcium influx can activate a program of signaling that propagates to the neuronal nucleus and regulates gene transcription. These nuclear events are crucial as inhibiting them prevents calcium-induced dendrite growth [3].

Although calcium signaling alters dendrite structure via action at two distinct cellular locales, the relative contribution of local and nuclear action of calcium signaling to dendrite morphology remains unresolved. The two mechanisms are not mutually exclusive. Nuclear action relies on signaling events initiated in the periphery. In fact, several of these calcium sensors, as well as calcium itself, have been implicated in mediating both local and nuclear actions of calcium signaling.

2. Molecular mechanisms of local and nuclear calcium signaling

Calcium signal propagation to the nucleus requires calcium influx primarily through \textit{N}-methyl-	extit{D}-aspartate (NMDA) type glutamate receptors and the \textit{L}-subtype of voltage sensitive calcium channels (VSCC) (Fig. 1). Two intracellular signaling pathways are preferentially activated in response to calcium influx, the calcium/calmodulin-dependent protein kinase (CaMK) and the Ras/mitogen-activated protein kinase (Ras/MAPK) pathway (reviewed in [9]). Activation of calmodulin is the first step in the subsequent activation of CaMK signaling. Calmodulin is bound directly to the VSCC or in a submembranous pool associated with VSCC and NMDA receptors. Upon entry via VSCC or NMDA receptors, calcium is bound by calmodulin. In this calcium bound form, calmodulin binds CaMKs leading to the ensuing phosphorylation and activation of the CaMK.

Several CaMKs have been identified and implicated in mediating both the local and nuclear effects of calcium signaling. Pharmacological perturbation of CaMKs inhibited calcium-induced dendrite growth indicating a role for this family in mediating activity initiated changes in dendrite morphology. Of the CaMKs, CaMKII has been most extensively studied in relation to a role in dendrite development and function. Two isoforms of CaMKII, CaMKII\(a\) and CaMKII\(b\), mediate contrasting outcomes on dendrites. CaMKII\(a\) has been reported to stabilize or restrict dendritic growth of frog tectal neurons in vivo and mammalian cortical neurons in vitro [2,3]. CaMKII\(b\), however, has a positive effect on filopodia extension and fine dendrite development mediated by direct inter-
growth and elaboration are maximal [3,12]. For example, early stages of dendrite development and peak when dendrite elaboration, are expressed in the developing brain during the week. CaMKII, which have a positive effect on dendrite growth. Interestingly, CaMKII is known to induce dendrite growth. Attenuating CaMKII activity with cytoskeletal actin [10]. CaMKI has also recently been shown to be important for neurite growth in cerebellar granule cells and hippocampal neurons [11]. CaMKIs that are localized in the cytoplasm and dendrites, such as CaMKI and CaMKII, likely alter dendrites via phosphorylation of nearby targets including synapsin and MAP2. However, both CaMKI and CaMKII can activate gene transcription in vitro. CaMKIV is also involved in mediating activity dependent dendritic development. Attenuating CaMKIV signaling reduces calcium-induced dendritic growth, and expression of an activated form of CaMKIV in cortical neurons mimics the dendrite growth induced by calcium influx [3]. Furthermore, expression of wild type CaMKIV dramatically potentiates the calcium-induced response, suggesting that dendrite growth may be regulated, in part, by levels of endogenous CaMKIV expression. Unlike CaMKI, CaMKIα, and CaMKII, CaMKIV is predominately localized in the nucleus. This nuclear localization of CaMKIV implies that it mediates an effect on dendrite growth via transcriptional events. In fact, interfering with the activity of transcriptional regulators that are known targets of CaMKIV blocks the ability of active CaMKIV to induce dendrite growth. Interestingly, CaMKIIα and CaMKIV, which have a positive effect on dendrite elaboration, are expressed in the developing brain during the early stages of dendrite development and peak when dendrite growth and elaboration are maximal [3,12]. For example, CaMKIV protein is expressed in the embryonic rodent cortex and reaches its highest expression in the second postnatal week. CaMKIα expression, however, peaks later in cortical development, as dendrite elaboration is completed.

CaMKI, a protein kinase (Ras/MAPK) signaling is also activated by calcium influx via NMDA and VSCCs. Ras/MAPKs activated at the site of calcium entry mediate local effects on dendrites including filopodial formation and stability [6]. In addition, Ras/MAPK signaling has been implicated in mediating growth of SCG dendrites in response to phosphorylating MAP2 [13]. In addition to these local actions, Ras/MAPK signaling has been implicated in mediating the nuclear action of calcium signaling on dendrite growth [3].

3. Calcium regulation of CREB–CBP mediated transcription in dendrite development

Both CaMK and Ras/MAPK signaling can regulate gene transcription via phosphorylation of cAMP response element binding protein (CREB). CREB is a transcription factor that is constitutively bound to DNA and when phosphorylated at serine 133, binds CBP. The ability of CaMKIV to induce dendrite growth is blocked by inhibition of CREB and the transcriptional coactivator, CREB binding protein (CBP). The mechanism by which CREB and CBP-mediated transcription regulates dendritic growth in response to neuronal activity is not well understood. However, the involvement of CREB and CBP is certain, as disruption of either correspondingly disrupts dendrite growth and activity-induced gene transcription (Fig. 2) [3,14].

How do these calcium activated signaling events control transcriptional activation? One clue was obtained by examining the “route” of calcium entry. Calcium influx via the L-subtype of VSCC versus ligand gated ion channels, contributes to the differential response of CREB–CBP mediated transcription [14,15]. Another intriguing clue lies in the kinetics of CREB activation and thus presumably gene transcription. The CaMK pathway contributes to a fast but transient phase of CREB phosphorylation while the Ras/MAPK pathway to a slightly slower but prolonged phosphorylation state [16]. In addition, the degree to which CaMK signaling is activated can also determine activation of a CREB phosphatase and signal termination [17].

CREB phosphorylation at serine 133 alone, although sufficient for binding CBP, is not sufficient in all instances to activate gene transcription [17]. This suggests that other critical regulatory events, such as additional modifications of CREB or CBP, or involvement of other regulatory proteins is needed. In fact, CREB is phosphorylated at three other sites, Ser129, Ser142, and Ser143 (reviewed in [9,18]). While Ser129 is phosphorylated as a result of cAMP signaling, phosphorylation at Ser142 and Ser143 is regulated by activity and calcium influx. The combination of phosphorylation at Ser133, Ser142 and Ser143 effectively activates CREB-mediated transcription but attenuates CREB–CBP interaction. The role of these additional phosphorylation events in CREB-dependent dendritic growth has not yet been explored.

In addition to phosphorylation events on CREB, CBP is also a direct target of activity-induced signaling. Calcium influx activates CBP-mediated transcription, and CaMKIV phosphorylates CBP, suggesting that some of the effects of CaMKIV might be mediated by CBP [14,19]. It should be noted, however, that CBP can still activate gene transcription when the CaMKIV phosphorylation site is mutated, indicating other potential mechanisms of activation [18,19].

Aizawa et al. [20] recently carried out a screen specifically designed to identify calcium-dependent transcription activators. They identified several genes that mediate activity-dependent transcription in neurons, including a novel gene called calcium-responsive transactivator (CREST), which is constitutively localized in the nucleus. Although CREST lacks an identifiable DNA binding domain, it directly binds CBP and p300. In nonneuronal cells, however, cAMP signaling does not regulate association of CREST with CBP and p300. Whether CREST–CBP binding in neurons is also constitutive remains unknown.

Although the mechanism of calcium-induced regulation remains unanswered at this time, it is clear that CREST activates transcription in response to calcium influx. Calcium responsiveness may be mediated directly by phosphorylation or via an interaction with CBP. Also unanswered is whether constitutive or activity-induced CREB–CBP mediated gene transcription is altered in CREST null neurons. Currently, CREST has only been shown to potentially participate in
gene transcription mediated by CBP and p300. Is activity-induced CREST signaling specific to an interaction with CBP and p300 or is CREST involved in other transcriptional complexes? If CREST does associate with other transcriptional complexes, would CREST lend calcium responsiveness to these complexes? Revealing the mechanisms of CREST activation and signaling will be important for understanding how it lends specificity to the genes transcribed.

4. Role of CREST in dendrite development

The first indication that CREST may contribute to dendrite development came from protein and mRNA expression studies. CREST protein expression in the developing cortex precedes and remains elevated during the period of dendrite growth [20]. CREST mRNA is also expressed in multiple nondividing neuronal populations in postnatal development during this period. This temporal expression of CREST positions it to regulate transcriptional programs of dendrite growth, elaboration, and remodeling in response to calcium signaling in multiple neuronal populations. CREST expression also remains elevated in areas known to undergo dendrite plasticity and remodeling in the adult, such as the cerebellum, hippocampus and olfactory bulb.

Evidence for CREST playing a role in dendrite growth and development comes from in vitro and in vivo studies of CREST null mice. Neurons cultured from CREST null mice are capable of dendrite development indistinguishable from that of wild type neurons under constitutive culture conditions. However, CREST null neurons fail to elaborate dendrites in response to calcium influx via VSCC or NMDA receptors. This deficient response to calcium signaling can be rescued by expression of CREST in the null neurons. Whether CREST mediated elaboration of dendrites in response to calcium is dependent on CREB or CBP remains to be determined.

Dendrites of both hippocampal and cortical neurons develop abnormally in CREST knockout mice. Both basal and apical dendrites of hippocampal neurons are less elaborated in CREST null mice compared to controls. Interestingly a deficit in basal but not apical dendrites is seen in layer 5 cortical pyramidal neurons of CREST null mice, suggesting that separate transcriptional programs regulate basal and apical dendrite development in layer 5 pyramidal neurons.

5. Other calcium sensitive nuclear mechanisms in dendrite development

Identification of CREST may just be the first glimpse toward understanding the transcriptional mechanisms mediating activity dependent dendrite development. Aizawa et al. [20] identified several gene pools that induced transcriptional activation similar in magnitude to that of the CREB control. In addition to CREST, two other genes identified in this screen displayed a greater transcriptional response to calcium than did CREB: LMO4 and NeuroD2. Like CREST, LMO4 does not contain a DNA binding region but acts as a transcriptional regulator through interactions with transcriptional co-factors. LMO4 is a LIM only (LMO) family member of the LIM homeodomain containing transcription factors. Previous studies on LMO4 suggest that it plays a role in negatively regulating neurite extension [21]. The other gene identified, NeuroD2 is a member of the large bHLH family of transcription factors. Unlike CREST and LMO4, NeuroD2 regulates transcription through direct
interaction with DNA. NeuroD2 is expressed in the early developing nervous system where it has been shown to be important for neuronal differentiation. However, NeuroD2 expression persists in the adult hippocampus and cerebellum, sites of adult plasticity [22]. Recently, a NeuroD2 family member, NeuroD was shown to play a role in dendrite development of cerebellar granule neurons [23]. In response to activity CaMKII phosphorylates NeuroD at a site homologous to sequences found in CREB and NeuroD2. This hints that the NeuroD proteins may function, as CREB, to identify specific genes whose transcription is regulated by activity. Whether NeuroD2 and LMO4, like CREST, function in activity dependent dendrite development awaits further study.

CREB and CBP have been the focus of attention as transcriptional mediators of calcium signaling. However, other calcium-dependent transcriptional regulators, such as downstream regulatory element antagonist modulator (DREAM) and nuclear factor of activated T cells (NFAT) may also contribute to the control of dendritic development [9]. NFAT is a transcriptional activator whose nuclear translocation is regulated by calcium activation of calmodulin signaling. While involvement of calmodulin implies involvement of CREB signaling, NFAT regulates transcription via interactions with other transcription factors and independent of an interaction with CREB. NFAT signaling has been implicated in axonal outgrowth [24] and may also influence dendritic growth.

Unlike previously discussed calcium signaling mechanisms, in the absence of calcium DREAM represses transcription. DREAM is also unusual in that it contains both DNA and EF-hand calcium binding domains. Nuclear calcium directly binds DREAM to relieve repression of DREAM targets. In the absence of calcium, DREAM can also interfere with CREB–CBP interaction to block transcription. Thus activation of DREAM and the CREB–CBP complex may be coordinately regulated. Exploring the roles of factors such as NFAT and DREAM may reveal additional mechanisms of dendritic growth control.

6. Conclusion

A wide range of putative molecular effectors of neuronal activity have been identified including calcium, protein kinases, transcriptional regulators, and proteins involved in regulating the structure and function of synapses (reviewed in [25]). Although transcription is clearly involved, the identities of genes transcribed in response to stimulation that influence dendrite morphology directly largely await identification. However, cytoplasmic and secreted proteins whose expression increases after activity have been identified and implicated in modulating dendrite morphology. These genes include candidate plasticity genes 15 (cpg15), Arc, Homer, and brain-derived neurotrophic factor (BDNF) [25]. In view of the proposed role of transcription in translating activity into morphological changes, it is gratifying that the upstream promoter regions of the cpg15 and BDNF genes contain CREB consensus binding sites. Furthermore, expression is regulated by CREB–CBP mediated signaling [9,26,27]. Whether CREST and other nuclear calcium effectors also contribute to the activity-induced expression of these genes awaits further study.

In development and in the adult nervous system, neuronal activity has profound effects on dendrite morphology and neuronal connectivity. Translation of information relayed by activity into changes in dendrite morphology involves multiple signaling mechanisms as described previously and depicted in Fig. 1. Decoding how these signaling events are coordinated to specify transcription of specific genes and how the proteins encoded by these genes exert effects on dendrite morphology are mysteries yet to be revealed.

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