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REVIEW

# Transcriptional regulators that differentially control dendrite and axon development

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**Abstract** **Neurons are the basic units of connectivity in the nervous system. As a signature feature, neurons form polarized structures: dendrites and axons, which integrate either sensory stimuli or inputs from upstream neurons and send outputs to target cells, respectively. The separation of dendritic and axonal compartments is achieved in two steps during development: 1) dendrite and axon specification: how neurites are initially specified as dendrites and axons; and 2) dendrite and axon commitment: how dendrites and axons are committed to distinct compartmental fates and architectures. To understand neural circuit assembly and to correct erroneous dendrite or axon growth in a compartment-specific manner, it is essential to understand the regulatory mechanisms underlying dendrite and axon commitment. Compared to extensive studies on dendrite and axon specification, little is known about the molecular mechanisms exclusively dedicated to dendrite or axon commitment. Recent studies have uncovered the requirement of transcriptional regulation in this process. Here, we review the studies on transcriptional regulators: Dar1, p300-SnoN, NeuroD, which have been shown to separate dendrite- and axon-specific growth of the same neuron type after compartmental fates are specified.**

**Keywords** dendrite and axon commitment, Dar1, p300-SnoN, NeuroD

## Introduction

Neuron polarization — the establishment of two morphologically and functionally distinct compartments of dendrites and axons — is a fundamental issue in the assembly of neural circuits. Temporally, neuron polarization requires two key steps. First, one of several neurites initiates a fast growth to become the axon, while the remaining neurites are later specified into dendrites. Second, the nascent dendrites and axon develop distinct architectures with specialized molecular features that commit them to their final dendritic and axonal identities.

The specification of dendrites and axons occurs immediately after neurons exit mitotic division *in vivo* (Pollarolo et al., 2011) or soon after neurons are plated in culture *in vitro* (Dotti et al., 1988). Extensive studies have been aimed at dissecting the roles of molecular signaling or cellular organelles in establishing dendrite and axon identities (Arimura and Kaibuchi, 2005; Barnes and Polleux, 2009; Tahirovic and Bradke, 2009). Signaling molecules essential for initial specification include kinases, such as PI-3K (Shi et al., 2003; Ménager et al., 2004), Akt (Yan et al., 2006), LKB1 (Barnes et al., 2007; Shelly et al., 2007), cyclic nucleotides (Shelly et al., 2010), and cadherin (Pollarolo et al., 2011; Gärtner et al., 2012). Moreover, motor proteins, including kinesins (Nishimura et al., 2004; Jacobson et al., 2006), dynein (Zheng et al., 2008) and myosins (Kollins et al., 2009) also contribute to specification of dendrites and axons.

In contrast to the initial specification step that occurs within a brief time window, the commitment step of dendrite and axon persists through a much wider time frame and is subject to a number of regulations, including axon guidance and synaptogenesis (Goldberg et al., 2002). For this reason, an important criterion for determining whether a particular molecular mechanism conveys dendrite- or axon-specificity is that the analysis must be carried out in the same types of neurons at the same developmental time. By this criterion,

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most studies of the molecular mechanisms underlying dendrite and axon development need to be revisited in order to conclude whether those mechanisms regulate dendrite and axon development differently.

Indeed, despite the large number of studies on dendrite or axon development, it has been rare that these two neuronal compartments are compared in the same study. For instance, studies in *Drosophila* da neurons, a major genetic system for studying dendrite development, have identified several transcription factors, such as Abrupt (Li et al., 2004; Sugimura et al., 2004), Cut (Grueber et al., 2003) and Knot (Hattori et al., 2007; Jinushi-Nakao et al., 2007; Crozatier and Vincent, 2008), to be important regulators of dendritic complexity. However, whether or not these transcription factors regulate axon development in the same neurons remains to be determined. Similarly, in mammals, CREST, a SYT-related transcription factor, promotes dendrite growth of cortical pyramidal neurons *in vivo* and mediates neuronal activity induced dendrite growth in cortical neuron culture (Aizawa et al., 2004). It will be interesting to determine whether transcription factors, such as CREST, also regulate axon growth.

Several studies have focused on dendrite-axon commitment step. From a genetic screen in *Drosophila*, Ye et al. (2007) identified several genes that are preferentially required for the growth of dendrites but not axons. These genes have been termed *dendritic arbor reduction* (*dar*) genes. The *dar2*, *dar3* and *dar6* genes are regulators of ER-to-Golgi transport. Further studies in both *Drosophila* and mammalian neurons suggest that dendrite and axon growth display distinct dependence on the secretory trafficking, which is possibly due to differences in the demand for membrane supplies or in the efficiency of membrane trafficking between growing dendrites and axons (Wang et al., 2011). The characterization of the *dar1* mutants from the same genetic screen led to the finding that a transcriptional program that involves the transcription factor Dar1 is dedicated to dendrite but not axon development.

Here we summarize the transcriptional regulators that have been shown to differentially regulate dendrite and axon growth in the same types of neurons, either independently of neural activity or in response to neural activity.

## A transcription factor dedicated to dendrite growth: *Dar1*

The *dendritic arbor reduction 1* (*dar1*) gene was isolated from a genetic screen that sought to dendrite- and axon-specific regulators in the class IV dendritic arborization (da) sensory neurons in *Drosophila*. *Dar1* encodes a novel Krüppel-like factor (KLF). The C-terminal region of Dar1, which contains three zinc fingers, shares similarity with those of mammalian KLF5, KLF7, and KLF9, which are known regulators of neurite growth and regeneration (Pearson et al., 2008; Moore et al., 2011). Using advanced genetic approaches that allow analysis at single cell resolution *in vivo*, Ye et al. (2011) showed that *dar1* mutant neurons exhibit dramatic reduction in dendrite growth in all types of da neurons. Overexpressing *dar1* leads to dendrite overgrowth — specifically, the growth of microtubule-based dendritic branches. In sharp contrast, axon growth is unaffected by either *dar1* mutations or overexpression, supporting the notion that *dar1* is dispensable for axon growth.

Further studies revealed that the transcription of the microtubule severing protein, Spastin, is upregulated in *dar1* mutant neurons. Consistently, overexpressing Spastin significantly decreases total dendritic length, which phenocopies *dar1* loss of function.

Taken together, Dar1 directs a transcriptional program, likely via changing expression levels of microtubule regulators, to promote dendrite growth. This study also raises several intriguing questions: 1) Does Dar1 interact with other transcription factors, i.e. another KLF, to orchestrate dendritic arborization? 2) Does Dar1 control transcription of additional target genes for dendrite-specific development? 3) Does Dar1 regulate dendrite growth in response to external cues, such as neural activity? Addressing these questions will elucidate how intrinsic molecules and extrinsic signals work in concert to control dendrite development, thus establishing a framework for understanding the regulatory network of dendrite-specific development.

## A transcriptional complex dedicated to axon growth: *p300-SnoN*

p300, a histone acetyltransferase and transcriptional activator, specifically controls axon, but not dendrite, growth in cultured granule neurons from rat cerebellar cortex (Ikeuchi et al., 2009). p300 binds to and forms a transcription-activating complex with another transcriptional regulator, SnoN (Ikeuchi et al., 2009). SnoN, a substrate of the ubiquitin ligase Cdh1-APC complex, promotes axon growth in cultured granule neurons in vitro and granule neuron parallel fibers *in vivo* (Stegmüller et al., 2006). Consistently, the Cdh1-APC complex restrains axon growth in the same neurons (Konishi et al., 2004). Overexpressing SnoN with a mutated D-box (SnoN DBM), which leads to resistance to Cdh1-APC regulation, induces axon overgrowth (Stegmüller et al., 2006). Knock-down of p300 reduces axon growth and suppresses axon overgrowth caused by overexpressing SnoN DBM. In contrast, dendritic total length is unaffected by p300 knock-down (Ikeuchi et al., 2009). These results suggest p300 specifically controls axon growth by collaborating with SnoN.

What are the transcriptional targets of the transcriptional *p300-SnoN* complex involved in its axon-specific function? Through microarray analysis, Ikeuchi et al. (2009) found that the actin binding protein Ccd1 (Wang et al., 2006) is reduced

by knock-down of either SnoN or p300. Ccd1 is specifically localized to axon terminals. It promotes axon, but not dendrite, growth of cultured granule neurons *in vitro* and the growth of parallel fibers associated with granule neurons *in vivo*. Ccd1 is also required for the axon overgrowth phenotype observed in neurons overexpressing SnoN DBM. Thus, Ccd1 is an axon-specific cytoskeleton regulator that mediates SnoN-p300 regulation in axon growth.

In mitotic cells, SnoN can be regulated by TGFβ-Smad signaling. Upon ligand binding, TGFβ receptors are activated and in turn activate Smad2 and Smad3. Activated Smad2 and Smad3 function as scaffold proteins that trigger SnoN degradation via the Cdh1-APC complex (Bonni et al., 2001; Stroschein et al., 2001; Wan et al., 2001). These observations raised the questions of whether the p300-SnoN complex in post-mitotic neuron responds to TGFβ signaling. Stegmüller et al. (2008) found that TGFβ-Smad also regulates SnoN in post-mitotic cerebellar granule neurons and is important for SnoN-mediated axon growth. This study links neuronal intrinsic p300-SnoN transcriptional complex with the external cue TGFβ, demonstrating how intrinsic signaling and extrinsic molecules are integrated to direct axon growth.

Taken together, p300, by forming a transcriptional complex with SnoN, activates the transcription of the actin binding protein Ccd1 to specifically promote axon, but not dendrite, growth. The p300-SnoN complex is regulated by the Cdh1-APC ubiquitin complex and TGFβ-Smad signaling. Whether SnoN also functions in dendrite growth remains to be determined. If so, it is likely that SnoN collaborates with a different transcriptional partner that is dedicated for dendrite development.

## A transcription factor required for neural activity-dependent dendrite-specific growth: NeuroD

Neural activity plays important roles in neuronal morphogenesis (Whitford et al., 2002; Wong and Ghosh, 2002; Saneyoshi et al., 2010; West and Greenberg, 2011; Yuan et al., 2011). Calcium signaling is a key mediator of neural activity-dependent dendrite and axon development (Ghosh and Greenberg, 1995; Dickson, 2002; Konur and Ghosh, 2005). Among the transcription factors downstream of $Ca^{2+}$ signaling, NeuroD, specifically promotes dendrite growth.

NeuroD belongs to the family of basic helix-loop-helix bHLH proteins. It was initially shown to promote neuronal survival during neurogenesis (Miyata et al., 1999). Gaudillière et al. (2004) studied NeuroD's function after neuronal fate has been specified. In rat cerebellar granule neurons and in developing cerebellar cortex, knock-down of NeuroD results in severe loss of dendrite growth throughout developmental stages without changing axon growth or the identities of axons and dendrites.

NeuroD regulation of dendrite growth occurs through an activity-dependent mechanism. Increasing neural activity by applying membrane-depolarizing concentrations of KCl induces dendrite overgrowth in cultured granule neurons, an effect that is blocked by NeuroD knock-down. Moreover, treatment of high KCl increases NeuroD-mediated transcription as shown by *in vitro* transactivator trap assay. These results suggest neural activity regulates NeuroD's function and influences dendrite growth. Indeed, neural activity induces phosphorylation of NeuroD at Serine 336 by CaMKII, which is required for NeuroD's function in dendrite growth (Gaudillière et al., 2004). Furthermore, dendritic length is significantly reduced in NeuroD knockout mice, as compared to wild-type neurons (Gao et al., 2009).

In conclusion, NeuroD serves as a transcription factor, which is regulated by neural activity and calcium, to specifically promote dendrite growth in mammalian granule neurons.

## Concluding remarks

Although a large number of transcriptional regulators have been shown to be important in neuronal morphogenesis (Jan and Jan, 2003; Goldberg, 2004; Jan and Jan, 2010; de la Torre-Ubieta and Bonni, 2011; Moore and Goldberg, 2011), most of them affect dendrites and axons in the same direction or are only characterized exclusively in dendrites or axons, and thus do not meet the necessary criterion for confirming their role in establishing dendrite or axon specificity. Thus far, three dendrite- and axon-specific transcriptional regulators: Dar1, p300-SnoN and NeuroD have been examined by comparing dendrite and axon development. Further characterization of these transcriptional regulators and identification of more transcriptional regulators that are dedicated for either dendrite or axon commitment will not only shed light on the signal transduction mechanisms that differentially regulate dendrite and axon development, but also provide tools to identify dendrite- and axon-specific regulators of cytoskeleton and membrane systems.

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