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·Review·

Regulatory mechanisms underlying the differential growth of dendrites and axons

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A typical neuron is comprised of an information input compartment, or the dendrites, and an output compartment, known as the axon. These two compartments are the structural basis for functional neural circuits. However, little is known about how dendritic and axonal growth are differentially regulated. Recent studies have uncovered two distinct types of regulatory mechanisms that differentiate dendritic and axonal growth: dedicated mechanisms and bimodal mechanisms. Dedicated mechanisms regulate either dendrite-specific or axon-specific growth; in contrast, bimodal mechanisms direct dendritic and axonal development in opposite manners. Here, we review the dedicated and bimodal regulators identified by recent *Drosophila* and mammalian studies. The knowledge of these underlying molecular mechanisms not only expands our understanding about how neural circuits are wired, but also provides insights that will aid in the rational design of therapies for neurological diseases.

Keywords: axonal growth; dendritic arborizations; developmental neurobiology

Introduction

Neurons are the building blocks of neural circuits. At the cellular level, each neuron typically forms an input compartment, the dendrites, which receive information, and an output compartment, the axon, which sends processed information to its target. These two different subcellular compartments are highly specialized so that each can perform its specific tasks. Dendrites and axons are distinguishable from each other in terms of electrical excitability, morphology, microtubule orientation, and distribution of specific molecules and organelles[1, 2] (Table 1). These structural and functional differences between dendrites and axons make neurons classical examples of polarized cells. The separation and differential growth of these two compartments are fundamental to the establishment and maintenance of neuronal polarity.

The sequence of events during neuronal morphogenesis, which seems to be evolutionarily conserved, has been described in detail in studies of mammalian neurons[1, 3, 4]. In general, the separation of the dendrites and axons requires two steps: specification of dendrites and the axon, followed by the differential growth of each compartment (Fig. 1). During the specification step, the dendrites and axon assume their respective compartmental identities to establish neuronal polarity[2, 4]. In the differential growth phase, the dendrites and axon develop the specific morphological characteristics that allow them to assume their specialized roles in the establishment of directional information transmission[1]. Following these two steps, many neuron types also undergo remodeling to assume their mature morphologies[5].

While significant effort has been aimed at understanding how dendrites and axons are specified[1, 6], less is understood about the molecular underpinnings of differential dendrite and axon growth. Although one might at first think that differential growth is solely controlled by the

Table 1. Commonly-used dendritic and axonal markers in different types of neurons

Neuronal types	Dendritic markers	Axonal markers
Mammalian hippocampal neurons	MAP-2	Tau
Mammalian cortical neurons	MAP-2	NF-H, Tau
Mammalian granule neurons	MAP-2	Tau
Drosophila sensory neurons	Nod::βGal, DenMark	Kin::βGal
Drosophila CNS neurons	Nod::βGal, DenMark	Syt::GFP

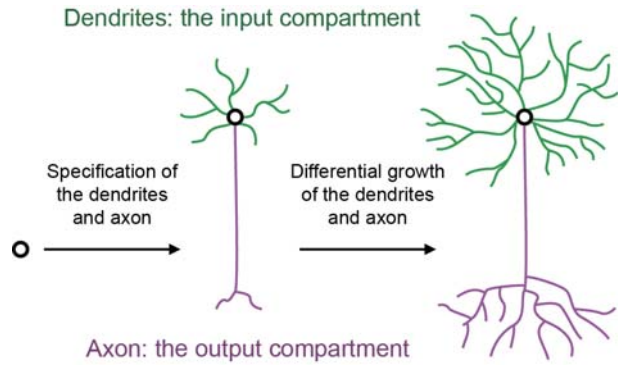

Fig. 1. A schematic illustration of the two steps of neuronal morphogenesis. Neuronal polarization is achieved in two steps. First, the nascent neuron (black circle) projects several processes, one of which commences rapid growth and becomes the axon[2, 3]. The remaining neurites then become dendrites as labeled by dendritic molecular markers[2, 3]. After acquiring their compartmental identities, the axon and dendrites extend additional branches to form the final branching patterns[2, 88]. The black circle indicates the soma; the green and purple processes indicate the dendrites and the axon, respectively.

compartmental differences set up during the specification step, this is unlikely to be the case for two reasons. First, the fact that different types of neurons exhibit distinct growth patterns of dendrites and axons argues for the existence of regulatory mechanisms that specifically control differential dendrite and axon growth. For example, during the differential growth phase, cerebellar Purkinje cells exhibit more dendritic growth than axonal growth, which leads to the formation of more elaborate dendritic than axonal arbors[7]. In contrast, cerebellar granule cells exhibit more axonal than dendritic growth, resulting a larger axonal than dendritic arbor[7]. Second, the existence of transcriptional programs that differentially regulate dendrite and axon development also supports the notion that the differential growth phase is controlled by *de novo* mechanisms and not simply by the cell-biological differences established during the specification phase[8-13].

Therefore, the regulatory mechanisms that operate in the differential growth phase play a major role in determining the final dendritic and axonal morphologies of mature neurons, and thus provide the basis for the morphological diversity observed in the nervous system.

Both mammalian and *Drosophila* neurons have been employed to identify the regulatory mechanisms that control differential dendritic and axonal growth. Mammalian neuronal cultures are robust systems for assessing dendritic and axonal arbor sizes, and are easily accessible to the application of pharmacological agents for manipulating the activity of molecules of interest. Despite this advantage, the cell culture environment differs from that *in vivo*. Thus, the roles of regulators identified in culture need to be further validated *in vivo*. Two major technical hurdles for the *in vivo* study of dendritic and axonal growth in the mammalian nervous system are the difficulty of achieving single-cell labeling and that of tracing the entire dendritic and axonal structures of a single neuron. As an alternative, the much smaller *Drosophila* nervous system offers an excellent system for studying the differential growth of dendrites and axons *in vivo*. Importantly, *Drosophila* is genetically tractable, allowing the use of advanced genetic mosaic techniques such as flip-out[14, 15] and mosaic analysis with a repressible cell marker (MARCM)[16]. Both of these techniques allow not only single-cell labeling, but also single-cell genetic manipulation.

In this review, we will focus on the roles of regulators identified in mammalian and *Drosophila* systems in

differentiating dendritic and axonal growth. Studies in these experimental systems have led to the discoveries of regulators dedicated to either dendrite or axon development ("dedicated mechanisms") and those that differentially direct dendritic and axonal development in opposite manners ("bimodal mechanisms") (Table 2). We will discuss these two major mechanisms separately.

Dedicated Mechanisms That Differentiate Dendritic and Axonal Growth

A number of molecular mechanisms operate in the differential growth phase[8, 9]. Although shared regulators, such as MAP1B (Futsch)[17] and histone deacetylase HDAC6[18, 19], are known to respectively promote or inhibit dendritic and axonal growth concurrently, other regulatory mechanisms are required to differentially regulate dendritic and axonal growth. For instance, differential regulation at the subcellular level can be achieved through "dedicated mechanisms", referring to regulators that specifically promote or inhibit the growth of one neuronal compartment without affecting the other[12] (Fig. 2). Based on their effect on dendritic and axonal growth, dedicated regulators can be further categorized into dendrite- or axon-dedicated regulators.

Dendrite-dedicated Regulators

In general, dendrite-dedicated regulators specifically control the growth of the dendritic compartment. These mechanisms can be either extrinsic, like growth factors, or intrinsic, like transcription factors. Extrinsic and intrinsic mechanisms may interact locally to promote the specific dendritic architectures of different neuron types. Besides *de novo* mechanisms, the cell-biological differences between axons and dendrites set up during the specification step may also influence the growth of one compartment and not the other. In this section, we will discuss our current knowledge of growth factors, transcription factors, and regulators of ER-Golgi transport in dendrite-dedicated regulation.

Table 2. Summary of dedicated regulators and bimodal regulators covered in this review

Molecules	Molecular function	Types of neurons studied	Role in dendritic growth	Role in axonal growth
Dedicated regulators				
BMP7/OP-1	TGF-β growth factor	Rat cultured sympathetic neurons/ cultured cerebral cortical neurons/ cultured hippocampal neurons	Positive regulator	None
NeuroD	bHLH transcription Factor	Cultured primary granule neurons	Positive regulator	None
Dar2	Homolog of Sec23	*Drosophila* da neurons	Positive regulator	None
Dar3	Homolog of GTPase, Sar1	*Drosophila* da neurons	Positive regulator	None
Dar6	Homolog of G-protein, Rab1	*Drosophila* da neurons	Positive regulator	None
Sar1	GTPase	Cultured hippocampal neurons	Positive regulator	None
Dar1	KLF transcription Factor	*Drosophila* da neurons	Positive regulator	None
SnoN-p300	Transcriptional complex	Cultured primary granule neurons	None	Positive regulator
Rac1	Small GTPase Rac	*Drosophila* PNS neurons and Purkinje cells	None	Positive/negative regulator
Bimodal regulators				
Sema3A	Secreted ligand	Cultured hippocampal neurons/ cortical neurons	Positive regulator	Negative regulator
CLASP2	Microtubule binding protein	Cultured cortical neurons	Positive regulator	Negative regulator
Rit	GTPase	Cultured hippocampal neurons	Negative regulator	Positive regulator
DLK	MAP Kinase Kinase Kinase	*Drosophila* C4da neurons	Negative regulator	Positive regulator

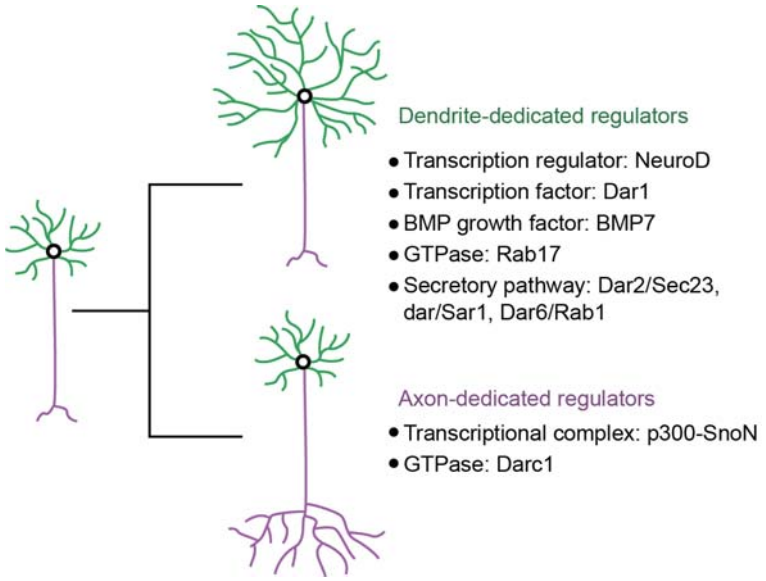

Fig. 2. Dedicated mechanisms of dendritic and axonal growth. Listed are known regulators that are dedicated to either dendrite-specific or axon-specific growth. The black circle indicates the soma; the green and purple processes indicate the dendrites and the axon respectively.

Growth factor BMP7 specifically promotes dendritic growth in mammalian cultured neurons The bone morphogenetic protein growth factor 7 (BMP7) (also termed osteogenic protein-1 or OP-1), a member of the transforming growth factor β (TGF-β) superfamily[20], is expressed in the nervous system. It induces the initial growth of dendrites in cultured rat sympathetic neurons, which typically develop a single axon without forming any noticeable dendritic structures in culture. Treatment of these neurons with recombinant human BMP7 leads to the formation of several dendrites without altering the number of axons[21]. BMP7 also selectively enhances dendritic arbor complexity after the initiation of dendrite formation. Exposure to BMP7 increases total dendritic length and the number of higher-order dendritic branches in CNS neurons *in vitro* without affecting axonal growth[22, 23].

How does BMP7 specifically promote dendritic growth? BMP signaling in general is transduced through ligand-receptor binding, which subsequently induces the phosphorylation of SMAD proteins and downstream transcriptional programs[24]. Consistent with this model, Garred and colleagues found that Actinomycin-D, a transcriptional inhibitor, blocks BMP7-induced dendritic growth in cultured sympathetic neurons. Microarray analysis of cultured sympathetic neurons treated with BMP7 for six hours showed changes in the transcript level of a number of transcriptional repressors belonging to the inhibitor of DNA binding (Id) family[25], an effect which may subsequently lead to the regulation of other transcriptional programs. BMP7 might also promote dendritic growth by enhancing the expression of the microtubule-associated protein MAP2[26]. Taken together, these studies suggest that the secreted molecule BMP7 may serve as an extrinsic mechanism that specifically promotes dendritic growth in mammalian neurons in culture.

Transcription factor NeuroD specifically promotes activity-dependent dendritic growth NeuroD is one of the basic helix-loop-helix (bHLH) transcription factors that control neuronal fate specification[27]. In addition to promoting neurogenesis[28], NeuroD expression persists in differentiated neurons[29] and controls dendrite morphogenesis in granule neurons[30, 31]. Gaudillière and colleagues found that knock-down of NeuroD inhibits dendritic growth but spares axonal morphogenesis in cultured primary granule neurons and granule neurons in cerebellar slices[30]. Furthermore, granule neuron dendritic branching is impaired in *NeuroD* conditional knock-out mice[31]. These results suggest that NeuroD specifically promotes dendritic growth.

NeuroD also plays a role in the neural activity-dependent patterning of dendritic arbors[32-34]. In cultured granule neurons, high neural activity induced by membrane-depolarizing concentrations of potassium chloride leads to more exuberant dendritic growth[30]. Knock-down of NeuroD blocks activity-induced dendritic overgrowth, suggesting that NeuroD may translate increased neural activity into a dendritic growth response[30]. Consistent with this notion, biochemical analysis revealed that NeuroD is phosphorylated by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)[30], a critical mediator of cellular responses to neural activity[35]. Gaudillière and colleagues further demonstrated that phosphorylation of NeuroD by CaMKII is indispensable for NeuroD to instruct activity-dependent dendritic growth[30]. Taken together, these studies suggest that NeuroD specifically mediates activity-dependent dendritic growth in granule neurons.

In addition to NeuroD, the calcium-responsive transactivator CREST[36] and the transcriptional complex AP-1[37] regulate activity-dependent dendritic growth in mammalian cortical and hippocampal neurons and *Drosophila* CNS neurons respectively. However, it remains unknown whether CREST and AP-1 function in axonal growth in these neuron types and thus whether they are dendrite-dedicated regulators.

Transcription factor Dar1 specifically promotes microtubule-based dendritic growth To perform a systematic search for genes that differentially regulate dendrite and axon development, Ye and colleagues used forward genetic screen that selected for mutants with dendrite- or axon-specific defects. To do this, they took advantage of the class IV dendritic arborization (C4da) sensory neurons in the *Drosophila* larva[38, 39]. In contrast to CNS neurons, C4da neurons directly sense multiple nociceptive stimuli[40-42] and their dendrites do not receive synaptic inputs. Nonetheless, the C4da neuron system has many advantages that make it well-suited for the study of dendrite and axon differential growth. First, the dendrites and axons of C4da neurons are easy to visualize with the help of a highly specific marker[43]. Second, unlike most invertebrate neurons, which are predominantly unipolar, da neurons resemble mammalian CNS neurons in terms of their multipolar morphology. Third, the dendrites and axons of these neurons exhibit similar cell-biological differences to those in mammalian CNS, including microtubule orientation[38, 44, 45] and organelle distribution[38, 46].

From their genetic screen of C4da neurons, Ye and colleagues isolated several mutants that displayed dendrite-specific growth defects, which they named *dendritic arbor reduction* (*dar*) mutants. The *dar1* gene encodes a *Drosophila* homolog of the Krüpple-like family of transcription factors (KLF), featuring three zinc-finger domains at the C-terminal region of the protein. Loss of *dar1* restricts dendritic growth in all classes of da neurons[47]. In sharp contrast, the growth of axons, including axon terminals, in these same neurons remains indistinguishable from wild-type controls[47]. Dar1 appears to preferentially promote microtubule-based, but not actin-based, dendritic growth. Overexpressing Dar1 specifically results in the appearance of microtubule-based higher-order dendritic branches. Moreover, loss of *dar1* function does not block the formation of F-actin-based dendritic filopodia caused by Rac1 overexpression. These results suggest that Dar1 preferentially regulates microtubules to promote microtubule-driven dendritic growth.

How does Dar1 influence the dendritic microtubule cytoskeleton? Ye and colleagues examined Spastin, a microtubule-severing protein, and found that the amount of *Spastin* mRNA was significantly elevated in *dar1* mutant neurons. These data indicate that Dar1 controls the transcription of *Spastin* to influence microtubules in the dendrites. Consistent with the change in *Spastin* transcript level, overexpression of Spastin impairs dendritic growth, leading to a phenotype reminiscent of that seen in neurons lacking *dar1*[47]. Further transcript profiling analysis may uncover additional Dar1 transcriptional targets involved in microtubule-based dendritic growth. In summary, these studies reveal Dar1 as a dendrite-dedicated mechanism that promotes dendritic growth *via* regulation of the microtubule cytoskeleton.

Regulators of ER-Golgi transport are preferentially required for dendritic growth Among the *dar* genes, three encode regulators of ER-to-Golgi transport: *dar2*, *dar3*, and *dar6*[38]. The mammalian homologs of these genes, Sec23, Sar1, and Rab1, respectively, are critical for ER-to-Golgi transport *via* COPII vesicles[48]. When mutations in *dar3* are introduced into single C4da neurons using the MARCM technique, not only do Golgi structures

become abnormal in the soma and dendrites, but total dendritic length is also markedly reduced[38]. When the Golgi apparatuses in the dendrites (termed dendritic Golgi outposts) are damaged using laser illumination, dendritic extension and retraction events become less dynamic. Moreover, redistributing the Golgi outposts in different parts of the dendritic arbor leads to a redistribution of dendritic branches. These findings highlight the idea that dendritic Golgi outposts may contribute locally to dendritic growth. Despite the changes in C4da neuron dendritic arbors, loss of *dar3* does not alter the axonal growth of these neurons[38]. Because the secretory pathway is a major source for the building blocks of the plasma membrane, these results suggest that growing dendrites have a greater demand for membrane supply during development than the axon.

Consistent with these findings in *Drosophila* C4da neurons, knock-down of the mammalian Dar3 homolog, Sar1, impairs dendrite-specific growth in cultured hippocampal neurons[38]. Taken together, these studies reveal a fundamental and evolutionarily-conserved difference in the reliance of dendritic *versus* axonal growth on the secretory pathway.

Implications of dendrite-dedicated mechanisms The diverse types of dendrite-dedicated mechanisms may form the basis for dendritic diversity in the nervous system. Since different neuron types require varied dendritic architectures to carry out their specific tasks, growth factors may induce the expression or activity of specific transcription factors to promote growing dendrites to assume the correct shapes. Because of the vast range of dendritic morphologies, it seems likely that many other dendrite-dedicated mechanisms that rely on growth factors and transcription factors remain to be discovered. In addition, the importance of secretory pathway regulators may be to transduce the signals provided by growth factors and transcription factors into physical changes in dendritic architecture.

Axon-dedicated Regulators

The axon-dedicated regulators identified so far include transcriptional and cytoskeletal regulators. These mechanisms are of particular interest for the development of axon regeneration therapies to treat spinal cord injuries and degenerative diseases. This section will discuss our current knowledge of axon-dedicated regulators, including transcription factors and cytoskeletal regulators.

The transcriptional complex SnoN-p300 specifically promotes axonal growth Ski-related novel protein N (SnoN) acts as a transcriptional repressor in TGF-β signaling[49]. In the nucleus of primary cerebellar granule neurons, SnoN is targeted for protein degradation by the Cdh1-APC ubiquitin ligase complex[50], which is indispensable for axonal growth in mammalian neurons[51]. Knock-down of SnoN inhibits granule neuron axonal growth. Conversely, elevated SnoN expression caused by either overexpression of a mutant form of SnoN resistant to degradation by the Cdh1-APC complex or by Cdh1-APC knock-down, results in elongated axons[50]. These results suggest that SnoN is both necessary and sufficient for axonal growth.

Further studies found that SnoN interacts with a histone acetyltransferase transcriptional activator, p300 or CREB-binding protein (CBP), to regulate axonal growth[52]. Knock-down of p300 impairs axonal growth without changing dendritic growth[52], suggesting that the SnoN-p300 complex is dedicated to axonal growth. Further, microarray analysis has led to the finding that expression of the actin-binding protein Ccd1[53] is reduced by knock-down of SnoN or p300[52]. Ccd1, like SnoN-p300, specifically promotes axonal growth in granule neurons[52]. Therefore, axon-dedicated regulation by the SnoN-p300 transcriptional complex is likely mediated by Ccd1.

Kirilly and colleagues found that knockdown of the *Drosophila* homolog of p300 leads to simplified dendrite arbors in pupal C4da neurons[54]. This suggests that the role of a specific regulator in differentiating dendritic and axonal growth could be cell-type specific or that p300 might mediate dendritic growth through a distinct mechanism.

The GTPase Rac1 specifically controls axonal growth The small GTPases of the Rac/Rho/Cdc42 subfamily are important regulators of the actin cytoskeleton in many cell types[55]. The *Drosophila* homolog of Rac, DRac1, plays an important role in the initiation and elongation of axonal growth in *Drosophila* PNS neurons[56]. Overexpressing either a constitutively active or a dominant-negative form of DRac1 inhibits axonal outgrowth and elongation without affecting the dendrites[56], suggesting that appropriate levels of actin polymerization are important for axonal growth.

The axon-dedicated role of Rac1 has been tested in mammalian Purkinje cells[57]. Consistent with the findings in *Drosophila*, overexpression of a constitutively-active

form of human Rac1 in cerebellar Purkinje cells leads to a reduction in axon terminals[57], while overall dendritic branching patterns remain normal. These results show that Rac1 is dedicated to axonal growth. It is noteworthy that constitutively-active Rac1 also reduces the size, while increasing the number, of dendritic spines on Purkinje cells[57]. Hence, although Rac1 is a dedicated regulator of axonal growth, it is also indispensable for organizing dendritic spine structures. This dichotomy may stem from an underlying imperative for proper actin regulation in both axonal growth and dendritic spine development.

Implications of axon-dedicated mechanisms Most current studies that aim to regenerate axons do not investigate the consequences of the interventions at the "other end" of the neuron—the dendrites. As a result, although many molecules are known to regulate axonal growth, very few are known to do so in an axon-specific fashion. Interventions that promote the regrowth of injured axons may not rescue defective dendrites or, even worse, may cause dendritic defects. Thus, it is imperative that we understand the intricacies of each growth program at both ends of the neuron to avoid unintended, adverse consequences of regenerative therapies.

Bimodal Mechanisms That Differentiate Dendritic and Axonal Growth

In addition to dedicated mechanisms, another strategy for differentially instructing dendritic and axonal growth is to direct their development in opposite manners at the same time. This mode of regulation is termed "bimodal regulation"[58] (Fig. 3). Unlike dedicated regulators, bimodal regulators might coordinate dendritic and axonal growth during development or in response to neuronal injury. This section discusses what is currently known about bimodal regulators.

Sema3A promotes dendritic growth but restricts axonal growth Semaphorin 3A (Sema3A) is a member of the Semaphorin family. Prior studies found Sema3A functions in an early step of neuronal polarization that specifies dendritic and axonal identities[59, 60]. In cultured hippocampal neurons, Sema3A inhibits cyclic adenosine monophosphate (cAMP) activity but enhances cyclic guanosine monophosphate (cGMP) activity[59]. cAMP, in turn, promotes axon initiation but suppresses the formation of dendrites; whereas cGMP has the opposite effect[61]. As a result, Sema3A preferentially promotes dendrite formation while suppressing axon formation. Similarly, Sema3A

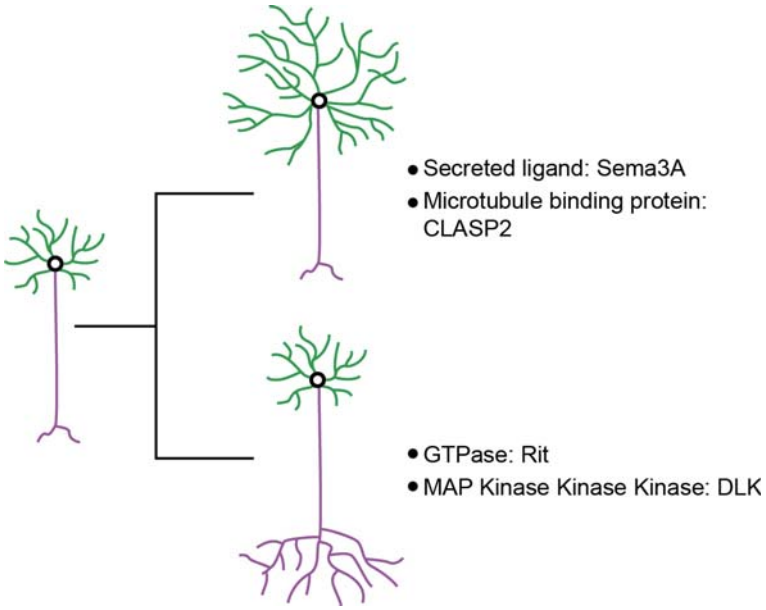

Fig. 3. Bimodal regulation of dendritic and axonal growth. Several bimodal regulators have been identified to oppositely alter dendritic and axonal growth. Sema3A and CLASP positively regulate dendritic growth but restrict axonal growth[59, 65], whereas Rit and DLK exert the opposite actions on these compartments[58, 68]. The black circle indicates the soma; the green and purple processes indicate the dendrites and the axon respectively.

acts as a chemoattractant for cortical apical dendrites but a chemorepellent for cortical axons[60]. The downstream effectors of Sema3A-cGMP/cAMP include protein kinase A (PKA), protein kinase G (PKG), and the serine/threonine kinase LKB1[59, 60, 62].

After dendrites and axon are specified, Sema3A-cAMP/cGMP continues to oppositely regulate the development of the dendritic and axonal compartments[59]. Exposure to Sema3A or cGMP results in more complex dendritic structures in cultured hippocampal neurons, and this is reversed by application of a PKG inhibitor[59]. These results demonstrate that Sema3A promotes the initiation and continued growth of dendrites while inhibiting these aspects of axonal growth.

CLASP2 promotes dendritic growth but restricts axonal growth Cytoplasmic linker protein (CLIP) and CLIP-associated protein (CLASP) bind to the plus end of microtubules and regulate microtubule dynamics in different cell types[63]. It is speculated that CLIP and CLASP proteins may be involved in the differential organization of the dendritic and axonal microtubule cytoskeleton[64]. In support of this, CLASP2 is reported to be a bimodal regulator. Knockdown of CLASP2 causes axonal over-branching but impairs dendritic extension in cultured cortical neurons[65].

It remains unknown how the bimodal function of CLASP2 is achieved. CLASP2 exhibits two microtubule-binding behaviors: it binds to the plus end of microtubules and also associates with microtubule lattices[63, 66]. The intriguing hypothesis that these two microtubule-binding activities may mediate the two opposite actions of CLASP2 on dendritic and axonal outgrowth remains to be tested.

Rit GTPase restrains dendritic growth but promotes axonal growth Rit is a member of the Ras GTPase family and is widely expressed in the mammalian nervous system[67]. Overexpression of a dominant-negative form of Rit inhibits axonal growth but leads to longer dendrites in cultured hippocampal neurons[68]. Conversely, overexpressing a constitutively active form of Rit markedly increases axonal length but reduces total dendritic length and number[68]. Lein *et al.* further found that extracellular signal-regulated kinase 1/2 (ERK1/2) mediates the bimodal regulation of Rit, as inhibition of mitogen-activated protein kinase/ERK 1 (MEK1) blocks the changes in both dendritic and axonal growth caused by constitutively active Rit. These data suggest that, in contrast to the axon-dedicated Rac1 GTPase, Rit GTPase functions as a bimodal regulator.

DLK pathway promotes axonal growth but restrains dendritic growth *in vivo* The evolutionarily-conserved dual leucine zipper kinase (DLK) pathway regulates axonal growth, regeneration, and degeneration[69-77], and organizes the presynaptic structures of axon terminals[78]. This pathway consists of two major components. The first is an E3 ubiquitin ligase named Pam/Highwire/RPM-1 (PHR). PHR targets DLK, a mitogen-activated protein kinase kinase kinase (MAPKKK), for protein degradation[69, 71]. Upregulated DLK expression, caused by either loss of PHR or overexpressing DLK, causes axon terminal overgrowth in various neuron types in *Caenorhabditis elegans*, *Drosophila*, and mammals[58, 69, 72, 79-82]. Moreover, loss of *DLK* blocks new axon outgrowth after nerve injury[70, 74-76, 83].

A recent study by Wang and colleagues demonstrates that overabundant DLK promotes axonal growth but negatively regulates dendritic branching in *Drosophila* C4da neurons[58]. The *Drosophila* homologs of the E3 ubiquitin ligase and DLK are named Highwire (Hiw)[81] and Wallenda (Wnd)[69], respectively. Either loss of *hiw* or overexpression of Wnd leads to exuberant axon terminal growth but markedly impairs dendritic growth in C4da neurons[58]. These dichotomous actions of the DLK/Wnd pathway are mediated by divergent downstream components. The transcription factor Fos and Down syndrome cell-adhesion molecule (Dscam) are required for axon growth in response to up-regulated DLK/Wnd[58, 84]. In contrast, dendritic regulation by DLK/Wnd is mediated by the transcription factor Knot[58]. It is noteworthy that in Knot-negative neurons, such as class I, II, and III da neurons, DLK/Wnd specifically promotes axonal growth and does not regulate dendritic growth[58].

The bimodal function of DLK/Wnd might serve to coordinate dendritic and axonal growth after nerve injury. Previous studies reported an increase in DLK/Wnd protein level after nerve crush injuries in both *Drosophila* motor neurons[75] and mouse optic nerves[74]. Based on the work of Wang and colleagues in C4da neurons, an elevated DLK/Wnd level likely restrains dendritic growth in injured neurons while promoting axonal regeneration. Indeed, it has been observed that axotomy not only triggers axon

regeneration but also causes more simplified dendrites in C4da[85] and mammalian neurons[86, 87]. These observations suggest that neurons may promote axonal regeneration at the expense of dendrites and that the bimodal regulator DLK/Wnd may coordinate these distinct dendritic and axonal responses to injury.

Implications of bimodal regulators The functional significance of bimodal regulation remains to be determined. We speculate that bimodal regulators might determine the ratio of dendritic arbor size to axonal arbor size[58]. For instance, high levels/activity of Rit or DLK might result in more elaborate axon branching but simpler dendritic structures; whereas high Sema3A and CLASP2 likely cause the opposite changes in dendritic and axonal patterns. It will be informative to determine whether bimodal regulators are differentially expressed in distinct neuron types and underlie the morphological diversity among them. Besides their functions during development, little is known about how these bimodal regulators control dendritic and axonal responses to injury or pathological conditions. Further investigation may shed light on how manipulating the activity of bimodal regulators might correct dendritic and axonal defects in disease conditions.

Summary

The differential growth of dendrites and axons is of fundamental importance to the establishment of connectivity and communication in neural circuits. It is also essential for generating the diverse neuronal morphologies that we observe in the nervous system. The molecular mechanisms that differentiate dendritic and axonal growth can be categorized into "dedicated" and "bimodal" mechanisms. Dedicated mechanisms specifically control the growth of only one compartment, while bimodal mechanisms promote the growth of one compartment while inhibiting the other. Moreover, it is likely that these distinct regulatory methods converge to pattern the distinct dendritic and axonal architectures of each neuron. Although just a few examples of each category have been discovered, our current knowledge hints at the possible complexity involved in patterning the nervous system. On one hand, many thousands of axon- and dendrite-dedicated regulators may be required to develop the diversity of neuronal architectures that we observe. On the other hand, this diversity may arise from various combinations and levels of just a few regulators. Furthermore, we do not yet fully appreciate the importance of having these various modes of regulation, especially bimodal regulation. Although we speculate that bimodal regulators function to coordinate axon and dendrite growth in both development and regeneration, further investigation is required to fully appreciate the role of these regulators. Further understanding of how these regulatory mechanisms operate during development and how to manipulate the activity of these regulators will also be instructive for designing strategies to restore defective neurons under pathological conditions. In some neurological disorders, only axons or dendrites are affected; in others, only a specific brain region or subset of neurons. Increasing our understanding of axon-specific, dendrite-specific, and bimodal regulators may allow us to specifically regrow, reshape, and regenerate many different types of neurons without adverse consequences for the remainder of the nervous system.

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