



# How do dendrites take their shape?

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Recent technical advances have made possible the visualization and genetic manipulation of individual dendritic trees. These studies have led to the identification and characterization of molecules that are important for different aspects of dendritic development. Although much remains to be learned, the existing knowledge has allowed us to take initial steps toward a comprehensive understanding of how complex dendritic trees are built. In this review, we describe recent advances in our understanding of the molecular mechanisms underlying dendritic morphogenesis, and discuss their cell-biological implications.

With their great complexity and variety, dendrites (Fig. 1) are wonders of nature's design. Built to receive and integrate inputs to neurons, dendrites occupy much of the brain's volume and have been the subject of studies since the days of Golgi and Cajal<sup>1</sup>. Over the course of much of the twentieth century, the prevailing belief that axons take the more active role in wiring the brain and in establishing synaptic specificity led researchers to focus on the development of axons more than that of the dendrites. In addition, the complexity and diversity of dendritic trees presented technical difficulties for conducting systematic studies of the mechanisms underlying their formation. Dendrites have been in the spotlight again recently, as technological advances have revealed new electrophysiological properties for dendrites and the activity dependence of dendritic form and function<sup>2</sup>. Recent technological advances have also enabled researchers to explore the cellular and molecular mechanisms of dendrite development. In this review, we outline the developmental steps that lead to mature dendritic structures and highlight and discuss recent progress made in understanding the molecular mechanisms that underlie these processes.

## Visualizing and genetically manipulating dendrites

To study mechanisms of dendrite formation, one first needs a reliable way of visualizing individual dendritic trees within intact nervous tissue. Much of our knowledge of dendritic structures in different parts of the nervous system, different developmental stages, and different animals, comes from observations using a single method, Golgi staining<sup>1,3</sup> (Fig. 1a). Recently, a variety of molecular biological methods have allowed a marriage between single neuron labeling and the ability to manipulate gene activity in these neurons. These methods include biolistic transfection<sup>4,5</sup> (Fig. 1b), and DiI labeling<sup>6</sup> and its coupling with virus transfection<sup>7</sup>. In addition, highly specific promoter elements can be used to achieve selective expression of markers and genes of interest in a small population of neurons<sup>8</sup> (Fig. 1c), and a genetic mosaic method has been developed that allows reliable labeling and genetic manipulation of identifiable individual neurons<sup>9</sup> (Fig. 1d). Visualization and manipulation of individual neurons both in primary cell culture<sup>10</sup> and on brain slices<sup>11</sup> have also helped researchers examine the process of dendritic development (see below). Some of these methods can be used to study the effects of extracellular ligands or intracellular expression of dominant mutants on dendritic development, whereas others, as an

added advantage, can be used to study loss-of-function mutations. Much of the progress reviewed in this article would not have been possible without these technological advances.

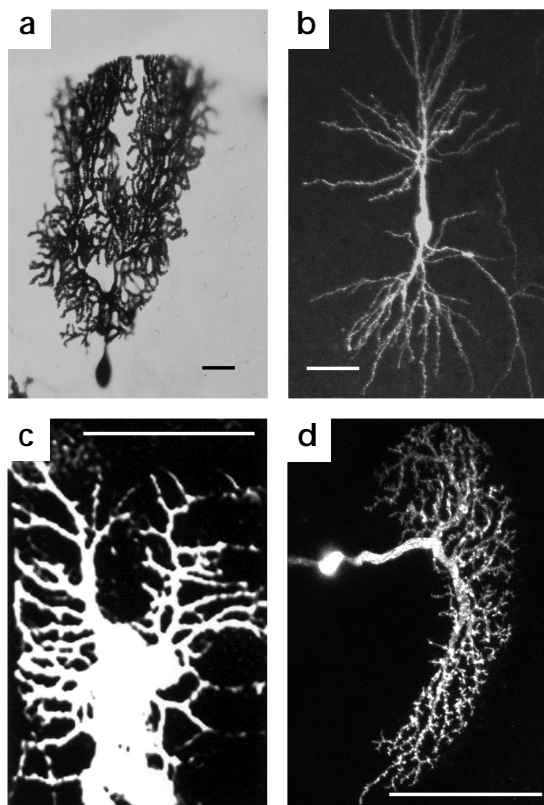
## Breaking down complex dendritic trees

Although the processes used to build dendritic trees are complex and diverse (Fig. 1), they can be broadly separated into several essential steps (Fig. 2). First, dendrites grow from morphologically unpolarized young neurons and attain characteristics such as length, diameter, growth rate and molecular composition that are different from those characteristics in axons<sup>12</sup>. Second, dendrites extend in a defined direction and increase in diameter as they undergo the third step, formation of branches at defined intervals. Fourth, as dendrites elaborate, many also generate small specialized protrusions known as dendritic spines, which are the sites of major excitatory synapses in the mammalian brain. Lastly, many neurons' dendrites stop growing at defined borders<sup>8,13</sup>, giving rise to their mature shape. Directing these complex developmental processes are a variety of cell-intrinsic programs and extrinsic cues from the environment (Box 1).

In the rest of the article, we summarize our current knowledge of the cellular and molecular basis of dendritic growth, guidance, branching and limiting dendritic growth. Despite the simultaneous and overlapping nature of some of these processes (Fig. 2, Table 1), we feel that dividing the overall process of dendritic development into discrete events will help provide cell-biological context for the functions of the molecules that we describe. Rather than providing a comprehensive review of the molecules involved in dendritic morphogenesis, we focus our discussions on selected proteins that we feel best illustrate the cellular processes. Other recent reviews provide more information on areas that we do not cover extensively, such as the establishment of neuronal polarity<sup>12,14</sup>, the structural plasticity of dendritic spines<sup>15-17</sup>, and the relationship of dendritic morphogenesis to synapse formation and synaptic activity<sup>18</sup>.

## Dendritic outgrowth

To form connections with the correct presynaptic axons, dendrites must extend away from their neuronal cell body and into their target field. Several extracellular factors affect dendritic growth in different classes of neurons. For instance, a GPI-linked candidate plasticity gene 15 (CPG 15), originally identified as being induced



**Fig. 1.** Visualizing individual dendrites. (a) A Golgi-stained Purkinje cell from the cerebellum of an adult mouse (unpublished image taken by L.L.). (b) A pyramidal neuron in rat hippocampal slice culture biologically transfected with mouse CD8 driven by an actin promoter, then immunostained<sup>48</sup> (copyright 2000, by the Society for Neuroscience). (c) Dendrites from a cluster of *Drosophila* multiple dendrite (md) neurons. The neurons are expressing GFP through the use of the GAL4-UAS system<sup>98</sup> and a GAL4 line specific to the md neurons<sup>8</sup> (copyright 1999, by the Cold Spring Harbor Laboratory Press). (d) The dendritic arborization of a single Vertical System neuron from the lobular plate of an adult *Drosophila*. The marked cell is a genetically modified clone expressing mouse CD8-GFP by use of the MARCM system<sup>9</sup> (unpublished image taken by E.K.S., GAL4 driver provided by T. Raabe and M. Heisenberg). Scale bars, 50  $\mu$ m for (a, b, d); approximately 25  $\mu$ m for (c).

by electrical activity, can promote neighboring tectal projection neurons to extend their dendrites when overexpressed in *Xenopus* tectal cells<sup>19</sup>. A bone morphogenic protein (BMP) family member, osteogenic protein 1 (OP-1), has the selective effect of inducing sympathetic neurons in culture to grow dendrites<sup>20</sup>, and it requires nerve growth factor (NGF) as a cofactor in this process<sup>20</sup>. NGF and other neurotrophins, including brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and NT4/5 themselves have been shown to be important in regulating dendritic growth of cortical neurons in a series of studies described below.

In developing ferret visual neocortex, neurotrophin application generally increases dendritic length and complexity, but this effect varies with different neurotrophins, from layer to layer, and between apical and basal dendrites<sup>21</sup>. In addition, a particularly dramatic effect of BDNF on layer 4 dendrites can be prevented if synaptic activity is blocked during BDNF treatment<sup>22</sup>, bolstering the idea that neurotrophins carry out their effects in an activity-dependent manner. Time-lapse imaging of live neurons in slice culture<sup>23</sup> showed that BDNF, along with causing dendrite sprouting in transfected cells, renders dendritic arbors highly unstable, causing them to both gain and lose dendrites at a rate much higher than in control cells. Therefore, the mechanism of BDNF could be to destabilize the dendrites, thereby allowing other signals to direct expansion or connectivity<sup>23</sup>. Neurotrophins bind to the trk family receptor tyrosine kinases with high affinity<sup>24</sup>. Soluble trk-receptor bodies that bind and neutralize neurotrophins block the effect of exogenously applied neurotrophins, and affect dendritic development by themselves<sup>23,25</sup>. This indicates that the effects of neurotrophins work through trk receptors, and, more impor-

tantly, that endogenous neurotrophins are necessary for dendritic growth. The signal transduction mechanisms are likely to be complex, as different splice variants of a given trk receptor (for example, trk B) are expressed differentially during development<sup>26</sup>, and can cell-autonomously mediate distinct dendritic effects for the same neurotrophic signal<sup>27</sup>.

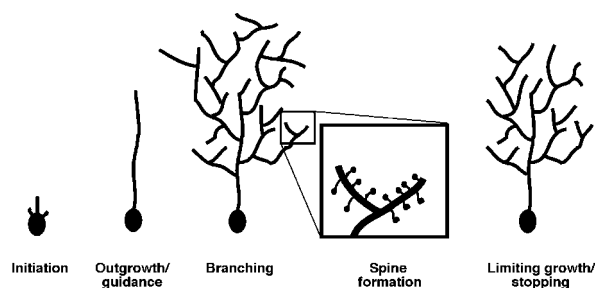
For the most part, the signaling pathways through which extracellular factors carry out their effects on dendritic growth have not been reported. It is not even clear whether these factors act by regulating gene expression to turn on a program for dendritic growth, or by directly regulating the cytoskeleton. To distinguish between these possibilities, one could examine the time course of the response to the application of these extracellular factors, or locally apply them to a small portion of the dendritic tree. This would be particularly interesting in the case of neurotrophins. In fact, NGF was the first protein shown to be able to induce the growth cones of cultured neurons to turn toward a high concentration source<sup>28</sup>. The time course and local effect of NGF on growth cone dynamics strongly suggest that NGF can regulate local cytoskeletal changes without the need for transcriptional regulation. It will be interesting to see whether local applications of neurotrophins can selectively induce nearby dendrites to grow and turn as the neurites of the cultured neurons do.

### Dendrite guidance

Dendrites, like axons, have to steer toward their eventual targets if the correct neural connections are to be made. One mechanism by which axons steer is by turning toward or away from point sources of a diffusible ligand<sup>29</sup>, and some dendrites also use this mechanism. An interesting example comes from the way in which axons and dendrites of cortical pyramidal neurons respond to a presumptive gradient of the diffusible signal semaphorin 3A (Sema 3A).

Sema 3A protein usually acts to collapse or repel axonal growth cones<sup>30,31</sup>. However, if the level of the cyclic nucleotide guanosine 3'/5'-monophosphate (cGMP) is raised within the axon, the repul-

**Fig. 2.** Major steps of dendritic development. For molecules involved in these different developmental steps, see the text and Table 1.





sion is converted into attraction in *Xenopus* spinal neurons *in vitro*<sup>32</sup>. Cortical pyramidal neurons send dendrites toward and axons away from the cortical plate, where *Sema 3A* is endogenously expressed<sup>33</sup>. In addition, mice that are mutant for *Sema 3A* show defects in dendrite and axon guidance of cortical pyramidal neurons<sup>11,34</sup>. It has further been shown that *Sema 3A*, along with serving as a repellent for axons<sup>34</sup>, serves as an attractant for the apical dendrites of the pyramidal neurons in cortical slices<sup>11</sup>. Guanylate cyclase (SGC), the enzyme that produces cGMP, is asymmetrically localized to the apical dendrites, and seems to be necessary for dendritic attraction<sup>11</sup>. This provides a potential mechanism by which high cGMP concentrations in the dendrites and low concentrations in the axons could account for their opposite responses to the presumptive *Sema 3A* gradient. This is consistent with the idea that axonal and dendritic growth cones share similar signal transduction machinery, although further experiments will be required to determine the extent of these similarities.

Another possible mechanistic parallel between axons and dendrites can be found in the *enabled (ena)* gene, mutation of which leads to a dendritic phenotype in *Drosophila* embryonic sensory neurons<sup>8</sup>. In wild-type embryos, dendritic lateral branches from the multiple dendrite (md) neurons grow in anterior and posterior directions from the segment boundaries (Fig. 1c). In *ena* mutant embryos, these neurites turn uncharacteristically dorsally, and often fail to reach the segment boundaries<sup>8</sup>. Whereas little is known about *ena*'s function in dendritic morphogenesis, it has been well characterized in the axon, where it is required for axon fasciculation and guidance<sup>35–37</sup>. *Ena* and the tyrosine kinase *Abl* likely act downstream of both receptor tyrosine phosphatase *Dlar*<sup>36</sup> and the axon guidance receptor *Roundabout*<sup>37</sup>. *Ena* and its mammalian homologs bind to a variety of actin cytoskeletal proteins and to actin itself, and are implicated in regulating actin dynamics<sup>38</sup>. It remains to be determined whether *ena*-class proteins use similar mechanisms for dendritic guidance.

### Dendritic branching

To cover the correct target fields, most neurons need to form extensive dendritic branches. Branching can be generated by two distinct mechanisms. The first mechanism is the splitting of growth cones, which has been observed to be the predominant mechanism

in cultured neurons under certain conditions<sup>39</sup>. The second mechanism is the emergence of branches from the side of established dendritic shafts (called interstitial branching), which might be the predominant mode in more physiological states. For instance, time-lapse studies of the dendrites of individual live pyramidal neurons from developing rat hippocampal slices<sup>6</sup> showed that new branches tend to form from existing dendritic shafts. Each branch first appears in the form of a single filopodium. Whereas most filopodia quickly retract into the dendritic shaft, some develop into growth-cone-like structures. Some of those growth cones, in turn, extend and become stable branches<sup>6</sup>. A similar process may also occur in the *Drosophila* md sensory neurons<sup>8</sup>.

Little is currently known about the molecular mechanisms underlying interstitial branching in dendrites. However, studies of axonal branching, which also occurs predominantly in an interstitial manner *in vivo* (for example, see ref. 40), may offer some insight. It is generally thought that local cues act on the cortical actin and membrane to cause cytoskeletal destabilization and filopodial protrusion. Filopodia can act as precursors of transient branches, the stability of which requires the invasion of microtubules (Box 2) that are derived from microtubules fragmented from the axon trunk<sup>41</sup>. Local destabilization of the microtubules also induces local exocytosis of vesicles, fueling the branch with new plasma membrane<sup>42</sup>.

Recent molecular studies of dendritic branching seem to be consistent with this working hypothesis. The Rho family of small GTPases, which are important regulators of actin polymerization (Box 2), are important for controlling different aspects of neuronal morphogenesis (for review, see ref. 43), including dendritic development (for review, see refs. 43, 44). Two recent live imaging studies in *Xenopus* tectal cells *in vivo*<sup>45</sup> and chick retinal ganglion neurons in explants<sup>46</sup> show that the small GTPase *Rac* is particularly important in regulating dendritic branch stability. Neurons expressing constitutively active *Rac* experience an increase both in the rate of sprouting and in the rate of retraction<sup>45</sup>, resulting in high turnover rate of dendritic branches<sup>45,46</sup>. In addition, dominant negative *Rac* has the opposite effect in branch turnover rate at least in retinal ganglion cells<sup>46</sup>. Whereas these sprouting and pruning events balance out to leave the neuron with a normal dendritic complexity at any given time, a

### Box 1. How autonomous is dendritic morphogenesis?

To what extent is dendritic development driven by intrinsic neuronal differentiation programs, and what extrinsic contributions are required from the environment for dendrites to develop normally? Studies of cerebellar Purkinje cells and their spectacular dendritic trees (Fig. 1a) have provided evidence supporting the importance of both cell-intrinsic programs and environmental influences. Early studies using X-irradiation or genetically mutant mice (for example, refs. 66, 67) showed that many features of Purkinje cells, including characteristic initial branching patterns and spine formation, can develop in the almost complete absence of its major presynaptic partners: the granule cells. However, these Purkinje cell dendrites have greatly reduced higher order branching and their orientation is severely disrupted. Because other aspects of the Purkinje cells' surroundings may be normal in these mice, these experiments are not stringent tests of the Purkinje cells' intrinsic capabilities, but they do indicate that some dendritic development can proceed without contact with or synaptic input from their normal presynaptic partners. More stringent tests are possible *in vitro*. When purified Purkinje cells in culture are provided with granule cells, their dendritic trees resemble those of *in vivo* wild-type cells much more closely, with appropriate synaptic connections on well-formed dendritic spines<sup>68</sup>. However, even these Purkinje cells never form the fully mature dendritic structures found *in vivo*. In all, it seems that Purkinje cells have intrinsic programs that allow for certain aspects of development, but that normal development requires environmental input from the granule cells and other sources.

Environmental cues can work through a variety of mechanisms, including secreted soluble factors, contact with glia or presynaptic axons, and synaptic activity from presynaptic partners (for review, see 17, 18, 79). Indeed, the *in vitro* Purkinje cells discussed above seem to survive and develop their dendritic complexity with the help both of soluble factors and electrical activity from the granule cells<sup>70,71</sup>. In theory, environmental signals may turn on dendritic differentiation programs by activating expression of genes necessary for dendrite development. Alternatively, environmental cues can induce local changes in dendritic structures. An eventual converging point between these global and local signals is the dendritic cytoskeleton, which controls the shape and form of the dendrites (Box 2).



Table 1. Molecules involved in dendritic and axonal morphogenesis.

Molecule	Function in dendrite development	Function in axons
<b>Extracellular</b>		
OP-1 (BMP family)	Induces dendritic growth in sympathetic neurons <sup>20</sup>	None <sup>83</sup>
CPG 15	Induces dendrite extension in neighboring neurons <sup>19</sup>	Determines growth rate and branch stability <sup>84</sup>
Neurotrophins	Generally induce growth and complexity <sup>21–23,25</sup>	Generally promote growth <sup>85</sup>
Sema 3A	Serves as attractive guidance cue <sup>11,34</sup> , see cGMP	Generally works as a repulsive guidance cue <sup>86</sup>
<b>Membrane receptors</b>		
Trk family	Receives neurotrophin signals <sup>24,25</sup>	Promotes growth <sup>87,88</sup>
Flamingo	Limits growth <sup>8</sup> , possibly through a homophilic interaction <sup>58</sup>	Supports fasciculation <sup>58</sup>
Notch	Inhibits growth <sup>64,65</sup> , promotes branching <sup>65</sup>	Regulates guidance <sup>89</sup>
<b>Cytoplasmic</b>		
Rac	Controls branch formation and stability <sup>45,46</sup> , spine formation <sup>47,48</sup>	Generally promotes growth <sup>43</sup>
Cdc42	Controls growth and branching <sup>8,47,90</sup>	Generally promotes growth <sup>43</sup>
RhoA	Limits growth <sup>45,46,48,60</sup> , at least partially through ROCK <sup>48</sup>	Limits growth or initiation <sup>43</sup>
Rho kinase (ROCK)	Limits growth <sup>48</sup>	Limits initiation and growth <sup>91</sup>
MAP2	Necessary for the initial formation of neurites <sup>75</sup>	Induces formation in culture <sup>75</sup>
CHO1/MKLP1	Forms and maintains dendrites <sup>76,77</sup>	None <sup>76,77</sup>
Lis1	Works with dynein to control growth, branching and maturation <sup>78</sup>	Regulates axonal transport <sup>78</sup>
Dynein	Promotes growth and branching <sup>78</sup>	Regulates axonal transport <sup>92</sup>
CaM KII	Stabilizes dendritic structures <sup>7,93</sup>	Limits growth and elaboration <sup>93</sup>
cGMP	May determine attractive versus repulsive response to Sema 3A, depending on concentration <sup>11</sup>	Regulates guidance <sup>32</sup>
Enabled	Serves in steering <sup>8</sup> , may regulate actin dynamics through profilin <sup>94,95</sup>	Regulates guidance <sup>35–37</sup>
Kakapo (Short stop)	Serves in branching <sup>8,49</sup> , likely through control of the microtubules <sup>49–51</sup>	Promotes growth and guidance <sup>9,54,55</sup>
<b>Nuclear</b>		
Notch	Inhibits growth <sup>64,65</sup> , branching <sup>65</sup>	Regulates guidance <sup>89</sup>
Prospero	Serves in guidance <sup>8</sup>	Promotes growth and guidance <sup>8,96,97</sup>

greater number of the dendrites are more transient than in wild-type neurons. The balance of increased extension and collapse may explain why in fixed samples, perturbation with Rac alone has a minimal effect on dendritic branching complexity (for example, see refs. 47, 48). It is conceivable that a Rac-mediated increase of dendritic branch dynamics, in coordination with regulation of microtubule invasion into these transient branches, eventually results in the formation of stable dendritic branches.

Another recent insight into the molecular mechanisms of dendritic branching comes from the genetic identification of *kakapo* (*kak*), also known as *short stop*, as particularly important for dendritic branching in *Drosophila* md neurons<sup>8</sup>. These *kak* mutants are also defective in motoneuron dendritic branching in the *Drosophila* CNS<sup>49</sup>. The *kak* gene encodes a cytoskeletal linker protein of over 5000 amino acids, with actin binding domains and a domain likely to associate with microtubules<sup>50,51</sup>. As a member of the plakin family of coiled-coil proteins, it is believed to serve as a linker between cytoskeletal elements and other cellular structures (for review, see ref. 52). Supporting this potential role of *kak* as a cytoskeletal linker, *kak* mutant embryos show disruption of microtubule structures in a variety of cell types<sup>49</sup>. Given these properties, it is not surprising that *kakapo/short stop* mutants have previously been identified as affecting axon extension and guidance<sup>9,53–55</sup>. The dendritic branching defects of *kak* mutants support the importance of interactions between the actin and microtubule cytoskeleton in the stabilization of dendritic branches.

What are the extracellular signals that trigger dendritic branching? Signals that regulate dendritic growth (such as neurotrophins,

see above) can also have the effect of increasing dendritic complexity, including branching. However, it is difficult to determine whether the primary cause of these factors is to stimulate dendrite extension, with branching as a secondary consequence to extension, or whether they trigger branching directly. The axon guidance protein Slit has been shown *in vitro* to stimulate axonal branching (and also to have a positive effect on growth)<sup>56</sup>. However, at least in *Drosophila* md neurons, Slit does not seem to affect dendritic branching<sup>8</sup>. It will be of great interest in the future to identify proteins that influence dendritic complexity and to study how they send their signals to the actin and microtubule cytoskeletons to orchestrate the often complex and highly ordered branching patterns that are hallmarks of dendritic trees.

Similar to dendritic branch formation, the emergence of dendritic spines involves lateral protrusions of membrane and cytoskeleton from the dendritic shafts. One interesting observation from live imaging of hippocampal pyramidal dendrites<sup>6</sup> is that dendritic branching and spine formation share the same initial stages of development. Both structures begin as transient filopodia, which can then either retract and disappear, extend to form a branch, or stabilize and assume the morphology of a spine. One potential molecular parallel between branching and spine formation is the involvement of the small GTPase Rac. In addition to affecting dendritic branching dynamics, Rac is important for the formation and maintenance of dendritic spines in cerebellar Purkinje cells in transgenic mice<sup>47</sup>, and pyramidal neurons in rat hippocampal slices<sup>48</sup>. One interpretation of Rac's drastic phenotypes in dendritic spines compared with its mild phenotypes in dendritic branching (steady state level) is the dif-



### Box 2. Cytoskeletal elements in dendritic morphogenesis.

The actin and the microtubule cytoskeleton determines the shape of the dendrites and provides the substrates upon which regulators of dendritic development act. Filamentous actin (F-actin) is distributed at the cortex of the dendrites, and is highly enriched in dendritic spines, whereas microtubules fill the interior of the dendrites<sup>72,73</sup>.

Just like the growing tips of axons, dendritic growth cones possess filopodia<sup>6</sup>. Filopodia are F-actin bundle-based structures that allow dendrites to explore signals in their environment. Not surprisingly, regulators of the actin cytoskeleton are important in many aspects of dendritic development. Rho family small GTPases (Rac, Rho, Cdc42), for example, are important regulators of the actin cytoskeleton<sup>74</sup>. These proteins serve as molecular switches, transducing signals when in their active GTP-bound state, but not when in their inactive GDP-bound state. They have a myriad of functions in neuronal development<sup>43</sup>, including dendritic development<sup>43,44</sup>. The readout of these signaling pathways includes, among other functions, *de novo* actin polymerization (Cdc42, Rac), and regulation of actin depolymerization (Rac) or myosin activity (Rho)<sup>43</sup>. Another actin regulator, enabled, mutations in which were identified in a dendritic screen<sup>8</sup>, is also intimately associated with the regulation of the actin cytoskeleton<sup>38</sup>. Differential participation of these regulators may underlie different aspects of dendritic development.

Microtubules distribute throughout the dendritic trunks and provide the structural integrity for dendrites. Illustrating this, cultured neurons treated with an antisense oligonucleotide of MAP2, a microtubule-associated protein that is specifically distributed in dendrites, failed to form dendrites<sup>75</sup>. In contrast to axonal microtubules that have unidirectional plus-end-distal arrangements, microtubules in dendrites have both plus-end-distal and minus-end-distal populations<sup>12</sup>. This bidirectional orientation of microtubules may be important for the differentiation and maintenance of dendritic structures. A kinesin-related motor protein, CHO1/MKLP1, is a strong candidate for transporting minus-end-distal microtubules into the dendrites<sup>76,77</sup>. When activity of this protein is inhibited by antisense oligonucleotide treatment of early-stage neurons in culture, dendrites fail to form<sup>76</sup>. When an antisense oligo of CHO1/MKLP1 is applied after dendrites are fully differentiated in culture, dendrites rapidly lose their minus-end-distal microtubules and acquire axon-like morphologies and organelle compositions<sup>77</sup>.

Another microtubule motor important for dendritic development is minus-end-directed cytoplasmic dynein. In *Drosophila*, single-cell knock-out of one dynein heavy chain (Dhc64) in mushroom body neurons results in defects in dendritic growth, branching and maturation<sup>78</sup>. Interestingly, a dynein-associated protein<sup>79</sup>, Lis1 (haploinsufficiency of the human ortholog causes a lissencephalic, or smooth brain, condition), exhibits a strikingly similar mutant phenotype to that resulting from dynein mutations in *Drosophila* neurons<sup>78</sup>. Lis1 and dynein also associate with Nudel, which is a substrate for the p35/Cdk5 kinase complex<sup>80,81</sup>. Activity of p35/Cdk5 can in turn be regulated by small GTPase Rac<sup>82</sup>, thus providing one potential link between regulators of the actin and microtubule cytoskeletons.

ference between the cytoskeletal elements. Whereas dendritic branches require microtubules to stabilize, actin is the dominant cytoskeletal element in spines (Box 2). Additionally, there seems to be a developmental progression from filopodia forming mostly branches to forming mostly spines<sup>6</sup>. Identifying the molecular mechanisms that underlie this developmental regulation will be of great interest.

### Limiting dendritic growth

Dendrites seem to know their territories; they stop growing if their territories are covered. One striking example is 'retinal tiling': ganglion cells stop growth upon contacting neighboring cells of the same kind. Thus, dendrites from each functional group of ganglion cells cover the entire retina only once<sup>13</sup>. A recent study demonstrated that *Drosophila* larval md neurons exhibit similar properties. When one md neuron is removed by laser ablation or by genetic manipulation, its territory can be occupied by a homologous md neuron on the other side of dorsal midline but not by other types of md neurons. These observations imply that a selective repulsion exists between dendrites of like neurons<sup>57</sup>.

What are the molecular mechanisms that prevent a dendrite from growing beyond its own territory? The *flamingo* (*fmi*) gene seems to be important in limiting dendritic growth in *Drosophila* md neurons. The *fmi* gene encodes a seven-pass transmembrane protein in the cadherin superfamily<sup>58</sup>. In *fmi* mutant animals, the dendrites of md neurons overshoot their normal regions, and the growing tips of the dendrites are abnormally dynamic, indicating that some signal limiting the motility and eventual boundary of the dendrite has been perturbed<sup>57</sup>. Moreover, dendrites from homologous md neurons on contralateral sides of the embryo cross the midline and overlap, indicating that a repulsive interaction has been lost<sup>57</sup>. The fact that the Flamingo protein shows a homophilic interaction when expressed in *Drosophila* cell lines<sup>58</sup> along with the fact that Flamingo is present in the dendrites<sup>57</sup> raises the possibility that

Flamingo interactions from the dendrites of one neuron to the dendrites of another mediate the establishment of a boundary. However, because all md neurons express Flamingo, additional specificity factors must exist to distinguish like versus non-like neurons.

Another gene involved in limiting dendritic growth is the small GTPase RhoA. Whereas Cdc42 and Rac1, two other Rho family members, have various effects that generally drive dendritic elaboration (Box 2), RhoA's major function seems to be to limit the extension of dendrites. To date, most studies of RhoA have used constitutively active (CA) and dominant negative (DN) forms. In a broad array of model systems, including chick retinal ganglion cells in explants<sup>46</sup>, *Xenopus in vivo*<sup>45,59</sup>, rat hippocampal slices<sup>48</sup> and *Drosophila* CNS<sup>60</sup>, constitutively active RhoA expression led to a reduction in dendrite length or volume covered by dendrites, whereas DN forms led to increased dendrite length. In addition, loss of function analysis of RhoA in the *Drosophila* mushroom body has shown that mutant neurons no longer respect their dendritic field and overshoot their normal boundaries<sup>60</sup>.

RhoA's signal for controlling dendrite length seems to be transduced through Rho-associated kinase (ROK or ROCK). Whereas expression of constitutively active RhoA leads to a marked reduction in dendrite length in the pyramidal neurons of the rat hippocampus, exposure to a ROCK inhibitor prevents this effect<sup>48</sup>. This suggests that ROCK is necessary for signal transduction from the activated RhoA. Furthermore, an activated form of ROCK can lead to a similar reduction in dendrite length<sup>48</sup>. Thus, ROCK is both necessary and sufficient for limiting dendritic growth mediated by RhoA. ROCK, in turn, seems to carry out its effects by controlling the phosphorylation of myosin light chains<sup>61,62</sup> and actomyosin contractility<sup>63</sup>, both of which could potentially be important for regulating dendritic growth.

Several studies have also indicated that the Notch receptor, a key regulator of cell fate during early development, may be



involved in limiting dendritic growth in post-mitotic neurons (for review, see 44). Using primary cultured neurons, it has been shown that high cell density, or treatment with the Notch ligand (Delta family proteins), activates the Notch receptor and results in inhibition of neurite growth<sup>64</sup>. Whereas no distinction between dendrites and axons was made in this study, it has been shown that activation of Notch results in inhibition of dendrite growth in culture<sup>65</sup>. Several lines of evidence indicate that Notch's effect on dendritic growth inhibition is exerted, at least in large part, through regulation of gene expression<sup>64,65</sup>. First, Notch is increasingly localized to the nucleus as cortical neurons differentiate *in vivo*. Second, expression of the intracellular domain alone (localized to the nucleus and unable to bind to the ligand) results in growth inhibition. Finally, Notch activation leads to a marked increase in reporter gene expression. These observations suggest that a change in the transcription program in neurons may accompany the cessation of global dendritic expansion.

### Summary and perspective

Research over the past few years has begun to give us a glimpse of the sorts of molecules involved in dendritic morphogenesis. As in axons, proper dendrite development relies on extracellular signals, and the signal transduction that lies downstream of them. This signal transduction includes local regulation of dendritic structures via the cytoskeleton, and global regulation mediated through changes in the transcription of target genes. By comparing what is known about axonal and dendritic development (Table 1), we can identify certain similarities and differences. However, we are far from a mechanistic understanding of the different steps of dendritic development.

The identification of molecules involved in dendritic morphogenesis is an essential step toward understanding how dendrites develop. A genetic screen for genes essential in dendritic development has already proven fruitful<sup>8</sup>, and additional screens will expand our list of proteins known to influence dendrite development. Candidate gene approaches using methods that allow visualization and genetic manipulation of individual dendrites (Fig. 1) will also continue to be productive. Candidates could be chosen based on their association with known genes important for dendritic development (Table 1). They could also be picked based on their functions in axonal morphogenesis, thus allowing researchers to study similarities and differences between the development of the two structures. Establishing cellular assays to dissect different steps of dendritic development and test candidate genes in these different processes will be crucial for sorting out the exact functions of genes in dendritic development.

Although we have presented dendritic development as a series of discrete steps, the processes of growth, branching and steering are actually simultaneous and overlapping (Fig. 2). These processes may also coincide with and be stabilized by interactions with axons and formation of synapses<sup>17</sup>. Some molecules seem to have specific functions in just one of these processes, whereas others are important for more than one (Table 1). The degree to which these processes share cellular and molecular mechanisms is still unknown, and will be an interesting topic for study in the future. Finally, whereas this review has focused on the initial development of dendritic structures, plasticity and remodeling of the dendrites are critical for our nervous system to deal with the changing world. There is every reason to believe that some of the mechanisms used in establishing the dendritic tree are also used to allow plasticity in the mature nervous system. Thus, studies of dendritic development may not only shed light on how dendrites are initially sculpted, but may also lend insight into how they are molded by experience.

### ACKNOWLEDGEMENTS

We thank Y.-N. Jan, S. Smith, A. Goldstein and J. Ng for their comments on this review. Work in our lab is supported by grants from the NIH.

RECEIVED 27 DECEMBER 2000; ACCEPTED 13 FEBRUARY 2001

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