The role of Notch and Rho GTPase signaling in the control of dendritic development

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Dendritic patterning exerts a profound influence on neuronal connectivity. Recent studies indicate that mammalian Notch receptors are expressed by postmitotic neurons and that Notch signaling has a considerable influence on dendritic growth and branching. Investigations into the intracellular effectors of dendritic development have revealed that dendritic growth and branching are differentially affected by activation of the Rho-family GTPases, RhoA, Rac1, and Cdc42. These observations suggest that the differential activation of Notch receptors and Rho-family GTPases by extracellular signals may be important in the generation of morphological diversity in the developing nervous system.

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Abbreviations

CBF1	c-promoter binding factor 1
Cdc42	cell division cycle 42
E(spl)	Enhancer of split
LIM	Lin-11, Isl-1, Mec-3
md	multiple dendrite
Notch IC	intracellular domain of Notch
N-WASP	neuronal Wiskott Aldrich syndrome protein
PAK	p21-activated kinase
ROCK	RhoA kinase
Su(H)	Suppressor of Hairless

Introduction

This review will focus on recent advances in our understanding of the molecular regulation of dendritic development. In the first part of the review, we will discuss experiments suggesting that Notch-mediated signaling, previously characterized as being involved in cell-fate decisions, plays an important regulatory role in the specification of dendritic morphology. In the second part of the review, we will consider the evidence that the Rho-family GTPases are critical mediators of dendritic growth and remodeling, and that RhoA, Rac1 and Cdc42 (cell division cycle 42) influence distinct aspects of dendritic patterning.

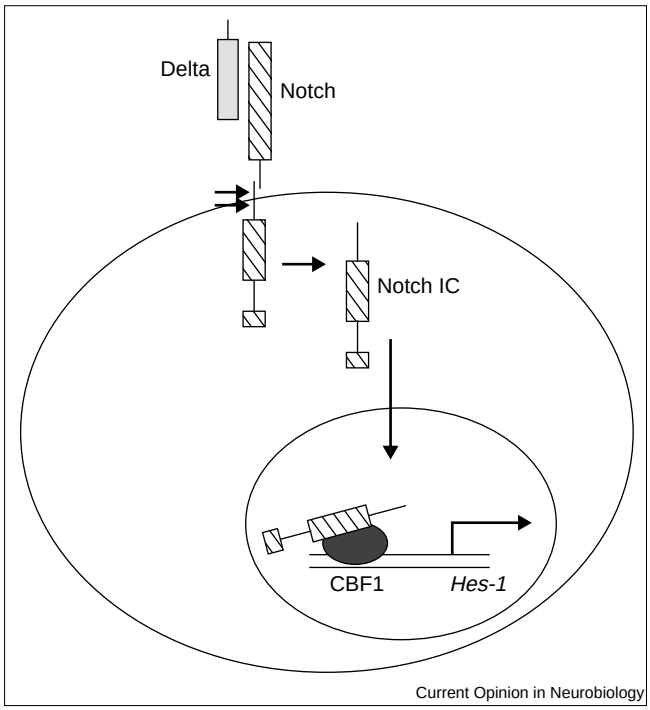
Regulation of dendritic development by Notch signaling

Originally identified in *Drosophila*, Notch is a type I cell-surface protein, approximately 300 kDa in size, which functions as a receptor (for reviews, see [1–3]). Proteolytic processing of full-length Notch generates two fragments that associate at the plasma membrane to form a receptor complex. The mechanism of Notch receptor activation involves cleavage and nuclear translocation of the intracellular domain of the receptor (reviewed in [4]; Figure 1). The intracellular domain of Notch (Notch IC) enters the nucleus and binds the transcription factor Suppressor of Hairless (Su[H]), activating gene transcription. In *Drosophila*, an important gene target of Notch signaling is the Enhancer of split (E[spl]) complex. These genes function as negative regulators of the Achaete-Scute transcription factors, which are known to act as proneural genes (reviewed in [5]). Mammalian homologs of Notch (Notch1–4), the Notch ligands Delta (Delta1–3) and Serrate (Jagged1, Jagged2), and the transcription factors Su(H) (c-promoter binding factor 1 [CBF1]) and E(spl) (Hes1–5) have been isolated ([6–15]; also see [2,5] and references therein). Several of these genes are expressed in the developing brain and spinal cord and are likely to control various aspects of neural development [8–12,16–20].

Notch function has been best characterized in the context of differentiation and cell-fate specification (for a review, see [1]). Despite the dominant view that Notch signaling functions to inhibit differentiation and restrict cell fates, recent observations suggest a role for Notch in regulating developmental events subsequent to specification of cell fate. For example, Notch is present on the growth cones of developing axons in *Drosophila*, and is required for axon guidance in the central nervous system (CNS) and peripheral nervous system (PNS) [21,22]. In addition, overexpression of the intracellular form of Notch in dorsal cluster (DC) neurons, a specific population of neurons in the *Drosophila* CNS, results in a severe reduction of axonal arborization [23•]. An opposite effect is observed by overexpressing the proneural gene, *atonal* [23•]. In a similar manner, the Notch-processing proteins kuzbanian and presenilin are required for proper axon extension and connectivity [24,25•]. In *C. elegans*, loss of presenilin function results in aberrant connectivity and neurite sprouting, defects similar to those seen in Notch mutants [25•]. These observations suggest that Notch signaling can exert an important regulatory influence on the development of postmitotic neurons in invertebrates.

A series of recent studies indicates that mammalian Notch homologs are also expressed in postmitotic neurons, and that Notch signaling plays an important role in the specification of dendritic morphology. Immunocytochemical localization studies indicate that mammalian Notch1 is expressed by both dividing cells in the ventricular zone (VZ) and postmitotic neurons in the cortical plate (CP) [26••,27••]. Interestingly, the subcellular localization of Notch1 appears to change during neuronal differentiation. Whereas Notch1

Figure 1



Diagrammatic representation of Notch signal transduction. Notch signaling involves a series of proteolytic processing events. The primary Notch translational product is cleaved in the secretory pathway by furin convertase to produce a heterodimeric receptor that interacts with ligands (such as Delta) on the cell surface. Two more cleavage events occur in a ligand-dependent manner (arrowheads), the last of which releases the cytoplasmic domain of Notch (Notch IC) from the plasma membrane. Once released, Notch IC translocates to the nucleus where it associates with the transcription factor Su(H)/CBF1 to activate transcription of genes such as *E(spl)/Hes-1*.

is largely excluded from the nucleus in VZ cells, Notch1 IC has a prominent nuclear localization in postmitotic neurons, suggesting that neuronal differentiation is associated with a nuclear translocation of Notch1 IC [26••] (Figure 2a).

Several observations suggest that Notch signaling might mediate contact-dependent inhibition of neurite outgrowth. For example, in postmitotic neurons there is an inverse correlation between Notch1 expression and total neurite length, and overexpression of a constitutively active Notch1 construct leads to a reduction in the total neurite length [27••]. Co-cultures of cortical neurons with Delta- or Jagged-expressing cell lines, or addition of soluble ligands, leads to a decrease in total neurite length, suggesting that Delta or Jagged are the relevant Notch1 ligands [27••]. In addition, overexpression of Numb and Numblike, intracellular modulators that inhibit Notch activation via Su(H)/CBF1, leads to an increase in total neurite length [27••]. Berezovska *et al.* [28••] have also found that expression of constitutively active Notch1 in hippocampal neurons leads to an inhibition of neurite outgrowth. A study examining Notch function in neuroblastoma cells came to a similar conclusion regarding the effects of Notch signaling on neurite length [29•]. Together, these observations indicate that Notch signaling has an inhibitory effect on process outgrowth.

Experiments by Redmond *et al.* [26••] indicate that, in addition to restricting length, Notch signaling in cortical neurons has a major influence on dendritic branching. Redmond *et al.* [26••] examined the effects of Notch1 signaling on dendrite morphology by measuring several parameters of dendrite complexity including process and branch-point number, dendrite length, and branching index. Inhibition of Notch1 signaling by overexpression of a dominant-negative Notch1 construct or with antisense oligonucleotide treatment leads to a decrease in dendritic branching in neurons (Figure 2b,c). Redmond *et al.* [26••] also report that overexpression of a constitutively active Notch1 construct decreases average dendrite length but increases the branching index, resulting in an overall increase in dendritic complexity. Taken together, these experiments reveal a positive role for Notch in dendrite branching and a negative role in dendrite and total-neurite length.

It is not yet known whether Notch regulation of dendritic development involves the same effectors that regulate cell-fate decisions, or if a different set of effector proteins mediate dendritic development. Some of the Notch effects on dendrites may be mediated by molecules previously implicated in cell-fate decisions, as Hes-1, neurogenin-1 and MASH-1 have all been shown to be involved in neuronal differentiation and neurite outgrowth [30–32]. An alternate mechanism for the effects of Notch signaling on process outgrowth has recently been proposed by Giniger [22]. In a series of genetic studies in *Drosophila*, Notch and the tyrosine kinase Abl were shown to interact synergistically in producing axonal defects. In addition, both Notch and Abl are present in the axon, and Notch can biochemically interact with the Abl-interacting protein Disabled. Because Abl and Disabled are thought to influence the actin cytoskeleton, they could provide a link between Notch activation and cytoskeletal changes, thus mediating the effects of Notch signaling on axonal and dendritic morphology.

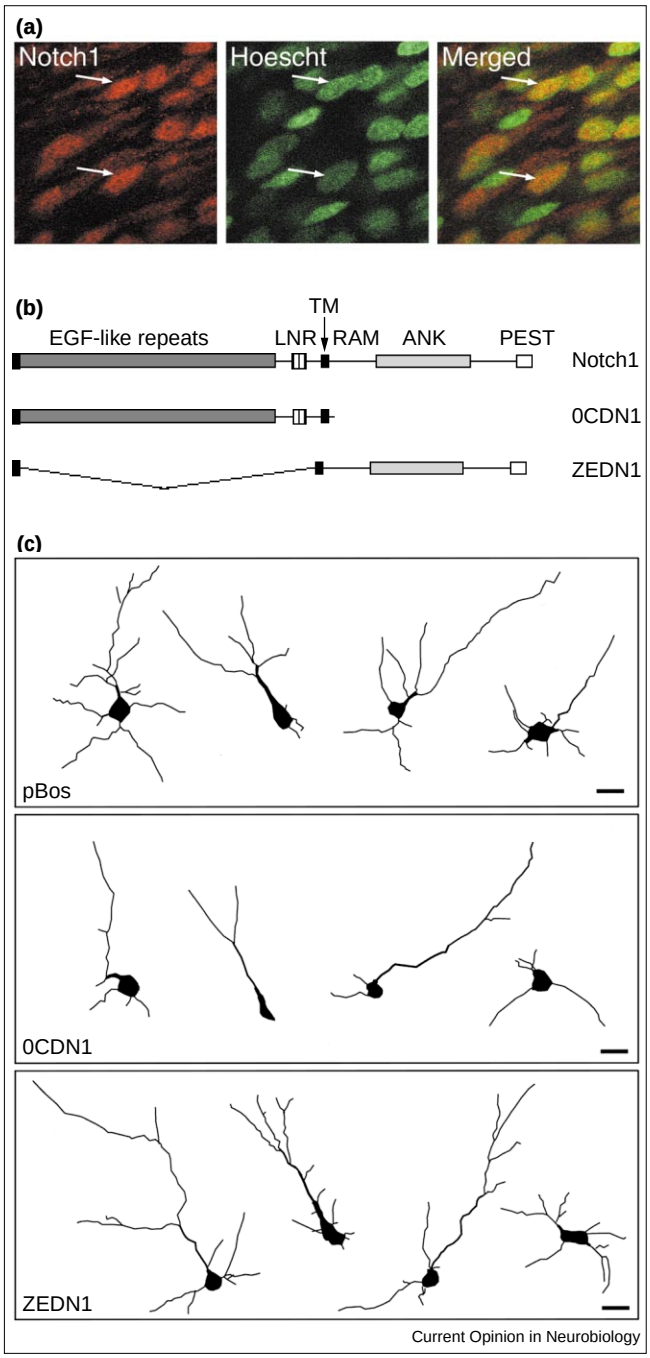
Genetic analysis of dendritic development

In an effort to identify additional genes that may influence dendritic patterning, Gao *et al.* [33••] have recently carried out a genetic screen for dendrite development defects in *Drosophila*. The dendrites of dorsal md (multiple dendrite) neurons in the *Drosophila* PNS are positioned between the body wall muscle and an epithelial layer. The proximity of dorsal md dendrites to the body surface allows them to be easily imaged. Normally, these dendrites extend in two dimensions: dorsal dendrites grow toward the dorsal midline, and secondary lateral dendrites extend along the anterior–posterior axis. These two aspects of dorsal md neuron dendrite development were used as an assay in a mutagenesis screen to identify genes that control different aspects of dendrite morphogenesis.

Figure 2 legend

Effects of Notch signaling on dendritic development (modified from [26••]). (a) Confocal images of sections taken from the cortical plate of a postnatal day 0 rat. The sections were labeled with anti-Notch1 IC (red) and with Hoechst 33258 to reveal the nucleus (green). The merged image shows localization of Notch1 in the nucleus (yellow). (b) Full-length (Notch1) and truncated Notch1 (0CDN1 and ZEDN1) cDNA constructs used in transfection experiments. 0CDN1 does not contain the cytoplasmic domain and acts to inhibit Notch1 signaling. ZEDN1 lacks the extracellular domain and acts as a constitutively active Notch1 receptor. ANK, ankyrin repeats; EGF, epidermal growth factor; LNR, Lin-12/Notch-related repeats; PEST, proline, glutamate, serine, threonine-rich; RAM, RAM23 region; TM, transmembrane domain. (c) Video camera-lucida drawings of the dendritic tree of cortical neurons transfected with β-galactosidase together with parent vector (pBos), 0CDN1, or ZEDN1 at two days *in vitro*, and processed for β-galactosidase immunoreactivity at 5 days *in vitro*. Scale bar is 20 μm.

Figure 2



This screen has led to the identification of several mutations that affect specific aspects of md dendrite growth. For instance, dorsal dendritic branches extend past normal boundaries in *sequoia* and *flamingo* mutants, and exhibit excessive growth in *tumbleweed* mutants. In other mutants such as *shrub*, the extent of dorsal branches are reduced. The extent and number of lateral branches are also reduced in several mutants including *kakapo* and *shrinking violet*. In addition, Dcdc42 overexpression increases the number of dorsal dendrites at the expense of lateral dendrite branches. This effect of Dcdc42 on dendrites is reminiscent of the role of Cdc42 in regulating dendritic development in cortical neurons [34]. This screen also identified genes (*enabled*, *yew* and *cypress*) that are involved in the proper routing of dendrites. One gene, *shrinking violet*, may even be involved in the stabilization of dendrites.

Although several of the genes identified primarily affect dendrite development, some of the mutations affect both axon guidance and dendrite morphogenesis. For example, mutations in genes that have been identified as regulators of the actin cytoskeleton (*Dcdc42*, *enabled* and *kakapo*) alter both axon and dendrite morphology. However, mutations in genes known to affect axon guidance (*roundabout*, *semaphorin*, and *slit*) do not affect md dendrite morphology, suggesting that not all molecular mechanisms are shared between axons and dendrites.

Regulation of dendritic development by Rho-family GTPases

Experiments from various groups over the past three years have provided compelling evidence that Rho-family GTPases (reviewed in [35,36]) are central regulators of dendritic morphology. Most of these experiments have examined the roles of RhoA, Rac1, and Cdc42 in regulating various aspects of dendritic development. Although Rho-family GTPases share significant homology, recent studies have revealed that different family members regulate distinct aspects of dendritic development, such as dendrite initiation, dendrite growth, dendrite branching, and spine formation (summarized in Table 1).

In cortical neurons, Rho-family GTPases play a central role in determining the number of primary dendrites in both pyramidal and non-pyramidal neurons [34]. Dominant-negative mutations of Rac1 lead to a marked decrease in the number of primary dendrites, suggesting that endogenous Rac1 is an important effector of dendrite initiation. Inhibition of Cdc42 also leads to a reduction in the number of primary dendrites, suggesting that dendrite initiation may be mediated by a common effector of Rac1 and Cdc42. The best characterized of these dual specificity effectors are the p21-activated kinase (PAK) family of serine threonine kinases (see Box 1), but PAK kinases have not yet

Table 1

	CA-RhoA	DN-RhoA	CA-Rac1	DN-Rac1	CA-Cdc42	DN-Cdc42
Dendrite initiation						
[34]	increased	no effect	increased	reduced	increased	reduced
Dendrite growth						
[40••]	**reduced**	**increased**	no effect	no effect	no effect	no effect
[41••]	**reduced**	**increased**	no effect	no effect	no effect	no effect
[39••]	**reduced**	**increased**	ND	ND	ND	ND
Dendrite branching						
[41••]	no effect	no effect	**increased**	no effect	no effect	no effect
[42••]	**reduced**	increased	no effect	**reduced**	ND	ND
[43••]	**reduced**	no effect	**increased**	**reduced**	ND	ND
Spine formation						
[43••]	no effect	no effect	**abnormal**	reduced	ND	ND
[47]	ND	ND	**abnormal/ reduced**	ND	ND	ND

Summary of the effects of altering the function of Rho-family GTPases using dominant-negative (DN) and constitutively active (CA) constructs (compared to controls or wild-type constructs) on different aspects of dendritic development. Consistent changes reported by two or more independent studies are indicated in bold. ND, not determined.

been demonstrated to regulate dendrite initiation. Another likely effector of the Rac1 effects on dendrites is LIM-domain-containing protein kinase, which can modulate actin dynamics by phosphorylation of cofilin [37]. The effects of Cdc42 on dendrite initiation may also be mediated by the Cdc42-specific effector neuronal Wiskott Aldrich syndrome protein (N-WASP). Consistent with this possibility, it has been shown that Cdc42 interacts with N-WASP, and that N-WASP enhances filopodia formation in transfected COS7 cells [38].

The role of Rho-family GTPases in dendritic growth and branching has been examined both in invertebrates and vertebrates. The role of Rho in dendritic development in *Drosophila* was examined by selectively visualizing cells lacking Rho function in the developing mushroom body. The most striking phenotype in these cells is an overextension of dendrites in cells lacking Rho function [39••], suggesting that endogenous Rho acts to restrict dendritic growth. Consistent with this possibility, expression of constitutively active mutants of Rho in the mushroom body leads to an inhibition of dendritic growth [39••].

The ability of Rho to restrict dendritic growth appears to be conserved in vertebrates. Expression of constitutively active mutants of RhoA in *Xenopus* retinal ganglion cells leads to a reduction in dendritic length [40••]. Similarly, in the *Xenopus* tectum, activation of RhoA, but not Rac1 or Cdc42, leads to a decrease in dendritic length [41••]. Conversely, expression of a dominant-negative form of RhoA leads to an increase in total dendritic length [41••]. In the chick retina, activated RhoA mutants inhibit dendritic growth in retinal ganglion cells [42••], and activated RhoA leads to an inhibition of dendritic growth in hippocampal neurons [43••]. Thus, activation of RhoA in a number of different cell types appears to limit dendritic growth.

The fact that the growth-restricting effects are associated with RhoA activation, but not with Rac1 or Cdc42 activation, suggests that the RhoA effects are mediated by a RhoA-specific effector. A good candidate for such an effector is RhoA kinase (ROK or ROCK), which is activated by RhoA, but not by Rac1 or Cdc42. Indeed, expression of ROCK in hippocampal slices leads to a dramatic reduction in dendritic growth [43••], and ROCK has been implicated in neurite remodeling in neuroblastoma cells [44–46].

In contrast to dendritic growth, which is most significantly affected by RhoA activity, dendritic branching and remodeling appear to be under the control of Rac1 and Cdc42. Expression of dominant-negative mutants of Rac1 and Cdc42, but not RhoA, leads to a reduction in the dendritic complexity of *Xenopus* retinal ganglion cells [40••]. Time-lapse imaging of *Xenopus* tectal cells has shown that constitutively active mutants of Rac1 lead to increased branch dynamics [41••]. Similar imaging experiments in chick retinal ganglion cells indicate that the addition of tertiary branches is enhanced by constitutively active Rac1 mutants [42••]. In contrast to Rac1 and Cdc42, which exert a positive effect on dendritic branching, RhoA suppresses branch formation [42••,43••]. Dendritic spine formation, which involves cytoskeletal changes similar to branch formation, appears to be regulated by Rac1. Expression of constitutively active Rac1 in Purkinje cells affects spine formation [47], and expression of a dominant-negative Rac1 in hippocampal neurons leads to a progressive elimination of spines [43••]. Thus, Rac1 and Cdc42 appear to be major positive effectors of dendritic branching and spine formation. It will be of interest to determine if the branching effects of Rac1 and Cdc42 are mediated by well-characterized effectors, such as PAK, LIM-kinase, and N-WASP, or if they involve novel effectors.

In addition to affecting particular aspects of dendritic growth, Rho-family GTPases can influence large-scale

Box 1
Regulation of the cytoskeleton by Rho-family GTPases

Much of our knowledge of how Rho-family GTPases are activated, and how they influence the cytoskeleton, is derived from experiments carried out in non-neuronal cells (reviewed in [53]). These mechanisms are also likely to be involved in the regulation of axonal and dendritic development by Rho-family GTPases. This section provides a brief overview of our current understanding of the molecular mechanisms that regulate Rho-family GTPase function.

In addition to RhoA, Rac1, and Cdc42, the Rho GTPase family includes Rnd1/Rho6, Rnd2/Rho7, Rnd3/RhoE, RhoD, RhoG, TC1, and TTF. The role of these GTPases in axonal and dendritic development remains to be explored. RhoA-family GTPases cycle between an active GTP-bound state and an inactive GDP-bound state. The transition from the GDP-bound state to the GTP-bound state is regulated by guanosine nucleotide exchange factors (GEFs; reviewed in [54]). Over 30 GEFs for Rho-family GTPases have been identified. Each of these proteins contains a Dbl-homology (DH) domain that encodes the catalytic activity and a pleckstrin homology (PH) domain. The PH domain is likely to be involved in targeting GEFs to the membrane via its interaction with the phosphoinositols, PIP2 and PIP3.

The Rho proteins have intrinsic GTPase activity, which can allow the GTP-bound molecule to transition to the GDP-bound state. The hydrolysis of GTP is enhanced by the action of GTPase-activating proteins (GAPs), which are closely associated with the GTPase (reviewed in [55]).

Effectors of Rho-family GTPases

During the past few years, a number of Rho GTPase effector proteins have been identified (reviewed in [35]). Most of these proteins interact with the GTP- but not the GDP-bound form of the GTPase. Typically, these effector proteins preferentially interact with one of the GTPases, and it is likely that the specificity of these interactions allows the different GTPases to mediate specific cellular responses. Many of the effectors for Rac1 and Cdc42 contain a CRIB (Cdc42/Rac1-interactive binding) motif that mediates interaction with the GTPases in the GTP bound state. Whereas Rac1 and Cdc42 share several effector proteins, RhoA largely interacts with a non-overlapping set of effectors. This difference in effector specificity may account for the observation that Rho inhibits dendritic growth, whereas Rac1 and Cdc42 principally affect dendritic branching.

The best-characterized RhoA effectors with respect to actin reorganization are ROCKα and ROCKβ (also referred to in the literature as ROK), and Dia1 and 2. Activation of ROCK appears to involve disruption of an autoinhibitory interaction upon binding to Rho-GTP. ROCK is a serine-threonine kinase that mediates at least some of its effects via phosphorylation of myosin light chain. Dia is a scaffolding protein that influences the cytoskeleton via its interaction with profilin.

A common effector for Rac1 and Cdc42 is the PAK family of serine-threonine kinases. PAK1, 2, and 3 have several substrates that might affect the cytoskeleton. One important effector is LIM-kinase, which can phosphorylate the actin-binding protein cofilin and is required for Rac1- and Cdc42-induced cytoskeletal changes [56].

Other than PAK, the major effectors for Cdc42 are WASP and N-WASP. WASP is the gene responsible for Wiskott-Aldrich syndrome. N-WASP is neuronally enriched and is a likely effector for Cdc42 effects on dendrites. N-WASP is a scaffolding protein that probably mediates cytoskeletal changes both by interactions with profilin and by direct interaction with actin.

dendritic remodeling. In the cortex, many neurons initially acquire a pyramidal morphology and undergo a developmentally regulated transformation into non-pyramidal neurons [48]. This transformation can be observed *in vitro* and involves the withdrawal of the apical dendrite and the extension of new primary dendrites [34]. This remodeling of the dendritic tree is largely inhibited *in vitro* by expression of dominant-negative Cdc42 and, to a lesser extent, by dominant-negative Rac1 [34], suggesting that the acquisition of cell-type-specific dendritic morphologies might also be under the control of Rac1 and Cdc42 signaling.

These observations suggest that extracellular signals might influence specific aspects of dendritic development by activating particular Rho-family GTPases. As described earlier, activation of Notch appears to restrict dendritic growth and promote dendritic branching. On the basis of the effects of individual Rho-family GTPases on dendritic development, one would predict that the effects

of Notch on growth inhibition may involve activation of Rho, whereas the effects on dendritic branching may involve activation of Rac1 and Cdc42. Similarly, it has been reported that glutamate receptor stimulation can induce local spine formation [42••,49,50]. Because activation of Rac1 also leads to spine formation [42••,47], it will be of interest to determine whether synaptic-activity-dependent spine formation is mediated by Rac1 signaling.

Conclusions

The molecular mechanisms that regulate dendritic development are gradually being elucidated. Recent studies indicate that Notch signaling can exert a major effect on dendritic growth and branching. It is not yet known whether the effects of Notch on dendrite growth and branching are mediated by a common set of effector molecules, or whether they involve different signaling pathways. The growth-restrictive effects of Notch contrast with the growth-promoting effects of some neurotrophins [51,52], and suggest that extracellular signals exert both positive and negative influences on dendritic growth. It is likely that the effects of extracellular signals on dendritic development are mediated at least in part by Rho-family GTPases, which have emerged as major regulators of dendritic growth and remodeling. It is now apparent that dendrite initiation, dendrite growth and dendritic branching are differentially affected by activation of RhoA, Rac1, and Cdc42 (Table 1), and it will be of interest to determine whether different extracellular signals selectively regulate the function of specific GTPases. In addition to these biochemical approaches, the genetic screens being carried out in *Drosophila* for mutations affecting dendrite development should also provide further mechanistic insight into the molecular control of dendritic development.

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