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Neuronal polarization *in vivo*: Growing in a complex environment

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Neurons are one of the most polarized cell types in the body. During the past three decades, many researchers have attempted to understand the mechanisms of neuronal polarization using cultured neurons. Although these studies have succeeded in discovering the various signal molecules that regulate neuronal polarization, one major question remains unanswered: how do neurons polarize *in vivo*?

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Recently, researchers have focused on exploring the molecular mechanisms that govern neuronal polarization *in vivo*. In this review, we first introduce the polarization process of embryonic cortical neurons and then discuss the microenvironment for neuronal polarization. Finally, we survey the responsible molecular mechanisms, including both extracellular and intracellular signals for neuronal polarization *in vivo*.

Introduction

Cell polarity is important for the development and maintenance of tissues and organs. Neurons appear to be the most highly polarized cell types in the body. In most cases, neurons possess two distinct types of processes: axons and dendrites. Axons and dendrites differ from each other in their morphology, function and composition of proteins and organelles. Axons are typically long and thin and contain synaptic vesicles to transmit signals to other neurons. Dendrites are relatively short and thick and contain neurotransmitter receptors that receive chemical signals from other neurons. Therefore, neurons are morphologically and functionally polarized. The mechanism by which neurons establish their polarity is a fundamental issue in neuroscience that is fascinating to many scientists.

Although the fundamental structure of neurons was described in the early 20th century, modern studies of neuronal polarization started in the late 20th century. The first paper that used the phrase 'neuronal polarity' was published in 1986 by Dr Banker and his colleagues [1], following which they described the morphological development of cultured neurons in detail [2]. They categorized cultured hippocampal neurons into five stages based on their morphology (stages 1–5). On the basis of these definitions, many researchers attempted to discover how neurons establish their polarity. In contrast to the extensive studies *in vitro* using cultured neurons, the studies of neuronal polarity *in vivo* are only now under exploration. Recently, the electroporation method was utilized for gene transfer to the embryonic rodent brain [3,4]. Using this method, several researchers have begun to investigate neuronal polarization in the embryonic cerebral cortex.

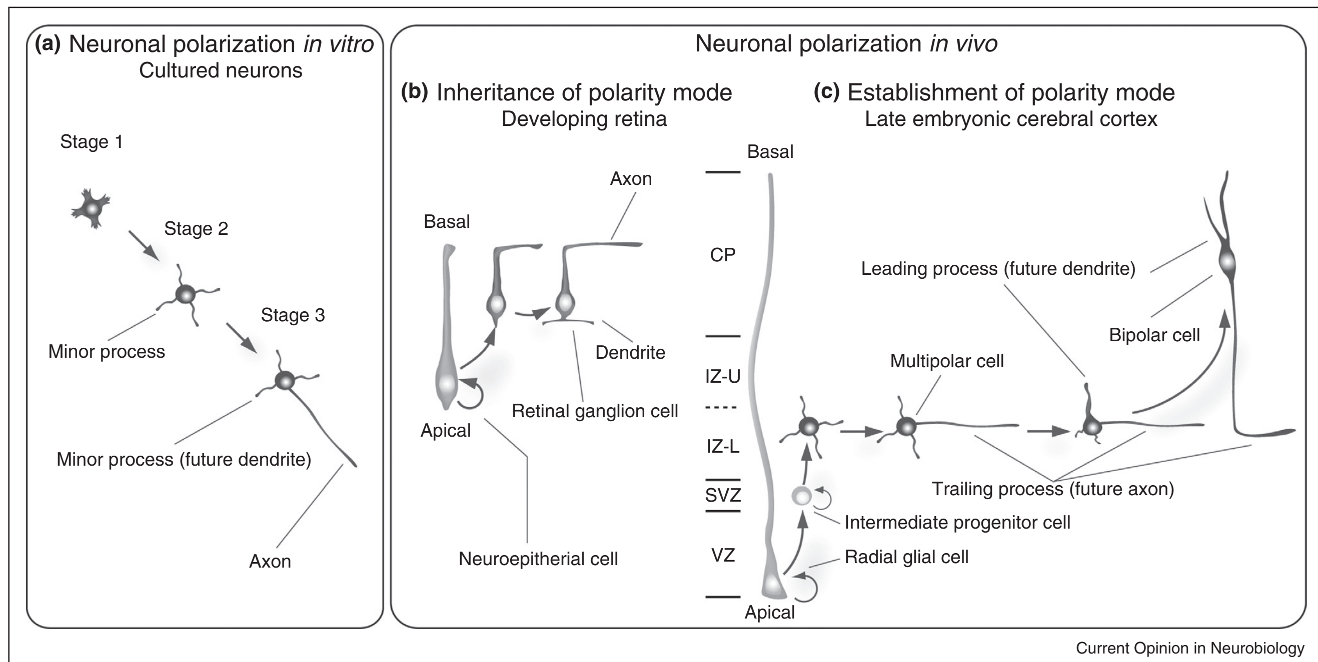
In this review, we summarize the current knowledge about the development of neuronal polarity *in vivo* and its underlying molecular network in the mammalian brain.

Development of neuronal polarity *in vivo*

Cultured embryonic hippocampal neurons have been frequently used to study neuronal polarity (Figure 1a). On the basis of Banker's definition, there are at least five developmental stages [2]. First, shortly after the plating of the dissociated neurons, hippocampal neurons form several thin filopodia (stage 1). Within several hours, the neurons form multiple immature neurites called minor processes (stage 2). The neurites are equivalent to each other and undergo repeated, random growth and retraction. One day after plating, the neurons begin to break their symmetric morphology. One of these minor processes initiates a rapid elongation and becomes much longer compared with the other neurites (stage 3). This neurite finally develops into an axon. Therefore, the initial event during neuronal polarization in cultured neurons is axon specification.

The developmental stages during the polarization processes of neurons in the brain differ from cultured neurons. The polarization processes are roughly divided into two modes: the 'inheritance of polarity' mode and the 'establishment of polarity' mode. The 'inheritance of

Figure 1



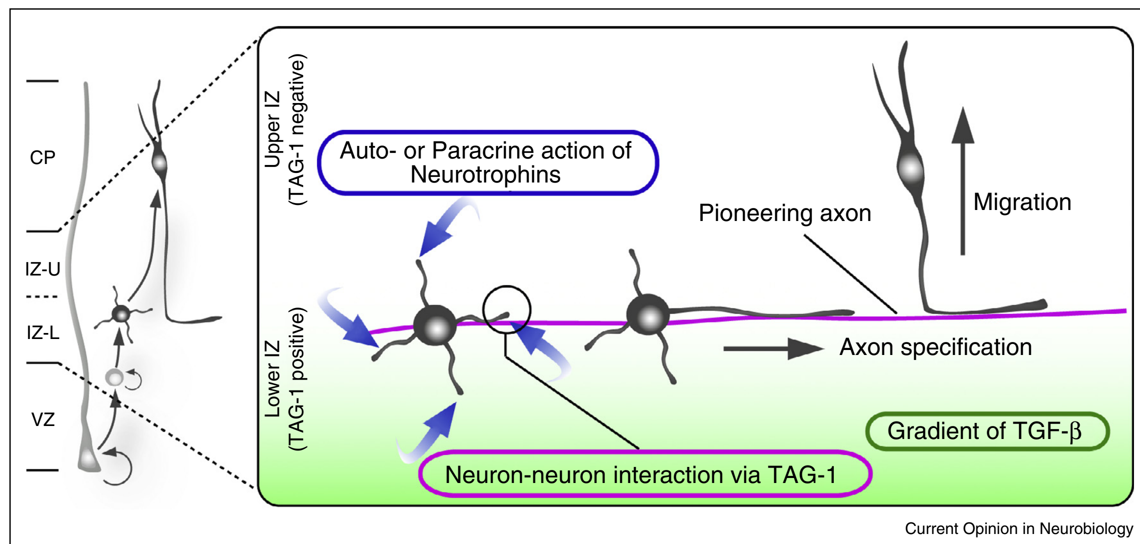
Neuronal polarization *in vitro* and *in vivo*. **(a)** The stage 1 neurons form several thin filopodia. The stage 2 neurons form multiple minor processes. The stage 3 neurons possess a single axon and several minor processes. Therefore, the initial event during neuronal polarization in cultured neurons is axon specification. **(b)** The retinal ganglion cells are directly derived from the retinal neuroepithelial cells. Then, the apical process eventually develops into a dendrite, and the basal process becomes an axon. **(c)** Most pyramidal neurons are generated from the radial glial cells in the ventricular zone (VZ) through intermediate progenitor cells in the subventricular zone (SVZ). The newly generated neurons then extend multiple neurites similar in the lower part of the intermediate zone (IZ-L). The multipolar cells first extend the trailing process tangentially and then generate the leading process. Finally, the multipolar cells transform into bipolar cells and migrate toward the cortical plate (CP) via the upper part of the intermediate zone (IZ-U). The trailing process (future axon) formation is the initial step of neuronal polarization.

polarity' mode exists in vertebrate retinal ganglion cells, which are directly derived from the retinal neuroepithelial cells and from which they inherit their apicobasal polarity [5,6] (Figure 1b). Therefore, the apical process eventually develops into a dendrite, and the basal process becomes an axon [6]. In this process, multipolar cells do not appear. Interestingly, when the polarity of the retinal neuroepithelial cells is impaired, the retinal ganglion cells show a multipolar morphology [6–8].

In the mid to late stage of neocortex development, neurons establish their polarity in the 'establishment of polarity' mode (Figure 1c). Most pyramidal neurons are generated from neural stem cells called radial glial cells in the ventricular zone (VZ) through intermediate progenitor cells in the subventricular zone (SVZ) [9]. First, the newly generated neurons extend multiple neurites similar to the stage 2 cultured neurons in the lower part of the intermediate zone (IZ); thus, they are called multipolar cells. Most of the multipolar cells first extend the trailing process tangentially, which develops into an axon, and then generate the leading process, a future dendrite [10••,11••,12]. These neurons are called bipolar cells. The bipolar cells migrate toward the cortical plate (CP) and develop into mature pyramidal neurons. The transition from multipolar to bipolar is a critical step of neuronal polarization *in vivo*. In most cases, the trailing process (future axon) formation is the initial step of neuronal polarization, which is similar to cultured neurons. The radial glial cells, which are the descendants of neuroepithelial cells, possess the same apicobasal polarity as the epithelial cells [9]. However, the intermediate progenitor cells detach from both apical and basal surfaces and therefore lose their polarity [13,14]. Because neocortical pyramidal neurons are derived from the unpolarized intermediate progenitor cells, they cannot inherit polarity and can form multipolar morphologies [9]. Thus, these neurons establish their polarity again during the multipolar-to-bipolar transition.

Recent studies suggest that several types of microenvironmental cues are involved in this process (Figure 2). Secreted factors, such as neurotrophins [15•] and transforming growth factor β (TGF-β) [16••], act in autocrine or paracrine manners to regulate neuronal polarization *in vivo* (see below). Another microenvironmental cue is cell-to-cell interactions [10••]. Here, we proposed a novel mechanism of neuronal polarization *in vivo* named 'Touch and Go'. As described

Figure 2



The microenvironment for neuronal polarization *in vivo*. Neurotrophins, such as BDNF and NT-3, act in autocrine or paracrine manners to regulate neurite formation (blue). A gradient of TGF-β concentration along the radial axis regulates the radial axon extension (green). The pioneering axons from early-born neurons play a critical role in neuronal polarization (magenta). Once one of the neurites of a multipolar cell contacts the pioneering axons through TAG-1, the neurite is stabilized and extends rapidly. Then, the multipolar cells transform into bipolar cells.

above, the multipolar cells repeatedly extend and retract immature neurites in the lower IZ, where many tangentially oriented axons of early-born neurons exist. Once one of the neurites of a multipolar cell 'touches' the pioneering axons from the early-born pyramidal neurons, the neurite is stabilized and extends rapidly (go). Finally, the longest neurite becomes an axon. A more recent study showed that an immunoglobulin superfamily cell adhesion molecule, named transient axonal glycoprotein-1 (TAG-1), is involved in this process. The downstream signal of TAG-1 will be discussed in the next section.

In summary, in addition to the basal signal from secreted factors, the cell-to-cell interaction between the immature neurites of multipolar cells and the pioneering axons might lead to the selection of one immature neurite to become an axon.

Signals of neuronal polarization

Cell extrinsic signals

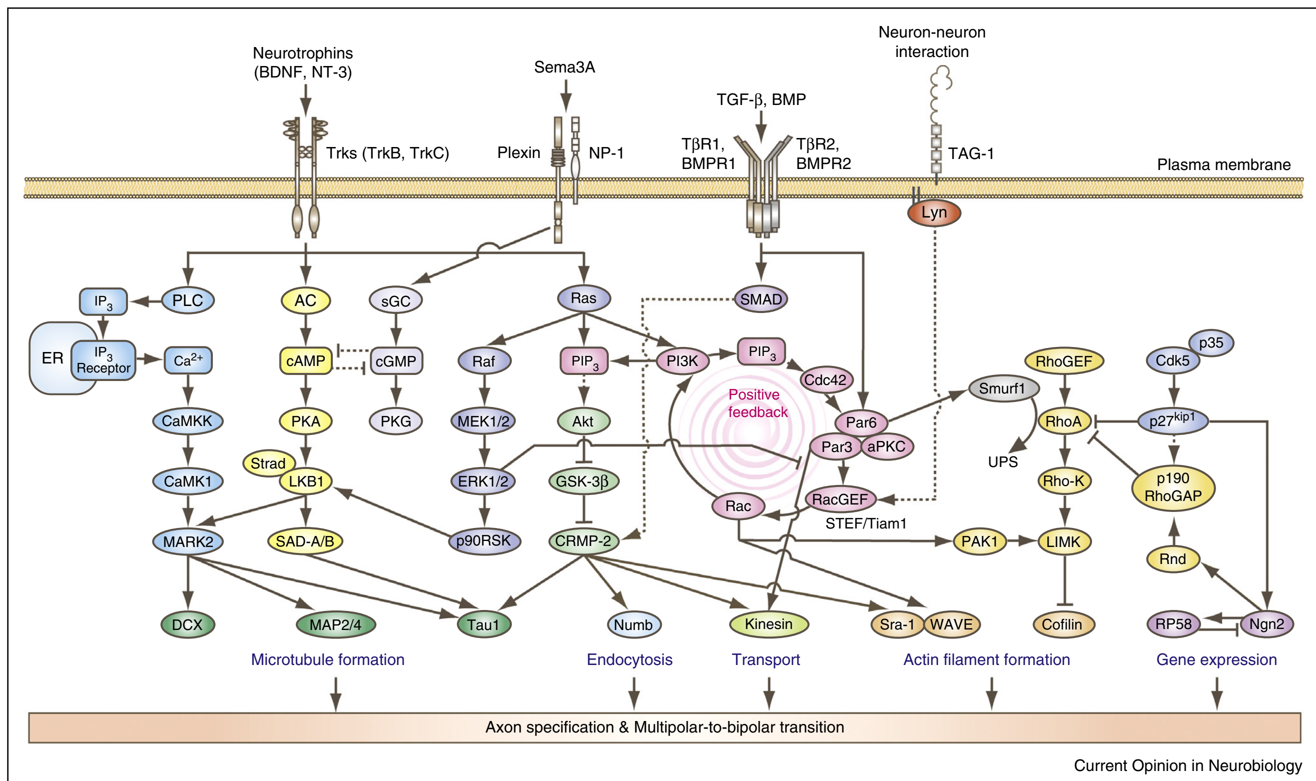
Accumulating evidence suggests that extracellular factors, such as neurotrophins, Wnts, insulin-like growth factor 1 (IGF-1), TGF-β, Semaphorin and Reelin, extracellular matrices, such as laminin, and cell adhesion molecules (TAG-1 and L1) promote axon specification in cultured neurons and *in vivo* [17–19] (Figure 3). Here, we focus on the neurotrophins, TGF-β and TAG-1.

Neurotrophins

Neurotrophic signaling pathways that underlie the establishment of neuronal polarity have been well studied using cultured neurons. Neurotrophins, such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), act on their tropomyosin-related kinase receptors (Trks) to activate phosphoinositide 3-kinase (PI3K), presumably through Ras, thereby producing phosphatidylinositol 3,4,5-trisphosphate (PIP_3) [20]. PIP_3 is concentrated at the tip of the nascent axon and inhibiting PI3K activity prevents axon specification in cultured neurons [21,22]. PIP_3 indirectly activates Akt, and activated Akt inactivates glycogen synthase kinase-3β (GSK-3β), leading to the activation of microtubule-associated proteins (MAPs), such as collapsin response mediator protein-2 (CRMP-2) and Tau1 [23,24]. It has been shown that CRMP-2 regulates tubulin polymerization, actin remodeling, endocytosis and axon-directed transport through its binding to tubulin, Sra-1, Numb and the kinesin light chain subunit of kinesin-1, respectively, thus contributing to axon determination and elongation in cultured neurons [17]. Tubulin polymerization and the subsequent microtubule stabilization are the critical events of axon specification. A previous study demonstrated that the future axon has more stable microtubules in its shaft and that the stabilization of microtubules induces axon formation in cultured neurons, which suggests that the stabilization of microtubules is sufficient for axon specification [25].

The functional analysis of neurotrophins and their downstream effectors in neuronal polarization *in vivo* has recently been developed. In the developing neocortex, neurons electroporated with the combination of the dominant

Figure 3



Signaling pathways involved in neuronal polarization *in vivo*. See text for details. AC, adenyl cyclase; aPKC, atypical protein kinase C; BDNF, brain-derived neurotrophic factor; BMP, bone morphogenetic protein; CaMKK, Ca^{2+}/calmodulin-dependent protein kinase kinase; cAMP, cyclic adenosine monophosphate; Cdc42, cell division cycle 42; Cdk5, cyclin-dependent kinase 5; cGMP, cyclic guanosine monophosphate; CRMP-2, collapsin response mediator protein 2; DCX, doublecortin; ER, endoplasmic reticulum; ERK1/2, extracellular signal-regulated kinase 1/2 (mitogen-activated protein kinase 1/2); GSK-3β, glycogen synthase kinase 3β; IP_3, inositol 1,4,5-trisphosphate; LIMK, LIM domain kinase; Lyn, v-yes-1 Yamaguchi sarcoma viral related oncogene homolog; MAP2/4, microtubule-associated protein 2/4; MARK2, microtubule affinity-regulating kinase 2; MEK1/2, mitogen-activated protein kinase kinase 1/2; Ngn2, neurogenin 2; NP-1, neuropilin-1; NT-3, neurotrophin-3; PAK, p21 activated kinase; Par3, partitioning defective 3; Par6, partitioning defective 6; PIP_3, phosphatidylinositol-3,4,5-trisphosphate; PI3K, phosphatidylinositol 3-kinase; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; PLC, Phospholipase C; $p27^{Kip1}$, cyclin-dependent kinase inhibitor 1B; p35, cyclin-dependent kinase 5, regulatory subunit 1; RSK, Ribosomal S6 Kinase; Sema-3A, semaphorin 3A; sGC, soluble guanylate cyclase; Smurf1, SMAD specific E3 ubiquitin protein ligase 1; Sra-1, specifically Rac1-associated protein; STEF, Sif and Tiam1-like exchange factor; Strad, STE20-related kinase adaptor; TAG-1, transient axonal glycoprotein-1; TβR1, the type I TGF-β receptor; TβR2, the type II TGF-β receptor; Tiam1, T-cell lymphoma invasion and metastasis 1; Trks, tropomyosin-related kinase receptors; UPS, ubiquitin–proteasome system; WAVE, Wiskott-Aldrich syndrome protein (WASP)-family verprolin homologous protein 1.

negative forms of TrkB and TrkC under the control of Cre recombinase and the Tα promoter failed to transition from the multipolar to bipolar stage [15•]. Furthermore, knockdown of TrkB impaired neuronal migration [26]. However, there is a lack of evidence for an *in vivo* role of the neurotrophins and Trks in axon specification using knockout mice. The inhibition of CRMP-2 function by gene knockdown or a dominant negative mutant impairs neuronal migration and multipolar-to-bipolar transition [27]. Furthermore, a recent study has shown that precise regulation of CRMP-2 phosphorylation downstream of TrkB is important for the multipolar-to-bipolar transition during neuronal migration [26]. Few *in vivo* studies of CRMP-2 using knockout mice are available. Although CRMP-2-S522A knock-in mice, in which CRMP-2 cannot be phosphorylated by cyclin-dependent kinase 5 (Cdk5), have been generated, no axonal defects have been reported [28]. In the CRMP-1-knockout background, CRMP-2-S522A knock-in mice show a severely abnormal dendritic patterning [28]. During the early developmental stage, all CRMPs (CRMP-1–5) are highly expressed in the nervous system. Therefore, a single knockout of CRMPs may not be sufficient to cause axonal defects most likely due to a compensatory regulation of the other CRMPs. Further studies using single and multiple knockout mice are required.

On the other hand, neurotrophins stimulate downstream effectors, including phospholipase C (PLC), which activates Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK) by the induction of Ca^{2+} [15•], and the production of cyclic AMP, which activates LKB1 via cAMP-dependent protein kinase (PKA) [29•]. The details are

shown below (cell intrinsic signals). These pathways may play pivotal roles in neurotrophin-dependent axon specification.

TGF-β super family proteins

TGF-β is a pleiotropic morphogen that governs a wide variety of cellular processes. Because the expression of TGF-β2 is highly restricted to the VZ, there is a gradient of TGF-β2 concentration along the radial axis [16••] (Figure 2). The TGF-β superfamily receptors are comprised of two groups, the type I and the type II serine/threonine kinases. Electroporating mouse embryos that harbored the homozygous floxed alleles of the type II TGF-β receptor (TβR2) with GFP and Cre recombinase in the developing neocortex retarded the neuronal migration and failed to form axons, despite the extension of a leading-edge process [16••]. The effect of TGF-β signaling on the neuronal migration and axon specification depends on the phosphorylation of partitioning defective 6 (Par6) at S345 by TβR2 [16••]. This phosphorylation of Par6 leads to the local recruitment of the ubiquitin ligase Smurf1 and the subsequent degradation of RhoA [30], which suggests that the local stoichiometry of Rho GTPases can be modified by TGF-β signaling to alter local actin organization. Bone morphogenetic protein (BMP) is one of the TGF-β superfamily members, and the BMP–SMAD signaling pathway suppresses the transcription of CRMP-2 during brain development [27]. Therefore, TGF-β signaling may regulate neuronal polarization in two independent pathways, including the local regulation of RhoA protein degradation and the transcriptional regulation of CRMP-2 expression.

Cell adhesion molecule

TAG-1 is a GPI-anchored protein that has been implicated in axon fasciculation and guidance [31,32]. TAG-1 is strongly expressed in the lower IZ, where neuronal polarization occurs [10••]. Both multipolar cells and pioneering axons express TAG-1, which suggests that TAG-1 might mediate cell-to-cell interactions between the multipolar cells and pioneering axons (Figure 2). The shRNA-mediated gene knockdown of TAG-1 in multipolar cells impaired axon specification and the multipolar-to-bipolar transition, and these phenotypes were rescued by the shRNA-resistant WT TAG-1, but not by the cell adhesion-deficient mutants of TAG-1 [10••].

Because TAG-1 is a GPI-anchored protein, it cannot activate intracellular signal molecules directly [31,32]. There are two candidate mechanisms of outside-in signaling by TAG-1: one mechanism involves cis-associated TAG-1 proteins and another mechanism involves Src family kinases (SFKs) in the lipid rafts [31]. Several cis-associated TAG-1 proteins, such as L1, NrCAM and contactin-associated protein-like 2 (CASPR2), have been identified; however, none of these molecules appear to regulate the TAG-1-mediated neuronal polarization *in vivo*. Several SFKs, such as Src, Fyn and Lyn, co-localize with TAG-1 in lipid rafts [33,34]. Of these SFKs, Lyn is activated by TAG-1 in cultured neurons [33] and mediates the TAG-1-mediated neuronal polarization *in vivo* [10••]. Because previous studies have suggested that Lyn activates the small GTPase Rac1, the TAG-1→Lyn signal activates Rac1 in neurons to induce polarization (Figure 3). In summary, homophilic TAG-1-mediated cell adhesion between the multipolar cells and pioneering axons induces neuronal polarization through Lyn and Rac1 [10••].

Cell intrinsic signals

Rho family of small GTPases

It is known that the Rho family of small GTPases, such as Rac1, Cdc42 and RhoA, act as key intracellular regulators in the morphological changes of various cells by reorganizing the cytoskeleton [35]. Rho GTPases serve as molecular switches by cycling between GDP-bound inactive and GTP-bound active states; once activated, they transduce signals to a variety of specific downstream effectors, thereby reorganizing the cytoskeleton (Figure 3).

In the developing neocortex, neurons electroporated with either constitutively active (CA) Rac1 or dominant negative (DN) Rac1 showed retarded migration and multipolar-to-bipolar transition [36]. Neurons electroporated with T-cell lymphoma invasion and metastasis 1 (Tiam1), Sif and Tiam1-like exchange factor (STEF/Tiam2) or P-Rex1, which are Rac1 guanine nucleotide exchange factors (GEFs), exhibited similar phenotypes, which suggests that the balance between Rac1 activity and GTPase cycling is required for polarization and migration *in vivo* [36–38]. In Rac1 conditional knockout mice crossed with Foxg1-Cre mice, Rac1-deficient neurons delayed the radial migration, although the migration defect was milder compared with the DN Rac1-electroporated brains [39]. In Rac1 conditional knockout mice crossed with Nestin-Cre mice, Rac1-deficient cerebellar granule neurons, which do not express other Rac isoforms, showed drastic changes in the localization of the WAVE complex and impaired neuronal migration and axon formation both *in vivo* and *in vitro* [40•].

Cdc42 also plays a critical role in axon formation. In Cdc42 conditional knockout mice crossed with Nestin-Cre mice, the ability of the neurons to form axons both *in vivo* and in culture was strongly suppressed [41•]. The disruption of axon formation may be due to increased levels of phosphorylated (inactive) cofilin, which is a regulator of actin dynamics and phosphorylated by LIM kinase. Because cofilin regulates neurite formation, cofilin-knockout neurons in the developing neocortex did not form a ‘multipolar’ morphology [42].

Partitioning defective 3 (Par3) is a key molecule in the Rac1 and Cdc42 signaling pathway [17–19]. Neuron-specific knockdown of Par3 by RNA interference

impaired neuronal polarization in cultured hippocampal neurons and cortical projection neurons *in vivo* [43,44•]. Par3 forms a complex with Par6 and aPKC, called Par-complex, and mediates Cdc42-induced Rac1 activation through the specific Rac GEFs Tiam1 and STEF/Tiam2 [45,46]. Because PI3K activates Cdc42 and the activated Rac1 binds to PI3K, the PI3K→Cdc42→Par-complex→Rac1→PI3K pathway is thought to be a positive-feedback loop for neuronal polarization. Par3 also regulates the selective transport of the Par-complex to the distal part of growing axons. The transport of the Par-complex into the axon is regulated by the direct interaction of Par3 and KIF3A [47]. A recent study showed that this interaction is regulated by the phosphorylation of Par3 by extracellular signal-regulated kinase 2 (ERK2) and is important for neuronal polarization *in vivo* [44•]. Because a mid-gestational embryonic lethality caused by a defective epicardial development was observed in the ubiquitous Par3 knockout mice [48], the cell autonomous roles of Par3 in neurons have not been frequently analyzed using the knockout mice. Therefore, neuron-specific Par3 knockout mice are required for the future analysis of Par3.

RhoA is typically associated with the destabilization of the actin cytoskeleton and myosin-based contractility. The constitutively active form of RhoA inhibits neuritogenesis, whereas a DN form of RhoA enhances neurite outgrowth [49]. Neurons electroporated with RhoA block radial migration, whereas interfering with RhoA function promotes migration. In RhoA conditional knockout mice crossed with FoxG1-Cre mice, a RhoA deficiency resulted in the disruption of adherens junctions, hyper proliferation of neural progenitors, and brain dysplasia [50]. However, there is still no analysis of the *in vivo* role of RhoA in axon specification. RhoA signaling is downregulated by Cdk5 and the Cdk inhibitor p27 (Kip1) during radial migration. Cdk5 phosphorylates and stabilizes p27 and maintains the amount of p27 in post-mitotic neurons [51]. The knockdown of p27 by *in utero* electroporation decreases the F-actin concentration, which leads to poor and thin multipolar processes [51,52]. The Cdk5-p27 pathway activates cofilin through the suppression of RhoA activity and regulates multipolar cell morphology [51].

The Rnd subfamily comprises a new branch of the Rho family of GTPases, and Rnd1, Rnd2 and Rnd3/RhoE have been identified as its members. Unlike other Rho family members, the Rnd proteins are constitutively active and are thought to be regulated primarily at the time of expression [53]. During embryonic neocortex development, the Rnd2 expression is intense in the basal/intermediate progenitors and the migrating neurons in the SVZ and IZ, weak in the VZ progenitors, and sharply downregulated in the CP [54,55]. Rnd2-deficient neurons failed to leave the IZ and displayed long processes and multipolar morphology, which suggests that Rnd2 is critical for the multipolar-to-bipolar transition [54,55]. The proneural transcription factor Neurogenin2 induces the expression of Rnd2, which is predominately localized in the early endosome and may regulate the trafficking of membrane-associated molecules that promote the multipolar-to-bipolar transition [55,56]. Recent studies have shown that the zinc-finger transcriptional repressor RP58 regulates the expression of Rnd2 through the negative regulation of Neurogenin2 expression. RP58-deficient neurons did not repress the expression of Neurogenin2 and Rnd2 and thereby failed to undergo the multipolar-to-bipolar transition [57,58].

Kinases

Par1/MARK2

Par1 is a Ser/Thr kinase [59], and its mammalian ortholog was originally identified as microtubule affinity-regulating kinase (MARK) [60]. MARK2 phosphorylates multiple substrates, some of which are MAPs, which change their affinity to microtubules following MARK2 phosphorylation. The substrates include MAP2/4, Tau1 and DCX [61]. Neurons electropolated with the MARK2 shRNA were stalled in the IZ with multipolar morphology *in vivo* [62•]. The addition of MARK2 kinase-dead enabled the transition from the multipolar stage to the bipolar stage, but did not rescue the migration phenotype, which suggests that MARK2 kinase independent activity is essential for the multipolar-to-bipolar transition [62•]. However, MARK2 knockout mice have no obvious phenotype in axon specification, which might be due to genetic redundancy with the other three MARKs (MARK1, 3 and 4) [63].

Par4/LKB1 and SAD kinases

The SAD-1 kinase was identified as a regulator of neuronal morphogenesis in *C. elegans* [64]. The SAD kinases phosphorylate Tau1 S262, which is highly phosphorylated in dendrites but not in axons. Double knockout mice for SAD-A and SAD-B kinases showed a loss of axons and abnormally orientated dendrites *in vivo* [65]. One of the upstream kinases of the SAD kinases is a Ser/Thr kinase named LKB1, a mammalian ortholog of Par4 [66]. The conditional disruption of the LKB1 gene using Emx-Cre mice led to a severe loss of axon formation in cortical neurons but did not impact neuronal migration [67•]. Additionally, the knockdown of LKB1 using siRNAs also prevented axon formation *in vivo* [29]. Notably, the depletion of LKB1 did not affect the multipolar-to-bipolar transition but did impair axon formation [29,67•]. Neurotrophins, such as BDNF and NT-3, can activate PKA or p90RSK. When LKB1 is activated by the phosphorylation at S431 by PKA or p90RSK and interacts with the pseudokinase STRAD, LKB1 phosphorylates SAD-A/B kinases and, most likely, MARK1-4 [29].

A recent study has shown that local cAMP and cGMP activities exert antagonistic actions on axon/dendrite polarization [68]. Semaphorin-3A (Sema3A), a secreted protein

of the class III semaphorin superfamily, elevated cGMP, but reduced cAMP and PKA and suppressed axon formation by the downregulation of the PKA-dependent phosphorylation of LKB1 [69]. Sema3A and BDNF exert antagonistic actions on axon/dendrite specification, and these effects are mediated by the reciprocal regulation of cAMP/cGMP signaling and downstream kinases, such as LKB1 [69].

CaMKK/CaMKI

Calcium is a ubiquitous second messenger involved in various developmental processes of neurons, including polarization. NT-3 induced a rapid increase in calcium ions (Ca^{2+}) in an inositol 1,4,5-trisphosphate (IP_3)-dependent fashion, and this increment activated CaMKK locally in the growth cone [15•]. CaMKK and its target CaMKI have been implicated in axon elongation [70,71,72] and neuronal polarization [15•] in cultured neurons. The accurate balance of CaMKK and CaMKI activation is important for the multipolar-to-bipolar transition *in vivo* [15•]. CaMKI phosphorylates MARK2 within its kinase domain, activates MARK2 kinase activity and promotes neurite outgrowth [70]. Furthermore, treatment with Muscimol, a $GABA_A$ receptor agonist, promoted axonal growth via the CaMKK-CaMKI pathway, which suggests that different upstream factors regulate CaMKK activity [73].

Conclusion

In contrast to cultured neurons, neurons *in vivo* are surrounded by a heterogeneous environment consisting of various cell types, extracellular matrices and secreted factors. These microenvironmental cues are finely coordinated and orchestrate intracellular signaling pathways. The question of how neurons integrate various extracellular signals and transform into polarized cells remains elusive. More comprehensive analyses, including proteomic analyses, genome editing and computational methods, will help us address this issue.

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