© 2015. Published by The Company of Biologists Ltd | Development (2015) 142, 2088-2093 doi:10.1242/dev.114454

DEVELOPMENT AT A GLANCE

Neuronal polarization

Tetsuya Takano, Chundi Xu, Yasuhiro Funahashi, Takashi Namba and Kozo Kaibuchi*

ABSTRACT

Neurons are highly polarized cells with structurally and functionally distinct processes called axons and dendrites. This polarization underlies the directional flow of information in the central nervous system, so the establishment and maintenance of neuronal polarization is crucial for correct development and function. Great progress in our understanding of how neurons establish their polarity has been made through the use of cultured hippocampal neurons, while recent technological advances have enabled *in vivo* analysis of axon specification and elongation. This short review and accompanying poster highlight recent advances in this fascinating field, with an emphasis on the signaling mechanisms underlying axon and dendrite specification *in vitro* and *in vivo*.

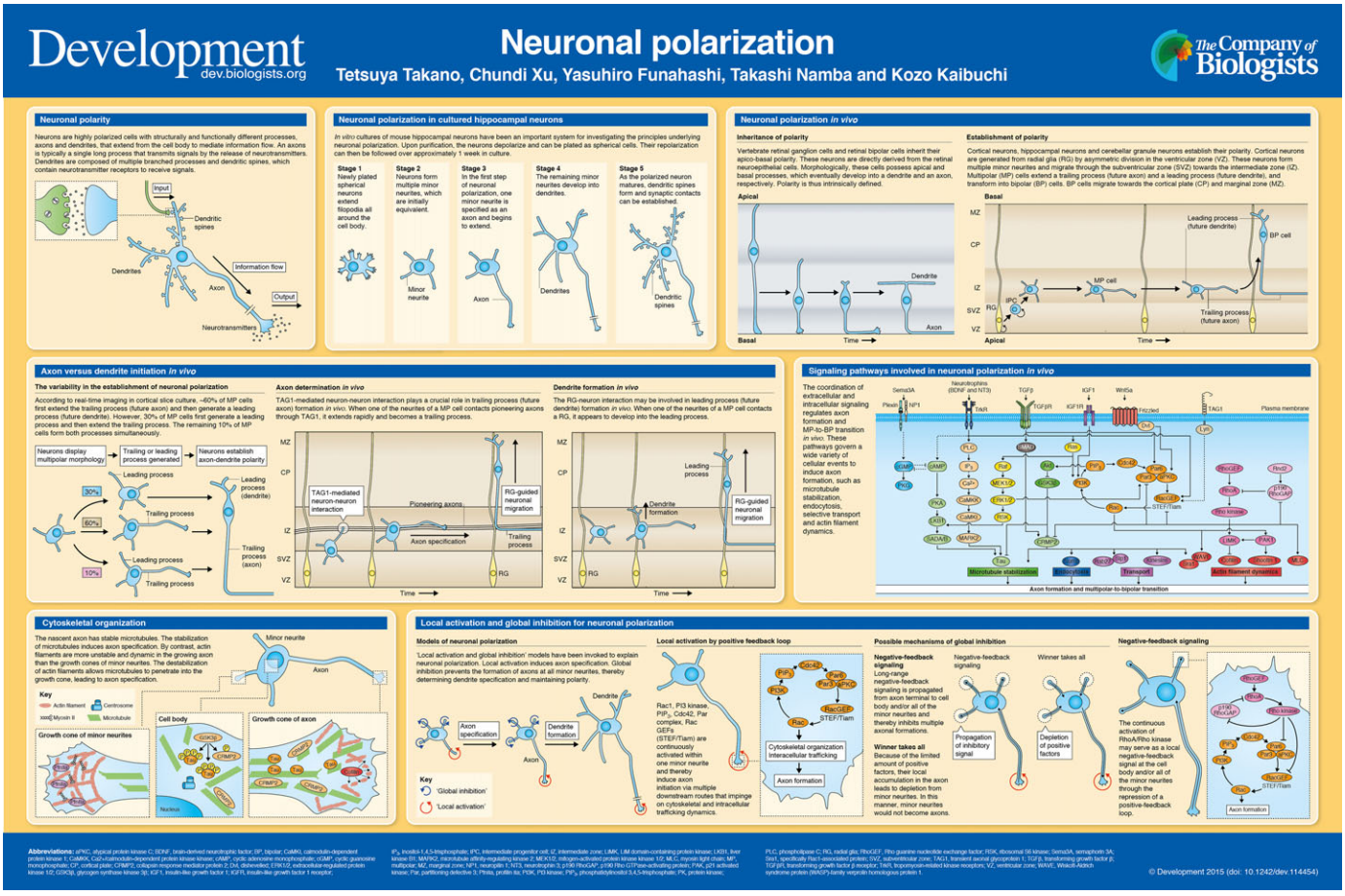
KEY WORDS: Neuronal polarity, Cellular asymmetry, Axon outgrowth, Dendrite outgrowth, Multipolar to bipolar transition

Department of Cell Pharmacology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan.

*Author for correspondence (kaibuchi@med.nagoya-u.ac.jp)

Introduction

Cell polarization is crucial for the development and correct functioning of many cell types, generating morphological and functional asymmetry in response to intrinsic and extrinsic cues. Neurons are one of the most highly polarized cell types, as they possess structurally and functionally different processes, axons and dendrites, that extend from the cell body (soma) to mediate information flow through the nervous system. An axon is typically a single long process that transmits signals to other neurons by the release of neurotransmitters. Dendrites are composed of multiple branched processes and dendritic spines, which contain neurotransmitter receptors to receive signals from other neurons. The formation and maintenance of such distinct cellular compartments are crucial for the proper development and physiology of the nervous system. How do neurons establish and maintain their polarity? Past decades have seen remarkable progress in understanding the molecular mechanisms responsible for mammalian neuronal polarization, predominantly through work performed on cultured hippocampal neurons (Arimura and Kaibuchi, 2007; Barnes and Polleux, 2009; Tahirovic and Bradke, 2009). More recent efforts have begun to explore the roles of



Development (2015) 142, 2088-2093 doi:10.1242/dev.114454

extracellular and intracellular signaling molecules, and their effectors, on neuronal polarization *in vivo*, using electroporation, and knockout and knock-in mice. It has become clear that both extracellular signaling and intrinsic mechanisms are responsible for the establishment and maintenance of neuronal polarity. Here and in the accompanying poster, we summarize the current understanding of how neuronal polarization generates functional neurons, focusing on the mammalian cerebral cortex and cultured hippocampal neurons as the primary model systems, and discuss the future challenges faced by this field.

Overview of neuronal polarization *in vitro* and *in vivo*

Banker and colleagues established dissociated rodent hippocampal neurons as a basic model system for neuronal polarity (Dotti et al., 1988; Craig and Banker, 1994). The morphological changes of cultured neurons are divided into five stages. Upon isolation, hippocampal neurons retract their processes, so that their development *in vitro* begins with round spheres that spread filopodia (stage 1; shortly after plating). These neurons subsequently form several minor neurites (stage 2; days 0.5-1.5), which show characteristic alternations of growth and retraction. The major polarity event occurs when one of these equivalent minor neurites grows rapidly to become the axon (stage 3; days 1.5-3). The next steps are the morphological development of the remaining short minor neurites into dendrites (stage 4; days 4-7) and the functional polarization of axons and dendrites, including dendritic spine formation (stage 5; >7 days in culture).

In contrast to cultured neurons, the polarization processes of neurons *in vivo* have different properties depending on brain region and developmental stage. For example, vertebrate retinal ganglion cells and retinal bipolar cells inherit their polarity (Barnes and Polleux, 2009). When born, they possess a neuroepithelium-like morphology, with apical and basal processes that eventually develop into a dendrite and an axon, respectively (Barnes and Polleux, 2009). By contrast, cortical and hippocampal pyramidal neurons, and cerebellar granule neurons establish their polarity during differentiation (Noctor et al., 2004; Solecki et al., 2006; Funahashi et al., 2014). Cortical pyramidal neurons are generated in the ventricular zone (VZ) and migrate through the subventricular zone towards the intermediate zone (IZ) (Miyata et al., 2004; Noctor et al., 2004). They extend multiple minor neurites and are called multipolar (MP) cells (Miyata et al., 2004; Noctor et al., 2004). One of the minor neurites grows rapidly to become a trailing process, and another develops into a leading process, which finally develop into an axon and a dendrite, respectively (Miyata et al., 2004; Noctor et al., 2004). The remaining minor neurites are retracted and MP cells subsequently transform into bipolar (BP) cells in the IZ. BP cells are completely polarized and migrate towards the cortical plate (CP). Although neuronal polarization can occur in parallel with neuronal migration, how these two processes are coordinated remains elusive. Polarization of neurons in the cerebral cortex serves as a well-studied model for polarity establishment *in vivo* (Funahashi et al., 2014), and the processes regulating it are discussed in detail below.

Axon versus dendrite initiation *in vivo*

The MP-to-BP transition is a crucial step during neuronal polarization *in vivo*. Time-lapse imaging of cortical slice cultures has revealed variability in the establishment of neuronal polarization in the developing neocortex. Almost 60% of MP cells first extend the trailing process (future axon) and then generate a leading process (future dendrite), whereas ∼30% of MP cells first generate a leading process and then extend the trailing process (Hatanaka and Yamauchi, 2013; Namba et al., 2014). The remaining 10% of MP cells form both processes simultaneously (Namba et al., 2014). We have recently proposed a novel model of axon initiation *in vivo* called the 'Touch & Go' model (Namba et al., 2014; Funahashi et al., 2014). According to this model, once a minor neurite of a MP cell 'touches' the pioneering axons of early born neurons, it extends rapidly ('goes') and develops into an axon. The cell-adhesion molecule transient axonal glycoprotein 1 (TAG1; CNTN2 – Mouse Genome Informatics) is involved in this process through Src family kinase Lyn-induced Rac1 activation. Thus, the TAG1-Lyn-Rac1 signal plays a role in axon specification through cell-to-cell interactions in the developing cortex (Namba et al., 2014).

However, the molecular mechanisms underlying the formation of a leading process in 30% of MP cells are largely unknown. Several reports suggest that the radial glial cell-neuron interaction is involved not only in neuronal migration but also in the formation of a leading process (Kawauchi et al., 2010; Jossin and Cooper, 2011; Gärtner et al., 2012). N-cadherin has been shown to regulate radial glial-guided neuronal migration (Kawauchi et al., 2010). In the developing cortex, the knockdown or expression of a dominant-negative mutant form of N-cadherin disrupts neuronal migration and the MP-to-BP transition (Kawauchi et al., 2010; Jossin and Cooper, 2011). Moreover, inhibition of N-cadherin by a dominant-negative mutant exhibits abnormal morphology of the leading process, thereby impairing neuronal polarization (Gärtner et al., 2012). Based on these results, the N-cadherin-mediated glial cell-neuron interaction may regulate the MP-to-BP transition, particularly formation of the leading process.

Signaling pathways involved in neuronal polarization

Neuronal polarization is precisely regulated by environmental cues in the extracellular matrix such as TAG1 and secreted factors such as neurotrophins [brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT3)], transforming growth factor β (TGFβ), Wnt5A, insulin-like growth factor 1 (IGF1) and semaphorin 3A (Funahashi et al., 2014). These secreted factors regulate axon specification in cultured neurons (Nakamuta et al., 2011). In the developing cortex, TGFβ and neurotrophins have been shown to be involved in neuronal polarization (Yi et al., 2010; Cheng et al., 2011; Nakamuta et al., 2011).

Type II TGFβ receptor (TβR2; Tgfbr2 – Mouse Genome Informatics) conditional knockout mice are defective in axon formation *in vivo* (Yi et al., 2010). TβR2 phosphorylates partitioning-defective 6 (Par6) at Ser345, contributing to axon formation (Yi et al., 2010). Par6 forms a protein complex with Par3 and atypical protein kinase C (aPKC), leading to axon formation through Rac1 activation (Nishimura et al., 2005; Arimura and Kaibuchi, 2007). The expression pattern of TGFβ in the developing cortex is graded, being highest in the VZ, and is thought to reflect the direction of axon initiation (Yi et al., 2010). However, time-lapse imaging of cortical slice cultures shows that MP cells extend their trailing process in any direction and then migrate towards the CP, leaving behind the trailing process and resulting in axon extension towards the VZ (Namba et al., 2014). Therefore, the gradient of secreted factors may be involved in the directional migration and axon elongation, rather than in axon specification *in vivo*.

Neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT3) are major regulators underlying neuronal polarization *in vivo* (Cheng et al., 2011; Nakamuta et al., 2011). Amplification of BDNF levels in an autocrine or paracrine manner

Development (2015) 142, 2088-2093 doi:10.1242/dev.114454

induces axon specification in cultured neurons (Cheng et al., 2011), whereas inhibition of the neurotrophin receptors TrkB and TrkC (Ntrk2 and Ntrk3) by the expression of a dominant-negative mutant or by knockdown disrupts the MP-to-BP transition (Nakamuta et al., 2011). More recent results show that local exposure to BDNF causes the accumulation of $p75^{NTR}$ (Ngfr – Mouse Genome Informatics) a receptor able to bind all of the neurotrophins, at a minor neurite, thereby leading to axon specification and elongation. Furthermore, knockout or knockdown of $p75^{NTR}$ disrupts axon formation *in vivo* (Zuccaro et al., 2014). These findings suggest that neurotrophin signaling has a vital role in neuronal polarization.

Intracellular signaling in axon specification

The neurotrophic signals that regulate neuronal polarization are transduced through at least four different pathways: the Rac1 activation pathway, the Ras-mediated-pathway, the cyclic adenosine 3′,5′-monophosphate (cAMP)-liver kinase B1 (LKB1; Stk11 – Mouse Genome Informatics) pathway and the Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK; Camkk1 – Mouse Genome Informatics)-calmodulin-dependent protein kinase I (CaMKI; Camk1 – Mouse Genome Informatics) pathway (Tahirovic and Bradke, 2009; Cheng and Poo, 2012; Funahashi et al., 2014). TrkB phosphorylates and activates Tiam1, a Rac1 guanine exchange factor (GEF; positive regulators of Rho family proteins), leading to axon specification through Rac1 activation *in vitro* (Miyamoto et al., 2006).

Ras is known to regulate axon specification in cultured neurons (Arimura and Kaibuchi, 2007). Activation of PI3 kinase by Ras produces phosphatidylinositol 3,4,5-trisphosphate (PIP_3). PIP_3 indirectly activates Akt, thereby inactivating glycogen synthase kinase 3β (GSK3β) through its phosphorylation at Ser9. The inactivation of GSK3β leads to axon specification through the activation of microtubule-associated proteins (MAPs) such as Tau (Mapt – Mouse Genome Informatics) and collapsin response mediator protein 2 (CRMP2; Dpysl2 – Mouse Genome Informatics) (Jiang et al., 2005; Yoshimura et al., 2005). CRMP2 governs a wide variety of cellular events, such as microtubule assembly, the endocytosis of adhesion molecules through Numb, the reorganization of actin filaments through the Sra1/WAVE1 complex and the trafficking of TrkB-containing vesicles during axon formation through interaction with the Slp1/Rab27/kinesin complex (Kawano et al., 2005; Arimura et al., 2009). In culture, expression of CRMP2 induces the formation of multiple axons, whereas the inhibition of CRMP2 impairs axon formation (Inagaki et al., 2001). In the developing cortex, expression of a dominant-negative mutant form or knockdown of CRMP2 impairs neuronal migration and MP-to-BP transition (Sun et al., 2010).

BDNF-induced elevation of cAMP leads to axon specification via protein kinase A (PKA)-dependent phosphorylation of the serine/threonine kinase LKB1 (Guo and Kemphues, 1995). Expression of LKB1 induces the formation of multiple axons, whereas knockdown of LKB1 prevents axon specification *in vitro* (Barnes et al., 2007; Shelly et al., 2007). Moreover, *Lkb1*-knockout mice also exhibit impairment of axon formation, while neuronal migration in the cerebral cortex is unaffected (Barnes et al., 2007). Phosphorylation of LKB1 at Ser431 by PKA induces its local activation in the nascent axon (Shelly et al., 2007). LKB1 then phosphorylates SADA/B kinases (Brsk2/Brsk1 – Mouse Genome Informatics) and microtubule affinity-regulating kinase 2 (MARK2) (Lizcano et al., 2004; Barnes et al., 2007). SADA/B kinase and MARK2 regulate microtubule dynamics through the phosphorylation of Tau. *Brsk1*/*Brsk2* double knockout neurons have a mixed axon/dendrite identity (Kishi et al., 2005; Barnes et al., 2007), and the developing cortex of *Brsk1*/*Brsk2* double knockout mice shows a loss of axons and abnormally orientated dendrites (Kishi et al., 2005). In addition, the downregulation of MARK2 by shRNA also impairs the MP-to-BP transition (Sapir et al., 2008). The cAMP-dependent phosphorylation of LKB1 is also involved in $GABA_B$ receptor-induced axon/dendrite outgrowth and neuronal migration *in vivo* (Bony et al., 2013). However, $LKB1^{S431A/S431A}$ knock-in mice exhibit no overt phenotype and unchanged SADA/B activity (Houde et al., 2014).

NT3 stimulates inositol-1,4,5-trisphosphate (IP_3)-induced Ca^{2+} release and thereby leads to the activation of the CaMKK-CaMKI pathway during neuronal polarization in culture (Nakamuta et al., 2011). A rapid increase in Ca^{2+} level induced by NT3 in an IP_3-dependent manner leads to the local activation of CaMKK at the tip of nascent axons (Nakamuta et al., 2011). The inhibition of CaMKK attenuates NT3-induced axon specification in cultured hippocampal neurons (Nakamuta et al., 2011). *In vivo*, developing cortical neurons expressing a dominant-negative form of CaMKK show impaired axon specification though neuronal migration is unaltered (Nakamuta et al., 2011). CaMKI, which acts downstream of CaMKK, has been shown to regulate axon specification and axon elongation (Wayman et al., 2004; Uboha et al., 2007). Together, these four pathways, perhaps along with others, play crucial roles in defining the future axon, and thus in determining neuronal polarity.

Rho family proteins in neuronal polarity

Members of the Rho family of small GTPases, such as Rac1, Cdc42 and RhoA, are major regulators of cytoskeletal dynamics (Hall et al., 1993; Fukata et al., 2003). Rac1 plays a vital role in axon specification through regulation of actin polymerization. The expression of either the constitutively active or dominant-negative form of Rac1 produces a delay in neuronal migration and a loss of leading and trailing processes (Kawauchi et al., 2003). Moreover, the expression of Rac-specific GEFs, such as STEF/Tiam1 or P-Rex1, also inhibits neuronal migration, suggesting that a balance of Rac1 activity is required for MP-to-BP transition and migration *in vivo* (Kawauchi et al., 2003). Conditional Rac1-deficient cerebellar granule neurons exhibit impaired neuronal migration and axon formation due to impaired localization of the actin regulator WAVE at the plasma membrane of the growth cone (Tahirovic et al., 2010).

The small GTPase Cdc42 is a key regulator of multiple aspects of neuronal development, including filopodial extension in growth cones (Schwamborn and Puschel, 2004; Arimura and Kaibuchi, 2007; Witte and Bradke, 2008). Conditional knockout of Cdc42 in the cortex and hippocampus leads to reduced embryonic cortex size and results in lethality at birth with impairment in axon formation (Garvalov et al., 2007). PI3 kinase regulates activation of Cdc42, and activated Cdc42 interacts with the Par complex (Nishimura et al., 2005). This interaction leads to Rac1 activation through Rac-specific GEFs (STEF/Tiam2 and Tiam1) and Rac1 then further activates PI3 kinase (Nishimura et al., 2005). Therefore, the PI3 kinase/Cdc42/Par complex/Rac1 pathway appears to represent a positive-feedback loop that functions as a driving force for axon formation (Arimura and Kaibuchi, 2007).

The small GTPase RhoA is another polarity regulator and modulates actin cytoskeleton and myosin-based contractility. Several lines of evidence suggest that RhoA is a negative regulator of neuritogenesis, including axon specification (Da Silva et al., 2003; Conde et al., 2010). A constitutively active form of RhoA inhibits the growth of minor neurite processes in hippocampal neurons (Threadgill et al., 1997; Conde et al., 2010),

Development (2015) 142, 2088-2093 doi:10.1242/dev.114454

whereas the inactivation of RhoA enhances neurite extension (Da Silva et al., 2003; Schwamborn and Puschel, 2004). Conditional knockout of RhoA in the midbrain results in the disruption of apical adherens junctions, hyper-proliferation of neural progenitors and massive dysplasia of the brain (Katayama et al., 2011). However, the role of RhoA in neuronal polarization *in vivo* remains to be elucidated. Inhibition of Rho kinase (ROCK), a downstream effector protein of RhoA, also enhances axonal elongation and induces the formation of multiple axons (Da Silva et al., 2003). Rho kinase phosphorylates and activates LIM domain-containing protein kinase (LIMK), which induces the inactivation of cofilin (Aizawa et al., 2001). The Rho GTPase Rnd2 regulates the MP-to-BP transition *in vivo* through the suppression of RhoA (Pacary et al., 2011). Therefore, the activity of RhoA is important for the establishment of neuronal polarity. Interestingly, RhoA activity is higher in the growth cones of minor neurites of polarized neurons than in the growing axon (Gonzalez-Billault et al., 2012). Rho kinase phosphorylates and inactivates p190 RhoGAP, a member of GTPase-activating proteins (GAPs; negative regulators of Rho family proteins), leading to sustained RhoA activation (Mori et al., 2009). Thus, the continuous activation of RhoA/Rho kinase might be required not only for axon specification but also for the maintenance of neuronal polarity by preventing the formation of multiple axons.

Cytoskeletal organization

Neuronal polarization is driven by cytoskeletal organization, primarily through microtubule and actin dynamics (Arimura and Kaibuchi, 2007; Barnes and Polleux, 2009). The stabilization of microtubules is crucial for the axon specification *in vitro* (Bradke and Dotti, 1999; Witte and Bradke, 2008). The stabilization of microtubules is regulated by MAPs such as Tau and CRMP2. Tau protects microtubules from the microtubule-serving proteins (Witte and Bradke, 2008). Phosphorylation of Tau by GSK3β suppresses its ability to bind microtubules and thereby inhibits the stabilization of microtubules (Wagner et al., 1996; Kimura et al., 2014). Phosphorylation of CRMP2 by GSK3β also inhibits its affinity to αβ-tubulin heterodimers (Yoshimura et al., 2005).

In contrast to microtubules, actin filaments are more unstable and dynamic in the growing axon than the growth cones of minor neurites *in vitro* (Bradke and Dotti, 1999; Witte and Bradke, 2008). The destabilization of actin filaments by severing proteins such as cofilin allows microtubules to penetrate into the growth cone, thereby leading to axon specification (Bradke and Dotti, 1999; Flynn et al., 2012). Conversely, myosin II and profilin IIa stabilize actin filaments at the minor neurites to prevent the formation of multiple axons through interfering with the penetration of microtubules (Kollins et al., 2009; Da Silva et al., 2003). Shootin 1 regulates axon outgrowth through actin dynamics in the growing axon in a p21 protein (Cdc42/Rac)-activated kinase 1 (PAK1)-dependent manner (Toriyama et al., 2013). Thus, the coordinated regulation of microtubules and actin filaments plays a crucial role in neuronal polarization.

Local activation and global inhibition for neuronal polarization

The involvement of 'local activation and global inhibition' models for establishment of neuronal polarity has been extensively proposed (Arimura and Kaibuchi, 2007; Inagaki et al., 2011). 'Local activation' is thought to induce axon specification and promote axon elongation, whereas 'global inhibition' is thought to prevent the formation of multiple axons, thereby determining dendrite specification and maintaining polarity. Several molecules, such as Rac1, PI3 kinase, PIP_3, Cdc42, Par complex and Rac-specific GEFs (STEF/Tiam), act as positive regulators of axon specification (Arimura and Kaibuchi, 2007). These positive regulators are continuously activated within one minor neurite and thereby induce axon initiation via multiple downstream routes that impinge on cytoskeletal and intracellular trafficking dynamics (Arimura and Kaibuchi, 2007; Takano et al., 2012).

However, the molecular mechanism of global inhibition remains a mystery. One of the major models proposed is long-range negative-feedback signaling, which is propagated from axon terminal to cell body and/or all minor neurites and thereby inhibits minor neurite outgrowth (Arimura and Kaibuchi, 2007). The continuous activation of RhoA/Rho kinase appears to be involved in this process. Rho kinase phosphorylates Par3 and in turn disrupts the Par complex, thereby abrogating Rac1 activation (Nakayama et al., 2008). Rho kinase also suppresses the STEF-induced Rac1 activation (Takefuji et al., 2007). Thus, RhoA/Rho kinase may serve as a local negative-feedback signal in the cell body and/or all minor neurites, through repression of the positive-feedback loop. However, the long-range negative-feedback signaling from the axon that leads to RhoA/Rho kinase activation remains largely unknown. Local elevation of cAMP in a neurite of the unpolarized neuron also might generate a long-range negative-feedback signaling that results in the reduction of cAMP in all other neurites (Shelly et al., 2010). Recently, a global inhibitory regulatory mechanism has been proposed: because growth-promoting factors are limited, a local accumulation of these factors in the nascent axon could deplete them in other minor neurites without a long-range negative-feedback signal (Inagaki et al., 2011).

According to these paradigms, dendrite specification could be a default pathway in the absence of axon specification in cultured neurons. In the developing cortex, however, one of the minor neurites of a MP cell develops into the leading process. Additional dendrite-specific processes caused by environmental cues might be required for neuronal polarization *in vivo* as described above. The elucidation of the molecular mechanisms inducing global inhibition to determine dendrite specification is a crucial issue in neuronal polarity research.

Conclusion

The establishment of neuronal polarization is essential for establishing proper neuronal circuits and functions. A large number of studies both *in vitro* and *in vivo* have uncovered a complicated signaling network regulating neuronal polarity (Arimura and Kaibuchi, 2007; Barnes and Polleux, 2009; Tahirovic and Bradke, 2009). However, despite intensive study, it is still unclear how neurons generate only one axon and multiple dendrites. In particular, the molecular mechanisms of global inhibition underlying the maintenance of neuronal polarity remain elusive, and there may be unknown molecular machinery functioning to prevent the formation of multiple axons and in turn to induce dendritic outgrowth. One reason for this major gap in our knowledge is the lack of suitable methodologies for investigating the spatiotemporal regulation of the signaling molecules responsible for negative-feedback signaling. Future challenges will entail exploring these issues using advances in imaging technology, genetic model systems and innovative experimental approaches.

Despite our incomplete understanding, the molecular mechanisms identified thus far seem to be widely used and evolutionarily conserved (Solecki et al., 2006; Doe and Kaibuchi,

Development (2015) 142, 2088-2093 doi:10.1242/dev.114454

2011). Therefore, our understanding of the molecular mechanisms leading to neuronal polarization is likely to provide new insights into the development of brain circuitry.

Acknowledgements
We apologize to authors whose work we are unable to cite due to space limitations.

Competing interests
The authors declare no competing or financial interests.

Funding
This work was supported by the Japan Society for the Promotion of Science KAKENHI (A) [25251021 to K.K.]; by the Ministry of Education, Culture, Sports, Science and Technology KAKENHI on Innovative Areas 'Neural Diversity and Neocortical Organization' [23123507 to K.K.]; and by the Japan Society for the Promotion of Science Grant-in-Aid for Young Scientists (B) [26830045 to T.T.]. Part of this study is the result of the 'Bioinformatics of Brain Sciences' carried out under the Strategic Research Program for Brain Sciences by Ministry of Education, Culture, Sports, Science and Technology [to K.K.].

Development at a Glance
A high-resolution version of the poster is available for downloading in the online version of this article at http://dev.biologists.org/content/142/12/2088/F1.poster.jpg

References

Aizawa, H., Wakatsuki, S., Ishii, A., Moriyama, K., Sasaki, Y., Ohashi, K., Sekine-Aizawa, Y., Sehara-Fujisawa, A., Mizuno, K., Goshima, Y. et al. (2001). Phosphorylation of cofilin by LIM-kinase is necessary for semaphorin 3A-induced growth cone collapse. *Nat. Neurosci.* **4**, 367-373.

Arimura, N. and Kaibuchi, K. (2007). Neuronal polarity: from extracellular signals to intracellular mechanisms. *Nat. Rev. Neurosci.* **8**, 194-205.

Arimura, N., Kimura, T., Nakamuta, S., Taya, S., Funahashi, Y., Hattori, A., Shimada, A., Ménager, C., Kawabata, S., Fujii, K. et al. (2009). Anterograde transport of TrkB in axons is mediated by direct interaction with Slp1 and Rab27. *Dev. Cell* **16**, 675-686.

Barnes, A. P. and Polleux, F. (2009). Establishment of axon-dendrite polarity in developing neurons. *Annu. Rev. Neurosci.* **32**, 347-381.

Barnes, A. P., Lilley, B. N., Pan, Y. A., Plummer, L. J., Powell, A. W., Raines, A. N., Sanes, J. R. and Polleux, F. (2007). LKB1 and SAD kinases define a pathway required for the polarization of cortical neurons. *Cell* **129**, 549-563.

Bony, G., Szczurkowska, J., Tamagno, I., Shelly, M., Contestabile, A. and Cancedda, L. (2013). Non-hyperpolarizing GABAB receptor activation regulates neuronal migration and neurite growth and specification by cAMP/LKB1. *Nat. Commun.* **4**, 1800.

Bradke, F. and Dotti, C. G. (1999). The role of local actin instability in axon formation. *Science* **283**, 1931-1934.

Cheng, P.-L. and Poo, M.-M. (2012). Early events in axon/dendrite polarization. *Annu. Rev. Neurosci.* **35**, 181-201.

Cheng, P.-L., Song, A.-H., Wong, Y.-H., Wang, S., Zhang, X. and Poo, M.-M. (2011). Self-amplifying autocrine actions of BDNF in axon development. *Proc. Natl. Acad. Sci. USA* **108**, 18430-18435.

Conde, C., Arias, C., Robin, M., Li, A., Saito, M., Chuang, J. Z., Nairn, A. C., Sung, C. H. and Caceres, A. (2010). Evidence for the involvement of Lfc and Tctex-1 in axon formation. *J. Neurosci.* **30**, 6793-6800.

Craig, A. M. and Banker, G. (1994). Neuronal polarity. *Annu. Rev. Neurosci.* **17**, 267-310.

Da Silva, J. S., Medina, M., Zuliani, C., Di Nardo, A., Witke, W. and Dotti, C. G. (2003). RhoA/ROCK regulation of neuritogenesis via profilin IIa-mediated control of actin stability. *J. Cell Biol.* **162**, 1267-1279.

Doe, C. Q. and Kaibuchi, K. (2011). Neuronal polarity in 2011. *Dev. Neurobiol.* **71**, 401-402.

Dotti, C. G., Sullivan, C. A. and Banker, G. A. (1988). The establishment of polarity by hippocampal neurons in culture. *J. Neurosci.* **8**, 1454-1468.

Flynn, K. C., Hellal, F., Neukirchen, D., Jacob, S., Tahirovic, S., Dupraz, S., Stern, S., Garvalov, B. K., Gurniak, C., Shaw, A. E. et al. (2012). ADF/cofilin-mediated actin retrograde flow directs neurite formation in the developing brain. *Neuron* **76**, 1091-1107.

Fukata, M., Nakagawa, M. and Kaibuchi, K. (2003). Roles of Rho-family GTPases in cell polarisation and directional migration. *Curr. Opin. Cell Biol.* **15**, 590-597.

Funahashi, Y., Namba, T., Nakamuta, S. and Kaibuchi, K. (2014). Neuronal polarization in vivo: growing in a complex environment. *Curr. Opin. Neurobiol.* **27**, 215-223.

Gärtner, A., Fornasiero, E. F., Munck, S., Vennekens, K., Seuntjens, E., Huttner, W. B., Valtorta, F. and Dotti, C. G. (2012). N-cadherin specifies first asymmetry in developing neurons. *EMBO J.* **31**, 1893-1903.

Garvalov, B. K., Flynn, K. C., Neukirchen, D., Meyn, L., Teusch, N., Wu, X., Brakebusch, C., Bamburg, J. R. and Bradke, F. (2007). Cdc42 regulates cofilin during the establishment of neuronal polarity. *J. Neurosci.* **27**, 13117-13129.

Gonzalez-Billault, C., Muñoz-Llancao, P., Henriquez, D. R., Wojnacki, J., Conde, C. and Caceres, A. (2012). The role of small GTPases in neuronal morphogenesis and polarity. *Cytoskeleton* **69**, 464-485.

Guo, S. and Kemphues, K. J. (1995). par-1, a gene required for establishing polarity in C. elegans embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* **81**, 611-620.

Hall, A., Paterson, H. F., Adamson, P. and Ridley, A. J. (1993). Cellular responses regulated by rho-related small GTP-binding proteins. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **340**, 267-271.

Hatanaka, Y. and Yamauchi, K. (2013). Excitatory cortical neurons with multipolar shape establish neuronal polarity by forming a tangentially oriented axon in the intermediate zone. *Cereb. Cortex* **23**, 105-113.

Houde, V. P., Ritorto, M. S., Gourlay, R., Varghese, J., Davies, P., Shpiro, N., Sakamoto, K. and Alessi, D. R. (2014). Investigation of LKB1 Ser431 phosphorylation and Cys433 farnesylation using mouse knockin analysis reveals an unexpected role of prenylation in regulating AMPK activity. *Biochem. J.* **458**, 41-56.

Inagaki, N., Chihara, K., Arimura, N., Ménager, C., Kawano, Y., Matsuo, N., Nishimura, T., Amano, M. and Kaibuchi, K. (2001). CRMP-2 induces axons in cultured hippocampal neurons. *Nat. Neurosci.* **4**, 781-782.

Inagaki, N., Toriyama, M. and Sakumura, Y. (2011). Systems biology of symmetry breaking during neuronal polarity formation. *Dev. Neurobiol.* **71**, 584-593.

Jiang, H., Guo, W., Liang, X. and Rao, Y. (2005). Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3beta and its upstream regulators. *Cell* **120**, 123-135.

Jossin, Y. and Cooper, J. A. (2011). Reelin, Rap1 and N-cadherin orient the migration of multipolar neurons in the developing neocortex. *Nat. Neurosci.* **14**, 697-703.

Katayama, K.-i., Melendez, J., Baumann, J. M., Leslie, J. R., Chauhan, B. K., Nemkul, N., Lang, R. A., Kuan, C.-Y., Zheng, Y. and Yoshida, Y. (2011). Loss of RhoA in neural progenitor cells causes the disruption of adherens junctions and hyperproliferation. *Proc. Natl. Acad. Sci. USA* **108**, 7607-7612.

Kawano, Y., Yoshimura, T., Tsuboi, D., Kawabata, S., Kaneko-Kawano, T., Shirataki, H., Takenawa, T. and Kaibuchi, K. (2005). CRMP-2 is involved in kinesin-1-dependent transport of the Sra-1/WAVE1 complex and axon formation. *Mol. Cell. Biol.* **25**, 9920-9935.

Kawauchi, T., Chihama, K., Nabeshima, Y.-i. and Hoshino, M. (2003). The in vivo roles of STEF/Tiam1, Rac1 and JNK in cortical neuronal migration. *EMBO J.* **22**, 4190-4201.

Kawauchi, T., Sekine, K., Shikanai, M., Chihama, K., Tomita, K., Kubo, K.-i., Nakajima, K., Nabeshima, Y.-i. and Hoshino, M. (2010). Rab GTPases-dependent endocytic pathways regulate neuronal migration and maturation through N-cadherin trafficking. *Neuron* **67**, 588-602.

Kimura, T., Ishiguro, K. and Hisanaga, S.-i. (2014). Physiological and pathological phosphorylation of tau by Cdk5. *Front. Mol. Neurosci.* **7**, 65.

Kishi, M., Pan, Y. A., Crump, J. G. and Sanes, J. R. (2005). Mammalian SAD kinases are required for neuronal polarization. *Science* **307**, 929-932.

Kollins, K. M., Hu, J., Bridgman, P. C., Huang, Y. Q. and Gallo, G. (2009). Myosin-II negatively regulates minor process extension and the temporal development of neuronal polarity. *Dev. Neurobiol.* **69**, 279-298.

Lizcano, J. M., Göransson, O., Toth, R., Deak, M., Morrice, N. A., Boudeau, J., Hawley, S. A., Udd, L., Mäkelä, T. P., Hardie, D. G. et al. (2004). LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *EMBO J.* **23**, 833-843.

Miyamoto, Y., Yamauchi, J., Tanoue, A., Wu, C. and Mobley, W. C. (2006). TrkB binds and tyrosine-phosphorylates Tiam1, leading to activation of Rac1 and induction of changes in cellular morphology. *Proc. Natl. Acad. Sci. USA* **103**, 10444-10449.

Miyata, T., Kawaguchi, A., Saito, K., Kawano, M., Muto, T. and Ogawa, M. (2004). Asymmetric production of surface-dividing and non-surface-dividing cortical progenitor cells. *Development* **131**, 3133-3145.

Mori, K., Amano, M., Takefuji, M., Kato, K., Morita, Y., Nishioka, T., Matsuura, Y., Murohara, T. and Kaibuchi, K. (2009). Rho-kinase contributes to sustained RhoA activation through phosphorylation of p190A RhoGAP. *J. Biol. Chem.* **284**, 5067-5076.

Nakamuta, S., Funahashi, Y., Namba, T., Arimura, N., Picciotto, M. R., Tokumitsu, H., Soderling, T. R., Sakakibara, A., Miyata, T., Kamiguchi, H. et al. (2011). Local application of neurotrophins specifies axons through inositol 1,4,5-trisphosphate, calcium, and Ca2+/calmodulin-dependent protein kinases. *Sci. Signal.* **4**, ra76.

Nakayama, M., Goto, T. M., Sugimoto, M., Nishimura, T., Shinagawa, T., Ohno, S., Amano, M. and Kaibuchi, K. (2008). Rho-kinase phosphorylates PAR-3 and disrupts PAR complex formation. *Dev. Cell* **14**, 205-215.

Namba, T., Kibe, Y., Funahashi, Y., Nakamuta, S., Takano, T., Ueno, T., Shimada, A., Kozawa, S., Okamoto, M., Shimoda, Y. et al. (2014). Pioneering axons regulate neuronal polarization in the developing cerebral cortex. *Neuron* **81**, 814-829.

Development (2015) 142, 2088-2093 doi:10.1242/dev.114454

Nishimura, T., Yamaguchi, T., Kato, K., Yoshizawa, M., Nabeshima, Y.-i., Ohno, S., Hoshino, M. and Kaibuchi, K. (2005). PAR-6-PAR-3 mediates Cdc42-induced Rac activation through the Rac GEFs STEF/Tiam1. *Nat. Cell Biol.* **7**, 270-277.

Noctor, S. C., Martínez-Cerdeño, V., Ivic, L. and Kriegstein, A. R. (2004). Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat. Neurosci.* **7**, 136-144.

Pacary, E., Heng, J., Azzarelli, R., Riou, P., Castro, D., Lebel-Potter, M., Parras, C., Bell, D. M., Ridley, A. J., Parsons, M. et al. (2011). Proneural transcription factors regulate different steps of cortical neuron migration through Rnd-mediated inhibition of RhoA signaling. *Neuron* **69**, 1069-1084.

Sapir, T., Shmueli, A., Levy, T., Timm, T., Elbaum, M., Mandelkow, E.-M. and Reiner, O. (2008). Antagonistic effects of doublecortin and MARK2/Par-1 in the developing cerebral cortex. *J. Neurosci.* **28**, 13008-13013.

Schwamborn, J. C. and Püschel, A. W. (2004). The sequential activity of the GTPases Rap1B and Cdc42 determines neuronal polarity. *Nat. Neurosci.* **7**, 923-929.

Shelly, M., Cancedda, L., Heilshorn, S., Sumbre, G. and Poo, M.-M. (2007). LKB1/STRAD promotes axon initiation during neuronal polarization. *Cell* **129**, 565-577.

Shelly, M., Lim, B. K., Cancedda, L., Heilshorn, S. C., Gao, H. and Poo, M.-M. (2010). Local and long-range reciprocal regulation of cAMP and cGMP in axon/dendrite formation. *Science* **327**, 547-552.

Solecki, D. J., Govek, E.-E., Tomoda, T. and Hatten, M. E. (2006). Neuronal polarity in CNS development. *Genes Dev.* **20**, 2639-2647.

Sun, Y., Fei, T., Yang, T., Zhang, F., Chen, Y.-G., Li, H. and Xu, Z. (2010). The suppression of CRMP2 expression by bone morphogenetic protein (BMP)-SMAD gradient signaling controls multiple stages of neuronal development. *J. Biol. Chem.* **285**, 39039-39050.

Tahirovic, S. and Bradke, F. (2009). Neuronal polarity. *Cold Spring Harb. Perspect. Biol.* **1**, a001644.

Tahirovic, S., Hellal, F., Neukirchen, D., Hindges, R., Garvalov, B. K., Flynn, K. C., Stradal, T. E., Chrostek-Grashoff, A., Brakebusch, C. and Bradke, F. (2010). Rac1 regulates neuronal polarization through the WAVE complex. *J. Neurosci.* **30**, 6930-6943.

Takano, T., Tomomura, M., Yoshioka, N., Tsutsumi, K., Terasawa, Y., Saito, T., Kawano, H., Kamiguchi, H., Fukuda, M. and Hisanaga, S.-i. (2012). LMTK1/AATYK1 is a novel regulator of axonal outgrowth that acts via Rab11 in a Cdk5-dependent manner. *J. Neurosci.* **32**, 6587-6599.

Takefuji, M., Mori, K., Morita, Y., Arimura, N., Nishimura, T., Nakayama, M., Hoshino, M., Iwamatsu, A., Murohara, T., Kaibuchi, K. et al. (2007). Rho-kinase modulates the function of STEF, a Rac GEF, through its phosphorylation. *Biochem. Biophys. Res. Commun.* **355**, 788-794.

Threadgill, R., Bobb, K. and Ghosh, A. (1997). Regulation of dendritic growth and remodeling by Rho, Rac, and Cdc42. *Neuron* **19**, 625-634.

Toriyama, M., Kozawa, S., Sakumura, Y. and Inagaki, N. (2013). Conversion of a signal into forces for axon outgrowth through Pak1-mediated shootin1 phosphorylation. *Curr. Biol.* **23**, 529-534.

Uboha, N. V., Flajolet, M., Nairn, A. C. and Picciotto, M. R. (2007). A calcium- and calmodulin-dependent kinase Ialpha/microtubule affinity regulating kinase 2 signaling cascade mediates calcium-dependent neurite outgrowth. *J. Neurosci.* **27**, 4413-4423.

Wagner, U., Utton, M., Gallo, J. M. and Miller, C. C. (1996). Cellular phosphorylation of tau by GSK-3 beta influences tau binding to microtubules and microtubule organisation. *J. Cell Sci.* **109**, 1537-1543.

Wayman, G. A., Kaech, S., Grant, W. F., Davare, M., Impey, S., Tokumitsu, H., Nozaki, N., Banker, G. and Soderling, T. R. (2004). Regulation of axonal extension and growth cone motility by calmodulin-dependent protein kinase I. *J. Neurosci.* **24**, 3786-3794.

Witte, H. and Bradke, F. (2008). The role of the cytoskeleton during neuronal polarization. *Curr. Opin. Neurobiol.* **18**, 479-487.

Yi, J. J., Barnes, A. P., Hand, R., Polleux, F. and Ehlers, M. D. (2010). TGF-beta signaling specifies axons during brain development. *Cell* **142**, 144-157.

Yoshimura, T., Kawano, Y., Arimura, N., Kawabata, S., Kikuchi, A. and Kaibuchi, K. (2005). GSK-3beta regulates phosphorylation of CRMP-2 and neuronal polarity. *Cell* **120**, 137-149.

Zuccaro, E., Bergami, M., Vignoli, B., Bony, G., Pierchala, B. A., Santi, S., Cancedda, L. and Canossa, M. (2014). Polarized expression of p75(NTR) specifies axons during development and adult neurogenesis. *Cell Rep.* **7**, 138-152.