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Par proteins and neuronal polarity

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Abstract

A hallmark of neurons is their ability to polarize with dendrite and axon specification to allow the proper flow of information through the nervous system. Over the past decade, extensive research has been performed in an attempt to understand the molecular and cellular machinery mediating this neuronal polarization process. It has become evident that many of the critical regulators involved in establishing neuronal polarity are evolutionarily conserved proteins that had previously been implicated in controlling the polarization of other cell types. At the forefront of this research are the partition defective (Par) proteins. In this review, we will provide a commentary on the progress of work regarding the central importance of Par proteins in the establishment of neuronal polarity.

Keywords

Neuronal polarity; PAR (for partitioning-defective) proteins; axon specification; cytoskeleton; signaling pathway

INTRODUCTION

Neurons develop two morphologically, molecularly and physiologically distinct subcellular compartments – axons and dendrites. Axon versus dendrite polarity is the basis of unidirectional information flow in the nervous system—axons are responsible for sending and dendrites are responsible for receiving signals. While the axon/dendrite polarity was recognized more than a century ago by Cajal, our understanding of how neurons develop polarity has only been recently addressed. A major breakthrough came from the success in *in vitro* culturing dissociated hippocampal neurons (Dotti et al., 1988), which, like neurons *in vivo*, polarize with a single axon and multiple dendrites. This highly accessible experimental preparation opened the door to the analysis of neuronal polarity at the cellular and molecular level. Using such preparations, extensive studies over the past decade have revealed a comprehensive network of molecular pathways that regulate the development of neuronal polarity. A number of excellent reviews on recent progress this area have been published (Arimura and Kaibuchi, 2007; Barnes and Polleux, 2009; Reiner and Sapir, 2009; Tahirovic and Bradke, 2009), here, we focus on the role of the partition defective (Par) proteins in regulating axon specification and the establishment of neuronal polarity.

IDENTIFICATION OF PAR PROTEINS

Par genes were first discovered in a classic genetic screen for regulators of cytoplasmic partitioning during mitosis in early embryos of the nematode *C. elegans* (Kemphues et al., 1988). The first division of a single cell embryo of *C. elegans* is asymmetric with regard to the partition of the cytoplasm. The daughter cell that becomes the anterior region of the adult is significantly larger than the other daughter cell that becomes the posterior region. In

Par mutant embryos, the division appeared symmetric. In total, six *par* genes, named *Par 1–6*, were identified in the screen (Table 1). The molecular identities of the Par proteins were revealed in subsequent studies. Par1 and Par4 encode serine/threonine protein kinases (Morton et al., 1992; Guo and Kemphues, 1995). Par2 has a RING finger domain that may act in the ubiquitination pathway (Levitan et al., 1994). Par3 and Par6 contain PDZ-domains, suggesting that they act as scaffold proteins (Etemad-Moghadam et al., 1995; Watts et al., 1996). Par5 is a member of the 14-3-3 protein family that binds to phosphorylated serines and threonines (Morton et al., 2002). The initial phenotypic analysis of *Par* gene mutations suggested that Par proteins are required for two related aspects of cell polarization and asymmetric cell division: the asymmetric positioning of the mitotic spindle that results in unequal cell division, and the asymmetric localization of a set of proteins and RNAs that are critical for distinct cell fate specification (Kemphues, 2000). While the cloning of *Par* genes provided crucial insights into their biochemical properties, the mechanistic link between Par proteins and cell polarization and/or asymmetric cell division was revealed only later through cell biological studies in *C. elegans* and other organisms.

MOLECULAR AND FUNCTIONAL CONSERVATION OF PAR PROTEINS

Par proteins, with the exception of Par2 (which is to date a nematode-specific protein), are highly conserved in diverse animals including *C. elegans*, *Drosophila* and mammals (Goldstein and Macara, 2007). Moreover, their functions in specifying cell polarity and regulating asymmetric cell division are also highly conserved across species. In one-cell embryos of *C. elegans*, Par3 and Par6 become selectively enriched at the anterior cortex, while Par1 and Par2 become selectively enriched at the posterior cortex. The asymmetric localization of Par proteins defines the anterior/posterior polarity of the embryo and is essential for proper positioning of the mitotic spindle and subsequent asymmetric cell division (Kemphues et al., 1988; Kemphues, 2000; Goldstein and Macara, 2007). Similarly, in *Drosophila* neuroblasts that divide to produce neurons and glia in the central nervous system (CNS), the selective enrichment of Bazooka (Par3 ortholog) and Par6 at the apical cortex is essential for proper localization of crucial cell fate determinants such as Numb and Miranda at the basal cortex and the correct orientation of the mitotic spindle to ensure asymmetric cell division (Kraut et al., 1996; Shen et al., 1997; Kuchinke et al., 1998; Schober et al., 1999). Recent studies in the developing mammalian neocortex suggest that the asymmetric distribution of Par3 in mitotic radial glial progenitor cells in the ventricular zone leads to activation of Notch signaling activation and differential cell fate specification in the daughter cells (i.e., asymmetric cell division) during the peak phase of neurogenesis (Bultje et al., 2009). Taken together, these studies demonstrate that evolutionarily conserved Par proteins play a central role in regulating asymmetric cell division in different species.

Another unifying theme regarding the molecular and functional conservation of Par proteins is in the control of epithelial cell polarization throughout metazoans (Shin et al., 2006). In *C. elegans*, by the end of the four cell stage of embryogenesis, Par3 and Par6 localize to the apical surface and are essential for the establishment of apical/basal polarity associated with gastrulation (Nance and Priess, 2002). In *Drosophila*, Bazooka and Par6, together with other evolutionarily conserved polarity proteins such as the Crumbs/Stardust/Patj protein complex, are required for establishing apical/basal polarity in epithelial cells (Tepass et al., 2001). In mammalian epithelial cells, Par3 (also called atypical protein kinase C (aPKC)-specific interacting protein, ASIP, or ephrin-interacting protein, PHIP) and Par6 form a ternary complex with atypical protein kinase C (aPKC), and this complex is selectively localized to tight junctions and is responsible for defining the apical/lateral boundary (Izumi et al., 1998; Lin et al., 1999; Suzuki et al., 2001; Suzuki and Ohno, 2006). These studies on the Par proteins in regulating epithelial cell polarization not only further demonstrate their

molecular and functional conservation across species, but also reveal insights into how Par proteins contribute to the establishment and maintenance of distinct cell morphologies.

PAR3/PAR6 PROTEINS AND NEURONAL POLARITY

Neurons are derived from progenitor cells in the neuroepithelium (Kriegstein and Alvarez-Buylla, 2009). Since the identification of Par proteins rapidly advanced our knowledge on the molecular basics underlying polarity establishment in epithelial cells, the lineage relationship between neuroepithelia progenitors and neurons raised the intriguing possibility that similar machineries in specified cell polarity are in the two cell types. The first experimental evidence to support this idea came from our observation that the ternary complex of Par3/Par6/aPKC plays a crucial role in axon specification and polarity establishment in cultured hippocampal neurons (Shi et al., 2003). Interestingly, as neurons polarize, the subcellular distributions of Par3 and Par6 become polarized, being selectively enriched at the tip of the future axon (Shi et al., 2003). Disruption of this polarized distribution of Par3 or Par6, by either ectopic expression or RNA interference (RNAi)-mediated depletion (Chen and Shi, unpublished data), impair axon specification and polarization of both mammalian hippocampal and cortical neurons in culture and *in vivo*. It is worth noting that the primary defect in neuronal polarization caused by disrupting the function of Par3 or Par6 is the prevention of neurites from differentiating into either dendrites or axons. Neuronal polarity defects manifest in different ways including no axon/dendrite differentiation of neurites, multiple dendrites with no axon, or multiple axons. These different phenotypes are often not carefully distinguished in neuronal polarity studies, even though the insights they reveal could be very important.

Since this initial report, there has been mounting evidence supporting a critical role of the Par3/Par6/aPKC protein complex in regulating axon specification and neuronal polarization (Fig. 1) (Nishimura et al., 2004; Shi et al., 2004; Nishimura et al., 2005; Higginbotham et al., 2006; Schwamborn et al., 2007; Vohra et al., 2007; Yi et al., 2010). These studies not only provide further insights into the function of the Par3/Par6/aPKC complex in developing neurons during polarization, but also reveal the important signaling pathways upstream and downstream of this protein complex. Proper polarization of neurons entails differential regulation of the actin and microtubule cytoskeletons at the tip of the growth cone in developing neurites so as to effect their growth rate (Baas, 1999; Bradke and Dotti, 1999; Witte et al., 2008). With microtubules in the central domain and actin cytoskeleton in the peripheral region, the growth cone of a fast growing neurite often harbors a dynamic actin cytoskeleton, but stable microtubules, thereby facilitating the invasion of microtubule bundles from the central domain to the peripheral region and thus promoting neurite growth (Geraldo and Gordon-Weeks, 2009). During the polarization process, one and only one neurite rapidly grows and becomes the future axon, while the remaining neurites grow less rapidly and later become dendrites. Therefore, the differential growth rate of neurites represents a key feature of neuronal polarization.

Both Par3 and Par6 are scaffold proteins, and Par6 forms a stable complex with aPKC and contains a semi-Cdc42/Rac interactive binding (CRIB) domain that specifically binds to the active GTP-bound form of the small GTPases, Cdc42 and Rac1 (Joberty et al., 2000). Par3, on the other hand, interacts with Par6 and with the T-lymphoma invasion and metastasis 1 (Tiam1), a guanine nucleotide exchange factor for Rac (Kunda et al., 2001; Nishimura et al., 2005). Small GTPases are central regulators of actin cytoskeleton dynamics (Hall, 1998), which is crucial to neuronal development (Govek et al., 2005). Polarization of the Par3/Par6/aPKC complex would facilitate the localized activation of small GTPases at the tip of the future axon to regulate local actin dynamics. While it has not been explicitly tested for the Rac family small GTPases, a recent study showed that the selective accumulation of Rap1 (a

member of the Ras family of small GTPases) in one neurite that becomes the future axon depends on proper Par3 localization (Schwamborn and Puschel, 2004; Schwamborn et al., 2007; Schwamborn et al., 2007). Further evidence for the role of Par3 and Par6 in the regulation of the actin cytoskeleton came from the studies showing that they control the morphogenesis of dendritic spines, a structure mostly supported by filamentous actin (Zhang and Macara, 2006; Zhang and Macara, 2008). While these studies link the Par3/Par6/aPKC complex to the local regulation of actin at the tip of the future axon, it is unclear whether the polarized distribution of the Par3/Par6/aPKC complex directly contributes to the differential regulation of microtubule dynamics in developing neurons during polarization, which will be interesting to explore in the future.

A number of signaling pathways have been implicated upstream of the Par3/Par6/aPKC complex in specifying neuronal polarity (Fig. 1). The most extensively studied pathway is the phosphoinositide 3-kinase (PI3K)/glycogen synthase kinase 3 β (GSK3 β) pathway. Previous studies showed that local activation of PI3K by receptor tyrosine kinases (RTKs, e.g. growth factor-mediated signaling) and accumulation of phosphatidylinositol-3,4,5-triphosphate (PIP3) at the tip of one neurite is important for axon specification and neuronal polarization (Menager et al., 2004). As a downstream effector, GSK3 β can be phosphorylated at Ser9 by elevated PI3K activity through Akt (also known as protein kinase B, PKB) and this phosphorylation results in the inhibition of GSK3 β activity (Doble and Woodgett, 2003). In dorsal root ganglion neurons, nerve growth factor-induced axonal growth depends on the PI3K/GSK3 β pathway and the microtubule plus-ending binding protein adenomatous polyposis coli (APC), a tumor suppressor that can be phosphorylated by GSK3 β (Menager et al., 2004; Zhou et al., 2004). In polarizing hippocampal neurons, both PI3K and GSK3 β activities exhibit a differential distribution in the axon versus the dendrites. Selective elevation of PI3K activity phosphorylates and in turn suppresses GSK3 β activity in the tip of the future axon (Shi et al., 2004). This reduction in GSK3 β activity in the tip of the future axon has been linked to a nonphosphorylated pool of collapsing response mediator protein-2 (CRMP-2), a critical player that regulates microtubule assembly and neuronal polarization (Yoshimura et al., 2005; Yoshimura et al., 2006). Interestingly, while inhibition of GSK3 β activity or genetic removal of GSK3 β has been found to inhibit axon specification and growth (Shi et al., 2004), inhibition of GSK3 β activity can also cause the formation of multiple axons (Jiang et al., 2005; Yoshimura et al., 2005; Oinuma et al., 2007). It is unclear what may underlie this apparent discrepancy; one possibility is the difference in the degree of the inhibition of the GSK3 β activity. Furthermore, it appears that the function of GSK3 β in regulating neuronal polarization may not depend on Akt/PKB serine phosphorylation (Gartner et al., 2006); nonetheless, these studies indicate the importance of the PI3K/GSK3 β pathway in axon specification and neuronal polarization.

A critical link between the PI3K/GSK3 β pathway and the Par3/Par6/aPKC complex comes from studies showing that the polarized distribution of Par3 in developing hippocampal neurons depends on the activity of PI3K and GSK3 β . Inhibition of either PI3K or GSK3 β activity prevents selective accumulation of Par3 in the tip of the future axon, a process which relies on microtubule-based protein trafficking involving APC and the kinesin motor KIF3A, and results in polarity defects (Nishimura et al., 2004; Shi et al., 2004). Besides the PI3K/GSK3 β pathway, the Wnt pathway has also been implicated in functioning upstream of the Par3/Par6/aPKC complex. Dishevelled (Dvl), a critical cytoplasmic mediator of Wnt signaling (Gao and Chen, 2010), associates with the Par3/Par6/aPKC complex and in turn stabilizes and activates aPKC (Fig. 1). Treatment with Wnt5a, a non-canonic Wnt, activates aPKC and promotes axon specification in hippocampal neurons in culture (Zhang et al., 2007). These studies suggest that non-canonic Wnt signaling may act as a source of extracellular cues in promoting axon specification and neuronal polarization.

While substantial evidence on the critical role of the Par3/Par6/aPKC complex in regulating axon specification and neuronal polarization in mammalian and *C. elegans* (Welchman et al., 2007) neurons has accumulated, this protein complex appears not to be essential for neuronal polarization in *Drosophila* (Rolls and Doe, 2004). Whether this discrepancy reflects a fundamental difference in neuronal polarization between *Drosophila* and mammals is unclear. It has been suggested that microtubule organization in *Drosophila* neurons differs from that in mammalian neurons (Stone et al., 2008). In general, little is known about neuronal polarization in the *Drosophila* nervous system; however, Par6 accumulates in areas of prospective dendritic formation, implicating a potential role in regulating dendrite versus axon specification and morphogenesis (Sanchez-Soriano et al., 2005). Further insights may be gained by a more direct comparison of the polarization processes of *Drosophila* neurons and mammalian neurons. In addition, while RNAi-mediated loss-of-function analyses on Par3 and Par6 have been carried out to demonstrate their crucial role in the polarization of mammalian neurons, studies using genetic ablation has been slow in coming forth. A mouse line carrying a conditional mutant allele of the *Par3* gene has been generated (Hirose et al., 2006). A ubiquitous disruption of *Par3* results in mid-gestational embryonic lethality with defective epicardial development (Hirose et al., 2006). However, it remains unclear whether selective disruption of *Par3* in the brain impairs the polarization of neurons. One potential complication of the genetic ablation study is the complexity of mammalian gene expression in general. Besides *Par3*, there is another gene, *Par3L*, which encodes for a long form of Par3 (Par3L for Par3-like or Par3 β) (Gao et al., 2002; Kohjima et al., 2002). In addition, both Par3 and Par3L have multiple splicing isoforms (Lin et al., 2000; Gao et al., 2002). Therefore, more comprehensive genetic ablation studies may be necessary to reveal the precise role of Par3/Par3L in controlling axon specification and neuronal polarization in the mammalian nervous system.

PAR1 AND PAR4 PROTEINS AND NEURONAL POLARITY

In addition to the Par3/Par6/aPKC complex, Par1 and Par4 also play important roles in regulating axon specification and neuronal polarization. Par1 is a serine/threonine kinase and its closest mammalian homologs are microtubule affinity regulating kinases (MARKs) (Drewes et al., 1997). Interestingly, the evolutionarily conserved kinase domain of Par1 is also present in the mammalian SAD kinase A and B (also known as brain related serine/threonine protein kinase, BRSK 2 and 1, respectively) (Kishi et al., 2005; Goldstein and Macara, 2007). Both MARKs and SAD kinases can phosphorylate microtubule associated proteins (MAPs) at the K-X-G-S motifs, typically resulting in a reduction in the affinity of MAPs to microtubules and thereby destabilizing microtubules (Fig. 1) (Drewes et al., 1997; Kishi et al., 2005). Local regulation of microtubules plays an important role in neuronal polarization (Baas, 1999; Witte et al., 2008). SAD kinase was discovered in *C. elegans* and demonstrated to be involved in the targeted delivery of vesicles to axons and in presynaptic differentiation (Crump et al., 2001). While it had been initially suggested that SAD kinase affects axonal-dendritic polarity, recent studies have provided more direct evidence on the role of SAD kinase in regulating neuronal polarity. In mammals, removal of both SAD-A and SAD-B kinases results in severe brain defects largely due to a loss of the ability of neurons to polarize (Kishi et al., 2005). In *C. elegans*, SAD-1 kinase regulates the specification of axonal identity through its interaction with Neurabin, a scaffolding protein not required for presynaptic differentiation (Hung et al., 2007). MARKs are also implicated in the establishment of neuronal polarity. It was reported that MARK2 is downstream of the mPar3/mPar6/aPKC complex since aPKC negatively regulates MARK2 through phosphorylation at threonine 595 (T595), inhibiting MARK2 from phosphorylating downstream MAP proteins (Hurov et al., 2004; Chen et al., 2006) (Fig. 1). The dephosphorylation of MAPs in the axon allows for local stabilization of microtubules that promotes axonal growth (Biernat et al., 2002; Chen et al., 2006). Another downstream target

of MARK2 is GAKIN/KIF13B (Yoshimura et al., 2010), a microtubule-based motor protein whose cargo includes PIP3 (Horiguchi et al., 2006). It has been suggested that MARK2 inactivation in the axonal tip through the Par3/Par6/aPKC complex promotes the disinhibition of GAKIN/KIF13B, permitting the transport and accumulation of PIP3 in the growing axon (Horiguchi et al., 2006; Yoshimura et al., 2010) (Fig. 1). Interestingly, MARK2 also regulates centrosome behavior and the polarization of cortical neurons *in vivo* (Sapir et al., 2008).

Par4 is a serine/threonine kinase, and its mammalian homolog LKB1, is a tumor suppressor (Morton et al., 1992; Baas et al., 2004). Activation of LKB1 relies on the formation of a complex with the pseudokinase STRAD (Ste20-related adaptor) and the scaffolding protein MO25 (Zeqiraj et al., 2009; Zeqiraj et al., 2009). In addition to an unusual allosteric mechanism of activation, LKB1 can be activated by phosphorylation at serine 431 (S431) by two known kinases: ribosomal S6 kinase (also known as p90RSK) or protein kinase A (PKA) (Sapkota et al., 2001). Upon activation, LKB1 translocates from the nucleus to the cytoplasm to phosphorylate and activate its substrates such as the adenosine monophosphate-activated protein kinase (AMPK) (Baas et al., 2003). Much like the mPar3/mPar6 complex, LKB1 and STRAD pseudokinase polarize to a single neurite of a non-polarized hippocampal neuron that develops into the future axon. The accumulation and polarization depend on the phosphorylation of S431 on LKB1 (Barnes et al., 2007; Shelly et al., 2007). This activation of LKB1 is necessary and sufficient for the proper establishment of cortical and hippocampal neuronal polarity. Interestingly, both SAD kinases and MARKs are downstream phosphorylation targets of LKB1 (Fig. 1) (Lizcano et al., 2004; Barnes et al., 2007). In the presence of STRAD pseudokinase, LKB1 activation initiates axonal differentiation and polarization through phosphorylating and activating SAD kinases in mammalian neurons (Barnes et al., 2007; Shelly et al., 2007). It is worth noting that in *C. elegans* while Par4 (LKB1 ortholog) and STRD-1 (STRAD ortholog) affect neuronal polarity, only STRD-1 functions in a linear genetic pathway with SAD-1 kinase. By contrast, Par-4 (LKB1 ortholog) appears to function through additional effectors other than SAD-1 kinase (Kim et al., 2010), indicating a difference in the action of Par4/LKB1 and SAD-1 in regulation of neuronal polarization between species.

Much like the Par3/Par6/aPKC complex, upstream signaling pathways regulating the activation of LKB1 have been suggested (Fig. 1). In particular, activation of PKA by cell permeable cyclic adenosine monophosphate (cAMP) or addition of brain derived neurotrophic factor (BDNF) is sufficient to drive axon specification and polarization of dissociated neurons in culture through activation of LKB1, again suggesting the interplay between extracellular signaling and intracellular polarity pathway in specifying neuronal polarity (Shelly et al., 2007).

PAR PROTEINS AND SIGNALING FEEDBACK LOOPS

Neurons in culture polarize in a rather homogeneous environment. This suggests the existence of positive and negative signaling feedback loops to ensure one and only one axon is specified (Andersen and Bi, 2000). While the precise nature of these signaling feedback loops remains obscure, a number of potential positive signaling feedback loops that involve Par proteins have been suggested. For example, PI3K activity is required for proper localization of Par3 that forms a complex with Par6, aPKC and the active forms of small GTPases, Cdc42 and Rac1 at the tip of the future axon (Shi et al., 2003; Nishimura et al., 2005). Given that Rac1 activates PI3K (Tolias et al., 1995; Keely et al., 1997; Chan et al., 2002), these molecular pathways form a positive feedback loop to drive axon specification and neuronal polarization. Another feedback loop to accumulate and sustain PIP3 in the growing axon has also been proposed through MARK2. PIP3 production triggered by

extracellular signals can activate aPKC (through PDK1 for example (Le Good et al., 1998)), which inhibits MARK2, thus relieving the inhibition of GAKIN/KIF13a to transport additional PIP3 to the growing axon (Yoshimura et al., 2010).

In addition, there is a proposed positive feedback mechanism that leads to the persistent activation of LKB1 in the nascent axon. Local elevation of cAMP levels followed by PKA activation through the action of an extracellular polarization factor like BDNF would activate LKB1 in a single neurite. This activation stabilizes the complex of MO25 and STRAD, promoting the accumulation of the heterotrimeric complex of LKB1/STRAD/MO25 at the future axon. Finally, one of the downstream phosphorylation targets of LKB1/STRAD/MO25 complex is LKB1 at S431 (Martin and St Johnston, 2003), the critical residue necessary for neuronal polarization (Shelly et al., 2007). While these feedback loop ideas are intriguing, further studies are needed to demonstrate their existence and functional importance during neuronal polarization.

CONCLUDING REMARKS

While both the pathways controlled by Par3/Par6/aPKC complex, Par4, and Par1 kinases have been found to be critical for neuronal polarization, it is unclear whether they are functionally linked to each other (Fig. 1). In the asymmetrically dividing *C. elegans* single cell embryo, where Par proteins were initially discovered, the Par3/Par6/aPKC complex accumulates at the anterior cell cortex and Par1 accumulates at the posterior cell cortex (Kemphues, 2000). Par4 remains symmetrically distributed at this time. The anterior enrichment of the Par3/Par6/aPKC complex is a prerequisite for the posterior localization of Par1. In contrast, in polarizing mammalian neurons both the Par3/Par6 complex and LKB1 (mammalian Par4) become selectively at the tip of the presumptive axon, while SAD kinases (mammalian Par1) are present in both the axon and dendrites (Shi et al., 2003; Kishi et al., 2005; Barnes et al., 2007; Shelly et al., 2007). It is unclear, therefore, whether the proper polarization of the Par3/Par6 complex and LKB1 depend on each other. Intriguingly, in the *Drosophila* oocyte, it has been suggested that Par1 can phosphorylate Bazooka/Par3 on two distinct serine residues within the binding site for Par5; this phosphorylation affects the formation of the Bazooka/Par6/aPKC complex and the localization of Bazooka (Benton and St Johnston, 2003). In addition, in *Xenopus laevis*, LKB1 activates PKC ζ which inactivates GSK3 β (Ossipova et al., 2003). These studies point out potential functional interactions between the Par3/Par6/aPKC complex with Par4, and Par1 kinases, which will be interesting to explore in the context of axon specification and neuronal polarization.

Neurons in culture polarize in random orientations. By contrast, neurons *in vivo* tend to polarize in the same orientation. For example, excitatory pyramidal neurons in the developing neocortex, an emerging model for studying neuronal polarization *in vivo*, polarize with their axons pointing towards the ventricle, to eventually form the white matter (Barnes and Polleux, 2009). This uniform organization in polarity suggests the existence of signaling gradient(s) that instructs the polarization of newly generated neocortical pyramidal neurons. A number of extracellular signaling molecules including neural growth factors (NGF, BDNF) (Shelly et al., 2007), insulin-like growth factor 1 (IGF1) (Sosa et al., 2006; Dupraz et al., 2009), Wnt (Zhang et al., 2007), integrins (Guo et al., 2007), and extracellular matrix proteins (Menager et al., 2004) have been implicated in functioning upstream of the Par proteins (Fig 1). However, the *in vivo* evidence of a functional gradient linked to the Par proteins has been lacking. Interestingly, a recent study suggests that TGF β may function upstream of the Par3/Par6 complex in regulating axon specification of neocortical pyramidal neurons *in vivo* (Yi et al., 2010).

Another interesting feature of mammalian neuronal polarization *in vivo* is the coupling of polarization and migration. Neurons often migrate long distances from their birth places to the final destination. In the developing neocortex, most, if not all newly generated pyramidal neurons undergo morphological switch from multipolar to bipolar morphology and at the same time change the mode of migration from non-directional to directional migration (Kriegstein and Noctor, 2004). While it remains uncertain when the axon-dendrite polarity of these neurons is established, the morphology switch likely represents an important step of the neuronal polarization process. Should this be the case, the polarization of these neurons would be crucial for their migration and the proper construction of brain tissues, e.g. the layer formation of the neocortex.

In addition, the localization of the centrosome has been suggested to be crucial for the selection/specification of the future axon (de Anda et al., 2005; Calderon de Anda et al., 2008; de Anda et al., 2010). An involvement of Par proteins in regulating centrosomal localization was already indicated in the original screen that led to their discovery, since they control the positioning of the spindle apparatus (Kemphues et al., 1988) – a structure nucleated by the centrosome. With regard to neuronal polarity, both LKB1 and MARK kinases appear to play a role in centrosomal localization in migrating neocortical pyramidal neurons (Asada et al., 2007; Sapir et al., 2008). Disruption of either of these proteins leads to mislocalized centrosomes and neurons exhibiting polarity defects. Recently, live imaging of embryonic neocortical slices demonstrated that the centrosome's proximity to the nascent axon is essential for initial axon formation *in vivo* as neurons are migrating into the cortical plate – the future neocortex (de Anda et al., 2010). With regard to Par proteins, mPar6a localizes to the centrosome of migrating cerebellar granule neurons and controls their polarized migration (Solecki et al., 2004). A direct role for mPar3 in centrosomal localization through the actions of dynein motor proteins has been demonstrated in migrating fibroblasts (Schmoranz et al., 2009); however, this aspect of Par3 function has not been examined in neurons. Future study to elucidate the function of the Par proteins in regulating centrosome localization and motility in polarizing and migrating neurons will be important to our understanding of neuronal polarization and migration.

Finally, a central aspect of the function of the Par proteins is their polarized distribution, which likely requires the interplay of extracellular signaling and intracellular machineries involved in protein trafficking, local protein synthesis and protein degradation. Certain parts of this interplay have been uncovered, yet our overall understanding of the regulation involved in Par protein polarization is far from complete. In stage 3 hippocampal neurons, Par3 accumulation in the nascent axon occurs through trafficking by the kinesin motor Kif3a and the microtubule plus-end binding protein, APC (Nishimura et al., 2004; Shi et al., 2004). LKB1 is speculated to polarize in response to extracellular factors (Barnes et al., 2007; Shelly et al., 2007). Another means of protein polarization is through local protein synthesis, and there is evidence for local translation of Par3 in neurons (Hengst et al., 2009), yet whether or not this contributes to the polarization of Par3 in developing neurons is unknown. Lastly, protein degradation is an important cellular process that contributes to protein polarization. A ubiquitinated pool of Par3 in hippocampal neurons has been reported (Yan et al., 2006), however, its functional importance remains to be tested. Therefore, future efforts in uncovering the polarization processes of the Par proteins will be crucial for understanding their functions in axon specification and neuronal polarization.

The discovery of Par proteins and subsequent studies across species and cell types has transformed our understanding of cell polarity – a fundamental feature of nearly all cell types, including neurons. While enormous progress has been made in revealing the central role of the Par proteins in controlling axon specification and neuronal polarization, many important questions remain unanswered. With some discussed here, as well as more to

come, Par proteins and neuronal polarity will continue to be an active and exciting field into the future.

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Figure 1. Summary of the signaling pathways involving the Par proteins in axon specification and neuronal polarization

Arrow heads at the end of lines indicate activation, whereas circles at the end of the lines indicate inhibition. Double-headed arrowheads indicate association. Broken arrows represent proposed interactions that have not been tested. Red stars inscribed with a yellow "P" indicate a phosphorylation event.

In *Drosophila* and mammalian orthologue columns, references are referring to the original reports on the discovery and cloning of the homologue. Some Par proteins have multiple homologs in mammals, which are separately listed here. In the case of LKB1 and mammalian 14-3-3 proteins, the original cloning of these proteins preceded the knowledge of their homology to Par5 and Par4, respectively. In these two cases, original identification papers are included as well as the reports recognizing their homology to the Par proteins.

Table 1
Summary of Par protein conservation and involvement in neuronal polarity

Original screen (Kemphues et al., 1988)	<i>C. elegans</i> protein identity	<i>Drosophila</i> homolog	Mammalian Homolog	Involved in neuronal polarity?
Par1	Serine/Threonine kinase (Guo and Kemphues, 1995)	Par-1 (Shulman et al., 2000; Tomancak et al., 2000)	MARK kinases (also called EMK) (Drewes et al., 1997) Kinase domain conserved in SAD kinases (also called BRSK) (Kishi et al., 2005)	Yes (Crump et al., 2001; Biernat et al., 2002; Kishi et al., 2005; Chen et al., 2006; Barnes et al., 2007)
Par2	Zinc-finger and ATP binding motif (Leviton et al., 1994)	Not conserved	Not conserved	Unknown
Par3	Scaffold (Etemad-Moghadam et al., 1995)	Bazooka (Kuchinke et al., 1998)	mPar3 (also called ASIP, PHIP) (Izumi et al., 1998; Lin et al., 1999) Par3L (also called Par3β) (Gao et al., 2002; Kohjima et al., 2002)	Yes (Shi et al., 2003; Nishimura et al., 2004; Schwamborn and Puschel, 2004; Shi et al., 2004; Nishimura et al., 2005; Higginbotham et al., 2006; Schwamborn et al., 2007; Schwamborn et al., 2007; Vohra et al., 2007; Zhang et al., 2007; Yi et al., 2010) No (Rolls and Doe, 2004)
Par4	Serine/Threonine kinase (Morton et al., 1992)	Par4 (Martin and St Johnston, 2003)	LKB1 (Hemminki et al., 1998; Jenne et al., 1998; Baas et al., 2004)	Yes (Asada et al., 2007; Barnes et al., 2007; Shelly et al., 2007)
Par5	14-3-3 protein (Morton et al., 2002)	Par5 (Benton et al., 2002)	14-3-3 proteins (Ichimura et al., 1988; Hurd et al., 2003)	Unknown
Par6	Scaffold (Watts et al., 1996)	Par6 (Petronczki and Knoblich, 2001)	mPar6 (Joberty et al., 2000)	Yes (Shi et al., 2003; Nishimura et al., 2004; Schwamborn and Puschel, 2004; Shi et al., 2004; Nishimura et al., 2005; Higginbotham et al., 2006; Schwamborn et al., 2007; Schwamborn et al., 2007; Vohra et al., 2007; Zhang et al., 2007; Yi et al., 2010) No (Rolls and Doe, 2004)