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REVIEW

Centrosome positioning and primary cilia assembly orchestrate neuronal development

Sneha RAO[1,2], Shaoyu GE[2], Maya SHELLY (✉)[2]

[1] *Program in Neuroscience, State University of New York at Stony Brook, Stony Brook, NY 11794-5230, USA*
[2] *Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, NY 11794-5230, USA*

Abstract **Establishment of axon and dendrite polarity, migration to a desired location in the developing brain, and establishment of proper synaptic connections are essential processes during neuronal development. The cellular and molecular mechanisms that govern these processes are under intensive investigation. The function of the centrosome in neuronal development has been examined and discussed in few recent studies that underscore the fundamental role of the centrosome in brain development. Clusters of emerging studies have shown that centrosome positioning tightly regulates neuronal development, leading to the segregation of cell factors, directed neurite differentiation, neuronal migration, and synaptic integration. Furthermore, cilia, that arise from the axoneme, a modified centriole, are emerging as new regulatory modules in neuronal development in conjunction with the centrosome. In this review, we focus on summarizing and discussing recent studies on centrosome positioning during neuronal development and also highlight recent findings on the role of cilia in brain development. We further discuss shared molecular signaling pathways that might regulate both centrosome and cilia associated signaling in neuronal development. Furthermore, molecular determinants such as DISC1 and LKB1 have been recently demonstrated to be crucial regulators of various aspects of neuronal development. Strikingly, these determinants might exert their function, at least in part, via the regulation of centrosome and cilia associated signaling and serve as a link between these two signaling centers. We thus include an overview of these molecular determinants.**

Keywords centrosome positioning, neuronal polarization/migration, primary cilia

Introduction

The assembly of the complex architecture of the mammalian nervous system requires the carefully coordinated timing for differentiation of distinct neuronal populations, migration, and neural circuit formation. The precise synchronization of these processes results from a tightly regulated process of cytoskeletal structuring, polarization and maintenance. A central cellular component of the neuronal cytoskeletal structure is the microtubule (MT) arrays and their organizing center (MTOC, centrosome). The dynamic centrosome positioning provides a structural foundation for the MT array-associated signaling, and the disassembly or assembly of primary cilia to respond to external cues during neuronal development, migration, maturation, circuit formation and maintenance.

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Correspondence: Maya SHELLY
E-mail: mshelly@notes.cc.sunysb.edu

Centrosome positioning in neuronal migration

Precise centrosome positioning and function have been demonstrated to be of crucial importance in neuronal migration in the developing neocortex. Newly born cortical neurons undergo extensive morphological changes and cytoskeletal structure transformations on their journey from their birth place at the ventricular zone to the cortical plate, which includes reversal in the direction of migration and transition from multipolar morphology to the stereotypical bipolar morphology with a leading and trailing processes (Barnes and Polleux, 2009; Kriegstein and Alvarez-Buylla, 2009). Although the cellular mechanisms and extrinsic cues that underlie these extensive morphological changes are

poorly understood, the forward and reverse neuronal migration is accompanied by a dynamic regulation of the centrosome position in a precise pattern (Bielas et al., 2004). Typically, neuronal migration occurs in a well-coordinated stepwise process where the leading process extends forward in the direction of the migration and the centrosome is positioned ahead of the nucleus in the direction of migration with movement of the centrosome into the leading process (Fig. 1B). This is followed by a rapid translocation of the nucleus toward the centrosome, resulting in the shortening of the leading process. The latter then elongates and the sequence of events repeats, resulting in cell migration. It has been hypothesized that the centrosome serves as the link between the driving forces of the dynamic MTs in the elongating leading process and the MT network that surrounds the nucleus (Tsai and Gleeson, 2005). Thus, MT-driven forces generated in the leading process transmit through the centrosome to the nucleus and result in the forward movement of the nucleus. In support of this notion it was shown that the MT network that surrounds the nucleus in cerebellar granule neurons converges at the centrosome, and that this network shows dynamic cycles of expansion and shrinkage along the axis of cell migration (Tsai and Gleeson, 2005).

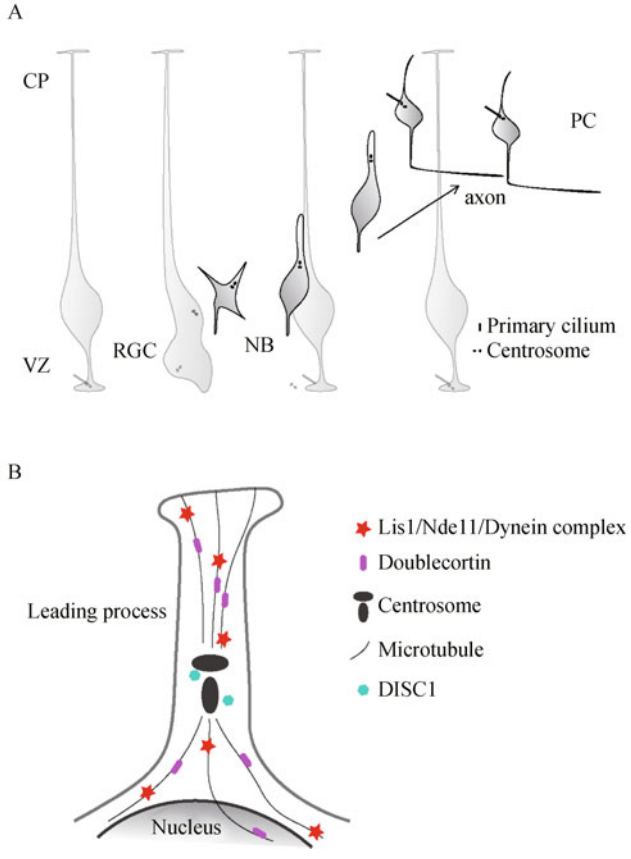

Figure 1 Schematic drawing of neuronal migration and potential mechanisms. (A) A simplified drawing to illustrate centrosome positioning and primary cilia dis- or assembly of radially migrating pyramidal neurons (PC) in the mammalian neocortex. Mouse cortical neurons are in general generated between E11 and E17 by radial glial progenitors (RGC) in the ventricular zone (VZ). Newly born neuroblasts migrate along RGCs and reach their final destination in cortical plate (CP). In migrating neuroblasts, the centrosome localizes in the leading process, which presumably form a microtube-based basket to translocate the nucleus. Two models have been previously proposed in a previous review (Tsai and Gleeson, 2005). During migration, most neurons establish their axon and dendrites. In these neurons, primary cilia are absent and formed upon the arrival of their desired destination. (B) Key molecules presumably involved in centrosome positioning and migration. Centrosome localizes in the leading process. Associated with dynein/LIS1/Ndel I complex, doublecortin and DISC1, positioned centrosome will pull the nucleus forward through microtubules.

Microtubule-associated proteins (MAPs) regulate centrosome positioning in neuronal migration

Further studies showed that the microtubule-associated proteins (MAPs) play a crucial role in the regulation of MT dynamics (Tsai and Gleeson, 2005). Among the MAPs, lissencephaly1 (LIS1) and doublecortin (DCX) have been extensively studied and found to be required for normal neuronal migration through their regulation of the coupling between the centrosome and the nucleus (Tanaka et al., 2004) (Fig. 1B). In particular, LIS1 is an evolutionary conserved regulator of the retrograde molecular dynein and dynactin motor complex (Hatten, 2005) and manipulation of LIS1 expression interfered with dynein activity (Xiang, 2003). LIS1 also interacts with the protein nudEL, which has two mammalian homologs, the nuclear distribution factor E-homolog 1 (NDE1), and NDE-like 1 (Nudel1) (Li et al., 2005) and this interaction has been shown to regulate dynein function in the promotion of neuronal migration in embryonic cortical neurons (Shu et al., 2004; Yamada et al., 2008). The regulatory role of this interaction has been reinforced by another study, in which downregulation of Ndel1 was shown to affect the coupling between the nucleus and the centrosome and genetic deletion of Nde1 affected both the proliferation of cortical progenitors and neuronal migration (Feng and Walsh, 2004). In accordance, Nde1 is a central component of the centrosome and has been shown to interact directly with γ-tubulin as well as other centrosome-associated proteins (Vergnolle and Taylor, 2007). Overexpression of Nde1 was shown to result in the dissociation of γ-tubulin from the centrosome and abnormal microtubule organization. Importantly, it was demonstrated that NDE1, Nudel1, and LIS1, bind directly to Disrupted in Schizophrenia 1 (DISC1), (Millar et al., 2003; Morris et al., 2003; Ozeki et al., 2003; Brandon et al., 2004; Camargo et al., 2007; Taya et al., 2007; Burdick et al., 2008; Bradshaw et al., 2009), an important regulator of the dynein motor complex that plays a critical crucial role in neuronal proliferation, migration, integration, and synaptic function within the developing and adult brain (Duan et al., 2007; Faulkner et al., 2008; Kvajo et al., 2008)

(see below, Fig. 1B). A recent study demonstrated that interaction of DISC1 and the cAMP-specific-hydrolyzing enzyme phosphodiesterase 4 (PDE4) modulate NDE1 phosphorylation by cAMP-dependent protein kinase A (PKA) (Bradshaw et al., 2011). Interestingly, the PKA-mediated phosphorylation of NDE1 inhibits neurite outgrowth in Rat Neuroscreen-1 cells (Bradshaw et al., 2011). Similar to LIS1, the DCX protein was also shown to complex with cytoplasmic dynein, and the two proteins regulate nuclear-centrosomal coupling. The MT-stabilizing protein DCX localizes to the centrosome and the MTs surrounding the nucleus in migrating neurons, and it interacts directly with LIS1 (Higginbotham and Gleeson, 2007). Importantly, ectopic expression of DCX in neurons that lack a single copy of the Lis1 gene rescues their migration defective phenotype. Furthermore, in cortical progenitor neurons isolated from the rostral migratory stream of DCX-deficient mice, nuclear translocation to the leading edge following the centrosome movement into the leading process, often fails, resulting in impaired migration (Ocbina et al., 2006), indicating that DCX is a crucial component of nuclear-centrosome coupling during neuronal migration. Importantly, MTs are not the only forces that control the forward nuclear translocation and neuronal cell movement, as recent studies have shown that myosin activity at the rear of the soma also contributes to the forward nuclear translocation. According to this model, the centrosome positioning in the leading process might create cytoplasmic expansion that allows nuclear translocation by contractile myosin forces at the rear of the cell (Higginbotham and Gleeson, 2007). Thus, it is conceivable that both MT- and myosin-based forces coordinate the sequential process of centrosome positioning and nuclear translocation to the leading process during neuronal migration.

Extracellular signals regulate centrosome positioning in neuronal migration

Of note, several extracellular factors including Slit and Semaphorin3A (Sema3A) are known to regulate the polarized extension of the leading process of a migrating neuron (Polleux et al., 1998; Hu, 1999; Wu et al., 1999; Polleux et al., 2000; McAllister, 2002; Chen et al., 2008). Apart from its role in serving as a link between the leading process and nucleus, the centrosome might also regulate the stabilization of the leading process while it extends in the direction of guidance cues. For example, it has been demonstrated that Slit serves as a chemorepellent to migrating neurons in the developing mammalian forebrain (Dickson and Gilestro, 2006), where it reverses the migration of cortical neurons and redirects them rostrally into the olfactory bulb (Wu et al., 1999). During this process of migration reversal, Slit induces the collapse of the leading process, and extension of a new leading process opposite from the source of Slit (Killeen and Sybingco, 2008). Interestingly, following the re-initiation of the leading process, the centrosome re-orients into the newly formed leading process while the nucleus remains immobile. These findings suggest that the centrosome might be necessary for the outgrowth and stabilization of the newly formed leading process rather than for its initiation. In support of this notion, it was shown that blocking centrosome reorientation does not inhibit the initiation of the new leading process, but that it causes for its instability, resulting in the failure of neuronal migration away from Slit (Higginbotham et al., 2006).

Although many studies to date have identified a central role of the centrosome in neuronal migration, here we should point out that another study using cultured cerebellar sections found that nuclear translocation was independent of centrosome positioning in radially migrating granule cells in mice (Umeshima et al., 2007). Similar observations for the lack of centrosome-nuclear coupling were found in migrating neurons in the zebrafish cerebellum (Distel et al., 2010). Whether these contradictory findings result from the specific model system used in these studies or the existence of an alternative migration pattern remains to be elucidated. Nevertheless, emerging evidence that downstream signaling associated with centrosome positioning regulates neuronal migration suggests the centrosome positioning likely plays an important role in neuronal migration.

Centrosome positioning in axon/dendrite development

As they migrate over long distances to populate different regions of the brain, most neurons establish axonal and dendritic identities. In some specific types of cells such as retinal ganglion cells and bipolar cells, post mitotic neurons may inherit the axon/dendrite polarity from the apical-basal polarity of their neuroepithelial progenitors (Hinds and Hinds, 1978; Morgan et al., 2006; Zolessi et al., 2006) reviewed by Barnes and Polleux (2009). However, the majority of neuronal subtypes undergo extensive stereotypical morphological changes, leading to polarized outgrowth of axon/dendrite, as shown by cerebellar granule neurons and cortical and hippocampal pyramidal neurons, three of the best-studied models of neuronal polarization *in vivo* (Rakic, 1971,1972; Shoukimas and Hinds, 1978; Gao and Hatten, 1993; Komuro et al., 2001; Hatanaka and Murakami, 2002; Noctor et al., 2004). The new born cortical neuron exiting from the asymmetric division has already acquired a bipolar morphology in the ventricular zone (VZ) with the long axis perpendicular to the cortical layers. With a brief transition to multipolar morphology in the subventricular zone (SVZ), the neuron resumes its bipolar morphology prior the onset of radial migration (Noctor et al., 2004). The leading process of the migrating cell becomes the main apical dendrite whereas the trailing process becomes the axon and grows rapidly toward the target. The exact timing for axon specification, whether it begins during the pre-migratory or migratory

phase, and whether it emerges from the bipolar or multipolar stage, remains unclear.

Centrosome positioning has been demonstrated to undergo changes during these different stages of neuronal development. During the transition from a bipolar to a multipolar morphology in the SVZ, developing cortical neurons might extend an axon before readopting a bipolar morphology and resuming migration to the cortical plate (CP) (Shoukimas and Hinds, 1978; Noctor et al., 2004). Interestingly, the centrosome is found to be located at the base of the nascent axon that might emerge from one of the multipolar processes, however, as cells leave the SVZ and regain their bipolar morphology, the centrosome relocates to the leading process that extends toward the CP (Shoukimas and Hinds, 1978). The positioning of the centrosome at the base of the nascent axon has been demonstrated in developing neurons *in vivo* and *in vitro* (Shoukimas and Hinds, 1978; Zmuda and Rivas, 1998; de Anda et al., 2005, 2010) and it might serve a crucial role to support the growth of the nascent axon, as growing axons require a steady delivery of membrane and MTs, as well as proteins such as guidance factor receptors, to the navigating growth cone to allow directed axon extension (Zmuda and Rivas, 1998; Baas and Yu, 1996). Thus, in developing neurons, centrosome positioning serves as a center for directed membrane trafficking and polarized MT-delivery to the developing axon and dendrite, as the centrosome is the primary source for MTs (Zmuda and Rivas, 1998; Baas and Yu, 1996), while the Golgi complex colocalizes with the centrosome (Zmuda and Rivas, 1998; Baas and Yu, 1996). Interestingly, in cultured hippocampal neurons, it was demonstrated that as a result of the last mitotic division, an intrinsic spatial polarization of the neuronal cell might determine the site for the onset of axon development and that asymmetric centrosome positioning plays a crucial role in this spatial decision (de Anda et al., 2005). In these neurons it was shown that the centrosomes, the Golgi apparatus and endosomes, cluster together opposite from the plane of the last mitotic division, and this spatial restriction marks the site of the emergence of the first neurite, which will consistently develop into the axon (de Anda et al., 2005). The polarized distribution of these organelles has been shown to be necessary and sufficient for neuronal polarization and the initiation of the axon. In support of the critical role of the centrosome in this process, it has been demonstrated that polarized MT polymerization and membrane transport precedes the initiation of the first neurite that is destined to become the axon. Furthermore, suppression of centrosome-mediated activities precludes neuronal polarization and axon development, whereas, neurons that harbor more than one centrosome develop multiple axons (de Anda et al., 2005). Developing cerebellar granule neurons undergo a typical developmental process in which, first, the neuron adopts a bipolar morphology and migrates tangentially with a leading and a trailing process, followed by emergence of a third process orthogonally from the cell body that becomes the leading process and redirects migration toward the inner granule layer. This newly formed leading process gives rise to the dendrite, whereas the trailing processes form a characteristic T-shaped axon. In the sequential formation of the T-shaped axon, the centrosome is initially located at the base of the first axon and then, as the cell forms the second axon, the centrosome reorients to the base of the newly forming axon (Zmuda and Rivas, 1998). The initiation of the second axon is abolished upon treatments that cause actin depolymerization, treatments that also result in the disruption of the typical centrosome positioning (Zmuda and Rivas, 2000). Importantly, actin depolymerization does not affect the extension of the second axon, if the actin-depolymerizing drugs are added following the initiation of this axon (Zmuda and Rivas, 2000). Taken together with the findings from cultured hippocampal neurons, it is thus possible to assume that the centrosome positioning at the base of the axon is required for the initiation of the axon but not for its extension. A different paradigm for the correlation between centrosome positioning and axon initiation has been found in the developing retinal ganglion cells (RGC) in zebrafish and mouse. Neuroepithelial progenitors have a typical apical and basal attachment, and they undergo asymmetrical cell division at the apical surface. Upon completion of mitosis, the nucleus loses its apical attachment and translocates basally, as the cell adopts a bipolar morphology with the extension of basal and apical processes. The axon develops from the basal process and the dendrite develops from the apical process. Interestingly, the centrosome was found to be localized to the apical side during nuclear translocation (Zolessi et al., 2006). Thus, unlike in cultured hippocampal and cerebellar granule neurons, in RGC neurons, the centrosome is localized in the trailing process, which becomes the dendrite, rather than in the axon. Nevertheless, these studies further confirmed the role of centrosome in neurite formation and extension.

Molecular mechanisms of centrosome positioning

The mechanisms for centrosome positioning at the leading edge were mainly studied in non-neuronal cells, primarily in astrocytes and fibroblasts. In these cells, the small Rho-GTPase Cdc42, acting through a Par6-aPKC complex, is required to establish cellular asymmetry during morphogenesis, asymmetric cell division and directed cell migration. In astrocytes, centrosome reorientation is regulated by integrin signaling that activates Cdc42, which regulates signaling from cell surface receptors to the actin–MT cytoskeleton (Etienne-Manneville and Hall, 2001; Raftopoulou and Hall, 2004). In a scratch-induced cell migration assay (Wound Healing Assay) using primary rat astrocytes and fibroblasts grown to a confluent monolayer, the cells extend their cytoskeleton into the wound and reorient the centrosome and Golgi complex in front of the nucleus in the direction of the

wound (Etienne-Manneville and Hall, 2003). In this directed cell migration for wound-closure, the Par6–PKCζ complex directly regulates the glycogen synthase kinase-3β (GSK-3β) activity to promote polarization of the centrosome and to control the direction of cell protrusion. The activity of the Rho-GTPase Cdc42 at the leading edge recruits and activates the Par6–PKCζ complex, whereas PKCζ phosphorylates and inactivates GSK-3β, specifically at the leading edge. Cdc42-dependent phosphorylation of GSK-3β induces the interaction of adenomatous polyposis coli (APC) protein with the plus ends of microtubules, an association that is essential for cell polarization (Etienne-Manneville and Hall, 2001; Etienne-Manneville and Hall, 2003). Of note, un-phosphorylated, active GSK-3β, does not allow the interaction of APC to MT plus-ends. Upon GSK-3β inactivation, APC binds to MT, causing their stabilization and facilitating MT capture at the leading edge by dynein, a retrograde molecular motor that is required for centrosome positioning in many cell types (Etienne-Manneville and Hall, 2001; Palazzo et al., 2001), whereby, the MT minus-end motor activity of dynein pulls captured MTs toward the leading edge and orients the centrosome within the protrusion into the wound.

PI3-kinase regulates neuronal development and migration through the Par protein complex

In neuronal development, this aspect of centrosome function was suggested by the study of the interplay between actin and centrosome-dependent MT cytoskeleton in axon extension and growth. For example, outgrowth of the newly specified axon was promoted by the collapsin response mediator protein (CRMP-2), an axon-specific microtubule-associated protein (Inagaki et al., 2001), and CRMP-2 overexpression induced the formation of multiple axons. Local activation of phosphatidylinositol 3′ kinase (PI3K) at the tip of the axon is important for neuronal growth and polarization (Shi et al., 2003), since PI3K induces activation of aPKC and several guanine-nucleotide-exchange factors (GEFs), which activate the Rho family of small GTPases Cdc42 and Rac-1, leading to enhanced axon growth (Arimura and Kaibuchi, 2007). Moreover, both atypical protein kinase C (aPKC) and Cdc42/Rac-1 interact with PAR-3/PAR-6, and thus may recruit the latter complex to localized regions of PI3K activity in the axon, to promote axon growth (Shi et al., 2003). The PAR-3/PAR-6 targeting to the axon tip might be mediated by APC (Shi et al., 2004). Importantly, recent studies have shown that BDNF and NT-3 could promote neuronal polarization by inducing PI3K-mediated phosphorylation at Ser-9 and inactivation of GSK-3β (Yoshimura et al., 2005; Jiang et al., 2005), indicating that inactivation of this constitutively active kinase at the growth cone is important for axon differentiation (Yoshimura et al., 2005).

A recent study that used local presentation of laminin and vitronectin demonstrated that these molecules polarize developing cerebellar granule neurons by regulating centrosome positioning downstream of PI3-kinase signaling (Gupta et al., 2010). Furthermore, a study that examined centrosome positioning in developing RGC neurons demonstrated that laminin at the basal lamina *in vivo* is essential for the polarized positioning of the centrosome and that Laminin acting directly on RGC neurons is sufficient for the polarized axon development in these neurons (Randlett et al., 2011). Another recent study demonstrated that N-cadherin expression accumulates at one pole of newborn cultured hippocampal neuron, to mark the site of the emergence of the first neurite (Gärtner et al., 2012). N-cadherin accumulation is followed by the relocation of the Golgi and centrosome toward the morphological pole marked by N-cadherin, indicating that organelle asymmetry might be a consequence of a polarized membrane-signaling event. This sequence of events for centrosome repositioning was demonstrated to be regulated by the PI3-kinase signaling (Gärtner et al., 2012). Conversely, another study highlighted an intriguing distinction between the role of centrosome in axon initiation as compared to the extension of an existing axon (Stiess et al., 2010), demonstrating that following axon initiation, acentrosomal MT nucleation might regulate axon growth in cultured hippocampal neurons.

Likely similar mechanisms operate in neurons to position the centrosome during their migration. For example, the chemorepellent Slit serves to redirect the migration of cortical neurons into the olfactory bulb, a process that involves centrosome repositioning into the new leading process that forms in the direction of the migration (Wu et al., 1999; Dickson and Gilestro, 2006). It was shown that the activity of Cdc42 is required in Slit-induced migration reversal (Wong et al., 2001). Furthermore, inhibition of GSK-3β or PKCζ blocks the action of Slit in migration reversal, an effect that is accompanied by prevention of centrosome repositioning and failure in the stabilization of the newly formed leading process (Higginbotham et al., 2006). Importantly, in migrating cerebellar granule neurons, Par6 and PKCζ localize to the centrosome and overexpression of Par6 disrupts PKCζ localization and centrosome integrity and positioning (Higginbotham et al., 2006).

LIS1/Disc1/DCX proteins regulate neuronal development and migration

Furthermore, the centrosome associated proteins LIS1 and DCX regulate axon extension (Koizumi et al., 2006; Taya et al., 2007). Dynein and its regulator, LIS1, are also localized to the centrosome, and the loss of function of these genes increases the nuclear-centrosome distance and results in defects in nuclear translocation (Feng et al., 2000; Tanaka et al., 2004). Downregulation of LIS1 or DCX impeded migration of cortical neurons, and arrested these neurons at the multipolar stage, abolishing the transition to the typical bipolar morphology (Bai et al., 2003; Tsai et al., 2005). These findings might imply that downregulation of DCX and LIS1

result in the failure of centrosome reorientation to the base of the emerging axon or the leading process, and that this centrosome miss-positioning precludes the transition from the multipolar to the bipolar morphology and impairs neuronal migration. In support of this notion it was demonstrated that DCX is necessary for MT stabilization and bundling in the growth cone of cortical neurons (Bielas et al., 2007). Another centrosome-associated protein, disrupted in schizophrenia 1 (DISC1), that caught extensive attention recently has been shown to be required during both normal migration and neurite development (Kamiya et al., 2005). It was shown that DISC1 anchors the dynein motor complex at the centrosome. The deletion of DISC1 in developing cortical neurons caused for the disruption of centrosome/nucleus coupling, an effect that resulted in the disruption of cortical neuronal migration and the miss-orientation of the apical dendrite. This centrosome–associated protein has recently been reported to play a similar role in the development of adult-born dentate granule cells (Duan et al., 2007). Furthermore, a recent study (Ishizuka et al., 2011) highlighted an important regulatory mechanism for DISC1 function that triggers a transition from DISC1-regulated maintenance of the proliferation of mitotic progenitor cells to DISC1-induced activation of migration of postmitotic neurons in mice. This transition in DISC1 function is regulated by specific phosphorylation of DISC1 at serine 710. This phosphorylation triggers the recruitment of Bardet-Biedl syndrome (BBS) proteins (see below) to the centrosome. These events downstream of DISC1 phosphorylation result in initiation of neuronal migration, whereas unphosphorylated DISC1 regulates proliferation of mitotic progenitors via a canonical Wnt signaling pathway (Ishizuka et al., 2011). The β-catenin is known to regulate cell-growth and differentiation downstream of Wnt signaling. Interestingly, a recent study examined the role of β-catenin in midbrain development by temporal, either complete or partial, deletion of β-cateninin in progenitor cells (Chilov et al., 2011). Cells with complete deletion of β-catenin did not contain centrosome or MT-network and failed to polarize. Importantly, this effect was shown to be associated with specific phosphorylation of β-catenin at Serine33/Serine34/Threonine41, a phosphorylation that targets β-catenin to the centrosome and regulates the β-catenin function (Chilov et al., 2011). Of note, the protein CAMDI (coiled-coil protein associated with myosin II and DISC1) interacts with DISC1 and translocates to the centrosome in a DISC1-dependent manner (Fukuda et al., 2010). This interaction, as well as the CAMDI-centrosome association was demonstrated to regulate neuronal migration as downregulation of CAMDI resulted in impaired radial migration and impaired centrosome positioning in cortical progenitor neurons. Interestingly, CAMDI associates with phosphomyosin II and induces the accumulation of phosphomyosin II at the centrosome in a DISC1-dependent manner, an association that is necessary for cortical radial migration (Fukuda et al., 2010).

Cross-talk between the Par protein complex and the LIS1/Disc1/DCX proteins

In addition to their localization to the centrosome, Par6–PKCζ complex, GSK-3β, LIS1, and dynein are also localized at the front edge of the leading process. This localization presumably regulates nuclear translocation and the outgrowth of the leading process. The emerging model for the molecular mechanisms in migrating neurons thus would be as follows: in response to an extracellular guidance cue such as Slit, activation of Cdc42 at the edge of a forming leading process would promote actin cytoskeleton dynamics. The localized recruitment of Par6–PKCζ complex to the leading edge will result in the phosphorylation and inactivation of GSK-3β, leading to MT capture and stabilization through APC. The LIS1 stimulates dynein motor activity that pulls captured MTs and the attached centrosome up into the leading process. Lastly, Par6 might facilitate the targeting of dynein and LIS1 to the centrosome, where dynein and LIS1 might regulate the translocation of the nucleus toward the centrosome (Tsai and Gleeson, 2005). These findings also demonstrate the important concept of the conservation of the molecular mechanisms that control cell polarization and migration in different cell types, and suggest that much is to be learned from other cell types as to how neurons reshape their cytoskeleton and regulate centrosome reorientation and positioning in response to extracellular signals.

LKB1 might regulate neuronal development and migration through centrosome positioning

Recent studies have demonstrated that the evolutionarily conserved serine-threonine kinase LKB1 plays a defining role in axon initiation during neuronal development (Barnes et al., 2007; Shelly et al., 2007). Interestingly, LKB1 might also regulate neuronal migration in a centrosome-dependent manner (Asada et al., 2007). Although the role of LKB1 in neuronal development and migration is only in its initial stages of elucidation, the current findings suggest that LKB1 might serve as an important molecular link between these two developmental processes.

The conserved role for LKB1 as an upstream regulator of cellular polarization in diverse cell types has inspired several recent studies on the role of LKB1 and its down-stream effectors in neuronal polarization, where LKB1 has emerged as an axon determinant during embryonic neuronal development. The localization and function of LKB1 is tightly regulated by the STE20-related pseudokinase STRAD, an adaptor protein that binds LKB1 and causes for its stabilization and activation (Baas et al., 2003). The ubiquitously expressed scaffolding protein MO25 is a third component of the trimeric LKB1-STRAD-MO25 complex (Boudeau et al., 2003). LKB1 is required for axon formation of cortical neurons *in vivo*. Because mice lacking LKB1 die

between E9 and E10, before the formation of the cerebral cortex (Ylikorkala et al., 2001), the role of LKB1 in neuronal development in the forebrain was examined by selective deletion of the LKB1 gene in dorsal telencephalic progenitors, which give rise to all pyramidal neurons in the cerebral cortex (Barnes et al., 2007). This specific deletion of the lkb1 allele resulted in striking absence of corticofugal axons and callosal axons in the mice (Barnes et al., 2007). Using *in utero* electroporation (Saito and Nakatsuji, 2001) of specific shRNAs to downregulate LKB1 expression in cortical progenitor cells in E18 rat embryos, (Shelly et al., 2007) also observed a striking absence of radially oriented axons in the cortical plate and horizontally oriented axons near the SVZ. Examination of the morphology of individual neurons during radial migration revealed that LKB1 is necessary during the early stages of axon initiation (Barnes et al., 2007). Interestingly, while the role of mammalian LKB1 in promotion of axon differentiation is now well established, a recent study of *C. elegans* motor neurons demonstrated a selective role for Par-4/LKB1 in dendrite growth, down-stream of UNC6/netrin signaling (Teichmann and Shen, 2011).

In utero electroporation studies have also suggested that overexpressing wild-type LKB1 resulted in increased axon formation in the cortical plate and SVZ (Shelly et al., 2007), with abnormal axonal branching and a higher number of long processes descending toward the white matter. Moreover, wild-type LKB1-transfected cells in the intermediate zone and SVZ showed complex morphology and apparent retarded migration, possibly due to multiple axon formation during radial migration, similar to the effects of LKB1 over-expression in cultured hippocampal neurons (Shelly et al., 2007). In cultured hippocampal neurons, LKB1/STRAD accumulation and Protein kinase-A (PKA)-dependent LKB1 phosphorylation and stabilization were shown to be an early signal for an undifferentiated neurite to become an axon (Shelly et al., 2007). In support of the role of LKB1-S431 phosphorylation for axon development *in vivo*, it was demonstrated that in the developing cortex, pLKB1-S431 levels were increased considerably between E15.5 and P1, the vital developmental period in which newly generated neurons extend axons (Barnes et al., 2007). Taken together, these *in vivo* findings indicate that LKB1 is required for axon formation *in vivo* and specific PKA-dependent phosphorylation of LKB1 is essential.

Several down-stream effectors of LKB1 are known to be involved in neuronal polarization. Association with STRAD and phosphorylation of LKB1 on S431 result in the activation of LKB1, and the activated LKB1 phosphorylates the PAR-1 related kinases, including the mammalian Synapses of the Amphid Defective, SAD-A and SAD-B (also known as BRSK1/2, the brain-specific kinases 1 and 2), and the Microtubule Affinity-Regulated Kinases MARK1-4. Both SAD-A/B and MARK-2 regulate axon formation (Kishi et al., 2005; Chen et al., 2006). Mice with targeted deletion of both SAD-A and SAD-B genes demonstrated striking absence of cortical axon formation (Kishi et al., 2005). Furthermore, SAD-A and SAD-B are known to phosphorylate the axonal microtubule binding protein Tau (Kishi et al., 2005; Barnes et al., 2007) on a specific serine residue S262, which regulates the binding of Tau to microtubules (Biernat et al., 1993), modifying the microtubule organization that is required for axon formation. LKB1 was shown to be necessary for the SAD-mediated phosphorylation of Tau (Barnes et al., 2007).

LKB1 likely regulates neuronal migration through the centrosome. In a study that addressed the role of LKB1 in developing cortical neurons, downregulation of LKB1 by in utero electroporation of specific shRNAs, was carried out at E14 and neuronal development was analyzed at E16, E17, and E18 (Asada et al., 2007). By E17, downregulation of LKB1 caused impeded radial migration and neuronal retention in the IZ, whereas most control neurons were found to migrate to the CP. Interestingly, this impeded migration resulted from disrupted centrosome positioning and nucleus-centrosome coupling following LKB1 downregulation (Asada et al., 2007). These observations reveal an important role of LKB1 in spatial positioning of the centrosome and in regulation of the nucleus-centrosome coupling during cortical migration. Furthermore, in neurons that did reach the CP following LKB1 downregulation, the neurons exhibited a defective axon/dendrite polarization, with high percentage of neurons exhibiting a thick dendrite-like neurite extending toward the ventricle and a single, thin, axon-like neurite oriented toward the pia (Asada et al., 2007). The inverted axon/dendrite orientation was accompanied by miss positioning of the centrosome to the base of the dendrite that oriented toward the ventricle instead of the apical localization of the centrosome in control cells (Asada et al., 2007). Thus, the migration defect was accompanied with the disruption of axon/dendrite polarity, resulting in reversed orientation of differentiating neurons. Interestingly, down-regulation of LKB1 in these neurons was accompanied by abolishment of GSK-3β phosphorylation at the axon tips, indicating that LKB1 might regulate axon formation and neuronal migration through phosphorylation of GSK-3β (Asada et al., 2007; Asada and Sanada, 2010). The LKB1-mediated phosphorylation of GSK-3β enables the localization of the MT-plus-end binding protein APC at the distal ends of microtubules in the tip of the leading process, resulting in MT-stabilization at the leading edge (Asada and Sanada, 2010). The LKB1-mediated phosphorylation of GSK-3β and the binding of APC to MT-distal ends are required for MT-stabilization at the leading edge, the forward movement of the centrosome, and thus in neuronal migration, indicating that LKB1-mediated regulation of the spatial activity of GSK-3β and APC are critical in these processes during embryonic neuronal development. Of note, downregulation of MARK2, the down-stream effector of LKB1, by in utero electroporation of specific shRNAs resulted in arrested neuronal migration at the IZ, with neurons exhibiting multipolar

phenotype (Sapir et al., 2008). This arrested migration following MARK2 downregulation was accompanied by increased distance between the centrosome and the nucleus, and slower and miss-oriented forward movement of the centrosome in migrating embryonic cortical neurons (Sapir et al., 2008). Importantly, MARK2 is a regulator of DCX activity during brain development (Sapir et al., 2008). Thus, MARK2 might regulate cortical neuronal migration by controlling the centrosome positioning and movement as well as MT dynamics, at least in part by phosphorylation of DCX (Sapir et al., 2008).

Cooperation between primary cilia and centrosome in neuronal development

Proper centrosome localization appears to be essential for primary cilia development. Furthermore, both centrosomes and cilia share common signaling components. Recent studies have demonstrated that cilia might be vital for normal neuronal migration and axon guidance in neuronal development, possibly via their tight connection with centrosome. Furthermore, several molecular participants have been identified that regulate neuronal development and migration through modulation of both centrosome positioning and cilia development. Indeed, several recent studies provide a greater insight into this connection and its role in neuronal development. We thus dedicate the next part of our review to discuss these findings on the role of cilia in neuronal development.

Cilia are protrusions of the cell membrane, supported by a microtubule framework that originates from the basal body, a modified centriole. They are versatile organelles that can be broadly classified into two types, motile and non-motile (primary) cilia, with a characteristic MT configuration. Here we focused on reviewing the current knowledge of the role of the cilium in neuronal development in particular. The importance of cilia in development came into focus with an increased awareness of conditions known as ciliopathies, where defects in ciliogenesis and genes that regulate ciliary functions and signaling lead to debilitating developmental disorders that involve neuronal pathologies (Fliegauf et al., 2007; Lancaster and Gleeson, 2009). Studies on the genes involved in these disorders and subsequently the signaling pathways affected by these genes provided clues about the contribution of cilia to normal developmental pathways (Lee and Gleeson 2011).

Motile cilia have a characteristic $9 + 2$ configuration of the axonemal microtubules originating from the basal body, with an outer ring of 9 microtubule doublets and a central doublet. These motile cilia exhibit a beating motion due to the movement of dynein arms that associate with the tubulin in the microtubules (Salathe, 2007). Motile cilia have been extensively studied and shown to have several functions, including regulation of cerebrospinal fluid flow by ependymal cells in cerebral ventricles (Ibanez-Tallon et al., 2004), establishing cell polarity in epithelia (Wallingford, 2010; Mirzadeh et al., 2010), and regulating right-left body-axis symmetry in embryonic development (Olbrich et al., 2002). Non-motile, or primary cilia, have a $9 + 0$ axoneme microtubule configuration and were formerly referred to as "rudimentary cilia," (Gardiner and Rieger, 1980). Originally, they were believed to only play a limited role in sensory transduction, a notion that has been challenged by more recent findings. (Pazour and Witman, 2003; Wheatley, 2005). Furthermore, most polarized cells in mammals possess a primary cilium at their apical surface. In the brain, primary cilia are ubiquitous and are present on neurons, neuronal stem cells and also glial cells (Louvi and Grove, 2011) and there is a growing body of evidence that demonstrates a variety of roles for these organelles, especially in development.

Role of cilia in neuronal development

Transport between the cytoplasm and cilia is mediated by a process known as intraflagellar transport (IFT), an essential process during the development of cilia that was first observed in flagella of the alga, *Chlamydomonas* (Kozminski et al., 1993). Essential components for cilia development are transported up the cilia along the axoneme as the cilia elongate (Ishikawa and Marshall, 2011). This movement can be bidirectional and is mediated by IFT particles that are driven by the molecular motors kinesin and dynein (Pedersen and Rosenbaum, 2008). IFT particles are composed of 17 proteins forming two complexes. Whereas complex B IFT particles carry cargo in the anterograde direction from the base to the tip of the cilium using a kinesin-2 motor, complex A particles move turnover products retrogradely with a dynein motor back to the base of the cilium (Louvi and Grove, 2011).

The IFT proteins that were first identified in *Chlamydomonas* have functional mammalian homologs (Pedersen and Rosenbaum, 2008). A pioneering study from 2000, showed that IFT88, an intraflagellar transport protein, is required for primary cilium assembly in *Chlamydomonas* (Pazour et al., 2000). Mice with a defect in the mammalian homolog of IFT88 (Tg737), lack primary cilia (Pazour et al., 2000) and demonstrate phenotypes that closely resemble those of human polycystic kidney disease, a disease that is associated with loss of polarity of renal epithalial cells. The IFT88-defective mice also show abnormal development of the telencephalon and abnormally upregulated Wnt signaling targets, along with disrupted Shh signaling (Willaredt et al., 2008). Mutation in a ciliary gene, Ttc21b, that codes for the putative ortholog of the complex A protein IFT39, leads to defects in dorsal forebrain development and an upregulation of Shh signaling (Stottmann et al., 2009). Furthermore, a loss of function mutation of the mouse Ift172 gene leads to abnormal ciliary morphology in the nervous system, with no ciliary microtubules, accompanied by defects in hindbrain and forebrain

development (Gorivodsky et al., 2009). Thus cilia and cilia mediated signaling pathways play essential roles in nervous system development. This has led to an increased interest in this formerly overlooked organelle.

Cilia-associated signaling regulates neuronal development

The development of the nervous system is regulated by morphogens such as Hedgehog and Wnt. The components of both these signaling pathways as well as other signaling molecules have been localized to cilia. This suggests that cilia act as sensors of a wide range of developmentally important cues in the nervous system as they provide a morphological and functional landmark for the localization of these essential signaling components.

Typical Hedgehog signaling involves binding of the ligand (Shh, Ihh and Dhh) to the transmembrane receptor, Patched (Ptch). In the absence of a ligand, Ptch inhibits the transmembrane protein, Smoothened (Smo) (Goodrich and Scott, 1998). Binding of Hh to Ptch causes receptor endocytosis and thus relieves the inhibition of Ptch on Smo, which then permits transcription of Hh target genes (Ho and Scott, 2002). Indeed, downregulation of Shh signaling in mice produces several developmental defects, including lack of hind limb formation, cyclopia and neural tube defects (Chiang et al., 1996). Furthermore, Hedgehog signaling plays a role in dorso-ventral patterning of the brain (Ekker et al., 1995). IFT protein mutations identified by a genetic screen in mouse embryos were shown to cause neural tube defects by blocking Shh signaling (Huangfu et al., 2003). Many ciliary proteins are part of the Shh signaling pathway and defects in these proteins can cause abnormal developmental effects, similar to those caused by defects in Shh signaling (Huangfu et al., 2003). Shh receptor-ligand interaction at the cilia upregulates expression of Smoothened (Smo), and removes Ptch1 from the ciliary cell membrane (Corbit et al., 2005; Rohatgi et al., 2007). The downstream effectors of Shh, the Gli transcription factors, are also localized at the tips of cilia (Haycraft et al., 2005). Taken together, these findings show that cilia, via Shh signaling, may be transducers of sensory information that is essential during neuronal development.

Wnt signaling is an important developmental regulator, known to mediate proliferation, cell-fate determination and stem cell maintenance in many cell types. The canonical Wnt/β-catenin signaling pathway involves binding of Wnt to Frizzled receptor proteins. This triggers a series of downstream events, including activation of Frizzled-LRP5 or LRP6 complexes, activation of the Wnt effector Disheveled, inactivation of the β-catenin degradation complex and cytosolic accumulation and nuclear translocation of β-catenin, where it activates transcription (Angers and Moon, 2009). The non-canonical pathway is mediated by downstream effectors other than β-catenin and contributes to many crucial developmental processes (Angers and Moon, 2009). Cilia have been linked to Wnt signaling by the protein Inversin, which is found in primary cilia and basal bodies (Shiba et al., 2009). Inversin causes degradation of Disheveled and inhibition of canonical Wnt signaling (Simons et al., 2005). Interestingly, Inversin mutations have been shown to cause nephronophthisis (NPH), a cystic kidney disease (Lienkamp et al., 2012; Phillips et al., 2004). In support of the inhibitory role of cilia in canonical Wnt signaling, it was shown that mouse embryonic fibroblasts with mutations in the ciliary components Kif3A and IFT88 had an upregulated response to Wnt3a isoform (Corbit et al., 2008). Recent studies have shown that cilia are required for the normal function of the mammalian target of rapamycin (mTOR) signaling pathway, an important regulator of translation, which was shown to regulate cell size as well as the size and function of the cilia (Yuan et al., 2012). Thus, cilia were shown to sense fluid flow and to regulate cell size by downregulating mTOR in zebrafish embryos (Yuan et al., 2012). Interestingly LKB1 was demonstrated to be localized to cilia and to negatively regulate mTOR signaling, thus modulating cell size in canine epithelial Madin-Darby Canine Kidney (MDCK) cells (Boehlke et al., 2010).

Another signaling pathway associated with cilia is the platelet derived growth factor (PDGF) pathway. In fibroblasts, primary cilia are required for PDGF signaling, which mediates growth, migration and proliferation in many mammalian cell types (Schneider et al., 2005). Cilia are also associated with many other signaling pathway components, including the adenylyl cyclase ACIII receptor and somatostatin receptor 3 (SSRT3) (Händel et al., 1999), which are present in almost all areas and cell types of the mouse embryonic cortex (Bishop et al., 2007; Arellano et al., 2012). Downregulation of the SSRT3 receptor, that showed minor effect on ciliogenesis, leads to impaired object recognition memory in mice (Einstein et al., 2010), indicating that signaling at neuronal primary cilia may play a role in modulating synaptic plasticity. Cilia were also shown to be important for cerebellar development likely through regulating Wnt signaling. Deletion of IFT88 or Kif3A were shown to cause abnormal cerebellar morphology and hypoplasia (Chizhikov et al., 2007), malformations that might result from disrupted proliferation of the granule cell population due to loss of ciliary signaling (Chizhikov et al., 2007; Spassky et al., 2008).

Studies on patients with ciliopathies further highlight the importance of ciliary function in the developing nervous system. For example, patients with Bardet-Biedl syndrome, an autosomal recessive ciliopathy, have smaller hippocampal volume; ventriculomegaly and a global reduction in cortical gray matter (Baker et al., 2011), indicating that cilia are essential for normal cortical and hippocampal development. Another autosomal recessive ciliopathy, Joubert's syndrome, is a rare condition that is characterized by abnormal or absent decussation in the superior cerebellar peduncles and corticospinal tracts, as well as heterotopia in many brain regions (ten Donkelaar et al., 2000; Juric-Sekhar et al., 2012) pointing

to a possible role for cilia in some aspects of axon growth and guidance. Taken together, the above findings suggest that neurological pathologies observed in ciliopathies like Bardet–Biedl and Joubert syndromes might be caused by disruption of essential developmental signaling pathways at the cilia.

Cilia-associated signaling regulates development of adult newborn neurons

Primary cilia are present in adult neural stem cells and were demonstrated to be essential for adult neurogenesis via Hh and Wnt signaling. Adult neural stem cells, also known as radial astrocytes, develop from granule neuron precursor cells during early postnatal life (Han et al., 2008). Ablation of ciliary genes or Hh signaling component Smo during embryogenesis prevents the expansion of granule neuron precursors in the subgranular zone in the hippocampus, blocking the development of an adult stem cell population (Han et al., 2008). Thus embryonic stem cell precursors cannot give rise to adult neural stem cells when Hh signaling at the primary cilia is blocked. Another study that used a conditional knockout for the gene Stumpy, which is essential for ciliogenesis (Breunig et al., 2008) showed additional evidence in support of the above results, whereby ablation of cilia leads to decreased Shh signaling and a concomitant decrease in neural precursor proliferation and radial astrocyte development (Breunig et al., 2008). The primary cilium is also an essential signaling center for the synaptic integration of adult-born neurons in the mouse hippocampus. A recent study demonstrated that newborn dentate granule cells lacking a primary cilium formed defective glutaminergic synapses with entorhinal cortical projections (Kumamoto et al., 2012). This study (Kumamoto et al., 2012) showed that ablation of primary cilia results in increased canonical Wnt signaling, which in turn, leads to defective dendritic refinement and synaptic integration. Interestingly, another recent study showed that ciliogenesis begins upon the completion of radial migration of embryonic cortical neurons (Arellano et al., 2012) (Tsai and Gleeson, 2005) (Fig. 1A). A similar observation was made in adult-born dentate granule cells (Kumamoto et al., 2012).

Shared signaling mechanisms regulate centrosome positioning, ciliogenesis and ciliary signaling

Several recent studies provide a greater insight into the connection between the cilia and centrosome in neuronal development. We conclude our review by highlighting these findings. A recent study (Gonçalves et al., 2010), showed that the tubulin cofactor TBCCD1, is localized to centrosomes and ciliary basal bodies, and that downregulation of this protein leads to changes in centrosome positioning in the retinal pigmental epithelial RPE-1 cells, where the centrosome shifts from the center of the cell to the periphery and primary cilia formation is disrupted (Gonçalves et al., 2010). Furthermore, when centriole generation is blocked in flies, they survive through the larval stages but die soon after they reach adulthood (Basto et al., 2006). Interestingly, these flies lack cilia in type 1 sensory neurons (Basto et al., 2006; Badano and Katsanis, 2006). Thus, proper centrosome localization appears to be essential for primary cilia development. Another study (Dammermann et al., 2009) focused on a protein that carries a single amino acid mutation in hydrolethalus syndrome, a perinatal lethal human ciliopathy. In *C. elegans* and *Xenopus* neurons, HYLS-1 is localized to developing centrioles and is stabilized at the centrosomes (Dammermann et al., 2009). Depletion of HYLS-1 leads to abnormal basal body localization and lack of cilia (Dammermann et al., 2009).

Another important group of signaling determinants that might link centrosome and cilia during neuronal development are the genes associated with Bardet-Biedl syndrome (BBS). Several BBS genes have been identified to date and have been found to be localized to both centrosomes and basal bodies of cilia (Ansley et al., 2003; Nachur et al., 2007; Kamiya et al., 2008; Jin et al., 2010). The BBS proteins form a complex called the "BBSome," which have a variety of functions. In cilia, this complex is essential in ciliogenesis and IFT (Blacque et al., 2004; Zaghloul and Katsanis, 2009; Jin et al., 2010). Furthermore, ciliary BBS proteins have been shown to modulate Wnt (Wiens et al., 2010) and Shh signaling (Zhang et al., 2012), which are important regulators of neuronal development (see above). BBS proteins appear to play a role in non-canonical Wnt signaling, which is involved in establishing cell polarity and have demonstrated roles in migration during development. One study (Ross et al., 2005) showed that mice with mutations in BBS proteins exhibit developmental defects similar to those observed in abnormal non-canonical Wnt signaling. Moreover, downregulation of BBS genes in zebrafish led to defects in gastrulation and neurulation (Ross et al., 2005).

In the centrosome, BBS proteins have been demonstrated to associate with the centrioles and pericentriolar material protein 1 (PCM1) (Kim et al., 2004). BBS4 was found to interact with the dynein-dynactin complex and to localize PCM1 to the centrosomes. This was shown to be essential for proper MT organization and cell cycle progression in several mammalian cell lines, including HEK293 and HeLa (Kim et al., 2004). Like BBS4, DISC1 was also shown to, localize PCM1 to the centrosome in cortical neurons (Kamiya et al., 2008). Furthermore, DISC1 was shown to directly associate with BBS1 and recruit it to the centrosome (Ishizuka et al., 2011). As described above, DISC1 plays an important role during the development and migration of cortical neurons by modulating centrosome positioning via other signaling components such as LIS1 and NUDEL (Kamiya et al., 2005). It was also shown that DISC1 is a regulator of neural progenitor proliferation in the embryonic brain as well as in the adult dentate gyrus by inhibiting GSK-3β signaling and thus stabilizing β- catenin (Mao et al., 2009). Interestingly,

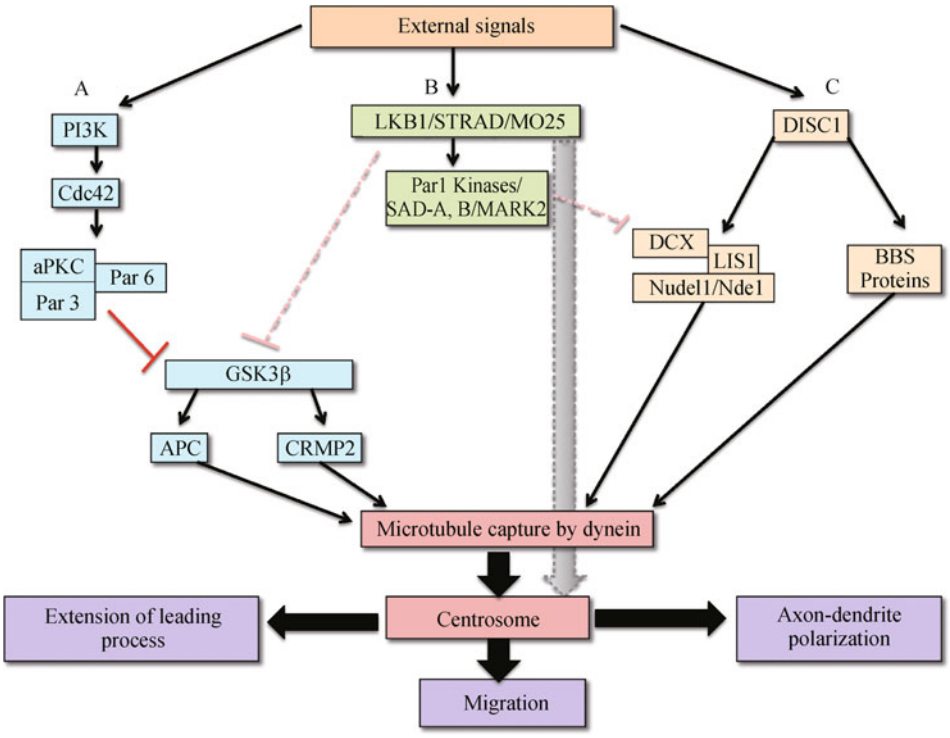

Figure 2 Schematic overview of the signaling mechanisms that regulate centrosome positioning during neuronal development and migration. External signals activate various intracellular signaling pathways that regulate centrosome positioning, which in turn regulate axon-dendrite polarization, extension of the leading process, and migration in developing neuronal cells. Solid arrows indicate the demonstrated links between the various signaling determinants and dashed arrows represent possible connections between signaling components. (A) External signals activate the PI3-Kinase pathway, which regulates centrosome position via the Par protein complex. The Par protein complex phosphorylates and inhibits GSK3β, which in turn, activates downstream effectors such as APC and CRMP2, allowing microtubule capture by dynein and regulating centrosome positioning. (B) LKB1, along with its co-factors STRAD and MO25 possibly regulates centrosome positioning (dashed grey arrow) and also phosphorylates and inhibits GSK3β (dashed red line), thus converging with the PI3-kinase pathway. Through downstream effectors such as Par1 Kinases SAD-A, SAD-B and MARK2, LKB1 may also inhibit DCX (dashed red line). (C) DISC1 is another important determinant of centrosome positioning by regulating the LIS1/DCX/Nudel1/Nde1 protein complex as well as the Bardet-Biedl syndrome (BBS) proteins.

downregulation of BBS4, PCM1 and DISC1 leads to defects in migration of cortical neurons (Kamiya et al., 2008), suggesting that the same molecular participants may regulate the switch between neuronal proliferation and migration. Indeed, a recent study demonstrated such a regulatory role for DISC1 in the transition between proliferation and migration of cortical neurons. In the process of migration, DISC1 was shown to recruit BBS proteins to the centrosome (Ishizuka et al., 2011).

Finally, LKB1 could also be a major component of the developmental processes by which cilia and the centrosome affect neuronal development. LKB1 has been shown to be closely associated with the centrosome during neuronal development (Asada et al., 2007; Asada and Sanada, 2010) (see above). LKB1 was also demonstrated to be localized to cilia and to modulate cell size in MDCK cells (Boehlke et al., 2010) (see above). Interestingly, a recent genome wide RNAi screen in cultured mouse fibroblasts (Jacob et al., 2011) showed that LKB1 is essential for cilia formation and that downregulation of LKB1 leads to absence of cilia and abnormal Shh and Wnt signaling. Taken together, these findings indicate that LKB1 may play a central role in connecting cilia and centrosome function in neuronal development.

Concluding remarks

Current evidence lines up various fundamental roles of centrosome positioning and its underlying mechanisms in neuronal development. Several signaling pathways and their molecular components have been demonstrated to play a role in centrosome positioning in neuronal development and migration (Fig. 2). Cilia, which are closely linked to the centrosome both structurally and functionally, are emerging as important signaling centers that regulate many crucial processes in neuronal development. Strikingly, recent studies have demonstrated that shared signaling determinants such as LKB1, DISC1 and BBS proteins might regulate neuronal development via both the cilia and the centrosome. Most studies to date have focused on embryonic neuronal migration and morphological development. Furthermore, recent studies

revealed some contradictory findings, and therefore, the signaling pathways that regulate centrosome positioning and its significance in these processes need to be further examined. Moreover, the mechanisms by which cilia regulate neuronal development need to be further dissected and future studies can further our knowledge of the links between the cilia and centrosome function in neuronal development. Recent studies have demonstrated a role for cilia in synaptic integration of adult newborn neurons. Future studies will further resolve whether the cilia and centrosome is involved in synaptic integration, circuit function and homeostasis. Moreover, the function of the centrosome and cilia in adult newborn neuronal development is still in the early stages of elucidation and much is still unknown. Therefore, although the current body of research underscores the importance of these organelles in neuronal development, the scope for future investigation is vast.

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