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Polarity Regulation in Migrating Neurons in the Cortex

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Abstract The formation of the cerebral cortex requires migration of billions of cells from their birth position to their final destination. A motile cell must have internal polarity in order to move in a specified direction. Locomotory polarity requires the coordinated polymerization of cytoskeletal elements such as microtubules and actin combined with regulated activities of the associated molecular motors. This review is focused on migrating neurons in the developing cerebral cortex, which need to attain internal polarity in order to reach their proper target. The position and dynamics of the centrosome plays an important function in this directed motility. We highlight recent interesting findings connecting polarity proteins with neuronal migration events regulated by the microtubule-associated molecular motor, cytoplasmic dynein.

Keywords Neuronal migration · Brain development · LIS1 · DCX · MARK2 · Par-1 · Polarity proteins · Centrosome · Microtubules · Actin

Neuronal Migration in the Cerebral Cortex

Neuronal migration is a necessary process required for proper brain architecture since most neurons are born in a position different from which they will reside in. The six layers of the cerebral cortex are composed of neurons that are born in different areas but are subsequently organized according to their birthdating [1, 2]. Neurons born relatively late during corticogenesis reside in more superficial layers on top of the older neurons, thus composing an inside-out organization. Neurons reach their target destination using different modes of migration. Neurons born in the germinal zones of the dorsal telencephalon migrate towards the pial surface of the cortex in a radial path. These neurons are the pyramidal or the excitatory neurons of the cerebral cortex. Neurons migrating along this route attach to radial glia, which provide a transient scaffold for directed migration [3–5]. Live cell imaging and *in utero* electroporation experiments have revealed that neurons undergo dramatic morphological changes during migration. During most of their migratory route, they exhibit a bipolar structure with a leading edge directed towards the pial surface and a trailing process pointed to the ventricular surface (Fig. 1). Within the subventricular zone and lower intermediate zone, an additional transient multipolar stage has been detected. This multipolar stage was described in several types of neurons as well as in neocortical neurons as a transient step preceding the migration along radial glia [6]. Neurons migrating along radial glia exhibit a bipolar structure, and once these neurons reach the pial surface, they detach from the radial glia and continue to move towards their correct laminar position.

Abnormal Neuronal Migration

Deficits in neuronal migration in humans and in mice have provided us with insights on the regulatory mechanisms involved in this process. Abnormal neuronal migration may result in cortical malformations that are responsible for a significant proportion of cases of mental retardation and epilepsy in children [7–9]. Lissencephaly (i.e., smooth brain) is a severe human neuronal migration disorder.

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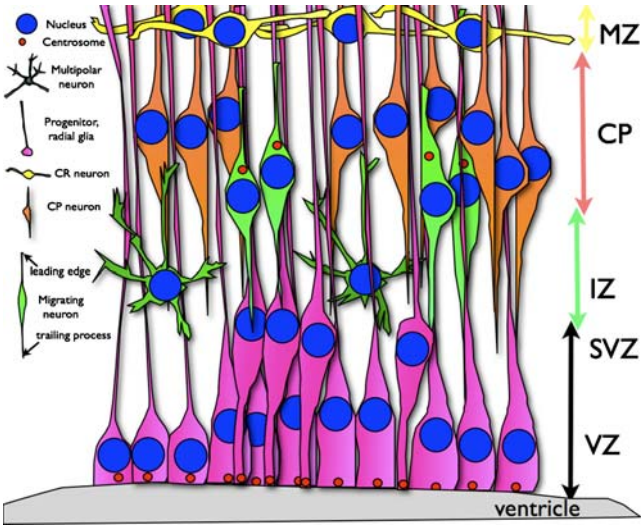

Fig. 1 Schematic presentation of some polarity changes neurons undertake during development. Neuronal progenitors (*pink*) are organized in a polarized fashion close to the ventricular zone (*VZ*). Their centrosomes (*red*) are oriented towards the ventricle. The cell nucleus (*blue*) in these cells move according to their position in the cell cycle. Migrating neurons (*green*) reorient their position in the boundary between the subventricular zone (*SVZ*) and the intermediate zone (*IZ*); they adopt a transient multipolar morphology which is followed by a transition to a bipolar morphology and migrate outwards towards the cortical place (*CP*). Once neurons reach the marginal zone (*MZ*), which is a cell-sparse area where Cajal–Retzius (*CR*) cells reside, they dissociate from the radial glia and move to their proper position in the cortex

Several genes have been identified that when mutated result in lissencephaly, among them; *LIS1* [10], the X-linked gene *DCX* [11, 12], and alpha-tubulin [13]. Lissencephaly is characterized by absent (agyria) or decreased (pachygyria) convolutions, producing a smooth cerebral surface with thickened cortex [14]. Subcortical band heterotopia (SBH) is a related disorder in which there are bilateral bands of gray matter interposed in the white matter between the cortex and the lateral ventricles. SBH (doublecortex) is very common among females with mutations in DCX [11, 12]. Lissencephaly and SBH have been observed in different regions of the same brain, defining an 'agyria–pachygyria-band' spectrum [15]. Both LIS1 and DCX affect microtubule dynamics [16–18], and their activity is regulated by phosphorylation [19–21]. Regulation of microtubules is thus likely to be an important feature in migrating neurons of the developing brain.

Neuronal migration phenotypes were observed in different *Lis1* mouse models [22, 23] as well as when using *in utero* electroporation for acute reduction of LIS1 levels [24–26]. In *Dcx* knockout mice, there was no observable radial migration phenotype [27]. Nevertheless, hippocampal lamination was disrupted [27] and the mice also exhibit epilepsy that is usually associated with the lissencephaly phenotype [28]. Mild phenotypes were observed in tangential migratory routes [29, 30]. The relatively mild phenotype has been attributed to gene redundancy from other DCX family members and there are 11 paralogs in mammals [31]. The closest family member, DCLK (doublecortin-like-kinase), is somewhat redundant to DCX, and also *Dclk*-deficient mice do not exhibit a radial migration phenotype [32, 33]. Nevertheless, DCLK also has a unique function and participates in proliferation of the neural progenitors [34]. Acute reduction of DCX using *in utero* electroporation showed severe neuronal migration impairments [24, 35–37]. The discovery of patients with mutations in Tubulin α1 followed characterization of hyperactive mice derived from a mutagenesis screen. These mice exhibited abnormalities in the laminar structure of the hippocampus and the cortex, accompanied by impaired neuronal migration [13].

The first mice to be described with a cortical malformation were the *reeler* mice [38–40]. *Reeler* mice have multiple abnormal cell positioning in different areas of the CNS [41, 42]. In the cerebral cortex, the typical layered organization is inverted in comparison with the normal organization [43, 44]. Furthermore, the splitting of the preplate, which occurs when waves of newly born neurons migrate through the first-born generation of neurons, does not occur. The mutated gene is reelin, a large extracellular protein [45–47], which binds to receptors and initiates a signaling pathway. The 'basic' reelin pathway includes the large extracellular reelin ligand, two receptors belonging to the family of lipoprotein receptors (VLDLR and ApoER2) [48–50], and an intracellular adaptor molecule, Dab1 [51–54]. Dab1 needs to be phosphorylated and is eventually degraded [55–57] in order to properly propagate the reelin signal [42, 58]. Mutations in the ligand, receptors, or the intracellular adaptor protein result in an indistinguishable phenotype known as the mouse 'reeler' phenotype. LIS1 has been found to interact with phosphorylated Dab1 and thus to converge to the reelin pathway [59]. As such, LIS1 may provide a link between reelin signaling and the regulation of the microtubule cytoskeleton. In humans, mutations in reelin are the cause for the Norman–Roberts type of lissencephaly [51].

Actin

Cell motility and cell polarization requires the polymerization of the actin cytoskeleton. The first example for the requirement for polymerizing actin in migrating neurons of the developing brain were mutations in the actin-binding protein Filamin A [60, 61]. The observed phenotype in brains of patients with mutation in Filamin A involves total failure of migration of some neurons and is known as periventricular heteropia (PH) [62]. Filamin A levels have

been associated with the acquisition of bipolar morphology: introduction of dominant negative Filamin A resulted in less motile and rounded cells whereas up-regulation of Filamin A levels promoted an elongated bipolar shape of migrating pyramidal cells [63, 64]. Filamin A expression is regulated by FILIP (FILamin A Interacting Protein) [63, 64] and by MEKK4 [65]. MEKK4 null mice exhibit a high incidence of PH that is usually bilateral, containing mostly postmitotic neurons [65, 66]. An additional actin-regulating protein, which has been shown to participate in neuronal migration, is N-cofilin [67, 68]. Cofilin is part of a family of actin-binding proteins, which disassembles actin filaments [69]. This protein family includes three members: n-cofilin and ADF, both of them are expressed in the brain, and m-cofilin (muscle specific). Cofilin is a ubiquitous actin-binding factor required for the reorganization of actin filaments. The activity of all three cofilins is regulated by phosphorylation of Ser3 [70]. However, the two cofilins expressed in the brain differ in their function; ADF null mice showed no gross brain abnormalities [67]; however, complete deletion of n-cofilin was embryonic lethal [71]. Conditional knockout of n-cofilin in brain progenitors resulted in a dramatic effect on brain structure. N-cofilin-deficient neuronal progenitors prematurely exited cell cycle, thus resulting in a depletion of the neuronal progenitor pool. In addition, there was an impairment of radial and tangential migration [67]. Additionally, n-cofilin has been proposed also to act as a downstream effector of the reelin pathway [68]. It has been shown that reelin signaling increases phosphorylation of cofilin, which disrupts its ability to bind to actin and thereby increases neurite stability and hinders migration. Since the main source of reelin is within the marginal zone (Fig. 1), it has been proposed to act there as a stop signal. According to this hypothesis, once the leading processes of migrating neurons contact reelin, they become stabilized and stop their migration.

In addition, the large group of small GTPases that mainly affect actin polymerization are involved in regulation of neuronal migration. The Rho GTPase family members include Rho, Rac and Rnd proteins, and Cdc42. These proteins are found either in an active GTP-bound conformation or inactive GDP-bound state. They are regulated by GAPs (GTPase activating proteins), GEFs (guanine nucleotide exchange factors), and GDIs (guanine nucleotide dissociation inhibitors). Their role in neuronal development and neuronal migration has been reviewed elsewhere [72, 73]. Of particular interest, functional suppression of Rac1 or its GAPs, STEF/Tiam1 or P-Rex1, inhibited neuronal migration *in vivo* [74, 75]. Furthermore, the role of Rnd2 in radial migration in the cerebral cortex has been also demonstrated [76, 77]. In addition, LIS1 and its interacting protein Ndel1 affect the activity of Cdc42, which may be related to the inhibition of neuronal migration [78–80].

Microtubules

Microtubules are one of the major components of the cytoskeleton and they are essential for cell division, cell migration, vesicle transport, and cell polarization. Proper regulation of the microtubule cytoskeleton is essential for successful neuronal migration. It is therefore not surprising that the dynamic properties of microtubules are regulated on multiple levels. Microtubules are polymers of α- and β-heterodimers of tubulin. The first level of regulation is the composition of tubulin dimers, which may include different tubulin isoforms. Microtubules assembled *in vitro* from specific tubulin isoforms exhibit different assembly, stability, and dynamic properties [81–83]. So far, only mutations in alpha-tubulin 1a have been associated with neuronal migration deficits in humans and in mice [13, 84–87]. One of the alpha-tubulin mutations associated with human disease has been studied in detail (R264C). The mutant tubulin was less efficiently incorporated into microtubule polymers due to reduced interaction with the cytosolic chaperonin CCT (chaperonin containing TCP-1) and a decreased interaction of the CCT intermediates to interact with one of the tubulin chaperones (TBCB) [88]. CCT is required for folding nascent actin and tubulin and interacts with a wide range of putative substrates. CCT binds substrate proteins and, through ATP-dependent conformational changes, encapsulates them in a central cavity. Upon release into the cytosol, the substrates may have folded to the native state or may require additional rounds of CCT binding and release. The next level of regulation includes post-translational modification of tubulins, which for only a few an *in vivo* role has been demonstrated. Tubulin acetylation is known to affect microtubule stability [89–91]. Furthermore, kinesin-1 and cytoplasmic dynein, which are important microtubule-associated molecular motors, prefer to move along acetylated microtubules [92, 93]. Phosphorylation has been found to affect the dynamic properties of microtubules during mitosis [94]. In addition, tubulin undergoes a special cycle of detyrosination/tyrosination in which the C-terminal tyrosine of alpha-tubulin is cyclically removed by a carboxypeptidase and re-added by a tubulin–tyrosine–ligase. Tubulin–tyrosine–ligase-deficient mice die within 1 day following birth, and exhibit multiple abnormalities in their nervous system, including abnormal neuronal migration [95]. A subset of proteins that bind to the plus tips of microtubules (CAP-Gly) are not recruited to the tips in an efficient way in the absence of tubulin–tyrosine–ligase, providing a possible mechanism of the effect of tubulin–tyrosine–ligase deficiency on microtubule dependent activities [96]. On top of the tubulin-based composite structure of microtubules, a large group of proteins termed microtubule-associated proteins (MAPs) dramatically modify microtubule dynamic behavior and function. One of the best-known conventional MAPs is

the tau protein (or MAPT). So far, the role of tau in neuronal migration has not been proven. There is a genetic interaction between the *Reelin* pathway and tau hyperphosphorylation [49, 97]. Mice deleted for tau exhibited a reduction in microtubule density in small caliber axons [98] as well as muscle weakness and memory disturbances [99]. It has been proposed that developmental functional redundancy by increase expression of other MAPs may explain the relatively mild phenotype [98]. This hypothesis has been substantiated by analysis of *Map1b/Tau* double mutant mice, where defects in axonal elongation and neuronal migration were observed [100]. The involvement of Tau in neuronal migration may also be inferred from analysis of human patients. Microdeletion of a region encompassing the *MAPT* gene results in moderate mental retardation with associated dysmorphic features [101–104]. The frequency of the microdeletion syndrome was estimated to be 1:20,000, thus a common underlying cause for mental retardation. It has been suggested that the deletion of Tau within this locus is responsible for the mental retardation phenotype.

MAP1B is another conventional MAP with an important role in the developing cortex [105–107]. Furthermore, accumulated data indicated that MAP1B participates in regulation of neuronal migration in several ways. MAP1B phosphorylation is controlled by Netrin 1 and the lack of MAP1B impaired Netrin 1-mediated chemoattraction *in vitro* and *in vivo* [108]. In addition, it has been proposed that Reelin can induce post-translational modifications on MAP1B that could correlate with its function in neuronal migration [109]. Furthermore, MAP1B may affect neuronal migration through its interaction with LIS1 and affecting the interaction of LIS1 with cytoplasmic dynein [110]. LIS1 and DCX, both of which are MAPs, will be discussed below. MAPs may even affect the orientation of microtubules within the neuronal axon. Microtubules in axons are polarized with the plus end pointing towards the growth cone. Cytoplasmic dynein and LIS1 were shown to affect microtubule polarity within the axon. In their absence, axonal microtubules exhibited a mixed polarity instead of the minus to plus end organization [111]. It is yet unknown whether this property directly affects neuronal migration.

LIS1 and DCX are MAPs and Complex with Dynein

MAPs play a significant role in regulation of microtubule dynamics and structural properties [112–114]. The MAPs LIS1 and DCX, which play a vital role in neuronal migration, have been the focus of intensive study during recent years [6, 20, 115–119]. LIS1 interacts with tubulin and can modulate MT dynamics *in vitro*. LIS1 is an evolutionary conserved regulator of dynein activity from yeast to mammals [120]. A tight relationship between LIS1, microtubule regulation, and MT-based motor proteins has been suggested for many organisms. The LIS1 ortholog in *Aspergillus nidulans* (NUDF) is a member of the NUD (nuclear distribution) family of proteins that are essential for distribution of the nuclei in the multinucleated hyphae. Several of the nud genes encode subunits of cytoplasmic dynein, and subunits of dynactin, which contributes to the motor processivity [121–124]. LIS1 interacts with distinct subunits of the dynein and dynactin complexes [125–128]. A schematic presentation of these protein complexes is shown in Fig. 2. Furthermore, modulation of LIS1 expression interfered with dynein functions [125, 127, 129]. An additional evolutionary conserved interaction is with NUDE, which has two mammalian paralogs Ndel1 and Nde1 [126, 130–132] (review [117]). Ndel1 and Nde1 cooperate with LIS1 in regulation of dynein function [126, 133]. Using *in utero* electroporation, it has been shown that Ndel1 sustains LIS1 activity on dynein, resulting in promotion of neuronal migration [25]. Furthermore, reduction in Ndel1 levels also affects the coupling between the nucleus and the centrosome. These interrelations have also been demonstrated using crosses between the corresponding *Lis1* and *Ndel1* knockout lines [134]. Ndel1 is a substrate to several kinases among which are Cdk5 [132], Cdc2, and Erk-1, -2 [133]. Phosphorylated Ndel1 binds to 14-3-3ε; this binding masks the Cdk5-phosphorylated Ndel1 sites and protects them from dephosphorylation [135]. 14-3-3ε belongs to the group of 14-3-3 proteins, which bind to phosphorylated sites and is equivalent to Par-5 mentioned below. Par-5 is part of an evolutionarily conserved PAR-aPKC (atypical protein kinase C) system involved in cell polarity in various biological contexts. Knockout of 14-3-3ε results in neuronal migration retardation in the mouse, and its deletion is

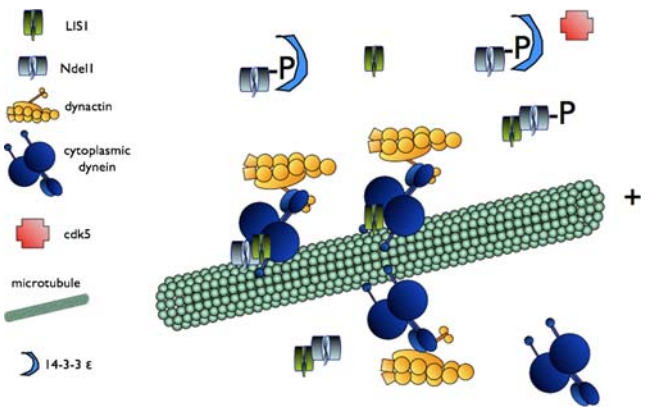

Fig. 2 Schematic presentation of protein complexes involved in regulation of cytoplasmic dynein. Cytoplasmic dynein is a microtubule associated motor moving towards the minus ends of microtubules. Dynactin interacts with dynein and increases its processivity. LIS1 and Ndel1 also interact with dynein and affects dynein's activity. Ndel1 undergoes phosphorylation by multiple kinases, including cdk5. Phosphorylated Ndel1 is recognized and bound by 14-3-3ε. All of the abovementioned proteins affect neuronal migration

associated with a more severe phenotype in the case of Miller–Dieker lissencephaly [135]. Knockout of Nde1 affected both the proliferation of cortical progenitors and neuronal migration [136]. Nde1 has been shown to be a central component of the centrosome; it interacts directly with γ-tubulin and additional five different centrosomal proteins [137]. Overexpression of Nde1 results in dissociation of γ-tubulin from the centrosome and abnormal microtubule organization.

DCX is a microtubule-associated protein (MAP) [17, 138, 139], which binds to microtubules in a unique position in between the protofilaments and stabilizes them [140]. DCX interacts directly with LIS1 [16] and its addition to neurons lacking one copy of *Lis1* rescue their phenotype [141]. Furthermore, DCX and its close family member DCLK were shown to complex with cytoplasmic dynein [34, 141]. Therefore, it is suggested that LIS1 and DCX are involved in regulation of the retrograde molecular motor. In addition, LIS1 and DCX regulate nuclear-centrosomal coupling in a coordinated manner [142]. The expression, phosphorylation, and dephosphorylation of DCX are regulated during brain development [138, 139, 143, 144]. The phosphorylation of DCX by at least three different kinases has been demonstrated: JNK [19], Cdk5 [142], protein kinase A (PKA), and the MARK (microtubule affinity-regulating kinase)/Par-1 family of protein kinases [20, 145]. The interaction of DCX with microtubules is particularly sensitive to phosphorylation by MARK/Par-1. Phosphorylated DCX seems to bind less efficiently to the microtubule polymer [145].

Polarity Proteins

Cellular polarization is required for multiple purposes including cell motility; one of the processes that require polarization is asymmetric cell division. Genetic studies first conducted in the simple organism *Caenorhabditis elegans* identified key players participating in cell polarity regulation [146, 147]. The first cell division in the *C. elegans* embryo is asymmetric and the size of the two daughter cells differs. The first group of mutants identified was deficient in the ability to specify an anterior–posterior axis in the early embryos and were designated as the Par genes (partition defective) [148] (Fig. 3). Initially, six Par genes were identified (Par-1 to -6) and the seventh member to this group was identified as atypical protein kinase C (aPKC) [149]. Par proteins are evolutionary conserved with the exception of Par-2. Par-1, -4, and aPKC encode for serine/threonine kinases that exhibit interesting and functional enzyme–substrate interactions. The mammalian orthologs of Par-1 are also known as MARK (microtubule affinity-regulating kinase) [150] or EMK (ELKL motif

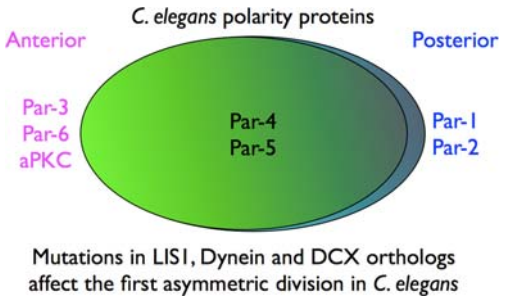

Fig. 3 Schematic presentation of Par (partition defective) proteins in the early embryo of the worm *C. elegans*. Par-1, a serine/threonine kinase, and Par-2, a ring-protein, are found in the posterior side. The scaffold proteins, Par-3 and Par-6, are found in the anterior side together with atypical protein kinase C (*aPKC*). Par-4, which phosphorylates and activates Par-1, and Par-5, which binds phosphorylated sites and is part of the 14-3-3 family of proteins, are distributed throughout the embryo. Mutations in any of the Par proteins as well as many other proteins including LIS1, dynein, and DCX orthologs affect the first asymmetric cell division in *C. elegans*

kinase) [151], which compose a small gene family in mammals [152]. The mammalian ortholog of Par-4 is also known as LKB1, PJS (Peutz–Jeghers syndrome), or STK11 (serine threonine kinase 11). Peutz–Jeghers syndrome, characterized by an increased risk for cancer, is caused by mutations in this kinase [153, 154]. Par-3 and -6 are scaffold proteins and Par-5 is the ortholog of 14-3-3 proteins, which bind discrete phosphorylated serine or threonine residues [155]. In the single cell worm embryo, the subcellular localization of the PAR proteins is important; Par-3, -6, and aPKC localize to the anterior cell cortex, Par-1 and -2 to the posterior cortex, and Par-4 and -5 are distributed throughout the cell (Fig. 3). High throughput screens in the same system identified additional genes that participate in the regulation of this process [156, 157]. Furthermore, mutations in the molecular motor cytoplasmic dynein and orthologs of its interacting proteins LIS1 and DCX affect the first asymmetric cell division in *C. elegans* [156–159]. Polarity generation results from a break in symmetry, which may be stochastic [160], or follow an external cue (Fig. 4). The general scheme presented in Fig. 4 is adapted to mammalian cells. Following this initial step, a signaling pathway is triggered; activation of CDC42 is one of the initial steps and it transmits its signal through Par-6 [161, 162]. In addition to evolutionary conservation properties, the findings that LIS1 and its interacting protein Ndel1 regulate CDC42 activation [78–80] and that DCX is a substrate of MARK2/Par-1 suggested that these proteins may be interconnected in the polarity pathway (Fig. 4). The polarity pathway participates in multiple processes during CNS development (reviewed by [163]). Furthermore, this pathway is also known to be involved in regulating the specification of the future axon in primary hippocampal neurons. The morphological changes occurring in primary

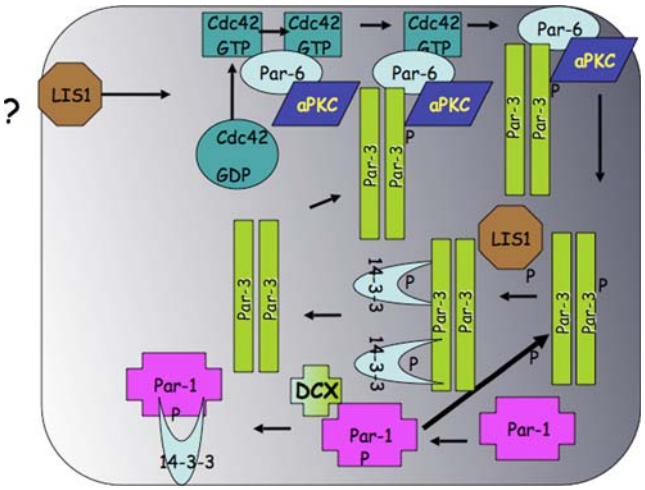

Fig. 4 Schematic presentation of the polarity pathway in a mammalian cell. An unknown cue (*?*) facilitates Cdc42 activation by LIS1. The scaffold protein Par-6 and aPKC are recruited to Cdc42 in the GTP-bound form. Par-6 binds Par-3, which is phosphorylated by aPKC. Par-3 is also phosphorylated by Par-1, and 14-3-3 (Par-5) binds to phosphorylated sites of Par-3 and Par-1. Par-1 phosphorylates DCX. LIS1 is found in multiple subcellular localizations

hippocampal cultures may be analogous to those observed in migrating neurons [164]. Activated CDC42 recruits the complex of Par-3, Par-6, and aPKC [165–168], which is followed by activation of MARK2/Par-1 [169]. In addition, kinases belonging to the MARK family (SAD-A and SAD-B) have been shown to regulate neuronal polarity *in vivo* [170, 171].

An interesting link between centrosomal localization and positioning of the future axon has been demonstrated in primary hippocampal neurons [172]. LIS1 [10] and DCX [11, 12], both of which are implicated in neuronal migration disorders, have been found to regulate the coupling between the centrosome and the nucleus in a dynein-dependent pathway [25, 26, 141, 173]. LIS1 is involved in activation of Cdc42 [78, 79, 174], which impacts a repertoire of targets including PAR-6, aPKC, PAR-3, and PAK5 [72]. In migrating cerebellar neurons *in vitro*, Par-6 and aPKC localize to the centrosome together with dynein/dynactin. Modulating Par-6 levels inhibited centrosomal motion and neuronal migration [175]. The above observations suggested that Par-1/MARK2 may play an important role during neuronal migration as a key node protein joining the polarity pathway and the dynein pathway.

The Polarity Kinase Par-1/MARK2 Affects Neuronal Migration

MARK2 is a member of a small family of proteins [152]; therefore, it is not surprising that no neuronal migration phenotype has been described in MARK2-deficient mice. *In utero* electroporation has been proven to be an efficient way to circumvent gene redundancy, as previously demonstrated in case of the *Dcx* family of proteins [33, 35]. However, MARK2 knockout mice do exhibit impairments in spatial learning and memory [176]. Based on the tight correlation between abnormal neuronal migration and mental retardation, it may be hypothesized that these mice exhibit a subtle, previously unappreciated phenotype in the developing brain. Reduction in the levels of polarity kinase Par-1/MARK2 in the developing brain using *in utero* electroporation of MARK2 shRNA resulted in a pronounced inhibition of neuronal migration (Fig. 5) [177]. Most of the shRNA-treated neurons were stalled at the boundary between the intermediate zone (IZ) and cortical plate (CP). The inhibited neurons mainly exhibited a multipolar morphology. Some of the neurons that managed to migrate towards the CP exhibited abnormal morphology with a curved or bifurcated leading edge pointing to the VZ [177]. As mentioned above, neurons adopt a multipolar morphology during their normal migratory route to the cortex [178]. This transient stage is sensitive to the levels of quite a few proteins; including DCX, LIS1, Filamin A, and others [6]. These proteins regulate cell polarity and motility in neocortical subventricular and intermediate zones during radial migration. Interestingly, adding MARK2 kinase-dead on top of MARK2 shRNA allowed neurons to change their morphology from multipolar to the bipolar. Nevertheless, this transition was not sufficient to allow for successful migration to the cortical plate (Fig. 5). This result strongly suggests that there may be different modes of regulation for each phase. This is the first example where a transition to the bipolar morphology occurred without subsequent migration. These results strongly suggest that there is an unknown kinase-independent activity involved in morphology change. Reduction of kinase activity on its own also retarded neuronal migration and cells did not reach their expected position in the cortical plate (Fig. 5). Reduction of kinase activity was achieved by expression of MARK2 kinase-dead which acts as a dominant negative and inhibits endogenous kinase activity, or via expression of PAK5, which binds to the catalytic domain of MARK2 and inhibits its activity [179]. Overexpression of MARK2 strongly inhibited neuronal migration; cells lost their polarity and adopted round rather than multipolar or bipolar morphologies (Fig. 5).

It is likely that the observed phenotypes can be attributed at least in part to modulation in the dynamics of microtubules (Fig. 6a–c). MARK2 phosphorylates multiple substrates, some of them are MAPs, which change their affinity to microtubules following MARK2 phosphorylation. The substrates include tau, MAP2, MAP4, and DCX [145, 150, 180]. Overexpression of MARK in cells leads to hyperphosphorylation of MAPs on KXGS motifs and to

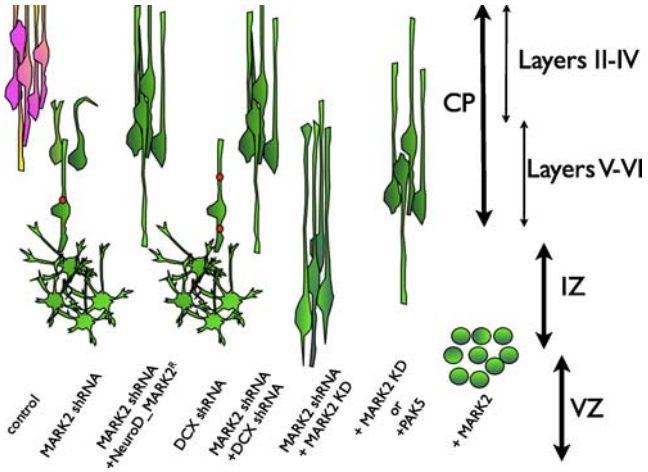

Fig. 5 Schematic presentation of *in utero* electroporation results showing the position and structure of neurons 4 days after electroporation, relating to MARK2 and DCX. Control neurons (*pink*) reach the superficial layers of the cortical plate (layers II–IV). MARK2 shRNA-treated neurons are stalled in the intermediate zone with multipolar morphology; some of the neurons that managed to migrate to the CP show abnormal polarity. The centrosome (in *red*) hardly moves. Neurons treated with DCX shRNA are inhibited in the IZ with multipolar morphology. In some of the neurons, the centrosome splits into two centrioles (in *red*) and neurons are inhibited in their migration. Partial rescue was observed when adding to MARK2 shRNA a specific amount of MARK2 expressed postmitotically (NeuroD_MARK2) or when both levels of MARK2 and DCX were reduced by shRNA. When MARK2 KD was added to MARK2, neurons exhibited a bipolar morphology, but did not migrate to the CP. Reduction in MARK2 kinase activity using MARK2 KD or PAK5 resulted in neurons that reached layers V–VI of the CP and failed to migrate to the more superficial layers. Increased MARK2 resulted in neurons that lost polarity in the VZ and IZ

disruption of the microtubule array [150]. As mentioned, *in vivo* overexpression of MARK2 resulted in loss of neuronal polarity (Fig. 5) [177]. Reduction in MARK2 resulted in more stable microtubules detected in primary neurons (Fig. 6b), and as a consequence *in vivo* neurons were stalled in the multipolar stage (Fig. 5). Reduction in DCX, one of the substrates of MARK2, resulted in an opposite effect with more dynamic microtubules (Fig. 6c), yet *in vivo* neurons are stalled in the multipolar stage (Fig. 5) [181] as previously reported [35, 37]. One clear conclusion from the above-described experiments may be that proper neuronal migration requires very accurate control of microtubule dynamics. Tipping the balance in either direction inhibits neuronal migration. Based on this conclusion, it was possible to postulate that simultaneous reduction of both DCX and MARK2 will allow for proper neuronal migration. Indeed, *in utero* electroporation of both DCX and MARK2 shRNA resulted in a partial rescue of

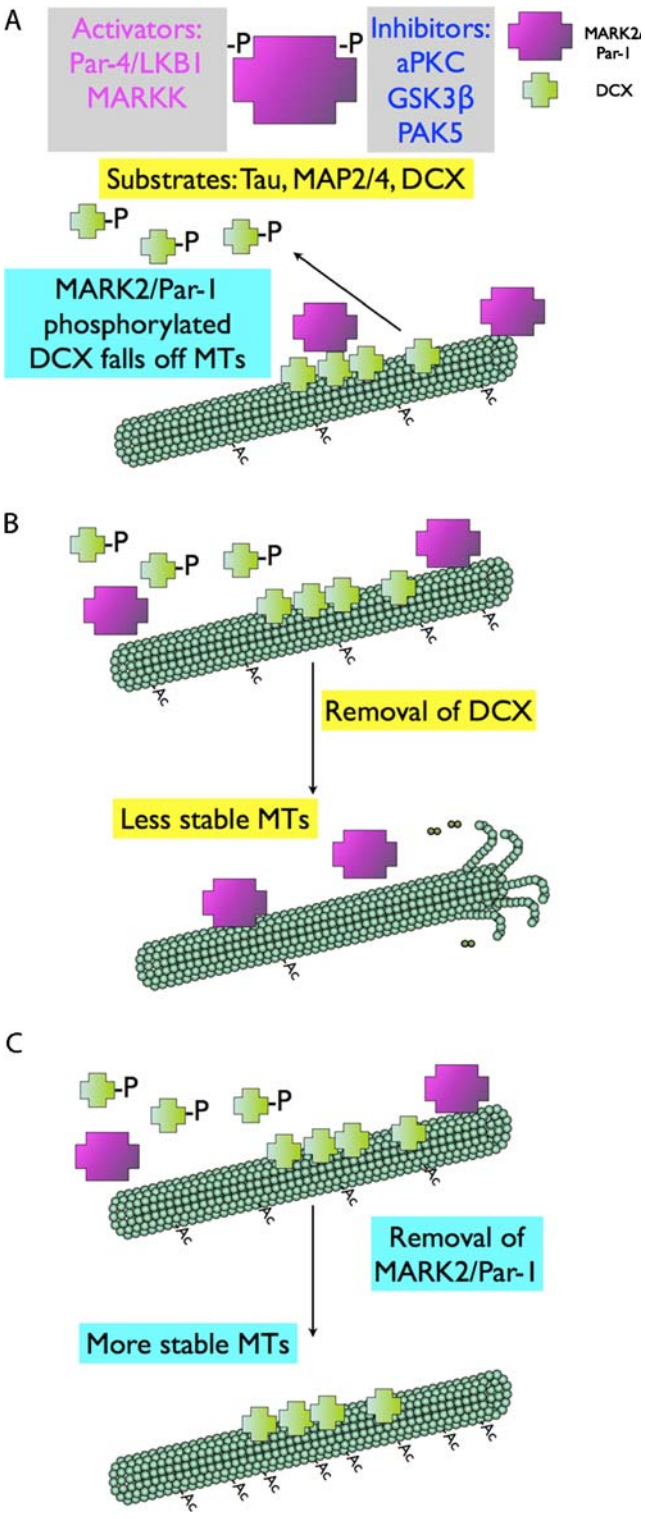

Fig. 6 **a** MARK2 regulation in respect to microtubules. MARK2/Par-1 is phosphorylated by either the upstream activators Par-4/LKB1 or MARKK or by the inhibitors aPKC, GSK3b. PAK5 binds to MARK2 and inhibits kinase activity in a phosphorylation-independent manner. MARK2 substrates include several microtubule associated proteins such as Tau, MAP2/4, and DCX. MARK2 phosphorylated DCX falls off microtubules. This in turn affects microtubule stability, which can be monitored by the level of tubulin acetylation (*Ac*). **b** Microtubule stability is increased when MARK2 levels are reduced. **c** Microtubule stability is decreased when DCX levels are reduced

neuronal migration (Fig. 5). These results have practical implications following the clear demonstration that a reduction in the levels of one gene may ameliorate the phenotype observed in case of mutation of another gene in the same pathway.

Previous studies indicated that centrosomal motility and the coupling between the centrosome and nucleus through microtubules is important for neuronal migration. Centrosomal motility requires the activity of molecular motors and cytoskeletal integrity. Furthermore, this process is subject to a delicate balance of opposing activities thus suggesting that reversible posttranslational modifications are likely to be involved in the regulation of dynamics of centrosomal motility and neuronal migration. For example, overexpression of FAK (focal adhesion kinase) mutated in its CDK5 phosphorylation site affected the structure of the microtubule fork in radially migrating cortical neurons which in turn reduced neuronal migration [182]. In addition, regulation of polarity is important for coupling of the centrosome and the nucleus. For example, manipulating the levels of the key polarity protein PAR-6, which localizes to the centrosome, impaired migration of primary cerebellar neurons [175]. Inhibition of the cell polarity factors GSK3 or PKCζ in migrating olfactory neurons resulted in impairment of centrosome reorientation and of process stabilization [183]. In addition, disruption of mouse Lis1, dynein, or Ndel1 leads to defective nucleus-centrosomal coupling [25, 141], and live imaging demonstrated slow and inefficient centrosomal motility [173]. When MARK2 levels were reduced, centrosomes moved very slowly [177] similar to the phenotype observed with disruption in dynein activity [173]. The dynamics of observed centrosomal behavior when DCX was reduced differed markedly [181]. The centrosome separated to two centrioles, which moved bidirectionally and fast (see Fig. 5). It has been proposed that the mammalian interphase centrosome consists of two independent units held together primarily as a result of the dynamic properties of the microtubule cytoskeleton [184]. Recent studies have indicated that the balanced activities of kinases and phosphatases play an instructive role in centrosomal splitting [185]. Therefore, it is possible to assume that reversible phosphorylation may also be involved in splitting of the centrosome in migrating neurons. DCX is a phosphoprotein and is dephosphorylated by phosphatases. The role of DCX kinases in neuronal migration has been well established, but phosphatases are likely to be as important. Of particular interest is the role of protein phosphatase 1 (PP1) in centrosomal splitting [186], since this phosphatase is capable of dephosphorylating DCX in a site-specific manner [143, 144]. Nevertheless, the role of actin and the associated molecular motor myosin in the maintenance of centrosomal integrity cannot be neglected. A basic role of the actin cytoskeleton in centrosomal splitting has been established [187–189]. In migrating neurons, the role of actin remodeling and the activity of myosin has proven to be essential for proper nuclear and cellular motility [173, 190–192]. Interestingly, opposed effects on centrosomal motility has been observed following elimination of either the Plexin-A2 receptor or its ligand Sema6A in tangentially migrating cerebellar neurons. In Plexin-A2 −/− granule cells, the centrosome turned around the nucleus before rapidly moving away from it, whereas in Sema6A −/− cells, the centrosome barely moved [193]. In both mutants, an obvious morphological effect suggesting abnormal actin regulation has been observed. Both DCX and MARK2 are capable of mediating a cross-talk between the microtubule and actin cytoskeleton [179, 194–196]. Therefore, although the role of the microtubule cytoskeleton has been emphasized here, it is likely that there is an active actin component in the observed phenotypes. In summary, seamless motility of the polarized centrosome requires a tight balance of factors involved in regulation of the molecular motors and the cytoskeleton.

Concluding Comments

Most neurons in the developing brain need to travel long distances prior to reaching their final destination. The ability to reach the destination relays on precise coordination between the definition of direction, which relays on polarity proteins, and polarization of the neuron itself, which requires polarized polymerization of the actin and microtubule cytoskeleton, and the action of the associated microtubule and actin-dependent motors, which will provide the force for motility. The centrosome, where microtubules originate, is a hub for many of these processes and many of the regulatory mechanisms take place there. Our current ability to follow and manipulate live neurons in the developing brain has opened endless windows of opportunities to understand these basic processes.

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