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Cytoskeleton in action: Lissencephaly, a neuronal migration disorder

Hyang Mi Moon and

Biomedical Sciences Graduate Program, Department of Pediatrics, Institute for Human Genetics, University of California, San Francisco, San Francisco, CA, USA

Anthony Wynshaw-Boris

Department of Pediatrics, Institute for Human Genetics, Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, San Francisco, CA, USA

Anthony Wynshaw-Boris: WynshawborisT@peds.ucsf.edu

Abstract

During neocortical development, the extensive migratory movements of neurons from their place of birth to their final location are essential for the coordinated wiring of synaptic circuits and proper neurological function. Failure or delay in neuronal migration causes severe abnormalities in cortical layering, which consequently results in human lissencephaly ('smooth brain'), a neuronal migration disorder. The brains of lissencephaly patients have less-convoluted gyri in the cerebral cortex with impaired cortical lamination of neurons. Since microtubule- and actin-associated proteins play important functions in regulating the dynamics of microtubule and actin cytoskeletons during neuronal migration, genetic mutations or deletions of crucial genes involved in cytoskeletal processes lead to lissencephaly in human and neuronal migration defects in mouse. During neuronal migration, microtubule organization and transport are controlled by PFAH1B1 (LIS1), DCX, YWHAE, and tubulin. Actin stress fibers are modulated by PFAH1B1 (LIS1), DCX, RELN, and VLDLR/LRP8 (APOER2). There are several important levels of crosstalk between these two cytoskeletal systems to establish accurate cortical patterning in development. The recent understanding of the protein networks that govern neuronal migration by regulating cytoskeletal dynamics, from human and mouse genetics as well as molecular and cellular analyses, provides new insights on neuronal migration disorders and may help us devise novel therapeutic strategies for such brain malformations.

Introduction

During mammalian central nervous system (CNS) development, the migration of newly born neurons to the appropriate areas within the brain, and the differentiation of post-mitotic neurons are essential for the proper establishment of synaptic circuits and their electrophysiological functions. Failure or delay in neuronal migration in the developing human neocortex results in cortical layer malformations and devastating neurological diseases. Human lissencephaly ('smooth brain') is a severe brain malformation disorder found in 1/30,000 births.^{1,2} Clinically, lissencephaly is characterized by a smooth cerebral surface of the brain without the convolutions known as gyri or sulci. Lissencephaly is always associated with mental retardation and lissencephaly patients suffer from epilepsy and motor function impairment. The most common type of lissencephaly is classical lissencephaly (Type 1), characterized by disorganized or less-defined layering of cortical

neuronal lamina rather than the well-defined distinct six neuronal layers formed during normal development. Reduced thickness of the cerebral cortex is also found in the brains of classical lissencephaly patients. This cortical malformation is mainly caused by the misregulation of neuronal migration during early development. During neocortical development, neurons are born from neural progenitors (NPs) in the ventricular zone (VZ) that divide again as they pass through the subventricular zone (SVZ) and intermediate zone (IZ), as intermediate or basal progenitors. After extensive migration toward the pial surface above the marginal zone (MZ), neurons become integrated into defined positions within neuronal layers in the cortical plate (CP) (Figure 1). Therefore, the defects in neuronal migration result in the mispositioning of neurons in the neocortex.

There are two different types of neuronal migration that occur during mammalian forebrain development: (1) radial migration; and (2) tangential migration. During radial migration, the excitatory cortical pyramidal neurons are born from NPs in the VZ and migrate to the CP. In a sequential migration stream, later-born neurons bypass early-born neurons and occupy more superficial layers of the CP, which generates an 'inside-out' pattern of cortical layers.³ Radial migration occurs extensively in the cerebral cortex and hippocampus during embryonic brain development. During tangential migration, inhibitory interneurons are generated from the different types of progenitors at the medial or lateral ganglionic eminence (MGE/LGE) and migrate to the neocortex (Figure 1). Radially migrating neurons generally display bipolar morphologies along radial glia with only a few branch points (although they do display transient multipolar morphologies in IZ layer). By contrast, tangentially migrating neurons frequently change the directions of migration and display dynamic morphological transitions. When post-mitotic neurons migrate, cytoskeletal remodeling of microtubules (MTs) and actin is evident in these cells. The volume of the nucleus (also called the soma) compared to those of extended processes is dramatically increased in the migrating neurons. The nucleus is surrounded by MT-enriched arrays in front of (fork-like MTs) and behind (cage-like MTs) the nucleus. In the leading process, the extended bundles of MTs emanate from the microtubule-organizing center (MTOC, also called centrosome) in front of the nucleus. Positioning of the centrosome defines the direction of movements of migrating neurons. The most anterior part of the leading process is the growth cone that senses extrinsic guidance cues and extends to the migration site. This protruded membrane structure is composed of filamentous actin (F-actin) stress fibers that establish new contacts with adhesion substrates (focal adhesion). Migration proceeds during neuronal locomotion in two modes of movements as a two stroke action, with asynchronous movements of the centrosome (C) and later the nucleus (N). The centrosome first moves into a swelling in the leading process and then the nucleus follows (nuclear translocation, also called somal translocation or nucleokinesis) due to a pulling force from MTs and dynein motors located at the centrosome. Centrosome movement forward into the leading process precedes nuclear movement and this coordinated relationship is called nucleus-centrosomal (N-C) coupling.^{4,5} Eventually, the trailing process is retracted due to actomyosin (F-actin + Myosin)-dependent motor functions, which leads to net movement of the neuron. Myosin-II-dependent motors play a dual role in nuclear movement by generating a forward MT pushing force and a pulling force from behind⁶ (Figure 2). This repetitive MT-actin remodeling is dynamically regulated during radial migration. Similarly, MGE-derived tangentially migrating cells also undergo MT- and actomyosin-dependent migratory cycles during nucleokinesis.^{7,8} These dramatic cytoskeletal changes are major common characteristics of neuronal cell migration.

A diverse collection of cytoskeletal proteins is involved in the tight control of neuronal migration during neocortical development. Extensive studies on the genes mutated in human neuronal migration defects have uncovered critical roles of their protein products in the regulation of cytoskeleton dynamics during neuronal migration and development. In this

review, we will focus on some of the genetic studies in humans and mouse model system that identified those genes. We will also highlight recent approaches focusing on cellular mechanisms of action of these key proteins working in neuronal migration in the context of the microtubule and actin cytoskeletons.

PAFAH1B1 (LIS1)

Human and mouse genetics—Human *PAFAH1B1* (platelet-activating factor acetylhydrolase isoform 1b regulatory subunit 1, formerly known as *LIS1*, Lissencephaly-1) was the first identified causal gene of neuronal migration disorders in any organism. Heterozygous *de novo* mutation or deletion of human *PAFAH1B1* gene on chromosome 17p13.3 is responsible for 40% of patients with isolated lissencephaly sequence (ILS).^{1,2,9,10} Thus far, more than 70 distinct intragenic heterozygous mutations have been identified from human genetic studies.¹¹ Severe brain malformations are the main characteristics of these patients, including simplified gyration in the cerebral cortex (agyria, pachygyria), disrupted cortical lamination, enlarged ventricles and neuronal heterotopias, leading to short lifespan of ILS patients.⁹

PAFAH1B1 (LIS1) protein is evolutionarily conserved from yeast to mammals. In order to study the *in vivo* function of mouse *Pafah1b1* (*Lis1*), our group generated *Pafah1b1* knockout (*ko*, null) and hypomorphic conditional knockout (*hc*) alleles by gene targeting. *Pafah1b1* heterozygous (*Pafah1b1*^{ko/+}) mice display mild neuronal migration defects in the cortex and hippocampus.¹² Further reduction of PAFAH1B1 (LIS1) protein levels by producing compound heterozygous (*Pafah1b1*^{hc/ko}) mice results in enhanced anatomical abnormalities in cortical and hippocampal structures. *Pafah1b1* compound heterozygous (*Pafah1b1*^{hc/ko}) mice display severe thinning of the cerebral cortex and broadly diffuse CP laminar organization. The hippocampal structures in these mice were also markedly disorganized due to neuronal migration defects during hippocampal development.¹³ These PAFAH1B1 dosage-dependent phenotypes in mouse animal models support a haploinsufficiency model for lissencephaly resulting from heterozygous deletion or mutation of *PAFAH1B1* in humans. Epileptic phenotypes and deficits in learning and motor function were also observed in these *Pafah1b1* mouse models, which have been seen in most human ILS patients.^{14,15}

PAFAH1B1 (LIS1), a member of NUD family proteins and a subunit of PAFAH—PAFAH1B1 (LIS1) is a 45kDa protein with a N-terminal, homodimerization and coiled-coil domain. The C-terminus of PAFAH1B1 contains seven WD-40 (tryptophan-aspartic acid-40) repeats that are required for dynein/MT binding. The *PAFAH1B1* homolog *nudF* was identified as one of the nuclear distribution *nud* mutants in the bread mold *Aspergillus nidulans*. The *nud* mutants exhibit defects in nuclear movement into fungal hyphae during sporulation. Several *nud* mutants were genetically identified in *Aspergillus nidulans* and one of the *nud* mutants was cytoplasmic dynein, a MT minus end-directed motor. Cytoplasmic dynein is a MT motor that plays a key role in generating the pulling power of retrograde cargo transport along MTs. In addition, dynactin, a dynein-regulatory accessory protein, was also identified as a *nud* mutant, confirming the participation of dynein in nuclear movements. Several NUD protein-dynein interactions are evolutionarily conserved, supporting the critical functions of PAFAH1B1-NUD proteins in cytoplasmic dynein regulatory pathways that control nuclear movement and nucleus positioning.

Among mammalian NUD family proteins, NUDE (*nudE* nuclear distribution gene E homolog) and NUDC (*nudC* nuclear distribution gene C homolog) were identified as PAFAH1B1 (mammalian *nudF* homolog protein)-binding partners. Mammalian *nudE* homologues are now termed *NDE1* (mammalian *NudE*, *mNudE*) and *NDEL1* (*mNudE*-like)

(see Box 1, NDE1 and NDEL1). NUDC directly binds to PFAH1B1 and dynein/dynactin complex and plays a chaperonin-like role for PFAH1B1 stabilization.^{16,17} A recent study demonstrated that *Nudc* siRNA-mediated knockdown (KD) in rat brain also results in neuronal migration defects.¹⁸

Box 1

NDE1, NDEL1: evolutionarily-conserved PFAH1B1 (LIS1)-binding partners

NDE1 (nuclear distribution gene E homolog 1) and NDEL1 (NDE1-like), mammalian homologues of NudE proteins, directly interact with PFAH1B1 through its conserved coiled-coil domain.^{22,44} PFAH1B1-dynein-NDE1 forms a complex that generates a persistent force of transport.⁴⁵ In recent human genetic studies, *NDE1* frame-shift mutations with protein decay have been reported and these mutations cause micro-lissencephaly (brain size reduction with cortical gyral simplification). *NDE1*-mutated patients have abnormal cortical gyral pattern and partial cortical layering defects in the brain. Similar mutation-carrying NDE1 proteins *in vitro* cannot localize to the kinetochores or centrosomes where normal NDE1 protein is recruited.^{46,47}

Nde1 homozygous knockout (KO) mice (*Nde1*^{-/-}) display microcephaly (small brain) with a fairly well-preserved CP. Detailed examination of the *Nde1* KO mouse cortex revealed that migrating neurons exhibited moderately retarded migration with thin superficial CP layers.⁴⁸ By contrast, *Ndel1* heterozygous (*Ndel1*^{ko/+}) mice display no obvious phenotype but further reduction of NDEL1 protein level in compound heterozygous (*Ndel1*^{hc/ko}) mice leads to cortical patterning abnormalities such as irregularly diffuse CP neuronal layering. In addition, *Ndel1* mutants (*Ndel1*^{hc/ko}) display significant splitting of the hippocampal pyramidal cell layer, suggesting neuronal migration defects in radial migration during hippocampal development.⁴⁹ *Nde1* and *Pafah1b1* double mutants (*Nde1*^{-/-}; *Pafah1b1*^{ko/+}) display synergistic effects on neuronal migration⁴⁹, while *Ndel1* and *Pafah1b1* double mutants (*Ndel1*^{hc/ko}; *Pafah1b1*^{ko/+}) display synergistic effects on brain size and organization.⁵⁰ Thus, the phenotypes of loss-of-function of NDE1 and NDEL1 are somewhat different, suggesting that NDE1 and NDEL1 may play distinct roles in PFAH1B1 pathway during brain development.

MT regulation

We recently demonstrated that *Ndel1* and *Pafah1b1* have distinct dosage-dependent effects on neuronal migration, neurite outgrowth and N-C coupling.^{51,52} However, cellular functions of NDEL1 in migrating neurons seem to be mediated by MT cytoskeleton regulation that is somewhat similar to PFAH1B1/dynein-dependent MT reorganization. In post-mitotic migrating neurons, CDK5-mediated phosphorylation of NDEL1 enhances PFAH1B1/dynein-regulated MT dynamics and promotes the binding to YWHAE. Importantly, both NDE1 and NDEL1 proteins are primarily localized to the centrosomes in mammalian cells, which suggests these NUD family proteins are important for MT reorganization by mediating central functions of PFAH1B1 at the centrosomes. Together, NDE1 and NDEL1 have key roles in cortical neuronal migration by integrating signals from centrosome/MT-associated proteins (Figure 4).

Interestingly, the PFAH1B1 protein was first discovered to be a non-catalytic subunit of PFAH (platelet-activating factor acetylhydrolase). The fully-functional PFAH complex is composed of two PFAH catalytic subunits (PFAH1B2 and PFAH1B3, formerly known as PFAH alpha 2 and PFAH alpha 1, respectively) and homodimers of regulatory subunits, PFAH1B1. This PFAH complex inactivates the intracellular messenger PAF (platelet-activating factor) by removing the acetyl moiety¹⁹ (Figure 3). The PAF receptor-encoding gene *PTAFR* is expressed in developing brain and PAF receptor-deficient mice

(*Ptafr*^{-/-}) show cerebellum disorganization. Importantly, the speed of neuronal migration is significantly slower in double mutants for *Pafah1b1* and the PAF receptor (*Ptafr*^{-/-}; *Pafah1b1*^{ko/+}) than *Pafah1b1* heterozygous (*Pafah1b1*^{ko/+}), suggesting the crucial role of PAF signaling pathway in neuronal migration.²⁰

MT regulation—PAFAH1B1 (LIS1) directly binds to tubulin and stabilizes MTs by regulating MT dynamics.^{21,22} Most importantly, PAFAH1B1 has been implicated in cytoplasmic dynein/dynactin-mediated MT cytoskeletal changes.¹⁰ Dynein is a MT minus end-directed motor protein that regulates MT dynamics and generates MT pulling forces in the cells. This dynein/MT modulation by PAFAH1B1 (LIS1) is evolutionarily conserved from *Aspergillus* to mammals. The *Aspergillus* PAFAH1B1 homolog (*nudF*) mutants exhibit less MT dynamics with reduced rates of MT polymerization at MT plus ends.²³ Mammalian PAFAH1B1 directly binds to several subunits of cytoplasmic dynein and dynactin.^{22,24,25} *Pafah1b1*-deficient cells display an increase in perinuclear localization of MT arrays and a decrease in MT plus end distribution at the cell periphery. These phenotypes seen in PAFAH1B1 deficiency are consistent with a loss of cytoplasmic dynein/dynactin complex at plasma membranes in these cells.^{22,25,26} In addition to this, PAFAH1B1 protein is enriched at MT-concentrated intracellular compartments such as the centrosome in mammalian cells.^{24,25} A decrease in PAFAH1B1 protein levels causes defects in N-C coupling with reduced distance between nucleus and centrosome during neuronal cell migration.^{4,27} The pulling force of nuclear movement during nucleokinesis is generated by the PAFAH1B1/dynein complex at the centrosome. Therefore, defects in N-C coupling of *Pafah1b1*-deficient neurons result in slower migration speeds of these cells. PAFAH1B1 and dynein KD cells display similar migration defects in rat cortical cultures, suggesting that PAFAH1B1 and dynein converge on the same MT regulatory pathways in cortical development.²⁸ PAFAH1B1 itself is an atypical MAP (microtubule-associated protein), and interacts with other MAPs such as DCX (doublecortin)²⁹ and MTAP1B (microtubule-associated protein 1b)³⁰. DCX binding to MTs elevates the rate of MT polymerization and stabilizes MT arrays. Similarly, MTAP1B binding on MTs enhances the growth of dynamic MTs.^{29,30} PAFAH1B1 also binds to CLIP1 (formerly known as CLIP170), a MT plus end binding protein that helps loading of vesicle cargoes along MT arrays to the cell periphery^{31,32} (Figure 3).

Actin regulation—Interestingly, PAFAH1B1-CLIP1 at MT plus ends is part of another large complex with CDC42 and RAC1, members of the small GTPase family proteins that function as actin regulatory proteins. An effector of these small GTPases, IQGAP1, participates in this interaction and further stabilizes this complex.³³ PAFAH1B1 promotes F-actin polymerization at distal ends of leading processes by modulating CDC42/RAC1-dependent actin stabilization. PAFAH1B1 has critical roles in regulating these small GTPases by activating CDC42/RAC1 and antagonistically inactivating RHOA (encoded by *RhoA* gene). *Pafah1b1* deficiency in heterozygous cells (*Pafah1b1*^{ko/+}) leads to the misregulation of actin cytoskeleton at the growth cones in the leading processes of migrating neurons. *Pafah1b1*-deficient neurons have reduced F-actin in the leading edge and less numbers of filopodia, which results in slower migration of these cells.^{33,34} This actin cytoskeleton remodeling by PAFAH1B1 also contributes to proper neuronal migration. Intriguingly, PAFAH1B1 binds to DAB1 (disabled homolog 1), a scaffold adaptor protein downstream of RELN (see RELN section). By interacting with phosphorylated-DAB1 (P-DAB1) in a phosphorylation-dependent manner, PAFAH1B1 controls F-actin polymerization in the growth cones of the migrating neurons³⁵ (Figure 3). DAB1-mediated crosstalk between PAFAH1B1 and RELN pathways has pivotal roles linking cytoskeletal dynamics of actin to those of MTs.

Deletion of human chromosome 17p13.3

Human and mouse genetics—Large deletions of human chromosome 17p13.3 cause Miller-Dieker syndrome (MDS), a severe form of lissencephaly (often complete agyria) accompanied by craniofacial dysmorphisms.² In contrast to ILS patients, where 40% display *PAFAH1B1* deletions, 100% MDS patients have larger contiguous gene deletions consisting of both *PAFAH1B1* and *YWHAE*. More than 20 genes are located in this deleted region. Among these genes, the human *YWHAE* gene is located near the telomeric tip of chromosome 17p about 1Mb from the *PAFAH1B1* gene. *YWHAE* gene encodes the 14-3-3 epsilon (tyrosine 3-monooxygenase 5-monooxygenase activation protein, epsilon peptide) protein, a member of 14-3-3 protein family that is an evolutionarily conserved signaling molecule.^{36,37} The location of mouse *Ywhae* gene is also conserved on mouse chromosome 11 where mouse *Pafah1b1* gene is located, and these are conserved synteny regions on human chromosome 17p13.3 region and mouse chromosome 11.

Mouse *Ywhae* null homozygous mutants display hippocampal and cerebral disorganization. Double compound heterozygous mice with both *Pafah1b1* and *Ywhae* mutations (*Pafah1b1*^{ko/+}; *Ywhae*^{-/-}) display more severe migration defects than those of single mutants, consistent with the more severe brain phenotypes seen in MDS patients.³⁸ This synergistic effects of reduced YWHAE protein levels on the severity of neuronal migration in *Pafah1b1*-deficient mice suggest that YWHAE may modulate components of the PAFAH1B1 pathway to control the motility of neurons.

YWHAE mediated MT regulation—YWHAE protein binds to CDK5-phosphorylated-NDEL1 (P-NDEL1) directly. CDK5 (cyclin-dependent kinase 5) plays a key role in the regulation of neuronal migration in mice (see Box 2. CDK5). YWHAE binding to NDEL1 protects P-NDEL1 from PPP2 (protein phosphatase 2, formerly known as PP2A)-dependent dephosphorylation and enhances P-NDEL1 stability.³⁸ These CDK5-mediated phosphorylation sites of NDEL1 are involved in dynein and PAFAH1B1 interaction. Hence, the P-NDEL1/YWHAE complex binds tightly to cytoplasmic dynein and PAFAH1B1 along MTs. Loss of the YWHAE protein results in mislocalization of NDEL1 and PAFAH1B1 offloaded from MT plus end tips³⁸, suggesting that YWHAE also converges on PAFAH1B1/NDEL1/dynein-regulated MT remodeling pathway during neuronal migration.

Box 2

CDK5: a key kinase to regulate neuronal migration in mice

CDK5 (cyclin-dependent kinase 5) is a multifunctional serine/threonine kinase involved in neuronal migration by regulating phosphorylation events of several substrates. Upon binding of neuronal activators CDK5R1 (CDK5 regulatory subunit 1, formerly known as p35) and CDK5R2 (CDK5 regulatory subunit 2, formerly known as p39), CDK5 becomes enzymatically active in the developing brain. *Cdk5* KO mice and *Cdk5r1/Cdk5r2* DKO mice display inverted cortical layering, suggesting that the CDK5-CDK5R1/CDK5R2 pathway is essential for normal cortical development and neuronal migration.⁷⁹

MT regulation

CDK5 phosphorylates NDEL1, a PAFAH1B1-binding MT/dynein regulator and P-NDEL1 increases peripheral MT polymerization.^{22,44} DCX is also a substrate of CDK5 phosphorylation. P-DCX reduces the affinity of DCX binding to MT arrays, which results in DCX localization at perinuclear MTs.²⁷ MTAP1B is phosphorylated by CDK5 and P-MTAP1B regulates MT-enriched axon elongation in migrating neurons.⁸⁰ Finally, CDK5-mediated PTK2 (protein tyrosine kinase 2, formerly known as FAK)

phosphorylation is required for MT organization, nuclear movement and neuronal migration.⁸¹

Actin regulation

Another important substrate of CDK5 in neuronal migration is CDKN1B (cdk inhibitor 1B, formerly known as p27^{kip1}), an actin-regulatory protein. P-CDKN1B (phosphorylated form of CDKN1B) is stabilized and suppresses RHOA-GTPase activity.⁸² RHOA has critical roles in actin cytoskeleton regulation by two major pathways: RHOA-ROCK(Rho kinase)-LIMK1-CFL1 phosphorylation and RhoA-ROCK-MLC (myosin light chain) phosphorylation. Therefore, CDK5-dependent CDKN1B phosphorylation is required for the inhibition of RHOA activity to reorganize the actomyosin network during neuronal migration (Figure 6).

VLDLR/LRP8 (APOER2)

Human *VLDLR* mutations cause severe lissencephaly with cerebellar malformation.⁸³ Homozygous mutant patients display mental retardation and gyral simplification in the cortex. As noted above, VLDLR and LRP8 are the members of the lipoprotein receptor family proteins at the plasma membrane and act as co-receptors for RELN.^{84,85} Compound DKO mice of VLDLR/LRP8 display perturbation of neuronal migration that phenocopies the *Reeler* mutant.⁸⁶ VLDLR/LRP8 play critical roles in actin-MT regulation by interacting with DAB1 through their cytosolic domain and VLDLR/LRP8-DAB1 interaction triggers cytoskeletal remodeling during neuronal polarization and migration (Figure 5) (see also RELN section).

CRK mediated Actin regulation—*CRK* (v-crk sarcoma virus CT10 oncogene homolog) is one of the genes located within the MDS critical region at the telomeric end of human chromosome 17p³⁹, adjacent to *YWHAE*, and additional loss of CRK may contribute to the more severe phenotype of MDS. CRK family proteins function as adaptor molecules to reorganize the actin cytoskeleton by regulating adhesion signals.⁴⁰ Intriguingly, CRK and CRKL (CRK-like) interact with P-DAB1 that is implicated in RELN-mediated actin regulatory pathway.⁴¹ *Crk* null mice display craniofacial defects during early development⁴² and *Crk/Crkl* KD prevents RELN-induced dendrite extension in hippocampal neurons⁴³, which are consistent with the severe craniofacial and neurological phenotypes seen in MDS patients.

DCX

Human and mouse genetics—*DCX* (doublecortin) is the most common genetic cause of X-linked lissencephaly and the human gene is located in Xq22.3-q23. Clinically, heterozygous *DCX* mutations in females (*DCX*^{+/−}) result in subcortical band heterotopia (SBH, misplaced neurons in the white matter rather than the CP), while hemizygous mutation in males (*DCX*^{y/−}) results in ILS. Female phenotypes with *DCX* mutation are variable due to random X-inactivation, resulting in genetic mosaicism in these patients.^{53,54} One distinct feature in *DCX*-associated ILS compared to that of *PAFAH1B1*-associated ILS is that *PAFAH1B1* has a more severe posterior (P) cortical abnormality, while *DCX* has a more severe anterior (A) phenotype (*DCX*^{P>A} vs. *PAFAH1B1*^{P<A}).

Mouse *Dcx* genetic male mutants (*Dcx*^{y/−}) display mild histological defects in the cortex and more notable disorganization of the hippocampus⁵⁵, probably due to compensatory mechanisms from doublecortin-like kinase (*Dclk*). In support of this, *Dcx/Dclk* double knockout (DKO) mice display severe defects in neuronal migration with abnormal lamination in the CP with the accumulation of multipolar neurons in the IZ.^{56,57} More careful analysis of neuronal migration by live imaging of brain slices from *Dcx* genetic male

mouse mutants (*Dcx*^{-/-})⁵⁸ or acute *Dcx* KD cells in rat brain slices⁵⁹ uncovered significant migration defects in *Dcx*-deficient migrating neurons.

MT regulation—DCX is a MAP expressed in NPs and differentiating neurons. DCX protein has two evolutionarily conserved tandem repeat domains that are required for MT binding and stabilization. DCX stabilizes MTs and enhances MT polymerization as well as the bundling capacity of MTs. *Dcx* mutant and *Dcx* KD immature neurons exhibit weakened N-C coupling and ultimately delayed centrosomal and nuclear movement.^{27,60} DCX is also a phosphoprotein that serves as a substrate of several kinases, including: MARK1 (microtubule affinity-regulating kinase 1) and PRKA (formerly known as PKA, protein kinase A) at residue Ser47⁶¹, and CDK5 mainly at residue Ser297⁶². MARK1- and PRKA-mediated phosphorylation of DCX reduces its MT binding activity. P-Ser47-DCX (phosphorylated form of DCX at Ser47) is required for proper localization of DCX protein at the leading processes of migrating neurons. Ser297 of DCX is the *in vivo* phosphorylation target of CDK5 and this phosphorylation event enhances the MT binding ability of DCX. DCX may directly interact with PAFAH1B1²⁹, and this interaction may contribute to MT stabilization near perinuclear regions in migrating neurons to provide power for nuclear translocation (nucleokinesis). Thus, DCX increases MT stabilization and MT nucleation by increasing tubulin polymerization, and is one of the key MT regulators in corticogenesis during early development.

Actin regulation—DCX is also phosphorylated by MAPK8 (mitogen-activated protein kinase 8, formerly known as JNK) at residue Thr321, Thr331, and Ser334. Phosphorylation of these sites of DCX are responsible for DCX recruitment to the growth cones of leading processes, the F-actin-rich zone. MAPK8-mediated DCX phosphorylation is essential for neurite outgrowth of migrating neurons.⁶³ Through binding to MAPK8IP1 (MAPK8-interacting protein 1, formerly known as JIP-1), DCX may control actin dynamics by regulating the RELN pathway, since JIP-1 directly binds to LRP8 (APOER2), a RELN receptor. DCX interaction with PPP1R9B (protein phosphatase 1 regulatory subunit 9B, formerly known as Spinophilin or Neurabin-II) may also provide a mechanism linking actin and DCX. DCX itself is co-sedimented with F-actin *in vitro* and adding PPP1R9B elevates DCX-F-actin binding affinity.⁶⁴ PPP1R9B is an actin-binding protein functioning as a protein phosphatase-1 (PPP1)-adaptor protein. PPP1R9B-mediated PPP1 targeting of DCX induces dephosphorylation of the residue Ser297. PPP1R9B and DCX colocalize at the growth cones where MT and actin dynamically interact. Dephosphorylation of Ser297 of DCX by PPP1R9B/PPP1 is important for DCX distribution at neurite tips during neuronal migration.⁶⁵

Tubulin – TUBA1A, TUBB2B, TUBB3—Given that PAFAH1B1, DCX and YWHAE are all MT-associated proteins, it is not a surprising finding that tubulin mutations themselves, including *TUBA1A*, *TUBB2B* and *TUBB3* can cause neuronal migration disorders and cortical malformations.

TUBA1A: *TUBA1A* (tubulin alpha-subunit 1A isoform) was first reported to have key roles in cortical development in both mouse and human.⁶⁶ The mouse model, first described in an ENU mutagenesis study, displayed hippocampal disorganization and behavioral deficits with no overt cortical phenotype, while in human *TUBA1A* mutation-harboring patients, cortical laminar organization was disrupted and hypoplastic cerebellum and brainstem regions were also observed. Mutations found in the mouse and humans were located either within GTP binding pocket or structurally close to the motif participating in beta-tubulin subunit interaction, suggesting that GTP-dependent incorporation of alpha- and beta-tubulin in heterodimer was impaired and resulted in defective MT polymerization.⁶⁶ A subsequent

study demonstrated that tubulin-related cortical malformations have a very broad phenotypic spectrum in human patients.⁶⁷

TUBB2B/TUBB3: Heterozygous mutation in human *TUBB2B* (tubulin beta-subunit 2B isoform) causes asymmetric polymicrogyria (PMG), characterized by multiple small gyri separated by shallow sulci. Halting of neuronal migration within IZ in *Tubb2b* KD rat embryo brains suggests that TUBB2B contributes to neuronal migration during development.⁶⁸ More recently, other beta-tubulin *TUBB3* (tubulin beta-subunit 3 isoform) human heterozygous mutations were identified that lead to cortical malformation and perturbation of axon guidance by altering MT dynamics.⁶⁹

RELN (Reelin)

Human and mouse genetics—RELN (Reelin), a large extracellular matrix glycoprotein (~400kDa), is expressed and secreted by Cajal-Retzius cells at the MZ layer of the developing brain. Mutation of the human *RELN* gene located on chromosome 7q22 causes an autosomal recessive form of lissencephaly syndrome with cerebellar hypoplasia as well as malformations in the hippocampus and brainstem.⁷⁰

The mouse *Reeler* mutant (*RELN* mutation) was the first mouse mutant described with neuronal migration defects and widely studied. Homozygous *Reeler* mice display ataxia, cerebellar hypoplasia and inverted ‘outside-in’ cortical layering, composed of superficial early-born neurons and deep positioning later-born neurons (opposite to the normal CP layering).^{71,72} The absence of a well-defined MZ layer is another characteristic of *Reeler* mouse cortex due to over-migration of neurons. This observation suggests that RELN functions as a ‘stop’ signal of migrating neurons.⁷³ The finding that RELN induces the detachment of migrating neuron from radial glial fibers⁷⁴ is consistent with the idea that RELN acts as a ‘stop and detach’ signal during radial migration.⁷⁵

MT regulation—RELN binds to the transmembrane receptors VLDLR (very low density lipoprotein receptor) and LRP8 (low density lipoprotein-related receptor 8, formerly known as APOER2). (see VLDLR/LRP8(APOER2) section). RELN enhances the interaction between RELN receptors and DAB1, an intracellular adaptor protein and further induces the phosphorylation of DAB1 by SRC family kinases (SRC, FYN) activation. Upon RELN binding, tyrosine phosphorylation of DAB1 activates the downstream PI3K pathway that mediates PI3K-AKT-GSK3beta signaling and consequently the CDK5-dependent phosphorylation of its substrates, MAPT (microtubule-associated protein tau) and MTAP1B is increased.^{76,77} Through this phosphorylation cascade, RELN-DAB1 regulates MT stability and dynamics in migrating neurons. Consistent with this, *Dab1* KO mice display a *Reeler*-like phenotype with neuronal migration defects.⁷⁸ Intriguingly, P-DAB1 also binds to PAFAH1B1, a key regulator of MT/dynein in phosphorylation-dependent manner. Compound heterozygous of *Dab1/Pafah1b1* mouse mutant (*Dab1*^{+/-}; *Pafah1b1*^{ko/+}) display more severe cortical migration defects than single heterozygous mutants, suggesting important crosstalk between RELN and PAFAH1B1 signaling pathways *in vivo* and *in vitro*³⁵ (Figure 5).

Actin regulation—RELN-induced activation of the DAB1-PI3K pathway elevates LIMK1 (LIM-domain containing protein kinase 1) kinase activity, which phosphorylates CFL1 (formerly known as cofilin), an F-actin-severing protein. Since P-CFL1 (phosphorylated cofilin) is an inactive form, the RELN-DAB1-PI3K-LIMK1-P-CFL1 signaling cascade increases the stability of F-actin stress fibers and consequently reduces actin cytoskeleton turnover. Therefore, *Reeler* mutants fail to transmit a DAB1-PI3K-LIMK1 signal and display reduced P-CFL1 levels in migrating neurons, resulting in misregulation of the actin

cytoskeleton⁷⁵ (Figure 5). P-CFL1 may be enriched in the leading processes when migrating neurons reach the MZ layer. By elevating P-CFL1, RELN stabilizes the actin cytoskeleton and helps anchor the leading processes to the basal lamina of developing cortex. Therefore, reduced P-CFL1 in the absence of RELN results in destabilization of actin dynamics in the leading processes and leads to somal translocation failure. The *Reeler* mutant mouse cortex display defects in somal translocation at the final stage of migration near the MZ, suggesting that the RELN pathway mediates a ‘detach and go (somal translocation)’ signal in this stage of radial migration.⁷⁵

ARX

ARX (aristaless-related homeobox) is an X-linked gene that encodes a transcription factor required for interneuron function and neuronal migration.⁸⁷ Missense and truncation mutations of *ARX* lead to X-linked lissencephaly with abnormal genitalia (XLAG), and patients display epilepsy and severe mental retardation. Mouse *Arx* KO mutants similarly display neuropathological phenotypes such as GABAergic interneuron dysfunction due to tangential migration defects.⁸⁸ *Arx* KO mice display disorganized pyramidal neuronal layering, suggesting ARX may play important role not only in tangential migration but radial migration as well.^{87,88} Since ARX can act as a transcriptional repressor and activator depending on promoter specificity, *Arx* mutation is accompanied by downregulation or upregulation of downstream target genes such as *Lmo1*, *Ebf3*, and *Shox2* to influence the regionalization process of the developing brain.^{89,90} Genome-wide Chip-ChIP (chromatin immunoprecipitation) promoter analysis from *Arx* mutant cells revealed that ARX binds to the promoters of genes in regulatory pathways important for axonal guidance and neurite extension⁹¹ suggesting that ARX is involved in cytoskeletal control of migration of post-mitotic neurons by transcriptional regulation.

Conclusion

Cytoskeletal remodeling of actin/MTs and the dynamic regulation of transport on these cellular structures are critical processes to induce neuronal polarization from the leading process to the trailing process and N-C coupling that drives nuclear and neuronal migration during CNS development. Several tubulin isoforms (TUBA1A, TUBB2B, TUBB3) are key components of neuronal MT arrays and DCX is tightly associated with MTs in migrating neurons. Loss of these proteins produces imbalances in MT dynamics, which causes lissencephaly or other cortical brain malformation in human. PFAFH1B1 (LIS1) is a key regulator of MT stability and dynamics by modulating cytoplasmic dynein localization and motor function, while YWHAE binds to P-NDEL1 to promote its function in dynein localization. Concomitant deletion of *PFAFH1B1* and *YWHAE* genes in chromosome 17p13.3 is the cause of MDS in human, suggesting that misregulation of MT stability and dynein dysfunction are the causes of MDS in human. Since deletion of CRK, an actin-regulatory protein on chromosome 17p, also occurs in MDS, actin dynamics may also play a role in this severe phenotype. Interestingly, PFAFH1B1, DCX, and RELN have central functions in the interplay between actin and MT cytoskeletons by affecting different downstream cellular pathways. PFAFH1B1 specifically activates RAC1/CDC42 GTPase activity to promote F-actin polymerization. The binding of RELN to its receptors, VLDLR/LRP8 (APOER2) recruits DAB1 adaptor protein to stabilize F-actin. Since P-DAB1 also interacts with PFAFH1B1, while DCX interacts with LRP8 through binding to MAPK8IP1 (JIP-1), the effects of PFAFH1B1/DCX on MT dynamics converge to RELN-VLDLR/LRP8-DAB1-actin regulatory pathway, which allows crosstalk between the actin and MT cytoskeletons. PFAFH1B1, DCX, and RELN are important organizational nodes to coordinate neuronal migration. Lastly, *ARX* mutation in human results in interneuron dysfunction and this gene is implicated in tangential migration of GABAergic neurons in the

developing brain. The finding that ARX binds to the promoters of genes in regulatory pathways important for axonal guidance and neurite extension⁹¹, suggests that ARX is involved in cytoskeletal control of migration of post-mitotic neurons by transcriptional regulation.

Although these previous studies have unraveled some key functions of several genes during neuronal migration in human and mouse, there are still many questions to be answered. It is likely that there is coordinate regulation between neuronal migration and neurogenesis/neuronal differentiation processes. Since many neuronal migration genes also encode centrosome-associated proteins (NDE1, NDEL1 and PAFAH1B1), cell cycle-dependent proliferation of NPs and the differentiation of daughter neurons and/or cell fates may be controlled by these centrosomal proteins. Recent studies of the human *WDR62* gene demonstrated that deficiency of this centrosome-associated protein also causes micro-lissencephaly.^{92,93,94} Emerging evidence strongly supports the notion that subcellular components other than centrosome (critical for N-C coupling) participate in neuronal migration processes during cortical development. These candidates include gap junction proteins, adhesion molecules and proteins involved in vesicle trafficking or recycling pathways. However, it is not clear whether these proteins contribute to neuronal migration through actin/MT cytoskeletal regulation or via distinct processes. To further understand the exact molecular and cellular mechanisms underlying neuronal migration during mammalian cerebral cortex development, additional *in vivo* and *in vitro* studies are needed for the accurate mapping of genetic and physical interactions in protein-protein networks and the proper positioning of individual protein components within detailed signaling pathways. Future studies of neuronal migration will likely explore many aspects of cytoskeletal regulation in migratory processes to further understand neuropathological pathways responsible for neurodevelopmental brain malformation disorders caused by neuronal migration defects in human. Delineation of these pathways will aid in the identification of potential new therapeutic targets to ultimately cure these devastating diseases.

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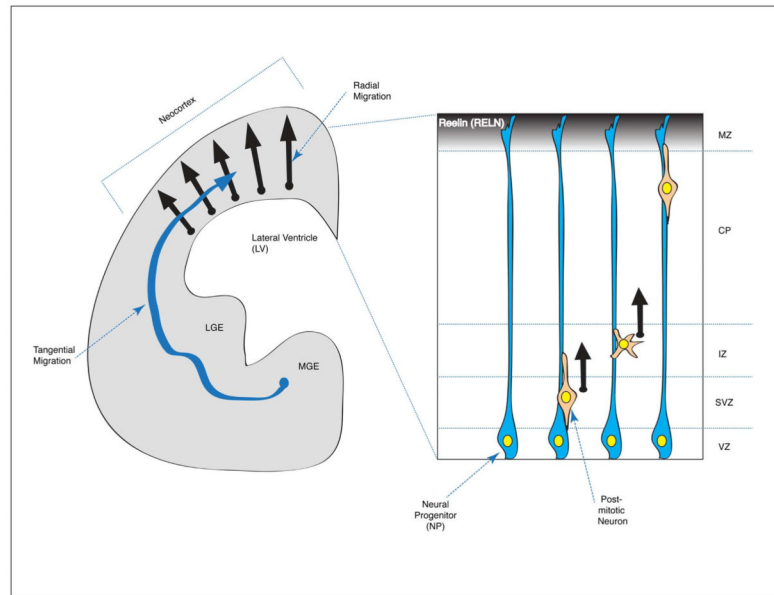


Figure 1. Schematic representation of the developing mammalian brain. (1) Coronal section of one half of the mammalian developing forebrain. There are two main migratory streams of post-mitotic neurons: the radial migration of excitatory cortical pyramidal neurons from the ventricular zone (VZ) to the cortical plate (CP) (black arrow) and the tangential migration of inhibitory GABAergic interneurons from lateral- and medial- ganglionic eminences (LGE/MGE) into the neocortex (blue arrow). (2) The developing cerebral cortex in mammals is multi-layered with different neuronal cell populations. Near the lateral ventricle (LV) surface, neural progenitors (NPs) reside in the ventricular zone (VZ). This progenitor zone is extended to subventricular and intermediate zones (SVZ and IZ, respectively). Newly born neurons from the division of NPs undergo extensive radial neuronal migration to enter the cortical plate (CP). The marginal zone (MZ) is a most superficial layer to contain Cajal-Retzius cells secreting the RELN (Reelin) glycoprotein.

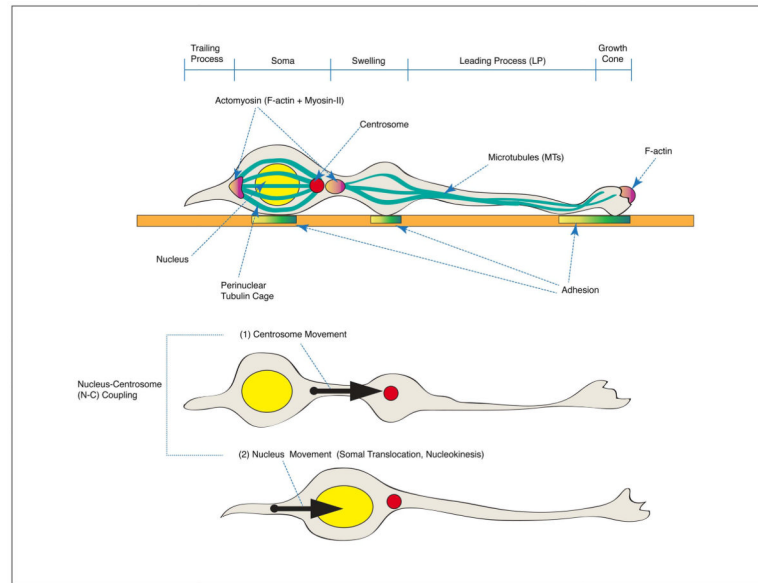


Figure 2.

Cytoskeletal features of migrating neurons during central nervous system (CNS) development. Migrating neurons are polarized from the growth cone, which is the migrating tip of leading process (LP) to the trailing process (TP). The nucleus is surrounded by a perinuclear tubulin cage and the rear side of nucleus is enriched with actomyosin (filamentous actin, F-actin + Myosin-II) that generates pushing forces of nuclear movement (somal translocation, nucleokinesis). Migration occurs in two distinct modes of movements in a two stroke model: (1) Centrosome (C) movement toward the swelling in the LP (2) Nuclear (N) movement in the direction of migration. This N-C coupling consequently provides the pulling force on microtubules (MTs) along the LP, which establishes new contacts to adhesion substrates.

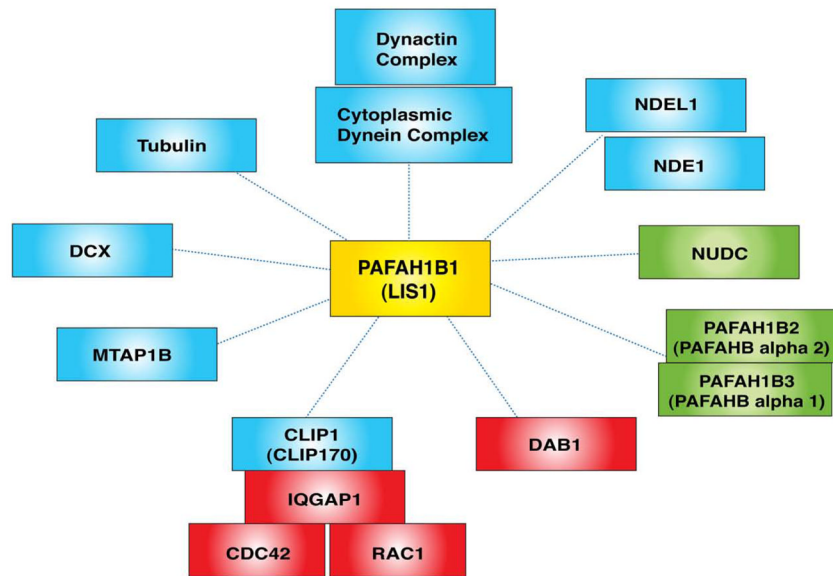


Figure 3.

PAFAH1B1 (platelet-activating factor acetylhydrolase 1 regulatory subunit 1, formerly known as LIS1)-binding partners. The binding of PAFAH1B1 (LIS1) to the cytoplasmic dynein/dynactin complex is evolutionarily conserved from fungi to mammals. Nuclear distribution proteins such as NDE1, NDEL1 and NUDC interact directly with PAFAH1B1. PAFAH1B1 itself is a microtubule-associated protein (MAP) and is localized at tubulin/MT-rich subcellular compartments in cells like centrosomes. Other MAPs, such as DCX and MTAP1B, are also PAFAH1B1-binding partners. PAFAH1B1 was also identified as a non-catalytic subunit of PFAH and PAFAH1B1 associates with PFAH catalytic subunits such as PAFAH1B2 (PAFAH alpha 2) and PAFAH1B3 (PAFAH alpha 1). When PAFAH1B1 binds to CLIP1 (CLIP170), a MT plus end protein, it forms a complex with IQGAP1 and CDC42/RAC1. Through this interaction, PAFAH1B1 participates in F-actin dynamics in growth cones during neuronal migration. Interestingly, PAFAH1B1 also binds to DAB1, an actin-regulatory protein acting in RELN signaling pathway. PAFAH1B1 has dual roles in distinct regulatory pathways of MT and actin cytoskeletons. (Blue proteins: MT regulators, red proteins: actin regulators, green proteins: Unclear detailed function in MT/actin regulation)

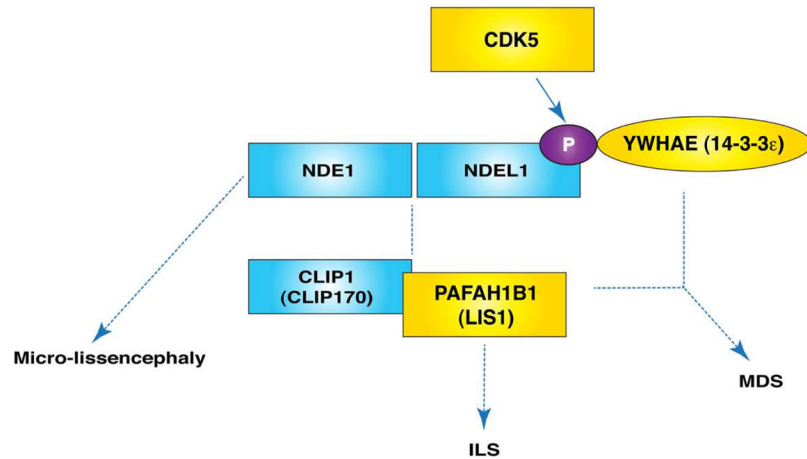


Figure 4.

NDE1 and NDEL1, key proteins integrating several signals in neuronal migration. *NDE1* and *NDEL1* are two mammalian homologues of *Aspergillus nidulans nude*. Upon CDK5 (cyclin-dependent kinase 5)-mediated phosphorylation of NDEL1, P-NDEL1 binds to YWHAE (formerly known as 14-3-3 epsilon). Isolated lissencephaly sequence (ILS) is caused by the haploinsufficiency of human *PAFAH1B1* (*LIS1*) gene. Simultaneous chromosomal deletion of the regions including *YWHAE* and *PAFAH1B1* in human causes Miller Dieker syndrome (MDS), a severe case of lissencephaly with craniofacial malformation. Human *NDE1* heterozygous mutations result in micro-lissencephaly.

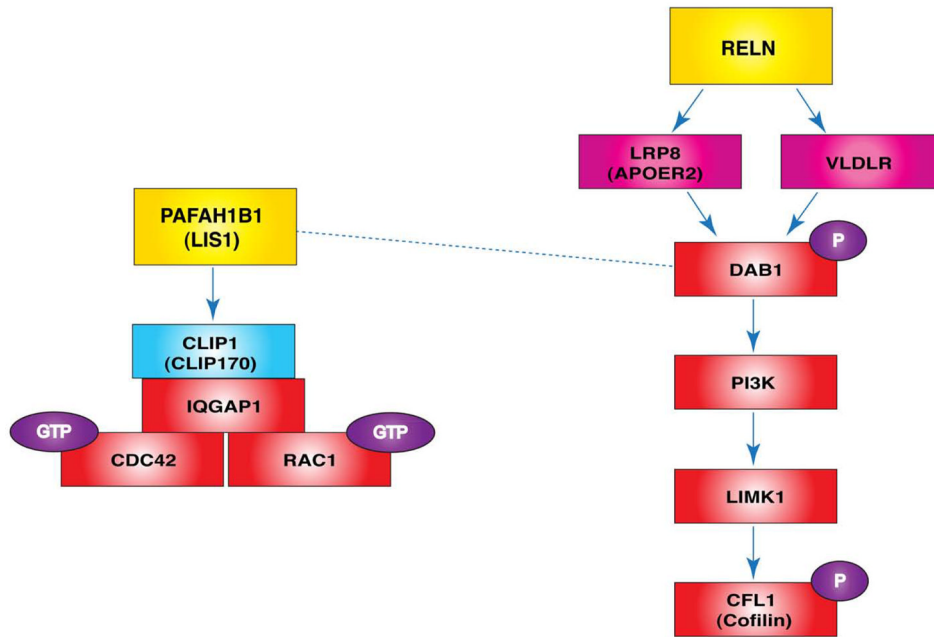


Figure 5.

PAFAH1B1 and RELN-mediated actin regulation. Formation of the PAFAH1B1-CLIP1-IQGAP1-CDC42/RAC1 complex stabilizes and sustains the GTPase activities of CDC42 and RAC1. RELN, a large glycoprotein secreted from the MZ area in the cortex, directly binds to lipoprotein receptors such as VLDLR and LRP8 (APOER2). This binding recruits the DAB1 adaptor protein to the membrane where DAB1 is phosphorylated. P-DAB1 activates PI3K-LIMK1 signaling and LIMK1 phosphorylates CFL1 (cofilin), an actin-severing protein, which keeps CFL1 in an inactive state. This further stabilizes F-actin stress fibers in migrating neurons. Since the dynamic regulation of the actin cytoskeleton is required for neuronal motility, a balance between actin polymerization and depolymerization is essential for neuronal migration processes. (Dashed line: direct interaction between PAFAH1B1 and P-DAB1, Blue protein: MT regulator, red proteins: actin regulators)

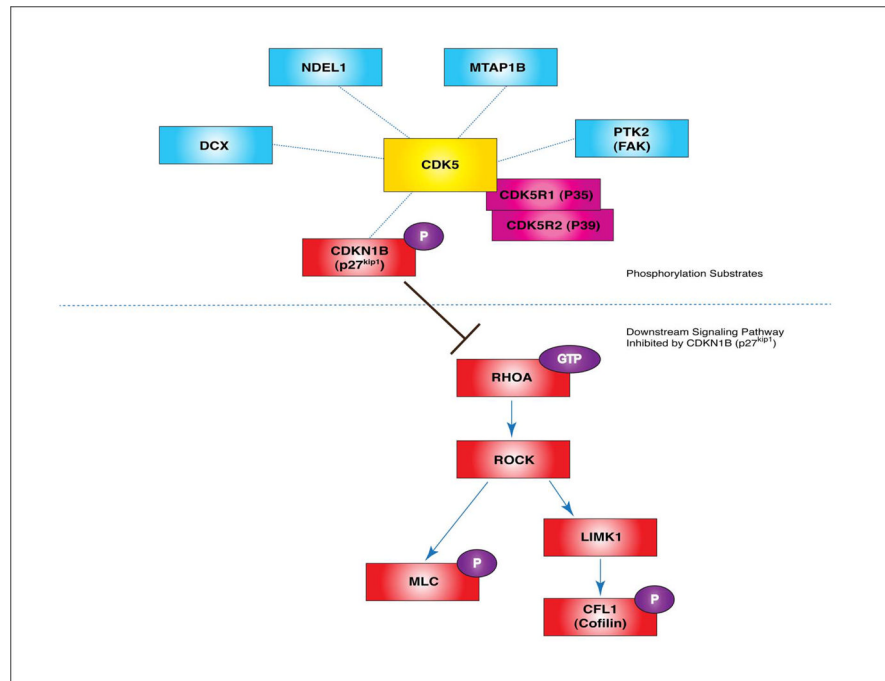


Figure 6. CDK5 phosphorylation substrates during neuronal migration. When CDK5 is activated by binding of CDK5R1 (p35)/CDK5R2 (p39), this kinase phosphorylates multiple substrates in migrating neurons. Among those substrates, DCX, NDEL1, MTAP1B and PTK2 (FAK) are MT-regulating proteins. A very interesting phosphorylation substrate of CDK5 is CDKN1B (p27^{kip1}). P-CDKN1B suppresses RHOA GTPase activity. RHOA-ROCK-LIMK1-CFL1 and RHOA-ROCK-MLC signaling pathway have been implicated in actin cytoskeletal remodeling during neuronal migration. CDK5 indirectly affects these actin-regulating signaling pathways by modulating CDKN1B function. (Blue proteins: MT regulators, red proteins: actin regulators)

Table 1

Gene	Chromosomal Locus in Human	Human Clinical Presentation	Migration Defects in Mouse	Regulation Mechanisms
<i>PAFAH1B1</i>	17p13.3	ILS	Abnormal lamination of cortex and hippocampus	MT/Dynein Actin
<i>YWHAE</i>	17p13.3	MDS (with LIS1 deletion)	Abnormal lamination of cortex and hippocampus	MT
<i>NDE1</i>	16p13.11	Micro-lissencephaly	Microcephaly Moderate lamination defects in cortex	MT
<i>DCX</i>	Xq22.3-q23	ILS (male) SBH (female)	Mild disorganization of hippocampus, Modest cortical lamination defects	MT Actin
<i>Tubulin (TUBA1A, TUBB2B, TUBB3)</i>	TUBA1A, 12q13.12 TUBB2B, 6p25.2 TUBB3, 16q24.3	ILS with cerebellar hypoplasia TUBB2B – Asymmetric PMG	Abnormal lamination of cortex	MT
<i>RELN (Reelin)</i>	7q22	ILS with cerebellar hypoplasia	Inverted layering of CP in cortex	MT Actin
<i>VLDLR</i> [LRP8 (APOER2), Mutations not found in human]	VLDLR, 9p24	ILS with cerebellar dysfunction	Inverted layering of CP in cortex	MT Actin
<i>ARX</i>	Xp21.3	XLAG	Absence of cortical interneurons and lamination defects	Transcriptional regulation

The genes implicated in neuronal migration. (ILS: isolated lissencephaly sequence, MDS: Miller-Dieker syndrome, SBH: subcortical band heterotopia, PMG: polymicrogyria, XLAG: X-linked lissencephaly with abnormal genitalia. CP: cortical plate, MT: microtubule)