PROTEIN–PROTEIN INTERACTIONS, CYTOSKELETAL REGULATION AND NEURONAL MIGRATION

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Neuronal migration, like the migration of many cell types, requires an extensive rearrangement of cell shape, mediated by changes in the cytoskeleton. The genetic analysis of human brain malformations has identified several biochemical players in this process, including doublecortin (*DCX*) and *LIS1*, mutations of which cause a profound migratory disturbance known as lissencephaly ('smooth brain') in humans. Studies in mice have identified additional molecules such as Cdk5, P35, Reelin, Disabled and members of the LDL superfamily of receptors. Understanding the cell biology of these molecules has been a challenge, and it is not known whether they function in related biochemical pathways or in very distinct processes. The last year has seen rapid advances in the biochemical analysis of several such molecules. This analysis has revealed roles for some of these molecules in cytoskeletal regulation and has shown an unexpected conservation of the genetic pathways that regulate neuronal migration in humans and nuclear movement in simple eukaryotic organisms.

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Protein–protein interactions are a little like human relationships. Some are dedicated, faithful and lifelong, while other relationships are brief flings with a promiscuous variety of partners that may leave no lasting trace or may induce profound changes. The problem of separating functional interactions from accidental ones has dogged the analysis of many proteins, especially those that regulate neuronal migration.

Neuronal migration is an almost universal feature of the development of the nervous system, since neurons are usually formed in specialized proliferative zones but ultimately reside in distinct layered structures or organized nuclei. The migration of neurons seems to involve many of the same aspects of cytoskeletal regulation that characterize the migration of non-neural cell types, as well as some specialized features. So, many of the proteins involved in neuronal migration ultimately seem to operate by regulating the neuronal cytoskeleton and associated molecular motors. However, identifying the signalling pathways involved has been a challenge. This article will review a flurry of recent progress in this area, which has begun to sketch some biochemical pathways that might be of general importance.

Human neuronal migration genes

The human cerebral cortex consists of a highly folded sheet that is composed of six neuronal layers. Classic lissencephaly (from the Greek 'lissos', meaning smooth, and 'enkefalos' meaning brain) is a severe human brain malformation characterized by an absence or reduction of the normal cerebral convolutions (FIG. 1). The cerebral cortex of the lissencephalic brain is abnormally thickened and lacks organized layers, and the normal morphology of cortical neurons is also disrupted[1–3]. Because the laminar structure of the cerebral cortex is formed during embryogenesis by a highly ordered process of neuronal migration, disruption of this process is believed to be the main cause of the cortical malformation seen in lissencephaly[4,5].

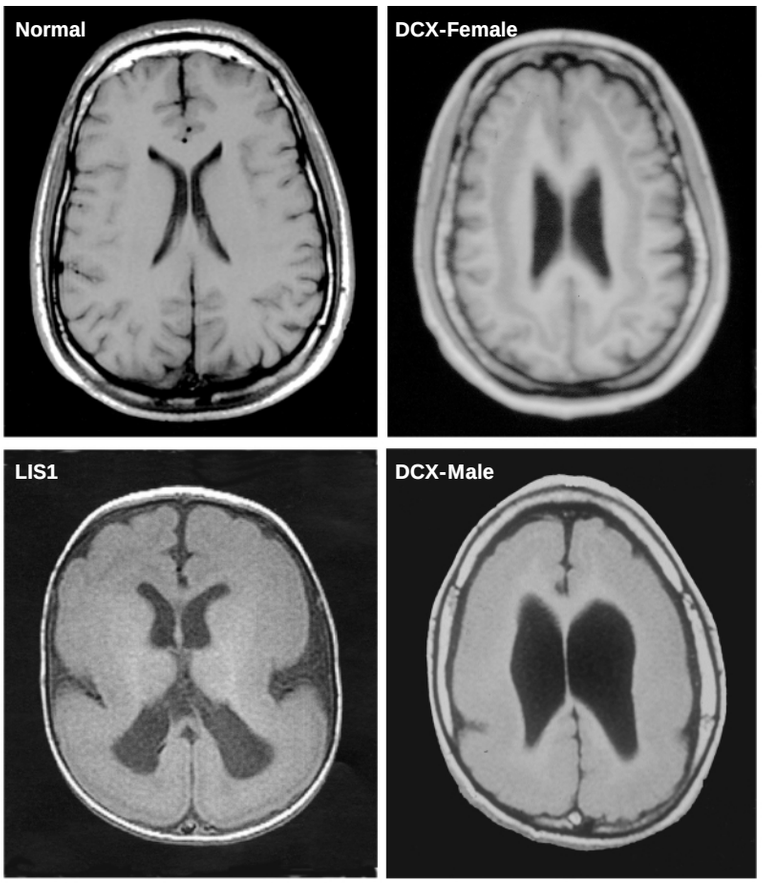


Figure 1 | **MRI appearance of human lissencephaly and double cortex syndrome.** MRI images of cerebral cortex in a normal human being (Normal), a patient with *LIS1* mutation (LIS1), a female patient with *DCX* mutation (DCX-Female) and a male patient with *DCX* mutation (DCX-Male). Notice that patients with mutations in *LIS1* and male *DCX* patients show strikingly similar lissencephalies, whereas female patients with *DCX* mutations present with 'double cortex' syndrome, in which a band of grey matter is embedded within the white matter beneath the normal cortex.

Clinically, classic lissencephaly may be found in patients that also show a characteristically dysmorphic face, referred to as the Miller–Dieker syndrome. Lissencephaly can also occur without peculiar facial features, in which case it is referred to as the isolated lissencephaly sequence (ILS). In both cases, affected individuals usually have profound mental retardation, intractable seizures and other neurological abnormalities[6].

Genetically, lissencephaly occurs as autosomal dominant or X-linked forms. Almost all patients with the Miller–Dieker syndrome have heterozygous deletions of chromosome 17p13, and ~40% of patients with ILS show submicroscopic deletions at the same locus[6,7]. A gene mapping to this critical lissencephaly locus was isolated in 1993. This gene, named *LIS1*, has been confirmed as the chromosome 17 lissencephaly gene that is responsible for a large percentage of autosomal dominant forms of lissencephaly[7,8].

Mouse models with *Lis1* mutations have a variety of dosage-dependent phenotypes that have been studied with HYPOMORPHIC and null alleles[9]. Whereas *Lis1* homozygous mutant mice die soon after implantation, mice with decreased levels of Lis1 protein showed dosage-dependent delay in neuronal migration and defects in cortical lamination. This suggests that *Lis1* is an essential gene and that a reduction in *Lis1* dosage is responsible for the neuronal migration arrest and cerebral cortex malformation.

More recently, another gene that is responsible for X-linked lissencephaly has been identified, and is referred to as doublecortin (*DCX*)[10,11]. Mutations in the *DCX* gene give rise to isolated lissencephaly in affected males with phenotypes strikingly similar to that arising from *LIS1* mutations (FIG. 1). On the other hand, female patients with heterozygous *DCX* mutations show double cortex syndrome (also known as subcortical band heterotopia). In double cortex syndrome, a population of neurons arrests halfway between the cortex and the ventricle, forming a subcortical band in the white matter. This condition presumably reflects a mosiac state in the female brain due to the random inactivation of the normal and mutant X-chromosomes[11].

DCX is a microtubule-associated protein

Of the two lissencephaly genes, the molecular mechanism of DCX protein function seems to be simpler. Compelling experimental evidence now suggests that DCX is a microtubule-associated protein (MAP). Independently, several groups have shown that DCX is expressed exclusively in postmitotic neurons, and colocalizes and co-purifies with polymerized microtubules[12–14]. *In vitro*, recombinant DCX protein can co-assemble with microtubules from brain, and can stimulate the polymerization of microtubules. Overexpression of DCX induces microtubules to form thick bundles which are resistant to colchicine treatment and cold; ordinarily, these treatments depolymerize microtubules[12].

The analysis of disease-causing human mutations further supports the role of DCX as a MAP. Missense mutations cause amino acid substitutions in the DCX protein that cluster in two tandemly repeated 90-amino-acid domains in the amino terminal end of the protein (FIG. 2a). Structure prediction of each of the DCX repeats suggests that they may form a β-grasp superfold, a structural motif shared by several proteins that bind GTPases (also known as G proteins) such as the Ras-interacting domains of c-Raf1 and Ral-GEF[15,16]. *In vitro* binding experiments have shown that each repeat can bind to purified tubulin, which consists of polymers of a GTPase-like structure. Two intact repeats together are necessary and sufficient for microtubule polymerization and stabilization[17]. Moreover, disease-causing mutations in DCX disrupt its microtubule bundling properties.

MAPs represent a structurally diverse group of proteins that share the property of regulating the dynamic transitions of microtubules. MAPs often bind to microtubules reversibly, promote tubulin polymerization and stabilize microtubules in the form of bundles[18,19]. Other MAPs identified so far include the neuronal proteins tau

HYPOMORPHIC MUTATION
A mutation that does not eliminate the wild-type function of a gene and gives a less severe phenotype than a loss-of-function mutation.

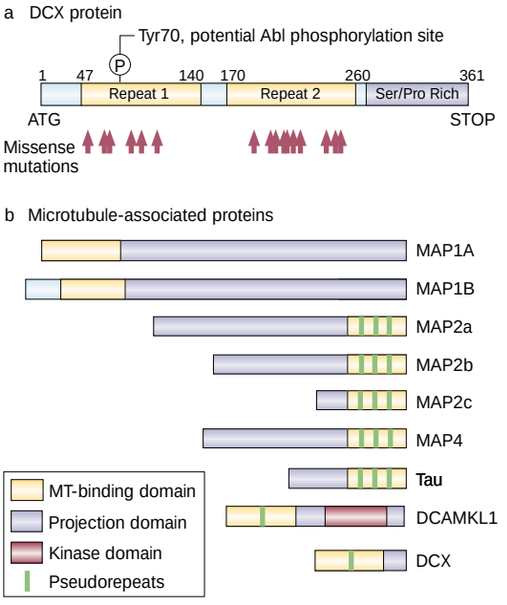


Figure 2 | **Structure and domain organizations of DCX and other major microtubule-associated proteins. a** | DCX protein is composed of two tandem repeats at the amino terminus and a serine/proline-rich region at the carboxy terminus. Missense point mutations have so far been identified in the DCX repeats and are indicated by arrows. **b** | Similar to MAP1A/1B, DCX contains a microtubule (MT)-binding domain at the amino terminus and a potential projection domain at the carboxy terminus. However, the DCX projection domain is much smaller, suggesting that the microtubule bundles induced by DCX are tightly spaced and therefore more stable. Similar to MAP2, MAP4 and Tau, the microtubule-binding domain of DCX contains pseudorepeats. However, the DCX repeats have a unique structure and are highly conserved in DCAMKL1.

and MAP2, which are localized to axons and dendrites, respectively. MAP4 is present in all non-neuronal vertebrate cells, whereas MAP1A and MAP1B are expressed at high levels in neurons and are also found in non-neuronal cells[20,21]. All of these proteins share common structural features: they are composed of one or more basic microtubule-binding domains and an acidic PROJECTION DOMAIN (FIG. 2b). The microtubule-binding domains of MAP2, tau and MAP4 contain a proline-rich sequence and three or four 31-amino-acid residue pseudorepeats. Different MAPs vary considerably in the structure and size of their projection domains. Overexpression of MAPs in cells can induce microtubule bundling, which greatly enhances the stability of microtubules. The spacing of the microtubules within these bundles seems to be controlled by the size of the projection domain of the overexpressed MAP[22].

Similar to other MAPs, the two tandem repeats of DCX have been shown to form a functional microtubule-binding domain[17,23], whereas the carboxy-terminal region of DCX is rich in serine and proline and might therefore be functionally equivalent to a MAP projection domain. The microtubule bundling phenotype observed in DCX-overexpressing cells strongly resembles the microtubule bundling induced by overexpression of other MAPs. However, the smaller size of the DCX projection domain (FIG. 2) predicts that DCX-induced microtubule bundles might be very tightly spaced, as observed for microtubules in the leading processes of migrating neurons *in vivo*[24].

DCX repeats are highly conserved in DCAMKL1, a protein with an amino-terminal domain 70% identical to DCX and a carboxy-terminal domain that is similar to Ca/calmodulin-dependent kinase. DCAMKL1 is preferentially expressed in the nervous system and has also been shown to be a MAP with microtubule stabilizing activity that is very similar to DCX[25,26]. The DCX repeats have more recently been identified in other proteins that might be predicted to function as MAPs as well. So, it is quite likely that DCX and DCAMKL1 define a new class of MAPs, and that they function in neurons by regulating the polymerizing, bundling and/or stabilization of microtubules in migrating neurons.

LIS1 is also a potential microtubule regulator

That mutations in *LIS1* and *DCX* cause almost indistinguishable phenotypes in humans raises the question of whether LIS1 is also involved in regulating microtubule stabilization. Accumulating evidence suggests that LIS1 and DCX might have related functions in neuronal migration. *LIS1* encodes a ubiquitously expressed 45 kDa protein with 7 WD40 repeats[8]. The WD40 repeat is a structure that has been found in a variety of proteins, including the β-subunit of heterotrimeric G proteins[27]. It is generally thought to be a structural motif that mediates protein–protein interactions. Consistent with its structural features, multiple protein–protein interactions centred on LIS1 have been detected over the years. LIS1 was first found to be a non-catalytic subunit of platelet-activating factor acetylhydrolase (PAFAH), a heterotrimeric enzyme that catalyses the inactivation of platelet-activating factor (PAF)[28]. However, the significance of this interaction in neuronal migration remains elusive, since there is no proof that the catalytic subunits of this enzyme play a role in cortical development. In rat spleen, LIS1 has also been found to tightly associate and co-purify with the activated tyrosine kinase Syk, a receptor-associated tyrosine kinase that plays an important role in B-cell receptor signalling[29,30]. Since Syk is a tyrosine kinase that is only expressed in haematopoietic cells, which tyrosine kinase (if any) LIS1 may bind in the brain is unknown.

More interestingly, similar to DCX, LIS1 is also reported to be a MAP that might bind microtubules directly. Sapir *et al.*[31] found that recombinant LIS1 co-sediments with polymerized microtubules from brain. In addition, LIS1 can interact with tubulin and inhibit the CATASTROPHE events of the dynamic assembly of microtubules *in vitro*[31]. This finding strongly suggests that, like DCX, LIS1 might also function in microtubule regulation in migrating neurons. However, LIS1 does not seem to share the common structural features of other MAPs, and a variety of studies suggest that the microtubule regulatory function of LIS1 could be more complicated than simple stabilization.

PROJECTION DOMAIN
Microtubule-associated proteins are usually organized into two domains: the microtubule-binding domain and a projection domain. By electron microscopy, the projection domain can be seen as a filamentous arm extending from the wall of the microtubule; this arm can bind to membranes, intermediate filaments or other microtubules. Its length controls the spacing between microtubules.

CATASTROPHE
The abrupt transition of a microtubule — a dynamic polymer — from growing phase to shortening phase.

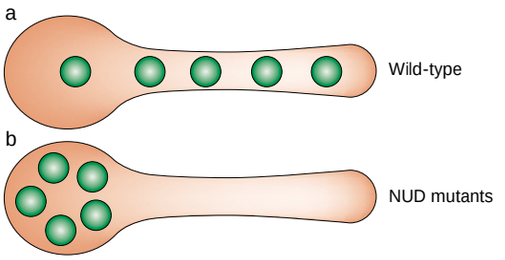


Figure 3 | **Characteristic phenotype of *Aspergillus* nuclear distribution mutations. a** | A spore of *Aspergillus* contains a single nucleus, which divides to form multiple nuclei that migrate from the rounded spore end towards the tip of the germ tube in coordinating with the growth of the tip. **b** | Phenotype of the nud (nuclear distribution) mutants. The germ tube extends and nuclear division proceeds, but the nuclei fail to move from the spore end of the germ tube.

A *LIS1* homologue is a nuclear migration gene

Remarkable insights into the microtubule regulatory function of LIS1 have come from studies of a *LIS1* homologue in the filamentous fungus, *Aspergillus nidulans*. During the development of the fungus, nuclear division is followed by the movement of daughter nuclei into the developing germ tube, and the cell then becomes multi-nucleated as the nuclei continue to undergo multiple rounds of division and migration. This uniform distribution of the nuclei is necessary for proper growth and development. Many genetic mutations that lead to defective nuclear migration have been isolated (FIG. 3). Analyses of these mutants (called nuclear distribution mutants, or nud mutants) have shown that nuclear migration is a microtubule-mediated event. Nuclear migration is disrupted by benomyl, an inhibitor of microtubule polymerization[32], and mutations in α-, β- and γ-tubulin genes in *Aspergillus* all lead to nuclear migration defects[33,34]. As well as microtubules, microtubule-based motor proteins such as dynein and dynactin are known to be essential for nuclear migration[35,36]. Moreover, mutations in the *nudF* gene provide another example of failure in transporting nuclei into the growing germ tube[37]. The *nud*F gene product shows 42% sequence identity (62% similarity) to the human LIS1 protein. Homology is observed throughout the length of the two sequences, suggesting that *nud*F and *LIS1* are ORTHOLOGUES.

Having an orthologue in *Aspergillus* allowed the function of LIS1 to be studied *in vivo* with the powerful tools of genetics. Studies of genetic interactions between *nud*F and other nuclear migration genes have provided important information on the molecular function of the NUDF protein. Interestingly, *nud*F and *nud*A, which encodes the heavy chain of cytoplasmic dynein, share a strikingly similar nuclear migration phenotype, and one allele of *nud*A is an extragenic suppressor of a heat-sensitive *nud*F mutation[38]. Moreover, *nud*F and *nud*A double mutations had the same phenotype as each of the single mutations. Together, these results suggest that dynein and *nud*F act very closely, perhaps by a direct physical interaction, in the same nuclear migration pathway in fungus.

The *Aspergillus* α-tubulin gene also showed genetic interactions with several nuclear migration genes including *nud*F and dynein. Willins *et al.*[39] showed that a mutation in α-tubulin that produces destabilized microtubules could suppress the dynein mutations *nud*A, *nud*G (which encodes the dynein light chain) as well as the *nud*F mutations. These data suggest that NUDF and dyneins might actually function via destabilization of microtubules during the process of nuclear migration in *Aspergillus*.

LIS1 interacts with two homologues of *nud*E

The *Aspergillus nudE* was identified in a screen for multicopy suppressors of a *nud*F temperature-sensitive mutation. Multicopy plasmids of the *nud*E gene improve the growth and nuclear distribution of the *nud*F mutant. However, suppression by *nud*E was not due to an effect on overall *nud*F expression, as extra copies of the *nud*E gene did not increase the level of NUDF protein in the mutant. The *nud*E null mutant was viable, but displayed defects in nuclear migration that were less severe than the *nud*F null and *nud*A null alleles[40]. These data indicate that the NUDE protein may function as a downstream effector of NUDF in regulating nuclear migration. Therefore, by homology, the function of vertebrate LIS1 in neuronal migration might be elucidated by identifying its analogous downstream effectors.

In mammals, two *nud*E homologues or *nud*E-like proteins, mNudE and NUDEL, were cloned from LIS1 TWO-HYBRID SCREENS[41–45]. mNudE and NUDEL share over 50% sequence identity, they are similar in size and structural organization, and both interact with LIS1 through a structurally similar amino-terminal region. The first 190 amino acid residues of *nud*E homologues are highly conserved from fungus to mammals, and are predicted to form a continuous coiled-coil stretch. In *Aspergillus*, a *nud*E construct encoding only the coiled-coil region complemented the *nud*E deletion and suppressed the *nud*F mutation. So, many, if not all, biological functions of the NUDE protein are carried out by its amino-terminal coiled-coils.

The LIS1 binding domain in mNudE can be further narrowed down to a 70-amino-acid region in the coiled-coil domain[41]. By itself, this region seems to interfere with endogenous LISL–mNudE/NUDEL function, since overexpression of the 70-amino-acid LIS1 binding domain as a truncated protein was found to block the LISL–mNudE/NUDEL interactions in *Xenopus* embryos, and resulted in severe defects in central nervous system lamination[41]. Moreover, missense mutations in LIS1 that disrupt human cortical development produce stable proteins that fail to bind mNudE (REF. 41). These studies provide strong evidence that LISL–mNudE/NUDEL interactions are important for CNS development.

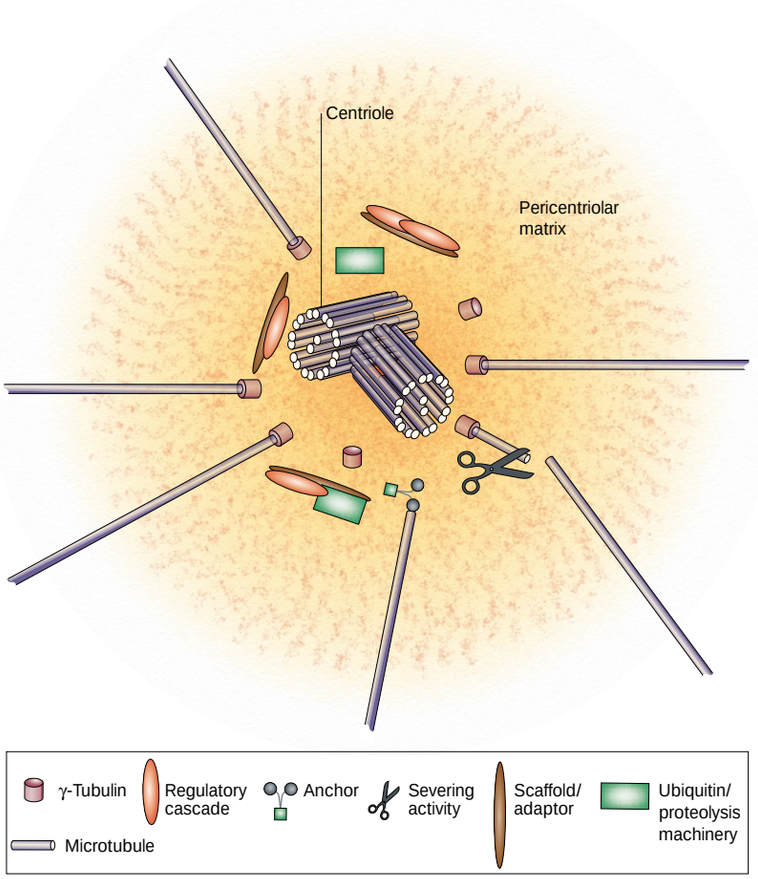
Expression studies of mNudE and NUDEL suggest an overlapping and, to some extent, complementary pattern of distribution of the two proteins. NUDEL is expressed more abundantly in brain, whereas mNudE could be detected in all tissues examined[41–43]. However, the expression of mNudE, particularly in the brain, is

ORTHOLOGUES
Genes belonging to different organisms that carry out similar functions.

TWO-HYBRID SCREEN
A system used to determine the existence of direct interactions between proteins. It involves the use of plasmids that encode two hybrid proteins; one of them is fused to the GAL4 DNA-binding domain and the other one is fused to the GAL4 activation domain. The two proteins are expressed together in yeast and, if they interact, the resulting complex will drive the expression of a reporter gene, commonly β-galactosidase.

Box 1 | Structure of the centrosome or microtubule-organizing centre

The centrosome is the major microtubule-organizing centre in animal cells. A centrosome is centred around a pair of centrioles that are oriented at right angles to one another. Surrounding the centrioles is a cloud of pericentriolar matrix (PCM), in which the minus ends of microtubules are embedded. Scaffolding proteins in the PCM organize multiple activities including microtubule nucleation and polymerization, microtubule anchoring and stabilization, as well as microtubule severing. These activities are presumably regulated by kinases, phosphatases, components of the ubiquitin degradation pathway and other regulatory molecules that have been localized to the centrosome.



highly regulated during the course of embryonic development. The brain mNudE messenger RNA level peaks at E12 in mouse, then is downregulated through later stages of embryogenesis to a much lower level in adulthood. By contrast, the mouse NUDEL message is first detectable around E13.5, increases during development, and reaches its peak around postnatal day 5. The temporal differences in expression suggest that mNudE and NUDEL might interact with LIS1 at different developmental stages. Both mNudE and NUDEL were detected throughout all cortical layers in the developing mouse brain. Whereas mNudE is expressed at high levels in the ventricular zone in proliferating neural progenitors, NUDEL shows stronger expression in postmitotic neurons of the cortical plate. In terms of subcellular localization, mNudE predominantly concentrates in the centrosome or microtubule-organizing centre (MTOC) (BOX 1). NUDEL can also be detected in the centrosome, but it seems to have a wider distribution throughout various cellular compartments including the axons of mature neurons.

Functionally, mNudE has been studied mostly for its role in regulating the centrosome/MTOC, which is the major site for nucleation and initial polymerization of microtubules in living cells (BOX 1). mNudE and LIS1 colocalize at the MTOC of interphase cells including cortical neurons, while mNudE also interacts with γ-tubulin, a key player for initiating microtubule polymerization at the MTOC (REF. 41). By forming a complex with γ-tubulin, mNudE regulates its distribution and thereby regulates microtubule organization. Overexpression of mNudE induces ectopic MTOCs and disrupts the normal interphase-cell microtubule organization. Moreover, mNudE interacts with multiple centrosomal proteins and might act as a structural and functional scaffold for the MTOC. In contrast to mNudE, the localization of LIS1 to the MTOC does not appear to reflect a direct interaction with γ-tubulin and other centrosomal proteins, but instead may occur through binding to mNudE.

The function of NUDEL has so far been emphasized in its potential role of regulating microtubule motors. NUDEL binds directly to dynein, regulating its subcellular distribution. NUDEL and mNudE may share functions in the centrosome, but NUDEL is enriched in the processes of mature neurons, pointing to additional roles in axon transport and neurite outgrowth[42,43]. The functions of both mNudE and NUDEL are likely to be more clearly elucidated by the creation of engineered mutant mice.

Interactions between LIS1 and dynein

In addition to interactions between LIS1 and mNudE/NUDEL, evidence in metazoans also points to important direct interactions between LIS1 and dynein motors. Genetic analyses in *Aspergillus* have indicated that NUDF and NUDA act in the same nuclear migration pathway, and that NUDF affects nuclear migration by acting on the dynein motor system. Very interesting recent findings have shown that LIS1 physically interacts and forms complexes with dynein in mammals. Faulkner *et al.*[46] showed that LIS1 co-immunoprecipitates with dynein and dynactin complexes, and LIS1 and dynein immunoreactivity colocalizes in the CELL CORTEX and mitotic KINETOCHORES. Overexpression of LIS1 induces spindle misorientation and mitotic arrest at M-phase, suggesting that a LIS1–dynein interaction might be important for cell division. Smith *et al.*[47] reported a similar interaction between LIS1 and the dynein–dynactin complex, and they also showed that overexpression of LIS1 alters the cytoplasmic distribution of dynein. The idea of a dynein–LIS1 interaction was further supported by recent studies in *Drosophila*, where Lis1 was shown to be essential for germline cell division and oocyte differentiation by acting as a cortical anchor for dynein during *Drosophila* oogenesis[48,49]. Furthermore, somatic null mutations in the *Drosophila Lis1* gene were shown to cause defects in neuroblast proliferation, dendritic development and axon transport[50]. The axon transport defect

CELL CORTEX
The part of the microtrabecular lattice that lies under the cell membrane.

KINETOCHORE
Specialized assembly of proteins that binds to the centromeric region of the chromosome.

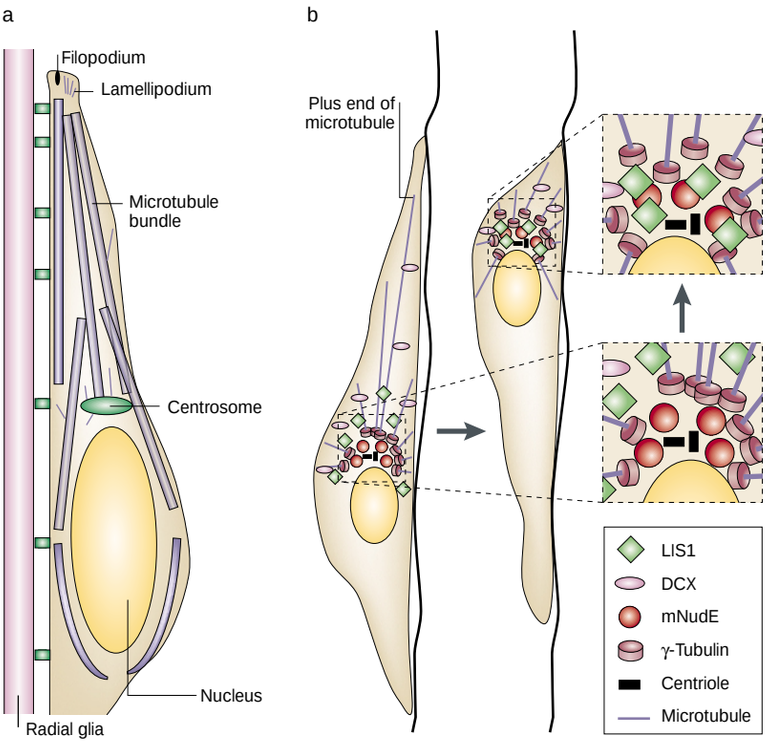


Figure 4 | **Model for microtubule-based nuclear translocation in neuronal migration.** It has been shown that neuronal migration along the radial glia starts with the extension of the leading process, which is followed by the translocation of the nucleus and the cytoplasmic content. **a** | Migrating nuclei often associate tightly with the centrosome. **b** | Studies on the function of DCX and LIS1 so far suggest the following simple model. Migration is initiated by microtubule extension in the leading process which might be regulated by DCX and other MAPs. This step is followed by a regulatory event that leads to the recruitment of LIS1 to the microtubule-organizing centre through binding to mNudE. Interaction of LIS1 with mNudE at the centrosome reduces the nucleation and polymerization of microtubules at the minus end, possibly by regulating the γ-tubulin-complex. As a result, microtubules shorten at the minus end and the nucleus is pulled towards the leading edge of the migrating neuron.For simplification, microtubules at the trailing end of the cell are not illustrated. Other key cytoskeletal elements that are generally required for cell migration, such as actin fibres and dynein motors, are not shown. The membrane of the radial glial fibre to which the migrating neuron is attached is depicted by a dark line.

was phenotypically similar to a dynein heavy chain mutation, further supporting a close interaction between LIS1 and dynein.

Is the role of LIS1 in neuronal migration mediated by dynein motors? Although there is no direct functional evidence to support a specific role for cytoplasmic dynein in neuronal migration, dynein is involved in various forms of intracellular motility, including separation of the spindle poles during mitosis, retrograde axonal transport, protein sorting and organelle redistribution. Dynein might be necessary for the transport of the nucleus and other cytoplasmic contents during the course of neuronal migration. Thus, it is possible that defects in LIS1–dynein interactions affect the translocation of the neuronal cell body. However, as indicated by genetic analyses in mouse and *Drosophila*, *LIS1* is also an essential gene that is important for cell division[48,50]. It is quite likely that the LIS1–dynein interaction also contributes to mitosis and cell-cycle progression. Therefore, additional studies on the role of LIS1–dynein interactions in cortical development are necessary to understand the contribution of dynein motors to neuronal migration.

MINUS END
The end of the microtubule that anchors at the microtubule-organizing centre. It is also the end that assembles more slowly.

A model of LIS1- and DCX-mediated migration

What are the roles of microtubules in migrating neurons and how do LIS1 and DCX fit in? Several experimental observations indicate that neuronal migration might critically depend on nuclear translocation. Using time-lapse video microscopy, Rakic and others showed that neuronal migration starts with an extension of the leading process of the cell, which is followed by translocation of the nucleus and the cytoplasmic content[24,51,52]. Rivas and Hatten[53] observed that in addition to the condensation of actin fibres, the leading process of a migrating neuron undergoes an accordion-like extension and retraction while microtubules form a cage-like web around the cell soma. They suggested that the microtubules of the leading process are highly unstable and the dynamic assembly of microtubules provides a net forward force for migration. The microtubule cage around the soma, which functions to position the nucleus and the longitudinal microtubules in the leading process, might serve to orient the directionality of flowing membranous organelles. Most recently, Nadarajah *et al.*[54] reported two distinct forms of radial movement of cortical neurons — locomotion and somal translocation. Somal translocation might be the predominant or only mode of radial migration at early stages, and is possibly controlled by microtubules and motors. These observations are consistent with the hypothesis that nuclear migration is a key step in cortical neuronal migration, and the role of LIS1 and DCX in neuronal migration might involve the translocation of the nucleus and other soma contents towards the direction of cell movement.

Together, multiple lines of experimental evidence suggest a model for LIS1- and DCX-mediated neuronal migration (FIG. 4). In this model, neuronal migration is driven by microtubules and can be dissected into several steps, with LIS1 and DCX acting at multiple stages of this process. First, neurons receive migration signals and microtubules extend in the leading process. This step might be regulated by DCX and other MAPs, including LIS1, and results in stable microtubule structures to support changes in cell polarity. Second, a regulatory event occurs and modifies LIS1 so that it is recruited to the MTOC and binds to mNudE and/or NUDEL. Third, the interaction of LIS1 with mNudE at the centrosome reduces the nucleation and polymerization of microtubules at the MINUS END. Finally, as a result, microtubules shorten at the minus end, and the nucleus is pulled towards the leading edge of the migrating neuron by microtubule-based motors, such as dyneins. The same molecular mechanisms of LIS1 depicted in this model might also apply to its role in promoting cell division and mitotic progression, where the action of LIS1–mNudE/ NUDEL and dynein complexes on microtubules/mitotic spindles controls the separation of sister chromatids and daughter nuclei.

SURFACE PLASMON RESONANCE An instrumental biosensor or device that detects alterations in the optical evanescent waves resulting from small changes in refractive index at the interface between the sample and the device. The instrument can measure biomolecular interactions in real time and allows the detailed analysis of the resultant signals.

Evidently, our model is speculative at this point, and many critical questions remain unanswered. What is the initiation signal for migration? What regulates the activity and binding of LIS1 to mNudE/NUDEL and the centrosome? How does LIS1 stabilize microtubules in the leading process and destabilize microtubules at the centrosome? How is microtubule shortening achieved by the LIS1–mNudE/NUDEL complex at the centrosome? How do interactions between LIS1 and dynein relate to centrosomal function? While answers to these questions await future investigation, emerging experimental evidence provides some clues.

One interesting possibility is a direct interaction between LIS1 and DCX, which is currently not included in our model. As has recently been described[55], DCX and LIS1 protein expression showed partial overlap in cultured neurons and transfected cells. Direct binding of recombinant DCX and LIS1 protein was detected *in vitro* with SURFACE PLASMON RESONANCE and co-precipitation experiments. Furthermore, specific binding domains on both DCX and LIS1 proteins were assigned through deletion and mutational analyses. Nonetheless, directed and non-directed yeast two-hybrid assays have not revealed direct binding and there is no evidence that the two proteins can be immunoprecipitated together from embryonic brain (Y.F. and C.A.W., unpublished observations), or that they interact *in vivo*. Perhaps a direct interaction is weak or transient but functionally important.

Another critical unanswered question concerns the role of LIS1 phosphorylation. LIS1 was reported to exist as a phosphoprotein[56]. Moreover, LIS1 was found to interact with tyrosine kinase Syk in rat spleen[29]. Syk plays a key role in propagating signals downstream of cell receptors by phosphorylating its substrates. Tubulin is known to be a major *in vivo* substrate of Syk[57], and the centrosome was also found to be a site for Syk phosphorylation in activated B cells[58]. The tight association of LIS1 and Syk suggests that LIS1 might be one of the phosphorylation targets of Syk or a Syk-equivalent tyrosine kinase. At the centrosome/MTOC, the function of mNudE/NUDEL might also be regulated by phosphorylation, since both proteins contain phosphorylation sites for multiple protein kinases, and since the *Xenopus* homologue of mNudE and NUDEL, MP43, was initially identified as a mitosis-specific phosphoprotein[59]. So, both LIS1 and mNudE/NUDEL interact with multiple proteins and both could be regulated by phosphorylation. These features potentially allow their functions to be regulated in many different ways in response to dynamic changes in the migrating neuron.

LIS/DCX and other neuronal migration genes

The Cdk5 kinase, which is also required for neuronal migration, is an attractive candidate for regulating LIS1/DCX phosphorylation and function, but how they relate to other molecules involved in neuronal migration is speculative. Cdk5 is a Ser-Thr kinase that is expressed relatively abundantly in neuronal cells. It associates with a neuronal-specific activator, P35, and has been shown to play a variety of roles in both neuronal development and degeneration. Mutations in the genes encoding both Cdk5 and P35 result in serious neuronal migration defects in mice[60,61]. Cdk5/P35 have been shown to phosphorylate a number of MAPs, including tau[62–64]. Moreover, NUDEL has been shown to be an *in vivo* substrate for Cdk5 and to be regulated by Cdk5 in terms of its subcellular distribution[43], and mNudE is also a potential Cdk5 substrate[41]. It is possible that Cdk5/P35 phosphorylates mNudE, LIS1 or DCX in addition to NUDEL.

Mutations in other mouse and human genes, such as *Reelin*, *Disabled*, the *ApoE2R* and *VLDLR* genes (which encode members of the low-density lipoprotein (LDL) receptor superfamily of endocytic receptors) and *FLN1* have also been found to cause defects in cortical neuronal migration[65–67]. Reelin is an extracellular matrix protein secreted by the earliest-born neurons in brain. Reelin seems to serve as a 'stop' signal for migrating neurons, at least in some contexts, and might regulate integrin-mediated adhesion between neurons and radial glial cells[68]. *Reelin* mutation in the mouse results in reversed neuronal layering in the cortex[69,70], apparently reflecting prolonged attachment of neurons to radial glial cells[71]. In humans, *Reelin* mutations cause lissencephaly, as well as malformations of the cerebellum[72], indicating possible links with other lissencephaly genes.

In the mouse, mutations in the *Disabled* gene, or double mutations in *ApoE2R* and *VLDLR* genes, show identical phenotypes to the *Reelin* mutation[65]. Functionally, the ApoE and VLDL receptors appear to serve as Reelin receptors (or co-receptors), whereas Dab1 functions as a cytoplasmic adaptor in the Reelin signalling pathway[66,73–75]. A potential link between this pathway and the microtubule cytoskeleton has come from observations that *reeler* mutant mice have abnormally high levels of phosphorylation of the MAP, tau[76]. This would imply that the Reelin pathway might ordinarily inhibit the phosphorylation of tau, which might, overall, tend to stabilize microtubules. Cdk5 is one of the major kinases that phosphorylate tau, and is therefore a possible, albeit unproven, intermediary between Reelin and tau. Although there are intriguing hints, at the moment there is no conclusive evidence showing that Cdk5, DCX or LIS1 are downstream targets of the Reelin signalling pathway. It would be interesting to look into the potential effect of this pathway on microtubule dynamics in neurons.

Concluding thoughts

The functions of LIS1 and DCX seem to focus on the stabilization of microtubules, although they might regulate microtubule stability by different mechanisms. Cdk5 might also regulate microtubule structures, although it is a multifunctional kinase that also seems to interact with pathways regulating actin[77]. In fact, actin regulation is certainly essential for neuronal migration, as illustrated by the mutations in the gene encoding actin-binding protein filamin (FLN1) that completely

arrest the migration of some neurons[67]. Therefore, further identification of molecular interactions centred on DCX and LIS1, as well as intensive genetic analysis of neuronal migration disorders in humans and mice, promise a continued dissection of cytoskeletal elements that are crucial to neuronal function.

Links

DATABASE LINKS *LIS1* | *Lis1* | *DCX* | tubulin | tau | MAP2 | MAP4 | MAP1A | MAP1B | DCAMKL1 | PAFAH | Syk | dynein | dynactin | *nud*F | *nud*G | *nud*E | mNudE | NUDEL | MP43 | Cdk5 kinase | P35 | *Reelin* | *Disabled* | *VLDLR* | *FLN1*

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