REVIEW

LIS1 and dynein motor function in neuronal migration and development

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Neuronal migration has been studied extensively for over 30 years in diverse mammalian species from the mouse to human. The sequence of events that occurs during cortical development is shared by all of these species (for reviews, see Gleeson and Walsh 2000; Walsh and Goffinet 2000). At the time of neurogenesis, neural precursors proliferate and differentiate into young postmitotic neurons. These postmitotic immature neurons migrate from the ventricular zone (VZ) to a layer called the preplate at the surface of the developing cerebral cortex. The first migrating neurons split the preplate and form the cortical plate, which develops into the cortex. As migration from the VZ continues, cortical lamination is established in an inside-out fashion. The earliest-born neurons end up deep in the cortex, as later-born neurons migrate past them toward the pial surface to establish more superficial layers of the cortex. The latest-born neurons reside near the pial surface. In the final stages of cortical development, synaptogenesis and apoptotic elimination of populations of neurons occur. Physically, the migration of neurons require the same three steps necessary for migration of any cell: The extension of the leading edge that explores its environment for attractive and repulsive signals; the movement of the nucleus into the leading process, called nucleokinesis; and the retraction of the trailing process.

The understanding of the process of mammalian neuronal migration resulted primarily from neurobiological studies of brain development in normal mammals and mutant mice such as *reeler*. These studies provided important insight into the laminar development of the CNS. However, they did not identify the genetic and developmental pathways that regulate neuronal migration. Cloning of disease genes for human neuronal migration defects and mouse mutants have provided critical entry points into these pathways, whereas genetic and cell biological studies have guided our understanding of the function of these gene products in neuronal migration. For example, cloning of the gene mutated in *reeler* and the identification of its protein product RELN

led to the elucidation of a RELN-dependent signal transduction pathway (for recent review, see Rice and Curran 1999). Other genes have been identified that are responsible for neuronal migration defects in the human (LIS1 and DCX) and mouse (Cdk5 and its required activators p35 and p39), but until recently, the relationship between these genes involved in migration were unknown.

Several recent studies have demonstrated that LIS1, the protein product of a gene mutated in the human neuronal migration defect lissencephaly, binds to and regulates dynein motor function in the cell. These studies place LIS1 in the midst of a well-studied motor complex important for several critical cell functions and perhaps provide a context for integrating several neuronal migration pathways. In this review, we will provide some background on the human and mouse studies that provided the entry points to neuronal migration pathways, the investigation of gene function in model systems, and what these studies tell us about the mechanisms regulating neuronal migration. We will focus on the recent studies of LIS1 function and how they can potentially integrate other known pathways of neuronal migration.

Human lissencephaly and LIS1

Lissencephaly (smooth brain) is a term used to describe the smooth surface of the cortex seen in human brain malformations caused by certain types of neuronal migration defects. Classical or type I lissencephaly defines a subgroup of human neuronal migration disorders characterized by generalized agyria (absence of gyri) and pachygyria (reduced numbers of broadened gyri), four abnormal cortical layers, enlarged ventricles, and generalized neuronal heterotopias (Barkovich et al. 1991; Dobyns and Truwit 1995; Dobyns et al. 1996). Isolated lissencephaly sequence (ILS) is a heterogeneous disorder consisting of variably severe lissencephaly with no other major malformations, whereas Miller-Dieker syndrome (MDS) consists of a generally more severe classical lissencephaly than ILS, characteristic craniofacial anomalies (microcephaly with bitemporal narrowing, a high forehead, a small nose with anteverted nares, thin vermilion border, and micrognathia), and occasionally other malformations (Dobyns et al. 1984). Children with ILS and MDS are severely retarded, have epilepsy, and usu-

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ally die early in childhood (Dobyns et al. 1992). MDS and some cases of ILS are the result of haploinsufficiency at human chromosome 17p13.3. Virtually 100% of the patients with MDS and 40% of patients with ILS have visible or submicroscopic deletions detectable by FISH (Dobyns et al. 1994; Chong et al. 1997).

Human LIS1 mutations are responsible for neuronal migration defects

The *LIS1* gene was cloned from 17p13.3 and was the first gene identified that was associated with neuronal migration defects (Reiner et al. 1993). *LIS1* was disrupted in an ILS patient with a translocation and in several other key MDS patients (Chong et al. 1997). Point mutations and an intragenic deletion in *LIS1* were identified in ILS patients who showed no gross structural chromosomal rearrangements (Lo Nigro et al. 1997; Pilz et al. 1998). These studies demonstrate that *LIS1* is responsible for classical lissencephaly in patients with ILS and contributes to the migration defects seen in patients with MDS. The more severe neuronal migration phenotype and facial dysmorphisms displayed by MDS patients suggest that genes other than *LIS1* are responsible for these phenotypes.

LIS1 consists of seven spaced WD-40 repeats and probably assumes the structure of a "propeller wheel," similar to other WD-40 repeat proteins (Neer et al. 1994; Garcia-Higuera et al. 1996). Bovine and murine *LIS1* are almost identical in amino acid sequence. In mammals,

LIS1 has at least two identified functions (Fig. 1). *LIS1* is a noncatalytic subunit of platelet-activating factor acetylhydrolase (PAFAH) isoform Ib, an inactivating enzyme for platelet-activating factor or PAF (Hattori et al. 1994), so the formal gene name for *LIS1* is *PAFAH1B1*. Only one nonmammalian homolog of the catalytic subunits of PAFAH1B has so far been identified in *Drosophila*, but it does not appear to possess PAFAH catalytic activity (Sheffield et al. 2000), suggesting that the PAFAH1B function of *LIS1* is a mammalian evolutionary adaptation. In addition, *LIS1* has a nonenzymatic function (Fig. 1). It is part of a highly conserved pathway that regulates nuclear migration in fungi (Morris 2000). The *LIS1* homolog in *Aspergillus*, NudF, is part of a signaling pathway that regulates nuclear migration along microtubules via the regulation of dynein motor function. As will be discussed below, this function is conserved in all eukaryotes from fungi to mammals. It is unclear how this function is related to PAFAH1B activity. One hypothesis that could link the enzymatic and nonenzymatic functions of *LIS1* is that the hydrolysis of PAF facilitates the release of *LIS1* from PAFAH1B so it can interact with other molecules involved in migration, such as dynein motors (Fig. 1).

Dosage-dependent neuronal migration defects in Lis1 mutant mice

Although human studies demonstrated that *LIS1* mutations are responsible for lissencephaly, it is not possible

Figure 1. The nuclear distribution pathway first described in *Aspergillus nidulans* (left) is conserved in mammals (center) and *Drosophila* (right). Direct interactions of NudF, LIS1, or DLis1 (red) with other proteins are indicated by a black solid arrow. Genetic interactions are represented by a dashed blue arrow. The directions of arrows represent the presumed direction of information transfer. Dotted lines connect proteins in a complex with no attempt to represent the molecular interactions. Genes discovered in an organism with no function assigned are indicated with a question mark. Mammalian LIS1 is part of a complex for an enzyme that inactivates PAF, PAFAH1B. This function appears to be unique to mammalian LIS1. The clear arrows to the right of LIS1 are meant to raise the possibility that PAF binding and inactivation to lyso-PAF by PAFAH1B may release LIS1 from the complex and may provide an added level of regulation of intracellular LIS1 levels. Homologs are in similar colors and assume similar levels in each organism. See text for details.

to study the neuronal migration defects that occur embryologically during human development. Besides obvious ethical constraints, LIS1 mutations occur de novo in affected children or are the consequence of mild parental mosaicism. To examine the consequences of dosage reduction and complete deficiency of *Lis1* in vivo, we produced two knock-out and one conditional knock-out *Lis1* mutant alleles by gene targeting in the mouse (Hirotsume et al. 1998). The two knock-out alleles are nulls, while the conditional allele is hypomorphic because of the disruption of transcription or splicing by the insertion of PGKneo in intron 3. By mating mice with various *Lis1* alleles, mice were produced with graded reduction in LIS1 dosage. Mice with decreased levels of *Lis1* exhibited dose-dependent disorganized cortical layers, hippocampus, and olfactory bulb because of cell autonomous neuronal migration defects (Hirotsume et al. 1998) and are a good model for the human disorder. Complete loss of LIS1 results in peri-implantation lethality, demonstrating that *Lis1* is an essential gene. Several lines of evidence support the conclusion that there are in vivo migrational defects in mice with reduction of LIS1 dosage (Hirotsume et al. 1998). These include histological analysis during development, BrdU birthdating experiments, and in vitro migration of granule cells from cerebellar cell reaggregates.

Further studies demonstrated impairments of motor function and cognition in *Lis1*-null heterozygotes (Paylor et al. 1999), the types of deficits expected based on the human phenotype. The disruption of hippocampal cellular and synaptic physiology was examined in detail in *Lis1*-deficient mice (Fleck et al. 2000). The hippocampal defects seen in the *Lis1* mutant mice were the result of neuronal migration defects, determined by BrdU birthdating studies. Golgi impregnation studies demonstrated that the dendritic arbor of neurons from both wild-type and *Lis1*+/-KO cells located within stratum pyramidale were not significantly different but that the dendritic arbor of heterotopic pyramidal cells was significantly smaller compared to both cell types, with stunted dendrites that make fewer dendritic branches. Electrophysiological studies demonstrated an enhanced excitability in the *Lis1*+/-KO CA1 pyramidal cell layer, disorganized field potentials in the *Lis1* mutant hippocampus, and hyperexcitability to electric stimulation. Finally, exposing *Lis1* mutant hippocampal slices to conditions of elevated $[K^+]_o$, the so-called "High- K^+ " model of epilepsy, a model for status epilepticus, resulted in increased interictal bursts. These data are consistent with the enhanced excitability and provide a potential explanation for the intractable seizures seen in ILS patients.

Other genes required for neuronal migration

Several other human and mouse proteins that participate in neuronal migration have been identified either by cloning the human and murine genes responsible for migration defects or on the basis of phenotypes displayed in gene-targeted mice. Some of these genes and pathways with particular relevance to LIS1 will be briefly de-

scribed before returning to the functions of LIS1 and what is known about LIS1-dependent pathways.

RELN pathway

The cloning of RELN, the product of the gene mutated in the most well-known and studied spontaneous recessive mouse mutant *reeler* (for reviews, see Caviness and Rakic 1978; Goffinet 1984; D'Arcangelo and Curran 1998; Rice and Curran 1999), subsequently led to the delineation of several members of the RELN signaling pathway. RELN is a large extracellular matrix (ECM)-like secreted protein (D'Arcangelo et al. 1995; Hirotsume et al. 1995) produced by the Cajal-Retzius cells lining the outer layer of the preplate (Ogawa et al. 1995). RELN may provide extracellular cues to guide neurons along a RELN-gradient toward the pial surface (Ogawa et al. 1995; D'Arcangelo et al. 1997). RELN binds at least three types of cell surface receptors: the lipoprotein receptors such as VLDLR and ApoER2 (D'Arcangelo et al. 1999; Hiesberger et al. 1999; Trommsdorff et al. 1999), the cadherin-related neuronal receptors (CNRs; Senzaki et al. 1999), and $\alpha\beta 1$ -integrin (Dulabon et al. 2000). The relative importance and interactions of these RELN receptors are currently unknown, although RELN binding to $\alpha\beta 1$ -integrin is an apparent stop signal for migration (Dulabon et al. 2000). *mDab1* (*mouse disabled-1*), the vertebrate homolog of the *Drosophila disabled* gene (*dab*), transduces the downstream signal of at least some of these RELN receptors. *mDab1*-deficient mice display a *reeler* phenotype (Howell et al. 1997a; Sheldon et al. 1997; Ware et al. 1997). *mDab1* is a phosphoprotein that binds to a number of tyrosine kinases, including the SH2 domain of c-abl (Howell et al. 1997b), as well as the transmembrane receptor Notch (Giniger 1998). It appears that lipoprotein receptor activation may directly activate *mDab1* as part of a RELN dependent pathway (D'Arcangelo et al. 1999; Hiesberger et al. 1999), whereas CNR binding to other tyrosine kinases such as fyn may also activate *mDab1* (Senzaki et al. 1999). Components downstream of *mDab1* in the RELN pathway are not known at this time, but this is an area of active investigation.

Cdk5 and its activators

Homozygous mice with targeted disruption of the serine-threonine kinase *Cdk5* (Oshima et al. 1996) or its regulatory subunit *p35* (Chae et al. 1997) also demonstrate severe defects in cortical lamination. Though these mice display a *reeler*-like phenotype, further analysis of the *Cdk5*^{-/-} mutants has demonstrated a distinct difference from *reeler* (Gilmore et al. 1998). In *reeler*, the preplate is never split by migrating neurons and the cortical neurons remain below it in a disorganized manner. In *Cdk5*^{-/-} mice, the preplate is split by a population of early cortical neurons, but later-born neurons accumulate below this layer. These data suggest that Cdk5 kinase activity is not required for early cortical develop-

ment but is crucial for migration of later-born neurons. The similar phenotype of Cdk5 and RELN pathway mutants suggests that many of these proteins participate in a common signal transduction pathway that is crucial for cortical lamination. A recent study identified Cables, a protein that may link the RELN pathway to Cdk5. Cables is an activator of Cdk5 that binds to Cdk5 in the brain along with the c-abl tyrosine kinase (Zukerberg et al. 2000). Active c-abl phosphorylates and activates Cdk5, and Cdk5 phosphorylation is enhanced by Cables. mDab1 also binds to c-abl and is activated by RELN pathway signals.

Doublecortin

Although *LIS1* was the first identified human gene involved in neuronal migration, others have since been isolated. One of these, *doublecortin* (*DCX*), has particular relevance to the pathways discussed in this review. This gene is responsible for X-linked subcortical band heterotopia (SBH; des Portes et al. 1998; Gleeson et al. 1998). *DCX* has a predicted kinase domain and may be part of an Abl-dependent transduction pathway involved in neuronal migration (Gleeson et al. 1998). Only males harboring *DCX* mutations have ILS, as females are mosaics because of X-inactivation and manifest SBH. Recently, it was reported that mutations and deletions of *LIS1* or *DCX* (also known as *XLIS*) account for 76% of cases of ILS (des Portes et al. 1998; Pilz et al. 1998). Recent studies have shown that *DCX* binds to microtubules (Francis et al. 1999; Gleeson et al. 1999; Horesh et al. 1999). In addition, an interaction between *DCX* and *LIS1* was reported (Caspi et al. 2000).

The nuclear distribution (nud) pathway of *Aspergillus nidulans*

The relationship among any of the proteins or pathways that participate in mammalian neuronal migration was unclear or unknown until recently. Surprisingly, studies of nuclear distribution mutants in the slime mold *Aspergillus nidulans* and other related fungi have helped to clarify *LIS1*-dependent pathways and their function, which in turn led to a more integrated view of neuronal migration pathways.

The biology of nuclear movement has been studied in several fungi including *A. nidulans*, *Saccharomyces cerevisiae*, *Neurospora crassa*, and *Nectria haematococca*. In the filamentous fungus *A. nidulans*, nuclei migrate toward the growing tip of hyphae to establish regular spacing, a process termed nucleokinesis (Oakley and Morris 1980; Xiang and Morris 1999). *A. nidulans* is amenable to genetic manipulation, and several nuclear distribution mutants (*nud* mutants) have been generated to study nucleokinesis in this organism (Morris 1975; Xiang et al. 1999). One of these, *nudF*, is the fungal homolog of *LIS1* (Fig. 1). The *nudF* gene was isolated as a multicopy suppressor of the *nudC* mutant that also complemented the *nudF6* temperature-sensitive mutant

(Xiang et al. 1995). Like the mammalian protein, NudF contains WD-40 repeats and, remarkably, is 42% identical and 64% similar to the human *LIS1* protein. *NudC* is an essential gene in *A. nidulans* (Chiu et al. 1997). *NudC* appears to regulate the level of NudF posttranscriptionally, but the biochemical function of NudC is unknown (Osmani et al. 1990). Homologs have been isolated in a number of eukaryotic organisms.

It has long been established that nuclear movement was a microtubule-dependent process (Oakley and Morris 1980, 1981). However, the identification of other *nud* mutants directly implicated cytoplasmic dynein and dynactin in this process. The *nudA* and *nudG* genes code for the heavy chain and the 8-kD light chain of cytoplasmic dynein, respectively (Xiang et al. 1994; Beckwith et al. 1998). A mutant allele of *nudF*, *nudF6*, can be suppressed by mutations in *nudA*, the cytoplasmic dynein heavy chain, providing genetic evidence that NUDF and NUDF are in the same genetic pathway (Willins et al. 1997) and suggests that *LIS1* might directly interact with cytoplasmic dynein. A large series of *nud* mutants has been generated, leading to the identification of at least 10 new loci involved in nucleokinesis, *nudI* through *nudR* (Xiang et al. 1999). The *nudK* gene codes for the actin-related protein Arp1. This 45-kD protein is part of the dynactin complex, an accessory complex of dynein (Schafer et al. 1994; Schroer 1994). Arp1 is concentrated at the centrosome and, hence, was originally named contractin (Clark and Meyer 1992; Lees-Miller et al. 1992). Dynactin may be a receptor required for dynein-mediated vesicle transport (for review, see Holleran et al. 1998), but the precise mechanism by which dynactin participates in movement of nuclei remains to be determined.

A screen for multicopy suppressors of the *nudF* phenotype identified *nudE* (Efimov and Morris 2000). NudE is a homolog of the nuclear distribution protein RO11 in *Neurospora crassa* (Minke et al. 1999), the mitotic protein MP43 in *Xenopus laevis* (Stukenberg et al. 1997), and two murine homologs of nudE named NUDEL (for NudE-like) and NudE (see below). The double mutants *nudF;nudE* and *nudE;nudA* were similar to the *nudF* and *nudA* (cytoplasmic dynein heavy chain) single mutants, indicating that NudF, NudE, and NudA are part of the same genetic pathway (Fig. 1). NudE physically interacts with NudF but not with *LIS1*. However, chimeric NudE proteins containing the conserved coiled-coil amino terminal domains of human or *Xenopus* MP43 did physically interact with mammalian *LIS1*.

Thus, several genes responsible for nuclear movement have been isolated from *Aspergillus*. The nud pathway clearly involves the fungal *LIS1* homolog, NudE, cytoplasmic dynein, and dynactin. The nud pathway is remarkably conserved in eukaryotes (Fig. 1). *NudF* is the *LIS1* homolog in *A. nidulans*. Cytoplasmic dynein and dynactin are conserved throughout eukaryotic evolution. Homologs have been cloned in *Drosophila* and several mammalian species.

The process of nuclear migration in fungi is strikingly similar to neuronal migration in mammals. Migrating

neurons initially extend leading process along radial glial fibers. After completion of this process, the nucleus of the migrating neurons must relocate their position from their birthplace to their final destination in the nervous system. LIS1 is required for mammalian neuronal migration in a dosage-sensitive manner (Hirotune et al. 1998) and for nuclear migration in *A. nidulans*. Nuclear distribution in *A. nidulans* is also similar to nuclear movement and cellular transport in *Drosophila*. Therefore, it is not surprising that the same molecular mechanism utilizing NUDEL, NudE, LIS1, and cytoplasmic dynein motors may underlie these processes in all eukaryotes.

Drosophila LIS1 (DLis1) mutants and function

In *Drosophila melanogaster*, nuclear movement and cellular transport are dependent on microtubules, cytoplasmic dynein, and dynactin. The *Drosophila LIS1* homolog, *DLis1* (Fig. 1), encodes a protein that is 70% identical to LIS1 and 41% to NudF (Liu et al. 1999). *DLis1* is highly expressed in the germarium and the developing oocyte. Homozygous *DLis1* mutant embryos hatched normally but soon experienced growth retardation and died, demonstrating a requirement for *DLis1* early in development similar to the mouse (Hirotune et al. 1998). In the normal *Drosophila* egg chamber, there is a germline stem cell that divides asymmetrically to produce 15 nurse cells and one oocyte. In *DLis1* germ-line mutant clones or cysts, an oocyte rarely develops, and there is a reduction in the number of nurse cells. In another study, the *DLis1* gene was isolated in an enhancer screen for oocyte determination and nuclear positioning (Swan et al. 1999), as well as for nurse-cell-to-oocyte transport. This is a phenotype similar to that of the *Dhc64C* cytoplasmic dynein heavy-chain loss-of-function mutant (McGrail and Hays 1997). The pupal lethal *DLis1* homozygous flies demonstrate nuclear migration defects in developing third-instar imaginal discs. A similar phenotype was seen in dynactin *Glued* subunit mutants (Fan and Ready 1997). Furthermore, *Drosophila* dynein physically interacts with the p150 subunit of dynactin encoded by *Glued* (McGrail et al. 1995), suggesting that *DLis1*, cytoplasmic dynein, and dynactin are associated. In support of this hypothesis, the *Dhc⁶⁻⁶* gain-of-function allele of cytoplasmic dynein heavy chain dominantly suppressed the *DLis1* homozygous phenotype in developing egg chambers, resulting in fertility, proper nuclear positioning, and nearly normal oocyte growth. These results are similar to those in *A. nidulans* discussed above.

Because of the early embryonic lethality of flies completely deficient for *DLis1*, it was impossible to address the function of this protein in somatic cells. *DLis1* function was further analyzed by completely eliminating *DLis1* in somatic clones of the *Drosophila* nervous system using mosaic analysis with a repressible cell marker (MARCM) technology (Lee and Luo 1999). These studies support a genetic interaction between *DLis1* and cytoplasmic dynein heavy chain in neuroblast proliferation, dendritic growth, and axonal transport (Liu et al. 2000). *DLis1* is highly expressed in the developing fly brain and

ventral nerve cord, so the analysis was focused on the developing mushroom bodies in the *Drosophila* central nervous system. Mutant *DLis1* mushroom bodies exhibited neuroblast proliferation defects and reduced dendritic arborization, similar to the dendritic arborization defects seen in the mouse. Similar phenotypes were observed in *Dhc64C* (cytoplasmic dynein heavy chain) loss-of-function MARCM clones, supporting the genetic interaction between *DLis1* and cytoplasmic dynein initially suggested by egg cell chamber phenotypes (see below). *DLis1* mutant neurons developed axonal swellings that suggest a defect in axonal transport, and similar swellings were observed in the *Dhc64C* MARCM clones. These elegant genetic experiments demonstrate a role for *DLis1* in neuroblast proliferation, dendritic elaboration, and axonal transport in *Drosophila*. The importance of the microtubule cytoskeleton in these processes and the similarities between the *DLis1* and *Dhc64C* mutant MARCM clones further supports a mechanistic association among *DLis1*, microtubules, cytoplasmic dynein, and dynactin.

Other experiments in *Drosophila* suggest that *DLis1* plays a role in regulating dynein localization (Swan et al. 1999). In stage 5 of oogenesis, *DLis1* is concentrated along the cortex of wild-type oocytes. This localization pattern is unchanged in dynein mutants. However, later in development, the oocyte-specific localization of dynein is abolished in egg chambers of the *DLis1* mutant, demonstrating that *DLis1* is important for the proper localization of dynein in the developing egg chamber. LIS1 binds the pleckstrin homology domain of β -spectrin, a membrane protein (Wang et al. 1995). In *DLis1* mutant egg cell chambers, there are aberrantly formed fusomes or cytoskeletal branched structures composed of membrane skeletal proteins α spectrin, β spectrin, ankyrin, and Hu-li tai shao (Hts; Lin et al. 1994; de Cuevas et al. 1996). Mutations in Hts or α spectrin eliminate fusomes and cause egg chambers with <16 cells, often with a missing oocyte (Yue and Spradling 1992; de Cuevas et al. 1996). The similarity of this and *DLis1* phenotypes suggest a possible interaction between *DLis1* and membrane cytoskeletal proteins and may explain the defects seen in the egg chambers of *DLis1* mutants.

These studies in *Drosophila* demonstrate several levels of interaction between *DLis1*, cytoplasmic dynein, and dynactin in various cellular processes, including nuclear movement in photoreceptor cells, oocyte development, neuroblast proliferation, dendritic growth, and axonal transport.

LIS1 regulates dynein motor function in mammals

The regulation of dynein motor function by LIS1 and microtubule organization is conserved in mammalian cells. It was shown previously that LIS1 associates with the microtubule fraction of cell supernatants (Sapir et al. 1997). Consistent with these findings, Smith et al. (2000) found that LIS1 is present in regions of high microtubule density in fibroblasts, especially at the centrosome and microtubule organizing center (MTOC). LIS1 colocalized

with the dynein intermediate chain (IC) and the dynactin subunit p150^{glued} in the ventricular zone and the cortical plate during brain development, and these three proteins were present in a complex in the brain. LIS1 was ~15× more abundant in neurons than Cos-7 cells. Overexpression of LIS1 in COS-7 cells resulted in an increase in LIS1 association with p150^{glued} and DIC and increased the signal intensity of LIS1 at the MTOC as well as the intensity of p150^{glued} and DIC, suggesting that LIS1 may regulate the distribution of these molecules by increasing retrograde dynein transport. Overexpression of LIS1 also resulted in the peripheral redistribution of microtubules and tighter packing of the Golgi complex around the nucleus. In contrast, reduction of LIS1 in *Lis1* +/KO cells resulted in a dispersal of p150^{glued} throughout the cell, enrichment of microtubules near the nucleus, and looser packing of the Golgi complex. Each of these processes is dependent on cytoplasmic dynein motor function and provides strong evidence that LIS1 dosage regulates dynein motor function in fibroblasts. It appears that high expression of LIS1 stimulates the plus-end-directed peripheral movement of microtubule segments by stationary cytoplasmic dynein–dynactin complexes (perhaps located at the cell cortex) as well as the retrograde transport toward the MTOC and centrosome of organelles such as the Golgi complex by mobile dynein–dynactin motors. Reduced levels of LIS1 result in the opposite phenotype.

LIS1 interaction with cytoplasmic dynein and dynactin appears to be important for mitotic cell division and cytokinesis (Faulkner et al. 2000). LIS1 was coimmunoprecipitated from bovine brain cytosol with antibodies to cytoplasmic dynein heavy chain and the dynein intermediate chain, as well as the p150^{glued}, p62, and Arp1 subunits of dynactin. LIS1 was localized at mitotic spindle poles, cell cortex, and kinetochores in cells, and it may have a function during mitosis. Perturbation of LIS1 levels in cultured cells disrupted mitotic progression, mitotic-spindle orientation, and chromosome attachment. Overexpression of LIS1 in MDCK cells increased the number of cells in mitosis and prometaphase, similar to overexpression of dynamitin, a subunit of dynactin. These LIS1-overexpressing cells contained multipolar spindles and unaligned chromosomes, which indicates a defect at the kinetochore. p150^{glued} staining was abolished from microtubule plus ends, and dynactin localization was disrupted. Dynactin was found in clumps at the cell cortex along with perturbed astral microtubules. Reducing LIS1 levels with an antisense construct resulted in the accumulation of cells in mitosis and chromosomal abnormalities. NRK cells injected with an anti-LIS1 antibody displayed delays in mitotic progression, delayed and unstable chromosome alignment, unattached or mono-oriented chromosomes, and anaphase delay.

These results suggest that LIS1 plays a critical role in cell division and chromosome segregation. They imply that defects seen in human lissencephaly caused by haploinsufficiency of LIS1 may be the result of altered neurogenesis in the proliferative ventricular zone as well as defective migration of neurons into the cortical plate. In

support of this, LIS1 dosage reduction in mice is associated with progressive thinning of the cortex, implying that LIS1 may be important for neuronal proliferation (Hirotsune et al. 1998). In addition, proliferation of cells in the ventricular zone of murine *Lis1* mutants is disrupted, and embryonic fibroblasts from *Lis1* mutants have slow doubling times (M.J. Gambello and A. Wynshaw-Boris, unpubl.).

Cloning of mammalian *Nude* homologs

The experiments discussed above provide strong evidence for a functional relationship between LIS1, cytoplasmic dynein, and dynactin in mammalian cells as well as fungi and flies. However, the nature of the physical interactions was unknown until recently. Several groups reported the cloning of mammalian *Nude* homologs from yeast two-hybrid screens using LIS1 as bait, and some of these studies provided a direct link between LIS1 and dynein motors (Feng et al. 2000; Kitagawa et al. 2000; Niethammer et al. 2000; Sasaki et al. 2000). There are at least two mammalian *Nude* homologs: mNude and NUDEL. Both are coiled-coil proteins of 344–345 amino acids and contain 10% serines. Many of these serines are consensus phosphorylation sites for serine-threonine protein kinases, including Cdk5 (see below), ATM, casein kinase II, and PKCs. As noted above, mNude and NUDEL proteins display a high degree of homology to each other (55% overall identity and 85% identity in the coiled-coil domain), to nude from *A. nidulans* (Efimov and Morris 2000), to the nuclear distribution protein RO11 in *Neurospora crassa* (Minke et al. 1999), and to the mitotic protein MP43 in *Xenopus laevis* (Stukenberg et al. 1997). The NUDEL protein is heterogeneous, with multiple bands between 42–45 kD detected in the brain (Niethammer et al. 2000; Sasaki et al. 2000), suggesting that it can be phosphorylated (see below), but mNude was detected as only a single 40-kD band in brain (Feng et al. 2000). LIS1 was immunoprecipitated from brain extracts with either anti-NUDEL or anti-mNude antibodies, demonstrating a strong in vivo interaction between LIS1 and the two *Nude* homologs.

mNude and NUDEL each bind to themselves and to LIS1 via the coiled-coil region (Feng et al. 2000; Niethammer et al. 2000; Sasaki et al. 2000), suggesting that hetero- and homodimerization can occur. The binding sites for LIS1 and mNude are distinct in the coiled-coil region (Y. Feng and C. Walsh, pers. comm.). LIS1 binding sites for other proteins could not be precisely determined, as only proteins with small truncations from the N terminus displayed any binding activity. Nearly the entire region of LIS1 consists of the WD repeat domain except for the N-terminal region, so the integrity of this domain may be crucial for normal LIS1 function or protein stability. Point mutations in LIS1 corresponding to those detected in human patients with lissencephaly (LoNigro et al. 1997) severely affected the interaction of LIS1 with itself, NUDEL, and mNude (Feng et al. 2000; Sasaki et al. 2000), consistent with the functional importance of these interactions.

There is some overlap in the embryonic patterns of expression of NUDEL and mNudE, but these two genes and proteins also have distinct spatial and temporal patterns of expression, suggesting that they may have distinct functions. NUDEL is expressed in increasing levels from the early embryo to birth and in the adult, especially in the brain (Niethammer et al. 2000; Sasaki et al. 2000). However, mNudE expression is high in the embryo but low after birth in most tissues including the brain (Feng et al. 2000). NUDEL and mNudE proteins colocalize with LIS1 at the centrosome or MTOC in mitotically dividing embryonic neuroblasts or fibroblasts (Feng et al. 2000; Niethammer et al. 2000; Sasaki et al. 2000). Thus, in mitotically dividing cells, LIS1 colocalizes with mNudE and NUDEL.

Once neurons mature, the MTOC undergoes morphological changes and participates in axonal projections and dendrite formation (Bass 1999). As neurons develop in vivo or in vitro, the distribution pattern of NUDEL and LIS1 together began to shift from the MTOC to the axon, and in the adult cortex, it was specifically localized to the axon (Sasaki et al. 2000). In primary neuronal cultures, the initial centrosomal distribution of LIS1 and NUDEL was lost, and these proteins localized to the axon, growth plate, and soma (Sasaki et al. 2000). Elegant studies using deconvolution microscopy using primary neuronal cultures demonstrated that NUDEL colocalized with the dynein IC near the cell body, while NUDEL colocalized with p150^{glued} in the growth cones (Niethammer et al. 2000). Similar experiments have not been done with mNudE.

In the adult, NUDEL and LIS1 are associated with dynein motors during retrograde transport (Sasaki et al. 2000). After ligation of the sciatic nerve, axonal proteins associating with a motor protein that moves anterogradely or retrogradely will accumulate at the proximal or proximal and distal region to the ligation, respectively (Okada et al. 1995; Hanlon et al. 1997). Six hours after ligation, LIS1, NUDEL, and the dynein IC (a retrograde motor protein) accumulated at both sides of the ligation. KIF5C (an anterograde motor protein) accumulated only at the proximal side of the ligation, as expected. These data support the interpretation that NUDEL and LIS1 are associated with the minus-end directed dynein motor during retrograde transport. It is unknown if mNudE is associated with motor proteins involved in axonal transport.

NUDEL and LIS1 regulate the function of cytoplasmic dynein motors

The cell biological experiments described above demonstrated that LIS1 and NUDEL were associated with dynein motor proteins in cells but did not prove a physical interaction. Biochemical studies were performed to confirm these studies in neurons. A taxol-treated microtubule fraction that contained the dynein IC from brain contained a significant fraction of tightly associated LIS1 and NUDEL (Sasaki et al. 2000). All three proteins were released by Mg-ATP treatment, which releases dynein

motor proteins, suggesting that LIS1 and NUDEL are tightly associated with dyneins. Similarly, LIS1 and NUDEL were contained in insoluble fractions enriched for membrane and cytoskeletal proteins (Niethammer et al. 2000), where Cdk5 and its activator p35 were also found (see below).

A direct interaction between both NUDEL and LIS1 with the CDHC was demonstrated by directed yeast two-hybrid assays and coimmunoprecipitation of relevant proteins expressed by transfection (Sasaki et al. 2000). NUDEL and LIS1 interact with P1-loop domain of CDHC (a region containing the ATP binding site), while only NUDEL interacted with the C-terminal domain of CDHC. Curiously, a distinct region outside of the coiled-coil domain of NUDEL (the region known to be important for self-association and interaction with LIS1) was found to be critical for binding the P1-loop domain and C-terminal domain of CDHC. LIS1-binding sites for NUDEL or the CDHC proteins have not been determined precisely. Thus, LIS1 and NUDEL bind directly and specifically with CDHC and provide a direct biochemical link between LIS1 and cytoplasmic dynein motors.

Niethammer et al. (2000) demonstrated that NUDEL distribution was affected by LIS1 dosage in fibroblasts. Overexpressing LIS1 resulted in tighter perinuclear distribution of NUDEL, while *Lis1* +/KO fibroblasts displayed a more diffuse localization of NUDEL. Similarly, Sasaki et al. (2000) showed that NUDEL distribution was affected in embryonic fibroblasts from mice with progressively reduced levels of LIS1 from 50% to 35% of wild-type levels. However, the opposite effect on NUDEL distribution was observed: As LIS1 dosage decreased, NUDEL became more tightly associated around the nucleus, as did several proteins associated with CDHC function such as LIS1 itself and β -tubulin. Similar to previous studies (Smith et al. 2000), reduction of LIS1 resulted in a more diffuse pattern of β -COP localization, a Golgi complex-associated protein. The reasons for these discrepancies are unclear. It is possible that cells were at different passages or grown under different conditions. In addition, two different antibodies to NUDEL were used. Although these details remain to be resolved, overall, these results suggest that LIS1 functions to localize NUDEL and CDHC (with associated proteins) to regulate dynein motor function and organelle transport in the cell.

If NUDEL also is important for localizing CDHC, then interference with NUDEL function by transfecting cells with constructs expressing full-length or truncated NUDEL proteins should result in a similar mislocalization pattern of dynein motors in the cell. COS cell expression of a C-terminal NUDEL fragment (257–345 amino acids [aa]) that binds to the P1-loop and C terminus of the CDHC resulted in a similar perinuclear redistribution of CDHC as observed in cells with reduced levels of LIS1 (Sasaki et al. 2000). Full-length NUDEL or an N-terminal NUDEL (1–256 aa) fragment that binds to NUDEL and LIS1 did not affect CDHC distribution. Although far from conclusive, these results suggest that

the binding of NUDEL to CDHC regulates its localization and its motor function. Of interest, overexpressed NUDEL colocalized with dynein IC at the cell periphery, while overexpressed LIS1 did not (Niethammer et al. 2000), suggesting that LIS1 and NUDEL may have distinct functions in the regulation of dynein motors. Overall, these results are consistent with the hypothesis that LIS1 and NUDEL are responsible for the localization of CDHC (with associated proteins) and regulate dynein motor function and organelle transport in the cell. Further expression studies, mouse knock-outs, and perhaps even *in vitro* dynein motor assays will be required to definitively understand the role of LIS1 and NUDEL in regulating dynein motor function.

mNudE interacts with dynein LC and functions at the centrosome

Similar to NUDEL, mNudE is localized to the centrosome. LIS1 and NUDEL together bind to CDHC, and this interaction presumably places these proteins with the CDHC at the centrosome. In contrast, it appears that mNudE alone binds to γ -tubulin and several other centrosomal proteins (Feng et al. 2000), as mNudE, but not LIS1, can be immunoprecipitated with antibodies to γ -tubulin. mNudE also interacts in yeast two-hybrid assays with the centrosomal proteins pericentrin, mitotin/CENP-F, TCP-1, CEP110 and SLAP and the dynein LC, suggesting that mNudE is a central component of the centrosome.

To determine an *in vivo* function for mNudE, the LIS1-binding domain (aa 88–156) of mNudE was injected into the right blastomere of *Xenopus* embryos at the two- to four-cell stage (Feng et al. 2000). Embryos injected with mRNA for this truncated mNudE displayed smaller anterior structures and smaller eyes on the injected side of the embryo. The retinas were smaller, and lamination was disrupted. The forebrain and midbrain on the injected side were thickened and disorganized, and abnormal optic tract projections into the tectum were observed. These results suggest that mNudE, through interactions with LIS1, plays an important role in migration or lamination of the central nervous system. As noted above, the binding sites for LIS1 and mNudE are distinct in the coiled-coil region (Y. Feng and C. Walsh, pers. comm.). It should be noted, however, that the region of mNudE used in these experiments is similar to the coiled-coil region of NUDEL, so this fragment of mNudE has the potential to disrupt interactions of mNudE, NUDEL, and any other mammalian NudE homolog with LIS1. Nonetheless, this experiment provides the best evidence that mammalian NudE homologs are involved in the development of the brain.

Overexpression of GFP-mNudE in COS cells resulted in disruption of the centrosomal-based microtubule network (Feng et al. 2000). γ -tubulin dissociated from the centrosome and displayed a disorganized cytosolic pattern. Disordered microtubules appeared to radiate from aggregates of ectopic GFP-mNudE protein and seemed to recruit γ -tubulin to these aggregates. This cellular phe-

notype is clearly distinct from the effect of overexpression of NUDEL in COS cells, which resulted in a redistribution of dynein motor components. These differences may be the result of distinct functions of NUDEL and mNudE or of differences in levels of overexpression. These issues can be directly addressed when similar NUDEL and mNudE expression constructs are introduced in the same cells and by mouse knock-outs.

NUDEL is a substrate of Cdk5

The mitotic phosphoprotein 43 (mp43), an ortholog of NUDEL in *Xenopus*, was originally identified as a protein recognized by the phospho-epitope antibody MPM-2 (Stukenberg et al. 1997). mp43 is phosphorylated in a cell cycle-dependent fashion and is a potential substrate of Cdk serine/threonine kinases. In addition, the NUDEL protein extracted from the brain consists of multiple species that could be reduced to a single one by phosphatase treatment, consistent with posttranslational modification by phosphorylation (Niethammer et al. 2000; Sasaki et al. 2000). NUDEL and Cdk5 were enriched in similar brain fractions and colocalized in the cell bodies and growth cones of embryonic cortical neurons (Niethammer et al. 2000). These findings suggested the intriguing possibility that NUDEL may be a substrate of the Cdk5/p35 and that NUDEL could provide a link between two neuronal migration pathways not previously known to intersect.

NUDEL is a bona fide substrate for Cdk5/p35 *in vitro* (Niethammer et al. 2000; Sasaki et al. 2000) and *in vivo* (Niethammer et al. 2000). The latter studies were particularly convincing. First, the slower-migrating NUDEL species detected in brain extract were reduced but not eliminated in Cdk5 single- or p35/p39 double-mutant mice. Cdk5 activity is not detectable in either of these mutants, suggesting that NUDEL is phosphorylated by this kinase complex as well as others. Second, cotransfection of each of five possible consensus serine/threonine sites' mutants (S198A, T219A, S231A, S242A, and T245A) with Cdk5/p35 into COS cells identified the first three sites (123-NUDEL) as substrates for the enzyme complex, and a triple mutant of these sites was not detectably phosphorylated. Third, embryonic cortical neurons transfected with the 123-NUDEL mutant or treated with roscovitine, a Cdk5 inhibitor, induced neuritic swellings or varicosities that contained dynein IC. As noted above, such defects have also been noted in DLis1 deficient neurons in *Drosophila* (Liu et al. 2000). Fourth, roscovitine decreased endogenous NUDEL staining at the centrosome/MTOC. Finally, NUDEL staining was more punctate in neurons expressing a dominant negative Cdk5 mutant. These data suggest that Cdk5 phosphorylation of NUDEL controls its cellular localization and probably influences dynein motor function.

These same serine residues are conserved in mNudE, suggesting that mNudE may be a target of Cdk5 as well. Unlike NUDEL, there is no evidence that mNudE is present as multiple bands in the brain. However, direct

in vitro and in vivo experiments can address whether or not mNudE is a substrate for the Cdk5 complex.

LIS1 regulation of dynein motor function and localization

The recent studies outlined in this review clearly demonstrate that LIS1 regulates dynein motor function and localization of dynein-associated proteins. Some of the proteins that participate with LIS1 have been identified, and others can be inferred based on the known proteins in complex with cytoplasmic dynein or from genetic studies in a number of eukaryotic organisms. Most notably, LIS1 binds two different NudE homologs to regulate dynein motor function and centrosomal localization. At least one of these homologs, NUDEL, is directly phosphorylated by Cdk5 and its coactivators, p35 and p39. It appears that each of these proteins regulates dynein motor function in a positive fashion, as decreasing the activity, decreasing the amount, or eliminating the protein completely results in decreased dynein motor function and mislocalization of dynein components and centrosomal proteins. Thus, the apparent function of the LIS1 pathway is to activate dynein motors and place them in the proper cellular location for function.

A unified nuclear migration pathway?

On the basis of the cloning of genes that are mutated in human and murine neuronal migration disorders and the understanding of the function of these gene products, there appear to be at least four genetic and biochemical pathways that regulate neuronal migration: first, the RELN pathway; second, the Cdk5 pathway; third, the LIS1 pathway; and fourth, the DCX pathway (Fig. 2). As noted throughout this review, several recent studies tie together what appeared to be distinct pathways to ultimately regulate dynein motor function via LIS1. Cdk5 phosphorylation of NUDEL (and perhaps mNudE) regulates its localization and may regulate the activity of these NudE homologs. These findings intimately link the Cdk5 and LIS1 pathways of neuronal migration. In fact, it is possible that NUDEL (and mNudE) are the major substrates for Cdk5 with respect to neuronal migration. Cables activates Cdk5 through interaction with c-abl and mDab1, which could link the RELN and Cdk5 pathways. Like LIS1, DCX is a microtubule binding protein, and one report describes an interaction between LIS1 and DCX (Caspi et al. 2000).

A plausible model based on these studies can be proposed to unify all of these pathways into a single pathway that regulates nucleokinesis during neuronal migration.

Figure 2. Integration of pathways that regulate nucleokinesis during neuronal migration. The RELN pathway is to the right, Cdk5/p35 in the middle, and the LIS1 pathway is on the left. Doublecortin (DCX) is placed close to the LIS1 pathway based on its interaction with LIS1 and its role in microtubule dynamics. See text for details.

tion through the regulation of dynein motor function (Fig. 2). RELN binds to the VLDLR/ApoER2 complex to activate mDab1. Activated mDab1 associates with c-abl and activates Cables, which in turn activates the phosphorylation of Cdk5 by c-abl. Activated Cdk5 then phosphorylates NUDEL (and perhaps mNudE). A LIS1–NudE homolog complex then positively regulates dynein motor function through direct association with CDHC and dynein LC. DCX may modulate this process via its association with microtubules and LIS1. On the basis of the conserved pathway in *A. nidulans*, it is likely that dynein activation will lead at least to the migration of the nucleus into the leading edge during neuronal migration. However, the localization of many of the components of these pathways in the axons and growth cones of neurons also suggests a role for this pathway in leading edge extension.

This model is undoubtedly an oversimplification of what is likely to be a process that is regulated in a highly ordered and complicated fashion. There may be many branch points to and from this basic backbone. For example, it is known that RELN binding to $\alpha 3 \beta 1$ -integrin apparently regulates adhesiveness of neurons and provides a stop signal for migration. It is also clear that regulation of the actin cytoskeleton is important for all forms of cell migration, including neuronal migration. However, the relationship among these diverse proteins into a single pathway regulating nucleokinesis during mammalian neuronal migration provides a simple framework for future studies. With the ability to make mice that are mutated in genes corresponding to critical proteins, as well as the current availability of mutants for a number of the proteins in this pathway, the questions can be most directly addressed in the mouse. The use of the mouse will allow examination of genetic interactions in vivo and provide critical cells for in vitro studies. A number of mouse mutants still need to be made or are in the process of being made, including knock-outs and conditional knock-outs for *Dcx*, *Nudel*, *mNudE*, *Cables*, the *Pafah1b* catalytic subunits, and conditional mutants for at least some of the dynein subunits. It will also be important to continue to search for proteins that interact with any component of these pathways.

With these tools and information in hand, the outstanding questions that remain can be addressed to understand the mechanisms and processes used to move the nucleus and to extend the leading edge of a migrating neuron. These questions can be addressed by the genetic, neurobiological, and cell biological approaches that have provided us with our current understanding of neuronal migration. What is the precise molecular mechanism of action of LIS1, NUDEL, and mNudE in the regulation and localization of dynein motor function? What is the precise effect of phosphorylation of NUDEL (and perhaps mNudE) on dynein motor function? Does the Cdk5/NUDEL/LIS1 pathway also regulate proliferation, survival, and axonal transport? Are there adult functions of mNudE? Is there a role for PFAH1B in regulating LIS1 function in neuronal migration and dynein motor func-

tion? How does DCX influence neuronal migration, and does it act via LIS1? Do other kinases than c-abl activate Cdk5? Do other kinases phosphorylate and regulate NUDEL and mNudE function? Does Cdk5 phosphorylate other proteins involved in neuronal migration? Is Cables the only link between Cdk5 and the RELN pathway? Does the RELN pathway activate migration pathways distinct from Cdk5? The answers to these questions will expand our current understanding of the pathways that regulate neuronal migration in mammalian development. This understanding is still in its rudimentary stage, but the convergence of these recent studies provide important connections that can now be experimentally exploited.

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