**Introduction**

This review will focus on the developmental and cellular activities of the lissencephaly type 1 gene product, LIS1.

**Human LIS1 associated diseases, lessons from human genetics**

Twenty years ago LIS1 (Lissencephaly 1) (officially known as PAFAH1B1) was the first gene to be identified as involved in a neuronal migration disorder [1†] (recent review [2]). The lissencephaly-pachygyria spectrum of diseases defines a variety of brain malformations that cause relative smoothness of the brain surface and includes lissencephaly (smooth brain surface), agyria (no gyri) and pachygyria (broad gyri) (Figure 1a). These brain malformations, which reflect the severe consequences of LIS1 hemizygote deletions of human chromosome 17p13.3, are partially due to impairment in the migration of neurons in the developing brain. Instead of the normal six-layered cortex, only four layers can be observed (Figure 1b) [3]. The position of neurons within defined layers of the cerebral cortex depends upon their birth-date and their proper movement from their place of birth to their accurate placement. The progenitors of the radially migrating cortical neurons residing in the ventricular zone (VZ) and the subventricular zone (sVZ) fall into three major categories: radial (or apical) progenitors, intermediate (or basal) progenitors and outer radial glia progenitors. Following the formation of the first layer, neurons that are born in the VZ and sVZ are layered in an inside-out manner, with later born neurons positioned above earlier born neurons [4,5]. In patients with deletions in LIS1 the mid-hindbrain appears normal in MRI scans [6†]. Deletions of additional genes adjacent to LIS1, including MYO1C, CRK, YWHAE and TUSC5, result in Miller-Dieker lissencephaly syndrome, which is manifested in a more severe brain malformation as well as typical facial dysmorphology (Figure 1c). Somatic mutations, affecting only a portion of the brain cells may result in mislocalization of some of the neurons in the brain [7]. When a relatively high proportion of cells are affected a band of misplaced neurons can be observed, also known as subcortical band heterotopia, or double-cortex (Figure 1a). Proper brain development is very sensitive to LIS1 dosage. As mentioned above, half dosage results in lissencephaly, whereas increased dosage delays development and may result in cognitive impairment [8]. A partial duplication of LIS1 resulted in a different phenotype; microcephaly, neurodevelopmental delays, and profound white matter atrophy in the absence of overt lissencephaly [9].

**LIS1 studies in mouse models**

Studies conducted in the mouse and rat revealed the importance of LIS1 in cell proliferation and neuronal migration. LIS1 affects cell proliferation in the developing brain at multiple stages [8,10–16]. Neuronal progenitors, knocked down for LIS1 failed to proliferate [15]. In addition, mosaic analysis demonstrated the requirement of LIS1 for the proliferation of all neuronal lineages and astrocytes [17]. Interkinetic motility, the cell cycle dependent nuclear oscillation within the apical cortical progenitors, is sensitive to LIS1 levels as well. Abnormal interkinetic motility was observed upon LIS1 knockdown, Lis1 knockout, as well as in brains with increased dosage of LIS1 [8,11,15]. LIS1 was found to be essential for precise control of mitotic spindle orientation in both early neuroepithelial stem cells and radial glial progenitor cells [18]. LIS1 genetically interacts with Nde1 in proliferating cells in the ventricular zone; mice with an allelic series of Lis1 and Nde1 double mutations displayed a striking dose-dependent size reduction and delamination of the cerebral cortex [19].
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Figure 1

![Diagram showing Normal Cortex, Subcortical band heterotopia, and Lissencephaly type I](image)

Lissencephaly type 1 associated diseases. (a) Schemes of brain MRI of normal, subcortical band heterotopia (double cortex) and lissencephalic brain. The ventricles are in black, the white matter in dark grey and the grey matter in light grey. In the lissencephalic brain note the marked paucity of sulci/gyri, thickening of the cerebral cortex and the loss of underlying white matter. In the doublecortex brain note the areas where ectopic neurons accumulate. (b) Presentation of a normal six-layered cortex (left) and a typical four-layered cortex in case of lissencephaly type Layer 1 is the outermost molecular layer contains Cajal-Retzius neurons. Layer 2 consists of pyramidal neurons with some disorganization. Layer 3 exhibits lower densities of neurons with myelinated axons. Layer 4 is a broad band of disorganized neurons of variable thickness. (c) Human chromosome 17 showing the relative genomic locations of LIS1 (PAFAH1B1), MYO1C, CRK, YWHAE and TUSC5.

Multiple lines of evidence link LIS1 to regulation of neuronal migration in the developing brain. Abnormal radial migration was noted in a mouse carrying a hypomorphic allele of Lis1 (Lis1/Lis1) [20], in Lis1−/+ [21], as well as with further reduction of the LIS1 dosage using a floxed allele [21], or by knockdown of the gene’s transcripts using in utero electroporation [15,22,23]. In migrating neurons with reduced LIS1 levels, the centrosome and the nucleus were less tightly coupled [22,24]. Lis1 shRNA inhibited somal movement but not process extension in the migrating cells [23]. LIS1 also affects the tangential migration of inhibitory neurons from the ganglionic eminence to the cortex. It was demonstrated that proper LIS1 levels are required for normal tangential migration in Lis1−/+ [25] as well as in a mouse model with increased LIS1 dosage [8].

**LIS1 structure and cellular functions**

Why is the LIS1 locus so dosage sensitive? LIS1 participates in multiple protein-protein interactions, thus the relative levels of this protein are key to multiple cellular activities (review [26]). The N-terminal part of LIS1 is composed of a LisH domain and a coiled coil domain, which are important for protein dimerization [27–29]. These are followed by seven WD repeats, which serve as an interaction platform with several proteins [30] (Figure 2). Biochemical analysis revealed that the interaction between LIS1 and the catalytic subunits of...
Schematic presentation of cytoplasmic dynein, LIS1, Nde1 interactions and movements along axonal microtubules. Top, schematic presentation of dynein (left), a six AAA containing molecular motor, LIS1 (middle), a seven WD repeat protein, Nde1 (right), a protein with coiled-coiled domains. (a) Schematic presentation of the LIS1–Nde1–dynein interaction according to McKenney et al., Nde1 strengthens the LIS1–dynein interaction. (b) Schematic presentation of LIS1–dynein interaction according to Huang et al. The position of LIS1 between the 3rd and 4th AAA domain and in proximity to dynein’s microtubule binding domain. Also in yeast Nde1 strengthens the LIS1–dynein interaction. (c) Schematic presentation of the molecular motor moving to the minus ends of microtubules. LIS1–Nde1–dynein interactions are more important in case of large cargoes.

LIS1 functions in normal development and disease

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How does LIS1 regulate cytoplasmic dynein activity? Studies using mammalian dynein suggest that Nde1 acts as a linker protein assisting the attachment of LIS1 to cytoplasmic dynein (Figure 2a) [46,47]. This tripartite interaction enabled the motor to remain attached to microtubules under high load conditions [48**]. Nevertheless, exactly how the Nde1–LIS1–dynein interaction is regulated is unclear and is likely to be of a complex nature. One study suggested that LIS1 might affect the Nde1–dynein interaction, since the addition of LIS1 caused dynein to be released from Nde1 more easily [44]. The results were unexpected in view of the known role of Nde1 in recruiting LIS1 to dynein [48–51]. The interaction between LIS1 and dynein may also be regulated by the nucleotide state of dynein [48,52].

Functional conservation and differences in LIS1 activity as a dynein regulator are highlighted in studies using other model organisms. A screen for suppressors of nuclear distribution mutants in Aspergillus nidulans supplied the initial evidence for the cellular mechanism of LIS1 action and its link to dynein and dynein regulators [53]. Studies using cytoplasmic dynein derived from budding yeast showed that LIS1 allowed individual dynein motors to remain attached to microtubules for extended periods of time [54**]. This effect is enhanced by Nde1 [54**], consistent with the proposed role for Nde1 in tethering LIS1 to dynein [48,50,51]. In these studies LIS1 did not affect dynein’s ATPase activity, showing that LIS1 can uncouple dynein’s ATP hydrolysis cycle from changes in microtubule affinity, consistent with studies of the mammalian protein [55]. Single particle electron microscopy demonstrated that the interface of the interaction between LIS1 and yeast dynein was located near AAA3/4 and the stalk (Figure 2b). Mutations in dynein at this predicted interface abolished specificity, in the case of dynein, its multiple interacting and regulatory proteins may alter its mechanochemical properties and/or dictate cargo specificity. Nevertheless, it should be noted that the activities of the plus end and minus end directed molecular motors are coordinated, and usually dynein perturbations will also affect anterograde motility [39–42]. The best-studied regulators of cytoplasmic dynein are LIS1 and dynactin, whose activities appear to be coordinated (reviews [35,43]). Cytoplasmic dynein regulation by either NudE–LIS1 or by dynactin was found to be mutually exclusive [44]. Differential activities of these dynein regulators were also noticed in the developing muscle in the fruit fly [45]. Dynine light chain and Partner of inscuteable (Pins), contributed to the regulation of both muscle length and to the positioning of nuclei. However, LIS1 contributed only to dynein-dependent muscle length determination, whereas the fly CLIP-170 ortholog and dynactin contributed only to dynein-dependent myonuclear positioning.

platelet-activating factor acetylhydrolase 1b (PAFAH-IB) cannot occur when LIS1 interacts with Nde1 [30]. LIS1 was identified as a regulatory subunit of PAFAH-IB, thus the naming nomenclature PAHAH-IB1 [31]. Nevertheless, most of the known LIS1 activities relate to its interactions with the molecular motor cytoplasmic dynein. Many LIS1 interacting proteins are dynein regulators; these include Ndel, Nde1 and CLIP-170 ([32], review [26]). Cytoplasmic dynein is a large and complex microtubule-associated molecular motor, which moves towards the minus ends of microtubules, and serves as the main retrograde motor in neurons (recent reviews [33*,34,35]). Many members of the kinesin superfamily of proteins, which include 45 genes in the mouse, are plus end directed molecular motors (reviews [36–38]). It has been hypothesized that while in the case of kinesins, the number of different members of the superfamily may allow for sufficient...
the LIS1–dynein interaction and caused defects in dynein function in living yeast.

LIS1 is an abundant protein that is expressed in many tissues and in wide developmental stages. Nevertheless, the observed human and mouse brain phenotypes suggest that there should be a higher sensitivity to LIS1 dosage in neuronal cells. Indeed, the role for LIS1 and one of its binding partners, Nde1, or for LIS1 and Nde1 in high-load transport mediated by dynein was very limited in non-neuronal cells but especially pronounced in neurons [56**,57**]. Acute LIS1 inhibition using injection of antibodies in rat cortical neurons or RNAs in hippocampal neurons severely affected the transport of large vesicles specifically in neuronal cells [56**]. Similar results were noticed in adult dorsal root ganglia neurons; preferential inhibition of the motility of large vesicles was observed following LIS1 depletion or in neurons derived from Lis1−/− mice [57**] (Figure 2c). LIS1 overexpression increased the average velocity and processivity of retrograde transport of both small and large organelles [57**]. Cargo size dependency was not observed in the regulatory activity of LIS1 on dynein in the fungus Aspergillus nidulans. LIS1 was required there for the proper distribution of three distinct dynein cargos; including both small cargo (endosomes) and larger cargo (peroxisomes and nuclei) [58*]. In Aspergillus nidulans LIS1 is required mainly for the initiation of dynein-driven motility, based on an analysis of cargo flux [58*]. In mammalian cells the relative contribution of LIS1 in cargo initiation vs. high load cargo transport remains to be determined; multicolor fluorescent of dynein, LIS1 and cargo will be an exciting future direction.

The regulatory interaction of LIS1 and dynein may explain some of LIS1 effects on neuronal function. In addition to cytoplasmic dynein, LIS1 was found in motile mammalian cilia [59]. Motile cilia are found on the surface of brain ventricles and are important for the circulation of the cerebral spinal fluid. The expression of a LIS1 ortholog in Chlamydomonas reinhardtii, which lack Nde1 and Nde1 orthologs, was highly dependent on the beat frequency and/or flagellar stiffness, thus suggesting a role for LIS1 in high-load activity also in conjunction with axonemal dyneins [60**].

In neurons, the role of LIS1 and Nde1 complex in regulation of dynein has been shown to affect synapses [61**]. It was found that the dynamic behavior of Post Synaptic Density (PSD) puncta before their recruitment to synaptic contact sites in interneuron dendrites, is distinct from the mobility of non-synaptic scaffold complexes. In interneuron dendrites, the motility and the final positioning of PSD-95, was found to be microtubule-associated and dependent on the activity of the LIS1/Nde1/dynein complex. It has been proposed that LIS1 and Nde1 may help dynein-dependent tethering of microtubules at the post-synaptic densities. Alternatively, LIS1 and Nde1 may increase the force of the molecular motor associated with the post-synaptic densities. This mechanism may be required to overcome large mechanical resistance generated by interaction of the post-synaptic densities with both presynaptic components and the surrounding cortical actin meshwork. These findings may be of importance taking into consideration that dysfunction of glutamatergic synapses on interneurons may lead to epilepsy, which is part of the pathophysiology of lissencephaly patients.

**Conclusions and future directions**

As the first gene to be identified in a human neuronal migration disease, LIS1 provided the scientific community a handle to better understand cortical development on a molecular level. After two decades of study, we understand that the highly conserved gene product is involved in basic cellular functions including proliferation, migration and cellular transport. We understand that its multiple activities results from LIS1 ability to serve as a hub for multiple partners but is also due to its function on a fundamentally important complex, the molecular motor dynein. Understanding the molecular mechanisms allowed us to go back to human genetics and where dosage sensitivity appears to be causing more than one condition. There is no doubt that the study on LIS1 did not exhaust itself and more cellular functions are to be discovered in the future.

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**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


A recent review article highlighting the roles in LIS1 and DCX during brain development.


Please cite this article in press as: Reiner O, Supr T. LIS1 functions in normal development and disease, *Curr Opin Neurobiol* (2013), http://dx.doi.org/10.1016/j.conb.2013.08.001


26. Reiner O: Lis1, let’s interact sometimes... (part 1). Neuro 2000, 28:633-636.


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49. An interesting study demonstrating how LIS1 with or without Nde1 affects the activity of cytoplasmic dynein.


56. An interesting study using protein purified from yeast demonstrating how LIS1 can enable prolonged attachment of cytoplasmic dynein to microtubules.


59. A study demonstrating a preferential role of LIS1 in neurons and in assisting axonal transport of large, but not small, vesicular structures.


61. A study demonstrating the role of LIS1 and the phosphorylated form of Nde1 in axonal transport of acidic organelles moving in axons of adult rat sensory neurons.


63. A study conducted in the filamentous fungus Aspergillus nidulans, showing that LIS1 is ubiquitously used for dynein-based transport, yet it was required primarily for the initiation of motility.


65. A study in Chlamydomonas reinhardtii, showing that the expression of the LIS1 ortholog in this species, which is a monomeric protein, and lacking Nde1 and Ndel1 orthologs, was highly dependent on the beat frequency or flagellar stiffness.


A study showing that immature dendrites of GABAergic interneurons form long protrusions which are important for retrograde translocation of synaptic contacts to the parental dendrites. This translocation process is dependent on microtubules and the activity of LIS1. Suppression of this retrograde translocation resulted in disorganized synaptic patterns on interneuron dendrites.