Molecular Neurobiology

ISSN0893-7648/99/20(2/3): 143–156/$13.50

The Unfolding Story of Two Lissencephaly Genes and Brain Development

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Abstract

Formation of our highly structured human brain involves a cascade of events, including differentiation, fate determination, and migration of neural precursors. In humans, unlike many other organisms, the cerebral cortex is the largest component of the brain. As in other mammals, the human cerebral cortex is located on the surface of the telencephalon and generally consists of six layers that are formed in an orderly fashion. During neuronal development, newly born neurons, moving in a radial direction, must migrate through previously formed layers to reach their proper cortical position. This is one of several neuronal migration routes that takes place in the developing brain; other modes of migration are tangential. Abnormal neuronal migration may in turn result in abnormal development of the cortical layers and deleterious consequences, such as Lissencephaly. Lissencephaly, a severe brain malformation, can be caused by mutations in one of two known genes: *LIS1* and *doublecortin (DCX)*. Recent in vitro and in vivo studies, report on possible functions for these gene products.

Index Entries: Neuronal migration; LIS1; *doublecortin*; brain development; PAF acetylhydrolase; microtubules.

The Convoluted Brain

During the evolution of the primate brain the cerebral cortex expanded substantially, greatly increasing its functional complexity (Tobias, 1996; Rakic, 1996). Structurally, the convoluted brain encompasses billions of highly organized interconnected neurons. The brain attains its general adult-like structure after months of prenatal development but continues to change and differentiate during the first years of postnatal life. In humans, the development of the nervous system begins with the formation of the embryonic disk. By d 18 of embryonic development, primordia of the forebrain can be identified in the rostal wall of the neuroectoderm of the embryonic disk. The forebrain develops from the anterior vesicle, and by d 36, the telencephalic vesicle is divided into two parts. By 3 mo, two smooth-

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walled cerebral hemispheres can be identified. As the hemispheres continue to increase in size by proliferation of cells, the neural wall expands and the surfaces of the hemispheres become convoluted. This process allows the cerebral cortex to greatly increase its surface area without changing the overall size of the brain (Netter, 1991). The formation of primary sulci and convolutions is genetically regulated by still unknown molecules and mechanisms.

The Production of Neurons

Neurons and glial cells are generated from a pool of progenitors that reside close to the ventricles, the ventricular epithelium. During the initial phases of neural development, the progenitor pool proliferates and expands (Caviness et al., 1997). With the initiation of neurogenesis, young neurons exit the cell cycle and start to migrate. Within the ventricular zone the position of the cell soma and nucleus is correlated with its phase in the cell cycle (Sauer, 1936). This phenomenon is known as interkinetic nuclear migration. Mitosis occurs at the ventricular surface, and during G1, nuclei rise from the surface. S-phase nuclei continue with this movement and are located in the outer side of the ventricular zone. At G2 the nuclei move toward the ventricular surface where mitosis occurs. The divisions within the cell cycle can be symmetrical (vertically oriented divisions, parallel to the ventricular epithelium) or asymmetrical. Time-lapse microscopy showed that the cleavage orientation predicts the fate of the daughters (Chenn et al., 1998). More specifically, vertical cleavages produce identical daughter cells, whereas horizontal cleavages produce two different cells. The cell in contact with the ventricular lumen can reenter the cell cycle, whereas the one on top of the lumen behaves like a postmitotic "neural cell" and migrates away. The onset of neurogenesis occurs in a gradient; the first neocortical neurons that undergo terminal division are in the rostrolateral area: the terminal divisions subsequently continues to the caudomedial area (Caviness et al., 1997).

Cell-cycle parameters differ in rodents and monkeys. Unlike the progressive slowing in cortical development that occurs in rodents, cell division accelerates during neurogenesis of the enlarged cortical layers in monkeys (Kornack and Rakic, 1998). The duration of the cell cycle in monkeys is about five times longer than that reported in rodents (Kornack and Rakic, 1998). Monkeys and humans differ not only in the size but also in the physiological properties of their brains. Interestingly, the total surface area of the brain in humans is about 10-fold larger than in monkeys and the relative proportion of some areas are different (Blinkov and Glezer, 1998; Rakic, 1990). This may indicate the presence of different ventricular progenitors.

Neuronal Migration and Cortical Layers

The first neurons to proliferate and differentiate are known as Cajal-Retzius cells (Cajal, 1899; Retzius, 1891; Sotelo and Soriano, 1997). Although, it has recently been proposed that Cajal cells are different from Retzius cells (Meyer et al., 1999), we shall still refer to them as Cajal-Retzius cells. These neurons and the subplate neurons form the preplate that is an early transient structure. Structurally, the preplate is subdivided by the appearance of the cortical plate into superficial layer I and the subplate (Marin-Padilla, 1998). It has been postulated that Cajal-Retzius cells regulate the phenotype of radial glial cells and the inside-out formation of the cortical plate (layers 2–6) (Marin-Padilla, 1984). Neuronal cell migration is essential for obtaining the correct six-layered structure of the cortex. Birthdating studies on the developing cerebral cortex have shown that precursors sequentially produce neurons destined for five layers (II–VI) in an inside-first, outside-last manner (Angevine and Sidman, 1961; Rakic, 1974). A large portion of cerebral-cortex neurons migrate along radial glia (first described by Rakic, 1971). Neurons

arriving at the cortical plate pass each other and become arranged radially in the form of ontogenic columns (Rakic, 1990). An ontogenetic column is defined as a group of neurons that originates from several progenitors of the same proliferative unit. The radial glia, the migrating pathway, and the ontogenetic column together form the radial unit that extends from the ventricular to pial surface. The radial unit hypothesis (Rakic, 1988a) was based on the observation that postmitotic cells preserve their topological relations during migration by remaining attached to a given radial glial fascicle once they initiate their movement (Rakic, 1972). Studies with transgenic mice have demonstrated that most neurons move and remain radially distributed in the cortex (Tan and Breen, 1993; Soriano et al., 1995; Rakic, 1995). However, multiple types of experiments have indicated that in addition to radial migration, a significant proportion of cortical neurons migrate along nonradial pathways (Walsh and Cepko, 1992; 1993; Fishell et al., 1993; Anderson et al., 1997; DeDiego et al., 1994; Meyer et al., 1998; Lavdas et al., 1999). The radial unit hypothesis provides an explanation for the large expansion of cortical surface resulting from a larger number of proliferative units without a significant increase in thickness that occurred during the phylogenetic and ontogenetic development (Rakic, 1988b).

The layers of the cerebral cortex segregate neurons that share characteristic dendritic morphologies, physiological properties, and axonal connections (reviewed in McConnell, 1988, 1991). Interestingly, transplantation studies suggest that the time of birth influences the ultimate fate of a neuron (McConnell, 1988b, McConnell and Kaznowski, 1991). For example, progenitor neurons that normally produce deep-layer neurons were transplanted into older brains at different stages of the cell cycle. When progenitor cells are transplanted during S-phase, they are multipotent. Their daughters, which have completed their final cell cycle in an older environment, take on a novel fate and migrate to the upper cortical layers, along with newly generated host neurons. However, when the precursors are transplanted late in the cell cycle, the daughters migrate specifically to layer six that is appropriate for their birth date. The fate of pyramidal or nonpyramidal cells can be determined by local cues within the ventricular zone (Luskin et al., 1993; Mione et al., 1994).

The cerebral cortex is also regionalized in the tangential plane into functionally distinct areas, each one subserving a specific function, such as the analysis of incoming sensory information and the coordination of motor outputs. Moreover, the different areas are characterized by distinctive cytoarchitectonic features (Brodmann, 1909), and the axonal connection of neurons in fact reflects their functional specificity. Thus, the regionalization of the cerebral cortex, is a complex genetically regulated process (Rubenstein et al., 1994), and the functionality of the regions requires proper thalamic inputs as well (Kennedy and Dehay, 1997).

Lissencephaly

Lissencephaly means smooth brain, and is considered a neuronal migration abnormality. Patients with this condition usually have both Agyria (synonymous to lissencephaly) and Pachygyria. Agyria denotes a smooth brain without secondary sulci, and Pachygyria denotes a brain with a thicker neocortex and a paucity of secondary sulci (Barth, 1987). There are two histologically distinct types of lissencephaly: (1) type I or classical lissencephaly and (2) type II or cobblestone lissencephaly (Barkovich et al., 1996). Whereas in type I we can describe the cerebral cortex as consisting of four layers, in type II no discrete layers are formed. Moreover, in type II lissencephaly, brain regions, such as the cerebellum and the brainstem, are also involved as well as other organs, such as eyes and muscles (Kurlemann et al., 1993). This review focuses on type I lissencephaly. Morphologically, in type I lissencephaly the cerebral surface is smooth and the cerebral cortex consists of four layers: a molecular layer, an external cellular layer, a cellular layer with lower density of cells, and an internal

cellular layer (Ferrer et al., 1987). Neurons with layers V and VI identity occupy the external cellular layer, whereas the internal cellular layer consists of neurons that are normally found in layers II–IV but fail to migrate to their final positions in patients with lissencephaly. In addition, in these patients a vertical arrangement of nerve cells is found throughout the cerebral cortical mantle, although some pyramidal cells are obliquely oriented and show distorted dendrites (Ferrer et al., 1987).

With such a severe malformation a poor prognosis is expected (Pavone et al., 1993). When examined neurologically, all patients have severe to profound mental retardation and abnormalities. In infants, variable hypotonia is common, whereas older children have a combination of hypotonia and spastic paralysis. Seizures, which are common, are often not responsive to therapy. It is possible to diagnose type I lissencephaly by computerized tomography (CT) or magnetic resonance imaging (MRI). Typically, the smooth brain appears as a figure eight shape on axial images: open sylvian regions resulting from underdeveloped opercula, thickened cortex, loss of interdigitations between the cortex and white matter, and enlargement of the posterior portions of the lateral ventricles. Lissencephaly occurs in about one in 30,000 live births (De Rijk-van Andel et al., 1991; Dobyns et al., 1993; Dobyns and Truwit, 1995).

LIS1, the Gene

LIS1 was cloned in 1993 as the gene involved in lissencephaly type I (Reiner et al., 1993). The human genome contains one functional *LIS1* gene on chromosome 17 (Reiner et al., 1993) and two pseudogenes on chromosome 2 (Reiner et al., 1995a; Fogli et al., 1999a). Similarly, the mouse genome contains one functional *Lis1* gene on chromosome 11 (Reiner et al., 1995b; Peterfy et al., 1998a) and two pseudogenes on chromosome 3 and 7 (Reiner et al., 1995b; Peterfy, 1998b). Large deletions that encompass additional genes in 17p13.3 region cause Miller-Dieker syndrome, in which patients have typical facial abnormalities in addition to the brain phenotype (Miller, 1963; Dieker et al., 1969). This syndrome is most likely due to deletions of additional gene(s) in the region, thus it is considered to be a contiguous gene disorder. HIC1 has recently been proposed as a possible candidate causing the facial abnormalities (Grimm et al., 1999). The first connection between lissencephaly and chromosome 17 was proposed in 1983 (Dobyns et al., 1983). LIS1 was initially associated with lissencephaly based on nonoverlapping deletions in one allele that is the most common mutation (Dobyns et al., 1993; Reiner, 1993). However, one point mutation, several C-terminal truncations, and an internal deletion of exon 9 also caused a severe form of lissencephaly (Lo Nigro et al., 1997; Pilz et al., 1998; Fogli et al., 1999b). The functional consequence of these mutations is most likely reduced levels of LIS1 protein owing to abnormal protein folding (Sapir et al., 1999a). It is plausible that other missense mutations or small deletions in the *LIS1* gene may cause other neuronal migration disorders different from classic lissencephaly (Barkovich et al., 1996). Recently, a boy was diagnosed with subcortical band heterotopia resulting from a missense mutation in exon 6 of *LIS1* (amino-acid substitution S169P) (Pilz et al., 1999). A patient with an in-frame N-terminal deletion was also recently reported (Fogli et al., 1999b) as well. He showed a less severe degree of cortical abnormality, with diffuse pachgyria and small areas of agyria in the posterior convexity. This patient also had mild hypotonia, but was able to walk unassisted from the age of 3 yr and 4 mo, had no epilepsy, was cognitively less impaired, and responded to simple requests.

LIS1, the Protein

The LIS1 protein contains seven WD (tryptophan–aspartic acid) repeats (Reiner et al., 1993; Neer et al., 1993; Reiner et al., 1996), a motif shared by at least 140 known proteins involved

in cell regulation, including the β subunits of G-proteins (Gβ) (Neer et al., 1994; Fong et al., 1986; Garcia-Higuera et al., 1996). This protein motif is likely to mediate protein-protein interactions. The crystal structure of Gβ, which contains seven WD repeats similar to those in LIS1, has been solved (Wall et al., 1995; Sondek et al., 1996). Interestingly, the WD repeats in Gβ from a sevenfold β propeller (Neer and Smith, 1996). It is very likely that the propeller structure in Gβ is shared by other WD-repeat containing proteins. Supporting this notion, some of the biochemical parameters of WD-containing proteins are very similar (Garcia-Higuera et al., 1996). LIS1 is a protein that is highly conserved during evolution; bovine (Hattori et al., 1994), mouse (Reiner et al., 1995b), and chicken proteins (Shmueli and Reiner, 2000) exceed 99% similarity. The homolog in *Drosophila* is 70% similar to the human protein (Liu et al., 1999; Swan et al., 1999; Sheffield et al., 2000). There is a *LIS1* homolog in *Aspergillus nidulans* NudF (55% similarity) that was isolated during a screen of mutants that are defective in nuclear migration (Xiang et al., 1995). It has been proposed that the association of LIS1 in nuclear migration along microtubules is conserved during evolution and may be part of the neuronal migration defect (Morris et al., 1998a, 1998b), and which may imply functional conservation.

LIS1 was cloned independently as a subunit of the heterotrimeric cytosolic platelet-activating factor acetylhydrolase (PAF-AH) isoform Ib (Hattori et al., 1994). PAF-AHs are enzymes that inactivate Platelet Activating Factor (PAF) by removing the acetyl moiety at the *sn*-2 position of PAF. Among its numerous known roles, PAF can act as an intercellular messenger (Hanahan, 1986; Harper, 1989; Chao and Olson, 1993; Koltai et al., 1991; Kornecki and Ehrlich, 1988; Minhas et al., 1996; Yue and Feuerstein, 1994). Importantly, among the three subunits of isoform Ib (Hattori et al., 1993), the β subunit is identical to the *LIS1* gene product (Reiner et al., 1993), and two highly homologous α (α1, α2) subunits form a catalytic heterodimer. The expression profile of α1 is different from α2. In the rat and in the mouse, the α1 subunit is expressed mainly during development, whereas the expression of the α2 subunit persists to adulthood (Manya et al., 1998; Albrecht et al., 1996). Expressed in many cell types, *Lis1* appears throughout development starting in the oocyte and zygote (Cahana and Reiner, 1999). Nevertheless, high levels of expression were observed in the developing CNS, particularly in the ventricular zone (Reiner et al., 1995b). The β subunit, LIS1, does not show any catalytic activity, suggesting a regulatory role for this subunit. However, LIS1 indeed affects catalytic activity, depending on the enzyme composition; it enhances the enzymatic activity of α2/α2 but reduces the activity of α1/α1 (Manya et al., 1999). Its interaction with the PAF-AH subunits is poorly conserved during evolution. The catalytic subunit homolog in *Drosophila* interacts neither with the LIS1 *Drosophila* homolog nor the mammalian one, although Drosophila LIS1 does interact with the mammalian PAF-AH catalytic subunits (Sneffield et al., 2000).

PAF-AH activity may be related to the recurrent seizures from which lissencephaly patients suffer. In a seizure model in rats we found that PAF-AH activity was markedly reduced as early as 30 min after seizures begun, and returned to control values after 1 wk. The expression of LIS1 and the PAF-AH α2 catalytic subunit changes in correlation with the enzymatic activity (Shmueli et al., 1999).

LIS1 was also shown to interact with tubulin and microtubules, which directly influences microtubule dynamics in vitro (Sapir et al., 1997). LIS1-tubulin interaction is conserved during evolution, suggested by the microtubule function of the LIS1 homolog in *Aspergillus nidulans*, NudF (Xiang et al., 1995). An additional interaction that is conserved during evolution is with nudC, a protein of unknown function, which in *Aspergillus nidulans*, controls the concentration of NudF (Xiang et al., 1995; Morris et al., 1998a, 1998b). Our finding implies that LIS1 may be one of the factors that regulate microtubule dynamics during neuronal differ-

entiation and migration. We assume that only a portion of LIS1 molecules in a cell is found associated with microtubules, whereas other LIS1 molecules act as the β subunit of PAF-AH(Ib), and that the two interactions do not occur simultaneously. Phosphorylation is one of the ways to control cellular localization and protein–protein interactions. Indeed, LIS1 is a phosphoprotein that is developmentally regulated (Sapir et al., 1999b). Localization of functional domains of the LIS1 protein is important. However, in generating a series of mutations that mostly resulted in premature termination (Sapir et al., 1999a), the resulting LIS1 protein was improperly folded. Therefore, we suggest that most LIS1 mutations reported (Lo Nigro et al., 1997; Pilz et al., 1998; Fogli et al., 1999b) result in a decrease in the amount of functional LIS1 protein.

The Role of LIS1 in the Developing Brain

In order to study the function of LIS1, Hirotsune and coworkers (Hirotsune et al., 1998) generated three different mutant alleles by gene targeting. It was suggested that these mutations result in a null allele. The heterozygote mutant mice in their study exhibited poor organization in the cortical layers. In addition, the hippocampus of the mutated mice demonstrated a split pyramidal cell layer. Abnormal neuronal migration was observed both in vivo and in vitro (Hirotsune et al., 1998). We mutated the mouse *Lis1* gene by deleting the first coding exon using conditional gene targeting (Cahana et al., submitted). Our mutation resulted in a shorter protein (sLIS1) that initiated from the second methionine. These mice had a less severe phenotype than the null allele, mimicking one lissencephaly patient with a less severe phenotype that expresses a short LIS1 protein (Fogli et al., 1999b). Developmentally, this mutation affected neuronal migration. Specifically, neurons of *Lis1*+/– that were labeled by BrdU at E13.5 migrated less than neurons found in wild-type littermates, especially in the dorsal telencephalon. Biochemically, the mutant protein is not capable of homodimerization, thus affecting some LIS1 functions.

Doublecortex, Lissencephaly, and the *doublecortin* Gene

The first link between lissencephaly and subcortical band heterotopia (SBH) was shown by Pinard et al., reporting two families with mothers and daughters with band heterotopia and sons with lissencephaly (Pinard et al., 1994). MRI of one of the female patients revealed that the frontal parenchyma appeared as a four-layered structure showing normal interdigitations with a layer of white matter. Below this was a thin, smooth layer of tissue with the appearance of gray matter. There was a fourth thick layer of white matter. The cortex was of normal thickness but slightly pachygyric. The bilateral-layered aspect appeared to consist of heterotopic tissue forming a band parallel to the cortex in the white matter, along with the gray matter of the frontal gyri, thus giving a double-cortex image (Pinand et al., 1994). These initial two families suggested an X-linked inheritance. Additional families were reported (Berg et al., 1998), allowing mapping of the gene to Xq22 (des Portes et al., 1997; Ross et al., 1997), and eventually to cloning of the *doublecortin* gene (des Portes et al., 1998a; Gleeson et al., 1998). The phenotype of X-linked lissencephaly (mutations in *doublecortin*) is very similar to chromosome 17-linked lissencephaly (mutations in *LIS1*). Nevertheless, a slight difference was noted: whereas the brain malformation owing to *LIS1* mutations was more severe over the parietal and occipital regions, *doublecortin* mutations produced the reverse gradient, which was more severe over the frontal cortex (Pilz et al., 1998). The clinical severity of SBH varies strikingly from the asymptomatic clinical presentation with heterotopic bands, determined by MRI, to severe mental impairment with intractable epilepsy. The relative thickness of the heterotopic band

seems to be correlated with the phenotype, since patients with thicker bands have more severe mental retardation and seizures (Barkorich et al., 1994).

Doublecortin (DCX) Expression and Function

The primary sequence of *doublecortin* (DCX) did not provide any clues for its function. Initially, the sequence of DCX revealed a similarity to a cDNA from human brain (*KIAA0369*) (Ohara et al., 1997). The deduced amino-acid sequence of human KIAA0369 protein contains two domains: an N-terminal segment similar to DCX (85% similarity) and a C-terminal domain nearly identical to the previously discovered kinase CPG16 in rat (Nediri et al., 1993; Herroni et al., 1998; Silverman et al., 1999; Vreugdenhil et al., 1999). Therefore, we suggested naming this protein Doublecortin-like kinase (DCLK) (Burgess et al., 1999). Human *KIAA0369* cDNA has been sequenced and mapped to human chromosome 13q12.3 (Nagase et al., 1997; Matsumoto et al., 1999). Because of the sequence similarity and the similar expression profile, DCX and DCLK may have functional interactions (Burgess et al., 1999). The expression of DCX initiated on mouse embryonic d 10.5, increases during development, and is observed in E18 in all brain regions examined (Francis et al., 1999; Gleeson et al., 1999a). Interestingly, the expression of DCX is restricted to the nervous system, in contrast to LIS1 expression, which is very wide, DCX's expression is downregulated in the adult (Francis et al., 1999; Gleeson et al., 1999a). The protein is expressed by both radially and tangentially migrating populations of neurons. In addition, DCX was shown to be colocalized with microtubules (Francis et al., 1999; Gleeson et al., 1999a). In order to study the function of DCX, we analyzed the protein upon transfection of COS cells. Doublecortin was found to bind to the microtubule cytoskeleton. In vitro assays (using biochemical methods, DIC microscopy, and electron microscopy) revealed that Doublecortin binds microtubules directly, stabilizes them, and causes bundling. Microtubule stabilization and bundling formation was also shown in in vivo assays. Doublecortin is a basic protein with an isoelectric point of 10, typical of microtubule-binding proteins. However, its sequence contains no known microtubule-binding domain(s). Together, these findings and the LIS1 interaction with microtubules emphasize the central regulatory role of microtubule dynamics and stability during neuronal morphogenesis (Horesh et al., 1999).

So far, many different mutations have been identified in DCX, resulting in nonsense, splice site, and missense mutations throughout the gene (Pilz et al., 1998, 1999; des Portes et al., 1998a, 1998b; Gleeson et al., 1998, 1999b; Kato, 1999). Single amino acid-substitution mutations in DCX cluster in two regions: amino acids 43–125 and amino acids 178–253 (Gleeson et al., 1999b). We performed a detailed sequence analysis of DCX and DCX-like proteins from various organisms and defined an evolutionarily conserved Doublecortin (DC) domain (Sapir, 2000). The domain typically appears in the amino terminal end of proteins and consists of two tandemly repeated 80-amino acid regions. In most of the patients, missense mutations in *DCX* fall within the conserved regions. We postulated that these repeats may be important for microtuble binding. Accordingly, we expressed DCX or DCLX (KIAA0369) repeats in vitro and in vivo. Our results suggest that the first repeat binds tubulin but not microtubules and enhances microtubule polymerization. In order to study the functional consequences of *DCX* mutations, we overexpressed seven of the reported mutations in COS7 cells and examined their effect on the microtubule cytoskeleton. The results obtained demonstrate that some of the mutations indeed disrupt microtubules. The most severe effect was observed in a mutation in amino acid 125, where tyrosine was substituted into histidine (Y125H). When overexpressed at high molar concentration, this mutation disrupts microtubules in vitro. In

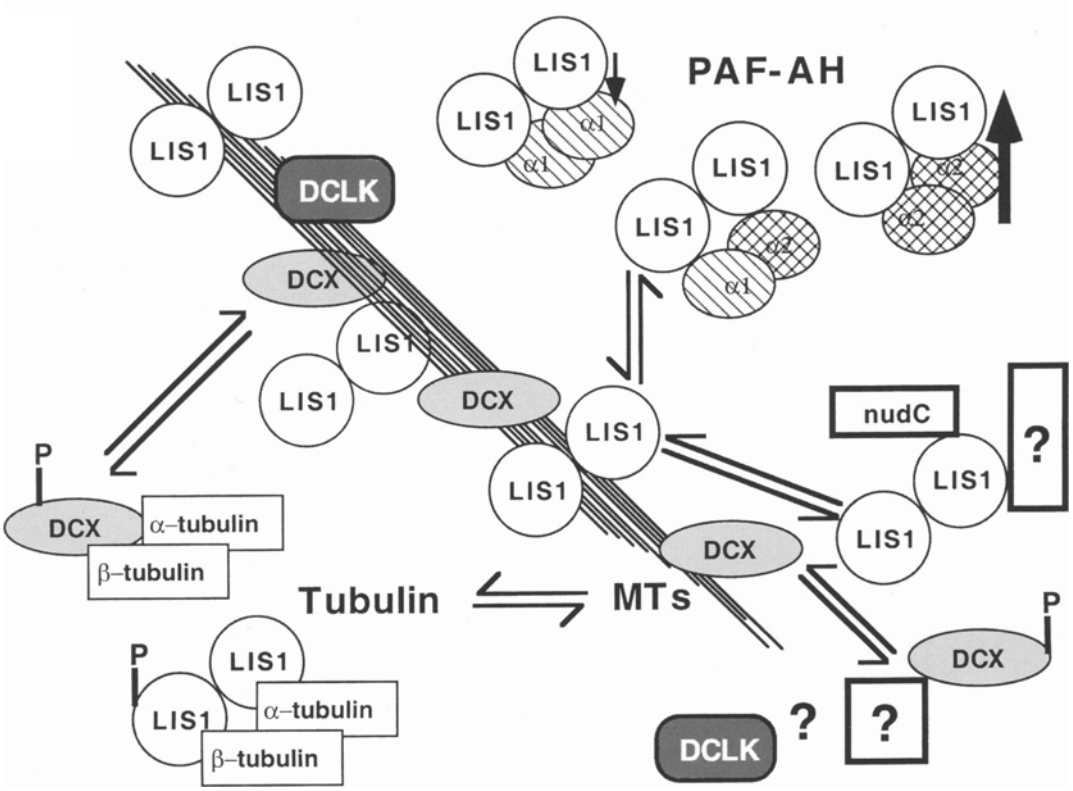

Fig. 1. Lissencephaly-associated proteins and cellular interactions. LIS1, DCX, and DCLK can be found bound to microtubules (MTs) or in the cytoplasm interacting with other proteins; possible movement of proteins are indicated by ↔. LIS1 or DCX can be phosphorylated (P added) or not. LIS1 increases ↑ enzymatic activity of the α2 dimer, but decreases ↓ activity of the α1 dimer. Unknown interacting proteins are indicated by? inside rectangular boxes, unknown substrates of DCLK are indicated by?

conclusion, these results emphasize the importance of DCX-microtubule interaction during normal and abnormal brain development.

Is There More to It?

Two of the gene products involved in lissencephaly affect the dynamic instability of microtubules. In the cellular model (Fig. 1), we tried to summarize the multiplicity of the possible interactions. LIS1 is found usually as a dimer and can interact with several proteins; it interacts with tubulin subunits and microtubules and its phosphorylation state possibly controls its intracellular localization. In addition, LIS1 interacts with the catalytic subunits of PAF-AH; it increases the activity of the α2 catalytic dimer, but decreases the activity of the α1 dimer and has no effect on the activity of the heterodimer. LIS1 was also shown to interact with nudC, a protein of unknown function. So far, DCX is only known to interact with tubulin subunits and microtubules possibly regulated by phosphorylation. DCX stimulates microtubule polymerization and causes the bundling of microtubules. DCLK can also interact with microtubules; however, its endogenous substrates are currently unknown. It is likely that these proteins can interact with

additional, unknown proteins and have direct or indirect regulatory roles. Furthermore, the expression/phosphorylation/intracellular localization of these proteins is highly regulated during development. Some of the genes may be involved in rare cases of recessive autosomal lissencephaly. Most likely, however, we are just getting a glimpse of the complex intracellular network that occurs in vivo during brain development.

Acknowledgments

I thank Prof. Salvador Martinez for very helpful suggestions and Michal Caspi for her critical reading of this manuscript. I thank current and previous members of the laboratory; their work enabled my writing this review. This research was partly supported by BSF grant No. 97-00014 (to O.R.), Volkswagen-Stiftung, HFSP grant No. RG283199, and the Minerva Foundation. O.R. is the Incumbent of the Aser Rothstein Career Development Chair in Genetic Diseases.

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