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Genes that regulate neuronal migration in the cerebral cortex

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Abstract

Malformations of cortical development are increasingly recognized as causes of mental retardation and epilepsy. However, little is known about the molecular and biochemical signals that control the proliferation, migration, and organization of the cells involved in normal cerebral cortical development. Analysis of genes required for cortical development will help elucidate the pathogenesis of some epilepsies. In humans, two striking examples of abnormal cortical development, with varying degrees of epilepsy and mental retardation, are 'double cortex' and lissencephaly. Double cortex (DC), also known as subcortical band heterotopia, shows an abnormal band of neurons in the white matter underlying a relatively normal cortex. In pedigrees, DC often occurs in females, whereas affected males show more severe lissencephaly (XLIS), i.e. an abnormally thick cortex with decreased or absent surface convolutions. We and others have identified a novel brain specific gene, *doublecortin*, that is mutated in Double Cortex/X-linked lissencephaly (DC/XLIS) patients. Although the cellular function of doublecortin (DCX) is unknown, sequence analysis reveals a cytoplasmic protein with potential MAP kinase phosphorylation sites, as well as a site that is likely to be phosphorylated by c-Abl, suggesting that doublecortin functions as an intracellular signaling molecule critical for the migration of developing neurons. Interestingly, the *scrambler* mouse mutant demonstrates abnormal lamination with some similarity to lissencephaly and reflects a mutation in the murine homolog of the *Drosophila disabled* gene, *mdab1*, which binds c-Abl. Although a direct interaction between doublecortin and mDab1 has not been demonstrated, it is plausible that these two proteins may be part of a common signaling pathway. Therefore, abnormalities in signal transduction may be an underlying mechanism for the neuronal migration defects in DC/XLIS and the *scrambler* mouse, but further research is necessary to determine how such abnormalities give rise to cortical malformations and epilepsy.

Keywords: Neuronal migration; Epilepsy; Double cortex; Lissencephaly; *Scrambler*; *Reeler*

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1. Introduction

Although the etiology and genetic basis of epilepsy are diverse, it is becoming increasingly evident that malformations of the cerebral cortex are responsible for a substantial number of neurological defects, such as epilepsies and mental retardation. Recent advances in neuroimaging allow accurate recognition of previously undiagnosed neuronal migration disturbances (Aicardi, 1994; Kuzniecky, 1994; Barkovich, 1996). Post-mortem examination of patients with epilepsy and pathological examination of resected brain tissue from patients with intractable epilepsy have demonstrated brain malformations in up to 40% of patients examined (Meencke, 1983; Hardiman et al., 1988; Farrell et al., 1992; Meencke and Veith, 1992; Vinters et al., 1992). However, the sequence of events leading from abnormal cortical development to epileptogenesis is not understood.

The formation of the cerebral cortex can be divided into three major developmental stages: (1) proliferation, (2) migration and (3) differentiation. Neural precursors proliferate in the ventricular zone, and post-mitotic neurons migrate to establish the mature layered cortex. The cells that migrate first form the preplate above the ventricular zone. As additional cells divide and migrate out of the ventricular zone they form a transient cortical plate which splits the preplate into an outer marginal zone (presumptive layer 1) and an inner subplate layer. The cortical plate is eventually filled with subsequent waves of cells migrating past the earlier cells in an 'inside-out' pattern (Fig. 1) (Rakic, 1974, 1990). Migration occurs radially along glial guides as well as independent of radial glia in a tangential pattern parallel to the pial surface or laterally within the ventricular (O'Rourke et al., 1992; Walsh and Cepko, 1992; Fishell et al., 1993; Tan and Breen, 1993; Walsh and Cepko 1993; O'Rourke et al., 1995).

Although the cellular events leading to the development of the mature cerebral cortex are well described, little is known about the molecular and biochemical signals involved. Several malformations of cortical development in human and mouse, including double cortex and lissencephaly in humans and *reeler* and *scrambler* in mice, in-

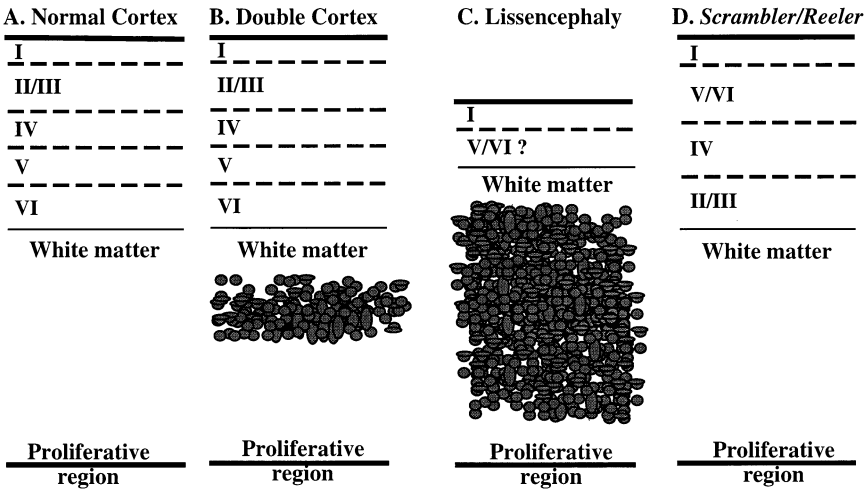

Fig. 1. Schematic diagram of normal cerebral cortex (A) and mutations affecting neuronal migration: double cortex (B), lissencephaly (C), and *scrambler/reeler* (D). The adult human cortex normally contains six layers of neurons. Cells are formed outside the cortex in the proliferative region along the ventricles and the cells migrate through the white matter into the cortex. In B and C, abnormal proliferation and/or blockage of normal migration results in heterotopic neurons (dark-shaded cells) which fail to reach their normal locations. In double cortex (B), there is an apparently normal six-layered cortex with a band of heterotopic neurons in the subcortical white matter. Lissencephaly (C) lacks normal lamination and results in a disorganized cortex with roughly four layers. The defect in neuronal migration in the *scrambler* and *reeler* (D) mouse mutants results in a reversal of the normal cortical layering.

volve incomplete or defective migration of cortical neurons. Identification of the genes and pathways involved in abnormal migration will advance our understanding of normal cortical development as well as perhaps help elucidate the pathogenesis of epilepsy.

2. Human neuronal migration disorders

Double cortex and lissencephaly are two distinct malformations that arise from incomplete neuronal migration from the ventricular zone to the cortex. In these disorders, the normal choreographed migration that generates the highly ordered laminar brain structures is defective. Double cortex, also known as subcortical band heterotopia or laminar heterotopia, is characterized by an abnormal band of neurons in the white matter underlying an apparently normal cortex (Fig. 1) (Raymond et al., 1995; Harding, 1996). The severity of the clinical manifestations, epilepsy and mental retardation, vary with the relative thickness of the heterotopic band of neurons (Barkovich et al., 1994).

Patients with lissencephaly display a more severe phenotype than double cortex. The deficient neuronal migration in lissencephaly is characterized by the absence (agyria) or decrease (pachygyria) in surface convolutions of the cortex (Aicardi, 1991). The cortex is abnormally thick and disorganized with a roughly four-layered cortex lacking normal lamination (Fig. 1) (Crome, 1956; Norman et al., 1995). Lissencephaly is associated with profound mental retardation, intractable seizures, feeding problems and a shortened lifespan. Lissencephaly may be manifest in several syndromes including isolated lissencephaly sequence (ILS) or Miller–Dieker syndrome (MDS) where it is associated with craniofacial dysmorphisms and other congenital abnormalities (Dobyns et al., 1991, 1992).

Lissencephaly and double cortex have been observed both sporadically and also within the same pedigrees. In several families, females with double cortex have sons with lissencephaly, severe mental retardation and epilepsy, while daughters have double cortex and milder mental impairment, suggesting the involvement of a single X-linked gene (Pinard et al., 1994). Most patients with double cortex are female, also supporting linkage to the X chromosome (Dobyns et al., 1996). The phenotypic differences between males and females are likely due to X inactivation. Cells in the brain of the hemizygous males would all express the affected X chromosome and hence have the more severe phenotype. Heterozygous females, on the other hand, presumably develop double cortex secondarily to X-inactivation and therefore possess two populations of cells; those which express a functionally normal X chromosome leading to proper migration, and cells which express the mutant X chromosome, terminating their migration prematurely in the subcortical white matter and populating the heterotopic band. The variability in phenotype expressed in females is presumably due to the degree of inactivation of the normal alleles, although this is unproved.

By collecting a handful of informative pedigrees, we performed linkage analysis, with a pooled multipoint LOD score of approximately 3.3, and revealed a candidate map location between Xq21–q24 (Ross et al., 1997). The gene was simultaneously mapped by des Portes et al. (1998a,b), giving a pooled LOD score of approximately 4.2. The candidate region was further refined using a de novo X-autosome translocation in Xq22.2–q23 in a girl with lissencephaly (Dobyns et al., 1992). Construction of a physical map allowed identification, by polymerase chain reaction (PCR) and fluorescent in situ hybridization (FISH), of yeast artificial chromosomes (YAC) and cosmids that span the translocation (Allen et al., 1998; Gleeson et al., 1998). Sequence analysis of the spanning clones revealed a gene, called *doublecortin*, that encoded a novel protein and which was disrupted by the translocation (Gleeson et al., 1998). Furthermore, *doublecortin* was also simultaneously identified by analysis of anonymous ESTs that mapped to the candidate region (des Portes et al., 1998a). The identification of *doublecortin* as the causative gene for double cortex and X-linked lissencephaly was verified by identification of mutations that cosegregated with the disease (des Portes et al., 1998a,b; Gleeson et al., 1998; Sossey-Alaoui et al., 1998; Gleeson et al., 1999).

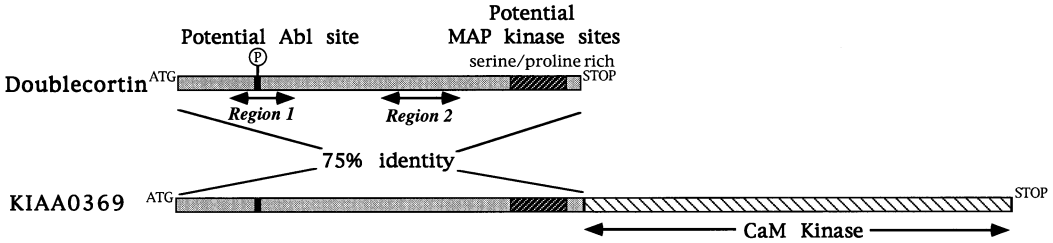

Fig. 2. Comparison of the sequence of Doublecortin and the brain specific EST, KIAA0369. The deduced amino acid sequence of Doublecortin reveals a potential Abl phosphorylation site at tyrosine residue 70 and potential MAP kinase sites within the serine/proline rich-region. Mutation analysis detected amino acid substitution mutations clustered in two regions represented by arrows below Doublecortin. The first region contains the potential Abl phosphorylation site. Doublecortin and KIAA0369 share a long stretch of strong amino acid identity but KIAA0369 also has an extended 3′ end with strong homology to CaM Kinase.

Doublecortin (*DCX*) consists of six coding exons that extend over 100 kb of genomic DNA and encodes a 40-kDa predicted protein (des Portes et al., 1998a; Gleeson et al., 1998). Northern blot, in situ hybridization and RT-PCR demonstrate expression primarily during brain development in neuronal precursors and migrating cells (des Portes et al., 1998a). Specifically, strong labeling is detected in neuronal precursors in the ventricular zone as well as in neurons of the cortical plate in human fetal brain. Moderately labeled neurons in the intermediate zone appear to be migrating based on their organization and orientation in chains (des Portes et al., 1998a). This suggests that *doublecortin* plays a role in the migration of neurons through the subplate to the cortical plate and is consistent with the hypothesis that heterotopic neurons migrate out of the ventricular zone but arrest when they fail to penetrate the subplate (Gleeson and Walsh, 1997). Doublecortin is also expressed at lower levels in the adult frontal cortex (Sossey-Alaoui et al., 1998) where the cortical abnormalities are more severe in some patients with DC/XLIS (Pilz et al., 1998; Gleeson and Walsh, unpublished observations).

Although the function of doublecortin is unknown, analysis of the deduced amino acid sequence reveals several potentially key domains including potential MAP kinase, protein kinase C and caesin kinase II phosphorylation sites (Fig. 2) (des Portes et al., 1998a; Gleeson et al., 1998). There is also one potential phosphorylation site for Abl at tyrosine residue 70 (Fig. 2), suggesting that doublecortin may be part of an Abl-dependent signaling pathway. Preliminary evidence demonstrates that a synthetic doublecortin peptide encoding the c-Abl site is efficiently phosphorylated by c-Abl in vitro (Gleeson and Fox, unpublished observation). This is intriguing in light of the potential role of Abl phosphorylation in the *scrambler* mutant mouse that also displays abnormal neuronal migration (discussed below). Doublecortin lacks identifiable transmembrane domains, consensus signal peptides, or hydrophobic segments suggesting that doublecortin is likely an intracellular protein rather than secreted or membrane-bound. The carboxy terminus contains a region rich in serine and proline residues which may be important for serine phosphorylation and the tertiary structure of the protein, respectively (Fig. 2). Whereas doublecortin does not have significant homology with any protein of known function, it is homologous over its entire length to the 5′-end of an anonymous, brain-specific EST, KIAA0369 (GenBank # AB002367) (Fig. 2) (des Portes et al., 1998a; Gleeson et al., 1998). The 3′-end of KIAA0369 is predicted to encode a calcium calmodulin-dependent kinase (CaM-kinase) domain. Although doublecortin does not share significant homology to the kinase, it may function by competitively modifying the activity of the CaM-kinase. The structural analysis of doublecortin therefore suggests that it functions as an intracellular signaling molecule critical for the migration of developing neurons. The role of the potential domains was further elucidated by the analysis of mutations in patients with DC/XLIS.

Extensive mutation analysis of the splice sites and coding exons of doublecortin was conducted by single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and direct sequencing (des Portes et al., 1998a,b; Gleeson et al., 1998; Sossey-Alaoui et al., 1998; Gleeson et al., 1999). Nonsense, missense, or splice site mutations were identified in 100% of pedigrees displaying double cortex and lissencephaly, and in approximately 50% of patients with sporadic double cortex. Sporadic patients tend to display mutations that result in premature protein truncations whereas pedigrees generally display single amino acid substitution mutations (Gleeson et al., 1999). Patients with sporadic mutations also have more severe clinical diagnoses and brain abnormalities as detected by MRI and no ascertained patients with truncation mutations have had children (Gleeson et al., 1999). This suggests that truncation mutations are more severe than single amino acid substitutions clinically and likely result in loss of doublecortin function. Single amino acid substitution mutations would result in a less severe clinical phenotype due to residual doublecortin gene function or dysregulation of doublecortin protein. Thus, there is a reproductive bias for the less severe substitution mutations. The failure to detect mutations in all the sporadic patients suggests the presence of non-coding region mutations which were undetectable by the screening methods used. Alternatively, double cortex might be genetically heterogeneous with additional genes responsible for the double cortex phenotype, although presently there is no evidence of this.

Characterization of the double cortex mutations may shed light onto the potential function of the protein. Amino acid substitution mutations were tightly clustered in two regions of doublecortin, near amino acid 75 (47–125) and near amino acid 200 (178–214), with only one mutation detected outside these two regions (Fig. 2) (Gleeson et al., 1999). Correlation of the mutation clusters with the predicted protein structure suggests that these domains may be critical for doublecortin function. The first cluster of mutations is near a potential consensus phosphorylation site for Abl, although none of the detected mutations alter the tyrosine residue predicted to be phosphorylated (Fig. 2). Abl is a non-receptor tyrosine kinase that has been implicated in cell adhesion and migration. The second region has no homology to any other proteins with defined function; perhaps these regions are involved in novel protein–protein interactions (Fig. 2). Hypotheses about doublecortin function must take into account the regions identified by mutation characterization.

Lissencephaly is also caused by mutations and deletions in a gene distinct from doublecortin, termed *LIS1*, located on human chromosome 17p13.3 (Ledbetter et al., 1992; Reiner et al., 1993; Lo Nigro et al., 1997). Large deletions of *LIS1*, detectable by cytogenetics, are the most common mutation observed in patients with lissencephaly (Pilz et al., 1998). *LIS1* encodes the β subunit of the brain isoform of platelet activating factor acetylhydrolase (PAFAH), an enzyme which regulates platelet activating factor (PAF) activity (Reiner et al., 1993; Hattori et al., 1994; Chong et al., 1997). Although the precise role of platelet activating factor (PAF) in neuronal migration has yet to be defined, it is involved in neuronal cell differentiation and the regulation of intracellular calcium concentrations (Kornecki and Ehrlich, 1988), synaptic plasticity and long-term potentiation (Clark et al., 1992) and neuronal growth cone collapse and neurite withdrawal (Clark et al., 1995). The LIS1 protein, PAFAH1B, contains WD40 repeats that are common to G protein β subunits suggesting that it may also be involved with signal transduction pathways critical for cortical development (Garcia-Higuera et al., 1996; Ho et al., 1997).

Lis1 is highly expressed in normal mouse brain in the ventricular zone, cortical plate and developing cortex (Mizuguchi et al., 1995; Reiner et al., 1995). Expression of *Lis1* in the cortex and hippocampus of adult mice correlates with brain regions affected in Miller–Dieker lissencephaly patients (Reiner et al., 1995). In addition, there is a reduction of *LIS1* immunoreactivity in the brains of human patients with Miller–Dieker lissencephaly (Mizuguchi et al., 1995; Isumi et al., 1997). Furthermore, animal studies with graded reductions in *Lis1* gene activity result in migration

defects (Hirotsune et al., 1998). Thus, *LIS1* plays an important role during differentiation and development of the cortex and defects in *LIS1*, as well as *DCX*, cause neuronal migration abnormalities.

Mutation analysis in a cohort of sporadic patients with lissencephaly without detectable deletions of chromosome 17 demonstrated *LIS1* mutations in approximately 50% and *DCX* mutations in up to 20% of male patients (Pilz et al., 1998). The location of *DCX* mutations in lissencephaly patients is similar to the clustering in double cortex patients, around the Abl phosphorylation site, supporting this region as functionally important. In addition, in two patients, a severe lissencephaly phenotype resulted from a mutation that eliminates only the 59 most carboxy terminal residues, implicating the importance of the C-terminal serine/proline-rich region. Similar to the results in double cortex patients, sporadic *DCX* mutations lead to a more severe lissencephaly phenotype than those with familial inheritance patterns. Since mutations in some patients are still unaccounted for, it is likely that additional genes for lissencephaly exist.

The localization of the most severely affected regions of the brain differs between patients with mutations in *DCX* and *LIS1* (Pilz et al., 1998). *DCX* mutations produced lissencephaly with more severe abnormalities over the frontal regions. The severity of the X-linked lissencephaly phenotype is consistent with the expression pattern of DCX in frontal cortex (Sossey-Alaoui et al., 1998). *LIS1* mutations, on the other hand, produce more severe gyral abnormalities over the parietal and occipital regions. The similarities in overall lissencephaly phenotype, lack of foliation and thickening of the cortex suggests that DCX and LIS1 are potentially involved in the same mechanisms controlling neuronal migration, although the difference in regions of the brain most severely affected suggests that they are part of related but distinct signal transduction pathways.

3. Animal models of neuronal migration

Besides human migration disorders, there are several animal models of abnormal neuronal migration including *reeler*, *scrambler*, and *yotari* mutant mice, as well as *cdk5*, *p35* and *lis1* knockout mice. In all these animal models, as in double cortex and lissencephaly, neurons fail to migrate to their appropriate locations. Although the genes responsible for each of these disorders have been identified, the relationship between the functions of the gene products is still not clear.

The most well characterized animal model of abnormal neuronal migration is the *reeler* mouse. The *reeler* mouse was identified several decades ago and displays an abnormal gait (Falconer, 1951). Further analysis revealed abnormalities in cortical lamination patterns creating a cortex that is roughly inverted from normal (Fig. 1), with additional defects of neuronal positioning in other brain regions, as well as profound cerebellar hypoplasia (Caviness, 1982; Goffinet, 1984, 1992). The *reeler* mouse harbors an autosomal recessive mutation mapped to mouse chromosome 5. The responsible gene was identified as a result of a transgene insertion into the *reeler* locus, as well as by physical mapping across the candidate gene region (D'Arcangelo et al., 1995; Hirotsune et al., 1995). The *reeler* protein, Reelin, is a large secreted polypeptide with homology to extracellular matrix molecules involved in cell adhesion (Hirotsune et al., 1995). A specific receptor system for this molecule remains unidentified. Reelin is synthesized and secreted by the Cajal-Retzius cells of layer 1 of the cerebral cortex, at a distance from the migrating cells which appear to require Reelin for appropriate guidance (Ogawa et al., 1995). The radial glial guidance system may be abnormal, leading to the failure of cells to split the preplate, although exactly how this defect in Reelin causes abnormal migration is uncertain.

More recently the *scrambler* mutant mouse (*scm*) was identified with a phenotype similar to *reeler*. The *scrambler* mutation arose spontaneously in an inbred colony at The Jackson Lab (Sweet et al., 1996). Like *reeler*, the mutant animals were initially identified at about 2 weeks of age by their unstable, ataxic gate and whole body tremors. In addition, *scrambler* brains have cytoarchitectural abnormalities in the layering of the

cerebral cortex and cerebellum. Histological analysis and birthdating experiments demonstrate the absence of a marginal zone as well as an inverted inside-out pattern of cortical development (Fig. 1) (Sweet et al., 1996; Gonzalez et al., 1997). The hippocampus is disorganized with the appearance of two indistinct pyramidal layers rather than one precise layer. The cerebellum is hypoplastic, reduced in size and lacking normal folia. Although there is a clearly discernible molecular layer in the cerebellum, there is marked disruption of the Purkinje and granule cell layers (Goldowitz et al., 1997). Despite these abnormalities, the *scm* mice do not display behavioral seizures or abnormal brain patterns of EEG activity (Sweet et al., 1996). Although the *scm* mice present with physical and morphological abnormalities indistinguishable from those seen in the *reeler* mice, *scm* maps to a location on mouse chromosome 4 distinct from the *reeler* locus on chromosome 5. Furthermore, *scm* mice express normal levels of Reelin mRNA and protein suggesting that the *scm* gene product may act downstream of Reelin as part of the same signaling pathway (Goldowitz et al., 1997; Gonzalez et al., 1997; Sheldon et al., 1997).

Another recently identified mouse mutant, *yotari* (*yot*), has a similar behavioral phenotype and neuropathology to *scrambler* and *reeler* mice (Yoneshima et al., 1997). The *yotari* mutation arose during the generation of mice carrying a disruption in the gene encoding the receptor for inositol-1,4,5-triphosphate. *Yotari* is caused by an autosomal recessive mutation independent of *reeler* based on genetic crosses between *yotari* and *reeler* mice. In addition, *yotari* mice have normal Reelin expression suggesting that, like *scrambler*, *yotari* acts as a downstream component in the same signaling pathway as *reeler*. Subsequently, *yotari* was shown to be allelic to *scrambler* (Sheldon et al., 1997).

Construction of a genetic and physical map across the *scm* candidate region allowed identification of the gene responsible for *scrambler* (Ware et al., 1997). The YACs which span the region were analyzed by interspersed repetitive sequence PCR (IRS-PCR) (Hunter et al., 1994). Amplification with primers specific to the IRS allowed analysis of the non-repetitive DNA sequences that lie between the repetitive sequences. An exon of the mouse *disabled* gene (*mdab1*) was identified by this method (Ware et al., 1997). A splice mutation was detected in *scm* mice near a retroposon-like element in *mdab1* (Sheldon et al., 1997; Ware et al., 1997). In another approach, *mdab1* was analyzed as a candidate gene because it mapped to the *scm* candidate region and is expressed almost exclusively in the brain during development (Sheldon et al., 1997). Furthermore, targeted disruption of *mdab1* resulted in disturbed lamination of the brain, indistinguishable from the phenotype seen in *scm* and *yot* mice (Howell et al., 1997a,b). *Scm* and *yot* express mutated forms of *mdab1* mRNA and little to no mDab1 protein (Sheldon et al., 1997; Ware et al., 1997). Taken together these results implicated *mdab1* as the gene responsible for the *scm* and *yot* phenotype.

Mouse *disabled 1* (*mDab1*) is the murine homolog of *Drosophila disabled* (*Dab*) which was originally identified as an enhancer of a disrupted *Drosophila abl* (*D-abl*) gene, thus the name *disabled* (Gertler et al., 1989). Dab and D-abl are co-localized within the *Drosophila* CNS and double mutants display serious defects in axonogenesis (Gertler et al., 1993). Mouse Dab1 was originally identified in a two-hybrid screen by virtue of its binding with c-Src, a non-receptor tyrosine kinase (Howell et al., 1997a,b). mDab1 contains a phosphotyrosine binding domain (PBD) and binds other non-receptor tyrosine kinases such as Fyn and c-Abl. The Dab1 protein is intensely expressed and tyrosine phosphorylated in developing neurons throughout the nervous system and is concentrated in developing neurites (Howell et al., 1997a,b). mDab1 has no known catalytic activity but appears to function as an intracellular component of a tyrosine kinase cascade. The ability of mDab1 to bind c-Abl directly suggests the existence of a c-Abl-dependent signaling pathway essential for normal neuronal migration.

3.1. Elucidation of the biochemical pathway in murine neuronal migration

Given the remarkable similarities in phenotype

between *reeler* and *scrambler* mice, it has been suggested that Reelin and mDab1 share a common biochemical signaling pathway with mDab1 downstream of Reelin. Like *reeler*, neuronal precursors in *scrambler* mice fail to properly penetrate the preplate and align with the cortical plate of the cerebral cortex. Both Reelin and mDab1 are expressed very early during the development of the cerebral cortex, hippocampus and cerebellum and Reelin is expressed in cells adjacent to the mDab1 expressing cells (Rice et al., 1998). These temporal and spatial expression patterns are prior to the appearance of histological abnormalities in both mouse mutants. Thus, Reelin and mDab1 likely play a role in initial stages of cortical lamination (Rice et al., 1998).

Analysis of the expression patterns of mDab1 during development suggested that mDab1 accumulates in the ectopically migrating neurons in *reeler* mice. Although the levels of *mdab1* mRNA are comparable, the mDab1 protein is overexpressed in the brains of *reeler* mice as compared to normal mice (Rice et al., 1998). The accumulation of mDab1 in the absence of Reelin evoked signal suggests that Reelin plays a regulatory mechanism in the levels of mDab1 during neuronal migration. The absence of Reelin may result in excess mDab1 due to failure of mDab1 degradation after fulfilling its signaling function or absence of a decrease in the rate of translation in response to Reelin. These results suggest a biochemical link between Reelin and mDab1 although a direct biochemical association has not yet been demonstrated. It is unlikely that there is a direct physical interaction between mDab1 and Reelin since Reelin is secreted while mDab1 is a cytoplasmic protein. Thus, an additional protein such as a Reelin receptor may be involved, with mDab1 acting as an adapter protein between the Reelin receptor and downstream signaling pathways. Further investigations are necessary to reveal the roles of Reelin and mDab1 in neuronal migration.

Additional genes that are crucial for normal neuronal migration include *cyclin-dependent kinase-5* (*cdk5*) and *p35*. Cdk5 is a serine threonine kinase that phosphorylates histone H1, tau, or neurofilaments, and is highly expressed in the axons of post mitotic neurons (Tsai et al., 1993; Delalle et al., 1997). Disruption of *cdk5* results in abnormal lamination of the cerebral cortex and altered foliation of the cerebellum similar to the *scrambler* and *reeler* phenotypes (Ohshima et al., 1996). P35 is a regulatory subunit that associates with Cdk5 to activate kinase activity (Tsai et al., 1994). Engineered mutations in *p35*, which eliminate Cdk5/P35 kinase activity, cause migration defects of the cerebral cortex, as well as sporadic seizures and adult lethality but spares the cerebellum (Chae et al., 1997). The *cdk5* and *p35* mRNAs are expressed in axons of the developing and mature nervous system and Cdk5/P35 kinase activity is essential for neurite outgrowth during neuronal differentiation (Nikolic et al., 1996; Delalle et al., 1997). Due to the similarities in cortical abnormalities, including the inverted cortical layering in *cdk5*/*p35* knockout mice, *cdk5* and *p35* are implicated as additional genes in the signal transduction pathway involving Reelin and mDab1. Unlike *reeler* and *scm* mice where cells fail to penetrate the cortical plate, the cortical lamination fails after the formation of the cortical plate in *cdk5* and *p35* null mice (Gilmore et al., 1998; Kwon and Tsai, 1998). This suggests that the Cdk5/P35 kinase functions downstream of mDab1 during neuronal migration (Rice et al., 1998).

4. Correlation between animal models and human disorders

Mutations in the genes responsible for the described neuronal migration defects in mice have not yet been identified in humans. Analysis of the syntenic regions in humans where the mouse genes map may elucidate the corresponding human disorders. Conversely, the construction of animal models of abnormal neuronal migration or in vitro assay systems by mutating genes involved in human neuronal migration disorders will allow more in depth analysis of gene function. Although *doublecortin* transgenic or knockout mice have not yet been described, *Lis1* animal models have been constructed (Hirotsune et al., 1998).

In order to analyze the function of *LIS1* in neuronal migration, Hirotsune et al. (1998) have created several mutations in the *Lis1* gene, *Pafahb1*, resulting in either residual *Pafahb1* function or a null phenotype. In contrast to other mouse mutants, heterozygous mice carrying the *Pafahb1* null allele show a phenotype, though they lack the inversion of the cortex that characterizes *reeler* and *scrambler*. Instead, they show somewhat delayed neuronal migration resulting in subtle layering defects. Although the defects are reminiscent of those in ILS and MDS patients they are subtler, suggesting that the greater severity of the heterozygous *LIS1* human phenotype might reflect the greater distance that neurons must migrate in humans. However, compound heterozygotes of the two *Pafahb1* alleles led to a much more severe phenotype than seen in the mice heterozygous for the null allele alone. Moreover, mice homozygous for the *Pafahb1* null allele die very early in embryogenesis. This implicates a broader role for *Pafahb1* in early stages of embryogenesis and neurogenesis. Since the *Pafahb1* mutant mice display phenotypes unique from the other models of neuronal migration, it likely participates in a distinct pathway from *DCX* or *mdab1* but this requires further study.

Molecular genetics has allowed the identification of DCX and mDab1 but the role that they play in neuronal migration is still being investigated. Since both these predicted proteins appear to lack intrinsic enzymatic activity their cellular mechanism of action presumably involves the interaction with other signaling molecules. Although a direct physiological interaction between DCX and mDab1 during neurogenesis has not been demonstrated, it is plausible that there are interactions between their protein products. They may be part of a common signal transduction pathway carrying signals from the plasma membrane to the cytoskeleton. Interactions with other signal transducing molecules may depend on their Abl phosphorylation sites. Therefore disorders of signal transduction may be the common denominator in the human and mouse disorders of neuronal migration. However, any interaction is currently speculative.

5. Conclusion

Although genetic approaches are enabling identification of new genes that regulate neuronal migration, the biological functions of these genes are still being deduced. Each gene needs to be analyzed independently and then in concert with the other genes to determine potential interactions. The analysis of human and animal neuronal migration disorders has allowed the identification of genes and elucidation of molecular mechanisms regulating normal brain development. The recent identification of the genes responsible for DCX/XLIS and the *scrambler* mutant mouse demonstrates the power of the molecular genetic approach and suggests a significant role for signal transduction pathways in cortical development. Further biochemical analysis of these pathways may lead to a greater understanding of the etiology of epilepsy and lead to new therapeutic treatments for intractable seizures.

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