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COMMENTARY

A New View of Early Cortical Development

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ABSTRACT. Recently, several genes that regulate the development of the cerebral cortex and are potential pharmacological targets have been cloned. Reelin, an extracellular matrix glycoprotein secreted by Cajal-Retzius cells in the marginal zone, instructs the radial organization of the cortical plate. The response of cortical plate cells to reelin requires the tyrosine kinase adaptor disabled-1 (Dab1). Cyclin-dependent kinase 5 and its activator p35 are necessary for the development of the cortical plate, probably at a later stage than reelin/Dab1. The transcription factor Tbr-1 is essential for differentiation of preplate and Cajal-Retzius cells and for formation of thalamocortical connections, while D1x-1/2 are required for tangential migration. Some neurotrophin systems such as neurotrophin 4, brain-derived neurotrophic factor, and neuregulin and its receptor ErbB are also thought to assist in the regulation of cortical development. In addition, a few genes implicated in human cortical dysplasias have been characterized. *LIS1* encodes a protein related to platelet-activating factor acetyl hydrolase that is defective in lissencephaly-1 of the Miller-Dieker type, while the double cortex malformation is related to mutations of a new gene dubbed *doublecortin*. BIOCHEM PHARMACOL **56**;11:1403–1409, 1998. © 1998 Elsevier Science Inc.

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The development of the mammalian cerebral cortex requires the orchestrated migration of postmitotic neurons from germinative, ventricular zones, through the intermediate zone, to the external field of the telencephalon where they form architectonic patterns. Although it is widely accepted that these morphogenetic events are under strict genetic control, for decades the only clues about this regulation have been provided by studies of the *reeler* mutant mouse [1, 2] and of human cortical malformations [3]. The last few years have witnessed the identification of several genes implicated in cortical development, that are potential pharmacological targets, namely *reelin* [4], *Dab1*† [5–8], *Cdk5* [9], *p35* [10], *Tbr-1* and *D1x-1/2* in mice [11, 12], and *LIS1*, for "lissencephaly I of the Miller-Dieker type" [13] and "*doublecortin*" [14, 15] in humans. Although the overall picture remains sketchy, a simplified model of early cortical development that incorporates some recent findings is schematized in Fig. 1. It is based primarily on observations of mouse development, but will hopefully serve as a guide for the interpretation of human data as well. Before discussing recent data, a brief background of early cortical development will be provided.

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† *Abbreviations:* Cdk5, cyclin-dependent kinase 5; CP, cortical plate; *Dabl*, disabled-1 gene; NRG, neuregulin; and NT4, neurotrophin 4.

EARLY NORMAL CORTICAL DEVELOPMENT

In mammals, cortical development begins with the formation of the preplate, a horizontal neuronal network that includes pioneer neurons in the marginal zone, Cajal-Retzius cells, and subplate cells. Some preplate neurons are thought to migrate radially from the ventricular zone, while others—such as Cajal-Retzius cells—may follow a tangential route [16]. The next step is the appearance of the CP, populated with radially oriented neurons. The majority of CP neurons are generated in cortical ventricular zones and migrate along radial fibers [1, 17]. Interestingly, early migrating cells have a stellate shape [18], while later migrating neurons assume a bipolarly elongated shape and are more closely apposed to radial guides. In addition, some neurons generated in the ganglionic eminence reach the cortex by tangential migration [11]. The CP splits the preplate population into two contingents. Some preplate cells settle in the marginal zone, while others are displaced inward, between the CP and the intermediate zone, and form the subplate [19]. The CP is the precursor of most of the cortex (layers II to VIa). It increases in thickness by addition of radially migrating neurons from the ventricular zones that migrate beyond previously established layers to settle at progressively more superficial levels. Thus, deep cortical layers V and VI are generated early, while progressively younger neurons form cortical layers IV, III, and II. This "inside-out corticogenetic gradient" is a general feature of the mammalian cortex.

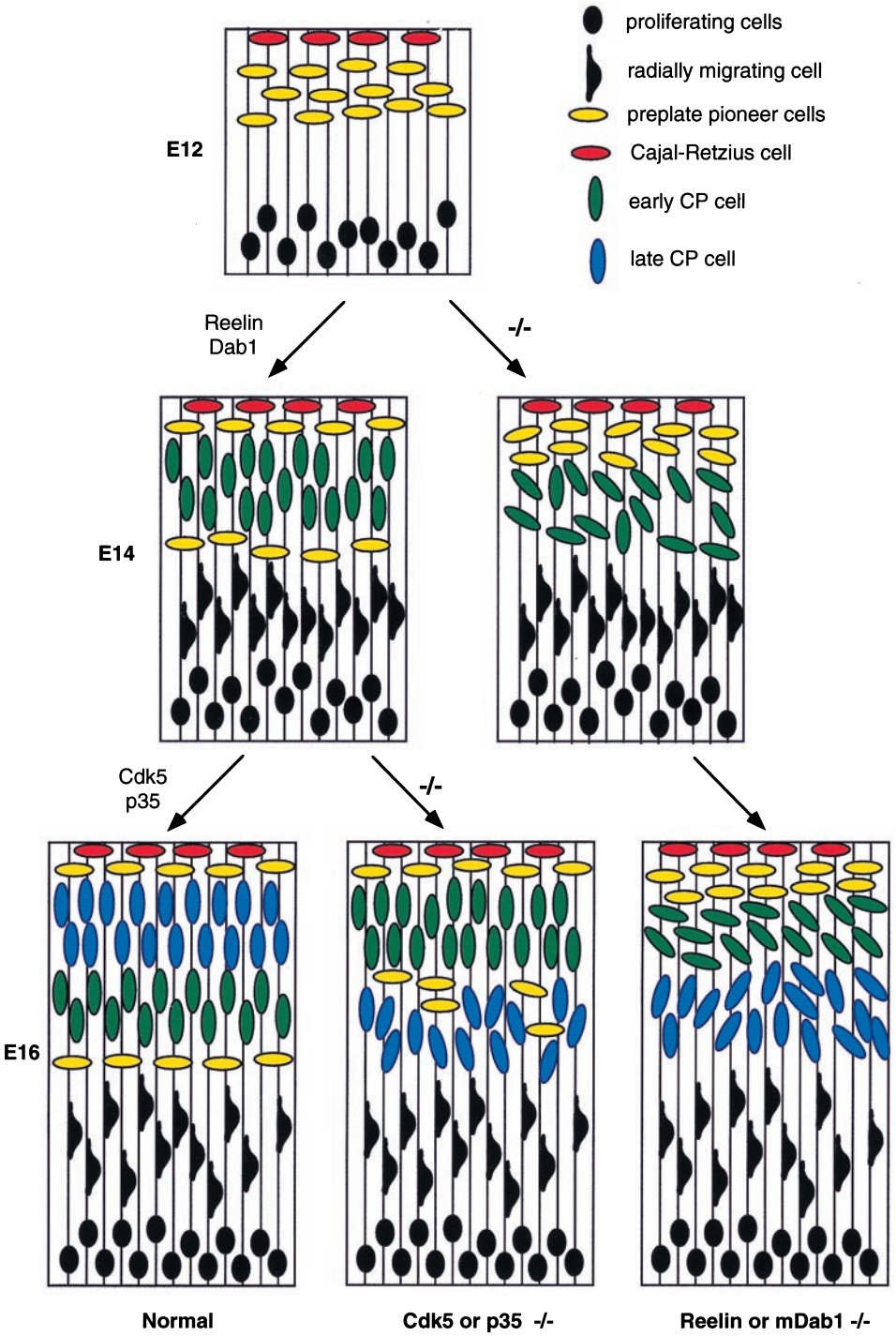

FIG. 1. Simplified model of early cortical development. The appearance of the CP (at E14) divides the preplate (E12) into two cell contingents: pioneer neurons in the marginal zone externally and subplate cells. Cajal-Retzius cells may derive from the preplate or invade the marginal zone by tangential migration. The early organization of the CP is defective in *reelin*- and *Dab1*-deficient mice but occurs normally in *Cdk5*- or *p35*-deficient mice. A second step consists in the migration of CP neurons according to an inside–outside gradient. At E16, for example, older neurons are found at a deep level, while younger cells settle more superficially. This gradient is defective in mice lacking *reelin/Dab1* and also in *Cdk5/p35*-mutant mice.

REELIN AND DISABLED-1 (DAB1)

In reeler mice, neurons are generated in normal numbers and at the normal time. The preplate forms normally, and CP cells migrate initially normally from the ventricular zones. However, when CP cells reach their destination, something prevents them from ordering into the normal architectonic patterns, and they assume abnormal orientations and shapes. The splitting of the preplate does not occur; instead, all preplate cells are displaced externally by the poorly organized CP. Furthermore, later-migrating CP

cells fail to pass previously established layers as they would in normal animals. As a result, the maturation of the reeler cortex proceeds from outside to inside. A detailed discussion of the reeler phenotype is beyond the scope of the present commentary, and the reader is directed to other reviews [2, 20].

The product of the *reeler* gene, reelin [4], is a large (more than 388 kDa) glycoprotein [21] secreted in the extracellular matrix. As deduced from the cDNA sequence, reelin begins with a signal peptide, followed by a stretch of weak similarity with F-spondin and a unique region. The rest of the protein is composed of eight repeats of 300–350 residues centered on an epidermal growth factor motif, and terminates with a short, highly basic sequence [22]. Reelin is expressed by several neurons, most intensely by Cajal-Retzius cells of the molecular layer, but also by mitral cells in the olfactory bulb, external cerebellar granule cells, retinal ganglion cells, and many others [23]. Surprisingly, there is no correlation between reelin expression and the reeler phenotype. Some cells, such as Cajal-Retzius cells or retinal ganglion cells, express large amounts of reelin, yet are almost unaffected by the *reeler* trait. By contrast, CP neurons, Purkinje cells, and other targets of the *reeler* mutation do not express reelin. This difference between reelin expression and the reeler phenotype suggests that reelin may act at a distance on target cells such as CP cells. For example, reelin could behave as a cell-repulsive molecule.

Scrambler, a recessive mutation unrelated to *reeler*, generates a phenotype identical to *reeler*, including the absence of a molecular layer with profuse malorientation of cortical neurons and an inversion of the corticogenetic gradient, which is directed from outside to inside as in *reeler* [24, 25]. The *yotari* mutation is allelic to *scrambler* [7]. Reelin expression is normal in scrambler mice, suggesting that *scrambler* acts downstream from *reeler*. While the *scrambler* gene was being approached using positional cloning, Howell *et al.* [5] showed that a mouse ortholog of the *Drosophila* disabled gene, (*Dab1*), maps in the *scrambler* region and is expressed in the developing brain. Using homologous recombination, a *Dab1* null mutation was constructed [6]. Surprisingly, the brains of homozygous *Dab1* mutant mice reveal a phenotype similar to *scrambler* and *reeler*. This result expedited the identification of *scrambler* and *yotari* as *Dab1* mutations [7, 8]. *Scrambler* results from the inclusion of a partial IAP (intracisternal A-type particle) sequence in the Dab1 mRNA, resulting in abnormal transcript and putative protein. The *yotari* mutation yields to skipping of a few coding exons from the Dab1 mRNA [7].

The *Dab1* gene encodes several transcripts and proteins. A p80 protein is the predominant Dab1 form in the brain and presumably corresponds to the Dab1-555 transcript. The aminoterminal region of Dab1-555 contains a phosphotyrosine binding (PTB) or protein interaction (PI) domain initially described in ShcA and IRS-1 adaptor proteins. Dab1-555 is tyrosine-phosphorylated during early brain development, at E10–E11; then the degree of tyrosine phosphorylation decreases progressively and is undetectable in the adult brain. The kinase(s) that participates in Dab1 phosphorylation has not been identified. However, Dab1-555 in its tyrosine phosphorylated state binds to the SH2 domain of Src, despite the fact that it does not contain a typical SH2-binding site. Conversely, Dab1-555 is capable of binding other, unidentified tyrosine-phosphorylated proteins via its PTB/PI domain. Taken together, these data strongly suggested that Dab1-555 functions as a tyrosine kinase adaptor protein [5].

Preliminary studies of Dab1 expression in the embryonic cortex and cerebellum at E13.5 reveal a strong expression in the neurons of the CP and in Purkinje cells [6, 7]. Interestingly, these two neuronal classes are the first elements to manifest the reeler phenotype and are thus primary targets of the *reeler* gene [2, 20]. Similarly, in the medulla, reelin-positive neurons are found in the reticular formation dorsal to the inferior olivary complex [23], whereas Dab1 mRNA is detected in olivary neurons that manifest the phenotype [20]. These observations suggest strongly that Dab1 is a key element in the response of target neurons to the message delivered by reelin [26]. In addition, a preliminary comparison of the expression pattern of reelin and Dab1 indicates that the reeler phenotype is most evident in regions where expression of reelin and Dab1 are topographically distinct, while the phenotype is more subtle in regions where expression of both proteins is concurrent. Of course, this hypothesis needs to be studied further, particularly with determination of the neuronal types that express reelin and Dab1.

Although the role of Dab1 in a reelin-initiated transduction cascade is likely, several elements in the transmission of a reelin signal to postmigratory neurons remain unclear. For example, no induced null mutation for cytosolic kinases such as Src, Abl, Fyn, and Yes generates a phenotype evocative of reeler. The most prominent missing link is the membrane component that senses the reelin signal. Clearly, the identification of this key element is high on the agenda of various groups.

CYCLIN-DEPENDENT PROTEIN KINASE 5 (Cdk5) AND ITS ACTIVATORS p35 AND p39

Cdk5 was isolated through its similarity to human Cdc2, a main regulator of the cell cycle [27, 28]. In adult mice, the *Cdk5* gene is expressed principally in the brain and at lower levels in testis. Cdk5 histone H1 kinase activity is present in adult and developing brain tissue and correlates with the differentiation of neuronal cells. No Cdk5 activity is found in cell lines, suggesting that a regulatory subunit is necessary for enzymatic activity. However, in primary cultures from E18 rat embryonic cortex, a constant level of Cdk5 protein is accompanied by increasing kinase activity that parallels increasing neuronal differentiation. A similar Cdc2-like kinase was also isolated by its ability to phosphorylate high and medium molecular weight neurofilament subunits, and the tau microtubule-associated protein.

Cyclin-dependent kinases are active as heterodimers of the kinase subunit and a cyclin. Accordingly, p35 was characterized as a Cdk5-associated phosphoprotein and a strong activator of Cdk5 [29]. Despite the analogy with regulatory cyclins, p35 does not display significant similarity to cyclins. The expression patterns of p35 and Cdk5 were studied in the embryonic mouse brain by *in situ* hybridization and shown to be largely similar, although not strictly identical. In the central nervous system, the distribution of p35 and Cdk5 RNA is restricted to post-mitotic neurons. p35 is expressed solely in the central nervous system, while Cdk5 is expressed in both the central and peripheral nervous systems. These and other observations suggest that the Cdk5/p35 complex belongs to a kinase family involved in processes other than cell-cycle control. Its activity of phosphorylation of neural-specific neurofilaments and tau proteins indicates that Cdk5/p35 could help stabilize the cytoskeleton, with influence on neuronal shape, axonal transport, and conduction properties.

The role of Cdk5 *in vivo* was assessed by generating *Cdk5* null mutant mice by homologous recombination [9]. More than 60% of *Cdk5*-null animals die *in utero*, and the others die during the first natal day. These mutant mice have unique anomalies of brain histogenesis and neuronal lesions with accumulation of neurofilament proteins, suggestive of a defective cytoskeleton. The developmental alterations affect principally the cerebral cortex, hippocampus, and cerebellum, and suggest abnormal neuronal migration, with lack of proper stratification in the cortex and hippocampus, and absence of foliation of the cerebellar cortex. These abnormalities are reminiscent of those in reelin and Dab1 deficient mice. Although the early death of affected animals complicates the analysis, the developmental alterations have been studied, using BrdU birthdating and various histological techniques [30]. These studies reveal interesting differences between *Cdk5* (−/−) and *reeler* mice, particularly in the development of the cerebral cortex. As explained above, in reelin and Dab1 deficient mice, first migrating CP neurons displace the whole preplate externally. By contrast, in *Cdk5* (−/−) embryos, the CP settles initially within the preplate and divides it into an external contingent in the marginal zone and an internal contingent in the subplate, almost as would occur in normal embryos. It is only at a later stage that migrating CP neurons do not pass the subplate and fail to migrate to the superficial level of the cortex, as they normally would. As a result, the corticogenetic gradient is directed from outside to inside. In a sense, this anomaly is more suggestive of a cell migration disorder than the malformation in reelin/Dab1 deficient animals and defines a new stage of cortical development.

The gene encoding p35, the Cdk5 activator, was also disrupted in mice using homologous recombination [10]. Compared with *Cdk5* (−/−) mice, *p35* (−/−) animals survive better and have less severe alterations. The phenotype consists mainly in a defect of commissural systems such as the corpus callosum and anterior commissure, and in a cortical architectonic anomaly that is reminiscent of that in *Cdk5* (−/−) animals. In p35-deficient embryos, first-generated CP neurons migrate normally and dissociate the preplate into the marginal zone and subplate, a feature that differs from *reeler/Dab1*. However, later-born cells fail to complete radial gliophilic migration and settle instead at a deeper level, among the subplate and at the periphery of the intermediate zone. The mature cortex has a well-defined molecular layer, but an inversion of cortical laminae, and the inner layers are somewhat mixed with subplate derivatives. The brain malformation in *p35* mutant mice, however, is more subtle than that in *Cdk5*-deficient animals, suggesting that cofactors other than p35 regulate the activity of Cdk5. In particular, the p39 Cdk5 regulator [31] seems to be expressed more strongly in the hindbrain and may account for the normal development of the hindbrain in *p35* (−/−) mice, while it is clearly affected in *Cdk5*-null mice. The observation that reelin expression is normal in Cajal-Retzius cells in *Cdk5* and *p35* (−/−) mice confirms that both genes act independently of the reelin cascade, perhaps at a later stage of cortical development. It would be most interesting to assess whether *Cdk5/p35* interact genetically with *reelin/Dab1*, for example by examining double mutants.

Although the details remain poorly understood, these recent data suggest the tentative model of cortical development schematized in Fig. 1. Reelin is an extracellular signal secreted by early generated Cajal-Retzius cells of the marginal zone, and sends—directly or indirectly—some message to migrating CP neurons. CP cells receive it, presumably through a cell-surface sensor/transducer system. The signal is then relayed within the target cell where it initiates a cascade of events, involving the Dab1 tyrosine kinase adaptor, and eventually instructing postmigratory neurons to detach from their radial guides, assume a radial orientation, and form a dense, well-organized cortical plate. Later-generated neurons migrate over larger distances, which requires a more intimate interaction with radial guiding fibers as well as a more refined machinery for migration, particularly at the level of the cytoskeleton. Cdk5, its p35 activator, and presumably other cofactors would be required for this later migration.

GROWTH FACTORS AND TRANSCRIPTION FACTORS

Although these fields are beyond the scope of this commentary, we wish to draw the reader's attention to a few recent findings that appear relevant to the formation of the early cortex. The administration of high doses of NT4 to the embryonic cortex results in increased cellularity of the marginal zone with local enlargement of the extracellular space mimicking an extraneous molecular layer. This effect is NT4-specific, as mice deficient in TrkB, the principal NT4 receptor, are resistant to this action [32]. To a certain extent, the alterations induced by NT4 are reminiscent of microgyric patterns (*vide infra*). It was further demonstrated

that the extraneous neurons in the marginal zone after NT4 treatment become reelin-positive with development [33]. The observation that cellularity of the marginal zone increases with NT4 treatment supports the hypothesis that some neurons in this layer migrate tangentially from other parts of the brain. A somewhat similar result was observed in mice transgenic for the neurotrophin brain-derived neurotrophic factor (BDNF) [34]. In those animals, in which large amounts of BDNF are secreted, the cerebral cortex does not develop normally but instead a complex malformative pattern, also evocative of polymicrogyria, is found. In this case, however, increased cellularity in the marginal zone is accompanied by a reduction in reelin mRNA and decreased reelin secretion. Another set of data suggests that the EGF-related NRG and the ErbB receptor proteins may be involved in the control of radial neuronal migration. Using *in vitro* models, radial gliophilic neuronal migration in the cerebellum (granule cells) and the cortex proceeds abnormally following perturbations of NRG-ErbB receptor signalling. Migrating neurons produce NRG, which activates ErbB receptors such as ErbB4, located on radial glial cells. Blocking of the ErbB receptor impairs the formation of radial glial fibers and interferes with migration [35, 36].

Several transcription factors are expressed in the embryonic forebrain and allow the definition of transverse as well as longitudinal compartments that form the basis of the so-called "prosomeric" model [37]. Gene targeting of some of these transcription factors provides particularly intriguing results. Mice deficient in the homeobox genes *D1x-1* and *D1x-2* have a deficiency in the tangential migration stream followed by immature neurons generated in the lateral ganglionic eminence to reach the cortex. These cells are believed to differentiate into γ-aminobutyric acid and calbindin-positive cortical interneurons [11] and provide a clear-cut demonstration of the importance of tangential migration in addition to the better-known radial, gliophilic migration pathway. Another putative transcription factor expressed by postmigratory cortical neurons is Tbr-1. Tbr-1-deficient mice have subtle abnormalities of cortical architectonics. Although the preplate develops normally at embryonic day 12.5 as in normal mice, no reelin-positive Cajal-Retzius cells are differentiated. The subplate does not form normally, and preplate derivatives are instead mixed with early CP cells. Furthermore, while thalamocortical and corticothalamic fibers grow initially normally towards the internal capsule, they stop at this level and fail to achieve normal connections. This malformation suggests that the absence of Cajal-Retzius cells (and of reelin) may be responsible for the cytoarchitectonic cortical malformation, and that the abnormal subplate fails to guide thalamic axons to the cortex [12].

HUMAN MALFORMATIONS

Although not exactly similar to any human disorder, *reeler* and related mutations nevertheless serve as physiopathological models for human developmental disorders. A common feature of human cortical dysplasias is that they are highly epileptogenic. In this respect, it is surprising that neither the *reelin* or *Dab1* deficiency leads to convulsions, while the *p35* (−/−) mutation apparently does. Cortical malformations are extremely diverse, and include diffuse neuronal migration disorders and/or cortical dysplasias. Their classification is difficult, and the various aspects are covered, for example in Friede [3] and Guerrini *et al.* [38].

Agyria or Lissencephaly type 1, including Miller-Dieker syndrome, is characterized by the absence of gyri and a thick cortical ribbon, usually with some preservation of radial neuronal organization. The agyric cortex has four layers: the molecular layer, a thin layer of nerve cells, a tangential plexus of myelinated fibers, and a thick layer of poorly organized neurons that blends with the white matter. The term pachygyria refers to a similar, albeit less severe malformation. These disorders are thought to develop early, around gestational weeks 11–13, and are attributed to failure of neuronal migration.

Polymicrogyria is characterized by excessive folding of the upper cortical layers and is often associated with other malformations such as agenesis of the corpus callosum. This disease group is heterogeneous, but most forms are acquired, and genetically determined malformations are rare. In lissencephaly type 2, including the Walker-Warburg syndrome, the agyric or pachygyric pattern is only apparent on the surface of the brain, while microgyration with gyral fusion is clearly seen in sections. This disorder is thus similar to, and classified together with, polymicrogyria. Polymicrogyric disorders are considered secondary to lesions that occur later in fetal life than type 1 lissencephaly, probably near the fifth month.

Other disorders of neuronal migrations are heterotopias, which can be nodular or laminar. Laminar heterotopias are rare, bilateral, and symmetric; they are closely related to agyria/lissencephaly type 1 and pachygyria. They can be quite modest or profuse, an alteration then referred to as "double cortex." Periventricular, nodular heterotopias are more common than laminar heterotopias. They are populated by neurons that did not achieve normal radial migration, for example following defective radial glial guidance. They are associated with a variety of malformations and can be induced in rodents by X-irradiation or methylazoxymethanol teratogenesis.

The etiology of cortical dysplasias is known in only a handful of cases. A gene associated with the Miller-Dieker type I lissencephaly, named *LIS1* [13, 39], encodes a protein with similarity to PAF (platelet activating factor) acetyl hydrolase. It is expressed in Cajal-Retzius cells [40], but also in neurons of the cortical plate [41]. Progress has also been made in our understanding of the "double cortex" malformation. Families were described with mothers and daughters that had "double cortex," and sons with lissencephaly. The responsible gene was mapped to chromosome Xq28, and it is thought that, due to lyonization, the double cortex phenotype reflects chimerism with two neuronal popula-

tions, one normal and the other migration-defective. The lissencephaly in affected boys is due to the presence of a neuronal population in which all cells are affected. This model also suggests that the defect is cell-autonomous. The gene associated with the double cortex malformation has been cloned and named *doublecortin*. It does not show obvious similarity to any other gene [14, 15]. Interestingly, a rat cortical migration disorder, named "tish" (telencephalic internal structural heterotopia), apparently inherited as a recessive trait, is associated with seizures and seems to be a model of the "double cortex" malformation [42].

Studies of the development of reelin-producing cells in the human marginal zone [43] suggest that, in addition to early generated, classical Cajal-Retzius cells, reelin-positive neurons are added from developmental week 12 to the end of the second trimester, apparently by differentiation of granule cells from the subpial granular layer (SGL). SGL neurons originate from the para-olfactory ventricular zones and migrate tangentially over the cortical surface. Since its initial description, the SGL has been implicated in the genesis of polymicrogyria [44, 45]. This suggests that a dysregulation of reelin expression may be involved in the genesis of polymicrogyria, in the sense that this pattern would perhaps not form in the absence of reelin. Conversely, the absence of reelin in humans may generate a lissencephalic cortex, an hypothesis that remains to be demonstrated, as no human *reeler*-like syndrome has been found thus far.

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