Smooth, rough and upside-down neocortical development

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Lissencephaly, which means 'smooth cortex', is caused by defective neuronal migration during development of the cerebral cortex and has devastating clinical consequences. 'Classical' lissencephaly seems to reflect mutations in regulators of the microtubule cytoskeleton, whereas 'cobblestone' lissencephaly is caused by mutations in genes needed for the integrity of the basal lamina of the central nervous system. Reelin, which is mutated in a third type of lissencephaly, may represent a unifying link because it encodes an extracellular protein that regulates neuronal migration and may also regulate the microtubule cytoskeleton.

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Abbreviations

ApoER2	ApoE receptor 2
CNR	cadherin-related neuronal receptor
DCX	X-linked doublecortin
ECM	extracellular matrix
FCMD	Fukuyama-type congenital muscular dystrophy
FKRP	Fukutin-related protein
LCH	lissencephaly with cerebellar hypoplasia
LDL	low-density lipoprotein
MEB	muscle–eye–brain
Nud	nuclear distribution locus
VLDLR	very low-density lipoprotein receptor
WWS	Walker–Warburg syndrome

Introduction

The human cerebral cortex is a highly folded sheet of six neuronal layers, each with characteristic histological and functional properties. These six layers are set up during embryonic development by the migration of neurons from the proliferative ventricular zone near the middle of the brain to the developing cortical layers near the surface (Figure 1a). At least 25 different human syndromes have been identified that disrupt this normal architecture and, with the increasing use of magnetic resonance imaging as a neurological diagnostic tool, this number is certain to increase [1].

A relatively common (~1 in 100,000 live births) congenital cortical disorder known as 'lissencephaly' is recognized by disruptions of the normal folding pattern of the cortex. At a microscopic level, lissencephaly is a surprisingly diverse disorder, although all lissencephalies share abnormalities of neuronal migration and the laminar architecture of the cortex. Lissencephaly syndromes also vary greatly in both severity in the neocortex and the involvement of other regions of the central nervous system, including the cerebellum, hippocampus and brain stem [2]. Our understanding of different lissencephaly syndromes is increasing rapidly with the emergence of greater clinical use of magnetic resonance imaging coupled with improved tools for human genetics. This review highlights some of the recent advances in the field of cortical development, focusing on the genes underlying human lissencephaly.

Classical lissencephaly is caused by genes that regulate microtubules

The importance of microtubule organization and dynamics to neuronal migration is underlined by two loci that cause 'classical' lissencephaly, *LIS1* and *DCX*. Hemizygous mutations in the X-linked doublecortin gene (*DCX*) [3,4] or heterozygous mutations in *LIS1* [5] produce similar abnormalities. The classical lissencephalic brain is characterized by a nearly complete absence of gyri (the technical term for the cortical folds), a severely thickened, histologically abnormal, four- layered cortex, and enlarged ventricles (Figures 1e and 2e).

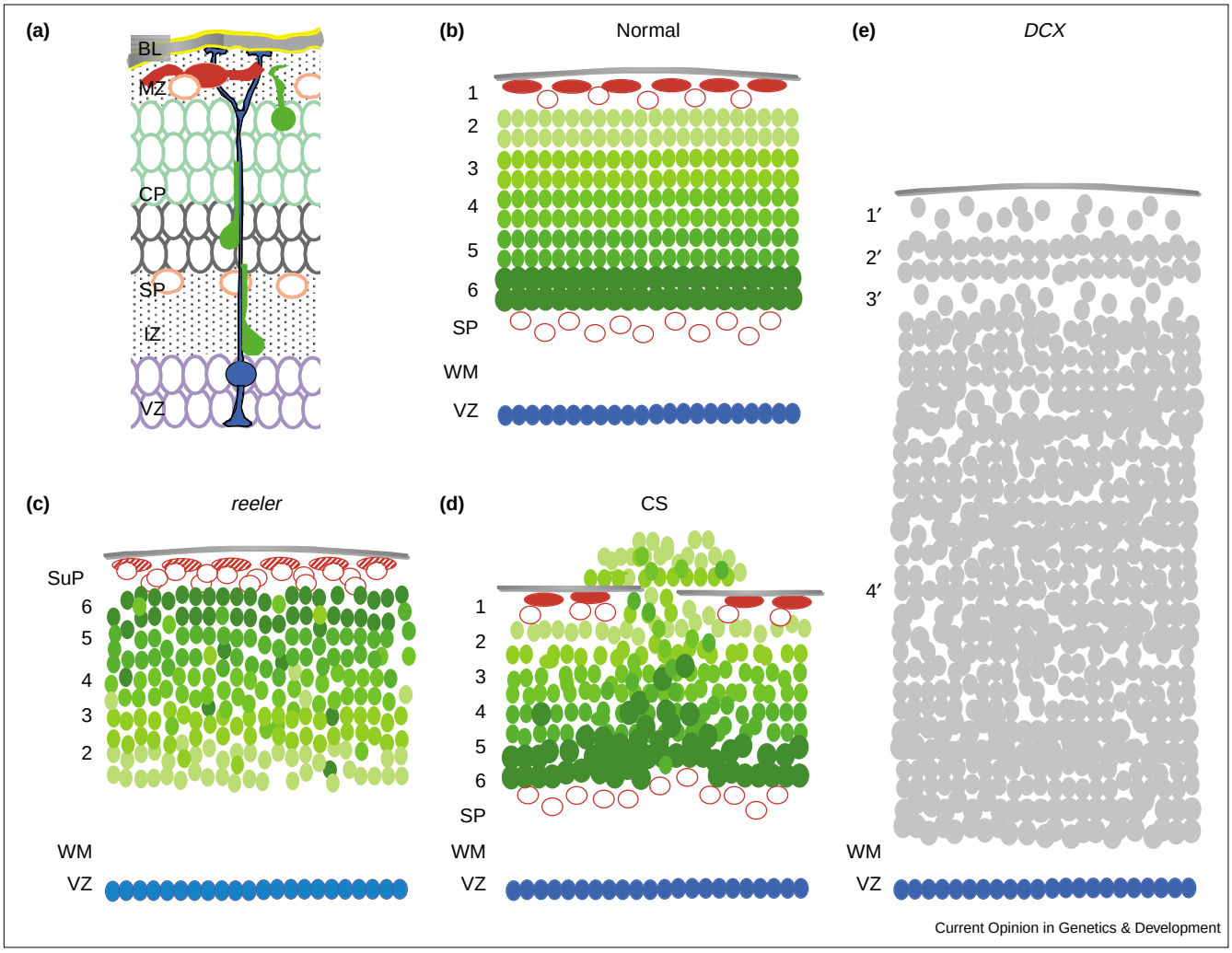
Although subtle differences between *LIS1* and *DCX* deficiency have been defined recently [6], these two genes both encode microtubule-associated proteins that are likely to function in the same biochemical pathway (Figure 3a) and that seem to interact physically [7•]. *DCX* encodes a novel microtubule-associated protein with a microtubule stabilizing function *in vitro* [8–10], whereas *LIS1* encodes PAFAH1b1 — a subunit of platelet-activating factor acetyl hydrolase [11], which also binds microtubules [12]. *LIS1* is homologous to *NudF*, a nuclear distribution locus (*Nud*) in the fungus *Aspergillus nidulans*, which is required for the distribution of nuclei along the multinucleate hyphae [13].

Intriguingly, the LIS1 protein interacts with the mammalian homologs of other Nud proteins (Figure 3a), including NudE [14•–17•] and NudC [18•]. This evolutionarily conserved complex [19•] seems to regulate microtubule dynamics by interacting with centrosomal components including γ-tubulin [15•] and the retrograde microtubule-based motor dynein (Figure 3a) [20,21]. Although several Lis1 functions have been identified [22,23], we remain mostly in the dark about the exact microtubule-based functions that the LIS1 protein complexes perform during the development of cortical layers, as well as the upstream signaling pathways that regulate them.

Cobblestone lissencephaly reflects abnormal extracellular matrix and basal lamina

A second form of lissencephaly, which was originally referred to as type II lissencephaly but is now called cobblestone cortex [2], results when neurons or neuronal precursors migrate out of the developing brain through breaches in the superficial neural basal lamina (Figures 1d and 2f). This aberrant migration produces bumpy neuronal

Figure 1

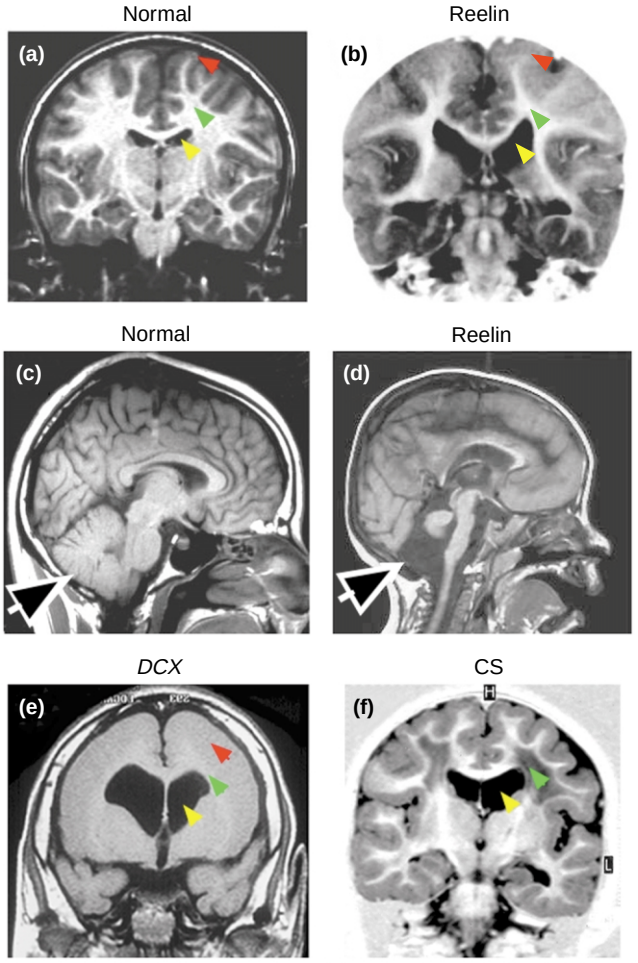


Histological defects of the neocortex that underlie different forms of lissencephaly. **(a)** Radial neuronal migration during cortical development. Neurons (solid green cells) migrate on a specialized elongated cell – the radial glial cell (solid blue cell) that spans the whole cortical wall from the ventricular zone (VZ) to the basal lamina (BL) of the pial surface, where the specialized glial endfeet terminate. Neurons migrate from the proliferative ventricular zone through the fiber-rich intermediate zone (IZ) into the developing cortical plate (CP). The process of migration here is understood poorly, but neurons arrest migration at the top of the cortical plate, immediately beneath the marginal zone (MZ) and the Reelin-expressing Cajal-Retzius cells (shown in solid red). Because newer layers of the cortical plate are added on top of older layers, this development is known as 'inside-out'. **(b)** The normal mammalian neocortex comprises six cellular layers overlying a band of white matter (WM). The cortical plate (green cells, layers 2–6) is sandwiched between layer 1 (upper red cells) and the subplate (SP; lower red cells). **(c)** Analysis of *reeler* mouse cortex shows that the cortical plate (green) develops beneath the subplate (now known as the superplate [SuP]). In addition, the cellular layering of the cortical plate is approximately inverted. In the *reeler* mouse the horizontally oriented Cajal-Retzius cells in the marginal zone (hatched red cells) do not express Reelin, a large secreted protein. **(d)** Description of cobblestone (CS) lissencephaly showing its two essential features: first, the basal lamina (gray line above layer 1) is broken; second, neurons have migrated through the breach and formed ectopic bumps on the surface of the brain. **(e)** Representation of classical lissencephalic cortex arising from a hemizygous X-lined doublecortin (*DCX*) mutation or heterozygous *LIS1* mutations shows a markedly thickened and simplified cortex with alternating bands of cell-sparse layers (1′ and 3′) and cell-dense layers (2′ and 4′). As the identity of these layers is not known the cells are shaded gray. In addition, the white matter is reduced.

'cobblestones' (ectopia) on the surface of the brain and is a feature of three distinct human disorders. Muscle–eye–brain (MEB) disease, Fukuyama-type muscular dystrophy (FCMD) and Walker–Warburg syndrome (WWS) are autosomal recessive disorders that encompass congenital muscular dystrophy, ocular malformations and cobblestone lissencephaly.

A specific allele of Fukutin, which is a predicted glycoprotein or glycolipid-modifying enzyme (Figure 3b) [24], underlies most cases of FCMD [25]; by contrast, MEB has been shown recently to be caused by mutations in *POMGnT1*, the gene encoding *O*-mannosyl-β-1,2-*N*-acetylglucosaminyl-transferase [26••]. POMGnT1 may glycosylate α-dystroglycan (Figure 3b), and this novel *O*-mannosyl glycosylation may

Figure 2



Magnetic resonance images of different lissencephaly syndromes with identified genetic causes. **(a–d)** Normal brains (a,c) show characteristic neocortical folding (gyri), which is simplified in the brains of individuals affected by mutations in Reelin (red arrows in b,d), who have lissencephaly with cerebellar hypoplasia (LCH). **(e)** Males hemizygous for mutations in X-linked Doublecortin (*DCX*) show classical lissencephaly, which is very similar to lissencephaly produced by autosomal dominant mutations in *LIS1* (not shown). Classical lissencephaly shows more severe agyria than LCH, a markedly thickened cortex (red arrows in [a,e]) and reduced white matter (green arrows in [a,e]). As in most lissencephalies, individuals with the *DCX* mutation show enlarged ventricles (yellow arrows in [a,e]). In addition to neocortical defects, individuals with LCH have a marked reduction in the size of the cerebellum (compare arrows in [c] and [d]). **(f)** Cobblestone (CS) lissencephaly is caused by *POMGnT1* or *Fukutin* mutations and is characterized by bumps of superficial ectopic neurons on the surface of the brain that are not normally resolved by magnetic resonance imaging. Radiographic findings of cobblestone lissencephaly show typically enlarged ventricles (yellow arrow) and reduced, aberrant white matter (green arrow). The term 'cobblestone cortex' is used increasingly to describe these cerebral disorders, because in many affected brains, such as this one, the cortex still has significant cortical folding (gyri and sulci). Panels (a–d) are reprinted with permission from [35•].

be required for α-dystroglycan binding to laminin, an extracellular matrix (ECM) protein [27].

Although Fukutin itself has not been shown to have enzymatic activity, the recently described Fukutin-related protein (FKRP) is a glycosyltransferase [28••], and individuals affected with FCMD have a deficiency in highly glycosylated α-dystroglycan [29••]. Therefore, both MEB and FCMD may be caused by a deficiency in the glycosylation of specific target proteins, including α-dystroglycan, which leads to a secondary deficiency in the basal lamina surrounding the developing brain. The cause of WWS is not known, but a recent analysis of 19 families with either WWS or MEB has indicated that these are distinct genetic and clinical disorders [30].

Normal mouse brain lacks gyri, so technically there are no murine lissencephaly loci. But some mouse mutants do show cortical migration defects with deficiencies in basal lamina integrity and also superficial bumps of neurons analogous to the cobblestone cortex observed in humans. Mice deficient in the laminin receptor, α_6 integrin [31], or in the ECM proteoglycan perlecan [32] show similar basal lamina breaches in the cortex and aberrant neuronal migration. The α_6 integrin phenotype can be exacerbated by mutations in another laminin receptor, α_3 integrin [33], which strongly implicates laminin-binding integrins in basal lamina integrity.

Studies with knockout lines of embryonic stem cells have shown that α-dystroglycan initially binds laminin to the surface of the cell and that integrins and perlecan are required for the subsequent assembly of laminins into large clusters [34•]. Cobblestone lissencephaly loci in mice and man may therefore identify steps in a common pathway that is needed for the correct assembly of laminin clusters in the neural basal lamina (Figure 3b). Little is known, however, about the structure of the basal lamina in the cortex, whether these basal lamina components and receptors act passively to organize neuronal precursors and/or to restrain migrating neurons, or whether these basal lamina components are dynamic and have active roles in signaling.

Reelin mutations in man and mice

Yet another form of lissencephaly is caused by mutations in the Reelin gene (*RELN*), and Reelin may represent a critical link that begins to connect the ECM with cytoskeletal regulation. Mutations in Reelin cause lissencephaly with cerebellar hypoplasia (LCH) in which the affected individuals show simplified cortical folding (pachygyria; compare Figure 2b,d with Figure 2a,c), which seems less severe than the near-complete lack of folding (agyria) caused by *LIS1* or *DCX* deficiency [35•]. Surprisingly, the cerebellum in LCH is much more severely affected (arrows, Figure 2c,d) than in classical lissencephaly, and individuals with LCH are severely ataxic, mentally retarded and suffer from epilepsy [36].

The human *RELN* gene is the ortholog of the extensively studied *Reln* gene in mice, which is mutated in the

naturally occurring neurological mutant *reeler* [37,38]. *Reeler* mice, which are named after their reeling gait, show abnormal cellular layering in the neocortex (Figure 1c), cerebellum and hippocampus. Although the histology of affected humans has not been characterized, the existence of pachygyria in these people strongly suggests that they have layering abnormalities. Thus, humans deficient in Reelin seem to share all of the main anatomical features of *reeler* mice.

Potential interactions between Reelin and ECM receptors

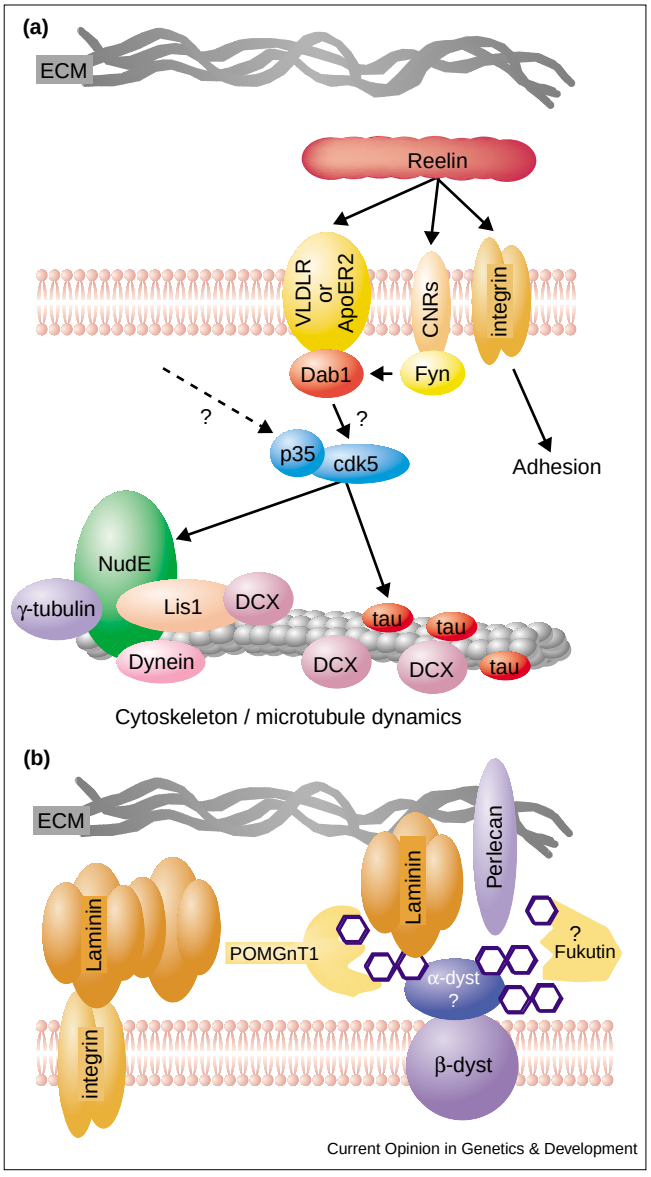
Given the involvement of integrins and integrin ligands in cobblestone cortex, it is intriguing that there are links between Reelin and integrins. Integrins comprise a large class of heterodimeric ECM receptors that consist of one α and one β subunit. Mice deficient in $\alpha_3\beta_1$ integrin show misregulation of Reelin protein and an excess of a 180-kDa amino-terminal fragment, which suggests that integrins may regulate proteolysis or clearance of Reelin [39•]. Immunoprecipitation studies indicate that $\alpha_3\beta_1$ integrin can bind Reelin and *in vitro* migration assays suggest that this binding may regulate the adhesion of neurons to glia [39•]. Recombinant Reelin induces migrating neurons to detach from radial glial cells *in vitro*, as do function-blocking antibodies against β_1 integrin [40]. This suggests that Reelin-dependent inhibition of integrin function may be required to detach the migrating neuron from the glial fiber.

When β_1 integrin is removed from the developing brain in a neural specific knockout mouse model [41••], the cortex shows abnormal clusters of Cajal-Retzius cells and a disordered cortical plate that is reminiscent of cobblestone cortex; however, unlike cobblestone cortex the neural specific β_1 integrin knockout does not show superficial bumps of ectopic neurons. Nor does the neural specific knockout show an obvious deficiency in neuron adhesion to the glia, because neurons migrate from the ventricular zone and form a 'wavy' but otherwise normally layered cortical plate. The neural specific knockouts of β_1 integrin show aberrant radial glial morphology [41••] as do *reeler* mice [42], which highlights a role for β_1 integrins in radial glial attachment to the neural basal lamina and in maintaining or remodeling of the basal lamina. Thus, although there are intriguing links between integrins, the basement membrane and the radial glial fiber, the precise mechanisms of Reelin integrin interaction is still not understood fully.

Potential Reelin signaling to the microtubule cytoskeleton

Recent work has identified a novel signaling pathway initiated by Reelin, and deficiency in several members of this pathway cause disorders of neuronal migration in mice. Reelin is predicted to be a 388 kDa secreted protein that is expressed strongly during corticogenesis by the Cajal-Retzius cells (Figure 1a, solid red cells) in the marginal zone adjacent to where new neuronal layers form [37,38]. Native Reelin forms a complex [43], and Reelin multi-merization may be required to initiate Reelin signaling. The antibody CR50, which blocks the function of

Figure 3



Possible biochemical interactions between neuronal migration proteins. (a) Reelin initiates signaling by binding protocadherins (CNRs) and members of the LDL superfamily (ApoER2 and VLDLR). Reelin binding may bring the cytoplasmic adaptor Dab1 into proximity with a non-receptor tyrosine kinase, possibly Fyn. Phosphorylated Dab1 may directly or indirectly regulate the serine/threonine kinase activity of p35/cdk5. Presumably cdk5 is regulated by other upstream cues as well as Reelin, and cdk5 phosphorylates several microtubule-binding proteins including tau and NudE to regulate the stability of microtubules. Reelin may also modulate neuron glial adhesion through interactions with the $\alpha_3\beta_1$ integrin and CNRs. (b) Diagram of a pathway that is essential for normal neural basal lamina structure and that may be perturbed in cobblestone lissencephaly. POMGnT1, the *O*-mannosyl glycosylase underlying muscle eye brain disease may glycosylate α-dystroglycan (α-dyst), which in turn permits the binding of laminin. Fukutin, a predicted glycosylase that is predicted to underly Fukayama-type muscular dystrophy, may perform a similar function and glycosylate α-dystroglycan. After the initial binding of laminins to dystroglycan, integrins and perlecan may facilitate the formation of larger laminin clusters that are required for normal basal lamina structure. The subcellular localization of Fukutin and POMGnT1 has not been characterized but is likely to be intracellular.

Reelin [44], specifically prevents Reelin multimerization [43]. Surprising new evidence shows that Reelin also has a serine protease enzymatic activity that cleaves laminin and fibronectin *in vitro* [45], raising the possibility that Reelin may modify the basal lamina directly. Reelin is itself processed proteolytically by an unknown zinc-dependent protease [46] but the activity of the resulting Reelin fragments is unknown.

To initiate signaling, Reelin binds members of the LDL receptor superfamily (ApoER2 and VLDLR) [47,48,49•] and members of the protocadherin superfamily (CNR1 to CNR8) [50•]. Complex formation leads to tyrosine phosphorylation of a cytoplasmic adapter protein Dab1 [51•] that is bound to the cytoplasmic tail of ApoER2 and VLDLR (Figure 3a). The brains of mice lacking both VLDLR and ApoER2 [49•], or Dab1 [52–54], are histologically indistinguishable from those of *reeler* mice (Figure 1b).

Downstream of the Reelin–receptor complex, the elements of the Reelin signaling pathway are less clear. Non-receptor tyrosine kinases phosphorylate Dab1 *in vitro* [55]. Although this phosphorylation is essential for Reelin signaling [56•], the identity of the specific kinase is unclear. One candidate kinase, Fyn binds protocadherins [57]; therefore, Reelin crosslinking of LDL receptors and protocadherins might directly assemble a phosphorylation complex (Figure 3a). In addition, LDL receptors bind the JNK-interacting proteins 1 and 2 [58•] — scaffold proteins that potentially link the Reelin receptors to mitogen-activated protein kinase pathways and to the microtubule motor kinesin [59•].

On the basis of phenotypic similarity, a probable component of the Reelin signaling pathway is the serine/threonine kinase cdk5 [60] and its activator p35 [61]. Mice that lack either cdk5 or p35 show inversions of cortical layering that are similar but not identical to the *reeler* phenotype, and studies with compound mutants of p35 and Dab1 indicate some genetic interaction [62•]. Because the many substrates of cdk5 include the Lis1-interacting protein NudEL [16•], as well as the microtubule-associated protein tau, cdk5 might connect Reelin signaling with other lissencephaly protein complexes such as the NudEL–Lis1 complex to control microtubule dynamics (Figure 3a). Notably, in *reeler* mice, or in mice lacking both LDL receptors, tau is hyperphosphorylated at two cdk5 sites [48], which suggests a link between Reelin, cdk5 and the microtubule cytoskeleton. Although a great deal of work remains to be done, the possibility of integrating many different neuronal migration proteins into a common pathway seems within reach.

Conclusions

The current catalog of neuronal migration mutants in mice and humans already presents a dizzying variety and is expanding rapidly. Although new mechanisms and pathways are likely to be discovered in the long term, in the short term further understanding of lissencephaly will come when the cell biology of Reelin, cdk5, and Lis1 signaling is more clearly described, and when the structure of the basal lamina of the developing cerebral cortex is clarified.

Update

A recent published study, using transgenic animals that express Reelin ectopically, suggests that appropriate Reelin localization may not be essential for the early development of the cortex [63•]. This study argues against simple models of Reelin signaling where Reelin acts either as an inhibitor or as an attractant to migrating neurons.

A second recent study showed that a splice variant of Dab1 called p45 can rescue Reelin–Dab1 signaling. However, unlike the wild-type allele p80, the p45 allele is haploinsufficient and neocortical and hippocampal disruptions are observed in the p45 heterozygote. The presence of later-born cortical neurons in the marginal zone of these p45 heterozygote animals supports the idea that Reelin signaling may be required for arresting some migrating cortical neurons [64•].

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