REELIN AND BRAIN DEVELOPMENT

Fadel Tissir and André M. Goffinet

Over the last 50 years, the reeler mutant mouse has become an important model for studying normal and abnormal development in the cerebral cortex and other regions of the brain. However, we are only just beginning to understand the actions of reelin — the protein that is affected by the reeler mutation — at the molecular and cellular level. This review discusses the most recent advances in this research field, and considers the merits of the various models that have been put forward to explain how reelin works.

Following their description more than 50 years ago, reeler mice were the only known animal mutants with malformations of the cerebral cortex. Understandably, they generated a lot of interest, which increased further after the cloning of the affected gene — reelin (Reln). As the reeler mouse model has been exhaustively reviewed, and the Reln signalling pathway has recently been discussed, we will not attempt to cover the field extensively, but instead will focus on recent findings that concern the role of Reln during brain development. Our understanding of the action of Reln remains sketchy, and we will try to point out caveats as well as advances in this field. After a presentation of the reeler malformation, we will summarize present views on the biochemistry of Reln signalling, and discuss observations and hypotheses about the putative mechanism of action of this interesting protein.

Reeler and reeler-like phenotypes

In rodents, Reelin deficiency results in the reeler phenotype, several alleles of which have been described in mice and rats. In humans, it is the cause of the Norman–Roberts type of lissencephaly. The human phenotype is only known from medical imaging, but it seems to be similar to its murine counterpart. In mice, mutations of Disabled 1 (Dab1) and double mutations of two lipoprotein receptors, very-low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor type 2 (ApoER2), generate similar phenotypes that will be referred to as reeler-like throughout this review.

In reeler and reeler-like mice, neurons are generated in germinative zones as in wild-type animals, and their migration is initially normal. However, as they approach their destination, reeler neurons fail to form normal architectonic structures. This is illustrated in Figs 1 and 2 for the embryonic cortex, and in Fig. 3 for the cerebellum and inferior olivary complex. It seems that there is an instruction at the end of migration that is lacking in reeler mutants, so that neurons do not recognize their proper location and orientation at the end of their migration pathway. The rest of the differentiation program is unaffected — normal neuronal classes are formed, dendritic trees and axons ramify and connect almost normally with their physiological targets, and gliogenesis and myelination are not directly altered. However, due to abnormal neuronal positioning, the dendritic trees and initial axonal pathways are often distorted. The defect is most severe in the cerebral cortex, hippocampus and cerebellar cortex, but subtle anomalies have been identified at every location that has been searched, including the inferior olive, olfactory bulb, cochlear nuclei, facial nerve nucleus, thalamus and tectum (reviewed in Ref. 3). Defects were also recently demonstrated in the retina and spinal cord.

Reelin, the protein that is defective in reeler mice, is secreted by some neurons, such as Cajal–Retzius cells in the cortical marginal zones and cerebellar granule cells, and it acts through the extracellular milieu on neighbouring target cells — cortical plate cells and Purkinje cells, respectively — to provide an architectonic signal. Reception of the Reelin signal requires the presence of at least one of two receptors of the lipoprotein receptor family on the surface of target cells, namely VLDLR and ApoER2. The signal is then transduced by tyrosine phosphorylation of the intracellular adaptor Dab1. In some
locations, such as the cerebral cortex, hippocampus, cerebellum and inferior olive, Reelin-producing and Reelin target cells are spatially segregated. In other regions, such as the retina and spinal cord, the source and target cells are close to each other or even mixed. In a few cases, for example in human Cajal–Retzius cells, the same cells are positive for Reelin and Dab1, implying that there is an autocrine loop. Intriguingly, the reeler phenotype is most evident when the source and target cells are segregated, and this might be relevant to the mechanism of action of Reelin. In addition to the brain, Reelin is also expressed at lower levels in peripheral organs, such as the liver, kidney and a few others. However, the function of the pathway in non-neuronal tissues is still largely unexplored.

**Reelin and its partners**

The Reelin gene is about 450 kb long and maps to mouse chromosome 5 and human 7q22. Two alternative splicing events have been found: a micro-exon of 6 nucleotides (exon 64), which encodes Val–Ser, is included in the neuronal mRNA but not in mRNA that is made in non-neuronal cells. The use of an alternative polyadenylation site in intron 63 produces an mRNA limited to exons 1–63 that codes for a protein that lacks a carboxy (C)-terminal region. Variability in the 5′ untranslated region (UTR) of the human RELN gene has been tentatively correlated with a genetic susceptibility to autism.

The Reelin protein is 3461 amino acids long. As schematized in Figure 4, the sequence begins with a signal peptide, followed by a region with similarity to F-spondin, a unique region and then eight repeats, each of around 350 amino acids. Each repeat contains an epidermal growth factor (EGF) motif at the centre, flanked by two subrepeats, A and B, which show weak similarity to one another. The protein terminates with a basic stretch of 33 amino acids, which are absent from the Reelin that is coded by alternatively polyadenylated transcripts. Although the predicted molecular mass of the Reelin polypeptide is 387,497 Da, the estimated size...
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Reelin can be studied using antibodies against N- and C-terminal epitopes\(^{29,30}\) (FIG. 4). The full-length protein predominates in the supernatant of transfected cells, but several other fragments are also consistently found. N-terminal antibodies disclose two fragments of around 180 and 320 kDa in length, whereas C-terminal antibodies disclose fragments of around 100 and 240 kDa. In adult and embryonic brain extracts and body fluids (such as cerebrospinal fluid or plasma\(^{31,32}\)), almost no full-length Reln is detected, and the main polypeptides are the N-terminal 180 kDa and the C-terminal 100 kDa fragments (N. Ignatova and A. G., unpublished data). Comparison with partial recombinant Reln constructs shows that Reln is cleaved at two main locations — between repeats 2 and 3, and between repeats 6 and 7 (arrows in FIG. 4). Unfortunately, there are no antibodies available to probe the central region. In embryonic brain explant cultures, Reln processing was blocked by zinc chelators but not by other proteinase inhibitors, implying that metalloproteinase activity is involved\(^{33}\). On the other hand, Reln cleavage is observed in different settings, implying that the processing pattern might reflect access of proteinases to exposed loops of the protein, whereas folding stabilizes other domains. The question of processing is not trivial, as the central region of Reln is essential for receptor binding and to trigger Dab1 phosphorylation (see later discussion).

Lipoprotein receptors: VLDLR and ApoER2. The VLDLR gene maps to mouse chromosome 19 and human chromosome 9p24. ApoER2, which is also named LRP8, maps to mouse chromosome 4 and human chromosome 1p34. The structure and biology of these receptors have been discussed elsewhere\(^{34,35}\), and only the features that are relevant to Reln signaling will be summarized here. The VLDLR and ApoER2 genes and proteins are similar to the low density lipoprotein receptor (LDLR) gene, although they are more similar to one another than to LDLR.

Box 1 | Reelin and susceptibility to psychoses

In addition to the well-established function of reelin (Reln) during brain development, some studies point to a possible role for the Reln gene in conditions such as schizophrenia\(^{36,37}\) and autism\(^{38,39}\). The human RELN 5'UTR contains a low and variable number of CGG repeats, and this has been tentatively correlated with genetic susceptibility to autism\(^{37}\). This association should be regarded as preliminary, however, as it was not confirmed by other studies\(^{40}\).

In brain samples from patients with chronic psychosis, Reln protein and mRNA levels were reduced by approximately 50%. There were no changes in the levels of disabled 1 (Dab1) mRNA and protein; this is unexpected, as a marked upregulation of Dab1 protein levels is observed in Reln- and lipoprotein-receptor-deficient mice. The proximal Reln promoter is CG-rich, and like other such promoters, methylation is associated with decreased expression. Therefore, it was proposed that hypermethylation of the promoter might be the origin of decreased expression in psychiatric patients\(^{33-35}\). Promoter methylation is known to influence long-term rather than dynamic regulation of expression, so this hypothesis needs to be investigated further.

Reln mRNA is expressed in a subset of GABA (\(\gamma\)-aminobutyric acid)-expressing cortical interneurons, indicating that downregulation of Reln expression might disturb the delicate balance of cortical excitability, although Reln-deficient mice have no overt epilepsy. Heterozygous reeler mice have a 50% reduction in Reln levels, and have some behavioural features that are evocative of human psychosis\(^{40}\). Given the difficulty of behavioural studies in mice, these results should be independently confirmed. Taken together, these findings can be tentatively interpreted within a neurodevelopmental/vulnerability 'multi-hit' model of schizophrenia\(^{100}\).

Figure 2 | Photomicrography of the normal and reeler telencephalon at embryonic day 14.5. 2 μm thick plastic section, stained with toluidine blue. Note the poor organization of the cortical plate (CP) and absence of a subplate (SP) in the reeler mouse cortex. CP, intermediate zone; MZ, marginal zone; PIA, pial surface; VZ, ventricular zone.
An allele that results in a reduction, but not the elimination, of wild-type levels of gene product or activity, often causing a less severe phenotype than a loss-of-function (or null) allele.

HYPOMORPHIC ALLELE

Specialized rafts that contain the protein caveolin and form a flask-shaped, cholesterol-rich invagination of the plasma membrane. They might mediate the uptake of some extracellular materials and are probably involved in cell signaling.

LIPID RAFTS

Cholesterol-rich lipid domains that are used to transport proteins around the cell and to organize signaling complexes on the membrane.

Figure 3 | The reeler phenotype in other brain regions. a | In the embryonic cerebellum, shown here at embryonic day 15.5, the reeler trait manifests as a poor organization of the Purkinje cell plate (PP). The external granular layer (EGL) is initially unaffected. Its inner region contains Reh-secreting cells, whereas Purkinje cells express Reh receptors and disabled 1 (Dab1). The rhombic lip (RL) is where the EGL is generated. b | The inferior olive complex (IOC) is populated with neurons generated in the rhombic lip that reach the ventral part of the hindbrain by tangential migration. After they reach their location, normal neurons (left), which are receptor- and Dab1-positive, form defined folds, presumably under the influence of Reh that is synthesized by surrounding neurons in the reticular (Ret.) formation. In reeler mice (right), olivary neurons reach the olivary anlage normally but fail to form a folded olive. PIA, pial surface.

VLDLR and ApoER2 bind ligands with widely divergent structures. For example, in egg-laying species, VLDLR is known as the vitellogenin receptor, and it plays a key part in the uptake of yolk precursors. Despite this variety, binding of all ligands requires the LA repeat region. Furthermore, in vitro, all ligand binding is displaced by receptor-associated protein (RAP), an intracellular protein that prevents the premature interaction of a subset of mammalian LDLR members with ligands in the endoplasmic reticulum, and which might act as a chaperone to assist in the folding of lipoprotein receptors in the endoplasmic reticulum. In accordance with their protein roles, VLDLR and ApoER2 are not solely expressed in the brain. In mice, VLDLR is most abundant in skeletal and heart muscle, where it might participate in triglyceride uptake, whereas ApoER2 is highly expressed in the testes and placenta. LDLR is expressed in many tissues, but mostly in the liver.

Reh can be endocytosed after binding to the surface of receptor transfected cells, but endocytosis is not required for phosphorylation of Dab1, which occurs at 4°C, a temperature that is incompatible with endocytosis. Tyrosine phosphorylation of lipoprotein-receptor-related protein (LRP), a cousin of VLDLR and ApoER2, occurs in caveolae and involves the platelet-derived growth factor receptor-β (PDGFRβ) and phosphoinositide 3-kinase. Together with the recent demonstration that ApoER2 receptors are localized to caveolae, these observations indicate that Reh signaling might occur at the level of lipid rafts (K. Nakajima, personal communication), although this remains to be confirmed.

Disabled 1 (Dab1). Targeted inactivation or spontaneous mutations of the Dab1 gene, such as in the scambler and yotari mutants, generate a reeler-like phenotype. Dab1 is an intracellular adaptor that is expressed in cells that respond to Reh, such as cortical plate or Purkinje cells. Through its N-terminal PI/PTB domain, Dab1 docks to an NPxY sequence in the intracellular region of VLDLR and ApoER2 (REF. 36), and becomes phosphorylated on key Tyr residues when Reh binds to its receptors.

The Dab1 gene, which maps to mouse chromosome 4 and human chromosome 1p32-p31 (REF. 44), has a similar and surprisingly complex organization in both species. The coding region is spread over more than 300 kb. The main Dab1 protein (FIG. 5) is 555 amino acids long, and is encoded in 15 exons. The sequence begins with an N-terminal PTB domain of around 180 amino acids, followed by a region that contains important Tyr residues (among which Y198 and Y220 are phosphorylated in response to Rehn45), and a 310 amino acid C-terminal region of unknown function. Alternative promoter use, polyadenylation and splicing generate several Dab1 isoforms. Most remarkably, the 5'UTR spreads over 900 kb, and it contains at least four different 5'UTRs and four associated promoters. One complex UTR, composed of combinations of ten different exons, is neuron-specific and developmentally regulated. Two internal protein-coding exons named 555 are consistently co-expressed in non-neural tissue and in stem cells in the ventricular zone, but are excluded from Dab1 mRNA in parallel with neuronal differentiation. The expression patterns and functional implications of different Dab1 isoforms, and the origin of this highly complex genomic organization, remain to be determined.

The fact that the tyrosine phosphorylation of Dab1 is essential for signalling was elegantly shown using knock-in mice in which the endogenous locus was replaced by normal or mutant Dab1 complementary DNA (cDNA) sequences. Mice that express a cDNA in which five important Tyr residues are replaced by Phe have a reeler-like phenotype. Reciprocally, most but not all features of the Dabl mutant phenotype are rescued by replacing the Dab1 gene by a partial Dab1 cDNA that codes for the PTB domain and the region with the Tyr residues, thereby generating a hypomorph of Dab1. So, tyrosine phosphorylation is essential but not sufficient for Dab1 function. The C-terminal region serves some unidentified function that might be related to the presence of consensus serine/threonine phosphorylation sites, some of which are substrates of the cyclin-dependent kinase 5 (Cdk5)/p35 kinase.
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Figure 4 | Schema of the reelin protein. The sequence begins with a signal peptide of 27 residues, followed by a region with similarity to F-spondin (segment ‘SP’, amino acids 28–190). A unique region (segment ‘H’) between amino acids 191 and 500 is followed by eight repeats of about 350 amino acids (repeat 1, residues 501–860; repeat 2, 861–1220; repeat 3, 1221–1596; repeat 4, 1597–1947; repeat 5, 1948–2314; repeat 6, 2315–2661; repeat 7, 2662–3051; repeat 8, 3052–3428). Each repeat contains an epidermal growth factor (EGF) motif at the centre, flanked by two subrepeats, A and B, which show weak similarity to each other. The protein terminates with a stretch of 33 amino acids that is rich in basic residues (+3429 to +3461,+). The epitopes recognized by antibodies 142, G10, CR50, 12 and 14 are shown, and the two arrows point approximately to the sites of processing in vivo. Adapted, with permission, from Nature REF. 2 © (1995) Macmillan Magazines Ltd, signal peptide.

Unravelling the network

The dissection of the Reelin signalling network is proving to be confusing, because it does not seem to conform to known models. However, progress was recently made on two fronts — the interactions between Reelin and its receptors, and the characterization of tyrosine kinases that have been implicated in Dab1 phosphorylation.

Reelin-receptor interactions. The N-terminal moiety of Reelin does not bind to the VLDLR or ApoER2 receptors29, but nevertheless there is evidence that the N terminus contributes to signalling. The function-blocking CR50 antibody, which is directed against an N-terminal epitope, interferes with the binding of Reelin to its receptors21 and inhibits aggregation of the protein — a process that is considered to be important for Reelin’s functional activity20,30.

To find out how Reelin interacts with its receptors, binding of partial Reelin recombinant proteins to extraacellular receptor regions was studied in vitro. The same recombinant proteins were also assessed for their capacity to stimulate Dab1 phosphorylation in primary neuronal cultures31. Proteins comprised of the N-terminal region, the first four repeats and part of repeat 5 do not bind significantly, confirming that the N-terminal moiety of Reelin is not directly involved31,32. Proteins that contain one, two or three repeats fail to bind detectably to VLDLR and ApoER2. By contrast, proteins comprised of repeats 3–8 or repeats 3–6, and a Reelin mutant with its 388 N-terminal amino acids deleted, all bind to VLDLR and ApoER2 with an affinity that is comparable to full-length Reelin. The ability of recombinant proteins to bind to receptors and stimulate Dab1 phosphorylation is correlated. These experiments indicate that at least four repeats are necessary to activate the Dab1 part of the signal, and that the repeats that are contained in the central region of Reelin are particularly important. It is interesting to note that this region corresponds to the central fragment that is generated by proteolytic processing32.

These results seem to contradict the observations that the N-terminal region is important for Reelin function31,32,33. However, this discrepancy could reflect differences between the in vivo and in vitro situations. In vivo, CR50-sensitive Reelin aggregation through the N-terminus30,35 could increase the effective Reelin concentration, thereby recruiting a larger set of receptors and signalling molecules, and enhancing signal efficacy.

Dab1 tyrosine phosphorylation — the elusive kinase.

Although tyrosine phosphorylation of Dab1 is known as a key step in Reelin signalling, Fyn has only recently been identified as the main kinase that is involved in this signalling event34–36. In vitro, the ability of the cytoplasmic tyrosine kinases Fyn, Src and Yes, as well as Abl, to phosphorylate Dab1 was shown at the time of cloning of the Dab1 gene37. Fyn can be co-immunoprecipitated with Dab1, Reelin and receptors from embryonic brain extracts (Y. Jossin and A.G., unpublished data). Defective Reelin signalling is marked by an increase in Dab1 protein levels and a decreased ratio of phosphorylated to unphosphorylated Dab1 (ref. 42). Similar modifications are seen in mice with different dosages of the Fyn, Src and Yes genes. The data indicate that Fyn is the main Dab1 kinase in vivo, followed by Src and to a lesser extent by Yes, whereas Abl does not play a significant part.

How the Fyn and other cytoplasmic tyrosine kinases are recruited to the signalling complex is not known, although an appealing model has implicated protein-tyrosine kinase receptors of the CNR family38. CNR1 binds Fyn in its cytoplasmic tail and was thought to bind the N-terminal region of Reelin in its extracellular domain, thereby enabling Reelin to bring Fyn into the receptor complex. However, the binding of Reelin to CNR1 has not been confirmed. Furthermore, the observation that Dab1 phosphorylation can be stimulated by a Reelin molecule that lacks an N terminus argues against this mechanism. Recent studies39–41 indicate that Reelin might assemble a prepared complex that regulates the proximity of Fyn to Dab1, so that Dab1 induces Fyn activation and is phosphorylated in return. Fyn might be activated by the interaction of Dab1 with its SH3 domain, or after tyrosine phosphorylation of Dab1 with the Src SH2 domain. A similar model has been invoked for the tyrosine phosphorylation of the T-cell receptor by the Src-family kinase Lck, which is associated with and activated by clustering with CD4 (ref. 57).

An objection to the proposed key roles for Fyn and Src is raised by the observation that neither Fyn+/−, Src+/−, nor Fyn+/−;Src+/− double mutants, possess a reeler-like phenotype42–45, possibly due to functional redundancy. Fyn+/−;Src+/− double mutants die perinatally and have not been studied. In favour of redundancy, there is preliminary evidence that a reeler-like malformation can be induced in brain slice cultures by PP2, a wide spectrum inhibitor of Src family kinases (Y. Jossin and A.G., unpublished).

In addition, the Reelin pathway might interact with another signalling complex (FIG 5). Proteoglycans, such as the heparan sulphates proteoglycans and syndecans, modulate signalling by growth factor–receptor complexes46. Although Reelin is not a proteoglycan, its large size indicates that it could have a somewhat similar role, for example by providing several binding sites and concentrating growth factors locally. Furthermore, LRP modulates the PDGFR pathway47,48 and another related
molecule, LRP6, is a key regulator of Wnt signalling. The failure to identify such a 'missing component' among the many spontaneous or induced mouse mutants could be due to embryonic lethality or redundancy. This view is admittedly highly speculative, and the identity of this putative signalling system, which might be coupled to Reln, remains open to debate.

The action of Reln in the developing brain

The observations summarized earlier leave the question of what Reln is actually doing in the developing brain relatively untouched. Although our view is still fuzzy, we will discuss recent observations that point to a possible role for Reln in radial precursor cells, before ending with some speculations on the cellular action of Reln.

Reln and neural precursor cells

In man and rodents, Dab1 mRNA, as well as mRNAs for VLDLR and the ApoER2 receptor, are expressed in the ventricular zone—presumably in neuronal precursor cells. The Dab1 protein is detected in the same cells as Dab1 mRNA, but data on the VLDLR and ApoER2 proteins are sketchy. The relative intensity of VLDLR and ApoER2 mRNA hybridization in the ventricular zone differs among brain areas, as well as between developmental stages. These preliminary results indicate that precursor cells possess the machinery to respond to Reln. One target of Reln in the ventricular zone could be the future olfactory interneurons that follow the rostral migratory stream. Interestingly, it was shown that Reln facilitates the detachment of these interneurons from the stream, and promotes their migration to the olfactory bulb. Expression of Dab1 and Reln receptors in the ventricular zone agrees with the proposed role for Reln in the maturation or migratory capacity of neural stem cells. Reln assists in the formation of the radial glial scaffold in the dentate gyrus and promotes branching of radial glial cells in vitro through a Dab1-dependent mechanism, further pointing to a role for Reln signalling in radial precursor cells.

Unlike other species such as chick and crocodile, no Reln-positive cells are detected in the vicinity of the telencephalic ventricular zone in mammals, raising the question of the origin of the ligand that binds to the receptors in precursor cells. Reln secreted from Cajal–Retzius cells could bind to lipoprotein receptors on the radial processes of precursor cells that expand through the whole thickness of the tissue. The observation that Reln promotes branching of these radial processes concurs with this view, which also predicts that VLDLR and/or ApoER2 protein(s) should be present on radial processes in the marginal zone, close to Reln-producing cells. Alternatively, Reln or its active central fragment could diffuse from the marginal zone to the ventricular zone. In principle, these ideas should be easy to check. However, so far, it has proved difficult to detect extracellular Reln reliably. In addition, antibodies are not yet available to allow immunohistochemical studies of the central fragment of Reln and the lipoprotein receptors.

Given the potential links between Reln signalling, psychoses and Alzheimer's disease (boxes 1 and 2), the effect of this pathway on neural stem cells is potentially important, especially if it continues to operate in the adult brain and influence regeneration, as indicated by the observation that decreased Reln expression seems to influence granule cell dispersion in epilepsy.

Cell biological mechanisms of Reln action

Reln is present in the nervous system in all vertebrates, from lamprey, and even Amphioxus (G. Meyer, personal communication), to fish, Xenopus, reptiles, birds and mammals, and its expression is widespread. Comparative studies of Reln expression in the embryonic cortex indicate that Reln-positive Cajal–Retzius cells are present in the marginal zone in all amniotes, indicating that these cells are evolutionarily homologous. However, mammalian Cajal–Retzius cells are characterized by a striking amplification of Reln production, pointing to the spatiotemporal control of Reln expression as a key feature of cortical evolution. Like Cajal–Retzius cells, cerebellar granule cells synthesize large amounts of Reln in all species, and this is consistent with the cerebellar malformation that is observed in Reln-deficient mice and humans. On the other hand, the reeler malformation is subtle, and until recently had not been detected in many areas where Reln expression is known to be strong, such as the retina and spinal cord. The same contrast between high Reln expression and almost no alterations in reeler mice is even seen in some laminar structures, such as olfactory mitral cells. However, the parsimony principle dictates that a single molecular mechanism should explain all observations, until proven otherwise.
Box 2 | Reelin and tau phosphorylation: a link with Alzheimer's disease?

Alzheimer's disease (AD) is the leading cause of dementia in ageing populations. It is characterized by progressive brain degeneration and two key pathological findings: extracellular amyloid plaques composed of the amyloid peptide Aβ — a processing product from the amyloid precursor protein (APP) — and the presence of intraneuronal tangles made up of phosphorylated forms of the tau microtubule-associated protein. Hereditary forms of AD are due to mutations in APP or presenilin1 and 2 — two cofactors of APP proteolytic processing. Non-hereditary forms of AD are more frequent in carriers of the E4 allele of the apolipoprotein E (ApoE) gene.

Several intriguing putative links have been noted between AD and the reelin (Reln) pathway. First, ApoE is present in the brain and is a ligand for Reln receptors, and it might modulate Reln signalling. Second, APP and disabled 1 (Dab1) interact in yeast (REF. 101). Third, Cdk5 (REF. 102), in complex with its cofactors p35 and p39 (REF. 104), is a key tau kinase that can phosphorylate Dab1 (REF. 48). Cdk5 is essential for neuronal migration and has been implicated in AD (REF. 105). Fourth, defective Reln signalling is associated with increased tau phosphorylation. Conversely, activation of the signal results in a reduction of tau phosphorylation, and this is due to reduced kinase rather than increased phosphatase activity (REF. 106). Another intriguing finding is that increased phosphorylation in mice with defective Reln signalling is under the influence of modifier genes, one of which maps to chromosome 17 in the vicinity of the APP locus (REF. 107). Recent results (see figure) showed that, among the main tau kinases, Cdk5 activity is unaffected by Reln, whereas glycogen synthase kinase 3β (GSK3β) is inhibited by Reln through activation of protein kinase B (PKB) (REF. 108). Reln activates PKB by phosphorylation at S473, one of two sites that are crucial for activation (the other is T308). The activation of PKB inhibits the activity of GSK3β by phosphorylation at S9. The Reln-induced phosphorylation of PKB on S473 requires activation of phosphatidylinositol 3-kinase (Pi3K), and is Dab1-dependent. Although it is impossible to present an integrated view at this stage, these observations strongly imply that the Reln pathway has a role in AD pathogenesis.

In the reeler cortex, cortical plate cells keep a prolonged contact with radial glial guides and invade the marginal zone to pile up close to the pia. This led to the proposal that Reln could provide a ‘stop’ signal to neurons at the end of their migration pathway. Like the migration of other cells, neuronal migration proceeds by the extension of a leading edge, followed by progression of the nucleus in this cytoplasmic furrow (nucleokinesis). Leading edge extension requires actin polymerization/depolymerization, with formation of filopodia and lamellipodia in some cells, followed by consolidation of stress fibres. These processes are controlled by the small GTPases Cdc42 (filopodia formation), Rac (lamellipodia formation) and Rho (consolidation of stress fibres). Nucleokinesis depends on microtubule dynamics, as well as microfilaments.

Recent findings on radial neuronal migration have introduced an additional level of complexity into the ‘stop signal’ model. The new data indicate that radial neuroepithelial or glial cells are direct neuronal precursors and that early migrating neurons might use somal translocation rather than gliophilic migration. The latter mode of radial migration seems to become more important at later stages, when migration distances increase.

Clearly, the idea of a ‘stop’ signal requires formulation in cell biological terms. Although reeler mice have abnormal entorhinal–hippocampal connections (REF. 109), this is probably secondary to architectural disturbances, as Reln does not seem to influence leading edge extension or growth cone progression. However, Reln could negatively regulate nucleokinesis, and influence the position of the nucleus relative to processes and ramifications. The idea that Reln might act as an inhibitor of nucleokinesis could account for several observations. For example, in the tectum and cochlear nuclei, inhibition of the progression of neuronal nuclei by Reln might explain why, in reeler mutants, abnormally orientated neurons invade a cell-poor subpial zone. In the reeler spinal cord, spinal presynaptic neurons are in an ectopic position, close to the central canal. However, the reason is not a failure to migrate, but rather a late back-movement from outside to inside. This back-movement does not occur in normal animals, in which a Reln-positive layer is found deep in migrated presynaptic neurons, implying that Reln inhibits this back nuclear translation. In the reeler inferior olive, the long tangential migration from the rhombic lip occurs normally, but neurons seem to migrate too far, too close to the midline. This indicates that Reln secreted by the reticular formation might govern the location of the olivary neuronal nucleus and soma in relation to their processes.

On the other hand, some aspects of the reeler phenotype do not fit easily with this action on nucleokinesis. In the hippocampal formation, the reeler trait consists mostly of a poor lamination of the pyramidal cell layer and dentate granule cell layer, but this is due to failure of neurons to reach their respective layers rather than invasion of the marginal zone. In the reeler cerebellum, radially migrating Purkinje cells stop migration prematurely and fail to condense into a normal Purkinje cell plate. The defect in the molecular layer is due to the secondary degeneration of granule cells and not due to over-migration of Purkinje cells. Similarly, in the reeler facial nucleus, ectopic neurons settle in the tegmentum, and there is little or no overmigration towards the pia. The subtle malformations in the reeler retina are also not obviously explained by an effect on nucleokinesis.

Furthermore, an action of Reln as a direct negative regulator of nucleokinesis is difficult to reconcile with two important sets of observations. First, expression of the Reln cDNA under the control of the nestin promoter, which results in ectopic Reln expression in precursor cells in the ventricular zone, can partially but significantly rescue the reeler phenotype. It is difficult to imagine how Reln, in the ventricular zone, could directly influence the terminal migration of neurons that have already left it. However, it raises the possibility that Reln could make neurons responsive to an unidentified signal in the marginal zone.

A second question has arisen from studies of normal and Dab1-/- chimaeric mice. In the cerebral cortex of chimaeras produced by blastocyst injection, Dab1-/- cells
Figure 6 | Putative mechanism of action of Reelin. Only the proximal end of the reelin signalling pathway (Fig. 4) is known at present. Signal transduction is not understood, and the final effect of signal activation on target cells is also not clear. As explained in the text, reelin could influence the cytoskeleton and regulate nucleokinesis, as well as modulate expression of cell-cell interaction molecules on the cell surface. ApoER2, apolipoprotein E receptor type 2; Dab1, disabled 1; VLDAR, very-low-density lipoprotein receptor.

segregate from Dab1-/- cells, thereby forming two superposed cortical plates. Although this supports a cell-autonomous function for Dab1, a few normal cells were consistently located in an ectopic position in the abnormal cortex85. Similarly, in the cerebellum and hippocampus of chimaeric mice produced by morula aggregation86, a few mutant Purkinje and pyramidal neurons were found in ectopic positions. If Reelin directly determines the position of the nucleus, it would be predicted that all of the ectopically positioned cells would be deficient in Dab1. These observations imply that the response to Reelin requires collaboration among several target cells — a ‘community effect’86 — and points to the importance of surface molecule distributions, as already proposed long ago87 and emphasized again recently88. One could imagine a Reelin signalling network that provides instructions to migrating cells by modifying the synthesis of some surface proteins and/or directing them to specific cellular compartments (Fig. 6). Such a signal would be indirect, with a delay due to signal transduction and response of the target cell. This response is probably complex and might involve cytoskeletal components that regulate nucleokinesis, the upregulation or downregulation of cell adhesion molecules89, the routing of surface recognition components to specific surface domains, and probably other unanticipated elements.

Future prospects
To conclude, we would like to propose two broad questions that we consider to be important, and that should be amenable to analyses with the available technology. First, we need to understand better the factors that regulate Reelin expression, particularly in cortical Cajal-Retzius neurons. This will provide molecular insight into the complex problem of cortical evolution. The ever-increasing availability of genomic sequences allows the definition of conserved DNA segments in homologous genes — for example, Reelin and its partners — in different species. Presumably, such evolutionarily conserved sequences contain regulatory elements that could then be tested by gene electroporation in vivo or in vitro, and in transgenic mice.

Second, we should try to define the modifications in synthesis and/or distribution of cell-cell interaction proteins that result from Reelin signalling. Progress in cDNA subtraction techniques and cDNA microarray screening should allow the definition of some genes that are downregulated or upregulated by Reelin signalling. Better in vitro models for radial neuronal migration could be developed to study the role of different signalling pathways that control this delicate process and also to interfere with them. We can only guess what the answers to these key questions will turn out to be, but they will undoubtedly be exciting, and most probably unexpected.


This paper shows that a Dab1 null mutation can be rescued fully by a knock-in of the normal Dab1 cDNA, and partially by a partial cDNA that contains the PI3K/PI4K domain. This region encodes key Tyr residues, but not by a cDNA in which five Tyr residues are replaced by Phe.


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