Cortical development: **Receiving Reelin**

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Recent genetic and biochemical studies indicate that lipoprotein receptors are components of the neuronal receptor for Reelin, mediating the glycoprotein's essential function in cortical development. At least eight cadherin-related neuronal receptors may also play a part in this signalling system.

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The factors that guide the development of the neocortex remain a mystery, despite many decades of intense investigation. Recently, as in most other areas of biomedical research, there has been rapid progress in the discovery of genes required to execute the full program of corticogenesis. Without doubt the best studied of these genes is the one whose mutation leads to the mouse

phenotype highlights most of the major events in normal cortical development. The identification of the wild-type *reeler* gene product, Reelin, as a large secreted glycoprotein provided a significant boost to the field [1]. Now two families of cell surface proteins have been identified as likely receptors for Reelin [2–5], and the insights provided by these findings will undoubtedly accelerate the pace of discovery even further.

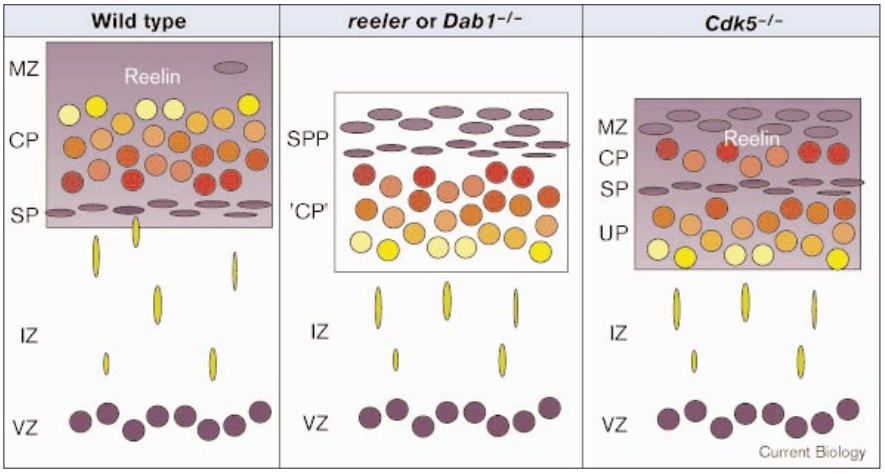
The normal neocortex originates from a thin layer of post-mitotic cells in the mantle of the early telencephalic vesicle (Figure 1, left panel). This layer is known as the preplate, and it contains the members of a primitive neuronal cell type, the Cajal-Retzius cell. During normal development, the cells that are destined to form the neocortex leave the ventricular zone, migrate into preplate and split it in two. The split results in a deep layer, known as the subplate, and a superficial layer known as the marginal zone. The Cajal-Retzius cells partition exclusively into the marginal zone, where they perform functions that are crucial for normal corticogenesis. Among these functions are the synthesis and secretion of the 400 kDa glycoprotein Reelin [1]. The importance of Reelin is illustrated by the phenotype of *reeler* mutant mice, where the Cajal-Retzius cells are unable either to make or to secrete a functional Reelin molecule [1,6]. In this situation, the migrating neocortical neurons are incapable of splitting the preplate and, instead, stack up beneath it in a broad band (Figure 1, center panel).

In the five years since the discovery of Reelin, there has been progress in identifying the intracellular signaling components that respond to its presence outside the cell. One member of this signaling pathway is Dab1, the mouse homologue of the *Drosophila* Disabled protein [7]. Dab1 appears to be a scaffolding protein that can interact with transmembrane glycoproteins, such as amyloid precursor protein and low-density lipoproteins, as well as some members of the Src tyrosine kinase family [7–9]. In the absence of Dab1 — as in mice with engineered mutations of the *dab1* gene or in the naturally occurring mutations *scrambler* and *yotari* — a phenotype indistinguishable from that of *reeler* mutants is found [10,11]. Although the kinase that is responsible for the modification is not known for certain, Dab1 is phosphorylated in the presence of extracellular Reelin, and its levels are higher in *reeler* mice, suggestive of a sensitization process [12,13]. Despite progress such as this in our understanding of the intracellular consequences of Reelin's presence, the extra-

The first substantial clue to the nature of the Reelin receptor came when the neurological consequences of two engineered mutations in the mouse were reported earlier last year by Trommsdorff *et al.* [2]. One of these mutations was in the gene encoding the receptor for low density lipoprotein (VLDL), and the other was in the gene encoding the type 2 receptor for apolipoprotein E (ApoE2). Each of the mutants was found to exhibit a mild but distinct central nervous system phenotype. The *vldlr*$^{-/-}$ mouse has a modest disruption in the structure of cerebral cortex and a significant cerebellar phenotype, with poor development of anterior lobules and large numbers of ectopic Purkinje cells. The *apoER2*$^{-/-}$ mouse has a complementary phenotype, showing significant displacement of neurons in the cerebral cortex but only modest defects in cerebellar structure.

More remarkable still was the observation that the double mutant — *vldlr*$^{-/-}$; *apoER2*$^{-/-}$ — has a phenotype indistinguishable from *reeler* or *scrambler* mice [2]. Both lipoprotein receptors have sequences that would enable them to bind Dab1 on their cytoplasmic tails, and the levels of Dab1 were found to be elevated in the double mutant, as they are in the absence of Reelin [8,13]. This led Trommsdorff *et al.* [2] to suggest that these two membrane proteins act in concert as components of the Reelin

Figure 1



The development of the cerebral cortex in wild-type and mutant mice. In wild-type mice (left), successive waves of migrating neurons populate the cortical plate (CP) in an 'inside-out' fashion, splitting the preplate into the subplate (SP) and marginal zone (MZ). The migrating cells are shown as yellow ovals, and where they stop to form the cortical plate the cells are represented by colored circles. In *reeler* or *Dab1*$^{-/-}$ mutants (middle), the migrating cortical plate cells appear incapable of splitting the preplate, which remains a single superficial layer, the 'superplate' (SPP). Mutant neurons stack up in inverted order with respect to their final cell division (indicated by the color of the cells). Note that, in the *Dab1*$^{-/-}$ mutants, normal quantities of Reelin are produced. Mice lacking Cdk5 activity (right) form cortices that are subtly different from those seen in *reeler* and *Dab1* mutants. The levels of Reelin are normal, but neuronal migration is disrupted nonetheless. The earliest emigrants from the ventricular zone (VZ) cross the intermediate zone (IZ), successfully split the preplate and assume an adult fate that is similar if not identical to the cells of cortical layer VI. Later immigrants, however, stack up *reeler*-like in an inverted layer that has been termed the underplate (UP).

receptor. This prediction has recently been validated by two biochemical studies [3,4]. D'Arcangelo *et al.* [3] used transfected cell lines producing one or the other receptor and directly measured their ability to bind Reelin. Hiesberger *et al.* [4] created chimeric receptors with the extracellular portion of the VLDL or ApoE2 receptor proteins coupled to the Fc portion of the immunoglobulin protein.

Although the two groups used different methodologies [3,4], they agree in concluding that the VLDL and ApoE2 receptors are necessary components of the Reelin receptor and calculate nearly identical binding constants. Nonetheless, they disagree on the exact configuration of the Reelin receptor. The controversy arises from the fact that an amino-terminal Reelin fragment, containing the part of the protein that binds CR-50, an antibody previously reported to block Reelin function, is incapable of binding VLDLR-Fc or ApoER2-Fc receptor chimeras [4]. This is surprising, as the expectation would be that CR50 blocks Reelin function by interfering with binding to its receptor. The location of the CR-50-binding epitope on Reelin is shown in Figure 2a. Hiesberger *et al.* [4] reason that this amino-terminal region of Reelin may bind to an unidentified co-receptor (see Figure 2b), while the carboxy-terminal repeat region of the protein may bind to the VLDL/ApoE2 receptors. They also speculate that the kinase activity that phosphorylates Dab1 may be provided by the as yet unidentified Reelin co-receptor (see Figure 2b).

D'Arcangelo *et al.* [3], however, found that the CR-50 antibody is capable of disrupting receptor binding, and subsequent phosphorylation of Dab1 in two different types of transfected cell — 293T and COS cells — producing the VLDL receptor [3]. This led them to conclude that a co-receptor is unlikely, as it would have to exist in two different cell types. One unifying hypothesis would be that Reelin undergoes a post-translational modification, requiring the carboxy-terminal region, that is necessary for VLDL/ApoE2 receptor recognition. The importance of the carboxy-terminal region of Reelin is demonstrated by the *Orleans* allele (*reln*^{Orl}). This mutation causes deletion of the last 205 residues of Reelin, and while the Cajal-Retzius cells are capable of synthesizing this truncated Reelin they are incapable of its extracellular export [6]. Sonic hedgehog is an example of an another protein for which a modification of the carboxy-terminal region is essential for its function [14].

Although two receptors might seem enough for any ligand, an entire family of cell surface molecules has now been proposed as additional Reelin receptors — members of the cadherin-related neuronal receptor (CNR) family [5]. The CNRs are produced specifically in neurons, and consist of six extracellular cadherin domains, a transmembrane region and carboxy-terminal intracellular sequences. The CNRs were initially identified through the interaction of their intracellular domain with Fyn, a member of the Src kinase family [15]. The CNRs are encoded at three genetic loci; at each locus there are 15–22 distinct first exons, only one of which is spliced at the RNA level to the first of three carboxy-terminal exons [16]. The mechanism of transcriptional regulation of these loci is currently not known.

The evidence suggesting that members of the CNR family might be Reelin receptors comes from the spatiotemporal expression patterns of *reelin* and eight CNR genes during mouse cortical development, as well as on

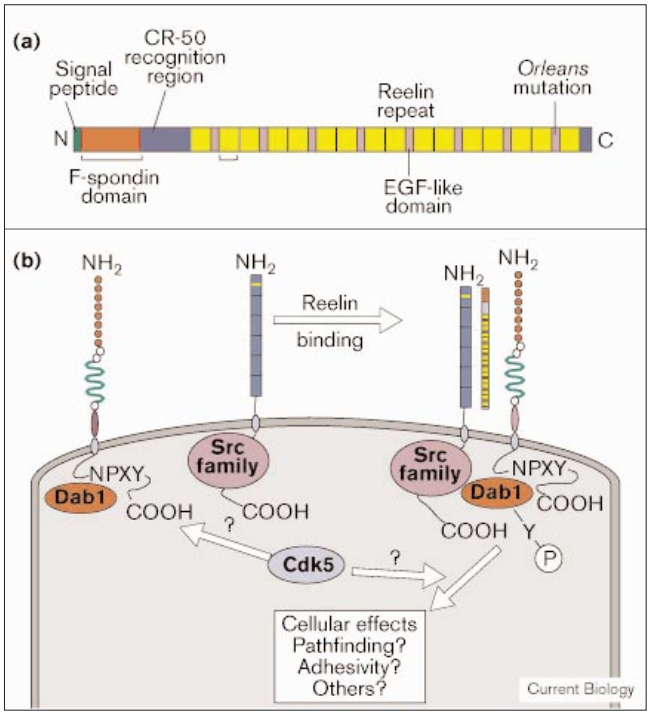
solution binding assays of chimeric molecules (similar to those carried out by Heisberger *et al.* [4] with the VLDL and ApoE2 receptors). Senzaki *et al.* [5] found that, in solution, Reelin appears to bind the first extracellular domain of CNR proteins, a domain which is highly conserved among the CNR family members used in the study. They found further that Reelin–CNR binding was disrupted by the anti-Reelin antibody CR-50 (which, as mentioned above, is known to block Reelin function). An additional piece of evidence that CNRs can transduce the Reelin signal is that an antibody against the Reelin-binding domain of the CNR protein disrupted Reelin-induced Dab1 phosphorylation in cultured cerebral cortical neurons [5].

The CNR-blocking antibody was raised against a region shown to be required for CNR binding to Reelin — specifically an RGD sequence. RGD epitopes are, however, common among many extracellular receptors, and there is concern that the observed effect of the anti-CNR(RGD) antibody on Dab1 phosphorylation may not be specific for CNR proteins. Furthermore, when the same anti-CNR(RGD) antibody was used in an assay in which developing cerebral cortical neurons were disassociated and reaggregated in culture, there was a disruption in the normal reaggregation pattern [5]. Reaggregates generated in the presence of the anti-CNR(RGD) antibody, however, were found to be structurally different from those found with the CR-50 antibody, or when *reeler* cerebral cortical neurons were used [17,18]. Overall though, while additional work is needed, the findings provide significant evidence to suggest that CNR proteins are acting as additional components of the Reelin receptor system.

How any of these potential receptors signals after binding Reelin on the cell surface is unclear. As outlined above, convincing lines of evidence demonstrate that VLDL/ApoE2 receptors are a component of the Reelin receptor. The inclusion of CNR as a Reelin co-receptor is an attractive suggestion, given that its intracellular domain has been shown to interact with Fyn, and this (or another) member of the Src family could be the kinase that phosphorylates Dab1. Dab1 was the first component of Reelin signaling to be discovered, and it is known to be phosphorylated in the presence of Reelin [12]. This post-translational modification might require that the two receptors be brought together in the presence of Reelin (Figure 2b).

It should be noted, however, that Dab1 has been reported to be phosphorylated normally even when numerous Src family members, including Fyn, are deleted individually (unpublished reports cited in [7]). Multiple kinases could be responsible for Dab1 phosphorylation, just as the ApoE2 and VLDL receptors are capable of replacing each other as components of the Reelin receptor. The lack of even a partial phenotype after deletion of individual Src

Figure 2



Reelin and models of Reelin receptor signaling. **(a)** Reelin is a large secreted glycoprotein [1] with a signal peptide (green), an F-spondin like domain (orange), and eight novel domains, known as Reelin repeats (yellow), each with an EGF like motif (purple). The site of the CR-50 epitope is indicated [6]. The *Orleans* deletion mutant results in the failure of Reelin export from the cell [6]. **(b)** The VLDL and ApoE2 receptors have a similar structure, including an NPXY domain capable of binding Dab1. The CNR proteins have six extracellular cadherin-like repeats (blue). The first EC repeat with its RGD sequence (yellow line) contains the Reelin-binding domain [5]. The CNR cytoplasmic domain is capable of binding Src family members [15]. Src family members can phosphorylate Dab1 [7], thus the idea that CNR and the VLDL/ApoE2 receptors form a receptor complex in response to Reelin is an attractive one. The similarities between the *Reelin*$^{-/-}$ and *Cdk5*$^{-/-}$ mutant phenotypes suggest that Cdk5 may play an as yet undefined role in Reelin signaling.

family members is still, however, curious. Furthermore, both VLDL and ApoE2 receptors are capable of internalizing Reelin in cultured non-neuronal cells in the presumed absence of CNR family members [3]. A complete picture of the Reelin response network must take into account the finding that loss of activity of the serine/threonine kinase Cdk5 also produces a *reeler*-like phenotype. *Cdk5*$^{-/-}$ mice have severe failures of neuronal migration in cerebrum, cerebellum and elsewhere (see Figure 1) [19–21]. It remains to be determined whether Cdk5 is involved in some way in Reelin signaling, and if so precisely how.

The biochemical mystery of Reelin action is paralleled by a biological one: what is the precise function of Reelin? Reelin might provide a 'stop' signal to migrating cortical neurons as they enter the cortical plate. It might also act as

a repellent to subplate neurons, thus accounting for the splitting of the preplate. But several lines of evidence argue that Reelin actually acts as a chemoattractant for migrating neurons. First, the protein is often produced at the destination site of the migrating neurons [1]. Second, cortical neurons are capable of binding Reelin, and Dab1 is phosphorylated when they do [2–4]. Finally, if external cerebellar granule cells expressing Reelin are placed in the fourth ventricle, from where the Purkinje cells normally migrate, many do not complete migration [18]. The suggestion is that migration of the Purkinje cells might be inhibited by their attraction to the Reelin-producing granule cells in the fourth ventricle.

There is, however, another possible function that Reelin might have. When embryonic neurons are disassociated and allowed to reaggregate, they normally have a stereotypical layering pattern. The absence of Reelin or the presence of the CR-50 antibody causes a disruption in this pattern, an observation that led to the hypothesis that Reelin regulates the adhesive properties of neurons [17,18]. Furthermore, the radial glia, thought to be used by many cortical neurons and Purkinje cells as a substrate for migration, are disrupted in both the cerebrum and cerebellum of developing *reeler* mice [22,23]. These various possible functions are not necessarily mutually exclusive. Reelin is a phylogenetically old protein that is found in many animals that do not have a neocortex, including turtles, chicken, lizards and *Xenopus* ([24,25] and A.M. Goffinet, personal communication). Reelin may have evolved over time to have a number of different functions.

A full understanding of the function of Reelin will require, among other things, a more complete picture of the patterns and mechanisms of neuronal cell migration. The rapid growth in the list of genes in which mutations perturb cortical cell migration have led to a new appreciation of the complexity of this process. For example, where once it was believed that all cells in the cerebral cortex are derived from the radial migration of cells found in the cerebral cortical ventricular zone, the phenotypes of *Dlx1* and *Dlx2* mutant mice have provided clear evidence that neurons of cortex that produce the neurotransmitter γ-amino-butyric acid (GABA) originate in the medial ganglionic eminence and migrate tangentially to the cerebral cortex during development [26].

The origin of the Reelin-secreting Cajal-Retzius cells is another example of the complexity of cortical cell migration. Presenilin-1 deficient mice lack Cajal-Retzius cells altogether, and additional evidence suggests that they are a mixed population with different sources, at least some of which migrate tangentially into the preplate from the ganglionic eminence [27,28]. Radial migration of neurons along radial glial guides is a common way for neurons to penetrate the cortical plate [29]. But the observations of Morest [30] and others, along with more recent evidence from the analysis of Cdk5-deficient mice [20], suggests that the earliest cortical plate neurons may translocate their nucleus within their own cell bodies, as opposed to using radial migration. Finally, a recent analysis using *in vitro* slice cultures supports the view that nuclear translocation is prominent within early precursors, while migration guided by radial glia is used by the later born cortical cells [31]. The mystery of cortical development thus remains a deep one, and while we have found many new pieces to the puzzle, we have not yet found the ways in which they fit together.

Acknowledgements

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