Go or Stop? Divergent Roles of Reelin in Radial Neuronal Migration

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Abstract

Neuronal migration is an essential step of brain development and is controlled by a variety of cellular proteins and extracellular matrix molecules. Reelin, an extracellular matrix protein, is required for neuronal migration. Over the past 10 years, the Reelin signaling cascade has been studied intensively. However, the role of Reelin in neuronal migration has remained unclear. Different Reelin fragments and different Reelin receptors suggest multiple functions of Reelin. In this review, the authors focus on Reelin effects on the actin cytoskeleton of migrating neurons.

Keywords

cerebral cortex, hippocampus, layer formation, actin cytoskeleton, cofilin

The mammalian cerebral cortex is a highly ordered structure composed of different classes of neurons that are arranged in a well-organized six-layered structure ranging from the pial surface to the white matter. During brain development, neurons are generated in the ventricular zone and migrate to their final destinations in the emerging cortical plate (Nadarajah and Parnavelas 2002). Defects in neuronal migration are involved in many neuronal disorders such as lissencephaly (smooth brain), epilepsy, mental retardation, and severe learning disabilities (Reiner and others 1993; Hong and others 2000; Lambert de Rouvroit and Goffinet 2001; Ayala and others 2007). Neuronal migration is precisely orchestrated by different signaling pathways; probably the best characterized signaling pathway controlling neuronal migration is the Reelin signaling cascade. After the discovery of the *reelin* gene in 1995 (D'Arcangelo and others 1995), extensive genetic, biochemical, and morphological studies have been carried out to determine the individual components of the Reelin signaling cascade. Reelin is an extracellular matrix protein secreted by Cajal-Retzius (CR) cells located in the marginal zone of the developing cerebral cortex (D'Arcangelo and others 1997; Alcántara and others 1998; Drakew and others 1998; Frotscher 1998). The Reelin signaling cascade includes two members of the low-density lipoprotein (LDL) family of lipoprotein receptors, apolipoprotein-E receptor 2 (ApoER2) and very low-density lipoprotein receptor (VLDLR; D'Arcangelo and others 1999; Hiesberger and others 1999; Trommsdorff and others 1999), the intracellular adaptor molecule Disabled 1 (Dab1) (Sheldon and others 1997; Howell and others 1997, 2000), and the Src family kinases Src and Fyn (Kuo and others 2005), most likely also $\alpha 3\beta 1$ integrins (Dulabon and others 2000; Schmid and others 2005) and cadherin-related neuronal receptors (CNRs; Senzaki and others 1999). Recent studies have shown that Crk, MAP1b, and Cullin5 are also involved in this signaling pathway (González-Billault and others 2005; Feng and others 2007; Park and Curran 2008). Binding of Reelin to ApoER2 and VLDLR induces tyrosine phosphorylation of Dab1 by Src and Fyn (Hiesberger and others 1999; Howell and others 2000; Keshvara and others 2001; Arnaud and others 2003; Benhayon and others 2003; Bock and Herz 2003).

It has been established that the Reelin signaling pathway is crucial for neuronal migration and positioning during corticogenesis. Thus, spontaneous mutation or targeted deletion of genes involved in the Reelin pathway were found to result in severe defects in brain architecture, especially in layered structures, such as the cerebral cortex, hippocampus, and cerebellum (Caviness 1982; D'Arcangelo and others 1995; Howell and others 1997; Sheldon and others 1997; Drakew and others 2002; Frotscher 1997, 1998; Trommsdorff and others 1999; Kuo and others 2005). The *reeler* mouse, a natural mutant lacking Reelin (Falconer

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1951; D'Arcangelo and others 1995), has been widely studied as a model opening insights into the molecular and cellular mechanisms governing neuronal migration and the lamination of cortical structures. Although many components of the Reelin signaling pathway have been identified and molecular interactions studied in some detail, the actual roles of Reelin in migration and the process of cortical lamination remain to be elucidated. The function of Reelin in the developing cortex has been difficult to ascertain because of the complexity of the *reeler* phenotype. Various models have been proposed based on the results of different experiments and observations. However, it has not been possible so far to develop a model that explains all the various defects of the *reeler* mutant or of mutants of the Reelin pathway, and different experiments have led to contradictory conclusions and concepts. In this review, we discuss these findings and elaborate divergent roles of Reelin in neuronal migration during corticogenesis.

Reelin Functions as a Permissive Factor during Early Corticogenesis

During early stages of corticogenesis, when the cerebral wall is relatively thin, early generated neurons migrate to their destinations via somal translocation, a relatively simple migration mode that is independent of radial glial fibers. During somal translocation, the extension of the leading process precedes the translocation of the cell body (Nadarajah and others 2001; Gupta and others 2002). The postmitotic neuron in the ventricular zone first extends a leading process directed toward the pial surface. Having reached the marginal zone, the leading process gives rise to several branches extending from a common branching point to attach the process to the extracellular matrix of the marginal zone and the basement membrane, respectively (Nadarajah and others 2001). The branched end feet of the leading processes in the marginal zone serve as traction sites for the subsequent translocation of the cell body (Gupta and others 2002) with the nucleus and perinuclear components being pulled through the elongated leading process. When the cell body reaches the common branch point, migration is terminated. Branching and anchoring of leading processes of migrating neurons to the marginal zone are essential steps for somal translocation. Loss of precise targeting of the leading process to its substrate may reduce the traction force and prevent somal translocation, eventually resulting in a failure of preplate splitting. In *reeler* mutants, the first wave of cortical neurons fails to split the preplate (Sheppard and Pearlman 1997), indicating that Reelin is required for early generated neurons to invade the preplate located in between CR cells and subplate cells. Almost all migrating neurons in the wild-type cortex have their long leading processes reaching deep into the preplate when they begin their journey in radial direction. In contrast, the leading processes of migrating neurons lacking Dab1 do not reach into the preplate but end underneath (Sanada and others 2004). In *reeler* mutants, the first wave of cortical neurons shows misoriented and less branched leading processes, which do not enter the preplate (Sanada and others 2004; Kuo and others 2005). In a stripe choice assay, glial fibrillary acidic protein (GFAP)–positive radial glial cells formed many more branches on Reelin-coated stripes than on control stripes (Förster and others 2002; Fig. 1). Similarly, it has been demonstrated that Reelin induces the branching of entorhinal fibers in the dentate gyrus (Del Río and others 1997). The hypothesis that Reelin induces branching was supported by a study showing that Reelin has a serine protease activity that cleaves laminin and fibronectin in vitro, indicating that Reelin may modify the basal lamina directly (Quattrocchi and others 2002). This may facilitate the branching and anchoring of the leading processes of migrating neurons to the preplate and the basement membrane, which is essential for subsequent nucleokinesis in migrating neurons. Together these studies indicated that Reelin may promote neuronal migration by instructing the end feet of the leading processes to move into the preplate, to induce their branching, and to attach them to the extracellular substrate, which facilitates somal translocation.

Direct evidence for Reelin to function as a permissive factor came mainly from two experiments: first, ectopic expression of Reelin under the *nestin* promoter in neuronal precursor cells of the *reeler* ventricular zone in vivo (Magdaleno and others 2002) and, second, incubation of *reeler* cortical slices from E13 in recombinant Reelin in vitro (Jossin and others 2004). Under these two different conditions, a major *reeler* phenotype could be rescued, the failure of preplate splitting that occurs in early corticogenesis when somal translocation predominates (Caviness 1982; Hoffarth and others 1995; Ogawa and others 1995; Sheppard and Pearlman 1997; Rice and Curran 2001). However, in both studies, another *reeler* phenotype, the inversion of cortical layers, which occurs in late corticogenesis when glia-guided locomotion predominates, could not be rescued. The results of both studies suggested that Reelin functions as a permissive factor for neurons undergoing somal translocation during early corticogenesis and that a specific location of Reelin is not critical for somal translocation to take place but may be essential for glia-guided locomotion.

When *reeler* cortical slices were incubated with recombinant Reelin (Jossin and others 2004), Reelin was ubiquitously distributed lacking a gradient. In situ hybridization and immunostaining showed that Dab1 and ApoER2

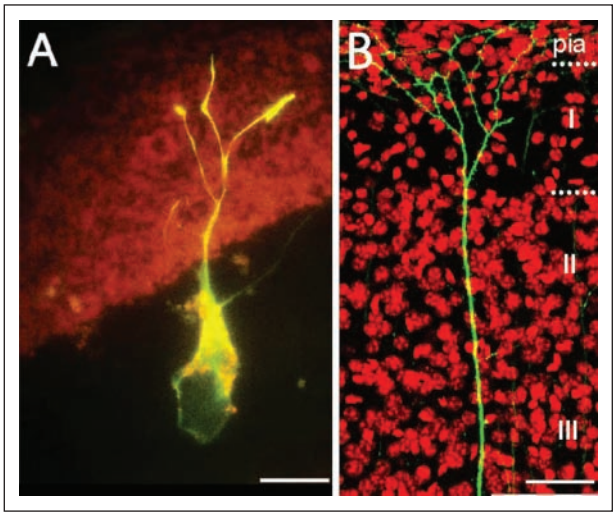

Figure 1. Reelin induces branching of glial cells and neurons. (*A*) Stripe choice assay stained for Reelin (red) and glial fibrillary acidic protein (GFAP; green). A GFAP-positive radial glial cell with its cell body located on a control stripe extends its main process to a Reelin-coated stripe (red) where it gives rise to several branches. Scale bar, 20 μm (from Förster and others 2002). (*B*) Cerebral cortex of a P14 Thy1–green fluorescent protein (GFP) transgenic mouse counterstained with propidium iodide (red). The apical dendrite of a layer V pyramidal cell (green), the former leading process of this neuron, branches intensely in layer I (the Reelin-containing marginal zone). Dotted lines demarcate layer I. Scale bar, 45 μm.

were expressed in the ventricular zone as early as at E11.5 (Rice and others 1998; Magdaleno and others 2002), indicating that the precursor cells are able to respond to Reelin (Tissir and Goffinet 2003). Ectopic Reelin in the *reeler* ventricular zone induced tyrosine phosphorylation of Dab1, indicating that the Reelin-Dab1 pathway is already active in the cells of the ventricular zone (Magdaleno and others 2002). Moreover, inhibition of Dab1 phosphorylation by the Src family kinase inhibitor PP2 (pyrazolopyrimidine) was found to induce a *reeler* phenotype in cortical slice cultures from E11 wild-type embryos, accompanied by a failure of preplate splitting (Arnaud and others 2003; Bock and Herz 2003). These results show that Dab1 is phosphorylated during early corticogenesis and indicate that phosphorylation of Dab1 is required for the permissive Reelin effect on migrating neurons and preplate splitting.

Divergent Roles of Reelin in Neuronal Migration during Late Corticogenesis

During late stages of corticogenesis when the cortical plate has formed and migration routes have become longer, neurons use glia-guided locomotion to reach their final destinations. Compared with somal translocation, the process of glia-guided locomotion appears to be more complex. At least four requirements are to be met for neurons undergoing glia-guided locomotion to reach their proper positions in the cortical plate. First, a radial glial scaffold needs to be established. Second, postmitotic neurons in the ventricular zone need to recognize this migratory substrate. Third, attractive and/or repellent signals are required to instruct the neurons to migrate toward the pial surface. Finally, migrating neurons are to be instructed when to detach from the radial glial fiber and stop their movement upon reaching their final destinations. During locomotion, the leading processes are motile; extension of the leading process and nucleokinesis occur almost simultaneously (Nadarajah and Parnavelas 2002). Late-generated neurons move most of the way through the cortex by locomotion and then detach from the radial glial fiber to switch to somal translocation. This way, they complete the final phase of their migration after the leading processes have reached the Reelin-rich marginal zone. They eventually position their cell bodies at the interface of the marginal zone with the top of the cortical plate (Nadarajah and others 2001; Nadarajah and Parnavelas 2002).

Is Reelin an Attractive Signal for Migrating Neurons before Their Leading Processes Have Reached the Marginal Zone?

In somal translocation, the extension of leading processes precedes nucleokinesis, and during translocation, the leading processes are anchored to the marginal zone. This suggests that the direction of migration is predetermined. During locomotion, the leading processes are not attached to the marginal zone but are free and motile (Nadarajah and Parnavelas 2002). However, both modes of migration show a clear directionality toward the cortical surface, suggesting that there are molecules in the superficial layers attracting them. Reelin is a very good candidate for such a function regarding its spatial and temporal expression patterns. This hypothesis is supported by observations in mutants of the Reelin pathway. In *reeler* mice, migrating cortical neurons extend their leading processes not toward the cortical surface as in wild-type animals but into various directions, and they fail to bypass early generated neurons and accumulate beneath the superplate, an abnormal cell accumulation formed in the absence of preplate splitting. Early generated neurons are found in superficial layers and late-generated neurons in deeper layers (Caviness and Rakic 1978; Tissir and Goffinet 2003), indicating that Reelin attracts cortical neurons to migrate past the subplate and the earliest cortical neurons of the cortical plate toward the Reelin-rich marginal zone.

The results of several recent studies employing molecular genetic approaches have also provided evidence that Reelin acts as an attractive factor for migrating neurons. The loss of Dab1 function or the expression of mutated Dab1 not only prevented neurons from reaching their proper positions but also markedly reduced their migrational speed (Sanada and others 2004). In addition, in utero electroporation of Dab1 shRNA at E18 caused neurons to lag behind their siblings, and their leading processes were less developed (Feng and others 2007). The leading processes of migrating neurons in *scrambler* mice, a natural Dab1 mutant, did not reach the preplate but accumulated underneath (Sanada and others 2004). In Dab1 mosaic chimeras, $Dab1^{+/+}$ cells migrated toward the Reelin-rich zone and formed a supercortex beneath the marginal zone, whereas $Dab1^{-/-}$ neurons formed a layer below the wild-type neurons (Hammond and others 2001), which implies that wild-type neurons have an affinity for a Reelin source near the pial surface. Together, these results argue in favor of not only a permissive function of Reelin but also an attractive effect. This attractive effect may be exerted by the various Reelin fragments. Reelin is cleaved into five different fragments, the smallest one being only 100 kDa (Lambert de Rouvroit and others 1999; Jossin and others 2004). It is reasonable to assume, but needs to be proven experimentally, that the smaller fragments diffuse more easily, thereby forming a top-down gradient from the marginal zone to the ventricular zone. This Reelin gradient may act as an attractant for neurons to migrate to the top of the cortical plate, thus enabling newly generated neurons to pass by earlier generated ones in the developing cortical plate. Indeed, Reelin fragments could be detected in the cortical plate by immunostaining (Jossin and others 2007). However, immunostaining might not be sensitive enough to detect low protein levels in deep portions of the developing cortex, intermediate zone, and ventricular zone. To confirm that Reelin does diffuse into deeper portions of the cerebral wall during development and form a gradient, Western blot analyses for Reelin in the different zones of the embryonic cortex—for example, the marginal zone, cortical plate, intermediate zone, and ventricular zone—have to be performed. Another possibility is that the attractive effect of Reelin on migrating neurons is indirectly exerted by radial glia, which is used as a scaffold by migrating neurons. Reelin in the marginal zone may modulate the density and distribution of adhesive molecules on the surface of radial glial fibers and thus instruct neurons to migrate toward the marginal zone. Several studies showed that the genes encoding for VLDLR, ApoER2, and Dab1, essential components of the Reelin signaling pathway, are expressed by radial glial cells (Förster and others 2002; Hartfuss and others 2003; Luque and others 2003) and that mutation of these genes resulted in radial glial defects (Weiss and others 2003).

Reelin Stops Migrating Neurons at the Marginal Zone

Upon reaching their destination, migrating neurons terminate the migration process and start to differentiate their definitive processes. The marginal zone, in which Reelin is highly expressed, is relatively cell free in wild-type animals but is densely populated by early generated neurons in the *reeler* mutant (Fig. 2). Incubation in Reelin-conditioned medium reduced the migratory activity of neurons and forced them to detach from the radial glia (Dulabon and others 2000). These observations showed that migrating neurons seemed to avoid Reelin-rich areas and suggested that Reelin acts as a stop signal for migrating neurons. Along this line, real-time imaging demonstrated that cortical neurons cease migration when they encounter Reelin in the marginal zone (Dulabon and others 2000). Migrating neurons entered the marginal zone when Reelin-producing CR cells were depleted, which resulted in a Reelin deficiency (Supèr and others 2000). Similarly, mutations involving molecules of the Reelin signaling cascade supported Reelin's function as a stop signal for migrating neurons. Thus, in mutants of the src family kinase Fyn, which is involved in Dab1 phosphorylation, some late-born neurons were found to overmigrate and invade the marginal zone (Kuo and others 2005). A single copy of the truncated *dab1* gene (Dab1-45) lacking the C-terminal region of Dab1 ($dab1^{p45/}$) caused the invasion of late-born cortical plate neurons into the Reelin-rich marginal zone of the neocortex (Herrick and Cooper 2002). Cullin5 binds to phosphorylated Dab1 and targets it for degradation, and Cullin5 knockdown in the cortex leads to increased Dab1 levels in vivo and a unique migration defect. Cullin5 knockdown neurons are located more superficially within the cortical plate relative to control neurons and partly invade the marginal zone (Feng and others 2007).

To stop the migration of neurons, two requirements are to be fulfilled: First, migrating neurons have to detach from the radial glial scaffold. Second, the cytoskeleton of migrating neurons needs to become stabilized. Evidence presented below suggests that Reelin does in fact contribute to both processes.

Reelin Induces the Detachment of Migrating Neurons from the Radial Glial Scaffold

In several studies, Reelin was assumed to function as a detachment factor. In an early electron microscopic analysis of the E17 *reeler* neocortex, postmigratory neurons

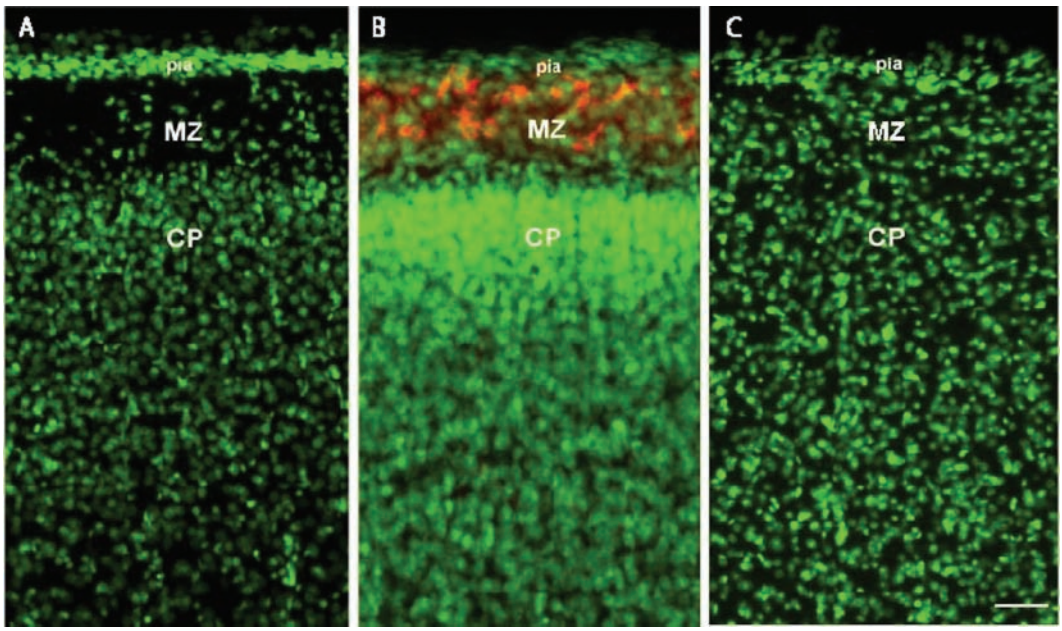

Figure 2. Reelin prevents neurons from invading the marginal zone. Cerebral cortices of (*A, B*) P2 wild-type mouse and (*C*) *reeler* mouse stained for Reelin (red) and Neurotrace (green). In layer I (marginal zone, MZ) of wild-type mouse, where Cajal-Retzius cells are located and Reelin is concentrated, only a few cells are seen. In contrast, the marginal zone of *reeler* is densely filled with cells. Scale bar, 50 μm. CP = cortical plate.

were found more closely associated with radial glial fibers than were wild-type neurons. This suggested differential adhesion properties of the migrating neurons to their radial glial fibers (Pinto-Lord and others 1982). In vitro aggregation assays demonstrated that embryonic cortical neurons in *reeler* mice are more adhesive to each other than their wild-type counterparts (Hoffarth and others 1995). Migrating neurons in the *scrambler* cortex remained attached to the processes of their parent radial glial cells during the entire course of radial migration, whereas wild-type neurons detached from the glial fiber in late migrational stages (Sanada and others 2004). In vitro migration assays showed chains of migrating neurons from the rostral migratory pathway to detach from each other and from the surrounding glia in cultures treated with Reelin (Hack and others 2002). This detachment function of Reelin is likely to depend on Reelin-α3β1 integrin interactions (Dulabon and others 2000).

Reelin Stabilizes the Cytoskeleton of Migrating Neurons

Neuronal migration is based on cytoskeletal dynamics that determine the speed of migration and coordinate components of the migration process, such as process extension and cell soma propulsion. These changes in cell shape require constant remodeling of the actin and microtubule cytoskeleton (Gupta and other 2002). Because Reelin is essential for neuronal migration, molecules that modulate cytoskeletal dynamics are likely to be targets of Reelin signaling in migrating neurons. Indeed, a few recent studies showed that Reelin signaling is linked to processes related to the reorganization of actin and microtubules in the cytoskeleton.

Cofilin is one of the actin-associated proteins that regulate actin polymerization and depolymerization via its actin-severing activity. The activity of cofilin is reversibly regulated by phosphorylation and dephosphorylation at Ser3, which occurs rapidly in response to various stimuli known to promote the reorganization of the actin cytoskeleton. The nonphosphorylated form is the active, actin-depolymerizing form of the protein, whereas Ser3 phosphorylation renders cofilin unable to depolymerize F-actin (Gungabissoon and Bamburg 2003; Moriyama and others 1996). An appropriate balance of Ser3 phosphorylation of cofilin is required for proper neuronal migration (Kawauchi and others 2006). Indeed, cofilin was found involved in cortical neuronal migration in vivo (Bellenchi and others 2007). Minamide and others (2000) showed that radial migration and layer formation are affected in *n-cof*$^{fl/fl,nes}$ embryos. Immunolabeling for p-cofilin revealed a much stronger staining in the Reelin-rich

marginal zone than in other cortical layers, and in *reeler* mutants, p-cofilin was down-regulated (Chai and others 2009). Exposure of cortical neurons from E17.5 *reeler* embryos to recombinant Reelin dramatically increased cofilin phosphorylation, mediated by Dab1 and LIM-kinase. Moreover, live imaging showed that Reelin inhibits the motility and extension of processes of dissociated neurons, likely by stabilizing the actin cytoskeleton (Chai and others 2009).

Another main cytoskeletal component is the microtubule, which controls the movement of the nucleus toward the centrosome. This mechanism, called nucleokinesis, is an essential step in neuronal migration. Microtubule-associated proteins (MAPs) are typically involved in controlling the microtubule-mediated physical interactions between the nucleus and the centrosome. MAPs stabilize microtubules, organize them into bundles, and connect them to membranes and intermediate filaments (Maccioni and Cambiazo 1995). The ability of MAPs to modulate microtubule dynamics is regulated by their phosphorylation (Huang and others 2004). The phosphorylation state of the microtubule-stabilizing protein tau is a well-established marker of microtubule stability. Hypophosphorylated tau binds to microtubules and stabilizes them, whereas hyperphosphorylation of tau leads to its dissociation from the microtubules and disruption of the axonal cytoskeleton (Merrick and others 1997). Genetic and biochemical studies have demonstrated that tau is one of the targets of the Reelin signaling pathway. Activation of the Reelin pathway leads to Akt activation and inactivation of GSK-3β, a well-known major tau kinase (Beffert and others 2002). In *reeler* as well as in *vldlr/apoer2* double mutant mice, the phosphorylation level of the microtubule-stabilizing protein tau was found dramatically increased, suggesting that a loss of the Reelin signal affects the assembly and stability of the neuronal cytoskeleton (Hiesberger and others 1999). Wild-type Dab1 also protects mice from tau hyperphosphorylation (Brich and others 2003). Thus, Reelin signaling may induce dephosphorylation of tau, which stabilizes the microtubule cytoskeleton of migrating neurons.

MAP1B is a neuron-specific microtubule-associated protein that binds to microtubules and actin microfilaments, contributing to their stabilization via a process that is believed to depend on type I MAP1B phosphorylation (Goold and others 1999). It has been shown that Reelin induces MAP1B phosphorylation through Gsk3β and Cdk5 activation.

Lis1 is one of the important MAPs involved in neuronal migration. Mutations in the human *lis1* gene cause a severe form of lissencephaly, named Miller-Dieker syndrome (Hattori and others 1994). Interactions between Lis1 and the Reelin pathway have been investigated in studies of compound mutant mice with disruptions in the Reelin pathway and heterozygous Lis1 mutations. Dab1 and Lis1 were found to bind in a Reelin-induced phosphorylation-dependent manner, thereby indicating molecular interaction. Loss of ApoER2 combined with Lis1 reduction resulted in a reeler-like phenotype, suggesting that Lis1 modulates Reelin signaling, being an important component of the Reelin pathway downstream of VLDLR (Assadi and others 2003).

Opposite Roles of Reelin in the Lamination of Dentate Granule Cells

Compared to the neocortex, the hippocampus shows a relatively simple cytoarchitecture. The principal cells, pyramidal cells and granule cells, form densely packed layers in the hippocampus proper and dentate gyrus, respectively. This contrasts with the neocortex, which is composed of six layers and a large variety of cell types. Thus, the hippocampus appears to be a useful model to study neuronal layer formation. In *reeler,* granule cells are not arranged in a compact cell layer but are loosely scattered throughout the dentate gyrus. In addition, the pyramidal layer in hippocampal region CA1 is doubled (Fig. 3). Incubation in recombinant Reelin partially rescued the *reeler* phenotype in the cortex and induced preplate splitting (Jossin and others 2004). However, incubation in recombinant Reelin did not rescue the *reeler* phenotype in the dentate gyrus (Zhao and others 2004). Intriguingly, when a *reeler* hippocampus was co-cultured with a wild-type hippocampus, the granule cells of the *reeler* dentate gyrus formed a densely packed cell layer in close vicinity to the Reelin-rich wild-type molecular layer (Zhao and others 2004, 2006; Fig. 3). These results strongly suggest that Reelin has to be present in a specific topical location to induce a proper granule cell lamination. Treatment with CR-50, a monoclonal antibody blocking Reelin function, abolished the rescue of granule cell lamination. Also, when *reeler* dentate gyrus was positioned next to stratum oriens of wild-type CA1 lacking Reelin, the granule cells remained scattered over the *reeler* dentate gyrus (Zhao and others 2004). These experiments strongly indicate that Reelin is the factor that induces layer formation of granule cells. Reelin is secreted not only by CR cells in the hippocampus and neocortex but also by mitral cells in the olfactory bulb and granule cell progenitors in the cerebellum. When *reeler* dentate gyrus was co-cultured to these Reelin-containing tissues, granule cell lamination in the *reeler* dentate gyrus was similarly rescued (Zhao and others 2006). Hippocampus, neocortex, olfactory bulb, and cerebellum are different with respect to their cell types and cytoarchitechture. A unique similarity of these different brain regions is that they all contain Reelin. This further confirms that it is Reelin that induces the layer formation of granule cells in the dentate gyrus.

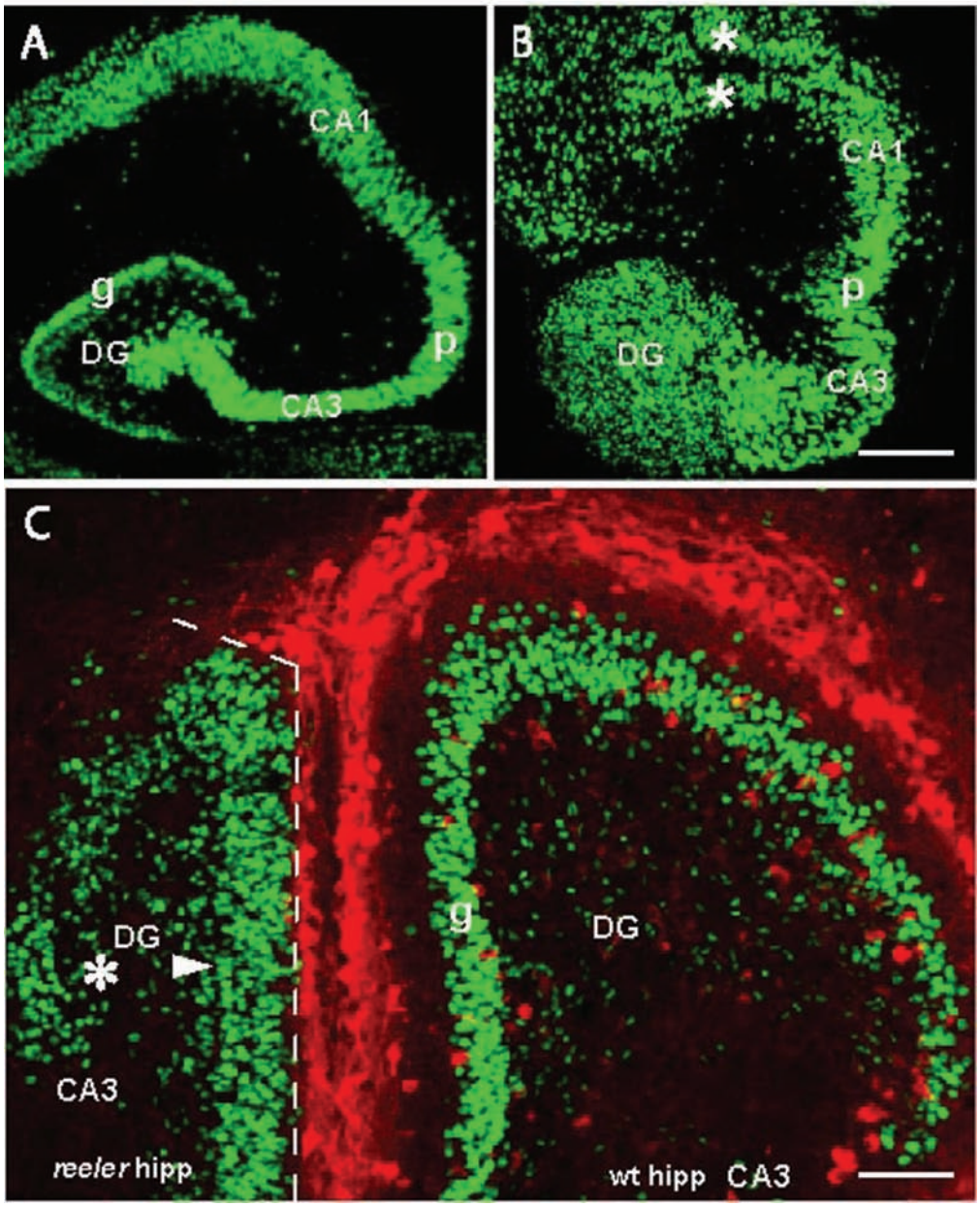

Figure 3. Rescue of the *reeler* phenotype in the dentate gyrus (DG) by wild-type co-culture. (*A, B*) Hippocampi of a (*A*) wild-type mouse and a (*B*) *reeler* mutant were stained with an antibody against NeuN, a neuronal marker. In the wild-type hippocampus, granule cells and pyramidal cells form densely packed layers (p = pyramidal layer; g = granule cell layer). In contrast, in the *reeler* mutant, granule cells are scattered over the entire dentate gyrus. In addition, pyramidal cells in CA1 form a double layer (asterisks). Scale bar, 150 μm. CA3, hippocampal region CA3. (*C*) Co-culture of *reeler* hippocampus with wild-type hippocampus stained for Reelin (red) and Prox1 (green), a specific marker of granule cells in the dentate gyrus. Dotted line indicates the border between the dentate gyrus of the *reeler* culture and the wild-type culture. Granule cells in the *reeler* dentate gyrus form a densely packed cell layer (arrowhead) close to the Reelin-positive outer molecular layer of the wild-type hippocampus. Note that cell density in the narrow region of the rescued granule cell layer (arrowhead) of the *reeler* dentate gyrus close to the Reelin-containing molecular layer of wild-type hippocampus is much higher than that in more remote positions (asterisk), indicating that most granule cells in the *reeler* dentate gyrus have moved toward the Reelin-containing wild-type dentate gyrus (compare with the loose distribution of the granule cells in *B*). Of note, *reeler* granule cells do not invade the Reelin-containing molecular layer of the wild-type dentate gyrus, suggesting that Reelin terminates the migration of granule cells. Scale bar, 60 μm (modified after Zhao and others 2004, with permission).

Looking carefully at the co-cultures of *reeler* hippocampus with wild-type hippocampus (Fig. 3C), we found that *reeler* granule cells moved from all areas toward the Reelin-rich zone of the wild-type dentate gyrus, resulting in fewer granule cells in remote regions and relatively more cells next to the wild-type dentate gyrus. However, *reeler* granule cells did not invade the Reelin-rich zone. These observations provide evidence that Reelin exerts opposite roles in the lamination of dentate granule cells: Reelin in the wild-type dentate culture first attracted *reeler* granule cells to move toward the wild-type dentate gyrus but then terminated their migration when their leading processes encountered the Reelin-rich zone of the wild-type dentate gyrus. These opposite roles of Reelin may be mediated by two different lipoprotein receptors, ApoER2 and VLDLR, which exert divergent functions in neocortical development (Hack and others 2007). To test this hypothesis, the hippocampus of single-receptor mutants has to be examined using specific markers of dentate granule cells.

Molecular Basis of Multiple Reelin Functions

Multiple Products of Reelin Processing

Reelin is a large extracellular matrix glycoprotein (>400 kDa) composed of three subdomains, the N-terminal F-spondin-like domain, the eight Reelin repeats (RR), and the short and highly basic C-terminal region (CTR; Tissir and Goffinet 2003). Reelin is secreted as a full-length protein, but it is then subjected to proteolytic cleavage at two sites, which produces five different fragments, two N-terminal fragments of approximately 180 kDa (N terminus to repeats 1 and 2) and 320 kDa (N terminus to repeats 1 and 6), a central fragment of approximately 120 kDa (repeats 3 to 6), and two C-terminal fragments of approximately 100 kDa (repeats 7 and 8) and 240 kDa (repeats 3 and 8) (Lambert de Rouvroit and others 1999; Ignatova and others 2004). Processing of Reelin is functionally important in vivo because inhibition of processing prevents signaling and perturbs cortical development in cultured embryonic brain slices (Jossin and others 2007); however, potential individual functions of these fragments have remained unknown. The different fragments are likely to have different diffusion properties. The full-length Reelin and larger fragments of Reelin may not diffuse over large distances but are anchored to the extracellular matrix in the vicinity of CR cells in the marginal zone, whereas the smaller fragments may diffuse into deeper portions of the cortex. Indeed, Reelin was detected among cortical plate cells with antibodies against the N-terminal region and central region but not with C-terminal-specific antibodies (Jossin and others 2007). This indicates that full-length Reelin is predominantly located in the marginal zone and that N-terminal and central fragments are both able to diffuse in the cortical plate after cleavage.

Binding of Reelin to Different Receptors Exerts Distinct Effects on Migrating Neurons

VLDLR and ApoER2 are components of the canonical Reelin signaling pathway. Mice deficient in both lipoprotein receptors have a phenotype indistinguishable from *reeler*, whereas mutations of either *vldlr* or *apoer2* generate subtle neurological phenotypes, indicating a redundancy (Trommsdorff and others 1999). Binding of Reelin to these two receptors induces tyrosine phosphorylation of Dab1 in neurons (Hiesberger and others 1999; Howell and others 1999; Benhayon and others 2003; Jossin and others 2004). However, Reelin associates more readily with ApoER2 than with VLDLR, and the level of Reelin-induced Dab1 phosphorylation was more severely reduced in neurons lacking ApoER2 than VLDLR (Andersen and others 2003; Benhayon and others 2003). In addition, VLDLR is selectively expressed in migrating neurons that are about to make contact with Reelin in the marginal zone. In contrast, ApoER2 is more ubiquitously expressed throughout the developing brain (Trommsdorff and others 1999). ApoER2, but not VLDLR, contains a unique insertion sequence of 59 amino acids in its cytoplasmic domain (Kim and others 1996) and binds to the family of JNK-interacting proteins (Stockinger and others 2000). Catalytic subunits of Lis1 specifically bind to the NPxYL sequence of VLDLR but not to ApoER2 (Zhang and others 2007). Differences in the expression pattern, different cytoplasmic binding domains, different affinities to Reelin, and a different potential to induce Dab1 phosphorylation suggest distinct physiological functions for these two receptors in neuronal migration. Indeed, single *vldlr* mutants and *apoer2* mutants show different phenotypes. The malformations in *apoer2* mutants are predominantly in the neocortex and hippocampus, whereas those in *vldlr* mutants mainly affect the cerebellum (Trommsdorff and others 1999). In *vldlr* mutants, the layering of the cerebral cortex is largely normal. However, many more neurons are found in the marginal zone, in which Reelin is highly expressed (Hack and others 2007; Fig. 4). These results indicate that VLDLR is involved in the inhibitory effect of Reelin on migrating neurons. Binding of Reelin to VLDLR prevents the neurons from invading the Reelin-rich marginal zone. In contrast, in *apoer2* mutants, only a few cells are detected in the marginal zone, comparable to the situation in wild-type animals (Fig. 4). However, in *apoer2* mutants, a large portion of late-generated neurons

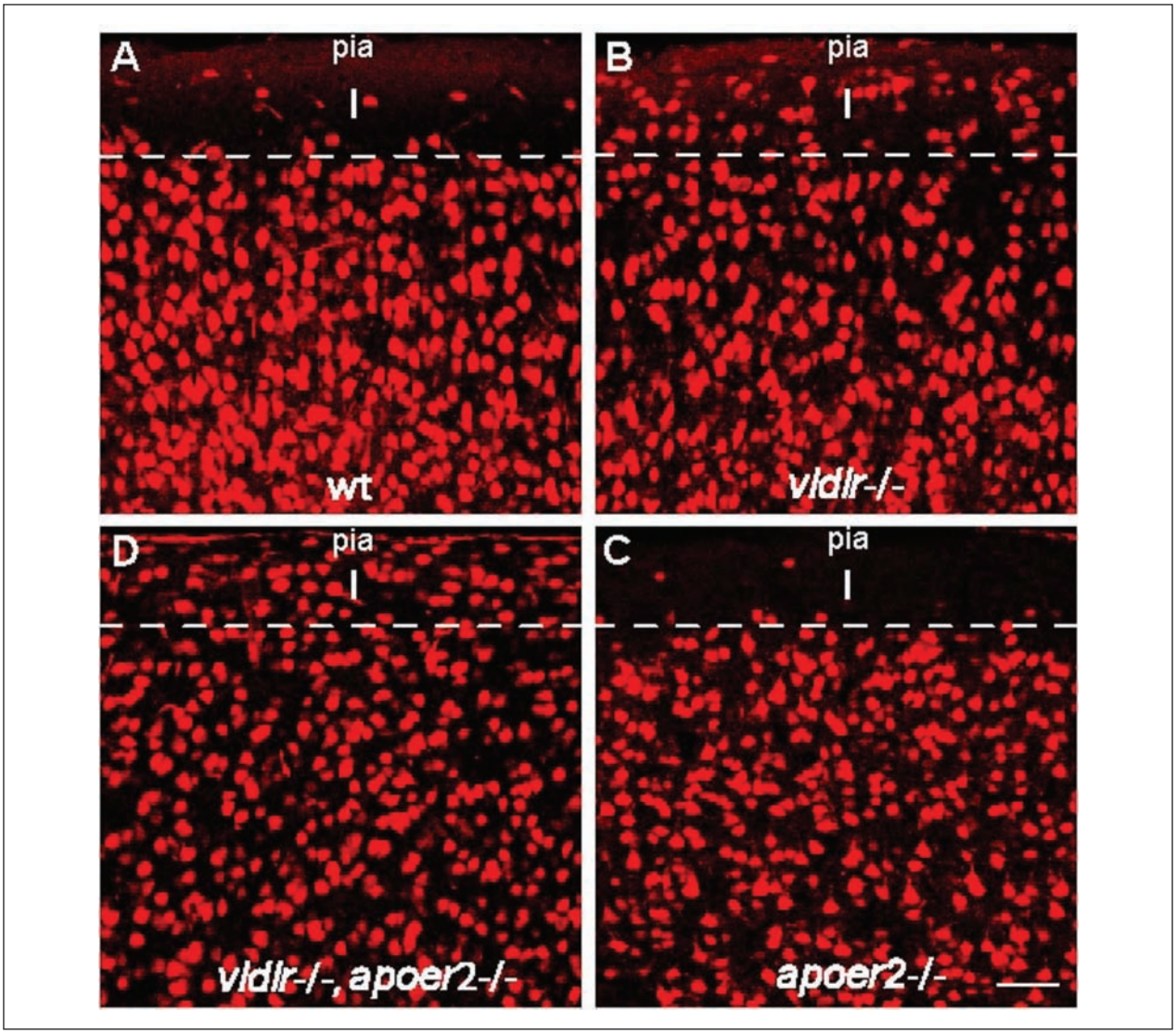

Figure 4. Very low-density lipoprotein receptor (VLDLR) is involved in the inhibitory function of Reelin, preventing the invasion of migrating neurons into layer I. Cerebral cortices from adult mice of different genotypes were stained with an antibody against NeuN. Dotted lines indicate the border between layer I and II. In the (*A*) wild-type mouse and the (*C*) *apoer2* mutant, only a few NeuN-positive cells were found in layer I (Reelin-containing marginal zone). In contrast, numerous NeuN-positive cells were seen in layer I of a (*B*) *vldlr* mutant and a (*D*) double-knockout mouse lacking both *apoer2* and *vldlr*, indicating that a loss of VLDLR leads to the invasion of neurons into the Reelin-containing marginal zone during development. Scale bar, 50 μm.

cannot complete their migration and are found in deeper layers, resulting in a partial inversion of cortical layers (Benhayon and others 2003; Hack and others 2007; Fig. 5). This suggests that ApoER2 is involved in the permissive or attractive effect of Reelin on migrating neurons. Binding of Reelin to ApoER2 attracts or promotes late-generated neurons to migrate past their predecessors to their proper destinations in the superficial layers.

Apart from ApoER2/VLDLR receptors, Reelin has also been shown to bind to a number of other cell surface molecules present in migrating neurons, including CNRs and α3β1 integrins (Senzaki and others 1999; Dulabon and others 2000), which are expressed in radially migrating neurons. Cells lacking α3 integrin are impaired in their detachment from radial fibers induced by Reelin in the marginal zone. Deficiency in functional α3β1 integrins leads to a reduction of Dab1 protein levels and to elevated expression of a 180-kDa Reelin fragment (Dulabon and others 2000). These results suggest that Reelin may regulate neuronal migration and layer formation through

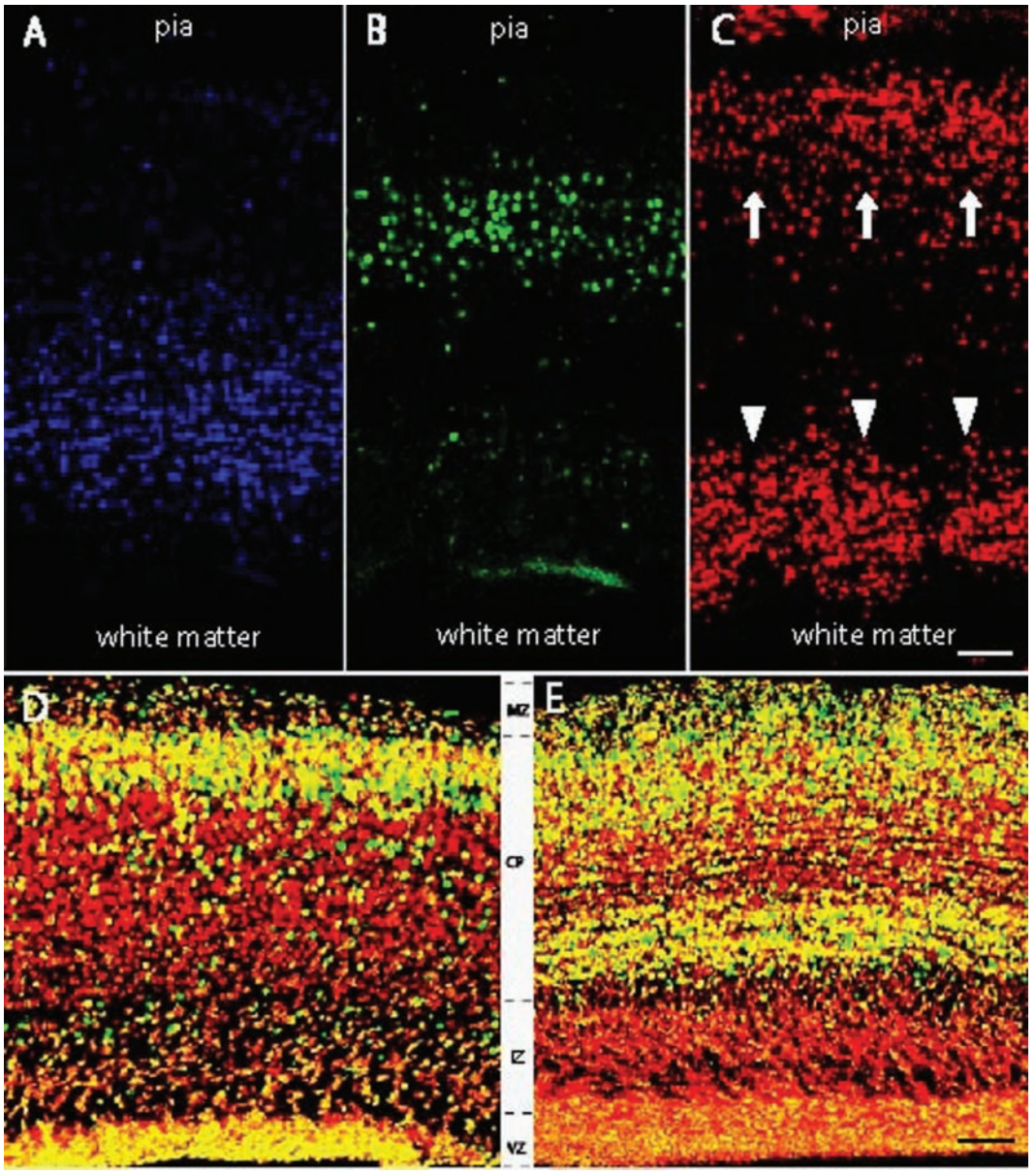

Figure 5. Impaired migration of late-generated cortical neurons in *apoer2* mutants. (*A–C*) Cerebral cortex of a P7 *apoer2* mutant stained with the layer-specific markers (*A*) Foxp2 (labeling early generated neurons in layer VI), (*B*) CTIP2 (labeling early generated neurons in layer V), and (*C*) Brn2 (labeling late-generated neurons in layers II–IV). CTIP2-positive cells are located above Foxp2-positive cells, indicating a normal inside-out lamination of early generated neurons. Although part of late-generated Brn2-positive neurons are located as normal in superficial layers (arrows), numerous Brn2-positive cells are found underneath the Foxp2-positive cells (arrowheads), indicating a migration defect of late-generated neurons and partial inversion of cortical layers in *apoer2* mutants. Scale bar, 100 μm. (*D, E*) BrdU (green) and propidium iodide (red) staining in the cerebral cortex of a (*E*) newborn *apoer2* mutant and a (*D*) wild-type littermate that were injected with BrdU at embryonic day 15.5 to label late-generated neurons. In the wild-type mouse, almost all BrdU-positive cells were detected in superficial layers, whereas two separated BrdU-positive cell layers were found in the *apoer2* mutant, one close to the marginal zone (MZ), another one close to the intermediate zone (IZ), indicating that part of late-generated neurons were unable to bypass layers of early generated neurons. Scale bar, 150 μm. CP = cortical plate; VZ = ventricular zone.

modulation of α3β1 integrin–mediated adhesion. The functional role of Reelin in modulating integrin-mediated adhesion is reflected in the abnormal adhesive phenotype of early born neurons in the *reeler* cortex (Hoffarth and others 1995) and the persistent apposition of *reeler* mutant neurons to radial glial fibers (Pinto-Lord and others 1982). The interaction between Reelin and α3β1 integrin receptors may be responsible for the separation of migrating neurons from radial glial fibers and, consequently, the establishment of laminar organization in the cortex.

CNRs are expressed in cortical plate neurons that are adjacent to the marginal zone (Senzaki and others 1999). CNRs bind Fyn at their cytoplasmic tails and also bind the N-terminal region of Reelin at their extracellular domains, thereby enabling Reelin to associate Fyn with the receptor complex (Senzaki and others 1999). Notably, Reelin-binding to CNRs depends on an RGD motif. As an RGD motif is also a feature of several integrin ligands (Pierschbacher and Ruoslahti 1984), integrins and Reelin might compete in their binding to CNRs. Reelin binding to CNRs might facilitate the detachment of migrating neurons from glia by separating CNRs from glial integrins. Thus, integrins and the CNR family might cooperate in modulating neuron-glia adhesion. This role of Reelin might be relevant at later stages of neocortical development, when locomoting cells need to detach from radial glial fibers to complete their journey by somal translocation.

Different Receptors Bind to Different Domains of Reelin

Jossin and others (2004) showed that the central fragment containing three to six Reelin repeats is sufficient to bind VLDLR and ApoER2 and induces Dab1 phosphorylation. Treatment with this central fragment could induce preplate splitting in *reeler* cortical slice cultures. The recombinant Reelin N terminus does not bind to VLDLR or ApoER2, nor does it stimulate tyrosine phosphorylation of Dab1 in primary neurons (Jossin and others 2004).

Interestingly, α3β1 integrin and CNR1 bind to the N-terminal region of Reelin, a site distinct from the region of Reelin shown to associate with other Reelin receptors such as VLDLR/ApoER2. Reelin fragments containing the rest of the Reelin repeats, including the C-terminus, did not associate with α3β1 integrin and CNR1 (Dulabon and others 2000; Senzaki and others 1999). Results obtained with the function-blocking antibody CR-50 point to a significant role of the N-terminal region. This monoclonal antibody against the N-terminal region of Reelin has been shown to block Reelin effects by interfering with Reelin aggregation that is essential for Reelin function (Ogawa and others 1995).

To date, the C terminus of Reelin has not been shown to bind to any receptors. However, it was shown that CTR can affect the structure of repeat 8 of Reelin, which is required for full signaling activity. Reelin mutants without CTR were less potent in activating downstream signaling in cortical neurons (Nakano and others 2007).

Altogether, the association of distinct regions of Reelin with different receptors implies different functional outcomes. At varying locations, the different fragments containing different Reelin domains bind to different receptors, thereby activating specific molecular events during the various stages of neuronal migration.

Summary and Conclusions

Reelin is secreted at a strategic position where neurons have to go and to stop. In this review, we have provided evidence for Reelin to have different effects on migrating neurons, on one hand providing an attractive signal that instructs the migration of neurons toward the pial surface. At the same time, Reelin acts as a positional cue to migrating neurons when they reach the marginal zone. Reelin instructs them to detach from their guiding glial fibers and stop their migration. In early stages of corticogenesis, Reelin-containing CR cells are relatively close to the ventricular zone. Subplate cells and a first wave of neurons destined to the cortical plate use somal translocation to migrate. Upon binding of Reelin to ApoER2, neurons extend leading processes to the pial surface. When these processes reach the marginal zone, Reelin binds to VLDLRs to promote their branching and anchoring to the extracellular matrix of the marginal zone and finally the translocation of their cell bodies toward the pial surface. Upon reaching a branching point, the cell bodies stop their movement and acquire their positions in the cortex.

In late stages of corticogenesis, after preplate splitting, the cerebral cortex becomes thicker. Now neurons move along radial glial fibers and adopt locomotion to migrate to the marginal zone. Small Reelin fragments containing central repeats diffuse from the marginal zone toward the ventricular zone and form a top-down gradient that binds to ApoER2 and drives newly generated neurons to migrate toward the marginal zone. Full-length Reelin and large Reelin fragments bind to integrin receptors, which leads to the detachment of neurons from their radial glial fibers. Simultaneously, full-length Reelin and large Reelin fragments bind to VLDLR to induce the branching and anchoring of the leading processes, followed by somal translocation during the final phase of migration. Late-generated neurons pass early formed layers until their leading processes reach the Reelin-rich marginal zone. Reelin-dependent signaling pathways converge at the level of the cytoskeleton and at the same time control

adhesion. Reelin regulates cytoskeleton dynamics by modulating the phosphorylation level of actin-associated proteins and MAPs and modulates the adhesive properties of migrating neurons by activating SFKs and integrins. The effects of Reelin on cytoskeleton dynamics and on adhesive properties of neurons are likely to be different (permissive or inhibitory) by binding to different receptors during different migration phases, which determines proper radial movement of migrating neurons: first go, then stop.

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