Review

# Role for Reelin in stabilizing cortical architecture

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**Reelin controls the migration of neurons and layer formation during brain development. However, recent studies have shown that disrupting Reelin function in the adult hippocampus induces repositioning of fully differentiated neurons, suggesting a stabilizing effect of Reelin on mature neuronal circuitry. Indeed, Reelin was recently found to stabilize the actin cytoskeleton by inducing cofilin phosphorylation. When unphosphorylated, cofilin acts as an actin-depolymerizing protein that promotes the disassembly of F-actin. Here, a novel hypothesis is proposed whereby decreased Reelin expression in the mature brain causes destabilization of neurons and their processes, leading to aberrant plasticity and aberrant wiring of brain circuitry. This has implications for brain disorders, such as epilepsy and schizophrenia, in which deficiencies in Reelin expression occur.**

## Reelin in brain development

During the development of the central nervous system (CNS), the extracellular matrix molecule Reelin controls the migration and laminar arrangement of neurons in various structures including the neocortex, hippocampus, cerebellum and spinal cord [1–9]. In the Reelin-deficient mouse mutant *reeler*, the migration of neurons is severely altered in these regions, resulting in a malformation of layers [10–14]. In the neocortex and hippocampus, Reelin is synthesized and secreted by early-generated Cajal–Retzius (CR) cells in the marginal zone underneath the pial surface [1,15]. The marginal zone is a cell-poor layer in wild-type mice, whereas in Reelin-deficient mice it is invaded by numerous neurons. Such a finding suggests that the Reelin located at the surface of the cortex acts as a "stop" signal, terminating the migration of neurons from the ventricular zone to the cortical plate during development [16,17].

It has long been known that early-generated neurons form deep layers in the cerebral cortex, whereas later-generated neurons populate the more superficial layers [18,19]. This early finding has two important implications: first, late-generated neurons migrate for longer distances, and second they have to bypass their predecessors on their way from the ventricular zone to the cortical surface. In the *reeler* mutant, this inside-out lamination of the cortex is severely altered and late-generated neurons fail to bypass their predecessors, resulting in an inversion of cortical layers. How does Reelin control neuronal migration and layer formation in the developing cerebral cortex?

At this point, it appears necessary to consider how cortical neurons migrate to reach their destinations in the cortical plate. Two principal modes of migration have been described, somal translocation and glia-dependent locomotion [7,20,21]. Somal translocation is used by early-generated neurons when the distance from the ventricular zone is short, so that it can be bridged by the leading process of the migrating cell. At later stages, migrating neurons use radial glial fibers as a guiding scaffold (glia-dependent locomotion) and only switch to somal translocation in the terminal phase of the migratory process when they have detached from the radial glial fiber and their leading process has reached the marginal zone of the cortex. Of note, the two modes of migration are not principally different. In both modes, the nucleus is moved towards the leading process and the trailing process is retracted. Moreover, during nuclear translocation the leading process needs to be stabilized and under tension [22], either attached to the guiding radial glial fiber (glia-dependent locomotion) or the marginal zone (somal translocation).

In the context of this article, it is important to note that in mutants with deficient Reelin signaling, the leading processes of migrating neurons are misoriented, and many of them do not reach the marginal zone [23]. The leading process becomes the apical dendrite of the neuron later on in development. It has been observed in early studies of the *reeler* mutant that many cortical pyramidal cells do not show the normal, uniform orientation towards the pial surface characteristic of wild-type animals, but instead are oriented in various directions [24–26]. Does Reelin in the marginal zone stabilize the leading processes of radially migrating cortical pyramidal neurons? Does Reelin signaling tense the leading process which is required for somal translocation to take place [22]? Does Reelin act on the actin cytoskeleton? Before considering these questions further, it appears appropriate to briefly discuss the Reelin signaling cascade and summarize what is known about the role of Reelin in the mature brain.

## Reelin signaling pathway

Much has been learned in recent years about the Reelin signaling cascade (Figure 1). Reelin was found to bind to two transmembrane lipoprotein receptors, the apolipoprotein E receptor 2 (ApoER2) and the very-low-density lipoprotein receptor (VLDLR) [27]. Binding to these receptors

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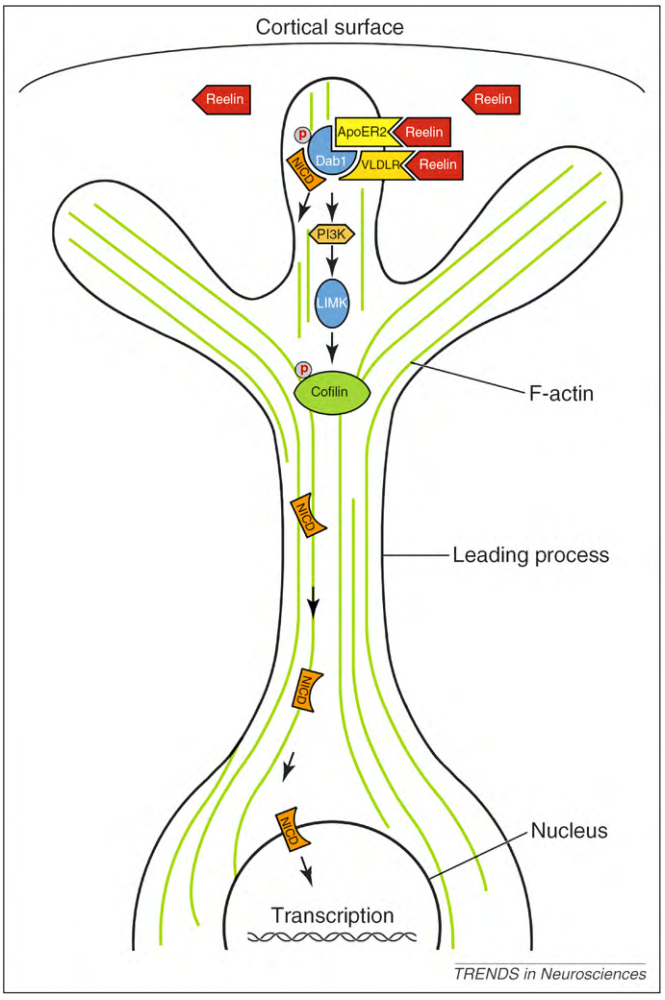


**Figure 1**. Schematic diagram illustrating Reelin-induced phosphorylation of cofilin in the leading processes of migrating cortical neurons. Binding of Reelin to the lipoprotein receptors ApoER2 and VLDLR results in the phosphorylation of Disabled-1 (Dab1), an adapter protein interacting with the intracellular domains of ApoER2 and VLDLR. Via the activation of phosphatidylinositol 3 kinase (PI3K), LIM kinase 1 (LIMK) is activated which, in turn, leads to the phosphorylation of cofilin. Phosphorylation of cofilin renders it unable to depolymerize F-actin. Thus, the F-actin cytoskeleton is stabilized, which anchors the leading process to the marginal zone underneath the cortical surface [53]. Tension of the leading process by actin cytoskeleton stabilization is important for the translocation of the nucleus of the cell in the terminal phase of the cortical migratory process [7,22]. Interestingly, a recently described Reelin–Notch interaction [62,63] points to an alternative way of neuronal process stabilization. The cleaved Notch intracellular domain (NICD) interacts with Dab1, which prevents NICD degradation [62]. NICD translocation to the nucleus induces target gene transcription leading to growth arrest and process stabilization [61].

induces phosphorylation of Disabled-1 (Dab1), an intracellular adaptor protein interacting with the intracellular domains of ApoER2 and VLDLR [27–29]. Reelin signaling involves phosphatidylinositol 3-kinase (PI3K) [30] and glycogen synthase kinase 3β [31], and there are reasons to assume that it eventually acts on cytoskeletal proteins such as F-actin. Mutations in the *dab1* gene and in the genes encoding for ApoER2 and VLDLR show a *reeler*-like phenotype [27,32–35].

## Roles for Reelin in the adult brain

Mutations in the human Reelin gene are associated with lissencephaly and cerebellar hypoplasia [36,37]. Several studies during the past decade have provided evidence for altered Reelin expression in a variety of neuropsychiatric diseases including schizophrenia [38], autism [39], bipolar disorder and severe depression [40], and Alzheimer's disease [41]. Does decreased Reelin expression in these different disorders imply that they all have a developmental component?

An alternate explanation arises from the finding that Reelin signaling via ApoER2 has effects on synaptic plasticity and memory processes in the adult brain [42]. In particular, modulation of synaptic plasticity was found to involve differential splicing of ApoER2 in an activity-dependent manner, a mechanism that is required for the Reelin-mediated enhancement of long-term potentiation (LTP), a form of synaptic plasticity associated with learning and memory. Remarkably, it has recently been shown that Reelin can counteract the suppression of LTP induced by β amyloid, which is a major component of plaques in Alzheimer's disease [43]. Thus, deficits in Reelin could result in memory impairments.

In accordance with a role in the mature brain, Reelin is expressed by a group of GABAergic interneurons after birth [44–47] in addition to being expressed by CR cells in the marginal zone. However, the functional significance of this late Reelin expression by interneurons is poorly understood. Before addressing other potential functions for Reelin in the mature brain, we will first discuss the role that Reelin plays in regulating the actin cytoskeleton.

## Reelin signaling stabilizes the actin cytoskeleton

Several observations in mutants deficient in Reelin signaling have pointed to a role for Reelin in the regulation of the actin cytoskeleton. First, it was observed in these animals that the leading processes of migrating neurons were less stable, misoriented and often not attached to the marginal zone [23]. Second, in *reeler* and Reelin receptor mutants, numerous neurons were found to "overmigrate" into the marginal zone [27,48]. Does Reelin stabilize the cytoskeleton, thereby anchoring the leading process to the cortical surface and providing a stop signal for migrating neurons that arrive at the marginal zone?

During migration and process extension, continuous remodeling of the actin cytoskeleton is required for the changes in cell shape that are associated with migrational events and the growth of processes. The actin cytoskeleton is subject to alterations by proteins such as cofilin that depolymerize F-actin, thereby supplying actin monomers for the formation of new actin filaments [49]. Thus, the activity of these proteins is essential for changes in cell shape which have been observed in migrating neurons or growth cones of extending axonal and dendritic processes [50]. The capacity of cofilin to depolymerize F-actin is terminated by phosphorylation of the protein at serine residue 3 by LIM kinase 1 (LIMK1) [51,52]. Could it be that Reelin exerts its stabilizing function by acting on cofilin or other actin-associated molecules?

It has recently been shown that Reelin signaling via ApoER2 induces cofilin phosphorylation at serine 3 [53]. Accordingly, levels of phosphorylated cofilin (p-cofilin) in *reeler* were significantly reduced, pointing to an enhanced capacity for reorganization of the actin cytoskeleton in this Reelin-deficient mutant. Incubation of *reeler* mouse cortical tissue in the presence of recombinant Reelin

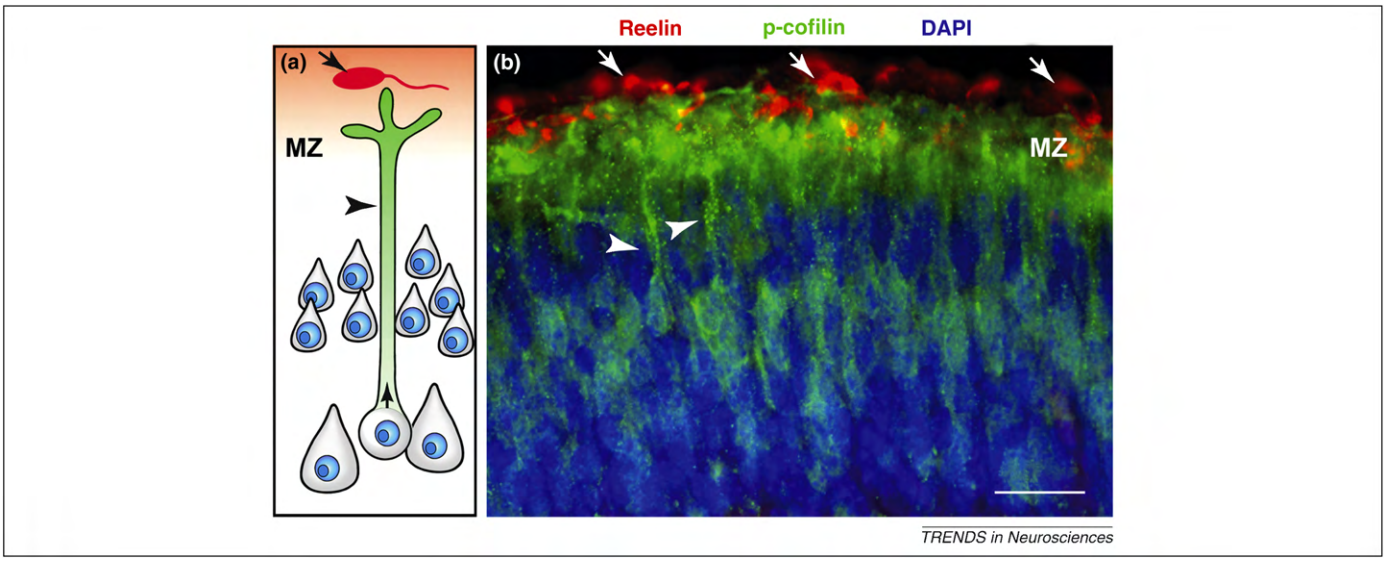


**Figure 2**. Immunostaining for phosphorylated cofilin (p-cofilin) labels the leading processes of late generated neurons destined for superficial cortical layers. **(a)** Schematic diagram illustrating a late-generated neuron in the terminal phase of its migration by nuclear translocation (arrow on top of the nucleus). Arrowhead denotes the leading process which extends to the marginal zone (MZ) and contains phosphorylated cofilin (green). Reelin (red) is synthesized and secreted by Cajal–Retzius cells in the marginal zone (arrow). Reelin-induced cofilin phosphorylation stabilizes the actin cytoskeleton in the leading process. This anchors the process to the marginal zone and allows for somal translocation to occur. **(b)** Double immunolabeling for Reelin (red) and p-cofilin (green) in a cortical section from a wild-type mouse (embryonic day 17.5), counterstained with DAPI (blue) to label cell bodies. Arrowheads point to immunolabeling for p-cofilin in the leading processes of neurons underneath the marginal zone. Arrows indicate Reelin expressing Cajal–Retzius cells. Scale bar: 40 μm. Adapted, with permission, from [53].

significantly increased p-cofilin levels. Moreover, Reelin signaling was found to activate LIMK1, the kinase which mediates cofilin phosphorylation. Reelin-induced p-cofilin levels were decreased when the tissue was incubated in the presence of a chemical inhibitor of src family kinases, which are known to phosphorylate Dab1, the adapter molecule downstream of ApoER2 and VLDLR in the Reelin signaling cascade [54] (Figure 1). Along this line, p-cofilin levels were significantly decreased following inhibition of PI3K, which interacts with Dab1 and is essential for layer formation in the cortex [30]. Finally, phosphorylation of cofilin was found to be significantly reduced in *apoer2* mutants and in *dab1* mutants. Together, these findings indicate that Reelin signaling induces phosphorylation of cofilin at serine residue 3, thereby stabilizing the actin cytoskeleton (Figure 1).

In agreement with these findings, immunostaining for p-cofilin was found to be particularly strong in the Reelin-containing marginal zone (Figure 2). Notably, p-cofilin was localized to late-generated radially migrating neurons destined for the superficial layers of the cortex. Immunostaining for p-cofilin was particularly evident in the leading processes of these cells, the future apical dendrites of cortical pyramidal neurons, with the strongest labeling in the tips approaching the marginal zone. These findings support the notion that the actin cytoskeleton becomes stabilized when the leading process arrives at the marginal zone.

## Functional significance of Reelin-induced cofilin phosphorylation

Reelin is a large 400 kDa extracellular matrix molecule which is concentrated in the marginal zone. By inducing cofilin phosphorylation and stabilization of the actin cytoskeleton, Reelin can anchor the leading processes of migrating neurons to the cortical surface, which seems to be required for somal translocation to occur (Figure 2). In this way, the uniform ascension of apical pyramidal cell dendrites of the cortex is likely to be maintained. This hypothesis is supported by the observation that in *reeler* mice, numerous apical pyramidal cell dendrites have lost their characteristic vertical orientation and run in various directions [24–26]. Lack of attachment of the leading process to the cortical surface and the resulting loss of process tension in the absence of Reelin signaling can render the neurons unable to migrate by somal translocation past their predecessors in deep cortical layers. As a result, the normal inside-out layering of the cortex is inverted in the *reeler* mutant. Such a prominent role of the leading process for migration is consistent with the "detach and go" model of Reelin function that has recently been proposed [7].

A role for Reelin in the stabilization of neuronal processes has been confirmed *in vitro*. Different processes of a single neuron behaved differently depending on whether they encountered a Reelin substrate or control substrate [53]. Whereas processes that encountered the Reelin substrate were stable over time, those that encountered only the control substrate often changed position and showed signs of increased motility and growth, manifested by the appearance of growth cones and lamellipodia. The decreased motility of processes that encountered the Reelin substrate was accompanied by increased immunoreactivity for p-cofilin [53].

Stabilization of the actin cytoskeleton by Reelin-induced cofilin phosphorylation could also be involved in the termination of the migratory process upon arrival of the migrating neuron at the marginal zone. As pointed out, the marginal zone is cell-poor in wild-type animals but densely populated in the *reeler* mutant, suggesting an overmigration in the absence of Reelin as a factor stabilizing the cytoskeleton (i.e. acting as a stop signal). Although

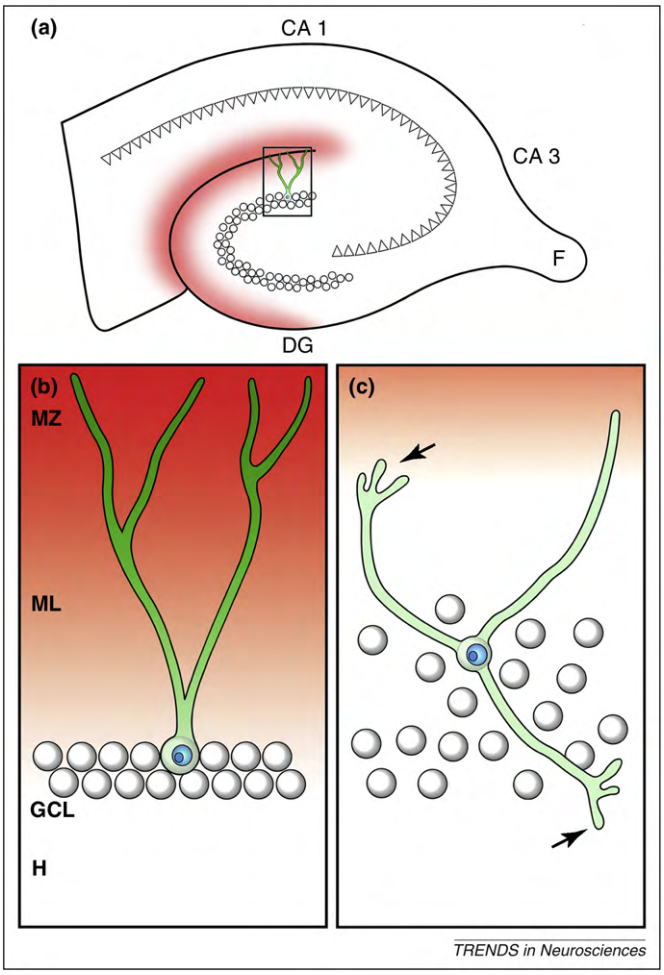


**Figure 3**. Effects of Reelin deficiency in adulthood on the structural organization of the dentate gyrus. **(a)** Schematic diagram of the hippocampus to indicate location of the granule cells illustrated in (b, c). CA1, CA3, hippocampal regions CA1 and CA3; DG, dentate gyrus; F, fimbria. **(b)** Normal anatomical organization of the DG. Granule cells are arranged as a compact band in the granule cell layer (GCL). One granule cell is shown with its dendrites extending towards the marginal zone (MZ) of the dentate gyrus, the outer molecular layer bordering the hippocampal fissure. The extracellular matrix of the marginal zone contains Reelin (red). Reelin binds to ApoER2 and VLDLR in the membranes of granule cell dendrites and induces phosphorylation of cofilin (green). This way, the cytoskeleton is stabilized and the granule cell dendrites are anchored to the marginal zone. A Reelin gradient in the molecular layer (ML) acting on the cell bodies of the granule cells keeps them in formation in a densely packed layer. Alternatively, Reelin signaling via Dab1 prevents degradation of NICD. Notch signaling, like cofilin phosphorylation, leads to cessation of process growth and stabilizing of the neuronal network. **(c)** In cases where Reelin activity is blocked or deficient, there is increased motility of the DG granule cells, which subsequently invade the molecular layer (granule cell dispersion). Granule cell dendrites are neither stabilized nor anchored to the marginal zone, but show signs of growth such as growth cones (arrows) and lamellipodia. Reelin deficiency in adulthood thus leads to structural reorganization including increased migratory activity of neurons and aberrant plasticity of their processes. These phenomena are likely to contribute to the pathological phenotypes of diseases, such as temporal lobe epilepsy, that are associated with Reelin deficiency. H, hilus.

this hypothesis appears plausible at first glance, some questions remain unanswered. Overmigration into the marginal zone was observed in *reeler* mice, *apoer2/vldlr* double knockout mice and in *vldlr* single knockout mice, but not in *apoer2* single mutants [27,48]. Of note, levels of p-cofilin were significantly reduced in *reeler*, *dab1* mutants and *apoer2* mutants, but were almost normal in *vldlr*-deficient mice [53]. Almost normal p-cofilin levels in *vldlr* mutants, mice which display strong overmigration of neurons in the cortex, do not preclude a role of cofilin phosphorylation in the arrest of migrating neurons arriving at the marginal zone but could indicate that additional mechanisms are involved.

It has been hypothesized that Reelin induces detachment of the migrating neuron from the radial glial fiber by involving integrins [55,56]. If detachment fails, in the absence of Reelin signaling, neurons would continue to migrate. As has been previously shown, degradation of Dab1 depends on Dab1 phosphorylation and on the E3 ubiquitin ligase component Cullin 5 (Cul5) [57]. Ablation of Cul5 in migrating neurons resulted in an accumulation of Dab1 and was associated with an overmigration of neurons at the top of the cortical plate. Could it be that reduced Dab1 phosphorylation in the absence of Reelin signaling via VLDLR prevents Dab1 ubiquitination and causes overmigration into the marginal zone?

It should be emphasized at this point that Reelin is only one among many other molecules that act on the cytoskeleton and can thus influence cell motility. As an example, overmigration into the marginal zone reminiscent of the *reeler* phenotype was observed when the expression of p21-activated kinase, a regulator of the cytoskeleton, was reduced [58].

## Stabilization of the cortical network by Reelin–Notch interaction

The Notch pathway is evolutionarily conserved and is involved in a variety of developmental processes [59,60]. Following binding of the Notch receptor ligands Delta and Jagged, the Notch intracellular domain (NICD) is cleaved by γ-secretase and translocated to the nucleus. By forming a transcriptional complex with CBF-1, NICD induces transcription of a variety of target genes.

In the context of this review, it has been shown that Notch signaling mediates the growth arrest of neuronal processes and contributes to network stabilization [61]. Upregulation of Notch activity was associated with the cessation of neurite growth, whereas inhibition of Notch signaling in mature neurons promoted neuronal process extension. Remarkably, two recent reports have provided evidence for a crosstalk of Reelin and Notch signaling [62,63], probably by physical interaction of NICD with Dab1, which was found to inhibit NICD degradation [62] (Figure 1). Inhibition of Notch signaling resulted in migration defects reminiscent of the *reeler* phenotype. Together, these studies point to an alternative way in which Reelin could stabilize the cortical network in addition to cofilin phosphorylation via LIMK1 activation.

## Reelin signaling in the epileptic brain

Human temporal lobe epilepsy (TLE) is associated with granule cell dispersion [64] and decreased Reelin expression in the hippocampus. Of note, the extent of Reelin deficiency in tissue samples from epileptic patients was found to correlate with the extent of granule cell dispersion [65]. This loss of compact granule cell layering is reminiscent of the scattered distribution of granule cells in the *reeler* mutant [12,14].

This observation that hippocampi from TLE patients show reduced Reelin expression and exhibit a phenotype in

the dentate gyrus that is strikingly similar to that of the *reeler* mutant once again raises important questions about the role of Reelin in normal and abnormal processes in the adult brain. For example, is the loss of granule cell lamination in TLE patients due to deficient Reelin signaling in the adult brain, or is the observed granule cell dispersion in epileptic patients caused by a migrational defect during development that leads to seizures later in life? More specifically, does deficient Reelin expression in the marginal zone of the dentate gyrus, namely the dentate outer molecular layer, allow numerous granule cells to invade the molecular layer, reminiscent of marginal zone invasion in the *reeler* mutant?

Recent studies using an animal model of TLE have begun to address these questions. Remarkably, decreased Reelin expression and granule cell dispersion can be induced in adult mice by the unilateral injection of kainate, an agonist of the excitatory transmitter glutamate, into the hippocampus [66]. In this well-characterized animal model of TLE, there is a significant decrease in Reelin expression along with the development of granule cell dispersion on the ipsilateral, but not contralateral, side of injection [67]. These findings show that seizures induced by kainate interfere with Reelin expression, which, probably by way of decreased levels of p-cofilin and altered Notch signaling, respectively, results in increased motility of the granule cells, thus leading to cell dispersion (Figure 3).

Interestingly, by using BrdU injections to label newly generated neurons, it has been demonstrated that the granule cells that invaded the molecular layer were not newborn cells but were fully differentiated neurons [67]. These findings imply that decreased Reelin expression could lead to destabilization of cortical architecture and pathological plasticity in the mature brain.

Further evidence to support this conclusion has come from experiments in which a Reelin-neutralizing antibody, which blocks Reelin signaling, was infused into the hippocampus of adult mice [67]. This resulted in significant granule cell dispersion. No dispersion of these cells was observed when unspecific IgG was infused in place of the Reelin-blocking antibody, demonstrating a causal relationship between deficits in Reelin signaling and granule cell layer malformation in the adult brain.

Importantly, recent studies have demonstrated that the granule cell dispersion observed after kainate injection can be prevented by the co-infusion of recombinant Reelin into the hippocampus at the same time [68]. This striking demonstration that Reelin can prevent the structural disorganization observed within the dentate gyrus after kainate injection further demonstrates the importance of Reelin not only during development but also in the adult brain.

## Stability of neuronal circuitry versus plasticity

The results summarized here indicate that Reelin signaling stabilizes the actin cytoskeleton. By phosphorylating cofilin, Reelin anchors the tips of leading processes to the marginal zone and controls the terminal phase of the migration process. Thus, the reduced phosphorylation of cofilin in the *reeler* mutant points to an increased capacity of cofilin to depolymerize F-actin, which, in turn, is associated with increased migratory activity and process growth. Indeed, there is an overmigration of neurons into the marginal zone and the tips of apical dendrites are no longer anchored to the marginal zone in *reeler* mutants. As observed in *in vitro* experiments, increased process motility was observed in cultured neurons that were not exposed to Reelin compared to those neurons that did have exposure to Reelin. And *in vivo*, increased migratory activity of mature hippocampal granule cells has been observed when Reelin signaling was blocked. Thus, it appears that Reelin stabilizes the actin cytoskeleton not only during development but also in the mature brain. This is likely to occur by the phosphorylation of cofilin at serine residue 3 or by an interaction between Reelin and the Notch signaling pathway, thereby resulting in a stabilized cortical and hippocampal architecture and counteracting aberrant neuronal migration and aberrant neuronal network organization.

Research over the past 30 years has accumulated numerous examples of an enormous capacity for plasticity in the adult brain. We have learned that molecules involved in synaptogenesis during development are involved in synaptic plasticity in the mature brain, thereby enabling the organism to cope with new environmental challenges [69]. We have reason to assume that it is this capacity of the brain for functional and structural changes at its synapses that underlies learning and memory [70]. It has in fact been shown that synaptic activity and plasticity are associated with structural changes at synapses, including *de novo* formation of dendritic spines, the major sites of excitatory synapses in the brain [71–77].

Although functional and structural plasticity at synapses appears to be important for fundamental brain functions such as learning and memory, the brain can otherwise be seen as a very stable organ. Unlike the cells in other organs, most neurons are formed prenatally and persist for the life span of the organism. The same is likely to hold true for many neuronal circuits that are established early in life and persist for extended periods of time. Examples of this are motor skills; having learned to ski early in life, we do not forget how to do it, even if we refrained from skiing for many years. Similarly, we precisely remember scenes of our early childhood when exposed to a similar situation as adults. It appears that the different forms of memory require a certain degree of stability in the neuronal network, while at the same time synaptic plasticity is needed to learn about new contexts that we were not exposed to in the past. Obviously, a balance between stability and plasticity is required for normal brain function, and there is evidence for spine stability to be associated with lifelong memories [78].

Although numerous studies over the past decades have focused on the plasticity of synapses and the enormous potential of neurons to adapt to novel environmental stimuli, relatively few studies have addressed the factors that control the maintenance of CNS organization. Energy is required to counteract entropy and an immune system is needed to control the myriads of microorganisms that otherwise have the potential to destroy our lives. What are the molecules and signaling cascades that control the maintenance of brain architecture, thereby keeping a

**Box 1. Outstanding questions**

- In which membrane compartments of the developing neuron (i.e. leading process, cell body and/or trailing process) are the two lipoprotein receptors for Reelin (VLDLR and ApoER2) located?
- Does the location of Reelin receptors vary during different developmental stages and in the mature brain?
- Can real-time microscopy be used to address the question of whether developing neurons and their processes are more motile in the *reeler* brain compared to in wild-type mice?
- What is the role of Reelin in postnatal cortical GABAergic interneurons?
- Does Reelin silencing (siRNA, conditional Reelin knockout) in the mature brain result in increased motility of neurons and their processes?
- Does Reelin silencing in the mature brain result in behavioral changes and deficits in learning and memory?
- Is deficient Reelin signaling in the mature brain associated with changes in neuronal connectivity and neuronal communication?
- Which factors lead to deficient Reelin expression in neuropsychiatric diseases? And is this deficit due to developmental defects or to secondary changes in the mature brain?
- Can Reelin application, or an enhancement in Reelin expression, become a therapeutic strategy?

balance between plasticity and stability? Could Reelin have such a stabilizing function in the adult brain, even though in concert with many other yet unknown molecules? The available data strongly argue in favor of such a hypothesis; experimentally induced decreases in Reelin expression and signaling function were found to induce granule cell dispersion and increase the migratory activity of mature neurons in the adult brain [67].

It remains to be shown to what extent decreased Reelin expression and aberrant neuronal plasticity contribute to mechanisms underlying neuropsychiatric diseases. Moreover, future lines of research will need to focus on the conditions under which Reelin expression is altered, whether or not such secondary network changes take place in response to decreased Reelin signaling, what the nature of these changes is and how they can contribute to disease outcomes (Box 1). Regarding human TLE, recent studies provide evidence that hypermethylation of the Reelin gene can lead to the observed decrease in Reelin expression [79].

## Outlook

A vast array of knowledge about the important function of Reelin in brain development has been attained since the phenotype of the *reeler* mouse was first described almost 60 years ago [10]. However, we are far from understanding the functions of Reelin in the mature brain. Beffert *et al.* were the first to show that Reelin has a functional role in synaptic plasticity in the mature brain by elucidating an interaction between ApoER2 and the NMDA receptor [42]. The latest findings, outlined above, propose an additional function for Reelin in the mature brain, namely a stabilization role in the maintenance of the differentiated neuronal circuitry via the stabilization of the actin cytoskeleton through phosphorylation of important actin depolymerizing proteins.

How can experimental research contribute to an understanding of the important functions that Reelin is involved in in the adult brain? Definitive studies that demonstrate that mature neurons and their processes become more destabilized under conditions of decreased Reelin expression are needed. One way to achieve this would be to block Reelin function experimentally in adult animals, monitor GFP-labeled neurons *in vitro* and *in vivo* [80–87] and document a quantitative increase in migratory activity and neuronal process motility. Such real-time microscopy studies of GFP-labeled neurons might eventually clarify to what extent experimentally induced Reelin deficiency in adult animals enhances neuronal motility and network reorganization, and whether or not this structural remodeling leads to impaired function.

In addition to antibody blockade of Reelin, siRNA experiments and conditional deletion of the Reelin gene could turn out to be very useful approaches compared to the complete knockout of the Reelin gene that is found in the *reeler* mouse model, because these approaches allow for normal brain development to take place in the presence of Reelin, before the Reelin gene is silenced during adulthood. Such approaches would determine whether an intermittent Reelin deficiency is associated with transient structural changes and functional deficits. Moreover, studies in conditional Reelin mutants would avoid specific developmental defects in *reeler* mice, such as cerebellar malformation associated with severe ataxia, thus enabling the investigator to subject these conditional mutants to a variety of behavioral experiments that are currently not possible.

Another issue concerns the exact location of extracellular Reelin in the adult brain, as well as the sorting of ApoER2 and VLDLR to lipid raft versus non-raft domains of the membrane [88], and to different cellular compartments (i.e. cell body, dendrite and/or dendritic spines). Previous attempts to localize Reelin receptors have been hampered by the low specificity of antibodies in aldehyde-fixed tissue. The disadvantages of conventional aldehyde fixation can probably be overcome by procedures such as high-pressure freezing [89–91], which avoids exposure of the tissue to fixatives. Learning about the precise temporal and spatial location of Reelin receptors will contribute substantially to our understanding of Reelin function in the developing and mature brain. Undoubtedly, we will continue to read new and exciting stories about Reelin, a molecule that was previously assumed to be only involved in layer formation during development.

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