Review Article

Reelin in the Years: Controlling Neuronal Migration and Maturation in the Mammalian Brain

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The extracellular protein Reelin was initially identified as an essential factor in the control of neuronal migration and layer formation in the developing mammalian brain. In the years following its discovery, however, it became clear that Reelin is a multifunctional protein that controls not only the positioning of neurons in the developing brain, but also their growth, maturation, and synaptic activity in the adult brain. In this review, we will highlight the major discoveries of the biological activities of Reelin and the underlying molecular mechanisms that affect the development and function of the mammalian brain, from embryonic ages to adulthood.

1. The *reeler* Mouse and *Reelin* Gene Discovery

Reelin was identified in 1995 as the gene disrupted in two mouse strains carrying the autosomal recessive mutation *reeler,* a transgenic and a spontaneous strain that originated in Edinburgh [1]. While a newly generated transgenic strain was instrumental in the cloning of the gene, the classic, spontaneous Edinburgh *reeler* mutant strain had been well known for decades to developmental neurobiologists for the distinct neurological phenotype observed in homozygous mice, which includes tremors, ataxia, cerebellar hypoplasia, and widespread disruption of cellular layers throughout the brain [2–10]. Upon cloning and mapping of the gene, it turned out that a large deletion in the *Reelin* gene had occurred in the Edinburgh strain, resulting in the complete loss of transcript expression [1]. Around the same time, a portion of the *Reelin* coding sequence was also identified in another spontaneous *reeler* strain that originated in Orleans [11]. In this strain, however, a truncated transcript predicted to encode a C terminal deletion mutant protein was stably expressed. To date, several additional *reeler* mouse mutant strains carrying mutations in the *Reelin* gene have been identified, all resulting in the loss of transcript expression. Like the original Edinburgh mutation (now called B6C3Fe

ala-Reln^{rl}/J), these strains exhibit essentially the same *reeler* phenotype and are made commercially available from The Jackson Laboratory. As *Reelin* gene orthologs were identified in other vertebrate species, other *reeler*-like mutations were quickly characterized. In the rat, for example, two mutant strains, the Shaking Rat Kawasaki and the Komed Zucker Creeping rat, that exhibited a *reeler*-like phenotype were reported to carry *Reelin* gene mutation [12, 13]. The human *REELIN* gene was also quickly cloned [14], and human subjects carrying *REELIN* mutations in homozygousity were identified few years later [15]. These patients exhibit lissencephaly with cerebellar hypoplasia (LCH), a phenotype that is very reminiscent of that observed in *reeler* mice.

One of the most intriguing aspects of the *reeler* phenotype is that many neuronal populations are born in normal numbers, at the right time and in the right place, but fail to reach the appropriate position and to form cellular layers in laminated cortical structures of the brain, such as the cerebral cortex, the hippocampus, or the cerebellum [6]. Additionally, failure of specific neuronal populations to reach appropriate locations and to assemble into distinct nuclei was reported in the brain stem and in the spinal cord [5, 16]. These observations strongly suggested that the gene mutated in *reeler* mice had to be an essential regulator of neuronal migration

and ultimately determines neuronal position within cortical structures and other anatomically distinct cellular aggregates of the central nervous system. Indeed, several studies have shown that *Reelin* plays important roles in the development of the spinal cord [16–18] and the retinal circuitry [19]. Here, we will discuss primarily its role in brain development and focus especially on recent advances related to the control of neuronal migration, which was the first recognized biological function of *Reelin* and, perhaps still to these days, its best-characterized activity. However, this is by no means its only function. In fact, it was noted early on that additional defects are present in the *reeler* mutant, including subtle connectivity abnormalities in the cerebellum [7] and fiber segregation abnormalities in the hippocampus [20, 21], suggesting that *Reelin* plays multiple roles in brain development.

How does *Reelin* control different aspects of brain development and function? Almost twenty years after its discovery, we still do not know everything to be known about this remarkable protein, but we have made tremendous progress in elucidating its structure, expression, and biochemical properties and ultimately in understanding how *Reelin* exerts its diverse functions, not only during embryonic and postnatal brain development, but also in the adult brain.

2. The *Reelin* Gene

Shortly after the identification of the *Reelin* coding sequence, the genomic organization of the mouse *Reelin* gene and its promoter region on chromosome 5 was unraveled [22]. This study revealed that the *Reelin* gene is very large and complex, containing 65 exons spanning approximately 450 kb of genomic DNA. The study also identified different *Reelin* transcripts generated by alternative splicing of a microexon although the significance of this splicing event remains obscure to these days. Furthermore, two different polyadenylation sites and a promoter region lacking canonical TATA and CAAT boxes, but containing putative recognition sites for transcription factors Sp1 and AP2, were defined. The promoter region of human *REELIN* gene was also cloned and characterized shortly thereafter, revealing the presence not only of Sp1 site, but also of binding sites for additional transcription factors, such as the cyclic AMP responsive element binding protein (CREB), T brain 1 (Tbr1), and paired homeobox 6 (Pax6) [23]. These and other follow-up studies led to the recognition that *REELIN* expression is heavily regulated by promoter methylation and that this epigenetic event may play an important role in controlling *Reelin* expression levels [23, 24]. Since reduced expression of *REELIN* has been reported in patients with schizophrenia [25], bipolar disorder [26], and autism [27], these findings have potentially important implications for the treatment of these disorders. The intriguing association between *REELIN* expression and cognitive disorders, as well as the possible use of pharmacological agents that decrease methylation and restore normal levels of *REELIN* in these patients, has been extensively discussed in several review articles [28–30]. Therefore, we will not address this topic here, except to underscore the importance of furthering our understanding

of *Reelin* activities in processes that take place during brain development and beyond.

3. The *Reelin* Protein

The cloning of mouse *Reelin* coding sequence predicted that the encoded protein was likely to be large and secreted. Indeed, a full-length *Reelin* protein of approximately 385 kDA was found to be secreted in the culture medium of wild type mouse neurons or clonal cells transfected with an expression vector containing the newly assembled coding sequence [31]. The secreted protein also was shown to be glycosylated. A remarkably similar protein was deduced from the sequence of the human *REELIN* cDNA, which resulted to be 388 kDA in size and 94.2% identical to the mouse protein at the amino acid level [14]. However, since the sequence of *Reelin* did not extensively match other known sequences in the database, the cloning of *Reelin* revealed little about its biochemical function. The predicted protein had a modular structure composed of a series of unique repeats, flanked by distinct N- and C-terminal regions. The N-terminal region contains a signal peptide, which is required for secretion, a small region of similarity to the extracellular protein F-spondin, and an epitope region that is recognized by the CR-50 monoclonal antibody. This antibody was developed concurrently with the cloning of *Reelin*, based on the presence of an antigen expressed by Cajal-Retzius cells which was absent in the embryonic forebrain of *reeler* mice [32]. Upon cloning of *Reelin*, the CR-50 antibody was shown to recognize the N-terminal of the native protein [31]. This antibody turned out to be an excellent reagent to study not only the distribution of the *Reelin* protein in brain tissue slices, but also its function, since it interfered with many *Reelin*-induced cellular and molecular events [20, 32–35]. Biochemical studies revealed that the interfering activity of the CR-50 antibody most likely lies in its ability to mask an N-terminal region of *Reelin* that is necessary for disulfide-linked homodimer formation [36]. The main body of the *Reelin* protein is composed of eight consecutive repeats, each composed of two subrepeats separated by a cysteine pattern commonly found in extracellular proteins, the EGF-like repeat [1]. The boundaries of these repeats have now been clearly defined, and the crystal structure of two central repeats 5 and 6 has been resolved in recent years [37]. The C terminal region of *Reelin* is highly conserved and contains a positively charged stretch of amino acids which appears to be important for optimal signaling [38]. Following the development of a battery of monoclonal antibodies [39], a biochemical analysis of the processing of *Reelin* became possible. It is now established that, after secretion in the extracellular environment, full-length *Reelin* is rapidly cleaved by proteases at two specific sites, generating three major proteolytic fragments [40]. Among these, the central fragment composed of repeats 3 to 6 is the only one that appears to be necessary and sufficient to induce layer formation in a cortical slice culture assay [40]. This fragment also contains amino acid residues that are required for receptor binding and is sufficient to induce signal transduction, albeit to a reduced extent compared to

the entire *Reelin* moiety [37, 40, 41]. Together, the data suggest that proteolytic processing is important *in vivo* to produce *Reelin* fragments, which can either diffuse away from the site of origin or assemble locally into large protein aggregates or homodimers capable of activating signal transduction to the full extent [36, 41, 42].

4. *Reelin* Expression during Brain Development

In situ hybridization experiments gave an initial overview of *Reelin* expression in the mouse brain and spinal cord [43, 44]. These studies also revealed that *Reelin* is expressed in many organs outside the central nervous system, during development and also in adult ages , suggesting that *Reelin* may be important not only for the initial cellular organization, but also for the stabilization and remodeling of these different tissues [43]. However, since only the brain was obviously disrupted in *reeler* mice, most studies of *Reelin* naturally focused on the development of this organ. Immunolabeling studies with the CR-50 antibody and double-labeling studies demonstrated that an early and transient neuronal population, the Cajal-Retzius cells, expresses high levels of *Reelin* during the embryonic development of forebrain structures [1, 20, 32, 44, 45]. These cells occupy the marginal zone of the cerebral cortex and hippocampus throughout embryonic development and die 1-2 weeks after birth, as neuronal migration and layer formation in these structures are completed. It is interesting to note that Cajal-Retzius cells had been described since the late 1800s, and their unique and complex morphology was carefully reconstructed, but it was not until the discovery of *Reelin* that the essential role of these cells in the control of neuronal migration during forebrain development was finally appreciated. In the embryonic cerebellum, *Reelin* is expressed by granule cell precursors in the external granule layer [1, 45, 46]. These cells eventually migrate inwardly to form the internal granule cell layer as the cerebellum continues to grow in size and becomes foliated at early postnatal ages. Thus, a similar scenario is present in all laminated embryonic cortical structures of the murine brain, where transient neurons express and secrete *Reelin* in superficial regions to promote neuronal migration and cellular layer formation in the cortical plate that is developing underneath them. This general pattern of expression is highly conserved in other rodents such as the rat [47, 48]. In this species, *Reelin* is expressed not only by Cajal-Retzius cells but also by another cell population of the forebrain marginal zone, the subpial pyriform cells. With the development of new monoclonal antibodies that recognized human *Reelin* [39], more immunochemical studies became possible. These studies showed that, like in the rat, two major cell populations of *Reelin*-expressing cells are present in the marginal zone of the prenatal human neocortex: classic Cajal-Retzius and Cajal-Retzius-like cells [49]. The presence of additional *Reelin*-producing cells types in the embryonic marginal zone correlates with a more complex and protracted development of the neocortex in more cognitively competent species.

5. Functions of *Reelin* during Brain Development

What is the role of *Reelin* during early brain development? Clearly, the expression of *Reelin* by Cajal-Retzius and Cajal-Retzius-like cells in the embryonic forebrain is linked to the control of neuronal migration in the cortical plate. In the embryonic cerebral cortex, it specifically directs the radial migration and the formation of cellular layers by principal, excitatory cortical neurons born in the ventricular zone. The cellular and molecular mechanisms that underlie this critical biological function of *Reelin* in the embryonic forebrain will be discussed in detail below. Similar to the cerebral cortex, *Reelin* also directs the radial migration and layer formation of pyramidal neurons in the embryonic hippocampus proper. The essential function of *Reelin* in the establishment of cortical cellular layers is maintained postnatally in the dentate gyrus. In this structure, neurogenesis and granule neuron migration continues after birth and into adulthood. Thus, in this unique brain region, *Reelin*, produced by Cajal-Retzius cells in the outer molecular layer, continues to function as a major regulator of neuronal migration and cellular layer formation throughout life [50]. The importance of *Reelin* in the postnatal dentate gyrus as a positional clue is underscored by the finding that loss of *Reelin* is associated with granule cell dispersion in patients, as well as in animal models of temporal lobe epilepsy [51, 52]. This observation has potential clinical implications, since follow-up studies have shown that addition of recombinant *Reelin* can rescue granule cell dispersion, at least in animal models [53]. Recent studies further suggested that granule cell dispersion results from impaired *Reelin* processing and the consequent lack of secretion by Cajal-Retzius cells of the dentate gyrus and not from cell death or expression deficits, raising renewed interest in the identification of the proteases that carry out *Reelin* proteolytic cleavage [51]. In the cerebellum, *Reelin* produced by granule cells in the external granule layer is required for the radial migration of Purkinje cells, which are born in the cerebellar ventricular zone and form initially a plate and then a single-cell layer. This view stems from the extensive analysis of cerebellar development in *reeler* mice [7, 54–56], from *Reelin* gene and protein expression data [1, 46], and from functional studies in organotypic cultures [34]. Taken together, this body of work indicates that *Reelin*, expressed by transient cells in superficial layers, specifically regulates radial neuronal migration in all developing laminated brain structures.

In addition to cellular layer formation, *Reelin* produced by Cajal-Retzius cells has been shown to promote the initial growth of axonal and dendrite processes. The formation of hippocampal fiber layers was first recognized as a perinatal function of *Reelin* [57]. In the early postnatal hippocampus, Cajal-Retzius cells continue to secrete large amounts of *Reelin* in the stratum lacunosum moleculare (SLM) and the outer molecular layer (OML) of the dentate gyrus. These regions represent the main targets of the entorhinohippocampal pathway. Using an organotypic coculture system, investigators elegantly demonstrated that *Reelin* is not essential for targeting these axons to the SLM and OML,

but it is required for their early growth and branching [20]. Furthermore, Cajal-Retzius-produced *Reelin* was shown to promote synaptogenesis by entorhinal afferents [58]. These studies implicated *Reelin* in axon maturation and indirectly implicated this protein in the formation of synaptic structures. However, the ability of *Reelin* to stimulate axon growth appears to be limited in time and place. Indeed, defects in the branching of entorhinohippocampal axons were only found in the developing, but not in the adult, *reeler* hippocampus [20]. Furthermore, no defects have been reported in other axonal pathways of *reeler* mice so far. Building on the early observation that dendrite growth and cellular orientation are abnormal in *reeler* [59–61], other studies implicated *Reelin* in the control of the growth of these cellular processes. Using a fluorescent transgene to facilitate visualization *in vivo*, the development of apical processes of dentate granule cells was found to be delayed in *reeler* mice [62]. Furthermore, using cultured immature hippocampal neurons of wild type and *reeler* mice, this study demonstrated that *Reelin* is directly required for normal dendritic elongation and branching. Altogether, these findings suggested that *Reelin* promotes the early growth of apical dendrites of hippocampal neurons, which grow toward the Cajal-Retzius-cell-containing layers at early postnatal ages. Similarly, in the developing cerebral cortex, *Reelin* signaling was shown to promote the outgrowth of apical dendrites originating from cortical neurons contacting Cajal-Retzius cells in the marginal zone [63, 64]. *In vitro* studies largely supported *in vivo* observations and helped defining the molecular mechanisms underlying dendrite outgrowth by forebrain neurons [62, 65]. Recent studies further demonstrated that *Reelin* induces the extension of the Golgi apparatus in apical dendrites, thus stimulating dendrite growth and cell polarization [66]. However, as for axonal processes, the role of *Reelin* may be limited to promoting the initial dendrite outgrowth that occurs during development. This idea is supported by the observation that Reelin signaling is not required for the full maturation of pyramidal hippocampal neurons in long-term *in vitro* cultures [67].

Besides growth of cellular processes, synapse formation represents an important step in postnatal brain development, which occurs during the late phase of dendrite and axon maturation. During these later times, approximately corresponding to 2-3 weeks after birth in rodents, the expression of Reelin in the forebrain is no longer exclusively localized to superficial layers but is widespread throughout all cellular layers due to the gradual appearance of Reelin-expressing interneurons [1, 32, 44, 45, 68, 69]. However, some Cajal-Retzius cells persist in the postnatal hippocampus and thus may play a role in later aspects of the development of this structure. Indeed, using fluorescence imaging techniques *in vivo* and *in vitro*, further studies demonstrated that Reelin promotes the development of dendritic spines [70]. This study specifically identified a defect in spine density in young adult heterozygous and homozygous *reeler* mice *in vivo* and in organotypic slice cultures *in vitro*, which was rescued by the addition of exogenous *Reelin*. However, as for the growth effect on hippocampal axonal and dendrites, spine density was only transiently impaired in young adult Reelin-deficient mice (less than 1 month old), was very slightly reduced at

2 months [71], and was no longer affected in adult animals older than 6 months of age.

Taken together, the evidence so far indicates that Reelin plays multiple roles in brain development: it plays a unique and major role in controlling radial neuronal migration in cortical structures during embryonic ages and also contributes to promoting the growth of some axonal projections in the hippocampus, apical dendrite development and synapse formation in forebrain cortical structures at postnatal ages. In the adult brain, it also modulates synaptic function and plasticity, as discussed later in this review (Figure 1).

6. Cellular Targets of *Reelin* Signaling during Early Brain Development

Decades of neuroanatomical analysis of the homozygous *reeler* mouse, which preceded the discovery of the *Reelin* gene, had set the stage for understanding the role of this protein in brain development. The reported widespread defects in cellular layer formation in all cortical structures implied that Reelin is essential for the migration of many neuronal populations, including most cortical and hippocampal neurons [2, 8, 59, 60, 72–75]. A careful examination of the progression of the anatomical defects during cerebellar development also indicated that the layer organization of Purkinje cells in the cerebellar cortex represented the step that was directly affected by the absence of Reelin [7, 54–56, 76]. Thus, the failure of granule cells to proliferate, which ultimately leads to the lack of foliation and cerebellar hypoplasia in *reeler* mice, was recognized as a secondary defect due to the malposition of Purkinje cells, which failed to enter the cerebellar cortex after leaving the ventricular zone and remained localized in a deep cerebellar mass. This insightful observation also suggested that Purkinje cells are the direct targets of Reelin and that Reelin primarily promotes the final steps in the radial migration of principal neurons in cortical structures. However, examination of the anatomical phenotype of *reeler* mice in other structures led to different interpretations. For example, in the *reeler* hippocampus, pyramidal neurons and dentate granule cells appeared to migrate past their normal position, leading to the hypothesis that Reelin represents a stop signal necessary to arrest radial migration rather than promoting it [57]. The cellular complexity of cerebral cortex made it even more difficult to predict, based on anatomical phenotype and expression data alone, the identity of the cellular targets of Reelin and its mechanism of action with regard to the control of radial migration. This determination required a deeper understanding of molecular mechanisms of Reelin activity on migration, which originated from advances in the wider field of brain development and the development of new genetic approaches that occurred over the course of two decades.

At the end of the 1900s, the prevailing view of neocortical development was that principal neurons, born in the ventricular zone, migrate radially into the cortical plate following the guidance of radial glial cells, which provide a scaffold for migration. Thus, a reasonable expectation around this time was that Reelin would affect the development or
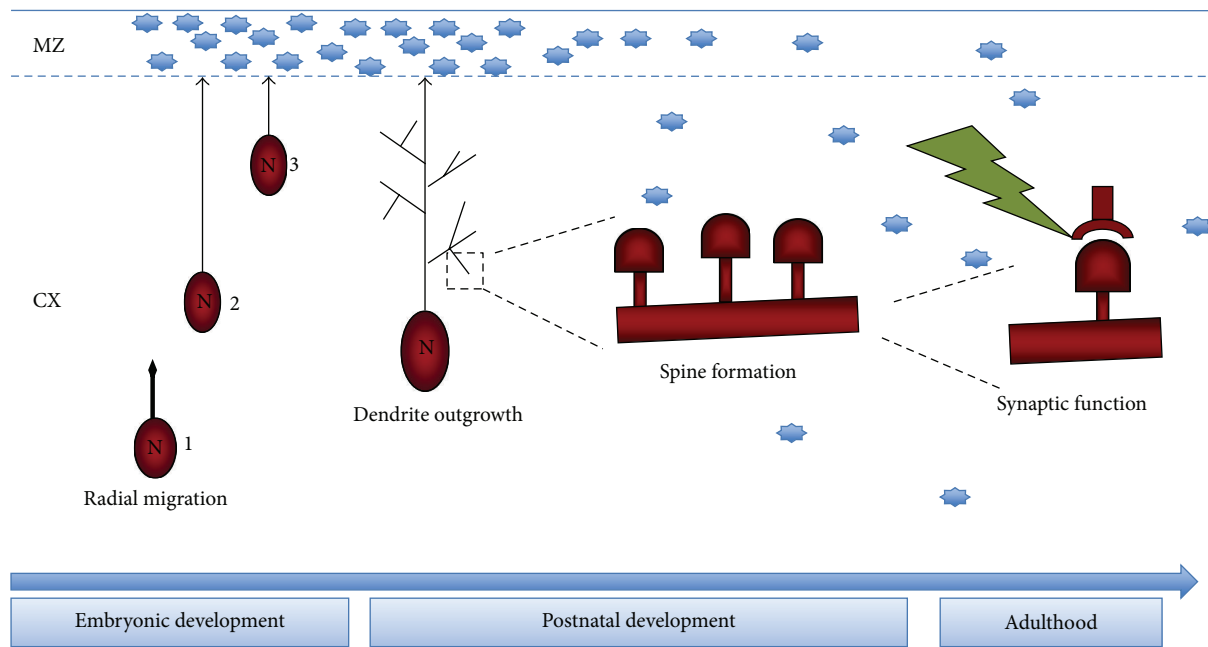
FIGURE 1: Schematic representation of the multiple activities of Reelin in the developing and adult forebrain. Blue octagons represent secreted Reelin molecules, and red ovals represent the cell bodies of principal forebrain neurons (N). During embryonic development, Reelin is mostly localized to the marginal zone (MZ) and directs radial migration by affecting multiple steps, such as radial orientation (1), attachment of the leading edge to the MZ (2), and somal translocation (3). During postnatal development, Reelin becomes expressed throughout cortical forebrain structures (CX), where it promotes mainly the growth of apical dendrites and spine formation. In the adult brain, Reelin modulates synaptic function and plasticity.

the integrity of radial glia scaffold and thus indirectly affect neuronal migration. Indeed, this scenario appears to be the predominant mechanism of action in at least one structure, the dentate gyrus of the hippocampus, where the radial glial scaffold is severely disrupted in *reeler* mutants [77]. In the *reeler* forebrain, some evidence of impaired radial glial cell development was also found [78]. However, based on the early observation that the CR-50 interfering antibody blocked neuronal aggregation in cortical explants lacking a true radial glia scaffold [32], the alternative hypothesis that Reelin may primarily affect principal cortical neurons directly, especially during the last phase of their migration inside the cortical plate, began to take hold [10]. Furthermore, new crucial discoveries in the field of cortical development provided a framework for this latter hypothesis. First, it became clear that radial glia cells are actually neural progenitor cells that give rise to many principal neurons of the neocortex [79, 80]. Second, new imaging studies demonstrated that, in addition to glial-guided locomotion, neurons could also migrate independently of radial support, by somal translocation or a multipolar mechanism [81, 82]. However, the clear identification of the cellular targets of Reelin during radial migration required a better knowledge of the molecules necessary to transduce the signal.

In addition to its major function in the control of radial neuronal migration in cortical structures, Reelin was also shown to affect the migration of neuronal precursors in the rostromigratory stream [83]. These precursors engage in tangential chain migration from the subventricular zone of the cerebral cortex on route to the olfactory bulb. Reelin causes the detachment of these precursors from their substrate, thus disrupting their migration. Thus, neuronal precursors in the rostromigratory stream appear to be direct cellular targets of Reelin, even though Reelin exerts a negative effect on their migration.

7. Molecular Mechanisms: The Discovery of the Dab1 Adaptor Protein

The first essential transducer of the Reelin signal to be identified was the mammalian homolog of Drosophila *Disabled-1* (Dab1), an intracellular adaptor protein that, when phosphorylated on tyrosine residues, was capable of interacting with Src-homology (SH2) domains of Src-family kinases (SFKs) [84]. Dab1 contains a phosphotyrosine binding (PTB) domain, which also enables this adaptor protein to bind not only tyrosine-phosphorylated proteins, but also phosphoinosites and receptor internalization domains [85–87]. Spontaneous mutations in the Dab1 gene were identified in *scrambler* and *yotari*, two independent lines of mutant mice that exhibited a phenotype remarkably similar to *reeler* [88–91]. Furthermore, an engineered Dab1 knockout mouse also exhibited the *reeler*-like phenotype [92]. The similarity between Dab1 and Reelin mutant mouse phenotypes strongly suggested that Dab1 functioned downstream of Reelin in the same linear signaling pathway. In addition, biochemical studies revealed that recombinant Reelin rapidly induces

the phosphorylation of Dab1 on multiple tyrosine residues in cultured cortical neurons [93–95]. This event is mediated mainly by Fyn and Src, two SFKs that are activated by Reelin [96, 97]. Dab1 phosphorylation on tyrosine residues is essential for signal transduction, since point mutations in these sites result in the appearance of a *reeler*-like phenotype *in vivo* [98]. Dab1 tyrosine phosphorylation is also coupled to its ubiquitination by the Cbl ligase and to its degradation by the proteasome system [99, 100]. This degradation represents a mechanism for signal termination and explains why Dab1 protein levels are elevated in the brain of *reeler* as well as Fyn/Src double knockout mice [89, 101, 102]. Taken together, these findings indicate that Reelin promotes the activation of Dab1 in target cells, but protein degradation mechanisms ensure that the signal is transient. This sequence of activation and shutoff may be crucial for the execution of multiple steps in neuronal migration. Indeed, different models have been proposed to explain how the Reelin function may change dynamically from a permissive, attractive clue during the initial phases of migration to a detachment from radial glia and thus a stop signal during later phases [103–106].

Expression studies indicated that Dab1 is expressed at high levels by principal neurons of the embryonic cerebral cortex and hippocampus and by Purkinje cells in the cerebellum, in a pattern that is largely complementary to that of Reelin [107, 108]. This pattern suggested that forebrain principal neurons and Purkinje cells are the direct targets of Reelin during migration and cellular layer formation. In the forebrain, however, Dab1 expression was also reported in radial glia cells [109], and the morphology of these cells was shown to be affected by Reelin [110], raising the possibility that Reelin may target both principal neurons and radial glia cells, to regulate migration and cellular layer formation.

8. Cellular Mechanisms of *Reelin*-Dependent Neuronal Migration

With the development of novel techniques that enabled investigators to manipulate gene expression *in vivo* and to observe neuronal migration in cultured organotypic slices, new functional studies became possible. Using the GFP-labeling retroviral and confocal imaging approach pioneered by the Kriegstein Lab to study cortical migration in rat cultures [80], investigators reported that Dab1 mutant (*scrambler*) neurons maintain an increased adhesion to radial glia fibers which prevents them from reaching the upper cortical layers [111]. This view seemed to lend support to earlier hypotheses postulating that the *reeler* defect was essentially due to excessive cellular adhesion, leading to an inability of newborn neurons to bypass previously generated cohorts and causing a traffic jam along radial fibers [9, 112]. This interpretation was challenged by later studies employing more sophisticated genetic tools [113]. Nevertheless, the first analysis of the migratory behavior of *scrambler* cortical neurons provided some important mechanistic insights into Reelin activity. Indeed, this study was the first to demonstrate that Dab1 is required to extend the leading processes of migrating neurons into the developing cortical plate. However, it did not identify

the target cells directly affected by Reelin, since the *scrambler* cortical slices lacked Dab1 in migrating cortical neurons as well as in radial glia. This issue was addressed in a following study, which utilized the newly developed technique of *in utero* electroporation [114] and RNA interference (RNAi) to suppress Dab1 expression specifically in a subset of migrating neurons [64]. Dab1 knockdown neurons were found to be deficient in their ability to extend the leading process and to translocate their cell bodies into the upper cortical layers. In addition to pointing out a mode of migration that may be exquisitely affected by Dab1 deficiency, this study also suggested that the apical processes of migrating neuron are essentially immature dendrites, and, in this context, Reelin stimulates the dendrite outgrowth, of neocortical pyramidal neurons, as it had been previously shown for hippocampal neurons [62]. Indeed, further studies demonstrated that Reelin controls neuronal orientation and polarized dendritic growth in the cortical plate as well as the developing cerebellum [63, 115, 116]. Together, these studies underscored the close functional link between cellular polarization, early dendrite outgrowth and neuronal migration. Despite these advances, many questions remained unanswered regarding the specific mode of migration affected by Reelin and Dab1 and the identity of the direct targets of Reelin during radial migration in the neocortex. These questions were finally answered through the development of a novel set of genetic tools, which enabled investigators to conditionally delete the *Dab1* gene in either early or late cortical neurons and examine their ability to undergo somal or glial-guide locomotion in cultured slices [113]. This study conclusively demonstrated that Reelin affects primarily somal translocation and that Dab1 is required in migrating neurons to extend their apical process into the superficial areas of the neocortex, thus enabling both somal translocation of early-born neurons from the ventricular zone and also the terminal translocation of late-born neurons that initially move by glial-guided locomotion past cohorts of previously generated neurons. A following study demonstrated that Reelin in the marginal zone promotes the adhesion, stabilization, and branching of Dab1-expressing apical processes by a cell-matrix adhesion mechanism mediated by Integrin $\alpha5\beta1$ [117]. However, this integrin may function in radial glia cells rather than in migrating neurons. Recently, another study documented heterotypic cell-cell interactions between migrating neurons and Cajal-Retzius cells, which are mediated by adhesion molecules nectin1 and nectin3 and by Cadherin 2 [118]. Cajal-Retzius cells express nectin1, which binds nectin3 on the cell surface of migrating neurons. Nectin3 and its associated effector afadin then mediate homophilic cell-cell adhesion via Cadherin 2 molecules, which are expressed by both cell types. These adhesion mechanisms allow for somal translocation to occur, bringing the cell bodies of migrating neurons just underneath the marginal zone, and explain why Reelin must be so strikingly localized to this zone during corticogenesis. They also reveal how Reelin (presumably in the form of a full-length protein, dimer, or insoluble multimer aggregate) conveys its short-range promigratory cue to neurons that come in contact with superficial layers via their apical process. But what about neurons that do not contact the marginal

zone and are located in the deep regions of the intermediate zone during the development of the cortical plate? Most of these neurons exhibit an initial multipolar morphology and normally acquire a bipolar morphology just prior to engaging in radial migration. Are they responsive to Reelin, and how do they receive the signal? Earlier work using cortical slices demonstrated that soluble Reelin added to the culture medium was able to promote migration and layer formation, albeit not as neatly as *in vivo* [40]. This activity seemed unlikely to be spatially restricted to the marginal zone, but how would Reelin come in contact with these distant neurons *in vivo*? With the development of novel epitope-specific antibodies, it became possible to visualize Reelin fragments in brain sections [41]. These immunolabeling studies revealed that the central fragment of Reelin could reach the depth of the cortical plate, suggesting that the cleavage of the Reelin protein generates signaling molecules that may function at long range, away from the marginal zone. Consistently, a recent study revealed that Reelin affects the migratory behavior of neurons that are located deep into the developing cortical plate, by promoting the conversion from a multipolar to a bipolar morphology [119].

In summary, the data so far indicate that Reelin in the marginal zone promotes somal translocation of Dab1-expressing principal cortical neurons directly from the ventricular zone, or terminal translocation from the developing cortical plate, and thus acts directly on migrating neurons as long as they make contact with the marginal zone through an apical process. This function may be induced by full-length Reelin or fragments that assemble into oligomers to maximize signaling activity through the phosphorylation of Dab1. Reelin fragments may also diffuse into the depth of the cortical plate to promote the bipolar transformation and the oriented migration of Dab1-expressing neurons that do not contact the marginal zone. While it seems likely that these direct effects of Reelin on migrating cortical neurons do not require a radial glia scaffold, the possibility that Reelin also functions by affecting the stability and branching of the radial glia end feet cannot be excluded, particularly in structures such as the dentate gyrus where the radial scaffold could significantly contribute to the efficiency of migration.

9. Dab1 Signaling in the Control of *Reelin*-Dependent Neuronal Migration

In the section above, we discussed the crucial role of the adaptor protein Dab1 in Reelin-dependent neuronal migration. Here, we will discuss the molecular mechanisms linking Reelin to Dab1 activation and the downstream events that link Dab1 to the execution of the migration function.

Early studies demonstrated that full-length Reelin or its central fragment alone induces the phosphorylation of Dab1 on specific tyrosine residues [40, 94, 95]. These phosphorylation events are mediated by the SFKs *Fyn* and *Src*, since they are blocked by the pharmacological inhibitor PP2 and by the genetic deletion of *Fyn* and *Src* genes [96, 101]. The appearance of a *reeler*-like phenotype in phospho-mutant Dab1 mice [98], as well as double Fyn/Src knockout

mice [101], further demonstrated that these tyrosine phosphorylation events are crucial for Reelin-induced neuronal migration and layer formation. Given the importance of this signaling event, considerable efforts have been devoted over the years to understand how Dab1 phosphorylation is stimulated and what are the molecular consequences of this protein modification. However, signal transduction is often the result of a nonlinear series of intersecting events, and it soon became clear that Reelin signaling was not going to be an exception. Indeed, Reelin was found to stimulate Src phosphorylation (presumably reflecting an activation of multiple SFKs) in a manner that is dependent on the presence of Dab1 [97, 120]. Thus, it appears that a positive feedback mechanism potentiates SFK and Dab1 activation in response to Reelin. SFK activation likely plays a major role in Reelin signal transduction. For example, it was shown to be required for the activation of other downstream events such as the activation of the PI3K by Reelin. The activation of PI3K was deduced from the induction of downstream events, such as the phosphorylation of Akt and the phosphorylation of GSK3β on an Akt-dependent site (Ser9) and from the ability of the PI3K pharmacological inhibitor LY294002 to block both of these events [121]. It was later demonstrated that Reelin also increases the levels of Akt phosphorylation on the Thr308 residue, a site which is directly phosphorylated by the PI3K-dependent kinase Pdk1 [71]. In addition, Dab1 was found to interact directly with the PI3K regulatory subunit p85α in response to Reelin stimulation [122]. Further downstream of Akt, other phosphorylation events were identified linking Reelin signaling to the mTOR pathway. The mTOR kinase participates in two complexes: mTORC1, which is primarily implicated in the control of protein translation and cell growth, and mTORC2, which is principally involved in the regulation of cytoskeletal dynamics [123]. Reelin was found to stimulate mTOR phosphorylation and to promote the mTORC1-dependent phosphorylation of p70S6K and the ribosomal protein S6 [65, 71]. Thus, Reelin stimulates mTORC1 activity likely through SFK, PI3K, and Akt since p70S6K and S6 phosphorylation is blocked by the SFK inhibitor PP2 and by the PI3K inhibitor LY294002, in addition to the mTORC1 inhibitor rapamycin [71]. However, it should be noted that, since biochemical studies identified mTORC2 as the complex that directly phosphorylates Akt on Ser473 [124] and Reelin stimulates this phosphorylation event, mTORC2 may also participate in Reelin signaling. The mechanism of activation of mTORC2 by many extracellular signals is not completely clear; however, SFK, PI3K, and Akt are also likely to mediate Reelin-induced mTORC2 activation, since Ser473 Akt phosphorylation is blocked by SFK and PI3K inhibitors. Despite the abundance of biochemical evidence, the biological significance of PI3K and Akt activation in Reelin signaling is not entirely clear. *In vitro* pharmacological studies using cultures cortical slices initially suggested that the activation of PI3K and Akt may be important for the control of neural migration during early brain development, while the activation of the mTOR pathway may be more relevant to postnatal functions of Reelin, such as the modulation of dendrite elongation [65].

However, a genetic requirement for PI3K and Akt in neuronal migration has not been demonstrated. Furthermore, the analysis of recent knockout models lacking PI3K modulators, such as the Pten phosphatase (a suppressor of PI3K) [125] or PIKE (an enhancer of PI3K) suggests that this kinase is likely to be primarily involved in the control of neuronal growth, dendritogenesis, and survival rather than migration or layer formation [126]. Finally, it should be noted that Akt phosphorylation defects have not been reported in the embryonic brain of *reeler* or Dab1 mutant mice. Recent studies provided evidence that mTORC1 is involved in the NMDA-dependent rescue of synaptic plasticity and behavioral defects in juvenile *reeler* heterozygous mice [127]. Finally, basal levels of Akt phosphorylation were shown to be reduced in adult forebrain-specific conditional Dab1 knockout mice, which also exhibit plasticity and behavioral defects [128]. Together, these findings suggest that activation of the PI3K/Akt/mTOR pathway by Reelin may not play a crucial role in the early events leading to layer formation and corticogenesis but is likely to be involved in the postnatal control of neuronal maturation and synaptic plasticity.

Early efforts to identify potential downstream effectors of Dab1 in the control of migration pointed to components of the platelet activating factor (PAF) acetyl hydrolase 1b (Pafah1b) complex. This complex functions as a phospholipase and catalyzes the PAF lipid through the activity of two catalytic α subunits. The complex also contains a regulatory subunit, Lis1, which is involved in neuronal migration [129] and human lissencephaly [130, 131]. Genetic interactions and biochemical experiments indicated that Reelin promotes the interaction between Dab1 and Lis1 and that the absence of these two proteins cooperated to increase the frequency of hydrocephalus [132]. Further studies also demonstrated that the catalytic subunit of Pafah1b α2 binds to Dab1 and suppresses the hydrocephalus phenotype of Reelin/Lis double mutant mice [133, 134]. However, Lis1 turned out to control many aspects of neurogenesis and neuronal migration through a mechanism that involves the coupling of the centrosome to the nucleus [135]. This mechanism appears to be independent of both the catalytic activity of the Pafah1b complex and Reelin. Furthermore, genetic ablation of the catalytic subunits of Pafah1b did not result in neuronal migration defects [133]. Thus, this complex does not appear to be involved in the control of migration or corticogenesis; however, it may play a role in as yet unidentified mechanisms that contribute to normal brain development by preventing the onset of hydrocephalus.

Other biochemical studies identified several molecules that bind Dab1 and thus may potentially function in Reelin signaling. These include proteins that affect the actin cytoskeleton, such as Nckβ [136] and N-WASP [100], proteins that modulate the activity of the Ras/Rap family of GTP-binding proteins, such as the GTPase activating protein (GAP) Dab2IP [137], and Crk family proteins [93, 138]. Recent RNA interference studies and knockout studies suggest that Dab2IP is required for neuronal migration in the neocortex [139] and for dendrite and synapse development

in the cerebellum [140]. However, its direct involvement in Reelin signaling has not yet been established. Crk family proteins, on the other hand, have been shown to play a significant role in Reelin-dependent migration, likely by binding the GTP exchange factor (GEF) C3G, which then activates Rap1 [93]. Three members of this family, CrkI, Crk II, and CrkL, were shown to bind phospho-Dab1, and Reelin was shown to induce the phosphorylation of C3G and the activation of Rap1. This study suggested that the CrkL/C3G/Rap1 pathway operates downstream of Dab1 in Reelin-stimulated neurons. However, CrkI and II, but not CrkL, were also shown to promote Dab1 phosphorylation in an SFK-dependent manner, suggesting a more complex signaling mechanism [138]. The biological relevance of Crk family proteins in neuronal migration was demonstrated *in vivo* by the conditional double knockout of Crk and CrkL in neural progenitor cells. In a situation analogous to that of Fyn and Src, single Crk or CrkL mutant mice displayed mild neuroanatomical phenotypes, but double Crk/CrkL mutant mice displayed a *reeler*-like phenotype [141]. The absence of Crk and CrkL did not affect Dab1 phosphorylation but, surprisingly, hindered the ability of Reelin to phosphorylate not only C3G, but also Akt on Ser473. Because this site is a target of mTORC2, these findings raise the possibility that Crks transduce Reelin signaling downstream to Rap1 as well as mTORC2.

The role of Rap1 in Reelin signaling and radial migration was further explored in multiple *in vivo* studies. In one study, Rap1 was shown to regulate the expression of cell adhesion molecules of the Cadherin family in migrating neurons, an event that is required for glia-independent migration [113]. Specifically, in this study, it was proposed that Rap1 promotes Cadherin expression, which in turn favors the extension and attachment of leading processes of migrating neurons and enables terminal translocation near the upper cortical plate. In a second study, Rap1 was shown to regulate the membrane localization of N-Cadherin [119]. This event was linked specifically to the acquisition of proper cellular orientation, which is also required for glia-independent neuronal migration, but occurs in the deeper regions of the developing cortical plate. Building on these observations, recent studies further investigated the role of the Crk/C3G/Rap1 pathway in Reelin-dependent migration and provided a comprehensive view of its molecular mechanisms [117, 118]. This work suggested that Rap1 has a dual function in migration. In deep regions of the cortical plate, Rap1 functions through N-Cadherins to orient cell bodies and enable entry into the cortical plate, but this activity may not be stimulated by C3G. Near the upper cortical plate, Reelin strongly activates C3G and Rap1, and this in turn leads to the activation of α5β1 integrins, which mediate the attachment of apical processes to fibronectin in the marginal zone, and the recruitment of cell adhesion molecules such as nectins and Cadherin 2, which mediate cell-cell interactions in the marginal zone. Together, these secreted and contact-dependent mechanisms enable terminal translocation and the completion of radial migration near the Reelin-rich marginal zone.

10. *Reelin* Receptors and the Control of Neuronal Migration

The body of work discussed above described in considerable detail the cellular and molecular mechanisms of neuronal migration which operate immediately upstream and downstream of Dab1 during Reelin signal transduction. This section will address the receptor activation mechanisms leading to Dab1 recruitment at the plasma membrane. What are the Reelin receptors, and how do they cause SFK activation and Dab1 phosphorylation?

Based on extensive genetic and biochemical studies, two high-affinity Reelin receptors have been identified and shown to play a major role in Reelin signaling. They are the apolipoprotein E receptor 2 (ApoER2) and the very-low-density lipoprotein receptor (VLDLR) [33, 142]. These two receptors are members of the lipoprotein receptor superfamily [143], and their involvement in Reelin signaling was first suggested by the observation that double Apoer2/Vldlr knockout mice exhibit a *reeler*-like neuroanatomic phenotype [102]. Single knockout mice displayed a milder layer phenotype, suggesting that they have partially overlapping functions in mediating the activity of Reelin on neuronal migration. Biochemical experiments *in vivo* and *in vitro* demonstrated that ApoER2 and VLDLR are each capable of binding Reelin with similar affinity [33, 142, 144]. Like all members of the lipoprotein receptor superfamily, ApoER2 and VLDLR can also bind lipoproteins and other extracellular ligands with lower affinity. Upon binding, these receptors internalize their ligand, including Reelin, a process that requires the NPxY motif, an internalization domain present on the cytoplasmic tail of the receptors. The receptors also bind Dab1 on their cytoplasmic tail near their NPxY motif [86, 145]. On the Dab1 side, binding to the receptors is mediated by the pleckstrin homology/phosphotyrosine binding domain (PH/PTB) [87, 145, 146]. ApoER2 and VLDLR actively traffic between the plasma membrane and the endosomes, and their translocation to the plasma membrane is facilitated by Dab1 binding [146]. Upon Reelin binding, the receptors cluster and internalize [147], thereby causing the activation of Fyn and Src, which then phosphorylate Dab1 and initiate downstream signaling events crucial for migration. Despite their partially redundant function, there are differences between ApoER2 and VLDLR signaling. Some of the differences can be attributed to relative receptor expression. For example, VLDLR is more highly expressed in the Purkinje cells of the cerebellum, and single VLDLR knockout mice exhibit defects in this structure, whereas ApoER2 is preferentially expressed in cortical and hippocampal neurons, correlating with defects in forebrain structures of single *ApoER2* knock out mice [102]. However, the two receptors can bind distinct signaling proteins, in addition to Dab1. Indeed, VLDLR, but not ApoER2, has been found to bind subunits of the Pafah1b complex [148], whereas ApoER2, but not VLDLR, can bind JNK and activate the p38MAP kinase pathway [149]. However, it is not clear whether these unique interactions participate in the control of neuronal migration. Both ApoER2 and VLDLR bind Reelin through a similar extracellular domain that contains a conserved lysine residue [37]. It is thought that the strongest signal activation is achieved when the receptors bind multimeric aggregates of full-length Reelin, presumably leading to massive receptor clustering and SFK activation [42]. However, it has been shown that ApoER2 and VLDLR can also bind a central fragment of Reelin that is generated by proteolytic cleavage [40]. Functional studies demonstrated that binding of this fragment is sufficient to induce Dab1 phosphorylation and lead to layer formation in cultured cortical slices. These findings raised the question of whether Reelin cleavage is an event that dampens activity by reducing the levels of the more active full-length protein or enables activity by releasing the active central fragment from a precursor full-length protein. This question has not yet been resolved and awaits the identification of the specific sites of cleavage, as well as the identification of the proteases responsible for Reelin cleavage. In the meantime, the fact that N- and C-terminal Reelin fragments are generated by cleavage and that they do not bind ApoER2 and VLDLR raised the possibility that other receptors or coreceptors may participate in Reelin signaling and contribute to migration control.

Some of the earliest candidates as alternative Reelin receptors were integrins. Integrins $\alpha 3/\beta 1$ and $\alpha 5/\beta 1$ in particular were implicated in Reelin signaling by binding experiments [150]. However, this finding has not been replicated by others, and the analysis of knockout mice lacking several integrin subunits suggested that, while these proteins contribute to migration and layer formation, they may not be directly required for Reelin-dependent migration. Rather, knockout studies suggested that $\beta 1$ integrins are primarily required for the integrity of the glia limitans [151]. Thus, their loss disrupts cellular layer formation through disruption of the radial scaffold; however, it does not directly affect neuronal migration, and it does not result in a *reeler*-like phenotype, suggesting that $\beta 1$ integrins are not essential for Reelin signaling in neurons [152]. Given the recent studies discussed above [117], the role of integrins in Reelin signaling cannot be completely dismissed. However, it seems likely that $\beta 1$-containing integrins in the cerebral cortex function in radial glial cells rather than in migrating neurons. This view is consistent with previous data demonstrating that Reelin and $\beta 1$ integrins are required for the formation of the radial glial scaffold in the hippocampus [153].

Another family of proteins that have been proposed to function as Reelin receptors is the Cadherin-related neuronal receptor (CNR) family, particularly CNR1 [154]. CNR proteins were found to be expressed in migrating neurons, bind the N terminal region of Reelin, and also associate with the SFK Fyn on the intracellular side. Their expression pattern and binding properties made them excellent candidates as Reelin receptors or coreceptors. However, the binding of CNR1 to Reelin was not replicated by other investigators [40].

In the past couple of years, new potential Reelin receptor systems have surfaced. Ephrin B proteins were reported to bind the N terminal region of Reelin and claimed to be essential components of the Reelin pathway in the control of neuronal migration [155]. However, as evident from the published corrigendum and the multitude of online comments related to this paper, many questions about

the validity of the findings have been raised. Nevertheless, further investigations of these receptors have been conducted. Since ephrin B proteins bind to the EphB family of transmembrane receptor tyrosine kinases, which transduce bidirectional tyrosine kinase-mediated signals to both the Eph receptor-expressing (forward signaling) and the ephrin-expressing cell (reverse signaling), the potential role of these kinases in brain development and Reelin signaling was also investigated. One study found that ephrin-B1 interacting with EphB2 controls the migration of dentate progenitor cells into the dorsal half of the developing dentate gyrus, perhaps in part by affecting Reelin expression [156]. Recently, a new study showed that the N terminal region of Reelin binds to the extracellular domains of EphB transmembrane proteins, inducing receptor clustering and activation of EphB forward signaling in neurons, independently of ApoER2 and VLDLR [157]. However, mice lacking EphBs displayed a very mild migration phenotype that was limited to a modest positioning defect of CA3 hippocampal-pyramidal neurons. Thus, even though there appears to be some signal integration between Reelin and ephrin/EphB receptors, the physiological significance of this interaction for neuronal migration is rather limited. Based on a recent study, it appears that ephrin/Eph signaling may be more important for mediating contact repulsion among Cajal-Retzius cells to ensure the even distribution of these neurons in the brain [158].

Overall, the data available so far indicate that the activity of Reelin in the control of neuronal migration is predominantly mediated by ApoER2 and VLDLR receptors, whose functions are overlapping but not identical. Both receptors are involved in the activation of SFKs, Dab1 phosphorylation on tyrosine residues, and the recruitment of Crk/CrkL, which lead to the activation of downstream events discussed above, which are essential for radial migration (Figure 2).

11. *Reelin*-Regulated Growth of Cellular Processes

In addition to controlling neuronal migration and layer formation in embryonic cortical structures, Reelin also plays an important role in promoting the growth and maturation of specific neuronal populations in the postnatal brain. As we mentioned above, the cellular source of Reelin in forebrain structures changes after birth. While Cajal-Retzius cells are the predominant population of Reelin-expressing cells in the marginal zone of the embryonic cerebral cortex and hippocampus, a subset of inhibitory, GABA-synthesizing neurons express Reelin and gradually become the predominant source of Reelin in the postnatal and adult forebrain [45, 68]. However, Cajal-Retzius cells are still present and remain the major source of Reelin during the first 1-2 weeks after birth in the early postnatal hippocampus. Transplantation studies demonstrated that this early postnatal source of Reelin is needed to promote the branching of axonal projections from the entorhinal-hippocampal pathway, which terminates in the marginal zone of the hippocampus and dentate gyrus (the outer molecular layer) [20, 58]. However, since Reelin is a secreted protein, its functional range needs not to be limited to the growth of cellular processes that develop in the same layers that contain Reelin-expressing cells, that is, the marginal layers. Indeed, early studies had revealed extensive dendrite abnormalities throughout the hippocampus of homozygous *reeler* mice [60]. However, it was unclear from these anatomical observations whether dendrite defects were due primarily to cellular ectopia or were the direct result of Reelin deficiency. This issue was revised in recent years with a combination of novel *in vivo* and *in vitro* approaches. Taking advantage of a fluorescent transgene that was expressed in the postnatal forebrain, developing apical dendrites of dentate granule neurons were visualized in *reeler* mutant and control littermates. From this comparison, it was apparent that dendrite growth was delayed in *reeler* mice [62]. Furthermore, *in vitro* studies demonstrated that dendrite elongation and branching proceed at a reduced rate in immature hippocampal neurons obtained from *reeler* or Dab1 knockout mice and kept for 3–6 days *in vitro* (DIV), irrespective of neuronal positioning [62]. In this experimental assay, the addition of recombinant Reelin to the culture medium rescued the *reeler* defect in a manner that was dependent on the activity of lipoprotein receptors and Dab1. Thus, the same signaling pathway that controls neuronal migration before birth also mediated an early postnatal function of Reelin in the control of dendrite outgrowth, at least in immature hippocampal neurons. Following *in vitro* studies further revealed that the trophic effect of Reelin on dendrite outgrowth is dependent not only on Dab1 and lipoprotein receptors, but also on other previously identified components of Reelin signaling, including Src kinases, Crks, PI3K, and Akt [65, 159]. These studies also demonstrated that Reelin, acting through the well-known PI3K/Akt pathway, positively modulates the activity of the mTOR kinase, which is required for the stimulation of dendrite outgrowth, and activates downstream proteins, such as the p70S6K, that are known to participate in the control of protein translation. Few years later, other studies confirmed the activation of mTOR by Reelin and further demonstrated the increased phosphorylation of p70S6K and the downstream ribosomal protein S6, events that are completely dependent on SFK and PI3K activity [71]. To these days, however, the effects of Reelin on protein translation and its significance in terms of cell growth and dendrite morphogenesis have not been fully investigated.

In addition to the previously identified signaling cascade, the analysis of the molecular mechanisms of cellular and fiber layer formation led to the identification of potentially new players in Reelin signal transduction. A study revealed that serum response factor (SRF), a transcription factor that regulates synaptic-activity-induced immediate-early gene (IEG) induction and cytoskeleton-based neuronal motility, is important for hippocampal lamination and that this factor mediates the effect of Reelin on dendrite development [160]. This finding raised the intriguing possibility that Reelin might enhance F-actin stability by elevating IEG mRNA levels via SRF-dependent gene transcription. All the studies mentioned above utilized immature hippocampal cultures (3–6 DIV) and demonstrated a trophic effect of Reelin on the initial growth of dendrites. However, it should
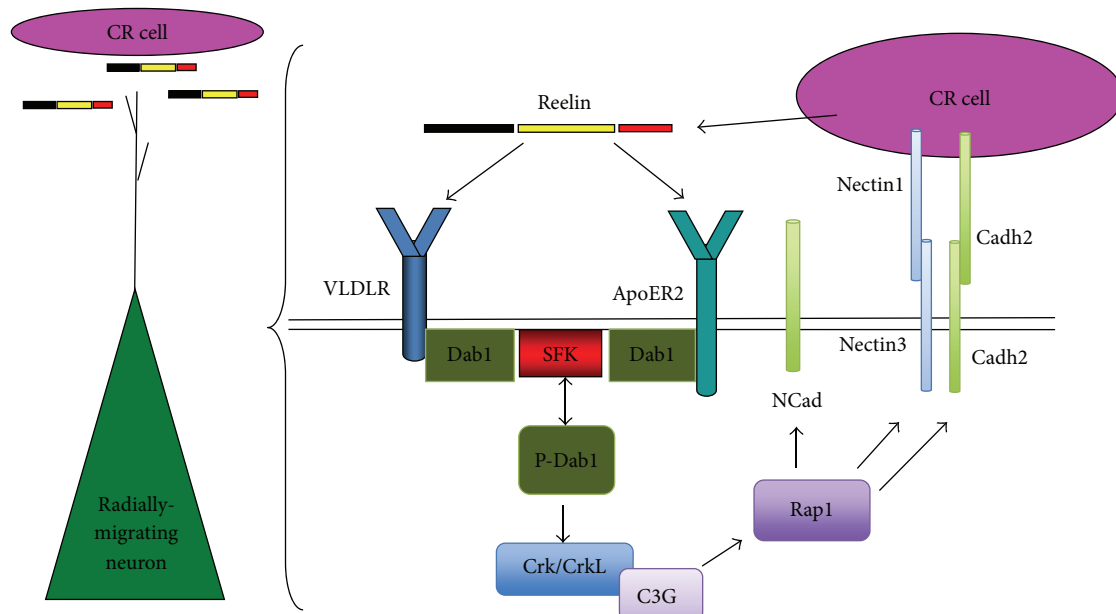
FIGURE 2: Diagram depicting the relationship between Reelin-secreting surface neurons and radially migrating neurons expressing the core signaling machinery (right). Surface neurons such as Cajal-Retzius cells (CR) secrete Reelin, which through its central region (yellow region) binds VLDLR and ApoER2 receptors on the surface of radially-migrating neurons. Ligand binding causes the activation of SFKs Src and Fyn, and the phosphorylation of the Dab1 adaptor protein. This event results in the recruitment of Crk/CrkL adaptor proteins mediate further downstream signaling through C3G and Rap1, resulting in the surface expression of N-Cadherin (Ncad), which is required for radial orientation, and activation of Nectin1-Nectin3, and homophilic Cadherin 2 (Cadh2) cell-cell interactions that are required to complete radial migration by somal translocation.

be noted that *in vitro* studies utilizing mature hippocampal cultures (18–20 DIV) obtained from Dab1 knockout mice demonstrated that, although Reelin-Dab1 signaling promotes initial hippocampal dendrite development, it is not required for neurons to reach full maturity [67]. Thus, as for entorhinohippocampal axons, hippocampal dendrite outgrowth is stimulated by Reelin during early neuronal development, but neurons do not require Reelin signaling to reach their full morphological maturity, as other growth factors likely compensate and promote the growth of these processes even in the absence of Reelin signaling.

What cellular mechanisms underlie the control of neurite development by Reelin? Recent studies showed that the Golgi apparatus is abnormal in *reeler* and Dab1 knockout mice and that Reelin treatment rapidly induced the deployment of the Golgi apparatus into dendrites of cultured neurons [66]. Mechanistically, it was demonstrated that Reelin-Dab1 signaling antagonizes the effects of Stk25, a scaffolding protein that links the LKB1-STRAD-GM130 complex to neuronal polarization. Other studies have shown that Reelin increases growth cone motility and filopodia formation, through activation of the Rho GTPase Cdc42, in an ApoER2-, Dab1-, and PI3K-dependent manner [161]. Building on these findings, a recent study further demonstrated that the Cdc42/Rac1 guanine nucleotide exchange factor αPIX/Arhgef6 promoted translocation of Golgi cisternae into developing dendrites of hippocampal neurons [162]. Reelin increased the αPIX-dependent effect and promoted the translocation of the Golgi apparatus into the dendrite that was most proximal to the Reelin source. These observations led to the interesting

conclusion that the spatial distribution of Reelin may contribute to the selection of the neuritic process that becomes the apical dendrite.

In addition to *in vitro* studies, *in vivo* studies demonstrated that the effect of Reelin on dendrite development is closely coupled to neuronal migration and is an important aspect of cellular layer formation. An examination of the morphology of Dab1 knockdown neurons in the late embryonic and early postnatal neocortex revealed simplified leading processes that were less likely to contact the Reelin-rich marginal zone [64]. These data confirmed a cell-autonomous role of Dab1 in dendritogenesis in the neocortex and suggested that remodeling of the leading process of a migrating neuron into a nascent dendrite by Reelin/Dab1 signaling plays an important role in final cell positioning. Furthermore, it was shown that Reelin injection *in vivo* caused deep cortical neurons to reorient their nuclei and polarize their Golgi toward the pia while initiating exuberant dendritic outgrowth within few hours [63]. Thus, Reelin exerts a direct role in promoting the rapid morphological differentiation and orientation of deep cortical neurons during early corticogenesis.

Finally, other *in vivo* studies indicated that Reelin not only promotes growth but also promotes the selective pruning of dendritic process in cortical neurons [163]. This study implicated serotonin 5-HT(3) receptor and Reelin signaling in the control of the complexity of apical dendrites of cortical layer II/III pyramidal neurons. Relevant to Reelin activity, these authors found that injection of Reelin antibodies resulted in increased dendritic complexity, whereas the application of recombinant full-length Reelin rescued

this defect. Surprisingly, however, they found that pruning was also achieved when the N-terminal fragment, but not the central fragment of Reelin, was injected. This study also suggested that the N terminal region of Reelin mediates dendrite refinement through receptors other than ApoER2 and VLDLR, consistent with previous observations that only the central fragment of Reelin can bind and engage these receptors [40]. The mechanisms of the Reelin-mediated regulation of dendrite growth may thus be more complex than initially envisioned, and it may vary depending on the cell type examined. For example, other investigators found that the amyloid precursor protein (APP) promotes the growth of cortical dendrites *in vivo* and is required for Reelin-induced dendrite growth in cultured hippocampal neurons [164]. This finding builds on previous reports that APP family members interact with Dab1 due to their NPxY motif [85, 86] and that Reelin signaling affects APP processing [165]. Mechanistically, the new study found that APP interacts with the central fragment of Reelin and with $\alpha 3\beta 1$ integrin. Since an $\alpha 3\beta 1$ antibody prevented APP and Reelin-induced neurite outgrowth, this study implicated integrins in Reelin signaling controlling neurite development.

12. Reelin Functions in Synaptogenesis and Spine Formation

As postnatal development continues and Cajal-Retzius cells progressively disappear from the forebrain, the expression of Reelin becomes predominantly localized to interneurons. A subset of GABAergic interneurons express Reelin throughout all cellular layers of the rodent neocortex and hippocampus at postnatal and adult ages [45, 68]. Since its early discovery, the physiological role of this new pattern of expression has been the subject of intense inquiry, given that the function of Reelin in the late postnatal and adult forebrain could no longer be related to neuronal migration or dendrite development in these brain regions. Attention turned to synapse development and function, since these biological processes predominate at later postnatal ages. The first documented evidence that Reelin plays a role in synapse formation in the forebrain related to the development of entorhinohippocampal connections [58]. Since Reelin had previously been implicated in the branching of this axonal projection, the finding appeared to be a logical extension of previous observations: the more axonal terminals are formed, the more they can synapse on hippocampal target cells. Therefore, the effect on synapse development initially appeared indirect and secondary to that on axonal branching. Nevertheless, adding support to a role for Reelin signaling in synaptogenesis, few years later came the observation that Reelin and Dab1 modulate the structure and function of retinal synaptic circuitry [19]. This paper showed that, even though there is no cellular ectopia in the highly laminated structure of the retina in *reeler* or Dab1 mutant mice, connectivity defects are present which ultimately lead to altered physiological responses. Thus, this paper was significant because it highlighted a role for the Reelin pathway in the formation and functional output of a specific synaptic circuitry, independent of cell position. In

the retina, Reelin is expressed not only by retinal ganglion cells, but also by some amacrine and cone bipolar cells, whereas Dab1 is expressed almost exclusively by AII amacrine cells [19]. These Dab1-positive cells play an essential role in mediating Reelin signaling and establishing retinal synaptic circuitry. A recent study revealed that, as in other parts of the central nervous system, ApoER2 and VLDLR function as Reelin receptors and are both required for the establishment and maintenance of normal retinal synaptic connectivity [166]. However, they appear to contribute differently to the development and maintenance of retinal synaptic connectivity. ApoER2 is predominantly expressed by both AII amacrine cells and other amacrine or bipolar cells in the developing retina and plays a role in the development of the rod bipolar pathway, whereas VLDLR is required for the establishment of oscillatory potentials [166].

In the forebrain, studies of heterozygous *reeler* mice emphasized a direct role for Reelin in the control of synaptogenesis, particularly in the formation of the excitatory postsynaptic structures. Heterozygous *reeler* mice express half the *Reln* mRNA levels of wild type mice and appear phenotypically normal in that they do not exhibit ataxia or layer defects in any brain region [1]. However, these mice exhibit behavioral and cognitive defects reminiscent of those found in human psychoses [167, 168] and are thus considered animal models for the disease [169]. The behavioral phenotype of heterozygous *reeler* mice has been challenged by other investigators [170] however, these mice do appear to have subtle anatomical abnormalities that would account for functional connectivity problems. For example, they exhibit delayed growth of dendritic processes in hippocampal neurons [62] and a reduction in the density of synaptic contacts in the frontal [171] and prefrontal cortex [127]. Defects in dendritic spine density were also found in the hippocampus of heterozygous *reeler* and Dab1 knockout mice, accompanied by altered molecular composition of the synaptosomes [70]. This study also showed that Reelin treatment rescued spine density defects of *reeler* organotypic hippocampal slices in a manner that was dependent on all core components of the signaling pathway, namely, the lipoprotein receptors ApoER2 and VLDLR, SFKs and Dab1 [70]. Follow-up studies revealed that defects in spine density are transient in heterozygous *reeler* mice and essentially disappear in adult mice by 2 months of age; however, the expression of postsynaptic proteins, including NMDA receptor subunits, remains altered [71]. In a complementary study, other investigators examined spine density and size in transgenic mice that overexpressed Reelin and found that Reelin overexpression caused an increase in spine size but did not change spine density in hippocampal regions [75]. Consistent with this view, a recent study in which a single dose of Reelin was injected in the hippocampus of live adult mice indicated that acute administration of Reelin alters dendritic spine morphology but not spine density [172]. In contrast, long-term administration (5 days) of Reelin *in vivo* also increases spine density [173]. Taken together, these studies suggest that subthreshold levels of Reelin are deleterious because they delay the normal development of excitatory synapses, but a chronic excess of Reelin may

also be disruptive because it alters spine morphology and number. Finally, it should be noted that reduced spontaneous inhibitory postsynaptic potentials have been reported in adult *reeler* heterozygous mice [167], suggesting that Reelin may play a significant role in the development of inhibitory synapses. However, this topic has not been yet investigated at a cellular and molecular level.

13. Adult Function of *Reelin* in the Modulation of Synaptic Activity

Reelin-expressing interneurons in the adult forebrain were identified as a heterogeneous population of bitufted, horizontal, and multipolar cells coexpressing either calretinin, calbindin, somatostatin, or NPY but not parvalbumin [45, 68]. A similar pattern of Reelin expression in selected interneurons was also observed in primates at postnatal ages [174]. In humans, the selective expression of Reelin in interneurons, together with the observed decrease in the expression of Reelin [25] and other interneuron markers in the postmortem brains of schizophrenic patients, led to the hypothesis that Reelin deficiency contributes to interneuron dysfunction in this disorder [30]. Reelin deficiency is recapitulated in the heterozygous *reeler* mouse, which exhibited subtle neuroanatomical abnormalities, such as decreased neuropil and dendritic spine density [70, 171], reminiscent of the changes reported in the brain of schizophrenia patients [175]. Heterozygous *reeler* mice also exhibit altered hippocampal synaptic function [167] and multiple behavioral abnormalities that parallel those noted in schizophrenia, such as defects in executive function [170, 176], contextual fear conditioning learning, and crossmodal prepulse inhibition [167, 177]. Thus, these mutant mice are useful models for understanding the cellular basis of behavioral and cognitive defects characterizing schizophrenia [169].

What is the significance of normal adult Reelin expression? Histochemical and ultrastructural studies revealed that extracellular Reelin accumulates around synapses [174, 178–180], leading to the suggestion that it may play a role in synaptic function. Indeed, a large body of evidence now suggests that this is the main function of Reelin in the adult brain. The first direct evidence that Reelin regulates synaptic function came from physiological studies using hippocampal slice cultures. In this acute *in vitro* system, Reelin treatment was shown to promote robust long-term potentiation (LTP) [181]. This study also showed that Reelin promotes hippocampal LTP in a manner that is dependent on both VLDLR and ApoER2. However, a following study further revealed that a particular splicing variant of ApoER2 capable of interacting with the NMDA receptor through PSD95 was required for Reelin-induced LTP [149, 181]. A number of electrophysiological studies using dissociated neuronal cultures demonstrated that Reelin increases synaptic activity by affecting postsynaptic NMDA and AMPA receptor activity [182–186] and also promotes neurotransmitter release [187]. Long-term Reelin treatment was found to alter the subunit composition of the NMDA receptor, favoring maturation of this receptor, and to increase AMPA receptor insertion

in the plasma membrane, thus reducing the number of silent synapses. Other studies further demonstrated that the LTP enhancement by brief Reelin stimulation is associated with increased Ca^{++} influx through the NMDA receptor [149, 183, 186]. Using an *in vivo* approach, the effect of chronic exposure was examined in transgenic mice that overexpressed Reelin under the control of the adult-forebrain specific CamKIIα promoter [75]. Reelin overexpressing mice displayed a dramatic dendritic spine hypertrophy but no changes in synaptic density, suggesting a specific function in the control of the size of excitatory synapses, which is highly related to their level of activity. Physiological recordings from hippocampal slices further documented an increase in LTP responses as well as neuronal activity during classical conditioning [75]. Using a different *in vivo* approach, other investigators injected Reelin directly into the hippocampus of adult mice and documented increases in synaptic plasticity, which correlated with enhanced cognitive performance in hippocampal-dependent tasks [173]. The role of Dab1 signaling in adult synaptic plasticity and learning was very recently addressed in a study that also utilized the adult-forebrain specific CamKIIα promoter, this time to drive conditional *Dab1* gene deletion [128]. This study demonstrated the critical role of Dab1, not only in mediating hippocampal LTP and especially the enhancing effect of Reelin on LTP, but also in modulating basal and plasticity-induced Erk signaling, and promoting hippocampal-dependent associative learning and the consolidation of long-term spatial memory.

Taken together, these findings indicate that Reelin-Dab1 signaling plays an important role in modulating synaptic function in the adult forebrain and significantly affects memory formation and cognitive function. The observed effects of Reelin signaling on synaptic development and function suggest that this pathway is potentially an excellent target for intervention in the treatment of cognitive diseases associated with Reelin deficiency, such as schizophrenia, autism, epilepsy, or Alzheimer's disease. The involvement of Reelin in neurodevelopmental or neurodegenerative disease and the potential implications for treatment have been recently reviewed and discussed in detail [28, 188]. However, further studies are needed to fully understand the impact of the Reelin signaling pathway on brain development and function in order to develop effective and safe therapeutic agents.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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