Chapter 9
Reelin and Cyclin-Dependent Kinase 5

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Contents

1 Introduction

Reelin, an extracellular signaling molecule, and cyclin-dependent kinase 5 (Cdk5), a cytoplasmic kinase, are key regulators of normal brain development, including establishment of the complex brain structure. Recent studies have indicated that both Reelin signaling and Cdk5 are also involved in synaptic plasticity and neurodegeneration. In this chapter, I shall describe the functions of Cdk5 in neuronal migration during brain development and present an overview of the relationship of Cdk5 with Reelin signaling based on analyses of mutant mouse models. I shall also

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refer to the functions of Reelin signaling and Cdk5 in dendrite development, synaptic plasticity, and neurodegeneration.

2 What Is Cdk5?

2.1 Cdk5 Is a Neuron-Specific Serine/Threonine Kinase

Cdk5 is a homologue of the Cdk protein family of serine/threonine (Ser/Thr) kinases (Meyerson *et al*., 1992; Hellmich *et al*., 1992). While the other Cdks are active in proliferating cells, Cdk5 is mainly active in postmitotic neurons (Hellmich *et al*., 1992; Tsai *et al*., 1993). The activity of Cdk5 is regulated by its binding with a neuron-specific regulatory subunit, either p35 (Lew *et al*., 1994; Tsai *et al*., 1994) or its isoform p39 (Tang *et al*., 1995); its activity is, therefore, correlated with the expression of p35 and p39. Expressions of p35 and p39 have been detected throughout the central nervous system (CNS), as well as in peripheral neurons such as the dorsal root ganglia (DRG) neurons (Zheng *et al*., 1998; Delalle *et al*., 1997). Cdk5 activity in the CNS has been shown to reach a peak from the late embryonic stage to the first week of the postnatal period, with later decline in the adult stage (Wu *et al*., 2000; Takahashi *et al*., 2003). However, some areas of the brain, including the hippocampus, have been shown to retain a relatively high expression level of p35 and high activity of Cdk5 (Wu *et al*., 2000; Takahashi *et al*., 2003).

2.2 Role of Cdk5 in Neuronal Positioning

Based on studies of the phenotypes of KO mice, it is considered that Cdk5 and p35 are critical for neuronal migration and positioning. Cdk5 KO mice exhibit perinatal lethality with disruption of the cortical laminar structures in the cerebral cortex, olfactory bulb, hippocampus, and cerebellum (Ohshima *et al*., 1996). p35 KO mice showed a milder phenotype than Cdk5 KO mice owing to the redundancy of p39 (Chae *et al*., 1997; Ohshima *et al*., 2001). Although p39 KO mice show no phenotype, double-null mice for p35 and p39 display a phenotype identical to that of the Cdk5 KO mice (Ko *et al*., 2001), confirming redundancy in these subunits. Birthdate labeling studies using BrdU confirmed that the migration defects of the neuronal subsets cause an abnormal laminar structure of the cerebral cortex in Cdk5 KO mice (Gilmore *et al*., 1998). In addition to cortical neurons, the formation of some nuclei, including the facial motor nucleus and inferior olive, in the hindbrain, is defective in Cdk5 KO mice (Ohshima *et al*., 2002).

How does Cdk5 regulate neuronal migration and positioning? Cdk5 modulates the actin cytoskeleton dynamics through phosphorylation of Pak1 (Nikolic *et al*., 1998; Rashid *et al*., 2001) and filamin 1 (Fox *et al*., 1998). Cdk5 also modulates the microtubule dynamics through phosphorylation of microtubule-associated proteins, including tau (Kobayashi *et al*., 1993), MAP1b (Paglini *et al*., 1998), doublecortin (Tanaka *et al*.,

2004), Nudel (Sasaki *et al*., 2000; Niethammer *et al*., 2000), and Collapsin Response Mediator Proteins (CRMPs) (Uchida *et al*., 2005). Among these substrates, defect of filamin 1 causes human periventricular heterotopia (Fox *et al*., 1998), and defects of Lis1 and doublecortin cause human lissencephaly type 1 (Reiner *et al*., 1993; des Portes *et al*., 1998; Gleeson *et al*., 1998). These findings indicate that Cdk5 regulates cytoskeletal dynamics that determines the speed of migration, extension of the leading processes, and cell-soma propulsion in migrating neurons. Cdk5 may also regulate cellular adhesion in neuronal–glial interaction through phosphorylation of β-catenin to regulate its interaction with N-cadherin (Kwon *et al*., 2000), and this Cdk5-mediated adhesion may also be important for the neuronal migration.

3 Relationship Between Reelin Signaling and Cdk5

3.1 Relationship in Neuronal Migration and Positioning

3.1.1 Similarities and Differences: Defects of Reelin Signaling Versus Defects of Cdk5/p35

During cortical development, the preplate splits into a marginal zone and the subplate in wild-type mice. However, in the *reeler* mice, the preplate remains undivided as a superficial layer (the "superplate"), and cohorts of cortical pyramidal neurons accumulate underneath the superplate (Caviness, 1982; Goffinet, 1984; Sheppard and Pearlman, 1997). In the case of Cdk5 KO mice, the preplate is formed normally, with the earliest-generated pyramidal neurons being positioned within the preplate. However, the subsequent cohorts of pyramidal neurons accumulate underneath the subplate (Gilmore *et al*., 1998), as shown schematically in Fig. 9.1.

In addition to those in the cerebral cortex, the *reeler* phenotype and Cdk5/p35 mice also share similarities in abnormalities of neuronal positioning in other areas of the CNS (Ohshima and Mikoshiba, 2002). For example, the cerebellum is another typical region of the CNS in which the *reeler* phenotype and Cdk5/p35 deficiency share a similar phenotype, although the similarity was only noted at first glance, and detailed analysis revealed significant differences. Both Reelin signaling and Cdk5/p35 are required for proper positioning of Purkinje cells (Mariani *et al*., 1977; Ohshima *et al*., 1996, 1999). Cdk5/p35 deficiency also results in failure of complete migration of the granule cells from the external granule cell layer (EGL) to the internal granule cell layer (IGL) (Chae *et al*., 1997; Ohshima *et al*., 1999). This inward migration of granule cells, however, occurs normally in the *reeler* mice. As summarized in Table 9.1, there is considerable overlap in the affected neuronal population between *reeler* mice and Cdk5 KO mice, with only some exceptions. For example, positioning of mitral cells in the olfactory bulb is abnormal in the Cdk5 KO mice, and positioning of the granule cells in the dentate gyrus is abnormal in the *reeler* mice (Stanfield and Cowan, 1979; Drakew *et al*., 2002);

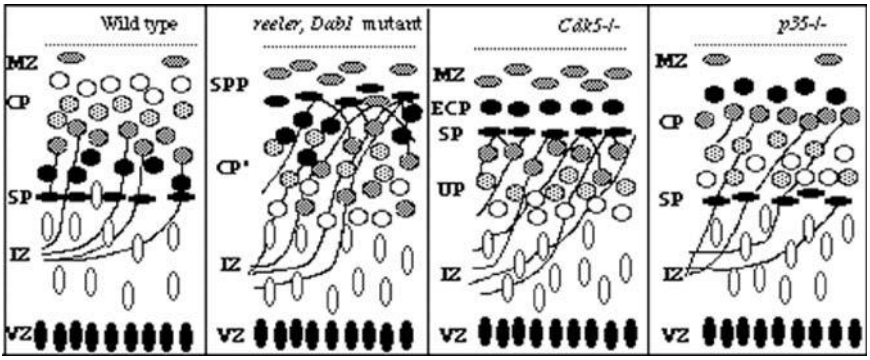

Fig. 9.1 Schematic representation of the cerebral cortex in the wild-type and mutant mice. In the wild-type mice, migrating neurons split preplate into a marginal zone (MZ) and subplate (SP), to form the cortical plate (CP). In *reeler* and Dab1 mutant mice, the preplate is not split and remains as a superplate (SPP) with mutant neurons stacked up in an inverse order. In the Cdk5-/- mice, the initial wave of migrating neurons (indicated by black circles) splits the preplate to form a narrow ectopic cortical plate (ECP), but later-born neurons (light gray and white circles) stack up *reeler*-like under the subplate in an inverted fashion as an underplate (UP). In the p35-/- mice, the later compensatory effects of Cdk5/p39 result in normal positioning of the subplate neurons. (Figure adapted with permission from Ohshima and Mikoshiba, 2002)

Table 9.1 Comparison of affected brain regions or neuronal subtypes in *reeler*/Dab1 mutant, Cdk5 KO, and p35 KO mice

Structure or neuronal type in CNS		*reeler*/Dab1 mutant	Cdk5 KO	p35 KO
Olfactory bulb	Mitral cells	−	+	−
Cerebral cortex	Subplate neurons	+	−	−
	Cortical neurons	+	+	+
Hippocampus	Pyramidal cell layer	+	+	±
	Dentate gyrus	+	±	±
Midbrain	Dopamine neurons in SN	+	+	−
Cerebellum	Purkinje cells	+	+	±
	Granule cells (inward)	−	n.d.*	+
Brainstem	Facial motor nucleus	±	+	−
	Inferior olive	±	+	−

+, affected; ±, mildly affected; −, unaffected. CNS, central nervous system; SN, substantia nigra.

*n.d., could not be determined because of perinatal lethality. This type of migration of granule cells is Cdk5-dependent (Ohshima *et al*., 1999).

on the other hand, only mild abnormality in the positioning of these neurons is observed in the p35 KO mice (Wenzel *et al*., 2001).

3.1.2 Genetic Interaction Between Reelin Signaling and Cdk5/p35 in Neuronal Positioning

To study the relationship between Reelin signaling and Cdk5/p35, double mutant mice with respect to these two signaling proteins were generated and their phenotypes

analyzed (Ohshima *et al*., 2001; Beffert *et al*., 2004). Because all of the Cdk5 KO mice die perinatally, the genetic interaction was examined by comparing the phenotype of those double knockout mice for p35 and Reelin (Ohshima *et al*., 2001), Dab1 (Ohshima *et al*., 2001), ApoER2 and VLDLR (Beffert *et al*., 2004) versus that in the respective single KO mice. Exaggerated neuronal migration defects in the hippocampus, as compared with that in the respective single KO mice, were typically observed in the double KO mice for p35/Dab1, p35/Reelin, p35/ApoER2, and p35/VLDLR (Ohshima *et al*., 2001; Beffert *et al*., 2004). Invasion of cells into layer I of the cerebral cortex is known to be a typical feature in the *reeler* and Dab1 mutant mice; this phenotype was observed in the double KO mice for p35/ApoER2 and p35/VLDLR, but not in the single KO mice for p35, ApoER2, or VLDLR (Beffert *et al*., 2004). Exaggerated defects of Purkinje cell migration in the cerebellum have also been reported in double KO mice for p35/Reelin and p35/Dab1 (Ohshima *et al*., 2001). These findings indicate that Reelin signaling and Cdk5 function together in a parallel manner to effect proper neuronal migration and positioning in the developing brain (Ohshima and Mikoshiba, 2002; Beffert *et al*., 2004).

3.2 Roles of Reelin Signaling and Cdk5 in Dendrite Development

Impairment of Reelin signaling during neuronal development has been shown to result in a reduced complexity of the dendritic tree (Borrell *et al*., 1999) and in lowering the synaptic complexity of the hippocampus (Del Rio *et al*., 1997). Another study, using cultured hippocampal neurons, has shown the important role of Reelin in the regulation of dendritic branching and demonstrated that this effect of Reelin was inhibited by the addition of a Dab1 phosphorylation inhibitor (Niu *et al*., 2004). The results of a recent Dab1 RNAi study in the developing cortex lent support to these findings (Olson *et al*., 2006). Cdk5 has also been shown to be involved in neurite outgrowth. Reduction of Cdk5 kinase activity by expression of the dominant-negative form of Cdk5 (Nikolic *et al*., 1996), or the addition of antisense oligonucleotides of Cdk5, p35, and p39 in a primary culture of neurons was shown to inhibit neurite outgrowth (Xiong *et al*., 1997). The synergistic effects of Reelin signaling and Cdk5 in dendritic development remain to be elucidated.

3.3 Roles of Reelin Signaling and Cdk5 in Synaptic Plasticity

Cdk5 and Reelin have both been shown to be involved in synaptic function. Cdk5 phosphorylates presynaptic proteins and may be involved in exocytosis (Matsubara *et al*., 1996; Shuang *et al*., 1998; Tomizawa *et al*., 2002) and endocytosis at presynaptic sites (Tan *et al*., 2003; Tomizawa *et al*., 2003). Cdk5 also phosphorylates

postsynaptic proteins including NMDA receptors (Li *et al*., 2001) and PSD-95 (Morabito *et al*., 2004), and may be involved in the regulation of synaptic plasticity. Both ApoER2 KO mice and VLDLR KO mice show defects of LTP induction and fear-conditioned associative learning (Weeber *et al*., 2002). p35 KO mice were shown to have defects of LTD induction and spatial learning (Ohshima *et al*., 2005). Interestingly, Reelin has been shown to enhance LTP of Schaffer collateral synapse at CA1 of hippocampus (Weeber *et al*., 2002). Such enhancement was, however, not observed in the hippocampus of the p35 KO mice, indicating that Reelin-dependent enhancement of LTP in the hippocampus may be dependent on Cdk5 activity (Beffert *et al*., 2004). The molecular mechanism underlying this relation would serve as an excellent subject for future study.

3.4 Roles of Cdk5 and Reelin Signaling in Alzheimer's Disease: Phosphorylation of Tau and Regulation of APP

Possible involvement of Cdk5 in Alzheimer's disease (AD) has been discussed. Accumulation of p25, which is a cleavage product of p35, and a stable protein, and elevated Cdk5 activity have been reported in the brains of AD patients (Patrick *et al*., 1999), although these observations have not been consistent among investigators (Takashima *et al.,* 2001;Yoo and Lubec, 2001). The pathological hallmarks of AD brains are amyloid plaques, which is caused by the abnormal processing of APP, and neurofibrillary tangles that result from abnormal phosphorylation and aggregation of tau protein. Increased tau phosphorylation has been demonstrated in the brains of *reeler,* Dab1 mutant, and ApoER2/VLDLR KO mice (Hiesberger *et al*., 1999; Brich *et al*., 2003). Dab1 can physically interact with intracellular domains of APP and APP-like proteins (Homayouni *et al*., 1999; Howell *et al*., 1999) and regulate their trafficking and proteolytic processing (Hoe *et al*., 2006). Interestingly, Cdk5 phosphorylates intracellular domain of APP at Thr668 (Iijima *et al*., 2000) and affects its intracellular trafficking and proteolytic processing (Lee *et al*., 2003).

4 Possible Molecular Mechanisms Underlying the Relation Between Reelin Signaling and Cdk5

4.1 Through the Phosphorylation of Dab1 by Cdk5

It has been reported that intracellular adapter protein Dab1 is phosphorylated at tyrosine sites, as well as Ser/Thr sites *in vivo* (Arnaud *et al*., 2003; Ohshima *et al*., 2007). *In vitro*, Cdk5 has been shown to phosphorylate Dab1 at multiple Ser/Thr sites in the carboxyl terminus (Ohshima *et al*., 2007). Among them, phosphorylation

of Dab1 at Ser491 by Cdk5 *in vivo* has been shown using phospho-specific antibody (Keshvara *et al*., 2002). However, functional significance of Cdk5-mediated Dab1 phosphorylation in Reelin signaling remains to be elucidated.

4.2 Sharing of Common Downstream Targets

Reelin signaling and Cdk5 may share downstream targets, such as the microtubule-associated protein, tau (Fig. 9.2). It had been shown that Reelin signaling induces Akt activation along with inactivation of GSK3β, a known major tau kinase (Beffert *et al*., 2002). Cdk5 is also known as tau kinase (Kobayashi *et al*., 1993; Paudel *et al*., 1993) and phosphorylates tau at several sites (Baumann *et al*., 1993). Interestingly, inhibition of Cdk5 can lead to increased phosphorylation of several neuronal proteins by GSK3β, including neurofilaments and kinesin light chain (Morfini *et al*., 2004).

Sharing of other downstream targets of Reelin signaling and Cdk5 may explain the synergic function of these two pathways (Fig. 9.2). Reelin signaling facilitates interaction between Dab1 and Lis1, and Lis1 is also associated with Nudel, which is a Cdk5 substrate as described above (Sasaki *et al*., 2000; Niethammer *et al*.,

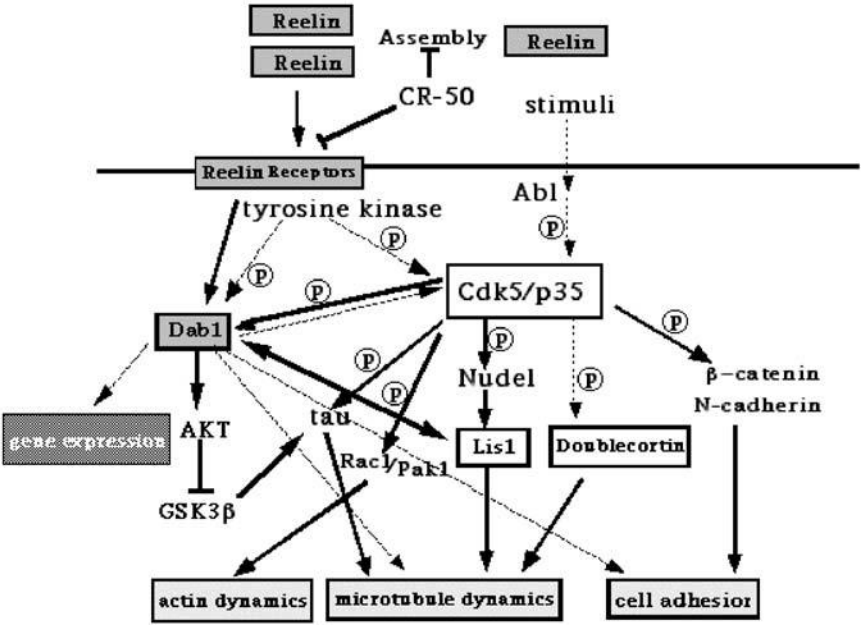

Fig. 9.2 Model of the signaling pathway of Reelin and Cdk5 in the control of neuronal positioning. Reelin and Cdk5 function in a parallel fashion. (Figure adapted with permission from Ohshima and Mikoshiba, 2002, with modification)

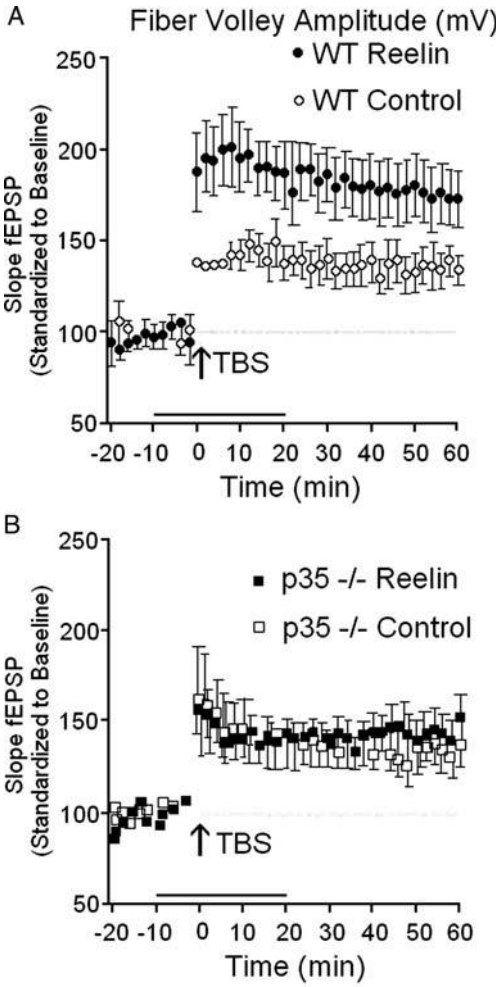

Fig. 9.3 Reelin treatment potentiates LTP in hippocampal slices in the wild-type (WT) mice (**A**). This potentiation is not observed in the p35-/- slice (**B**) indicating Cdk5/p35-dependency. TBS, theta burst stimulation. (Figures adapted with permission from Beffert *et al*., 2004)

2000; Assadi *et al*., 2003). Cdk5-mediated phosphorylation of Nudel might be important for the interaction of these proteins to activate nucleokinesis during neuronal migration. CRMPs are also candidates of downstream molecules shared by Reelin signaling and Cdk5, since CRMP1 has recently been shown to be involved in Reelin signaling, and it is a substrate of Cdk5 (Uchida *et al*., 2005; Yamashita *et al*., 2006).

5 Closing Remarks

As described above, Reelin signaling and Cdk5 are involved together in many aspects of brain development and functions. Genetic studies using double mutant mice indicate synergistic function of Reelin signaling and Cdk5 in brain development. The relationships between Reelin signaling and Cdk5 in other functions of the brain, synaptic plasticity, and neurological diseases remain to be precisely elucidated in future studies. Reelin seems to enhance LTP in the hippocampus and this enhancement appears to be Cdk5-dependent (Fig. 9.3). This is a good example indicating the close relationship between Reelin signaling and Cdk5 in the execution of brain function.

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Chapter 10
Reelin and the Cerebellum

Robert F. Hevner

Contents

1 Introduction

The cerebellum is a large, complex brain structure that mediates essential functions for movement, balance, cognition, and language (Ito, 2005). Development of the cerebellum critically depends on Reelin signaling. Complete deficiency of Reelin causes a severe cerebellar malformation, with extensive cellular disorganization and hypoplasia. Identical cerebellar defects are observed in mice lacking downstream components of the Reelin signaling pathway, including Reelin receptors VLDLR and ApoER2, adapter protein Dab1, or kinases Fyn and Src. The brain malformation results in ataxia and loss of balance, manifesting as a reeling gait in mice (hence the

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name Reelin) and multiple neurological problems in humans. More subtle abnormalities of Reelin signaling may underlie important neurobehavioral disorders in humans. In particular, some studies have linked *RELN* gene polymorphisms and reduced Reelin expression to autism. Since cerebellar defects are frequently observed in autistic brains, an attractive hypothesis is that Reelin signaling abnormalities may cause autism by perturbing cerebellar development or plasticity.

Despite growing biomedical significance, our understanding of how Reelin regulates cerebellar morphogenesis is far from complete. Reelin, its receptors, and downstream effectors are expressed by different cohorts of cells at different time points throughout cerebellar development. Most Reelin-producing cells are located near the surface of the developing cerebellar cortex, including cells of the rostral rhombic lip migratory stream (RLS), the nuclear transitory zone (NTZ), and the external granular layer (EGL). Other Reelin-producing cells are located deeper in the cerebellum, including some neurons of the deep cerebellar nuclei (DCN) and internal granular layer. Much evidence suggests that one important function of Reelin is to promote detachment of Purkinje cells from radial glia in the mantle zone of the embryonic cerebellar cortex, thus allowing multiple Purkinje cells to migrate along the same radial glia. The migrating Purkinje cells respond to Reelin signaling by activating a signaling cascade that includes Reelin receptors (VLDLR and ApoER2), adapter protein Dab1, and kinases Src and Fyn. Besides promoting Purkinje cell detachment from radial glia, Reelin may also regulate Purkinje cell spreading and monolayer formation, radial glia morphology, granule cell proliferation, unipolar brush cell migration, DCN cytoarchitecture, axon guidance, dendrite morphology, and synaptic plasticity. These mechanisms will require further basic research.

2 Overview of Cerebellar Development

Cerebellar development involves a complex sequence of events in neurogenesis, cell migration, axon pathfinding, dendritogenesis, synaptogenesis, and other mechanisms (Sotelo, 2004). Recent studies have made substantial progress in defining the cell types that express Reelin, and in characterizing neuron sources, lineages, and migrations. New discoveries that have changed the context for understanding Reelin functions are emphasized here.

2.1 Patterning and Rotation of the Cerebellar Anlage

The cerebellum develops from the dorsal part of hindbrain rhombomere 1 (r1), which undergoes a complex process of growth, flexure, and rotation to generate the cerebellar plate and upper rhombic lip, which together represent the cerebellar anlage (Fig. 10.1). The cerebellar plate and upper rhombic lip are distinct neurogenic compartments that express different genes and produce different sets of cerebellar neurons. Interestingly, most or all Reelin-producing cells are produced in the

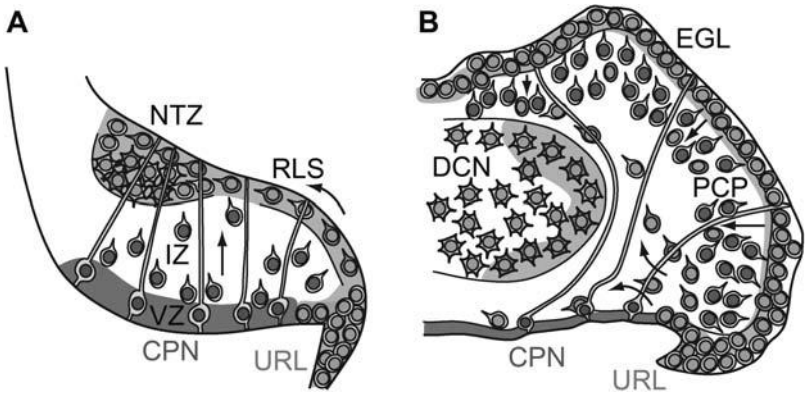

Fig. 10.1 Reelin signaling and cell migrations in cerebellar development. The diagrams show schematic views of the developing cerebellum in sagittal sections through the vermis, oriented with rostral to the left and dorsal to the top. (**A**) Early stage of cerebellar development (mouse E13.5). Cells derived from the upper rhombic lip (URL) (green nuclei) migrate nonradially (curved arrow) through the rostral rhombic lip migratory stream (RLS) to the nuclear transitory zone (NTZ). Reelin (blue) is expressed by many cells in the RLS and NTZ. At the same time, Purkinje cells (red nuclei) migrate radially (straight arrow) from the ventricular zone (VZ) of the cerebellar plate neuroepithelium (CPN) along radial glial cells (gray) through the intermediate zone (IZ), toward the RLS and NTZ. The Purkinje cells express cytoplasmic Dab1 (yellow). (**B**) Later stage of cerebellar development (mouse E17.5). The Purkinje cell plate (PCP) has formed, and the external granular layer (EGL) has replaced the RLS. Cells from the EGL migrate radially inward through the PCP (straight arrows), while unipolar brush cells migrate directly from the URL into the IZ (curved arrows). The deep cerebellar nuclei (DCN) contain neurons derived from the NTZ that have migrated radially inward toward the VZ (*See Color Plates*)

upper rhombic lip, while Reelin-responsive Purkinje cells are produced in the cerebellar plate (Fig. 10.1). Fate mapping and molecular expression studies indicate that the upper rhombic lip and cerebellar plate are initially patterned by signals from the roof plate and isthmic organizer (Zervas *et al*., 2004; Sgaier *et al*., 2005; Chizhikov *et al*., 2006). Initially, in the hindbrain neural tube, the upper rhombic lip primordium is located in dorsomedial r1, adjacent to the roof plate, while the cerebellar plate primordium is located in dorsolateral r1. Their relative positions subsequently shift as a result of cerebellar plate rotation and pontine flexure, so that the cerebellar plate comes to be located rostral to the upper rhombic lip. The cerebellar plate is a conventional neuroepithelium with radially organized ventricular zone (VZ), intermediate zone (IZ), and mantle zone (Fig. 10.1A). In contrast, the upper rhombic lip is a pure progenitor compartment, producing postmitotic neurons and precursors that migrate tangentially out of the rhombic lip and into the cerebellar plate, as well as to some brainstem nuclei. Accordingly, regional subdivisions within the cerebellum (longitudinal stripes and transverse domains) appear to be specified primarily by patterning of the cerebellar plate, which occurs prior to the start of neurogenesis (Zervas *et al*., 2004; Sgaier *et al*., 2005; Chizhikov *et al*., 2006).

2.2 *Different Types of Cerebellar Neurons Are Produced Sequentially*

The first neurons to be generated in the embryonic cerebellum are Purkinje cells and DCN projection neurons, which are produced from embryonic day (E) 11 to E13 in mice (Miale and Sidman, 1961). Although they are generated concurrently, Purkinje cells and DCN projection neurons are fundamentally different types of neurons. They differ by morphology, migration pathways, axon connections, and, most significantly, neurotransmitter systems. Purkinje cells release GABA, an inhibitory neurotransmitter, while DCN projection neurons release glutamate, an excitatory neurotransmitter. Studies in forebrain and spinal cord have demonstrated that transmitter phenotype is a fundamental aspect of neuron identity and is determined very early in neuronal fate specification (Schuurmans and Guillemot, 2002; Cheng *et al*., 2005). In the forebrain, glutamatergic and GABAergic neurons are produced in separate progenitor compartments, known as the cortical neuroepithelium (pallium) and ganglionic eminences, respectively. This suggests the possibility that Purkinje cells, DCN projection neurons, and other cerebellar neuron types might likewise originate from separate glutamatergic and GABAergic progenitor compartments. This hypothesis was recently supported (Machold and Fishell, 2005; Wang *et al*., 2005; Englund *et al*., 2006; Fink *et al*., 2006).

Following Purkinje cells and DCN projection neurons, the next neurons to be produced are local inhibitory interneurons (GABAergic) and unipolar brush cells (glutamatergic). Inhibitory interneurons differentiate into diverse morphological subtypes and migrate to locations throughout the cerebellar cortex and DCN. Unipolar brush cells differentiate into at least two subtypes and migrate only to the internal granular layer. The last neurons to be produced are granule cells (glutamatergic), which are the most abundant neuron type in the brain. Granule cells are produced from amplifying precursors in the EGL, which are, in turn, derived from the upper rhombic lip via migration through the RLS. Neurogenesis of granule cells covers a relatively long interval, lasting through postnatal day (P) 15 in mice (Miale and Sidman, 1961).

2.2.1 Purkinje Cells and Inhibitory Interneurons Are Derived from the Cerebellar Plate VZ

Efforts to determine the origins and lineages of cerebellar neurons have recently been enhanced by the introduction of new experimental approaches and genetic technologies. Specific progenitor compartments have been identified on the basis of molecular expression, and have been linked to the separate production of different classes of neurons. The new findings have important implications for understanding cerebellar development, and for dissecting the functions of Reelin signaling.

Purkinje cells have long been thought to arise from the cerebellar plate neuroepithelium (Altman and Bayer, 1985a,c). Recent studies have indeed confirmed and

expanded this view. Purkinje cells are produced from progenitors in the cerebellar plate VZ that specifically express Ptf1a (pancreas transcription factor 1a), a basic helix–loop–helix (bHLH) transcription factor that is essential for the development of Purkinje neurons (Hoshino *et al*., 2005). Mice that lack Ptf1a expression in the cerebellum, known as *cerebelless* mutants, completely lack cerebellar cortex due to decreased production of Purkinje cells and other GABAergic neurons, and secondary deficiency of granule neurons (Hoshino *et al*., 2005). Interestingly, *cerebelless* mutants survive up to 2 years, indicating that the cerebellum is virtually dispensable for mice in laboratory conditions.

Inhibitory interneurons of the cerebellum likewise originate from Ptf1a$^+$ progenitor cells in the cerebellar plate VZ (Hoshino *et al*., 2005). However, many inhibitory interneurons are not produced directly from VZ progenitor cells, but instead are generated by division of precursor cells migrating through the developing white matter (Zhang and Goldman, 1996). These precursor cells express transcription factor Pax2 (Maricich and Herrup, 1999) and require Ptf1a for their development (Hoshino *et al*., 2005). Since Purkinje cells and inhibitory interneurons are produced from the cerebellar plate VZ while glutamatergic neuron types are not, the cerebellar plate VZ may be regarded as a distinct compartment for GABAergic neuron production.

2.2.2 DCN Projection Neurons, Unipolar Brush Cells, and Granule Neurons Are Derived from the Upper Rhombic Lip

DCN projection neurons, like Purkinje cells, were thought to arise from progenitors in the cerebellar plate VZ (Altman and Bayer, 1985a,b). But surprisingly, recent experiments have demonstrated that DCN projection neurons actually arise from progenitors in the upper rhombic lip. These progenitors express Math1 (mouse atonal homolog 1), a bHLH transcription factor and specific marker of rhombic lip lineages in the cerebellum (Machold and Fishell, 2005; Wang *et al*., 2005). Newly generated DCN projection neurons migrate rostrally from the upper rhombic lip to the nuclear transitory zone (NTZ), a transient cell mass that is subsequently partitioned and organized to form the DCN (Fig. 10.1). The migration pathway from upper rhombic lip to NTZ, known as the rostral rhombic lip migratory stream (RLS), traverses a nonradial route along the subpial surface of the cerebellar plate (Wang *et al*., 2005). The migrating DCN projection neurons form a thin, almost continuous sheet across the dorsal surface of the cerebellum. Importantly, many cells in the RLS express Reelin, which thus accumulates at high levels throughout the subpial zone of the early embryonic cerebellum (Miyata *et al*., 1996). In addition, Reelin is expressed by some cells in the NTZ and later DCN, which may contribute to overall Reelin signaling in the cerebellum (Jensen *et al*., 2002). As discussed further below, Reelin evidently acts as a signal to regulate the radial migration of Purkinje neurons from the VZ toward the cerebellar cortex.

Unipolar brush cells are a unique type of glutamatergic interneurons in the cerebellar cortex. Their origins were revealed in 2006 by a careful study of transcription

factor expression patterns and cell migration in organotypic slice cultures (Englund *et al*., 2006). As shown in that study, unipolar brush cells are generated in the upper rhombic lip and migrate tangentially through the IZ or developing white matter to the internal granular layer (Fig. 10.1B). Like DCN projection neurons, unipolar brush cells arise from a Math1$^+$ lineage, although they can be identified more specifically by high-level expression of Tbr2, a T-domain transcription factor (Englund *et al*., 2006). The discovery that unipolar brush cells come from the upper rhombic lip supported the hypothesis that glutamatergic and GABAergic neurons are produced in separate progenitor compartments. Interestingly, many unipolar brush cells express Reelin, but the significance of this for cerebellar development is unknown.

Granule neurons are produced from proliferating precursor cells in the EGL, which are in turn derived from the upper rhombic lip. These origins were postulated many years ago on the basis of histological observations, and have been fully supported by newer approaches (Machold and Fishell, 2005; Wang *et al*., 2005). The EGL develops by replacement of the RLS with granule neuron precursors migrating from the upper rhombic lip (Fig. 10.1). Thus, granule neurons are related to DCN projection neurons and unipolar brush cells by common origins from Math1$^+$ lineages in the upper rhombic lip. Notably, newly differentiated granule neurons express Reelin in the inner part of the EGL and in the internal granular layer (Miyata *et al*., 1996). This source of Reelin may regulate Purkinje cell spreading during middle to late stages of cerebellar development.

2.3 Cerebellar Cell Migrations and Interactions

The five major types of cerebellar neurons (Purkinje cells, inhibitory interneurons, DCN projection neurons, unipolar brush cells, and granule neurons) undergo complex migrations and interactions during morphogenesis. The migrating cells interact with each other and with radial glia by cell–cell contacts and by production of extracellular factors that are detected by specific receptors. Reelin is one example of an extracellular factor that mediates important interactions in cell guidance (and possibly other processes as well). Another important extracellular factor is Sonic hedgehog, which is produced by Purkinje cells to stimulate the proliferation of granule cell precursors. A basic knowledge of these migrations and interactions is essential to understanding the role of Reelin signaling in cerebellar development.

2.3.1 Radial Migration of Purkinje Cells and Inhibitory Interneurons

At early stages of neurogenesis, the embryonic cerebellum lacks folia and instead exhibits a relatively flat surface topography (Fig. 10.1A). The cerebellar plate VZ appears to map directly onto the mantle (subpial) zone in a point-to-point manner,

defined by radial glial cells whose processes span the distance from ventricular to pial surface (Fig. 10.1A). This cellular organization resembles that in the embryonic neocortex, where radial glial cells organize the cortex into columnar units and guide radial migration of pyramidal neurons from VZ to cortical plate (Rakic, 1988, 1995). Similarly, radial glia in the embryonic cerebellum guide radial migration of Purkinje cells from the VZ to the mantle zone, where they form the Purkinje cell plate (Goffinet, 1983; Yuasa *et al*., 1993, 1996) directly beneath Reelin-expressing cells in the RLS and EGL (Miyata *et al*., 1996, 1997). Initially, the Purkinje cell plate is several cell layers thick, but the Purkinje cells subsequently spread out to form a monolayer, concurrent with the entry of granule cells from the external granular layer (Altman and Bayer, 1985c). Reelin signaling may play an important role in the tangential spread of Purkinje cells and monolayer formation (Miyata *et al*., 1997).

The radial phase of Purkinje cell migration appears to be guided mainly by contacts with radial glia. Electron microscopy has revealed adherens junctions (puncta and macula adhaerentia) between Purkinje cells and radial glia in the IZ (Yuasa *et al*., 1996). During this phase, the Purkinje cells express high levels of Reelin receptors as well as Dab1 mRNA and protein, which are downstream mediators of Reelin signaling (Trommsdorff *et al*., 1999; Rice and Curran, 2001). As the Purkinje cells enter the mantle zone and accumulate in the Purkinje cell plate, they lose adherens junctions to radial glia, suggesting that the Purkinje cells release their hold on radial glia in order to facilitate spreading (Yuasa *et al*., 1993, 1996).

Like Purkinje cells, inhibitory interneurons initially migrate radially from the cerebellar plate VZ into the IZ (Maricich and Herrup, 1999; Hoshino *et al*., 2005). However, the inhibitory interneuron precursors continue to proliferate as they migrate through cerebellar white matter, undergoing widespread tangential as well as radial dispersion. The inhibitory interneurons eventually settle in the DCN and all layers of cerebellar cortex (Zhang and Goldman, 1996; Maricich and Herrup, 1999). The signals that guide interneurons are unknown.

2.3.2 Tangential (Nonradial) Migration of Rhombic Lip Derivatives

The upper rhombic lip, also known as the germinal trigone of the cerebellum, gives rise to a massive efflux of neurons and neural progenitor cells that migrate rostrally into the cerebellar plate and brainstem. Genetic lineage tracing with *Math1* reporter mice revealed that cells exit the rhombic lip in a subpial stream, the RLS (later replaced by EGL), which delivers DCN projection neurons and granule cells to the cerebellum, and many additional neurons to diverse brainstem nuclei (Machold and Fishell, 2005; Wang *et al*., 2005; Fink *et al*., 2006). The subpial migration of cells in the RLS/EGL is regulated in part by the chemoattractant activity of stromal cell derived factor 1 (SDF-1), a chemokine secreted from the meninges (Zhu *et al*., 2002). The SDF-1 is detected by chemokine (C-X-C motif) receptor 4 (CXCR4) on RLS/EGL cells (Zou *et al*., 1998). The signals that control partition of RLS cells into DCN, the granular layer, and brainstem nuclei are not known.

The RLS is not the only pathway out of the rhombic lip. Unipolar brush cells migrate through a distinct narrow channel between the Purkinje cell plate and the cerebellar plate VZ into the cerebellar plate IZ (Englund *et al*., 2006). Having entered the IZ, the unipolar brush cells disperse widely in the developing white matter and enter the internal granular layer from below. The signals that guide this migration have not been studied.

2.3.3 Inward Radial Migration of Granule Neurons

Much evidence indicates that granule neurons use radial glia as guides to migrate from the EGL though the Purkinje cell plate to the internal granular layer (Hatten, 1999). Notably, this migration proceeds in the opposite direction as the earlier radial migration of Purkinje cells, i.e., inward rather than outward. The inward migration of granule neurons may be directed by repellent factors from the meninges (Zhu *et al*., 2002), presumably associated with downregulation of SDF-1 in the meninges and/or CXCR4 in granule neurons. Interestingly, the radial glia that mediate granule cell migration display a specialized phenotype with processes that extend to the pial surface but not the ventricular surface. These unique radial glia in the cerebellum are known as Bergmann glia (Hatten, 1999).

2.3.4 Sonic Hedgehog from Purkinje Cells Promotes Granule Cell Neurogenesis and Cerebellar Foliation

Adequate production of granule neurons critically depends on an interaction with Purkinje cells, which produce factors that stimulate proliferation of granule cell precursors in the EGL. This interaction presumably serves to optimally regulate the ratio of granule neurons to Purkinje cells, which is approximately 1000:1 in humans (Nolte, 1999). The main proliferative signal produced by Purkinje cells is Sonic hedgehog, which strongly stimulates granule cell proliferation (reviewed by Ruiz i Altaba *et al*., 2002). The proliferative effect of Sonic hedgehog is further potentiated by other factors (Mills *et al*., 2006). In turn, the proliferation of granule neurons determines the number of cerebellar folia, as demonstrated by experiments in which Sonic hedgehog signaling was manipulated genetically (Corrales *et al*., 2006). The interaction between Purkinje cells and granule cell precursors is severely impaired by Reelin deficiency, as explained in subsequent sections of this chapter.

3 Reelin Signaling in the Cerebellum

The Reelin signaling pathway involves the same receptors and downstream mediators in the developing cerebellum as in other regions such as developing cerebral cortex (reviewed in Rice and Curran, 2001; Förster *et al*., 2006). The receptors for

Reelin are ApoER2, VLDLR, and $\alpha 3\beta 1$ integrin, and downstream signaling mediators include Dab1 adapter protein and Src family tyrosine kinases (Rice and Curran, 2001; Kuo *et al*., 2005; Förster *et al*., 2006). The expression of Reelin and other signaling components is extremely complex in the developing cerebellum, and not yet fully understood. For example, Miyata *et al*. (1996) detected Reelin protein at various expression levels in different cell types and extracellular zones throughout cerebellar development. The importance of such subtle gradations of Reelin expression and accumulation is unknown. Nevertheless, the broad outlines of Reelin expression are clear enough to support the hypothesis that Reelin signaling regulates the migration of Purkinje cells, and probably other aspects of cerebellar development as well.

3.1 Purkinje Cell Radial Migration from VZ to Mantle Zone

The first cells to express Reelin during cerebellar development (~E13 in mouse) are located along the RLS and scattered in the NTZ (Miyata *et al*., 1996). These cells are derived from the rhombic lip and differentially express transcription factors Zic1, Pax6, Tbr2, and Tbr1 (Miyata *et al*., 1996; Fink *et al*., 2006). Since the RLS defines the subpial surface of the embryonic cerebellum (Fig. 10.1A), the early Reelin$^+$ cells are well positioned to signal Purkinje cells that they should cease migration and remain in the mantle zone to form the Purkinje cell plate. Consistent with this interpretation, much evidence indicates that migrating Purkinje cells express Reelin receptors and downstream mediators, including VLDLR, ApoER2, and Dab1 (Trommsdorff *et al*., 1999; Rice and Curran, 2001; Perez-Garcia *et al*., 2004). The role of Reelin signaling from the NTZ is somewhat unclear and problematic. As the NTZ grows and partitions to form the DCN, its cells migrate radially inward, toward the VZ (in the opposite direction to Purkinje cells) and ultimately come to reside near the roof of the fourth ventricle (Altman and Bayer, 1985a,b). It is unclear whether Purkinje cells migrate around or between these NTZ/DCN cells to reach overlying regions of cerebellar cortex. It is likewise unclear whether radial glia pass through the NTZ/DCN or curve around them (Fig. 10.1B).

The mechanisms of Purkinje cell migration that are regulated by Reelin have been studied very little. The available evidence suggests that Reelin may promote detachment of Purkinje cells from radial glial fibers. In *reeler* mice, which lack Reelin protein due to a genetic mutation, there are increased numbers of adherens junctions (puncta adhaerentia) between migrating Purkinje cells and radial glia and between pairs of migrating Purkinje cells (Yuasa *et al*., 1993). The prolonged attachment between Purkinje cells and radial glia may obstruct the arrival of following Purkinje cell cohorts migrating on the same radial glial fibers, and thus block formation of the Purkinje cell plate (Goffinet, 1983). Additional support for this hypothesis comes from studies of the cerebral cortex, where evidence indicates that Reelin/Dab1 signaling promotes detachment from radial glia and inhibits neuron migration (Dulabon *et al*., 2000; Sanada *et al*., 2004).

It has also been suggested that Reelin might regulate Purkinje cell migrations indirectly, by effects on radial glia morphology (Yuasa *et al*., 1993). This hypothesis was based on the observation that radial glia have abnormally curved and disorganized processes in *reeler* mice (Yuasa *et al*., 1993). On the other hand, it is equally possible that the radial glia abnormalities may be secondary effects of prolonged Purkinje cell attachment and impaired migration.

3.2 Purkinje Cell Spreading and Monolayer Formation

Once Purkinje cells have completed their radial migration into the mantle zone (by the day of birth in mice), they coalesce in the Purkinje cell plate, a continuous cellular stratum that is ~2–4 cells thick in newborn mice. During the subsequent prolonged period of granule cell neurogenesis and migration, which continue during the first 3 postnatal weeks in mice (Miale and Sidman, 1961), the Purkinje cells spread into a highly convoluted monolayer that defines the contours of the cerebellar folia (Goffinet, 1983). The signals that regulate Purkinje cell spreading and monolayer formation are unknown. Interestingly, Reelin protein is expressed by numerous EGL cells and accumulates in the extracellular matrix of the EGL during this period, and then is downregulated after monolayer formation is complete (Miyata *et al*., 1996). Thus, Reelin is expressed in appropriate patterns to guide Purkinje cell spreading. However, this aspect of cerebellar development has been studied very little, and the proposed function of Reelin signaling during this stage has not yet been confirmed experimentally. In mutant animals, such as *reeler*, the early defects of Purkinje cell radial migration are so severe that no useful information can be obtained about the role of Reelin in Purkinje cell spreading.

3.3 Regulation of Reelin Expression in Cerebellum

Reelin is expressed by a limited set of cerebellar neuron types, implying that expression is stringently regulated by upstream factors. Interestingly, all Reelin$^+$ cell types in the developing cerebellum share common origins from the upper rhombic lip. Cells in the upper rhombic lip lineage express numerous specific transcription factors, including Math1, Zic1, Pax6, Tbr2, and Tbr1. Of these, Zic1, Pax6, Tbr2, and Tbr1 have been co-localized with Reelin in subsets of rhombic lip-derived cells (Miyata *et al*., 1996; Englund *et al*., 2006; Fink *et al*., 2006). In the developing cerebral cortex, Reelin appears to be regulated by Tbr1. *Tbr1* null mutant mice have a severe cortical malformation with marked reduction of Reelin mRNA and protein (Hevner *et al*., 2001). In the cerebellum, however, *Tbr1* inactivation has no apparent effect on Reelin expression (Fink *et al*., 2006). Thus, despite similar roles played by Reelin signaling in controlling radial migration of neurons, the upstream regulation of Reelin evidently differs between cerebral cortex and cerebellum.

4 Cerebellar Malformations Caused by Reelin Deficiency

Under conditions of complete Reelin deficiency, as first described in *reeler* mutant mice, many brain structures develop abnormally. The most severe malformation is observed in the cerebellum, which is affected by both neuronal disorganization and marked hypoplasia. The cerebral cortex is also malformed, but unlike the cerebellum, shows no significant hypoplasia. The *reeler* brain malformations are beginning to be understood at a mechanistic level (Rice and Curran, 2001; Förster *et al*., 2006). Here, I focus on the mechanisms of cerebellar defects caused by Reelin deficiency.

4.1 Neurological Defects Caused by Cerebellar Malformations

The cerebellum is primarily a motor center, although some cognitive and language functions are also mediated by cerebellar circuits (Ito, 2005). In humans, cerebellar lesions cause mainly ataxia (incoordination) and vertigo (inappropriate sensation of movement or spinning). Since the cerebellum is small and malformed in rodents and humans with Reelin deficiency, one would expect to observe ataxia in affected individuals. Indeed, ataxia is a prominent symptom in Reelin-deficient mice (*reeler*) and rats (*SRK*), along with tremor or "shaking" of the body (Aikawa *et al*., 1988). Affected rodents generally die young, usually around the end of the first postnatal month (Mariani *et al*., 1977; Aikawa *et al*., 1988). In humans with *RELN* mutations, ataxia is not so prominent. Rather, the major symptoms are neurodevelopmental delay, hypotonia ("floppy baby"), language deficit, and generalized seizures (Hong *et al*., 2000; Chang *et al*., 2007). Presumably, ataxia would be more apparent if not masked by severe hypotonia. With appropriate medical care, affected humans can survive for many years, despite the severe neurological problems.

4.2 Cerebellar Hypoplasia and Disorganization in reeler and SRK

SRK rats and *reeler* mice exhibit virtually identical cerebellar phenotypes (Kikkawa *et al*., 2003). In *reeler* mice, the first defect to appear (by E14) is absent formation of the Purkinje cell plate (Goffinet, 1983). By E17, when normal embryos begin to show evidence of foliation, *reeler* mutants exhibit not only defective Purkinje cell migrations, but also reduced tangential growth of the cerebellar surface and absent foliation. These defects progress, and by the time cerebellar development is complete (~3 postnatal weeks in mice), the *reeler* cerebellum is only 25–33% of normal size, contains only 12–14% of the normal DNA amount, has no foliation, and has severe neuronal disorganization throughout most regions of cortex (Fig. 10.2). The disproportionate decrease of DNA relative to cerebellar mass in *reeler* is attributed

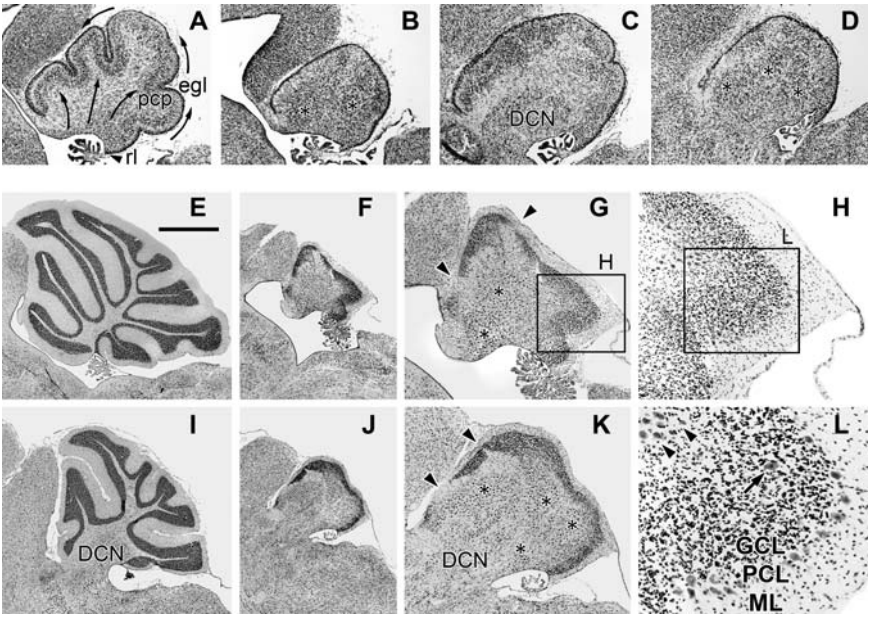

Fig. 10.2 Cerebellar histology in control and *reeler* mice. Sagittal sections through the cerebellar vermis (**A**, **B**, **E–H**, **L**) or hemisphere (**C**, **D**, **I–K**) of control and *reeler* (**B**, **D**, **F–H**, **J–L**) mice were stained with cresyl violet on P0.5 (**A–D**) or P22 (**E–L**). The boxed area in **G** is enlarged in **H**, and the boxed area in **H** is enlarged in **L**. In P0.5 controls, Purkinje cells had migrated to the Purkinje cell plate (pcp), and folia were developing by migration and proliferation of cells in the external granular layer (egl). In P0.5 *reeler* mice, the cerebellum was hypoplastic, no folia were developing, and Purkinje cells formed large, centrally located ectopic clusters (asterisks). The hypoplasia and defective foliation of the *reeler* cerebellum became even more obvious by P22. Most Purkinje cells in the P22 *reeler* cerebellum are located in the large central clusters, although some are isolated ectopically in the granule cell layer (GCL), and others form a nearly normal Purkinje cell layer (PCL) below the molecular layer (ML). In **L**, arrowheads indicate Purkinje cells in deep ectopia, and the arrow indicates a Purkinje cell in the GCL. The GCL in *reeler* consistently shows gaps (arrowheads in **G**, **K**), which may be related to the presumptive locations of fissures (Goldowitz *et al*., 1997). The deep cerebellar nuclei (DCN) in *reeler* are located near the normal location, but somewhat distorted by the Purkinje cell ectopia (Goffinet, 1983; Goffinet *et al*., 1984). Sections oriented as described for Figure 1. Scale bar (in **E**): **A–D**, 400 µm; **E**, **F**, **I**, **J**, 1000 µm; **G**, **K**, 500 µm; **H**, 200 µm; **L**, 100 µm (*See Color Plates*)

to selective depletion of granule neurons (Mariani *et al*., 1977). Despite the absence of foliation, some small regions of the cerebellar surface display essentially normal cortical cytoarchitecture, including formation of a monolayer by ~7% of remaining Purkinje cells (Mariani *et al*., 1977; Goffinet *et al*., 1984; Goldowitz *et al*., 1997).

The DCN develop relatively normally in *reeler* mice, although the organization of the lateral (dentate) nucleus is perturbed (Goffinet, 1983), and the medial (fastigial) nucleus is displaced laterally (Goffinet *et al*., 1984). Cerebellar patterning into longitudinal and transverse compartments, and cerebellar axon connections likewise

show only mild abnormalities, presumably secondary consequences of distortion caused by altered Purkinje cell migrations and hypoplasia (Mariani *et al*., 1977; Goffinet *et al*., 1984). Mice with deficiencies of downstream Reelin signaling molecules including VLDLR/ApoER2, Dab1, and Src/Fyn exhibit essentially identical cerebellar malformations as in *reeler* (Rice and Curran, 2001; Kuo *et al*., 2005).

The primary defect of cerebellar morphogenesis in Reelin deficiency is thought to be abnormal migration of Purkinje cells. This conclusion is supported by several observations. First, a defect of Purkinje cell migration (agenesis of the Purkinje cell plate) is the earliest morphological abnormality to appear in *reeler* mice (Goffinet, 1983). Second, Dab1 protein is upregulated in *reeler* Purkinje cells, indicating a biochemical response to decreased Reelin signaling (Sheldon *et al*., 1997; Rice *et al*., 1998). Third, the impairment of granule cell proliferation, and consequent hypoplasia and lack of foliation, can be adequately explained as a secondary consequence of abnormal Purkinje cell positioning. Granule cell precursors in the EGL require Sonic hedgehog, which is produced by Purkinje cells, to stimulate mitosis and neurogenesis (Ruiz i Altaba *et al*., 2002). Any decrease of Sonic hedgehog signaling reduces the neurogenesis of granule cells, which in turn leads to reduced foliation (Corrales *et al*., 2006). Since most Purkinje cells in *reeler* are located in abnormally deep positions far from the EGL, granule cell precursors are presumably exposed to lower concentrations of Sonic hedgehog and thus proliferate less. The abnormal organization of neuronal processes, such as Purkinje cell dendrites, may also impair transport of Sonic hedgehog to the EGL (Ruiz i Altaba *et al*., 2002).

Other cerebellar defects that have been reported in *reeler* include disorganized arrangement of radial glia processes and cell bodies (Yuasa *et al*., 1993), reduced numbers of unipolar brush cells (Ilijic *et al*., 2005; Englund *et al*., 2006), altered synaptic organization and physiological responses of some cerebellar neurons (Mariani *et al*., 1977), and mild defects of axon connections (Goffinet *et al*., 1984). Some or all of these abnormalities may be secondary to malpositioning of Purkinje cells. On the other hand, Reelin signaling regulates axon growth and branching independently of cell migration in the hippocampal formation, and thus might do so in the cerebellum as well (Del Río *et al*., 1997; Borrell *et al*., 1999).

4.3 Human Reelin Deficiency: Lissencephaly with Cerebellar Hypoplasia

Genetic studies have demonstrated that Reelin deficiency in humans causes a severe brain malformation involving the cerebellum, brainstem, and cerebral cortex. The malformation is classified as a form of lissencephaly (reduced number of cerebral cortical gyri) with cerebellar hypoplasia (Hong *et al*., 2000; Chang *et al*., 2006). The malformation syndrome is inherited in an autosomal recessive pattern and is caused by mutations affecting both copies of the *RELN* gene, located on chromosome 7q22. On neuroimaging scans, the cerebellum appears severely hypoplastic and lacks folia (Hong *et al*., 2000; Chang *et al*., 2007). In addition, the cerebral

cortex exhibits a simplified gyral pattern; cortical thickness is increased; the hippocampus appears malrotated and flat; and the brainstem is hypoplastic. The brain phenotype caused by Reelin deficiency is unique and can easily be distinguished from other forms of lissencephaly or cerebellar hypoplasia by neuroimaging (Hong *et al*., 2000; Chang *et al*., 2007). No histologic studies of the human brain phenotype have been reported.

Humans with a heterozygous mutation affecting only one copy of the *RELN* gene show no apparent neuropsychiatric abnormalities (Hong *et al*., 2000; Chang *et al*., 2007). Thus, the proposed role of Reelin signaling in diseases, such as schizophrenia and autism (Fatemi, 2005; Fatemi *et al*., 2005), may involve changes that are more complex than a partial reduction of mRNA and protein levels due to gene dosage effect.

4.4 Autism, Reelin, and the Cerebellum

Several studies have suggested that alterations of Reelin signaling may contribute to the pathogenesis of neurobehavioral disorders, especially autism (Fatemi, 2005; Fatemi *et al*., 2005). Genetic studies have linked autism to specific polymorphisms of the *RELN* gene, and decreased levels of Reelin mRNA and protein have been found in autistic brains relative to controls, particularly in the cerebellum (reviewed in Fatemi, 2005). Intriguingly, abnormalities of cerebellar structure are among the most consistent neuropathologic findings in autism (Kemper and Bauman, 1998; Palmen *et al*., 2004; Bauman and Kemper, 2005; Pickett and London, 2005). Specifically, the autistic cerebellum is smaller, contains fewer Purkinje cells (~41% loss), has atrophic Purkinje cells (~24% decrease of cell size), shows variable gliosis, and expresses increased levels of glial fibrillary acidic protein (Kemper and Bauman, 1998; Carper and Courchesne, 2000; Fatemi *et al*., 2002; Palmen *et al*., 2004; Laurence and Fatemi, 2005; Pickett and London, 2005). Defects of cerebellar development have been further implicated in autism by linkage to the *EN2* gene, which regulates embryonic patterning of the cerebellum (Kuemerle *et al*., 2007). These associations support the hypothesis that some symptoms in autism may be caused by cerebellar defects, sometimes arising from perturbations of Reelin signaling. Further studies will be necessary to clarify how abnormal Reelin signaling in humans may cause a reduction in the number of Purkinje cells, versus ectopic migration as observed in *reeler* mice and *SRK* rats.

5 Conclusion

The role of Reelin signaling in cerebellar development and disease is not yet fully understood. Existing data strongly suggest that Reelin signaling regulates radial migration of Purkinje cells by modulating their adhesive properties and contacts

with radial glia. Some data also suggest that other cell types and developmental mechanisms may be regulated by Reelin signaling, although the current evidence is inconclusive. For example, DCN neurons reportedly express Dab1 mRNA and protein during development, which would imply that they can respond to Reelin (Rice *et al*., 1998), but the role (if any) of Reelin signaling in regulating DCN cell migrations remains unclear. Molecular expression patterns further suggest that Reelin signaling may regulate Purkinje cell spreading into a monolayer. Likewise, the possible role of Reelin signaling in regulating axon pathfinding of cerebellar efferent and afferent axons deserves more study. Future studies along these lines would benefit from more sophisticated genetic models, such as conditional *reelin* knockout mice, to investigate the role of Reelin signaling in different cell types and at different stages of development. Such analysis will be essential to fully understand how Reelin signaling affects all aspects of cerebellar development, and ultimately lay the scientific foundation for interpreting links between Reelin signaling, cerebellar development, and autism.

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Chapter 11
Reelin and Radial Glial Cells

Eckart Förster, Shanting Zhao, and Michael Frotscher

Contents

1 Introduction

Defects of the radial glial scaffold in reeler mice were detected and characterized after the radial neuronal migration defects in this mutant had been described (Caviness and Rakic, 1978; Caviness *et al*., 1988; Pinto-Lord *et al*., 1982). Based on these findings, it has been hypothesized that radial glial defects contribute to the malpositioning of radially migrating neurons. Experimental evidence that Reelin may directly influence the development of radial glial cells is quite recent (Förster *et al*., 2002; Weiss *et al*., 2003; Hartfuss *et al*., 2003; Luque *et al*., 2003). The question as to why Reelin should simultaneously act on two different cell types, neurons

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and radial glial cells, turned out to be a semantic problem when radial glial cells were shown to be precursors of radially migrating neurons (Malatesta *et al*., 2000; Noctor *et al*., 2001; Miyata *et al*., 2001). Thus, when discussing a role of Reelin in the formation of the radial glial scaffold, the changing view of radial glial cell function in cortical development has to be taken into account.

2 Definition of Radial Glial Cells: Changing Views in History

By the end of the nineteenth century, different hypotheses existed on the nature of radial glial cells. Wilhelm His (1889) was the first to show that glial cells are generated in the anlage of the central nervous system and are not of mesenchymal origin, as suggested by Virchow (in: Jacobson, 1991). His and Ramón y Cajal (1911) assumed that glial cells and neurons were derived from different precursor populations. In contrast, Magini (1888a,b) and Kölliker (1896) had shown that the majority of neuroepithelial cells form long radially oriented processes toward the pial surface. The predominance of these cells already suggested that they might be neuronal precursors. Correspondingly, this cell type was termed "radial neuroglia" by Magini (1888a). Nonetheless, for more than 100 years, the generally accepted view of the nature of neuroepithelial cells was based on His's idea that glial cells and neurons were derived from different progenitor cells. Only recently, independently obtained results from different laboratories clearly demonstrated that neurons in the neuroepithelium originate from dividing radial glial cells (Malatesta *et al*., 2000; Noctor *et al*., 2001; Miyata *et al*., 2001). Thus, asymmetric division of a radial glial cell generates a neuron that migrates along the radial glial process of its own precursor cell (Alvarez-Buylla *et al*., 2001; Nadarajah and Parnavelas, 2002; Noctor *et al*., 2002). In view of these findings, the hypothesis of the different origins of radial glial cells and neurons in the ventricular zone had to be revised and the term "radial neuroglia," proposed more than 100 years ago by Magini (1888a), came to late honors. Radial glial cells were shown to transform into astrocytes when their role in neuronal migration is accomplished (Schmechel and Rakic, 1979; Mission *et al*., 1991).

3 Role of Radial Glial Cells in Neuronal Migration: Different Models

How do radial glial cells guide neuronal migration? In the current chapter, different models are discussed, thereby providing a base for the interpretation of Reelin's role in organizing the radial glial scaffold.

The earliest morphological evidence, suggesting that newly generated neurons could migrate along radial glial processes, was provided by Magini (1888b), and numerous later studies support this model (Rakic, 1971, 1972, 1988; Sidman and Rakic, 1973; Noctor *et al*., 2001).

By contrast, a different migration mode was proposed by Berry and Rogers (1965). After nuclear division within the perikaryon of the neuronal precursor cell in the neuroepithelium, one nucleus migrates within the radial process of the cell toward the cortical plate, whereas the second nucleus remains in the cell body close to the ventricular zone. A similar migration mode had been suggested by Morest (1970). According to Morest's model, the newly generated neuron first loses its contact to the ventricular surface, the cell body then translocates, being "pulled" by its radial process, toward the cortical plate.

Only recently, the existence of both modes of migration, i.e., migration of the neuron along a radial glial process, as well as translocation of the cell body connected to a radial glial process, could be confirmed by using video microscopy to monitor the migration of fluorescently labeled neurons in living slices of embryonic cortex. Thus, Noctor *et al*. (2001) found the generation of neurons by asymmetric division of radial glial cells and migration of the newly generated neuron along the radial glial process of its mother radial glial cell. These observations confirm Magini's interpretation (1888b), and the model suggested by Rakic (1972). By contrast, Miyata *et al*. (2001) could show that a radial glial cell divides asymmetrically and generates a neuron that keeps the existing radial process, whereas the perikaryon detaches from the ventricular zone. These observations are in line with the model suggested by Berry and Rogers (1965) and Morest (1970). Translocation of the cell body was documented for ontogenetically earlier generated neurons, and migration along radial glial processes was shown predominantly for neurons that were generated late in ontogenesis (Nadarajah and Parnavelas, 2002).

4 Reelin-Secreting Cajal-Retzius Cells Organize the Radial Glial Scaffold

Reelin is expressed by Cajal-Retzius (CR) cells in the marginal zone of the developing cortex (Fig. 11.1). Several studies have shown that CR cells play an important role in the organization of radial glial cells. Thus, application of 6-hydroxydopamine (6-OHDA), a toxin that causes CR cells to degenerate (Del Rio *et al*., 1996, 1997; Supér *et al*., 1997), induces disorganization of radial glial cells and astrocytes in the dentate gyrus (Hartmann *et al*., 1992). A dramatic decrease in the number of radial glial apical processes was observed after ablation of CR cells with domoic acid (Supér *et al*., 2000), indicating that CR cell-specific factors are required to maintain the radial glial phenotype and to anchor radial glial processes to the marginal zone.

In the homozygous reeler mutant, CR cells are present in the marginal zone but do not express Reelin. Absence of Reelin causes, in addition to malpositioning of neurons, an impaired development of radial glial cells (Caviness and Rakic, 1978; Pinto-Lord *et al*., 1982; Yuasa *et al*., 1993; Caviness *et al*., 1988; Hunter-Schaedle, 1997). Alterations include defasciculation of radial glial fibers (Caviness *et al*., 1988) and malformation of their apical processes (Pinto-Lord *et al*., 1982).

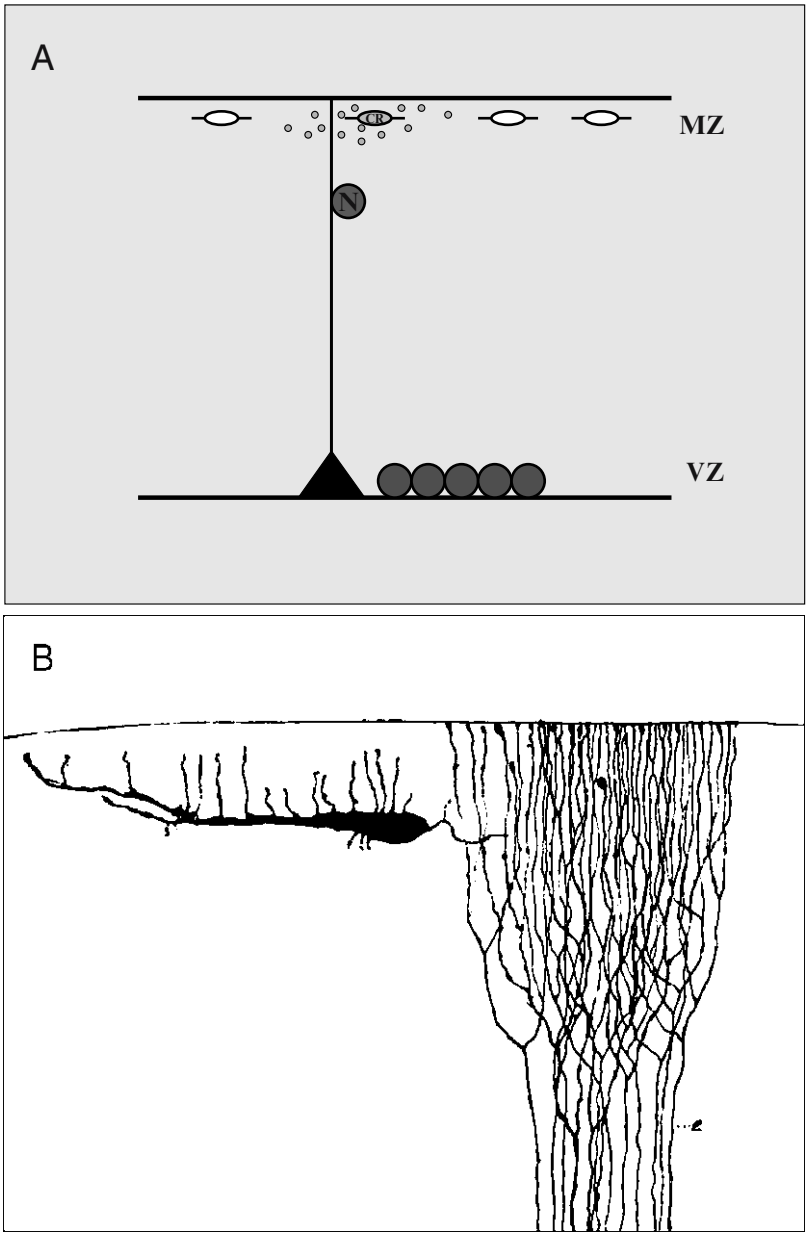

Fig. 11.1 (**A**) Schematic view of the developing cortex. A radial glial cell (black) is shown, extending a radial process from its perikaryon in the ventricular zone (VZ) toward the marginal zone (MZ). Neurons (red) in the ventricular zone are generated by asymmetric division of radial glial cells. A newly generated neuron (N) migrates along the radial glial process toward the marginal zone. Cajal-Retzius cells (CR; green) located in the marginal zone, secrete the glycoprotein Reelin (green dots) into the extracellular matrix. Reelin controls the positioning of radially migrating neurons by acting on both radial glial cells and migrating neurons (*See Color Plates*). (**B**) Detail of a drawing by Gustav Retzius from a silver-stained preparation of developing cortex (modified, Retzius, 1893). The drawing shows a Cajal-Retzius cell (horizontal cell, left) in the marginal zone below the pial surface and radial glial processes (right) that reach the pial surface. Note that radial glial fibers branch when entering the marginal zone

An intrinsic glial cell defect has been proposed to contribute to the radial glial malformations in the reeler mutant (Hunter-Schaedle, 1997).

Migrating neurons detach from radial glial fibers before they reach the most apical portions of these processes. In wild-type mice, apical radial glial processes give rise to multiple terminal branches when they reach the marginal zone of the neocortex (Fig. 11.1B; Retzius, 1893; Pinto-Lord *et al*., 1982) or the dentate gyrus (Rickmann *et al*., 1987). In contrast, apical radial glial processes in the reeler mutant do not branch and often do not reach the marginal zone (Pinto-Lord *et al*., 1982). An oblique or horizontal course of radial glial apical processes has also been described in the cerebellum of reeler mutants (Yuasa *et al*., 1993). These malformations indicate that Reelin is recognized by apical radial glial processes, induces their branching, and is required to anchor the ramified processes in the marginal zone. In most cortical regions, alterations of the radial glial scaffold in the reeler mutant are subtle when compared to neuronal migration defects.

Where may radial glial cells encounter Reelin? Immunocytochemistry with antibodies against Reelin suggests that Reelin is predominantly localized in the marginal zone (Ogawa *et al*., 1995). Furthermore, the finding that Reelin molecules assemble to form a large protein complex (Utsunomiya-Tate *et al*., 2000) suggests that Reelin exerts its action locally, i.e., in the marginal zone rather than as a freely diffusible factor. Thus, a direct action of Reelin on radial glial cells, particularly on the branching of their terminals and on the anchorage of their endfeet to the pial surface, could be a means to indirectly influence migrating neurons. The severe radial glial malformations in the dentate gyrus of mutants with defects in the Reelin signaling pathway suggest that this mode of Reelin action may be particularly important in the dentate gyrus (Förster *et al*., 2002; Weiss *et al*., 2003). However, phenotypic morphological studies could not answer the question as to whether Reelin may directly act on radial glial cells.

5 Reelin Signaling via Reelin Receptors and Dab1 Controls Radial Glial Differentiation in the Dentate Gyrus

Due to its simple cytoarchitecture, the dentate gyrus of the hippocampal formation is a particularly suitable model to study the formation of neuronal layers in the cerebral cortex (Stanfield and Cowan, 1979a,b; Cowan *et al*., 1980, 1981; Förster *et al*., 2006). Newly generated dentate granule cells were shown to migrate along radial glial fibers from the ventricular zone toward the dentate anlage (Rickmann *et al*., 1987), reminiscent of radial migration of neurons in the neocortex. Granule cells that are later generated from precursors in the secondary proliferation zone of the hilus were suggested to migrate along a specialized radial glial scaffold to their final positions in the dentate gyrus (Rickmann *et al*., 1987).

In rodents, the majority of dentate granule cells are born postnatally (Angevine, 1965; Schlessinger *et al*., 1975; Bayer, 1980), and radial glial cells in the dentate gyrus of rodents are known to persist after birth and to express GFAP (Woodhams *et al*., 1981; Levitt and Rakic, 1980; Eckenhoff and Rakic, 1984; Rickmann *et al*., 1987).

In contrast to neocortical cell layers, the dentate granule cell layer is not formed in an inside-out gradient, but later-generated granule cells are apposed beneath the earlier-formed granule cells (Stanfield and Cowan, 1979a). In the reeler mutant, dentate granule cells fail to migrate and accumulate in the hilar region. Granule cells are scattered in the hilar region of VLDLR/ApoER2-deficient mice, similar to the Reeler dentate gyrus (Caviness and Sidman, 1973; Stanfield and Cowan, 1979b; Deller *et al*., 1999; Drakew *et al*., 2002), demonstrating that the Reelin signaling cascade is required for the correct positioning of dentate granule cells. Dab1 has been shown to function downstream of Reelin. Dab1-deficient mice also develop a reeler-like phenotype (Sweet *et al*., 1996; Howell *et al*., 1997; Sheldon *et al*., 1997; Ware *et al*., 1997). Dab1 mRNA is predominantly expressed in neurons; however, GFAP-positive cells in the hippocampus may also express Dab1 mRNA (Förster *et al*., 2002). Like in reeler mutants, a regular radial glial scaffold does not develop in mutants deficient in Dab1 (Förster *et al*., 2002; Frotscher *et al*., 2003; Zhao *et al*., 2004, 2006), suggesting that Reelin may directly act on radial glial cells via Dab1 interaction. Mutant mice deficient in the Reelin receptors ApoER2 and VLDLR display phenotypically similar defects of the dentate radial glial scaffold. A gradual expression of the radial glial scaffold defects is seen in the dentate gyrus of mice deficient in only ApoER2 or VLDLR, in accordance with the gradual expression of granule cell migration defects in these mutants (Drakew *et al*., 2002; Gebhardt *et al*., 2002; Weiss *et al*., 2003). Thus, the Reelin signaling pathway is required for the formation of the dentate radial glial scaffold. Dentate radial glial defects in mutants lacking Reelin, Dab1, or ApoER2 and VLDLR, are likely to contribute to the granule cell migration defects seen in these mutants.

In the stripe choice assay, originally developed to study axonal repulsion or attraction, hippocampal GFAP-positive glial cells preferred Reelin-coated stripes to control stripes not containing Reelin (Förster *et al*., 2002; Frotscher *et al*., 2003). Moreover, long GFAP-positive processes, supposedly radial glial processes, branched significantly more often on the Reelin stripes than on control stripes (Förster *et al*., 2002).

These findings further support Reelin signaling in radial glial cells via ApoER2, VLDLR, and Dab1. There is also evidence for an additional involvement of β1-integrins, putative Reelin receptors (Dulabon *et al*., 2000), in radial glial cell differentiation in the hippocampus. In β1-integrin-deficient mice, subtle malformation of the radial glial scaffold in the dentate gyrus and similar migrational defects of the granule cells were described in the dentate gyrus (Förster *et al*., 2002).

6 Rescue of the Reeler Radial Glial Scaffold in Slice Cultures

How does Reelin act on radial glial cells? By using hippocampal slice cultures as a model, the question could be addressed as to whether Reelin has to be secreted in the marginal zone to exert its role in granule cell positioning. Zhao *et al*. (2004) have added recombinant Reelin to the incubation medium of reeler hippocampal

slices not expressing Reelin. In these Reelin-treated cultures, the length of GFAP-expressing glial fibers was significantly increased when compared to untreated reeler slices. However, the elongated GFAP-positive glial fibers did not form the characteristic radially oriented glial scaffold of the dentate gyrus. In line with this, recombinant Reelin in the medium did not rescue the formation of a compact dentate granule cell layer, and granule cells remained scattered over the dentate gyrus (Zhao *et al*., 2004).

The result of the experiment was different when a reeler hippocampal slice was co-cultured with a wild-type hippocampal slice, such that the Reelin-containing dentate marginal zone was closely apposed to the reeler slice. In this co-culture, Reelin secreted by the wild-type slice induced a growth of radial glial fibers in the reeler dentate gyrus that was oriented toward the source of Reelin, namely, the wild-type marginal zone. Moreover, in parallel with the rescued radial glial scaffold in reeler slices co-cultured with wild-type, a dense granule cell layer had formed in the reeler slice (Zhao *et al*., 2004; Förster *et al*., 2006). These findings suggest that Reelin has to be present in a specific topographic position, i.e., in the marginal zone, in order to exert its effect on granule cell positioning.

7 Reelin Acts Directly on Radial Glial Cells in the Developing Neocortex

Radial glial cell defects in the reeler neocortex are less severe than in the hippocampus. This raises the question as to whether a similar Reelin action, as in radial glial cells in the dentate gyrus, may also be attributed to the radial glial cells in the neocortex.

When labeling radial glial cells in reeler with the lipophilic dye DiI from the ventricular surface, the number of ventricular zone cells with long radial processes was shown to be significantly reduced compared to wild-type (Hartfuss *et al*., 2003). This reduction in process length was accompanied by a reduced expression of brain lipid binding protein (BLBP), a radial glial marker protein. Interestingly, only radial glial cells of the dorsal telencephalon displayed these defects, whereas radial glial cells of the ventral telencephalon, or the ganglionic eminence, were not altered in reeler mutants, pointing to regional differences in radial glial cell populations (Hartfuss *et al*., 2003).

In vitro, Reelin treatment increased both the BLBP content and process extension of isolated cortical radial glial cells, but not of dissociated radial glial cells isolated from the ventral telencephalon, thereby confirming the *in situ* observations. This Reelin effect was dependent on the presence of Dab1, suggesting that the Reelin signaling cascade is also active in radial glial cells and mediates these effects. Along this line, isolated radial glial cells from embryonic cortex (E14) expressed mRNA coding for the Reelin receptors. In addition, these cells were immunopositive for ApoER2 and VLDLR (Hartfuss *et al*., 2003). Localization of components of the Reelin signaling cascade in radial glial cells has also been shown by Luque *et al*. (2003), thereby confirming the above interpretations.

BLBP expression in the E12 to E16 reeler cortex was shown to remain constant, whereas an increase in the number of BLBP-expressing cells was observed in the wild-type cortex until almost all precursor cells contained BLBP. Thus, maturation of radial glial cells in the dorsal telencephalon, but not the ventral telencephalon, seems to depend on the presence of Reelin.

By contrast, immunoreactivity for the radial glial cell marker RC2 was similar in wild-type and reeler, suggesting that RC2 expression is not regulated by Reelin (Hartfuss *et al*., 2003).

In line with these findings, Magdaleno *et al*. (2002) have shown that ventricular zone cells in the neocortex are competent to respond to Reelin, likely by their long radial fibers extending toward the marginal zone, and that the Reelin signaling pathway is already activated in these cells before they start to migrate. Thus, Reelin expression in ventricular zone precursor cells was sufficient to rescue some of the migrational defects seen in the reeler mutant (Magdaleno *et al*., 2002). These findings support the idea that Reelin may directly act on radial glial cells.

As radial glial cells can transform into neurons that keep the radial fiber (Miyata *et al*., 2001), Dab1-mediated Reelin signaling in neurons may be required to maintain the radial orientation of the radial fiber toward the marginal zone. In fact, phosphorylation of Dab1 by Reelin binding to VLDLR and ApoER2 modulates cytoskeletal proteins (Hiesberger *et al*., 1999). Similar mechanisms may operate while these cells are still neuronal precursors, i.e., radial glial cells.

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Chapter 12
Reelin and Cognition

Shenfeng Qiu and Edwin John Weeber

Contents

1 Introduction

The cognitive function of the human brain refers to the mental manipulation of all the information acquired by the sensory system. A more physiological definition of cognition by Ulric Neisser states, "…the term cognition refers to all processes by which the sensory input is transformed, reduced, elaborated, stored, recovered, and used." It is our unique cognitive ability acquired from our distinct experience that ultimately makes us who we are; as Rene Descartes said, "I think, therefore I am." The authors have no intention or expertise to embark on a philosophical discussion of cognition in this chapter; we rather safely rely on our assertion that normal cognition requires proper function of the central nervous system, which constitutes hundreds of millions of neurons and supporting cells and is correctly "wired" during development and, equally as important, "rewired" in response to our postnatal experiences.

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The correct construction of neuronal circuits of the embryonic brain provides a structural basis for the CNS to carry out its normal function throughout life. This process requires a myriad of proteins encoded by numerous genes that are properly expressed spatiotemporally during development. For example, the expression of the extracellular matrix protein Reelin by specialized Cajal-Retzius cells located in the marginal zone is required for the ventricular migrating neurons to form a highly laminated structure in the neocortex, hippocampus, and cerebellum, which is indispensable for the formation of a functional network and activity-driven synaptogenesis (D'Arcangelo *et al*., 1995; Aguilo *et al*., 1999; Soda *et al*., 2003). Other proteins, whether they are located in the same [e.g., apolipoprotein E receptor 2 (ApoER2) and very-low-density lipoprotein receptor (VLDLR), disabled-1 (Dab1)] or distinct pathways (e.g., cyclin-dependent kinase 5 and its activators p35 and p39), are also required for the correct lamination (Ohshima *et al*., 1996; Howell *et al*., 1997; Trommsdorff *et al*., 1999; Ko *et al*., 2001). This typical gross laminar arrangement of neurons is necessary, albeit not sufficient, to carry out the normal flow of information, which is achieved by numerous chemical and electrical synapses and the electrical pulses generated there. It is perceivable that disruption of cortical, hippocampal, and cerebellar lamination, as observed in the Reeler mouse, would result in profound cognitive deficits that are similar to those observed in human lissencephaly patients bearing a *RELN* gene mutation (Hong *et al*., 2000). However, the severe motor deficits in these mutant mice preclude the performance of meaningful cognitive analyses on these animals.

Although much of our knowledge on the actions of Reelin comes from investigation of embryonic CNS development, recent studies have indicated that Reelin signaling also plays an important role in synaptic function in the postnatal brain (Weeber *et al*., 2002; Beffert *et al*., 2005; Ramos-Moreno *et al*., 2006). Reelin-expressing neurons are widely distributed in the adult brain (Pesold *et al*., 1998; Martinez-Cerdeno *et al*., 2002; Abraham and Meyer, 2003; Roberts *et al*., 2005; Ramos-Moreno *et al*., 2006), at a period long after the decrease in number of Cajal-Retzius cells (Marin-Padilla, 1998; Meyer *et al*., 1998; Sarnat and Flores-Sarnat, 2002). The functional significance of the persistent expression of Reelin after the completion of cell migration remains enigmatic in most brain regions. However, in the hippocampus, Reelin and its lipoprotein receptors are required for proper function of synaptic transmission and plasticity, and this signaling system profoundly modifies mammalian learning and memory behavior (Weeber *et al*., 2002; Beffert *et al*., 2005; D'Arcangelo, 2005). This chapter will focus on existing experimental evidence supporting the requirement of Reelin for normal synaptic function in the hippocampus as well as mammalian cognitive ability.

2 Reelin in the Adult Hippocampus

2.1 Expression in the Hippocampus

The hippocampus is a structure within the brain's limbic system and is one of the most comprehensively studied regions in the CNS due to the fundamental role it plays in many forms of learning and memory (Scoville and Milner, 1957), and its distinctive

laminar structure that exhibits the readily identifiable "trisynaptic" neuronal circuitry (Anderson *et al*., 1971). The developmental lamination of hippocampal principal neurons and the correct formation of innervating fibers require Reelin (Del Rio *et al*., 1997; Forster *et al*., 2002). In the postnatal mouse hippocampus, Reelin is expressed primarily by a group of interneurons (Alcantara *et al*., 1998; Pesold *et al*., 1998). Immunohistochemical labeling using the monoclonal G10 antibody demonstrates that Reelin-expressing neurons are located primarily in the hilar region of dentate gyrus and the stratum lacunosum-moleculare layer of the hippocampus proper. Reelin-positive cells can also be found in stratum oriens and stratum radiatum of the CA1 and CA3 regions (Fig. 12.1). Reelin is also required for dentate granule cells to form a compact layer during embryonic development (Drakew *et al*., 2002; Frotscher *et al*., 2003; Zhao *et al*., 2004). In the postnatal dentate gyrus, Reelin seems to promote radial glial fiber differentiation and may support the neurogenesis of granule cells in the hilus region and their migration and integration into the granule cell layer (Forster *et al*., 2002; Frotscher *et al*., 2003). In agreement, a recent study has shown that Reeler mice exhibit impaired neurogenesis in the dentate gyrus (Won *et al*., 2006). However, it is currently not known

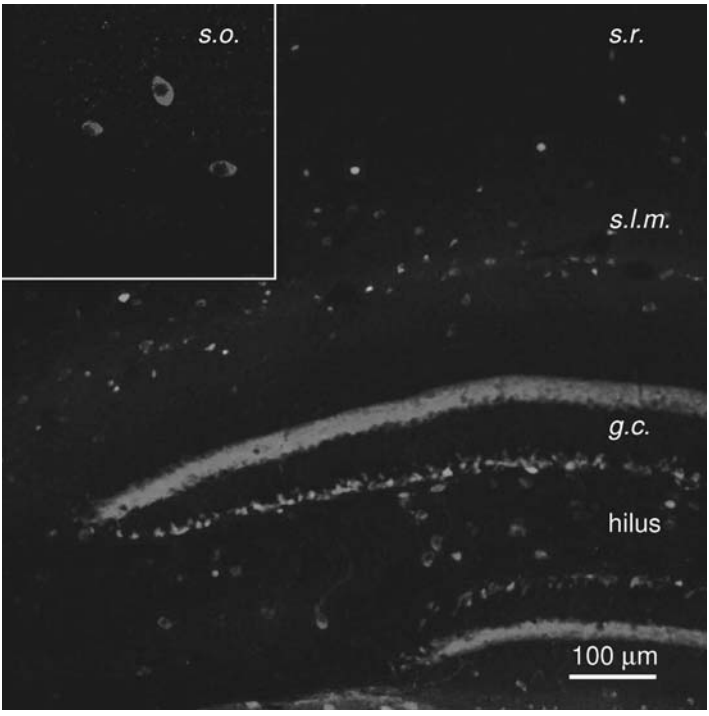

Fig. 12.1 Reelin-expressing cells in adult mouse hippocampus. Double immunofluorescent staining of a hippocampus cryosection obtained from a 6-week-old wild-type mouse. Note that Reelin-containing cells (red) were primarily distributed in the dentate hilar region (hilus) and stratum lacunosum-moleculare (*s.l.m.*) but also can be found in stratum oriens (*s.o.*) and stratum radiatum (*s.r.*) of CA1 region. Immunostaining of the calcium-binding protein calretinin (green) was used to visualize the dentate gyrus layers (*See Color Plates*)

whether Reelin signaling contributes to the synaptic function in the dentate gyrus, which receives heavy innervation from the perforant fibers and sends its mossy fibers to synapse on CA3 pyramidal cells.

2.2 Electrophysiology

The initial effort made to identify a putative role for Reelin in plasticity and memory was rather straightforward by using electrophysiological and behavioral studies in mice to identify and measure the effects of different components in the Reelin signaling pathway. The electrophysiological studies were carried out on acute hippocampal slices prepared from 4- to 6-week-old adult mice. Synaptic transmission and plasticity were tested in the Schaffer collateral-CA1 synapses, primarily because this area of the hippocampus is the most well characterized in the murine brain and due to the remarkable property of activity-dependent synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD) (Bliss and Lomo, 1973; Nicoll and Malenka, 1995). Both LTP and LTD are thought to underlie the cellular mechanisms of learning and memory behavior and are correlated with positive results as measured by cognitive tests in animals (Giese *et al*., 1998; Lee *et al*., 2003).

In electrophysiological studies using hippocampal slices, a single electric stimulus evokes action potentials on the presynaptic fibers and causes release of glutamate neurotransmitter at axon terminals. The postsynaptic glutamate receptors, which are ligand-gated ion channels, open in response to glutamate to allow ionic influx and efflux (Dingledine *et al*., 1999). One major type of ionotropic glutamate receptors, named α-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid receptors (AMPARs), mediates fast synaptic transmission. Another major type of ionotropic glutamate receptors, called the *N*-methyl-D-aspartic acid receptors (NMDARs), serve as co-incidence detectors and play a major role in the induction of synaptic plasticity. The opening of NMDAR-type ion channels requires both glutamate binding and membrane depolarization as a result of AMPAR activation. These receptors also have high permeability for calcium, whose intracellular dynamics affect the polarity of synaptic plasticity. Some NMDAR subunits, such as NR1, NR2A, and NR2B, are also subjected to modulatory phosphorylation at serine/threonine or tyrosine residues. These sites of phosphorylation not only affect channel kinetics but also control their trafficking to synaptic sites (Wang and Salter, 1994; Lau and Huganir, 1995; Yu *et al*., 1997; Chung *et al*., 2004). The most well characterized form of LTP in area CA1 induced by high-frequency stimulation relies on NMDA receptor activation and the subsequent modification of AMPA receptors. AMPAR modification can be achieved either by increased subunit phosphorylation (Barria *et al*., 1997; Banke *et al*., 2000; Lee *et al*., 2003) or by increased subunit synthesis and trafficking to synaptic sites (Shi *et al*., 1999; Hayashi *et al*., 2000), leading to long-lasting changes in synaptic strength. This artificially induced change in synaptic strength is thought to incorporate similar biochemical and structural changes as those that underlie the mechanisms involved in memory formation *in vivo*.

In order to investigate the potential effect of Reelin on synaptic transmission and plasticity, slices were perfused with recombinant Reelin isolated from HEK293 cells stably transfected with full-length Reelin cDNA (D'Arcangelo *et al*., 1997). Synaptically evoked field excitatory postsynaptic potentials (fEPSP) were recorded and the slope of fEPSP was used to quantify the strength of synaptic transmission. The input–output curve was then constructed by using fEPSP slope as a function of presynaptic fiber volley. It was observed that a short perfusion of Reelin medium did not change the baseline response, nor did it change the input–output curve (proportional increase in the fEPSP with increased stimulus intensity). However, it dramatically elevated the magnitude of LTP induced by two trains of 1-sec high-frequency stimulation (Fig. 12.2E). These results revealed that Reelin perfusion "preconditions" the CA1 synapses to favor LTP induction and maintenance. The mechanisms were largely speculative at the time of observation. It was proposed that Reelin activation of its lipoprotein receptors, ApoER2 and VLDLR, leads to Dab1 and Src kinase phosphorylation, and subsequently Src kinases phosphorylate NMDA receptors on tyrosine residues (Weeber *et al*., 2002). Therefore, the increased tyrosine phosphorylation of NMDA subunits leads to increased receptor conductance and enhanced calcium influx during LTP induction and thus leads to elevated LTP magnitude or lowered LTP threshold.

2.3 Reelin Signaling and Glutamate Receptors

This hypothesis was supported by two recent functional studies at cellular levels (Beffert *et al*., 2005; Chen *et al*., 2005). The enhanced NMDAR-mediated response in CA1 neurons following Reelin perfusion was confirmed in subsequent studies (Beffert *et al*., 2005). Isolated NMDAR-mediated whole cell currents were recorded in CA1 pyramidal neurons in the presence of bicuculline to block GABA responses and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) to block AMPAR responses, and the NMDAR currents were evoked at +30 mV to remove the voltage-dependent NMDAR magnesium block. It was observed that perfusion of Reelin significantly increased NMDAR-mediated charge transfer, indicating that there is a functional connection between Reelin signaling and enhanced glutamatergic response in CA1. Moreover, in NR2A-immunoprecipitated CA1 tissues, Reelin perfusion significantly increased the level of phosphotyrosine, indicating that there was enhanced NMDAR subunit tyrosine phosphorylation. Therefore, increased tyrosine phosphorylation and unitary conductance at least partially account for the enhanced NMDAR-mediated responses, although it is likely that phosphorylation-dependent trafficking of NMDAR subunits (Hayashi and Huganir, 2004) may also play a role. In a complementary study, Chen *et al*. (2005) have shown that increased tyrosine phosphorylation of another NMDAR subunit NR2B is increased. Moreover, in the presence of Reelin, glutamate-induced calcium influx through NMDA receptors is markedly enhanced, an effect that is dependent on lipoprotein receptors and Src family tyrosine kinases. These studies provide

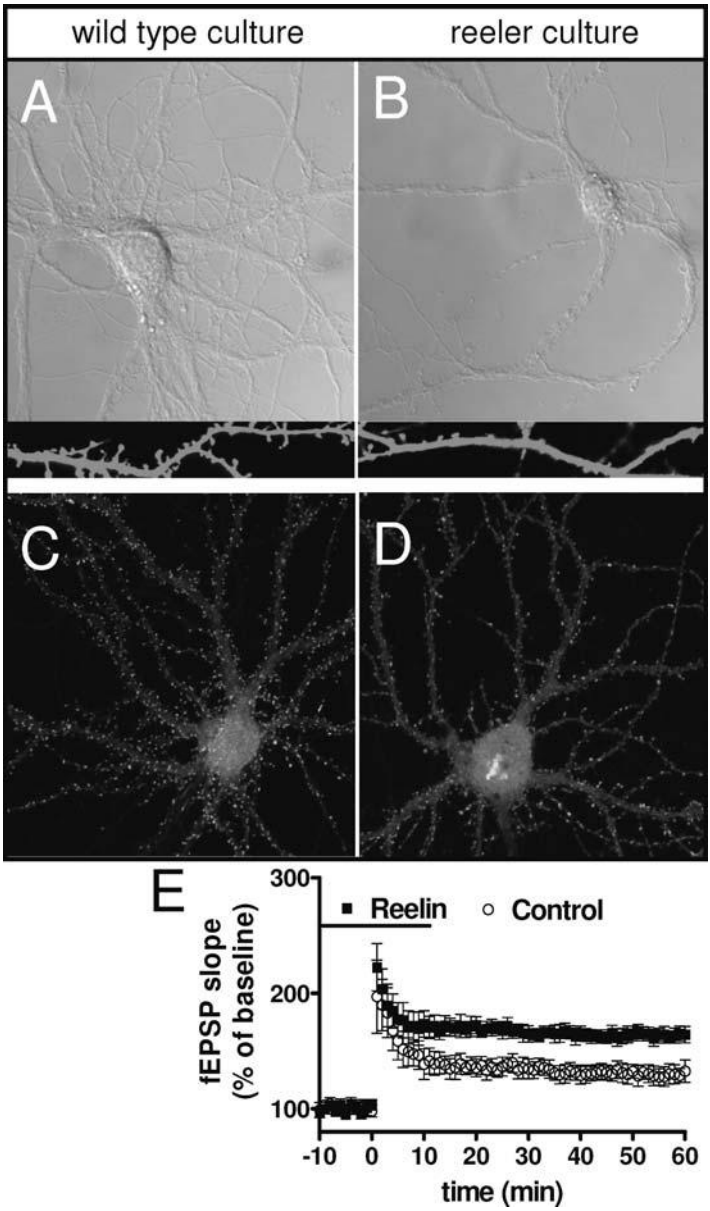

Fig. 12.2 Reelin signaling enhances glutamatergic function in the hippocampus. (**A**, **B**) In cultured embryonic mouse hippocampal neurons derived from homozygous Reeler embryos, stunted neurite growth and fewer neurite ramifications are seen; in addition, when neurons were filled with fluorophores to reveal dendritic spines, it was observed that neurons from wild-type cultures show significantly more spines in their primary dendrites. (**C**, **D**) Neurons from both wild-type and Reeler embryos are cultured for 2 weeks and then immunostained with NMDA receptor subunit NR1 and AMPA receptor subunit (GluR1) antibodies. A larger number of puncta that are positive for both NR1 and GluR1 were observed in wild-type cultures compared with Reeler cultures. (**E**) Long-term potentiation experiments using acute hippocampal slices prepared from 6-week-old mice. A 20-min perfusion of Reelin dramatically elevated the magnitude of tetanus-induced LTP (*See Color Plates*)

functional evidence that Reelin signaling in the adult hippocampus is connected with enhanced glutamatergic function.

Recent studies reveal that Reelin signaling not only enhances NMDA receptor function but also increases AMPA receptor-mediated synaptic responses through distinct mechanisms. Specifically, perfusion of purified recombinant Reelin for an extended time increased whole cell synaptic responses when CA1 neurons were voltage-clamped at −70 mV, indicating that increased AMPA receptor response was not secondary to enhanced NMDAR function. Moreover, this increase was not associated with changes of subunit phosphorylation. Further nonstationary fluctuation analysis of AMPA receptor-mediated miniature excitatory postsynaptic currents (mEPSCs) revealed that this increase was due to increased receptor numbers at synaptic sites. Therefore, elevated Reelin levels in the hippocampus would result in a profound enhancement of glutamatergic function at CA1 synapses. During *in vivo* conditions, this elevated level of Reelin may be achieved through increased activity of Reelin-expressing interneurons. However, obtaining a selective activation of these interneurons and dissecting the effects of endogenous Reelin on synaptic activity present a major technical challenge.

3 Reelin and Postnatal Hippocampus Development

Dendritic spines are small protrusions that cover the surface of dendrites and bear the postsynaptic structures that form excitatory synapses. Abnormally shaped or reduced numbers of dendritic spines are found in a number of cognitive diseases, such as fragile X syndrome, Williams syndrome, Rett syndrome, Down's syndrome, Angelman syndrome, and autism (Irwin *et al*., 2000; Kaufmann and Moser, 2000; Barnes and Milgram, 2002; Weeber *et al*., 2003). In cultured hippocampal neurons, Reelin signaling is required for normal development of dendritic structures. In the absence of Reelin or the intracellular adapter protein Dab1, neurons show stunted dendritic growth and fewer number of branches, similar to neurons of double knockout mice lacking both ApoER2 and VLDLR (Niu *et al*., 2004). This can also be seen using a patch electrode filled with fluorescent dyes to dialyze cultured neurons and analyze dendritic spine density. This technique reveals that hippocampal neurons cultured from homozygous Reeler embryos had significantly fewer dendritic spines, a phenotype that can be rescued by adding exogenous recombinant Reelin to the culture (Fig. 12.2A,B; Qiu *et al*., unpublished observations). The reduced number of dendritic spines indicates that a normal constitutive level of Reelin-lipoprotein receptor-mediated signaling is required for development of dendritic structures, which are crucial for intensive information processing by the neurons. This notion is in agreement with studies showing that heterozygote Reeler mice exhibit reduced dendritic spine densities and impaired performance in certain learning and memory behaviors (Tueting *et al*., 1999; Liu *et al*., 2001; Pappas *et al*., 2001; Qiu *et al*., 2006), which will be discussed later in this chapter.

Two major forms of NR2 subunits in adult hippocampus, NR2A and NR2B, are developmentally regulated (Monyer *et al*., 1994; Sheng *et al*., 1994; Chavis and Westbrook, 2001), subjected to activity- and behavior-induced changes (Carmignoto and Vicini, 1992; Quinlan *et al*., 2004; Barria and Malinow, 2005), and also help to determine the polarity of synaptic plasticity under certain circumstances (Liu *et al*., 2004; Massey *et al*., 2004). Recently, it has been shown that Reelin signaling is required for maturation of glutamate receptor function during postnatal development. For example, Reelin is required for a normal developmental switch from NR2B to NR2A of somatic NMDA receptor subunits in cultured hippocampal neurons (Sinagra *et al*., 2005), a process that is dependent on ApoER2, VLDLR, and the intracellular Dab1 and Src. NMDAR function is critically dependent on the composition of its subunits. Because both NR2A and NR2B can be tyrosine phosphorylated in response to synaptic activity (Lau and Huganir, 1995; Yu *et al*., 1997; Lu *et al*., 1998; Salter and Kalia, 2004) and Reelin signaling (Beffert *et al*., 2005; Chen *et al*., 2005), changes of NMDAR activity through developmental alteration of subunit composition and/or tyrosine phosphorylation by Reelin could have a dramatic impact on synaptic strength and plasticity. The study by Sinagra *et al*. (2005) investigated the maturation of somatic NMDA receptors in cultured neurons and found that Reelin signaling is required for normal developmental switch of somatic NMDAR subunits from NR2B to NR2A in cultured hippocampal neurons. However, a caveat should be noted that the contribution of Reelin signaling in neuronal maturation in the hippocampus during *in vivo* conditions may differ, due to the fact that *in vivo* maturation and subunit switch of NMDA receptors is critically dependent on neuronal activity that cannot be mimicked under culture conditions (Monyer *et al*., 1994; Quinlan *et al*., 1999; Lu and Constantine-Paton, 2004; Nakayama *et al*., 2005). In contrast to using neuronal culture, examination of the effect of Reelin on glutamatergic maturation was performed using *in vitro* cultured hippocampal slices obtained from 6- to 7-day-old mice. When slice cultures are prepared from young rodents, neural circuitries of the hippocampal formation are known to mature and to maintain a surprising three-dimensional, organotypic organization for many weeks *in vitro* (Zimmer and Gahwiler, 1984; Gahwiler *et al*., 1997). Using this preparation, it was found that Reelin signaling also facilitates a functional switch of synaptic NMDA receptor subunits from NR2B to NR2A. Additionally, Reelin promotes AMPA receptor surface expression and reduces the number of silent synapses in cultured hippocampal slices (Qiu and Weeber, unpublished observations). Therefore, Reelin-mediated signaling may play a previously unappreciated role to facilitate maturation of glutamate receptors at early postnatal stages.

4 Reelin Receptors and Synaptic Function

4.1 ApoER2 and VLDLR

Genetic ablation of both ApoER2 and VLDLR recapitulates the Reeler phenotype and precludes the ability to perform any meaningful cognitive tests on these animals (Trommsdorff *et al*., 1999). In comparison, knockout of either ApoER2 or

VLDLR does not produce the severe neuronal migration defects of the Reeler mouse, allowing behavioral tests that rely on normal locomotor ability for the tests (Trommsdorff *et al*., 1999; Weeber *et al*., 2002). It was found that mice lacking either ApoER2 or VLDLR showed hippocampus-dependent contextual fear conditioning deficits. In addition, these mice had impaired LTP and diminished responses to the Reelin enhancement of LTP in the hippocampal slices. Therefore, it seemed that both ApoER2 and VLDLR are required to carry out the normal synaptic function in the hippocampus. It must be noted that both ApoER2 and VLDLR knockout mice exhibited some degree of anatomical abnormalities, such as a distinct split of area CA1 pyramidal neurons and a less compact granule cell layer in dentate gyrus, which suggests that the contextual fear conditioning deficits in these animals may still be attributed to defects in developmental lamination and neuronal network connections. However, the observation that receptor-associated protein (RAP), a functional antagonist to LDLR family member, blocked hippocampal LTP favors the hypothesis that ApoER2- and VLDLR-mediated signaling *per se* is required for LTP induction and normal cognitive function (Zhuo *et al*., 2000; Weeber *et al*., 2002).

The importance of one of the Reelin receptors, ApoER2, in cognitive function was further underscored by two recent studies from Joachim Herz's research group. Using a homologous knockin gene replacement approach, Beffert *et al*. (2005) have shown that an alternatively spliced intracellular domain of ApoER2 plays an important role in normal cognition and Reelin modulation of synaptic plasticity. This intracellular domain, encoded by exon 19, is required for Reelin enhancement of LTP, maintaining normal associative and spatial learning, but not required for developmental lamination of cortical structures. Moreover, the tyrosine kinase activation does not require this alternatively spliced insert, because the NPXY-containing Dab1 binding motif is not affected by splicing. The connection of ApoER2 with cognitive function was supported by several lines of additional evidence. For example, the alternative splicing of exon 19 causes changes in neuronal activity and behavior. Additionally, ApoER2 and NMDAR are physically associated and coimmunoprecipitate in heterologous expression cell lines and colocalize to CA1 postsynaptic densities (Beffert *et al*., 2005; Hoe *et al*., 2006). Moreover, like NMDARs, ApoER2 also binds to PDZ domains of PSD-95 (Hoe *et al*., 2006), and therefore may recruit Src through postsynaptic scaffolding proteins and result in NMDAR tyrosine phosphorylation. Lastly, in mice carrying the mutations in the Dab1 binding sites on ApoER2, where the normal NFDNPVY motif was replaced by EIGNPVY, disrupted Dab1 binding leads to moderate lamination defects and inability to maintain late-phase LTP (Beffert *et al*., 2006). Therefore, there seems to be a mechanistic divergence downstream of Reelin binding to ApoER2, whereby one mechanism is coupled with proper lamination and the other coupled with glutamate receptor-mediated function and cognition. These seemingly divergent mechanisms may be coherently connected as more signaling components downstream to lipoprotein receptors are revealed, such as those that enlist MAP kinases (Senokuchi *et al*., 2004), tyrosine kinases (Arnaud *et al*., 2003; Ballif *et al*., 2003; Bock and Herz, 2003), lipid kinases (Beffert *et al*., 2002; Bock *et al*., 2003), and ligand-gated ion channels (Beffert *et al*., 2005; Chen *et al*., 2005; Sinagra *et al*., 2005).

4.2 Reelin Receptors and ApoE

In addition to being the major receptor for Reelin, ApoER2 also serves as a receptor for apolipoprotein E (apoE) in the CNS (Herz and Bock, 2002). ApoE is a small (34-kDa) secreted glycoprotein that associates with lipoproteins and mediates uptake of these particles into target cells via receptor-mediated endocytosis by the low-density lipoprotein (LDL) receptor family. Three commonly occurring isoforms have been identified in the human population due to single nucleotide polymorphisms on the APOE gene on chromosome 19. The apoE3 isoform (Cys112, Arg158) occurs at the highest frequency, followed by apoE4 (Arg112, Arg158) and apoE2 (Cys112, Cys158).

There exists a genetic connection between apoE isoform expression and Alzheimer's disease. ApoE4 allele inheritance increases the risk of early onset Alzheimer's disease, while apoE2 allele inheritance decreases the risk (Strittmatter *et al*., 1993; West *et al*., 1994). ApoE is known to play an important role in neural development, regeneration, and synapse remodeling. Consistently, apoE knockout mice show severe deficits in learning and memory (Oitzl *et al*., 1997), and mice with targeted replacement of the human apoE isoforms under the control of the mouse apoE promoter produce differential spatial memory performance depending on isoform expression (Grootendorst *et al*., 2005). Moreover, strong experimental evidence supports different neurotoxicity properties of distinct isoform-specific apoE microdomains (Brecht *et al*., 2004; Chang *et al*., 2005; Ye *et al*., 2005; Mahley *et al*., 2006). It is not clear whether these distinct learning and memory effects or susceptibility to cytotoxicity can be attributed to different signaling pathways mediated by apoE receptors in the CNS. However, evidence exists that relates apoE signaling to neuronal maturation. For example, apoE-mediated glia-derived cholesterol transport is required for the functional maturation of cultured central neurons (Mauch *et al*., 2001). This role of cholesterol can be attributed to neurons' limited capability for cholesterol synthesis and the fact that cholesterol directly participates in neuronal remodeling and synaptogenesis (Goritz *et al*., 2005). Again, it cannot be ruled out that apoE initiates signaling through ApoER2 and/or other CNS lipoprotein receptors that induce neuronal growth and functional maturation. These potential signaling effects by apoE may also profoundly modulate Reelin signaling, due to the fact that apoE affects the Reelin signaling through binding to ApoER2 and VLDLR and imposes a steric hindrance for Reelin binding. Because apoE4 preferentially associates with larger lipoprotein particles, it may have the greatest inhibition on Reelin signaling (Ohkubo *et al*., 2003).

Reelin-mediated signaling may contribute to the pathogenesis of Alzheimer's disease through the interaction with apoE receptors and intracellular adapter proteins. Mice lacking either Reelin, or both ApoER2 and VLDLR, exhibit hyperphosphorylation of microtubule-stabilizing protein tau (Hiesberger *et al*., 1999). One of the histopathological hallmarks of Alzheimer's disease, i.e., neurofibrillary tangles, contains hyperphosphorylated tau as the basic structural component. The tau protein is associated with the stabilization of microtubules and phosphorylation of tau

resulting in the destabilization of microtubules and allowing cytostructural remodeling. The hyperphosphorylated tau protein disrupts neuronal axonal transport and cytoarchitectural integrity and leads to impaired neuronal synaptic function and neurodegeneration. A putative pathway for Reelin-mediated control of tau phosphorylation is via Dab1 and Src activation, which leads to protein kinase B/AKT activation and inhibition of GSK3β, a key kinase involved in tau phosphorylation (Beffert *et al*., 2002). Therefore, changes in Reelin signaling can, in turn, lead to altered GSK3β activity and subsequent increased tau phosphorylation. The observation of a Reelin–tau connection reveals an interesting possibility that Reelin signaling may represent a new therapeutic target for regulating tau phosphorylation in Alzheimer's disease. In agreement with a potential role of Reelin signaling in Alzheimer's disease, a recent study has shown that Reelin level is elevated, and its glycosylation pattern is altered in the brains of Alzheimer's patients, which nicely correlates with changes in tau protein phosphorylation in the CSF of these patients (Botella-Lopez *et al*., 2006).

4.3 Integrins

In addition to the well-characterized ApoER2 and VLDLR, Reelin also binds to integrins. The functional significance of Reelin binding to these receptors is poorly understood, especially in the postnatal brain. However, it is well established that integrin-mediated signaling plays an important role in synaptic function. Integrins constitute a large family of heterodimeric membrane-spanning receptors for some RGD-motif-containing extracellular matrix proteins. Their interaction activates a myriad of intracellular signaling cascades, leading to reorganization of cytoskeleton actin filaments (Tozer *et al*., 1996; Milner and Campbell, 2002). This signaling mediated by integrins is also crucial for lamination of cortical structures (Graus-Porta *et al*., 2001), and together with Reelin signaling, is required for the formation of the radial glial scaffold in the hippocampus (Forster *et al*., 2002). The correct lamination effects of Reelin may be partly attributed to inhibitory effects on neuronal migration upon Reelin binding to integrins (Dulabon *et al*., 2000). Notably, multiple studies implicate integrins in cognitive ability and synaptic function. For example, integrins can modulate NMDA receptor maturation and function and play an important role in LTP and spatial memory function (Chavis and Westbrook, 2001; Chan *et al*., 2003, 2006). In addition, the levels of integrin-associated protein (IAP) mRNA correlate with performance in the inhibitory avoidance learning task, which is a hippocampus-dependent learning paradigm (Lee *et al*., 2000). Moreover, mice deficient in α3, α4, and α8 integrins exhibit impaired LTP in hippocampal CA1 field and impaired spatial memory performance (Chan *et al*., 2003). Additionally, in conditional β1 integrin knockout mice, with forebrain excitatory neuron-specific knockout of β1 integrin, they exhibit impaired synaptic transmission through AMPA receptors and diminished NMDA receptor-dependent LTP. These mutant mice also displayed normal hippocampus-dependent spatial and

contextual memory but showed impairment in a hippocampus-dependent working memory task (Chan *et al*., 2006). Therefore, β1-integrins may function as potential regulators of synaptic glutamate receptor function and working memory.

It is currently not clear how Reelin may activate integrin-mediated signaling in the postnatal brain. However, Reelin signaling through ApoER2/VLDLR may be overlapping with the integrin pathways. Integrins and Reelin receptors are both found at the dendritic spine and postsynaptic densities in CA1 neurons (Bi *et al*., 2001; Beffert *et al*., 2005), and like Reelin and apolipoprotein receptors, integrin-mediated signaling is also required for the maturation of cultured hippocampal neurons. For example, Chavis and Westbrook (2001) have shown that chronic blockade of integrins with RGD peptide prevents the developmental switch of NMDA receptor subunit NR2B to NR2A; similarly, chronic blockade of Reelin signaling with Src inhibitor or LDL receptor antagonist also delays the functional switch of somatic NMDA receptors (Sinagra *et al*., 2005). Moreover, the formation of dendritic spines during neuronal development and their structural plasticity also require integrin. In cultured hippocampal neurons, integrins are required for activity-dependent spine remodeling through mechanisms involving NMDA receptors and calcium–calmodulin-dependent kinase II (Shi and Ethell, 2006). For example, reduced dendritic spine numbers and hypoplasticity were correlated with decreased cortical Reelin expression in human schizophrenic patients (Eastwood and Harrison, 2006). Integrin-mediated signaling has also been shown to lead to tyrosine phosphorylation of Crk family adapter proteins, resulting in cytoskeletal rearrangement through recruitment of guanine-nucleotide exchange factors and small GTPases. Crk proteins bind to Dab1 only when Dab1 is tyrosine phosphorylated at Y232 and Y220. Reelin application leads to C3G tyrosine phosphorylation and the downstream small GTPase Rap1 activation in Dab1-dependent manner (Ballif *et al*., 2004). This pathway employed by Reelin signaling involving C3G and Rap1 is also operating to regulate integrin-dependent cell adhesion and motility. Therefore, it is possible that Reelin and integrin affect synaptic function through discrete but interacting pathways involving ApoER2/VLDLR and other extracellular matrix proteins, and these two signaling systems may overlap and cooperate in dictating synaptic function and plasticity.

5 The Heterozygote Reeler Mouse

The role of Reelin in cognition is best supported by research showing that gene dosage of Reelin is correlated with learning and memory in mice (Qiu *et al*., 2006). In contrast to the Reeler phenotype, a single allele inactivation of the *RELN* gene, as happens with the heterozygote Reeler mouse (HRM), results in some subtle yet distinct neuroanatomical, neurochemical, and behavioral features compared to their wild-type counterparts. The HRM exhibits ~50% overall Reelin reduction in the brain. Phenotypically, the HRM shows reduced dendritic spine density in the parietal-frontal cortex pyramidal neurons and the basal dendrites of CA1 pyramidal neurons, reduced cortical thickness, and decreased number of glutamic acid decar-

boxylase 67-kDa-positive cells. Moreover, the HRM also exhibits a reduced density of nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH) -positive neurons in cortical gray matter and a decreased prepulse inhibition behavior (Tueting *et al*., 1999; Liu *et al*., 2001; Salinger *et al*., 2003; Qiu *et al*., 2006). These unique features of the HRM are also partially shared by human schizophrenic patients and other cognitive disorders, such as lissencephaly, bipolar disorder, and autism (Fatemi *et al*., 2000, 2001, 2005a,b; Hong *et al*., 2000; Assadi *et al*., 2003), suggesting a potential role of Reelin in modulating cognitive function in the postnatal brain and a reduced Reelin signaling causing deficiency in synaptic function.

A single functional *RELN* allele in the HRM does not seem to change the gross anatomy in the mouse hippocampus, nor does it change the overall levels of glutamate receptor subunits including GluR1, GluR2/3, NR1, NR2A, and NR2B (Qiu *et al*., 2006). The HRM also does not differ from wild-type mice in rotorod, open field testing, hot plate nociception, light/dark preference, elevated plus platform tests, startle response thresholds, and water maze tests. However, the HRM exhibits a pronounced deficit in prepulse inhibition, which is a measure of sensory-motor gating of the nervous system (Tueting *et al*., 1999; Qiu *et al*., 2006). The HRM also exhibits contextual fear conditioning deficit, a hippocampus-dependent functional test. Examination of the synaptic transmission and plasticity in CA1 revealed a surprising plethora of phenotypic differences: the HRM exhibited decreased input–output responses, reduced paired pulse facilitation, and impaired LTP and LTD in CA1 (Qiu *et al*., 2006). The impaired LTP was reproducible at the single-cell level when using a theta burst stimulation protocol. Strikingly, there was a disrupted excitatory–inhibitory network balance in CA1 in the HRM. Although CA1 pyramidal neurons receive similar excitatory input, the inhibitory postsynaptic currents onto these neurons are greatly reduced. These phenotypes of the HRM strengthen the similarity of the HRM and human schizophrenic patients, in that the latter show diminished inhibition (Escobar *et al*., 2002; Lewis *et al*., 2005). The reduced inhibition of the HRM may reflect the failure of inhibitory interneurons to reach their final destination and form functional connections with pyramidal neurons, or failure of release of GABA neurotransmitters due to less excitatory drive onto these neurons. Although these phenotypes manifested by the HRM could be caused by the subtle developmental effects resulting from Reelin haploinsufficiency, a reduced signaling of Reelin through ApoER2/VLDLR may also account for at least some of these deficits.

6 Summary

The expression of Reelin protein persists in the postnatal brain at a stage when neuronal migration is long completed. The functional significance of this continued presence of Reelin is underscored by recent findings that Reelin- and ApoER2/VLDLR-mediated signaling play a novel role in synaptic function in the hippocampus (Fig. 12.3). This signaling system is coupled with enhanced glutamatergic function, facilitates glutamatergic maturation at postnatal stages, modulates synaptic plasticity,

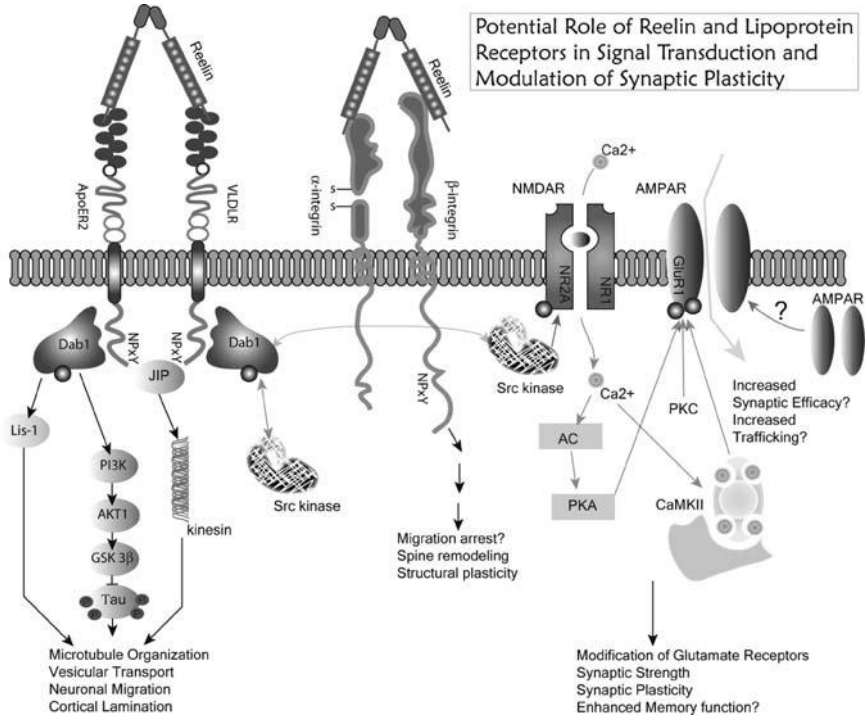

Fig. 12.3 Schematic representation of Reelin signaling and the subsequent enhancement of synaptic function in the adult hippocampus. Reelin binds and activates ApoER2/VLDLR and leads to tyrosine phosphorylation and activation of Dab1 and Src family protein tyrosine kinases. Src kinases phosphorylate NMDA receptor subunits and lead to enhanced channel conductance, augmented Ca^{2+} influx during activation, and increased synaptic plasticity. This increased synaptic plasticity may involve changes of AMPA receptor phosphorylation and trafficking as well. In response to Reelin signaling, PI3K and PKB/AKT can be activated as well, resulting in inhibition of tau phosphorylation. In addition to ApoER2/VLDLR, Reelin also activates integrins (*See Color Plates*)

and enhances memory performance in experimental animals. Considering the presence of Reelin-expressing cells in other parts of brain, it is possible that Reelin signaling is operating in various CNS structures with similar effects on the glutamatergic synapses. Because much of our current knowledge on the postnatal role of Reelin is acquired through perfusion of recombinant Reelin on the hippocampal slices, a selective activation of Reelin-expressing neurons in the hippocampus and subsequent study of adjacent synapses would more faithfully reflect the *in vivo* roles of Reelin signaling, although currently this is not technically feasible. Alternatively, a selective ablation of Reelin-expressing neurons (i.e., conditional knockout) in the postnatal hippocampus without the pandemic developmental disruption of overall hippocampal synaptic connection would be more informative than the promiscuous pharmacological blockade of Reelin receptors to address the consequences of reduced Reelin signaling in the adult brain. Given that aberrant Reelin signaling is

implicated in many human cognitive diseases, the authors consider these novel approaches to represent promising future directions to further understanding of the role of Reelin signaling in cognitive function.

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