Chapter 1
The *Reelin* Gene and Its Functions in Brain Development

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1 Introduction

Brain development and function requires the coordinated genesis, migration, and maturation of all of its cellular components. The product of the *Reelin* (*Reln*) gene has been identified as a major determinant of neuronal migration that also plays a significant role in cellular maturation and synaptic function. Thus, the *Reln* gene controls multiple aspects of brain development over the entire life span of a mammalian organism, from pre- to postnatal ages, and exerts distinct functions on migrating neuroblasts, radial progenitors, and postmigratory neurons. Some of the molecular mechanisms that mediate these functions have been elucidated by the analysis of mutant mice and biochemical interactions, but much remains to be discovered. In this chapter we will summarize the current state of our knowledge of *Reln* and its function in brain development.

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2 The Reelin Gene and Its Product

The *Reln* gene was discovered based on the genetic analysis of *reeler* mutant mouse strains. These classical neurological mutants exhibit in homozygosity a distinct phenotype characterized by ataxia and the disruptions of all layered structures of the brain (reviewed by Lambert de Rouvroit and Goffinet, 1998). The *reeler* mutant trait is recessive and it maps to the distal region of mouse chromosome 5. Identification of *Reln* as the gene disrupted in two independent *reeler* mutant strains that completely lack expression (D'Arcangelo *et al*., 1995) led to the full characterization of its genomic structure, transcript expression pattern, and amino acid composition of the predicted encoded product. The murine *Reln* gene is composed of 65 exons spanning a region of approximately 450 kb (Royaux *et al*., 1997). The N-terminal exons are separated by large introns whereas the remaining exons are closer to each other. One microexon encoding just two amino acids near the C terminus is alternatively spliced to generate two *Reln* transcripts, but the significance of this alternative splicing event is not known. In addition, two major transcription initiation sites and two polyadenylation sites have also been identified (Royaux *et al*., 1997). The only *Reln* mRNA detectable by Northern blot analysis is approximately 12 kb, is highly and predominately expressed in the brain, and is developmentally regulated (D'Arcangelo *et al*., 1995). It first becomes detectable in the embryo, peaks between 1 and 2 postnatal weeks, and then declines at lower levels in the adult brain. The encoded Reelin protein is a large secreted protein that consists of an N-terminal region followed by eight unique repeats each containing an EGF-like motif (D'Arcangelo *et al*., 1995). The N-terminal region contains the signal peptide and a small domain similar to F-spondin. Each Reelin repeat is composed of two related subrepeats, A and B, separated by an EGF-like motif. The C-terminal region contains a stretch of positively charged amino acids (D'Arcangelo *et al*., 1997). Deletion of this region resulting from a retroviral insertion is responsible for the lack of secreted, functional Reelin and the appearance of the mutant phenotype in the naturally occurring *reeler* Orleans strain (de Bergeyck *et al*., 1997; Takahara *et al*., 1996). The full-length mouse protein is composed of 3461 amino acids, which is subjected to N- and O-glycosylation, resulting in a secreted protein of approximately 450 kDa (D'Arcangelo *et al*., 1997). This protein is rapidly cleaved in the extracellular environment at two cleavage sites. The N-terminal site, located between repeats 2B and 3A, is cleaved by a metalloprotease, whereas the C-terminal site, located between repeats 6B and 7A, is cleaved by an unknown protease (Lambert de Rouvroit *et al*., 1999; Jossin *et al*., 2007). The activity of these proteases results in the generation of three major fragments, N terminal (N terminus to repeat 2; ~180 kDa), central (repeats 3 to 6; 190 kDa), and C terminal (repeats 7 and 8; 80 kDa) detectable with distinct specific antibodies (Jossin *et al*., 2007). In addition, the full-length protein, as well as intermediate fragments of 370 kDa (N terminus to repeat 6) and 270 kDa (repeats 6 to 8) resulting from partial processing can also be detected in Reelin-containing culture medium or tissue lysates (Jossin *et al*., 2007). Functional studies have demonstrated that the central (190 kDa) Reelin fragment is sufficient to activate

a tyrosine phosphorylation-dependent signal transduction and to induce layer formation in cortical slice cultures, whereas the N-terminal and the C-terminal fragments appear to be inactive (Jossin *et al*., 2004, 2007). The N-terminal region contains an epitope recognized by the functionally interfering antibody CR50 (D'Arcangelo *et al*., 1997; Ogawa *et al*., 1995). This region mediates the formation of stable homodimers composed of full-length Reelin proteins (Kubo *et al*., 2002; Utsunomiya-Tate *et al*., 2000). These homodimers appear to stimulate the tyrosine phosphorylation-dependent signaling pathway more efficiently than cleaved central fragments. On the other hand, the cleaved central fragment of Reelin can diffuse farther into the developing cortical plate than can full-length homodimers (Jossin *et al*., 2007). Thus, proteolytic processing may modulate the strength and the range of Reelin activity *in vivo*.

Reln mRNA and protein are expressed at high levels in superficial layers of embryonic cortical structures (Alcantara *et al*., 1998; D'Arcangelo *et al*., 1995; Ogawa *et al*., 1995; Schiffmann *et al*., 1997). Cajal-Retzius cells in the marginal zone of the cortex, stratum lacunosum moleculare of the hippocampus, and outer marginal layer of the dentate gyrus are the major source of Reelin in the embryonic neocortex and early postnatal hippocampus. Young granule cells in the external granular layer of the embryonic and early postnatal cerebellum also synthesize high levels of Reelin. The pattern of *Reln* expression, however, changes dramatically during postnatal development. In the adult brain, this protein is no longer confined to superficial cortical layers, but it is expressed in all layers of the cortex and hippocampus by a subset of GABAergic interneurons (Alcantara *et al*., 1998; Pesold *et al*., 1998). In the adult cerebellum, Reelin continues to be expressed by granule cells, which have migrated inwardly to form the internal granule layer. This shift in expression pattern most likely reflects the different functions exerted by Reelin in the pre- and postnatal brain.

Reelin activities are mediated by a signaling pathway that has been partially elucidated through the genetic analysis of mutant mouse strains. Mutations or deletions in the *reelin* gene account for the majority of identified mouse strains that exhibit a *reeler* phenotype. However, mutant mice indistinguishable from *reeler* have also been identified or generated by disrupting genes that are essential to Reelin signal transduction (Fig. 1.1). In some cases, more than one gene has to be disrupted to overcome functional redundancy and reveal the phenotype. Double knockout mice lacking both the apolipoprotein E receptor 2 (ApoER2) and the very-low-density lipoprotein receptor (VLDLR) (Trommsdorff *et al*., 1999), and double knockout mice lacking the two src-family kinases (SFKs) Fyn and Src (Kuo *et al*., 2005) are similar to *reeler*, whereas single mutants only exhibit various degrees of cortical layering defects. On the other hand, disruption of a single gene encoding the nonredundant adapter protein Disabled-1 (Dab1) in the spontaneous mutants *scrambler* and *yotari* (Sheldon *et al*., 1997; Ware *et al*., 1997; Yoneshima *et al*., 1997), the Dab1 null knockout (Howell *et al*., 1997), or the knockin mutant Dab5F lacking SFK-dependent phosphorylation sites (Howell *et al*., 2000), all result in a *reeler*-like phenotype. These genes thus define a signal transduction pathway that is crucial for Reelin activity on neuronal migration. Secreted full-length Reelin, or its active central

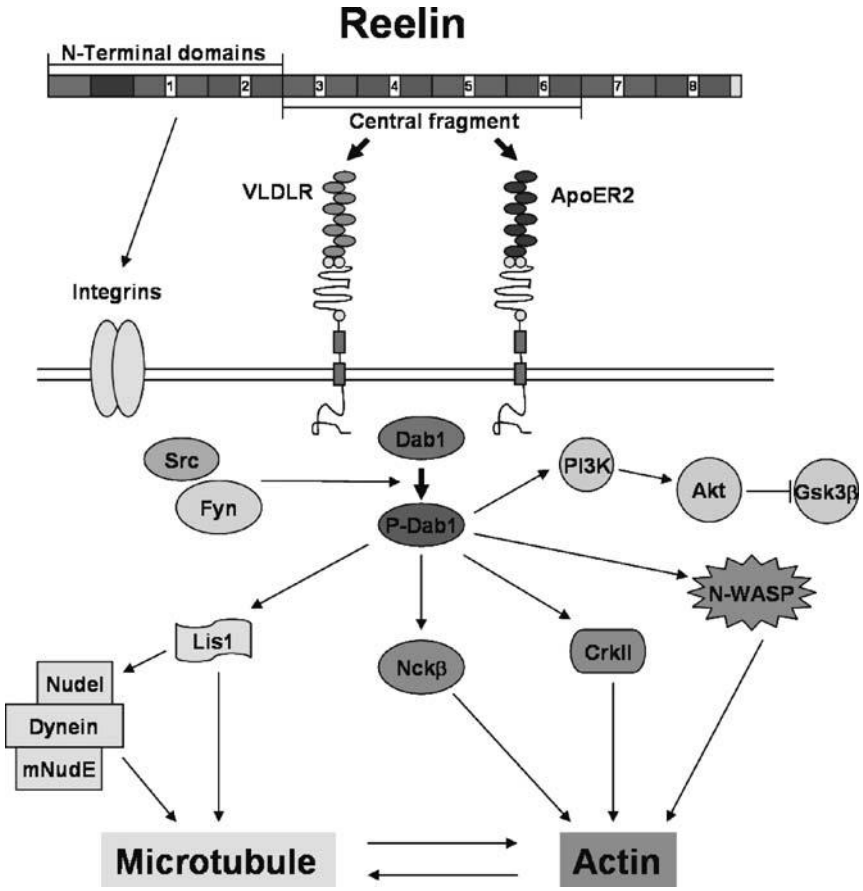

Fig. 1.1 The Reelin signaling pathway (*See Color Plates*)

fragment, bind to ApoER2 and VLDLR on the surface of target cells such as migrating neurons. These two Reelin receptors are members of the lipoprotein receptor superfamily and each is capable of binding Reelin with similar affinity (Benhayon *et al*., 2003; D'Arcangelo *et al*., 1999; Hiesberger *et al*., 1999). They also bind lipoproteins and other extracellular ligands with lower affinity. Like all members of the lipoprotein receptor superfamily, ApoER2 and VLDLR internalize their ligand, including Reelin, using an internalization domain, the NPxY motif, present on their cytoplasmic tail. The Reelin receptors actively traffic between the plasma membrane and the endosomes. Their translocation to the plasma membrane is facilitated by the binding of Dab1 to the cytoplasmic tail of the receptors near their NPxY motif by virtue of its pleckstrin homology/phosphotyrosine binding domain (PH/PTB) (Morimura *et al*., 2005; Trommsdorff *et al*., 1998). Upon Reelin binding, the receptors cluster (Strasser *et al*., 2004) causing the activation of Fyn and Src (Arnaud

et al., 2003b; Bock and Herz, 2003), which in turn phosphorylate Dab1 on specific tyrosine residues (Ballif *et al*., 2004; Howell *et al*., 1999; Keshvara *et al*., 2001). This event results in the ubiquitination of Dab1 by the Cbl ubiquitin ligase and its degradation by the proteasome system (Arnaud *et al*., 2003a; Suetsugu *et al*., 2004). Thus, Reelin promotes the phosphorylation, as well as the degradation of Dab1. This observation explains why Dab1 protein accumulates in the brain of *reeler*, double Apoer2/Vldlr, and double Fyn/Src knockout mice (Kuo *et al*., 2005; Sheldon *et al*., 1997; Trommsdorff *et al*., 1999). The short-lived phosphoDab1 is thought to function as a hub as it binds several intracellular signal transduction proteins including the PI3K regulatory subunit p85α (Bock *et al*., 2003), the actin-binding N-WASP (Suetsugu *et al*., 2004), Nckβ (Pramatarova *et al*., 2003), Crk family proteins (Ballif *et al*., 2004; Huang *et al*., 2004), and the neuronal migration gene product Lis1 (Assadi *et al*., 2003). All of these proteins potentially contribute to Reelin function in the control of neuronal migration by affecting cytoskeletal dynamics that determine cell motility and morphology. The binding of Dab1 to p85α correlates with Reelin-induction of PI3K activity, the downstream phosphorylation and activation of Akt, and the phosphorylation and inhibition of Gsk3β, which in turn results in a suppressed level of tau phosphorylation (Ballif *et al*., 2003; Beffert *et al*., 2002; Bock *et al*., 2003; Ohkubo *et al*., 2003). Consistent with a physiological role for Reelin in the regulation of this pathway, elevated levels of phosphorylated tau have been reported in *reeler*, double *Apoer2*/*Vldlr,* and *Dab1* mutant mice (Brich *et al*., 2003; Hiesberger *et al*., 1999). These findings could be important for the pathology of neurodegenerative disorders such as Alzheimer's disease, which are associated with accumulation of hyperphosphorylated tau. Binding of Dab1 to Crk family proteins such as CrkI, CrkII, and CrkL leads to the activation of a signaling pathway involving the GTP-exchange factor C3G and Rap1 (Ballif *et al*., 2004). Activated Rap1 could be important for cytoskeletal changes required during neuronal migration. Similarly, Dab1 interactions with the actin-binding proteins N-WASP and Nckβ or with Lis1, a protein that associates with the microtubule dynamin–dynactin motor complex and with the Pafah1b enzymatic complex, could be important for neuronal migration. In the case of Lis1, there is also genetic evidence that this protein is important for Reelin-dependent cortical layer formation, and that it likely functions downstream of VLDLR through interactions mediated by the Pafah1b complex (Zhang *et al*., 2007). In addition, β1 integrins have also been reported to bind Dab1 in a Reelin-dependent manner (Schmid *et al*., 2005). Together with the finding that α3 integrins bind the N-terminal region of Reelin, these data suggest that α3β1 integrins may participate in Reelin functions. However, because genetic deletion of β1 integrin does not severely disrupt neuronal migration (Graus-Porta *et al*., 2001), it is possible that these integrins may either stabilize newly formed cellular layers by promoting cell–cell adhesion (Schmid *et al*., 2004) or participate in other postnatal functions of Reelin such as synaptogenesis or synaptic activity (Dong *et al*., 2003; Rodriguez *et al*., 2000). Alternatively, the binding of Dab1 to α3β1 integrins may promote their degradation in response to Reelin, thus allowing the detachment of neurons from radial fibers and the formation of cellular layers (Sanada *et al*., 2004).

3 Functions of Reelin in Brain Development

The best-characterized function of Reelin is the control of radial neuronal migration and the formation of cellular layers during prenatal brain development. Layer formation is a distinct feature of all cortical structures including the cerebral cortex, the hippocampus, and the cerebellum. In the neocortex, principal neurons are born from the asymmetric division of progenitor cells near the ventricular zone, the radial glia (Malatesta *et al*., 2000; Noctor *et al*., 2001). These young neurons then migrate radially toward the pial surface, and stop just underneath the marginal zone, a relatively cell-free superficial layer, to form tight cellular layers with other neurons born approximately at the same time. The first cohort of radially migrating neurons splits an earlier transient structure called the preplate, consisting of Cajal-Retzius cells and subplate cells. Each new cohort of migrating neurons bypasses older layers en route toward the marginal zone generating the typical six cellular layers of the mammalian cortex in an inside-out fashion (Angevine and Sidman, 1961). Inhibitory neurons originating from extracortical regions such as the ganglionic eminences enter the developing cortical plate by tangential migration and distribute in specific cellular layers according to their specific subtype, thus contributing to the establishment of the cortical circuitry (Anderson *et al*., 1997; Lavdas *et al*., 1999). Similar mechanisms operate in other regions of the brain, such as the hippocampus or the cerebellum. In the hippocampus, principal (pyramidal) neurons are born near the ventricle and migrate radially to form a single, multicellular but compact layer at a considerable distance from the pial surface. In the cerebellum, principal neurons (the Purkinje cells) are born near the ventricle and migrate radially along so-called Bergmann fibers to form a single cell layer underneath a superficial layer composed initially by tangentially migrating granule cells, the external granular layer. Later in development, these cells actually migrate inwardly to form an internal granular layer underneath the Purkinje cell layer. The observation that layer formation in all cortical structures is disrupted in *reeler* and *reeler*-like mutants demonstrated that Reelin and its signaling pathway are crucial for this function (Caviness and Sidman, 1973; Caviness, 1973; Goffinet, 1983) (Fig. 1.2). However, the exact mechanisms governing layer formation are still not entirely understood. A current model suggests that a soluble form of Reelin (such as its central fragment) is produced near the pial surface and diffuses down into the developing cortical plate, hippocampus, or cerebellum in a smooth or step-gradient manner (D'Arcangelo, 2005; Jossin *et al*., 2007). There, at low levels, it promotes the extension of a leading edge and the radial migration of Reelin target cells such as cortical and hippocampal principal neurons or cerebellar Purkinje cells. Once these cells reach the top of the cortex, they encounter either high levels or a matrix-immobilized form of Reelin (such as the full-length homodimers), which prompt them to stop migration, detach from radial fibers, and associate into tight layers. Indeed, Reelin has been shown to cause migration arrest and detachment from radial glia *in vitro* using cortical imprint assays, and *in vivo* when injected focally into the neocortex using immobilized beads (Dulabon *et al*., 2000). Thus, Reelin could either increase or arrest motility, depending on the concentration or other

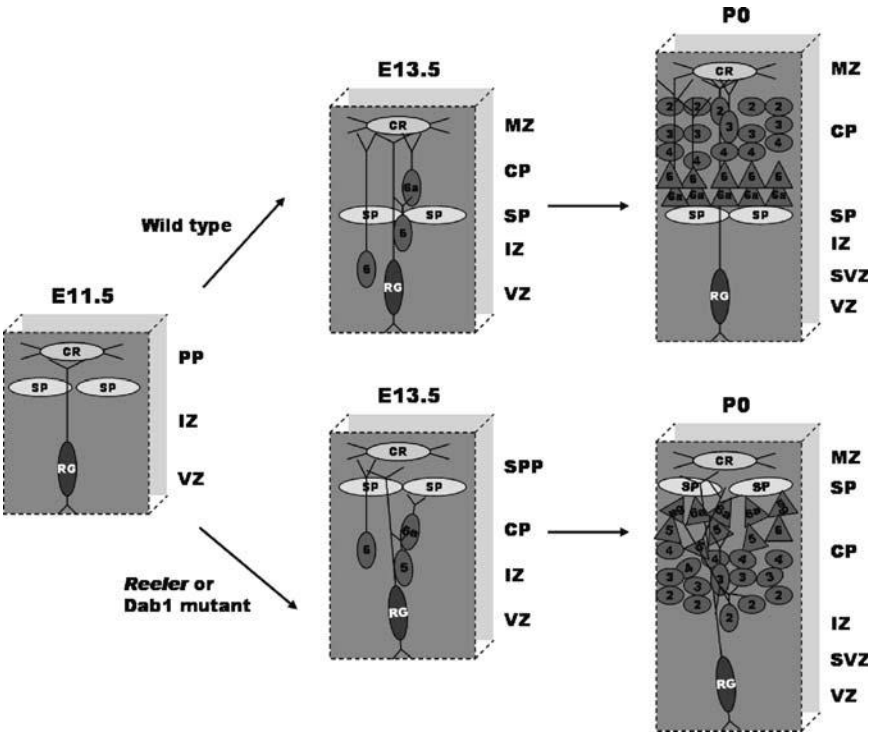

Fig. 1.2 Cortical development in normal and *reeler* and Dab1 mutant mice. In the embryonic cortex of normal mice, the preplate (PP) is split by the arrival of early radially migrating neurons, whereas in the *reeler* cortex, this does not happen and cells form a superplate structure (SPP). Cellular layers in the cortical plate (CP) are also disrupted in *reeler*. Other abbreviations: MZ, marginal zone; IZ, intermediate zone; VZ, ventricular zone; SVZ, subventricular zone; RG, radial glia; CR, Cajal-Retzius cells (*See Color Plates*)

environmental factor controling its diffusion. The different signal could be mediated by the differential recruitment of Dab1-interacting proteins that interact with the actin and microtubule cytoskeleton.

In recent years, it has become clear that Reelin not only controls neuronal migration during embryogenesis but also promotes neuronal maturation and function at postnatal ages. Heterozygous *reeler* mice express half the *Reln* mRNA levels of wild-type mice and are phenotypically normal, that is, they do not present any cortical layer defect nor are they ataxic like the homozygous mutants. However, they display a variety of behavioral and cognitive defects reminiscent of those found in human psychosis (reviewed by Tueting *et al*., 2006). Anatomically, they exhibit a stunted growth of dendritic processes in hippocampal neurons (Niu *et al*., 2004) and a reduction in the density of synaptic contacts in the frontal cortex (Liu *et al*., 2001). Because cellular layers are intact in heterozygous *reeler* mice, these developmental defects reflect a direct function of the *Reln* gene product on neuronal

maturation and synaptic formation. This postnatal function of Reelin appears to be mediated by the same signaling pathway that mediates its function in neuronal migration, that is, involves the ApoER2/VLDLR receptors and Dab1 (Niu *et al*., 2004). The importance of Reelin for the normal development of synaptic connectivity and function was initially demonstrated in the retina (Rice *et al*., 2001), but it is now appreciated in the brain as well (reviewed by Herz and Chen, 2006). Addition of recombinant Reelin promotes hippocampal LTP and this function requires the activity of both lipoprotein receptors (Weeber *et al*., 2002). A splicing variant of Apoer2 containing exon 19, which is capable of interacting with the postsynaptic density protein 95 (PSD95) and the JNK interacting protein (JIP), has been shown to be important for Reelin-induced LTP and the formation of spatial memory (Beffert *et al*., 2005). The role of Reelin in synaptic function is mediated in part through interactions between ApoER2 and the NMDA receptor (Beffert *et al*., 2005; Hoe *et al*., 2006). These proteins form a synaptic complex that controls Ca^{2+} entry through the NMDA receptor and thus regulate synaptic plasticity. In addition, Reelin signaling is also important for the regulation of NMDA receptor subunit composition during hippocampal neuronal maturation (Sinagra *et al*., 2005), and the NMDA receptor-mediated activity in cortical neurons (Chen *et al*., 2005). Recent physiological studies revealed that Reelin also enhances glutamatergic transmission through AMPA receptors. The enhancement of AMPA receptor responses is mediated by increased surface expression and increased amplitude of AMPA receptor-mediated excitatory postsynaptic currents (Qiu and Weeber, 2007; Qiu *et al*., 2006b). These results demonstrate that Reelin functions in the developing postnatal, as well as in the adult hippocampus by affecting synaptic strength and plasticity.

4 Reelin in Human Diseases

Reelin is highly conserved among vertebrate species, especially in mammals, suggesting a conserved function related to the development of layered structures that are particularly prominent in mammalian species. Reelin homologues have been identified in humans, rat, chicken, turtle, and *Xenopus*, but not in invertebrate species such as *Drosophila*. The amino acid sequence of human Reelin is 94.8% identical to that of the murine homologue indicating striking functional conservation (DeSilva *et al*., 1997). Homozygous mutations in the *RELN* gene in humans result in a phenotype strikingly similar to that of *reeler* mice, featuring severe ataxia, cognitive dysfunction, cerebellar hypoplasia, and cortical neuronal migration defects leading to a reduced number of cortical gyri (lissencephaly) (Hong *et al*., 2000). This severe phenotype reflects the essential function of Reelin in neuronal migration during prenatal brain development. However, even reduced levels of *RELN* expression may be deleterious for human brain development. For example, *RELN* expression is downregulated in inhibitory cortical neurons of patients with schizophrenia and psychotic bipolar disorder (Guidotti *et al*., 2000; Fatemi *et al*.,

2000; Impagnatiello *et al*., 1998). This decrease is due to epigenetic mechanisms involving the increased expression of DNA methyltransferase (DNMT1) (Grayson *et al*., 2005; Veldic *et al*., 2004). Reduced Reelin expression has also been reported in other cognitive disorders, such as autism (Fatemi *et al*., 2001, 2005), nonpsychotic bipolar disorder, major depression (Fatemi *et al*., 2000), and Alzheimer's disease (Chin *et al*., 2007), suggesting that a dysfunction in Reelin-regulated neuronal maturation and synaptic activity in the postnatal brain may contribute to these disorders. These subtle defects are recapitulated in the heterozygous *reeler* mice, which exhibit defects in learning and memory (Larson *et al*., 2003; Qiu *et al*., 2006a), as well as behavioral performance (Krueger *et al*., 2006; Ognibene *et al*., 2007; Tueting *et al*., 1999). Thus, heterozygous *reeler* mice may serve as models to investigate the molecular and physiological basis of cognitive dysfunction linked to Reelin deficiency. These animal models may now be just as valuable as homozygous *reeler* mice have been during the past few decades in aiding our understanding of the molecular mechanisms of cortical layer formation.

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References

Alcantara, S., Ruiz, M., D'Arcangelo, G., Ezan, F., de Lecea, L., Curran, T., Sotelo, C., and Soriano, E. (1998). Regional and cellular patterns of *reelin* mRNA expression in the forebrain of the developing and adult mouse. *J. Neurosci*. 18:7779–7799.

Anderson, S. A., Eisenstat, D. D., Shi, L., and Rubenstein, J. L. R. (1997). Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science* 278:474–476.

Angevine, J. G., and Sidman, R. L. (1961). Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* 192:766–768.

Arnaud, L., Ballif, B. A., and Cooper, J. A. (2003a). Regulation of protein tyrosine kinase signaling by substrate degradation during brain development. *Mol. Cell. Biol*. 23:9293–9302.

Arnaud, L., Ballif, B. A., Forster, E., and Cooper, J. A. (2003b). Fyn tyrosine kinase is a critical regulator of disabled-1 during brain development. *Curr. Biol*. 13:9–17.

Assadi, A. H., Zhang, G., Beffert, U., McNeil, R. S., Renfro, A. L., Niu, S., Quattrocchi, C. C., Antalffy, B. A., Sheldon, M., Armstrong, D. D., Wynshaw-Boris, A., Herz, J., D'Arcangelo, G., and Clark, G. D. (2003). Interaction of reelin signaling and Lis1 in brain development. *Nat. Genet*. 35:270–276.

Ballif, B. A., Arnaud, L., and Cooper, J. A. (2003). Tyrosine phosphorylation of disabled-1 is essential for reelin-stimulated activation of Akt and Src family kinases. *Brain Res. Mol. Brain Res*. 117:152–159.

Ballif, B. A., Arnaud, L., Arthur, W. T., Guris, D., Imamoto, A., and Cooper, J. A. (2004). Activation of a Dab1/CrkL/C3G/Rap1 pathway in reelin-stimulated neurons. *Curr. Biol*. 14:606–610.

Beffert, U., Morfini, G., Bock, H. H., Reyna, H., Brady, S. T., and Herz, J. (2002). Reelin-mediated signaling locally regulates protein kinase B/Akt and glycogen synthase kinase 3beta. *J. Biol. Chem*. 277:49958–49964.

Beffert, U., Weeber, E. J., Durudas, A., Qiu, S., Masiulis, I., Sweatt, J. D., Li, W.-P., Adelmann, G., Frotscher, M., Hammer, R. E., and Herz, J. (2005). Modulation of synaptic plasticity and memory by reelin involves differential splicing of the lipoprotein receptor ApoER2. *Neuron* 47:567–579.

Benhayon, D., Magdaleno, S., and Curran, T. (2003). Binding of purified reelin to ApoER2 and VLDLR mediates tyrosine phosphorylation of disabled-1. *Mol. Brain Res*. 112:33–45.

Bock, H. H., and Herz, J. (2003). Reelin activates SRC family tyrosine kinases in neurons. *Curr. Biol*. 13:18–26.

Bock, H. H., Jossin, Y., Liu, P., Forster, E., May, P., Goffinet, A. M., and Herz, J. (2003). PI3-kinase interacts with the adaptor protein Dab1 in response to reelin signaling and is required for normal cortical lamination. *J. Biol. Chem*. 278:38772–38779.

Brich, J., Shie, F. S., Howell, B. W., Li, R., Tus, K., Wakeland, E. K., Jin, L. W., Mumby, M., Churchill, G., Herz, J., and Cooper, J. A. (2003). Genetic modulation of tau phosphorylation in the mouse. *J. Neurosci*. 23:187–192.

Caviness, V. S. J. (1973). Time of neuron origin in the hippocampus and dentate gyrus of normal and reeler mutant mice: an autoradiographic analysis. *J. Comp. Neurol*. 151113–120.

Caviness, V. S. J., and Sidman, R. L. (1973). Time of origin of corresponding cell classes in the cerebral cortex of normal and reeler mutant mice: an autoradiographic analysis. *J. Comp. Neurol*. 148:141–152.

Chen, Y., Beffert, U., Ertunc, M., Tang, T. S., Kavalali, E. T., Bezprozvanny, I., and Herz, J. (2005). Reelin modulates NMDA receptor activity in cortical neurons. *J. Neurosci*. 25:8209–8216.

Chin, J., Massaro, C. M., Palop, J. J., Thwin, M. T., Yu, G. Q., Bien-Ly, N., Bender, A., and Mucke, L. (2007). Reelin depletion in the entorhinal cortex of human amyloid precursor protein transgenic mice and humans with Alzheimer's disease. *J. Neurosci*. 27:2727–2733.

D'Arcangelo, G. (2005). Reelin mouse mutants as models of cortical development disorders. *Epilepsy Behav*. 8:81–90.

D'Arcangelo, G., Miao, G. G., Chen, S. C., Soares, H. D., Morgan, J. I., and Curran, T. (1995). A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* 374:719–723.

D'Arcangelo, G., Nakajima, K., Miyata, T., Ogawa, M., Mikoshiba, K., and Curran, T. (1997). Reelin is a secreted glycoprotein recognized by the CR-50 monoclonal antibody. *J. Neurosci*. 17:23–31.

D'Arcangelo, G., Homayouni, R., Keshvara, L., Rice, D. S., Sheldon, M., and Curran, T. (1999). Reelin is a ligand for lipoprotein receptors. *Neuron* 24:471–479.

de Bergeyck, V., Nakajima, K., Lambert de Rouvroit, C., Naerhuyzen, B., Goffinet, A. M., Miyata, T., Ogawa, M., and Mikoshiba, K. (1997). A truncated reelin protein is produced but not secreted in the "Orleans" reeler mutation (*Reln*$^{rl\text{-}Orl}$). *Mol. Brain Res*. 50:85–90.

DeSilva, U., D'Arcangelo, G., Braden, V. V., Chen, J., Miao, G., Curran, T., and Green, E. D. (1997). The human reelin gene: isolation, sequencing, and mapping on chromosome 7. *Genome Res*. 7:157–164.

Dong, E., Caruncho, H., Liu, W. S., Smalheiser, N. R., Grayson, D. R., Costa, E., and Guidotti, A. (2003). A reelin–integrin receptor interaction regulates Arc mRNA translation in synaptoneurosomes. *Proc. Natl. Acad. Sci. USA* 100:5479–5484.

Dulabon, L., Olson, E. C., Taglienti, M. G., Eisenhuth, S., McGrath, B., Walsh, C. A., Kreidberg, J. A., and Anton, E. S. (2000). Reelin binds alpha3beta1 integrin and inhibits neuronal migration. *Neuron* 27:33–44.

Fatemi, S. H., Earle, J. A., and McMenomy, T. (2000). Reduction in reelin immunoreactivity in hippocampus of subjects with schizophrenia, bipolar disorder and major depression. *Mol. Psychiatry* 571:654–663.

Fatemi, S. H., Stary, J. M., Halt, A. R., and Realmuto, G. R. (2001). Dysregulation of reelin and Bcl-2 proteins in autistic cerebellum. *J. Autism Dev. Disord*. 31:529–535.

Fatemi, S. H., Snow, A. V., Stary, J. M., Araghi-Niknam, M., Reutiman, T. J., Lee, S., Brooks, A. I., and Pearce, D. A. (2005). Reelin signaling is impaired in autism. *Biol. Psychiatry* 57:777–787.

Goffinet, A. M. (1983). The embryonic development of the cerebellum in normal and reeler mutant mice. *Anat. Embryol. (Berl.)* 168:73–86.

Graus-Porta, D., Blaess, S., Senften, M., Littlewood-Evans, A., Damsky, C., Huang, Z., Orban, P., Klein, R., Schittny, J. C., and Muller, U. (2001). Beta1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. *Neuron* 31:367–379.

Grayson, D. R., Jia, X., Chen, Y., Sharma, R. P., Mitchell, C. P., Guidotti, A., and Costa, E. (2005). Reelin promoter hypermethylation in schizophrenia. *Proc. Natl. Acad. Sci. USA* 102:9341–9346.

Guidotti, A., Auta, J., Davis, J. M., DiGiorgi Gerevini, V., Dwivedi, Y., Grayson, D. R., Impagnatiello, F., Pandey, G., Pesold, C., Sharma, R., Uzunov, D., and Costa, E. (2000). Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. *Arch. Gen. Psychiatry* 57:1061–1069.

Herz, J., and Chen, Y. (2006). Reelin, lipoprotein receptors and synaptic plasticity. *Nature Rev*. 7:850–859.

Hiesberger, T., Trommsdorff, M., Howell, B. W., Goffinet, A. M., Mumby, M. C., Cooper, J. A., and Herz, J. (1999). Direct binding of reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron* 24:481–489.

Hoe, H. S., Tran, T. S., Matsuoka, Y., Howell, B. W., and Rebeck, G. W. (2006). DAB1 and reelin effects on amyloid precursor protein and ApoE receptor 2 trafficking and processing. *J. Biol. Chem*. 281:35176–35185.

Hong, S. E., Shugart, Y. Y., Huang, D. T., Al Shahwan, S., Grant, P. E., Hourihane, J. O. B., Martin, N. D. T., and Walsh, C. A. (2000). Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nature Genet*. 26:93–96.

Howell, B. W., Hawkes, R., Soriano, P., and Cooper, J. A. (1997). Neuronal position in the developing brain is regulated by mouse disabled-1. *Nature* 389:733–736.

Howell, B. W., Herrick, T. M., and Cooper, J. A. (1999). Reelin-induced tyrosine phosphorylation of disabled 1 during neuronal positioning. *Genes Dev*. 13:643–648.

Howell, B. W., Herrick, T. M., Hildebrand, J. D., Zhang, Y., and Cooper, J. A. (2000). Dab1 tyrosine phosphorylation sites relay positional signals during mouse brain development. *Curr. Biol*. 10:877–885.

Huang, Y., Magdaleno, S., Hopkins, R., Slaughter, C., Curran, T., and Keshvara, L. (2004). Tyrosine phosphorylated disabled 1 recruits Crk family adapter proteins. *Biochem. Biophys. Res. Commun*. 318:204–212.

Impagnatiello, F., Guidotti, A. R., Pesold, C., Dwivedi, Y., Caruncho, H., Pisu, M. G., Uzunov, D. P., Smalheiser, N. R., Davis, J. M., Pandey, G. N., Pappas, G. D., Tueting, P., Sharma, R. P., and Costa, E. (1998). A decrease of reelin expression as a putative vulnerability factor in schizophrenia. *Proc. Natl. Acad. Sci. USA* 95:15718–15723.

Jossin, Y., Ignatova, N., Hiesberger, T., Herz, J., Lambert de Rouvroit, C., and Goffinet, A. M. (2004). The central fragment of reelin, generated by proteolytic processing in vivo, is critical to its function during cortical plate development. *J. Neurosci*. 24:514–521.

Jossin, Y., Gui, L., and Goffinet, A. M. (2007). Processing of reelin by embryonic neurons is important for function in tissue, but not in dissociated cultured neurons. *J. Neurosci*. 27:4243–4352.

Keshvara, L., Benhayon, D., Magdaleno, S., and Curran, T. (2001). Identification of reelin-induced sites of tyrosyl phosphorylation on disabled 1. *J. Biol. Chem*. 276:16008–16014.

Krueger, D. D., Howell, J. L., Hebert, B. F., Olausson, P., Taylor, J. R., and Nairn, A. C. (2006). Assessment of cognitive function in the heterozygous reeler mouse. *Psychopharmacology* 189:95–104.

Kubo, K., Mikoshiba, K., and Nakajima, K. (2002). Secreted reelin molecules form homodimers. *Neurosci. Res*. 43:381–388.

Kuo, G., Arnaud, L., Kronstad-O'Brien, P., and Cooper, J. A. (2005). Absence of Fyn and Src causes a reeler-like phenotype. *J. Neurosci*. 25:8578–8586.

Lambert de Rouvroit, C., and Goffinet, A. M. (1998). The reeler mouse as a model of brain development. *Adv. Anat. Embryol. Cell Biol*. 150:1–108.

Lambert de Rouvroit, C., de Bergeyck, V., Cortvrindt, C., Bar, I., Eeckhout, Y., and Goffinet, A. M. (1999). Reelin, the extracellular matrix protein deficient in reeler mutant mice, is processed by a metalloproteinase. *Exp. Neurol*. 156:214–217.

Larson, J., Hoffman, J. S., Guidotti, A., and Costa, E. (2003). Olfactory discrimination learning deficit in heterozygous reeler mice. *Brain Res*. 971:40–46.

Lavdas, A. A., Grigoriou, M., Pachnis, V., and Parnavelas, J. G. (1999). The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. *J. Neurosci*. 99:7881–7888.

Liu, W. S., Pesold, C., Rodriguez, M. A., Carboni, G., Auta, J., Lacor, P., Larson, J., Condie, B. G., Guidotti, A., and Costa, E. (2001). Down-regulation of dendritic spine and glutamic acid decarboxylase 67 expressions in the reelin haploinsufficient heterozygous reeler mouse. *Proc. Natl. Acad. Sci. USA* 98:3477–3482.

Malatesta, P., Hartfuss, E., and Gotz, M. (2000). Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. *Development* 127:5253–5263.

Morimura, T., Hattori, M., Ogawa, M., and Mikoshiba, K. (2005). Disabled1 regulates the intracellular trafficking of reelin receptors. *J. Biol. Chem*. 280:16901–16908.

Niu, S., Renfro, A., Quattrocchi, C. C., Sheldon, M., and D'Arcangelo, G. (2004). Reelin promotes hippocampal dendrite development through the VLDLR/ApoER2-Dab1 pathway. *Neuron* 41:71–84.

Noctor, S. C., Flint, A. C., Weissman, T. A., Dammerman, R. S., and Kriegstein, A. R. (2001). Neurons derived from radial glial cells establish radial units in neocortex. *Nature* 409:714–720.

Ogawa, M., Miyata, T., Nakajima, K., Yagyu, K., Seike, M., Ikenaka, K., Yamamoto, H., and Mikoshiba, K. (1995). The reeler gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons. *Neuron* 14:899–912.

Ognibene, E., Adriani, W., Granstrem, O., Pieretti, S., and Laviola, G. (2007). Impulsivity-anxiety-related behavior and profiles of morphine-induced analgesia in heterozygous reeler mice. *Brain Res*. 1131:173–180.

Ohkubo, N., Lee, Y. D., Morishima, A., Terashima, T., Kikkawa, S., Tohyama, M., Sakanaka, M., Tanaka, J., Maeda, N., Vitek, M. P., and Mitsuda, N. (2003). Apolipoprotein E and reelin ligands modulate tau phosphorylation through an apolipoprotein E receptor/disabled-1/glycogen synthase kinase-3beta cascade. *FASEB J*. 17:295–297.

Pesold, C., Impagnatiello, F., Pisu, M. G., Uzunov, D. P., Costa, E., Guidotti, A., and Caruncho, H. J. (1998). Reelin is preferentially expressed in neurons synthesizing g-aminobutyric acid in cortex and hippocampus of adult rats. *Proc. Natl. Acad. Sci. USA* 95:3221–3226.

Pramatarova, A., Ochalski, P. G., Chen, K., Gropman, A., Myers, S., Min, K. T., and Howell, B. W. (2003). Nck beta interacts with tyrosine-phosphorylated disabled 1 and redistributes in reelin-stimulated neurons. *Mol. Cell Biol*. 23:7210–7221.

Qiu, S., and Weeber, E. J. (2007). Reelin signaling facilitates maturation of CA1 glutamatergic synapses. *J. Neurophys*. 97:2312–2321.

Qiu, S., Korwek, K. M., Pratt-Davis, A. R., Peters, M., Bergman, M. Y., and Weeber, E. J. (2006a). Cognitive disruption and altered hippocampus synaptic function in reelin haploinsufficient mice. *Neurobiol. Learning Memory* 85:228–242.

Qiu, S., Zhao, L. F., Korwek, K. M., and Weeber, E. J. (2006b). Differential reelin-induced enhancement of NMDA and AMPA receptor activity in the adult hippocampus. *J. Neurosci*. 26:12943–12955.

Rice, D. S., Nusinowitz, S., Azimi, A. M., Martinez, A., Soriano, E., and Curran, T. (2001). The reelin pathway modulates the structure and function of retinal synaptic circuitry. *Neuron* 31:929–941.

Rodriguez, M. A., Pesold, C., Liu, W. S., Khrino, V., Guidotti, A., Pappas, G. D., and Costa, E. (2000). Colocalization of integrin receptors and reelin in dendritic spine postsynaptic densities of adult nonhuman primate cortex. *Proc. Natl. Acad. Sci. USA* 97:3550–3555.

Royaux, I., Lambert de Rouvroit, C., D'Arcangelo, G., Demirov, D., and Goffinet, A. M. (1997). Genomic organization of the mouse *reelin* gene. *Genomics* 46:240–250.

Sanada, K., Gupta, A., and Tsai, L. H. (2004). Disabled-1-regulated adhesion of migrating neurons to radial glial fiber contributes to neuronal positioning during early corticogenesis. *Neuron* 42:197–211.

Schiffmann, S. N., Bernier, B., and Goffinet, A. M. (1997). Reelin mRNA expression during mouse brain development. *Eur. J. Neurosci*. 9:1055–1071.

Schmid, R. S., Shelton, S., Stanco, A., Yokota, Y., Kreidberg, J. A., and Anton, E. S. (2004). alpha3beta1 integrin modulates neuronal migration and placement during early stages of cerebral cortical development. *Development* 131:6023–6031.

Schmid, R. S., Jo, R., Shelton, S., Kreidberg, J. A., and Anton, E. S. (2005). Reelin, integrin and Dab1 interactions during embryonic cerebral cortical development. *Cerebral Cortex* 15:1632–1636.

Sheldon, M., Rice, D. S., D'Arcangelo, G., Yoneshima, H., Nakajima, K., Mikoshiba, K., Howell, B. W., Cooper, J. A., Goldowitz, D., and Curran, T. (1997). *Scrambler* and *yotari* disrupt the *disabled* gene and produce a *reeler*-like phenotype in mice. *Nature* 389:730–733.

Sinagra, M., Verrier, D., Frankova, D., Korwek, K. M., Blahos, J., Weeber, E. J., Manzoni, O. J., and Chavis, P. (2005). Reelin, very-low-density lipoprotein receptor, and apolipoprotein E receptor 2 control somatic NMDA receptor composition during hippocampal maturation in vitro. *J. Neurosci*. 25:6127–6136.

Strasser, V., Fasching, D., Hauser, C., Mayer, H., Bock, H. H., Hiesberger, T., Herz, J., Weeber, E. J., Sweatt, J. D., Pramatarova, A., Howell, B., Schneider, W. J., and Nimpf, J. (2004). Receptor clustering is involved in reelin signaling. *Mol. Cell Biol*. 24:1378–1386.

Suetsugu, S., Tezuka, T., Morimura, T., Hattori, M., Mikoshiba, K., Yamamoto, T., and Takenawa, T. (2004). Regulation of actin cytoskeleton by mDab1 through N-WASP and ubiquitination of mDab1. *Biochem. J*. 384:1–8.

Takahara, T., Ohsumi, T., Kuromitsu, J., Shibata, K., Sasaki, N., Okazaki, Y., Shibata, H., Sato, S., Yoshiki, A., Kusakabe, M., Muramatsu, M., Ueki, M., Okuda, K., and Hayashizaki, Y. (1996). Dysfunction of the Orleans *reeler* gene arising from exon skipping due to transposition of a full-length copy of an active L1 sequence into the skipped exon. *Hum. Mol. Genet*. 5:989–993.

Trommsdorff, M., Borg, J. P., Margolis, B., and Herz, J. (1998). Interaction of cytosolic adaptor proteins with neuronal apolipoprotein E receptors and the amyloid precursor protein. *J. Biol. Chem*. 273:33556–33560.

Trommsdorff, M., Gotthardt, M., Hiesberger, T., Shelton, J., Stockinger, W., Nimpf, J., Hammer, R. E., Richardson, J. A., and Herz, J. (1999). Reeler/disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* 97:689–701.

Tueting, P., Costa, E., Dwivedi, Y., Guidotti, A., Impagnatiello, F., Manev, R., and Pesold, C. (1999). The phenotypic characteristics of heterozygous reeler mouse. *Neuroreport* 10:1329–1334.

Tueting, P., Doueiri, M. S., Guidotti, A., Davis, J. M., and Costa, E. (2006). Reelin down-regulation in mice and psychosis endophenotypes. *Neurosci. Biobehav. Rev*. 30:1065–1077.

Utsunomiya-Tate, N., Kubo, K., Tate, S., Kainosho, M., Katayama, E., Nakajima, K., and Mikoshiba, K. (2000). Reelin molecules assemble together to form a large protein complex, which is inhibited by the function-blocking CR-50 antibody. *Proc. Natl. Acad. Sci. USA* 97:9729–9734.

Veldic, M., Caruncho, H. J., Liu, W. S., Davis, J., Satta, R., Grayson, D.R., Guidotti, A., and Costa, E. (2004). DNA-methyltransferase 1 mRNA is selectively overexpressed in telencephalic GABAergic interneurons of schizophrenia brains. *Proc. Natl. Acad. Sci. USA* 101:348–353.

Ware, M., Fox, J. W., Gonzales, J. L., Davis, N. M., Lambert de Rouvroit, C., Russo, C. J., Chua, S. C. J., Goffinet, A. M., and Walsh, C. A. (1997). Aberrant splicing of a mouse *disabled* homolog, *mdab1*, in the *scrambler* mouse. *Neuron* 19:239–249.

Weeber, E. J., Beffert, U., Jones, C., Christian, J. M., Forster, E., Sweatt, J. D., and Herz, J. (2002). Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. *J. Biol. Chem*. 277:39944–39952.

Yoneshima, H., Nagata, E., Matsumoto, M., Yamada, M., Nakajima, K., Miyata, T., Ogawa, M., and Mikoshiba, K. (1997). A novel neurological mutation of mouse, *yotari*, which exhibits *reeler*-like phenotype but expresses *reelin*. *Neurosci. Res*. 29:217–223.

Zhang, G., Assadi, A. H., McNeil, R. S., Beffert, U., Wynshaw-Boris, A., Herz, J., Clark, G. D., and D'Arcangelo, G. (2007). The PAFAH1B complex interacts with the reelin receptor VLDLR. *PlosOne* 2:e252.

Chapter 2
Apolipoprotein E Receptor 2 and Very-Low-Density Lipoprotein Receptor: An Overview

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1 The LDL Receptor Gene Family

1.1 Functions of Lipoprotein Receptors in Neurobiology

It is now well established that members of the low-density lipoprotein (LDL) receptor gene family are crucial regulators of different aspects of neuronal development, synaptic plasticity, maintenance of neuronal homeostasis, and neurodegeneration. This was highlighted in particular by the discovery that the lipoprotein receptors apolipoprotein E receptor-2 (ApoER2) and very-low-density lipoprotein receptor (VLDLR) function as receptors for the neuronal signaling protein Reelin. In this

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chapter, we will briefly introduce the family of LDL receptor-related proteins and review the functions of its members ApoER2 and VLDLR as Reelin receptors and their role in the developing and adult brain.

1.2 Structural Organization and Common Features of LDL Receptor Gene Family Members

The LDL receptor gene family consists of seven core family members in vertebrates that share common structural features. They are type I transmembrane receptors with an extracellular domain containing complement-type repeats and epidermal growth factor (EGF) homology domains in a modular arrangement followed by the single transmembrane-spanning sequence and a comparatively short cytoplasmic domain that interacts with adapter and scaffolding proteins through various sequence motifs (Fig. 2.1A; reviewed by Herz and Bock, 2002; Nykjaer and Willnow, 2002). The intracellular domains of all core members contain at least one Asn-Pro-X-Tyr (NPXY, where X designates any amino acid) tetra-amino acid motif, which is crucial for their involvement in receptor-mediated endocytosis and cellular signal transduction (reviewed by May and Herz, 2003; Schneider and Nimpf, 2003; Stolt and Bock, 2006). Another commonality of all LDL receptor-related proteins (LRPs) is their binding to the receptor-associated protein (RAP), an endoplasmic reticulum-associated chaperone that acts as a universal antagonist of all LRP ligands (Bu and Schwartz, 1998; Herz, 2006). Other ligands that are bound by members of the receptor family include lipoproteins, protease–protease inhibitor complexes, matrix metalloproteinases, plasma proteins, growth factors, and cytokines (reviewed by Willnow *et al*., 1999; May *et al*., 2005).

1.2.1 Intracellular Adapter Proteins Bind to the NPXY Motif in the Intracellular Domains of LRPs

The presence of at least one NPXY motif, which was discovered as a signal for receptor-mediated endocytosis (reviewed by Bonifacino and Traub, 2003), in the intracellular domains of all LDL receptor gene family members suggested that they might function as mere cargo receptors. This view was challenged by observations that a range of modular cytosolic adapter proteins bind to the NPXY motifs of different receptors with their phosphotyrosine binding (PTB) / protein interaction domains (Gotthardt *et al*., 2000), including the neuronally enriched proteins FE65 and Disabled-1 (Dab1). These studies (Trommsdorff *et al*., 1998; Howell *et al*., 1999b) set the stage for the seminal discovery that two family members, ApoER2 and VLDLR, directly act as signal transduction receptors for the neuronal signaling molecule Reelin (D'Arcangelo *et al*., 1999; Hiesberger *et al*., 1999; Trommsdorff *et al*., 1999).

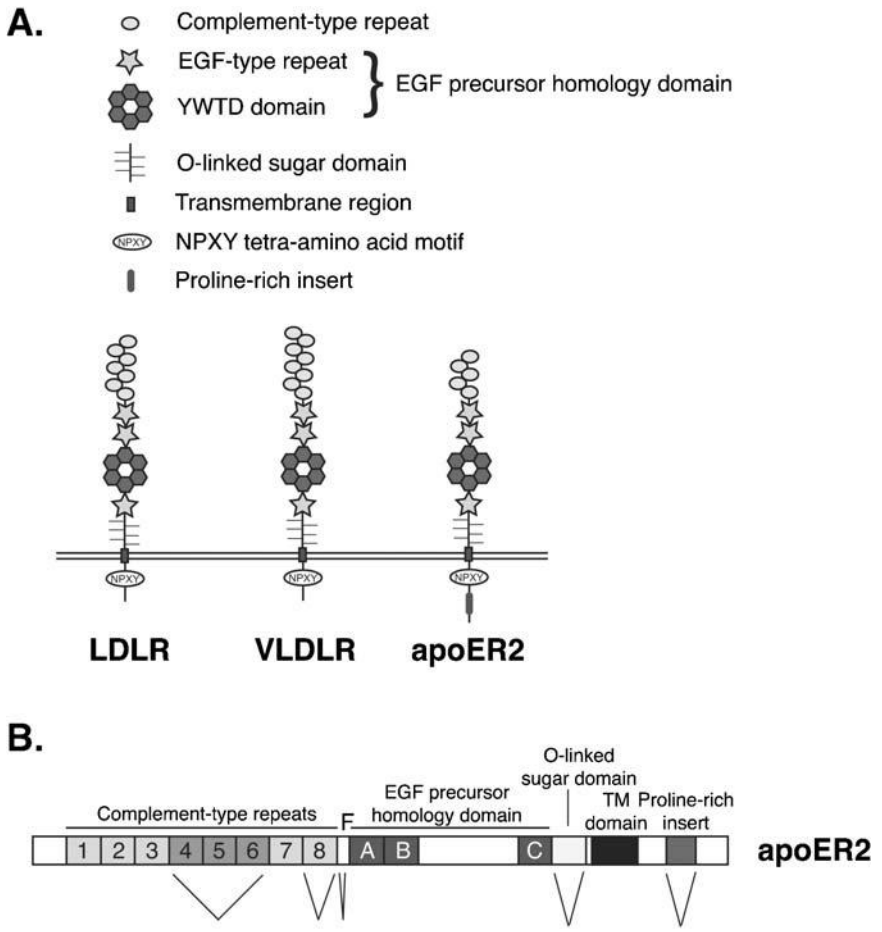

Fig. 2.1 Structure of ApoER2 and VLDLR. (**A**) ApoER2 and VLDLR, two close relatives of the LDL receptor. ApoER2 and VLDLR are two members of the LDL receptor gene family that closely resemble the namesake of the family, the LDL receptor. They are modular type I transmembrane proteins that consist of one ligand-binding domain comprising several complement-type (a.k.a. ligand-type or type A) repeats, an epidermal growth factor (EGF) precursor homology domain, and an O-linked sugar domain followed by a single transmembrane stretch and a short cytoplasmic tail. The EGF precursor homology domain consists of three EGF-like (type B) repeats flanking a domain containing modules of consensus YWTD tetrapeptides (ß-propeller). The NPXY motif in the cytoplasmic tail participates in endocytosis and signal transduction. Additional, larger members of the LDL receptor gene family that share this modular structure are the LDL receptor-related proteins LRP1, LRP1b, megalin (a.k.a. gp330), and MEGF7 (not depicted here). The more distantly related receptors LRP5, LRP6, and LR11/SorLA display different domain structures. **(B)** ApoER2 undergoes extensive tissue-specific splicing. Spliced exons are indicated below the bar representing the mRNA encoding murine ApoER2. The exon encoding the complement-type repeats 4–6 is constitutively spliced out in mouse. Other alternatively spliced exons encode the ligand-type repeat 8, a small insertion including the furin cleavage site (F) just upstream of the first EGF-like repeat (A), the O-linked sugar domain, and the proline-rich cytoplasmic insert. The type I and type II variants of VLDLR differ from each other by the presence or absence of the O-linked sugar domain, which is always present in the LDLR -TM domain, transmembrane domain

1.2.2 LRPs Are Receptors for Apolipoprotein E

All members of the receptor family also interact with the cholesterol transport protein apolipoprotein E (ApoE); therefore, they are commonly referred to as apolipoprotein E (ApoE) receptors (Herz and Beffert, 2000). ApoE was discovered as a polymorphic lipid transport protein that exists in three major isoforms in humans, named ApoE2, ApoE3, and ApoE4 (Mahley *et al*., 1984; Mahley and Rall, 2000; Strittmatter and Bova Hill, 2002). Besides its functions in lipoprotein metabolism and cardiovascular physiology, ApoE plays important roles in neurobiology (Mahley *et al*., 2006). Although neurons are capable of synthesizing ApoE, astrocytes are the main cell type secreting ApoE-containing high-density lipoprotein (HDL)-like lipid particles in the brain (Boyles *et al*., 1985; Pitas *et al*., 1987), which participate in lipoprotein receptor-mediated lipid redistribution during neuronal maintenance, plasticity, and repair (Ignatius *et al*., 1987; Boyles *et al*., 1989; Mauch *et al*., 2001). The seminal finding that the ε4 allele of *APOE* is a major risk factor for Alzheimer's disease (reviewed by Roses, 1996) together with the unanticipated discovery that ApoE receptors serve as pivotal mediators of neuronal signaling cascades (reviewed by Herz and Beffert, 2000) fueled an enormous interest in isoform-specific effects of ApoE on neuronal homeostasis beyond mere lipid redistribution.

1.2.3 Proteolytic Processing of LRPs by Gamma Secretase

One of the neuropathological hallmarks of Alzheimer's disease is the accumulation of amyloid plaques in the cerebrovasculature and brain parenchyma (Goedert and Spillantini, 2006). A major component of these plaques is the 42-amino-acid amyloid-beta (A-beta) peptide, which is derived from the type I transmembrane amyloid precursor protein (APP) by a sequence of proteolytic processing steps (Sinha and Lieberburg, 1999). In the last step, which is mediated by a complex of integral membrane proteins called gamma secretase (Wolfe, 2006), the APP intracellular domain (AICD) is released into the cytosol after regulated intramembrane proteolysis of the membrane-attached carboxyl-terminal stub. Clearance of amyloid-beta from the extracellular space and amyloid plaque formation might both be influenced by its isoform-specific interaction with ApoE (Mahley and Rall, 2000). Remarkably, several members of the LDL receptor gene family, including the Reelin receptors ApoER2 and VLDLR, are cleaved by gamma secretase as well (May *et al*., 2002, 2003; Zou *et al*., 2004; Hoe and Rebeck, 2005). This, together with the observation that several of the intracellular adapter proteins that bind to LRPs also interact with the cytoplasmic domain of APP, adds to a growing body of evidence for a critical role of ApoE receptors in the pathogenesis of Alzheimer's disease that involves different but converging cellular mechanisms (Fig. 2.3; discussed by Andersen and Willnow, 2006; Herz and Chen, 2006).

2 APOE Receptors as Mediators of Reelin Signaling in the Brain

In this section, we will review the molecular and cellular mechanisms of how ApoER2 and VLDLR might regulate neuronal positioning during neurodevelopment and discuss evidence for a role of these receptors in the control of synaptic transmission in the adult brain.

2.1 Structural Characteristics of ApoER2 and VLDLR

ApoER2 and VLDLR are of a comparable size and display the same overall structure as the LDL receptor (Fig. 2.1A). Both receptors display a high degree of conservation across species and are highly expressed in the brain (Schneider *et al*., 1997). In contrast to the LDL receptor, the VLDLR exists in two major splice forms designated type I and type II that differentially express the O-linked sugar domain just outside the plasma membrane, and its ligand binding domain contains eight, rather than seven, complement-type repeats (Fig. 2.1A). The type I VLDLR containing the O-linked sugar domain is the preferentially expressed form in the brain (reviewed by Takahashi *et al*., 2004). ApoER2, on the other hand, occurs in various species- and tissue-specific splice variants (Fig. 2.1B) (Schneider and Nimpf, 2003). These include constitutive deletion of the complement-type repeats 4–6 in the murine *apoer2* gene by alternative splicing, the variable expression of a furin cleavage site that allows for the secretion of the ligand-binding domain, splicing of the O-linked sugar domain, and a differentially expressed unique proline-rich insert in the cytoplasmic tail that specifically interacts with JNK-interacting proteins (JIP) and the postsynaptic density protein PSD-95 (Brandes *et al*., 1997, 2001; Clatworthy *et al*., 1999; Koch *et al*., 2002). Of note, tissue-specific glycosylation due to the presence or absence of the O-linked sugar domain turned out to be an essential regulator of ApoER2 cleavage by gamma secretase (May *et al*., 2003).

2.2 Reelin Signaling During Neurodevelopment

2.2.1 Phenotype of ApoER2- and Vldl-Receptor-Deficient Mice

Due to the structural similarities of ApoER2 and VLDL receptor to the LDL receptor, their closest relative which is defective in familial hypercholesterolemia, it was initially assumed that both receptors might function primarily as uptake receptors for cholesterol in the brain and other tissues (see, e.g., Frykman *et al*., 1995; Yamamoto and Bujo, 1996). A major breakthrough in the understanding of VLDLR and ApoER2 function in the brain came from a study by

Trommsdorff and colleagues. They showed that simultaneous inactivation of both receptors by gene targeting in mice resulted in a reeler-like phenotype, which is characterized by severe neuronal positioning defects in laminated structures of the brain (for details on the phenotype of reeler mice, see chapters by Mienville, Huang and D'Arcangelo, Förster *et al*., Hevner), suggesting a direct involvement of ApoE receptors in neuronal signaling (Trommsdorff *et al*., 1999). Subsequent studies demonstrated that Reelin directly binds to VLDLR and ApoER2 (D'Arcangelo *et al*., 1999; Hiesberger *et al*., 1999), with the latter exhibiting a slighty higher apparent affinity for Reelin (Benhayon *et al*., 2003). Analysis of single receptor mutant mice revealed milder neuroanatomical phenotypes, with neuronal positioning defects in the neocortex and hippocampus in the absence of ApoER2, and Vldlr deficiency resulting in mild cerebellar abnormalities (Trommsdorff *et al*., 1999; Benhayon *et al*., 2003; Beffert *et al*., 2006b). These observations might be explained by a combination of mere expression differences of both receptors in diverse regions of the developing brain and different binding affinities for Reelin. However, assuming that both receptors are functionally redundant with regard to the transmission of the Reelin signal, this leaves open the question why two structurally closely related receptors with different but overlapping expression patterns for the same ligand should have evolved (Cooper and Howell, 1999). Possible alternative explanations for the different neurodevelopmental phenotypes of single receptor mutants include region- and/or receptor-specific modulators of the Reelin response, e.g., co-receptors, co-ligands, or intracellular adapter proteins. Of note, ApoE receptor-independent effects of Reelin on neuronal migration of hindbrain efferent nuclei and gonadotropin-releasing hormone-secreting hypothalamic neurons have been described (Cariboni *et al*., 2005; Rossel *et al*., 2005).

2.2.2 Nonneuronal Manifestations of ApoER2 and Vldlr Deficiency

Besides their neurodevelopmental abnormalities, ApoER2- and Vldlr-deficient mice display additional defects according to their expression pattern in peripheral tissues. Loss of Vldlr in mice, which is expressed in the heart, muscle, adipose tissue, endothelium, and on macrophages, leads to reduced body weight (Frykman *et al*., 1995), resistance to diet-induced obesity (Goudriaan *et al*., 2001), and altered triglyceride metabolism in the concurrent absence of the LDL receptor (Tacken *et al*., 2000). Moreover, subretinal neovascularization is common in Vldlr-deficient mice (Heckenlively *et al*., 2003). An autosomal-recessive human genetic defect of the *VLDLR* gene was recently identified in the Hutterite population (Boycott *et al*., 2005). Patients homozygous for a large deletion including the *VLDLR* locus present with inferior cerebellar hypoplasia, nonprogressive ataxia, mild cerebral gyral simplification, and mental retardation, reminiscent of, but less severe than, the lissencephaly syndrome caused by a null mutation of the human *RELN* gene (Hong *et al*., 2000). In addition, a polymorphic triplet repeat in the 5′ untranslated region of *VLDLR* might predispose to cognitive impairment and Alzheimer's disease

(Okuizumi *et al*., 1995; Helbecque and Amouyel, 2000; Helbecque *et al*., 2001). Finally, homozygous deletion of *VLDLR* by genomic loss or promoter hypermethylation was observed in gastric cancer cell lines (Takada *et al*., 2006). This suggests a possible role as a tumor suppressor gene and adds to the finding that RELN is frequently silenced in pancreatic cancers (Sato *et al*., 2006; see chapter by Walter and Goggins).

ApoER2 is mainly expressed in the brain and in reproductive organs. ApoER2-deficient male mice are infertile as a result of abnormal sperm morphology and motility, which could be linked to the reduced expression of a selenoperoxidase important for spermatogenesis (Andersen *et al*., 2003). A significant association between a maternal *APOER2* polymorphism and fetal growth restriction was recently reported among African-American women (Wang *et al*., 2006). As ApoER2 is highly expressed in the placenta, this might reflect a requirement for ApoER2 in the regulation of the microenvironment for fetal growth. Other than for VLDLR, a human ApoER2 brain malformation syndrome has not been described. However, a possible association of *APOER2* with Alzheimer's disease may exist (Ma *et al*., 2002).

2.3 The Reelin–ApoER2/VLDLR–Dab1 Signaling Cascade

2.3.1 Evidence for a Linear Signaling Pathway Involving Reelin, ApoE Receptors, and Dab1

Biochemical and genetic evidence resulting from independent studies of several groups led to the delination of a signaling cascade (Fig. 2.2) where the extracellular protein Reelin binds to ApoER2 and VLDLR, thereby inducing the tyrosine phosphorylation and activation of the intracellular adapter protein Dab1 (see chapters by Olson and Walsh and Cooper *et al*.). This was based on the reeler-like phenotype of Dab1-mutant mice (Howell *et al*., 1997b; Sheldon *et al*., 1997; Ware *et al*., 1997), the identification of Dab1 as an intracellular binding partner of ApoER2 and VLDLR (Trommsdorff *et al*., 1999), the observation that Dab1 protein levels are upregulated in brains lacking Reelin or Vldlr and ApoER2 (Rice *et al*., 1998; Trommsdorff *et al*., 1998), the development of a neuronal Reelin signaling assay (Howell *et al*., 1999a), and the demonstration that mice expressing a nonphosphorylatable form of Dab1 recapitulate the reeler phenotype (Howell *et al*., 2000). Expression of these components in a heterologous cell line proved sufficient to reconstitute the signaling pathway (Mayer *et al*., 2006).

2.3.2 Modulation of Cytoskeletal Components in Response to Reelin

The identification of an ApoE receptor-dependent Reelin signaling cascade involving tyrosine phosphorylation of Dab1 raised the urgent question how this might translate

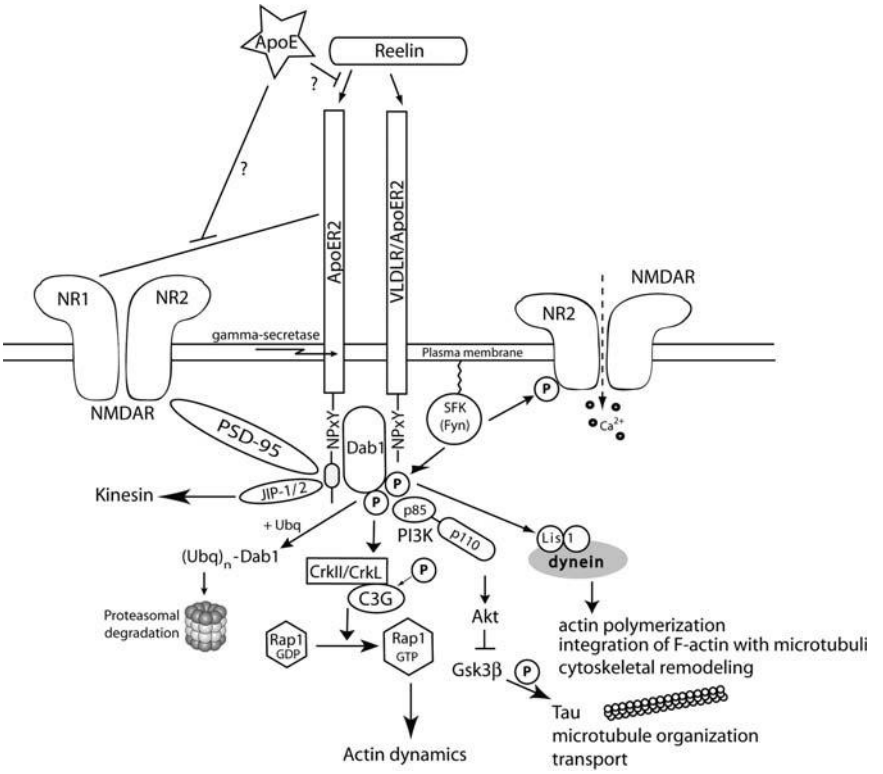

Fig. 2.2 Molecular mechanisms of Reelin signaling. The diagram summarizes mechanisms of ApoE receptor-mediated Reelin signaling that are important during neurodevelopment and for synaptic transmission in the adult brain (for details, see Sections 2.3 and 2.4). The cytoplasmic tails of ApoER2 and VLDLR serve as scaffolds for different signaling complexes, many of which target the cytoskeleton. Gamma secretase-mediated release of the receptor tail might therefore profoundly influence these signaling cascades. The adapter protein Dab1, which interacts with the NPXY motif of different transmembrane proteins, including the Reelin receptors, plays a crucial role as a signaling relay whose binding preferences are regulated by its Reelin-dependent phosphorylation state. Clustering of different transmembrane receptors by Reelin or other ligands like F-spondin represents another possibility of fine-tuning the signaling responses mediated by ApoER2 and VLDLR

into cytoskeletal rearrangement required for neuronal positioning (Feng and Walsh, 2001) in response to Reelin. An important clue came from the observation that the microtubule-associated protein tau is hyperphosphorylated in mice with genetic defects in components of the Reelin signaling cascade (Hiesberger *et al*., 1999). This led to the identification of a continuous Reelin-dependent pathway from its receptors to the cytoskeleton, involving activation of class I phosphatidylinositol-3 kinase (PI3K), a central regulator of diverse cellular functions, and Akt/PKB, which results in the downregulation of the tau kinase glycogen synthase kinase-3β

(GSK3β) (Beffert *et al*., 2002; Ballif *et al*., 2003; Bock *et al*., 2003). The modulation of tau phosphorylation by Reelin adds to the growing evidence linking ApoE receptors and Alzheimer's disease (see Section 1.2.3 and Fig. 2.3), where neurofibrillary tangles that result from the aggregation of abnormally phosphorylated tau are a neuropathological feature (Goedert and Spillantini, 2006). Of note, Reelin did not regulate the activity of Cdk5 (Beffert *et al*., 2002), another major tau kinase and regulator of neuronal positioning (reviewed by Dhavan and Tsai, 2001; see chapter by Ohshima). Further *in vivo* evidence for an independent but synergistic effect of Reelin and Cdk5 signaling on neuronal migration came from the phenotypic analysis of compound mutant mice lacking the Cdk5 activator p35 and Dab1 (Ohshima *et al*., 2001) or p35 and either Vldlr or ApoER2 (Beffert *et al*., 2004). On the other hand, Cdk5 signaling might modulate the Reelin response by phosphorylating Dab1 independently of Reelin signaling (Ohshima *et al*., 2007), and both signaling cascades converge on the microtubule-associated Ndel1/Lis1/dynein complex (Niethammer *et al*., 2000; Sasaki *et al*., 2000; Assadi *et al*., 2003) that regulates nucleokinesis, a critical cellular event in neuronal migration (Tsai and Gleeson,

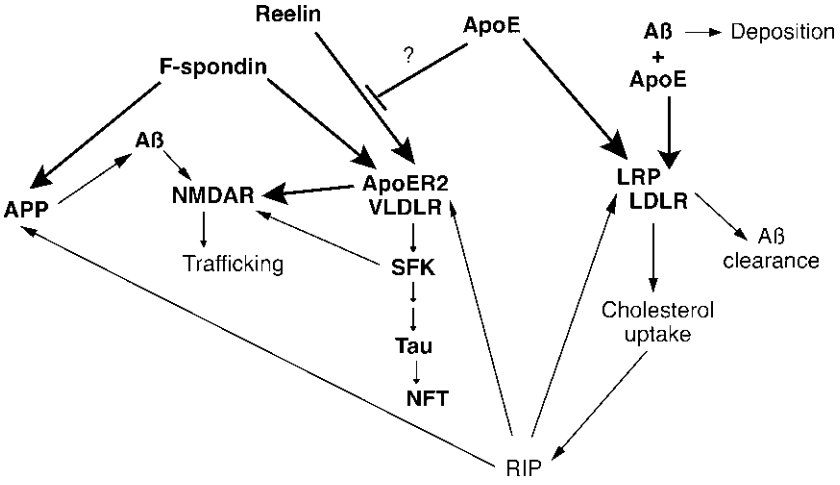

Fig. 2.3 Possible interactions of ApoE and amyloid-beta at the synapse. This diagram highlights possible interactions between ApoE, ApoE receptors, amyloid-beta, and NMDA receptors at the level of the synapse. Isoform-specific interactions of ApoE with its receptors might modulate Reelin signaling and endocytic uptake of ApoE-containing lipid particles. This would influence NMDAR-mediated neurotransmission, as well as the formation of neurofibrillary tangles and amyloid-beta deposition. ApoE-mediated uptake of extracellular cholesterol contributes to cholesterol homeostasis, which in turn has a marked impact on the regulated intramembrane proteolysis (RIP) of APP, ApoER2, and LRP. Proteolytic cleavage of APP yields amyloid-beta, the main component of amyloid plaques. Soluble amyloid-beta regulates the trafficking of NMDA receptors, which interact with Reelin receptors both intra- and extracellularly. The accumulation of subtle dysregulations of these complex events during the lifetime of an individual might contribute to neurodegenerative processes

2005). Another cytoskeletal element, the actin network, is also targeted by Reelin signaling. This is accomplished by the interaction of tyrosine-phosphorylated Dab1 with SH2/SH3 domain-containing scaffold proteins of the Nck and Crk families (Pramatarova *et al*., 2003; Ballif *et al*., 2004; Chen *et al*., 2004; Huang *et al*., 2004), which involves the Reelin-dependent phosphorylation at the positions Y220 and 232 (Howell *et al*., 2000; Keshvara *et al*., 2001) in a cluster of tyrosine residues proximal of the PTB domain of Dab1 (see chapter by Cooper *et al*.). Binding of phospho-Dab1 to CrkL promotes the phosphorylation of the guanine nucleotide exchange factor C3G (see Fig. 2.2), which results in the activation of the small GTPase Rap1 (Ballif *et al*., 2004) and subsequent modulation of the actin cytoskeleton (reviewed by Bos, 2005). The interaction of a splice variant of ApoER2 with the molecular motor kinesin is mediated by the adapter proteins c-jun NH2-terminal kinase (JNK)-interacting proteins (JIPs) JIP1 and JIP2 (Verhey *et al*., 2001), which bind to the spliced proline-rich insert of the intracellular domain (Gotthardt *et al*., 2000; Stockinger *et al*., 2000). Recruitment of a JIP1-JNK3 module to the ApoER2 insert plays a role in the control of neuronal survival in the normal aging brain (Beffert *et al*., 2006a).

2.3.3 Molecular Mechanism of ApoE Receptor-Mediated Dab1 Activation by Reelin

Since LRPs do not display an intrinsic kinase domain within their intracellular tails, it remained elusive how Reelin activates Dab1 *in vivo*, which had been shown before to be a substrate of nonreceptor tyrosine kinases of the Src family *in vitro* (Howell *et al*., 1997a). A series of genetic and biochemical studies from several groups (Arnaud *et al*., 2003b; Bock and Herz, 2003; Jossin *et al*., 2003) established that the Src family kinase (SFK) Fyn is the physiological Dab1 kinase (Fig. 2.2). Whereas Fyn knockout mice (Grant *et al*., 1992; Yagi *et al*., 1993), which express increased neuronal Dab1 levels, display only minor cortical lamination defects (Yuasa *et al*., 2004), simultaneous inactivation of Fyn and Src results in a reeler-like phenotype (Kuo *et al*., 2005), providing evidence for functional redundancy among SFKs during brain development. The activation of SFKs requires clustering of ApoER2 and VLDLR at the plasma membrane by oligomeric Reelin or receptor-activating antibodies, which brings the receptor-bound Dab1 into close proximity of SFK-enriched membrane subdomains (Jossin *et al*., 2004; Strasser *et al*., 2004). The resulting reciprocal activation of SFKs and Dab1 (Bock and Herz, 2003) is limited by the targeting of tyrosine-phosphorylated Dab1 for polyubiquitination and proteasomal degradation (Arnaud *et al*., 2003a; Bock *et al*., 2004). This highly efficient feedback regulation explains the observed accumulation of Dab1 protein in mice deficient for components of the Reelin signaling cascade.

Structural analysis of the Dab1 PTB domain bound to NPXY-containing peptides corresponding to parts of the ApoER2 (Stolt *et al*., 2003) or APP (Yun *et al*., 2003) intracellular domains and complexed with phosphoinositides provided a mechanistic insight into the simultaneous and noncooperative interaction of the PTB domain

with both ligands (Stolt *et al*., 2004). Further investigations using lentiviral transduction of primary neurons with Dab1 mutant constructs (Stolt *et al*., 2005) demonstrated the importance of phosphoinoside binding for the membrane targeting of Dab1 in neurons (Bock *et al*., 2003) and showed that both receptor binding and phosphoinositide binding of the Dab1 PTB domain are important for Reelin signal transduction (Stolt *et al*., 2005). Along with two subsequent studies (Huang *et al*., 2005; Xu *et al*., 2005), these data suggest that phosphoinositides target Dab1 to the plasma membrane, where its binding to the ApoER2 and VLDL receptor tails facilitates the Reelin-induced oligomerization and interaction with membrane-associated Src family kinases (reviewed by Stolt and Bock, 2006). Mice carrying a site-directed disruption of the Dab1 interaction motif in the ApoER2 intracellular domain display a neurodevelopmental phenotype that is virtually indistinguishable from reeler when bred on a *vldlr* knockout background, providing compelling *in vivo* evidence for the importance of the ApoER2–Dab1 interaction (Beffert *et al*., 2006b).

According to the model that emerges from these studies, tyrosine phosphorylation of Dab1, a key event in Reelin-mediated signal transduction, does not require the presence of an additional co-receptor providing tyrosine kinase activity. Interestingly, VLDLR- or ApoER2-dimerizing antibodies stimulated Dab1 tyrosine phosphorylation but failed to rescue the reeler phenotype in an *in vitro* embryonic slice culture assay of cortical plate development (Jossin *et al*., 2003). This suggests a functional role for other Reelin-binding, Dab1-interacting receptors like $\alpha 3\beta 1$ integrin (Dulabon *et al*., 2000; Calderwood *et al*., 2003) in a developmental, cell-type or tissue-specific context (Forster *et al*., 2002; Sanada *et al*., 2004).

2.3.4 Trafficking of Reelin Receptors

In addition to its interaction with ApoE receptors, Dab1 also binds to the NPXY motif in the intracellular tail of APP and APP-like proteins (Trommsdorff *et al*., 1998; Homayouni *et al*., 1999; Howell *et al*., 1999b). Further evidence for a functional relationship between APP and the Reelin–ApoE receptor–Dab1 pathway came from genetic studies. A gene-mapping approach for quantitative trait loci (QTLs) that influence the variation of tau hyperphosphorylation in Dab1 knockout mice on different strain backgrounds identified a highly significant QTL on mouse chromosome 16 centered over the *App* gene (Brich *et al*., 2003). In support of this, a genetic interaction between *appl* (amyloid precursor protein-like), the *Drosophila* homologue of *APP*, and Disabled has been described in two studies (Merdes *et al*., 2004; Pramatarova *et al*., 2006). Interestingly, Reelin and F-spondin, an extracellular matrix protein that binds to APP (Ho and Sudhof, 2004) and ApoER2 (Hoe *et al*., 2005), alter the trafficking and proteolytic processing of ApoER2 and APP (Morimura *et al*., 2005; Hoe *et al*., 2006b). Of note, other ApoE receptors, such as LRP1, LRP1b, and the distantly related receptor SorLA, regulate the endocytosis and intracellular trafficking of APP in a partly opposing manner as well (Andersen *et al*., 2005; Andersen and Willnow, 2006; Cam and Bu, 2006). An additional level of complexity in the interactions between ApoE, its receptors, and APP comes from the putative

transcriptional activity of the intracellular transmembrane receptor tails released by gamma secretase activity (Cao and Sudhof, 2001; Kinoshita *et al*., 2003).

2.4 Control of Synaptic Functions by ApoER2 and VLDLR in the Adult Brain

Most of the studies on the functions of ApoER2 and VLDLR as Reelin receptors have focused on the role of this signaling cascade in the developing brain. However, the molecules that are essential to this pathway continue to be highly expressed in the adult brain, suggesting additional functions beyond neurodevelopment. In this section, we will describe how Reelin and its ApoE receptors contribute to glutamatergic signaling at the synapse.

2.4.1 ApoER2 and VLDLR Modulate Neurotransmission

The emerging link between ApoE, cholesterol, and brain physiology (Mahley, 1988; Dietschy and Turley, 2004) sparked increasing interest in the possible regulation of neurotransmission by ApoE receptors, especially by LRP1, a multifunctional LDL receptor gene family member that is highly expressed in the brain (Bacskai *et al*., 2000; Zhuo *et al*., 2000; May *et al*., 2004). Defects in long-term potentiation (LTP), considered as an electrophysiological correlate of learning and memory, were recorded in hippocampal slices of mice lacking either Vldlr or ApoER2, with ApoER2 knockouts showing a more pronounced decay (Weeber *et al*., 2002). These defects were attributable to a defect in Reelin signaling, because perfusion of hippocampal slices with Reelin augments baseline LTP in wild-type, but not in ApoER2- or Vldlr-deficient mice. Both mutants also displayed behavioral deficiencies in fear-conditioned memory tests (Weeber *et al*., 2002). Mice that express a mutated form of ApoER2 with a defective intracellular Dab1 binding site show a severe LTP deficit and lack a Reelin-induced LTP enhancement, indicating a critical role for the Dab1 adapter protein (Beffert *et al*., 2006b). However, Reelin prevented the rapid degradation of LTP seen in mice expressing Dab1 binding-mutant ApoER2, which suggests the involvement of additional signaling mechanisms in the Reelin-mediated, ApoER2-dependent increase of synaptic plasticity (Beffert *et al*., 2006b).

2.4.2 Molecular Mechanisms of ApoE Receptor Signaling at the Synapse

The generation of knockin mice that constitutively either express or lack the proline-rich intracellular sequence encoded by the alternatively spliced exon 19 (plus-insert and minus-insert mice) provided important insights into the molecular mechanisms that are involved in the Reelin-dependent modulation of LTP and behavior

(Beffert *et al*., 2005). The ApoER2 insert mediates the interaction of ApoER2 with the modular adapter proteins JIP1 and JIP2 (Gotthardt *et al*., 2000; Stockinger *et al*., 2000), which can aggregate components of a mitogen-activated protein (MAP) kinase module on the intracellular receptor tail (Yasuda *et al*., 1999) and can also bind to the NPXY motif of APP with their carboxyl-terminal PTB domain (Taru *et al*., 2002). Furthermore, it mediates the splice form-specific interaction of ApoER2 with postsynaptic density-95 (PSD95) (Gotthardt *et al*., 2000; Beffert *et al*., 2005), a multidomain synaptic scaffolding protein that also interacts with another LDL receptor gene family member, LRP1 (May *et al*., 2004). Whereas baseline LTP was not dependent on the constitutive absence or presence of the alternatively spliced exon 19, the Reelin-mediated increase in LTP was absent in the minus-insert mice (Beffert *et al*., 2005). Interestingly, the alternatively spliced ApoER2 tail insert is dispensable during neurodevelopment, as indicated by the absence of neuronal positioning defects in the minus-insert knockin mice; accordingly, the activation of Dab1 by Reelin was unaltered in these mice (Beffert *et al*., 2005). The intracellular proline-rich sequence of ApoER2 seems to be required for the Reelin-induced and Dab1-dependent activation and recruitmemt of the SFK Fyn to the *N*-methyl-D-aspartate (NMDA) receptor complex, whose activity is regulated by the tyrosine phosphorylation of its subunits NR2A and NR2B (Beffert *et al*., 2005; Chen *et al*., 2005; Qiu *et al*., 2006). In addition to the PSD95-dependent recruitment of the insert-containing receptor variant to the postsynaptic density (Beffert *et al*., 2005), coupling of ApoER2 to the NMDA receptor complex can also occur in the absence of the insert, by interactions of the extracellular domains of ApoER2 and the NR1 subunit of NMDAR (Hoe *et al*., 2006a). The regulation of exon 19 alternative splicing by physical activity (Beffert *et al*., 2005), the possible interference of secreted soluble ApoER2 (Koch *et al*., 2002) with NMDAR, the coupling of the insert-containing form of ApoER2 with the kinesin motor via JIP1/2, the isoform-specific modulation of ApoER2–ligand interaction by ApoE, and the association of additional LDL receptor gene family members, including LRP1 (May *et al*., 2004), with postsynaptic NMDA receptors illustrate the high level of complexity of ApoE receptor signaling at the synapse (Fig. 2.3). Potential implications of this emerging field for neurological and psychiatric human disorders, including neurodegeneration, schizophrenia, autism, and temporal lobe epilepsy, are discussed in detail in several other chapters of this volume (see chapters by Lintas and Persico, Fatemi *et al*., Costa *et al*., Qiu and Weeber, and Abdolmaleky *et al*.).

3 Perspective

Although significant progress in the understanding of the role of ApoER2 and VLDLR as Reelin receptors during neurodevelopment and in the regulation of synaptic plasticity has been achieved within the past few years, and ascribing novel and unanticipitated functions to LDL receptor gene family members in signal transduction, important questions remain unsolved. For example, it is not clear why both

ApoER2 and VLDLR are required for Reelin-induced enhancement of LTP, whereas both receptors can compensate at least partially for each other during neuronal positioning in the developing brain. Another unresolved issue is the involvement of co-factors, which could represent co-receptors, co-ligands, or additional intracellular adapter molecules, in the modulation of the Reelin response. As mentioned above, induction of Dab1 tyrosine phosphorylation, albeit absolutely essential for the regulation of neuronal positioning in the neocortex (Howell *et al*., 2000), is not sufficient to correct the reeler phenotype in embryonic neocortical slices (Jossin *et al*., 2004). How do the Reelin–Dab1 and the Cdk5 signaling pathways interact? Interestingly, genetic ablation of either of the Cdk5 activators p35 or p39 inhibits the effect of Reelin on LTP, although no discernible effect of Cdk5 inhibition on Reelin signaling was observed (Beffert *et al*., 2004).

Finally, the biochemical and functional interaction of ApoER2, VLDLR, and LRP1 with the NMDA receptor at the synapse points toward the crucial question of how the different isoforms of ApoE, its ApoE receptors, and modulating ligands such as Reelin or amyloid-beta, which regulates NMDA receptor trafficking (Snyder *et al*., 2005), cooperate to control synaptic transmission at the molecular and cellular level (Fig 2.3).

References

Andersen, O. M., and Willnow, T. E. (2006). Lipoprotein receptors in Alzheimer's disease. *Trends Neurosci*. 29:687–694.

Andersen, O. M., Yeung, C. H., Vorum, H., Wellner, M., Andreassen, T. K., Erdmann, B., Mueller, E. C., Herz, J., Otto, A., Cooper, T. G., and Willnow, T. E. (2003). Essential role of the apolipoprotein E receptor-2 in sperm development. *J. Biol. Chem*. 278:23989–23995.

Andersen, O. M., Reiche, J., Schmidt, V., Gotthardt, M., Spoelgen, R., Behlke, J., von Arnim, C. A., Breiderhoff, T., Jansen, P., Wu, X., Bales, K. R., Cappai, R., Masters, C. L., Gliemann, J., Mufson, E. J., Hyman, B. T., Paul, S. M., Nykjaer, A., and Willnow, T. E. (2005). Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. *Proc. Natl. Acad. Sci. USA* 102:13461–13466.

Arnaud, L., Ballif, B. A., and Cooper, J. A. (2003a). Regulation of protein tyrosine kinase signaling by substrate degradation during brain development. *Mol. Cell. Biol* 23:9293–9302.

Arnaud, L., Ballif, B. A., Forster, E., and Cooper, J. A. (2003b). Fyn tyrosine kinase is a critical regulator of disabled-1 during brain development. *Curr. Biol*. 13:9–17.

Assadi, A. H., Zhang, G., Beffert, U., McNeil, R. S., Renfro, A. L., Niu, S., Quattrocchi, C. C., Antalffy, B. A., Sheldon, M., Armstrong, D. D., Wynshaw-Boris, A., Herz, J., D'Arcangelo, G., and Clark, G. D. (2003). Interaction of reelin signaling and Lis1 in brain development. *Nature Genet*. 35:270–276.

Bacskai, B. J., Xia, M. Q., Strickland, D. K., Rebeck, G. W., and Hyman, B. T. (2000). The endocytic receptor protein LRP also mediates neuronal calcium signaling via N-methyl-D-aspartate receptors. *Proc. Natl. Acad. Sci. USA* 97:11551–11556.

Ballif, B. A., Arnaud, L., and Cooper, J. A. (2003). Tyrosine phosphorylation of disabled-1 is essential for reelin-stimulated activation of Akt and Src family kinases. *Brain Res. Mol. Brain Res*. 117:152–159.

Ballif, B. A., Arnaud, L., Arthur, W. T., Guris, D., Imamoto, A., and Cooper, J. A. (2004). Activation of a Dab1/CrkL/C3G/Rap1 pathway in reelin-stimulated neurons. *Curr. Biol*. 14:606–610.

Beffert, U., Morfini, G., Bock, H. H., Reyna, H., Brady, S. T., and Herz, J. (2002). Reelin-mediated signaling locally regulates protein kinase B/Akt and glycogen synthase kinase 3beta. *J. Biol. Chem*. 277:49958–49964.

Beffert, U., Weeber, E. J., Morfini, G., Ko, J., Brady, S. T., Tsai, L. H., Sweatt, J. D., and Herz, J. (2004). Reelin and cyclin-dependent kinase 5-dependent signals cooperate in regulating neuronal migration and synaptic transmission. *J. Neurosci*. 24:1897–1906.

Beffert, U., Weeber, E. J., Durudas, A., Qiu, S., Masiulis, I., Sweatt, J. D., Li, W. P., Adelmann, G., Frotscher, M., Hammer, R. E., and Herz, J. (2005). Modulation of synaptic plasticity and memory by reelin involves differential splicing of the lipoprotein receptor apoer2. *Neuron* 47:567–579.

Beffert, U., Nematollah Farsian, F., Masiulis, I., Hammer, R. E., Yoon, S. O., Giehl, K. M., and Herz, J. (2006a). ApoE receptor 2 controls neuronal survival in the adult brain. *Curr. Biol*. 16:2446–2452.

Beffert, U., Durudas, A., Weeber, E. J., Stolt, P. C., Giehl, K. M., Sweatt, J. D., Hammer, R. E., and Herz, J. (2006b). Functional dissection of reelin signaling by site-directed disruption of disabled-1 adaptor binding to apolipoprotein E receptor 2: Distinct roles in development and synaptic plasticity. *J. Neurosci*. 26:2041–2052.

Benhayon, D., Magdaleno, S., and Curran, T. (2003). Binding of purified reelin to ApoER2 and VLDLR mediates tyrosine phosphorylation of disabled-1. *Brain Res. Mol. Brain Res*. 112:33–45.

Bock, H. H., and Herz, J. (2003). Reelin activates Src family tyrosine kinases in neurons. *Curr. Biol*. 13:18–26.

Bock, H. H., Jossin, Y., Liu, P., Forster, E., May, P., Goffinet, A. M., and Herz, J. (2003). Phosphatidylinositol 3-kinase interacts with the adaptor protein Dab1 in response to reelin signaling and is required for normal cortical lamination. *J. Biol. Chem*. 278:38772–38779.

Bock, H. H., Jossin, Y., May, P., Bergner, O., and Herz, J. (2004). Apolipoprotein E receptors are required for reelin-induced proteasomal degradation of the neuronal adaptor protein disabled-1. *J. Biol. Chem*. 279:33471–33479.

Bonifacino, J. S., and Traub, L. M. (2003). Signals for sorting of transmembrane proteins to endosomes and lysosomes. *Annu. Rev. Biochem*. 72:395–447.

Bos, J. L. (2005). Linking Rap to cell adhesion. *Curr. Opin. Cell Biol*. 17:123–128.

Boycott, K. M., Flavelle, S., Bureau, A., Glass, H. C., Fujiwara, T. M., Wirrell, E., Davey, K., Chudley, A. E., Scott, J. N., McLeod, D. R., and Parboosingh, J. S. (2005). Homozygous deletion of the very low density lipoprotein receptor gene causes autosomal recessive cerebellar hypoplasia with cerebral gyral simplification. *Am. J. Hum. Genet*. 77:477–483.

Boyles, J. K., Pitas, R. E., Wilson, E., Mahley, R. W., and Taylor, J. M. (1985). Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. *J. Clin. Invest*. 76:1501–1513.

Boyles, J. K., Zoellner, C. D., Anderson, L. J., Kosik, L. M., Pitas, R. E., Weisgraber, K. H., Hui, D. Y., Mahley, R. W., Gebicke-Haerter, P. J., Ignatius, M. J., and Shooter, E. M. (1989). A role for apolipoprotein E, apolipoprotein A-I, and low density lipoprotein receptors in cholesterol transport during regeneration and remyelination of the rat sciatic nerve. *J. Clin. Invest*. 83:1015–1031.

Brandes, C., Novak, S., Stockinger, W., Herz, J., Schneider, W. J., and Nimpf, J. (1997). Avian and murine LR8B and human apolipoprotein E receptor 2: differentially spliced products from corresponding genes. *Genomics* 42:185–191.

Brandes, C., Kahr, L., Stockinger, W., Hiesberger, T., Schneider, W. J., and Nimpf, J. (2001). Alternative splicing in the ligand binding domain of mouse ApoE receptor-2 produces receptor variants binding reelin but not alpha 2-macroglobulin. *J. Biol. Chem*. 276:22160–22169.

Brich, J., Shie, F. S., Howell, B. W., Li, R., Tus, K., Wakeland, E. K., Jin, L. W., Mumby, M., Churchill, G., Herz, J., and Cooper, J. A. (2003). Genetic modulation of tau phosphorylation in the mouse. *J. Neurosci*. 23:187–192.

Bu, G., and Schwartz, A. L. (1998). RAP, a novel type of ER chaperone. *Trends Cell Biol*. 8:272–276.

Calderwood, D. A., Fujioka, Y., de Pereda, J. M., Garcia-Alvarez, B., Nakamoto, T., Margolis, B., McGlade, C. J., Liddington, R. C., and Ginsberg, M. H. (2003). Integrin beta cytoplasmic domain interactions with phosphotyrosine-binding domains: A structural prototype for diversity in integrin signaling. *Proc. Natl. Acad. Sci. USA*100:2272–2277.

Cam, J. A., and Bu, G. (2006). Modulation of beta-amyloid precursor protein trafficking and processing by the low density lipoprotein receptor family. *Mol. Neurodegener*. 1:8.

Cao, X., and Sudhof, T. C. (2001). A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science* 293:115–120.

Cariboni, A., Rakic, S., Liapi, A., Maggi, R., Goffinet, A., and Parnavelas, J. G. (2005). Reelin provides an inhibitory signal in the migration of gonadotropin-releasing hormone neurons. *Development* 132:4709–4718.

Chen, K., Ochalski, P. G., Tran, T. S., Sahir, N., Schubert, M., Pramatarova, A., and Howell, B. W. (2004). Interaction between Dab1 and CrkII is promoted by reelin signaling. *J. Cell Sci*. 117:4527–4536.

Chen, Y., Beffert, U., Ertunc, M., Tang, T. S., Kavalali, E. T., Bezprozvanny, I., and Herz, J. (2005). Reelin modulates NMDA receptor activity in cortical neurons. *J. Neurosci*. 25:8209–8216.

Clatworthy, A. E., Stockinger, W., Christie, R. H., Schneider, W. J., Nimpf, J., Hyman, B. T., and Rebeck, G. W. (1999). Expression and alternate splicing of apolipoprotein E receptor 2 in brain. *Neuroscience* 90:903–911.

Cooper, J. A., and Howell, B. W. (1999). Lipoprotein receptors: signaling functions in the brain? *Cell* 97:671–674.

D'Arcangelo, G., Homayouni, R., Keshvara, L., Rice, D. S., Sheldon, M., and Curran, T. (1999). Reelin is a ligand for lipoprotein receptors. *Neuron* 24:471–479.

Dhavan, R., and Tsai, L. H. (2001). A decade of CDK5. *Nature Rev. Mol. Cell Biol*. 2:749–759.

Dietschy, J. M., and Turley, S. D. (2004). Thematic review series: Brain lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *J. Lipid Res*. 45:1375–1397.

Dulabon, L., Olson, E. C., Taglienti, M. G., Eisenhuth, S., McGrath, B., Walsh, C. A., Kreidberg, J. A., and Anton, E. S. (2000). Reelin binds alpha3beta1 integrin and inhibits neuronal migration. *Neuron* 27:33–44.

Feng, Y., and Walsh, C. A. (2001). Protein–protein interactions, cytoskeletal regulation and neuronal migration. *Nature Rev. Neurosci*. 2:408–416.

Forster, E., Tielsch, A., Saum, B., Weiss, K. H., Johanssen, C., Graus-Porta, D., Muller, U., and Frotscher, M. (2002). Reelin, disabled 1, and beta 1 integrins are required for the formation of the radial glial scaffold in the hippocampus. *Proc. Natl. Acad. Sci. USA*99:13178–13183.

Frykman, P. K., Brown, M. S., Yamamoto, T., Goldstein, J. L., and Herz, J. (1995). Normal plasma lipoproteins and fertility in gene-targeted mice homozygous for a disruption in the gene encoding very low density lipoprotein receptor. *Proc. Natl. Acad. Sci. USA* 92:8453–8457.

Goedert, M., and Spillantini, M. G. (2006). A century of Alzheimer's disease. *Science* 314:777–781.

Gotthardt, M., Trommsdorff, M., Nevitt, M. F., Shelton, J., Richardson, J. A., Stockinger, W., Nimpf, J., and Herz, J. (2000). Interactions of the low density lipoprotein receptor gene family with cytosolic adaptor and scaffold proteins suggest diverse biological functions in cellular communication and signal transduction. *J. Biol. Chem*. 275:25616–25624.

Goudriaan, J. R., Tacken, P. J., Dahlmans, V. E., Gijbels, M. J., van Dijk, K. W., Havekes, L. M., and Jong, M. C. (2001). Protection from obesity in mice lacking the VLDL receptor. *Arterioscler. Thromb. Vasc. Biol*. 21:1488–1493.

Grant, S. G., O'Dell, T. J., Karl, K. A., Stein, P. L., Soriano, P., and Kandel, E. R. (1992). Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science* 258:1903–1910.

Heckenlively, J. R., Hawes, N. L., Friedlander, M., Nusinowitz, S., Hurd, R., Davisson, M., and Chang, B. (2003). Mouse model of subretinal neovascularization with choroidal anastomosis. *Retina* 23:518–522.

Helbecque, N., and Amouyel, P. (2000). Very low density lipoprotein receptor in Alzheimer disease. *Microsc. Res. Tech*. 50:273–277.

Helbecque, N., Berr, C., Cottel, D., Fromentin-David, I., Sazdovitch, V., Ricolfi, F., Ducimetiere, P., Di Menza, C., and Amouyel, P. (2001). VLDL receptor polymorphism, cognitive impairment, and dementia. *Neurology* 56:1183–1188.

Herz, J. (2006). The switch on the RAPper's necklace. *Mol. Cell* 23:451–455.

Herz, J., and Beffert, U. (2000). Apolipoprotein E receptors: linking brain development and Alzheimer's disease. *Nature Rev. Neurosci*. 1:51–58.

Herz, J., and Bock, H. H. (2002). Lipoprotein receptors in the nervous system. *Annu. Rev. Biochem*. 71:405–434.

Herz, J., and Chen, Y. (2006). Reelin, lipoprotein receptors and synaptic plasticity. *Nature Rev. Neurosci*. 7:850–859.

Hiesberger, T., Trommsdorff, M., Howell, B. W., Goffinet, A., Mumby, M. C., Cooper, J. A., and Herz, J. (1999). Direct binding of reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron* 24: 481–489.

Ho, A., and Sudhof, T. C. (2004). Binding of F-spondin to amyloid-beta precursor protein: a candidate amyloid-beta precursor protein ligand that modulates amyloid-beta precursor protein cleavage. *Proc. Natl. Acad. Sci. USA* 101:2548–2553.

Hoe, H. S., and Rebeck, G. W. (2005). Regulation of ApoE receptor proteolysis by ligand binding. *Brain Res. Mol. Brain Res*. 137:31–39.

Hoe, H. S., Wessner, D., Beffert, U., Becker, A. G., Matsuoka, Y., and Rebeck, G. W. (2005). F-spondin interaction with the apolipoprotein E receptor ApoEr2 affects processing of amyloid precursor protein. *Mol. Cell Biol*. 25:9259–9268.

Hoe, H. S., Pocivavsek, A., Chakraborty, G., Fu, Z., Vicini, S., Ehlers, M. D., and Rebeck, G. W. (2006a). Apolipoprotein E receptor 2 interactions with the N-methyl-D-aspartate receptor. *J. Biol. Chem*. 281:3425–3431.

Hoe, H. S., Tran, T. S., Matsuoka, Y., Howell, B. W., and Rebeck, G. W. (2006b). DAB1 and reelin effects on amyloid precursor protein and ApoE receptor 2 trafficking and processing. *J. Biol. Chem*. 281:35176–35185.

Homayouni, R., Rice, D. S., Sheldon, M., and Curran, T. (1999). Disabled-1 binds to the cytoplasmic domain of amyloid precursor-like protein 1. *J. Neurosci*. 19:7507–7515.

Hong, S. E., Shugart, Y. Y., Huang, D. T., Shahwan, S. A., Grant, P. E., Hourihane, J. O., Martin, N. D., and Walsh, C. A. (2000). Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nature Genet*. 26:93–96.

Howell, B. W., Gertler, F. B., and Cooper, J. A. (1997a). Mouse disabled (mDab1): a Src binding protein implicated in neuronal development. *EMBO J*. 16:121–132.

Howell, B. W., Hawkes, R., Soriano, P., and Cooper, J. A. (1997b). Neuronal position in the developing brain is regulated by mouse disabled-1. *Nature* 389:733–737.

Howell, B. W., Herrick, T. M., and Cooper, J. A. (1999a). Reelin-induced tryosine phosphorylation of disabled 1 during neuronal positioning. *Genes Dev*. 13:643–648.

Howell, B. W., Lanier, L. M., Frank, R., Gertler, F. B., and Cooper, J. A. (1999b). The disabled 1 phosphotyrosine-binding domain binds to the internalization signals of transmembrane glycoproteins and to phospholipids. *Mol. Cell Biol*. 19:5179–5188.

Howell, B. W., Herrick, T. M., Hildebrand, J. D., Zhang, Y., and Cooper, J. A. (2000). Dab1 tyrosine phosphorylation sites relay positional signals during mouse brain development. *Curr. Biol*. 10:877–885.

Huang, Y., Magdaleno, S., Hopkins, R., Slaughter, C., Curran, T., and Keshvara, L. (2004). Tyrosine phosphorylated disabled 1 recruits Crk family adapter proteins. *Biochem. Biophys. Res. Commun*. 318:204–212.

Huang, Y., Shah, V., Liu, T., and Keshvara, L. (2005). Signaling through disabled 1 requires phosphoinositide binding. *Biochem. Biophys. Res. Commun*. 331:1460–1468.

Ignatius, M. J., Shooter, E. M., Pitas, R. E., and Mahley, R. W. (1987). Lipoprotein uptake by neuronal growth cones in vitro. *Science* 236:959–962.

Jossin, Y., Ogawa, M., Metin, C., Tissir, F., and Goffinet, A. M. (2003). Inhibition of SRC family kinases and non-classical protein kinases C induce a reeler-like malformation of cortical plate development. *J. Neurosci*. 23:9953–9959.

Jossin, Y., Ignatova, N., Hiesberger, T., Herz, J., Lambert de Rouvroit, C., and Goffinet, A. M. (2004). The central fragment of reelin, generated by proteolytic processing in vivo, is critical to its function during cortical plate development. *J. Neurosci*. 24:514–521.

Keshvara, L., Benhayon, D., Magdaleno, S., and Curran, T. (2001). Identification of reelin-induced sites of tyrosyl phosphorylation on disabled 1. *J. Biol. Chem*. 276:16008–16014.

Kinoshita, A., Shah, T., Tangredi, M. M., Strickland, D. K., and Hyman, B. T. (2003). The intracellular domain of the low density lipoprotein receptor-related protein modulates transactivation mediated by amyloid precursor protein and Fe65. *J. Biol. Chem*. 278:41182–41188.

Koch, S., Strasser, V., Hauser, C., Fasching, D., Brandes, C., Bajari, T. M., Schneider, W. J., and Nimpf, J. (2002). A secreted soluble form of ApoE receptor 2 acts as a dominant-negative receptor and inhibits reelin signaling. **EMBO** J. 21:5996–6004.

Kuo, G., Arnaud, L., Kronstad-O'Brien, P., and Cooper, J. A. (2005). Absence of Fyn and Src causes a reeler-like phenotype. *J. Neurosci*. 25:8578–8586.

Ma, S. L., Ng, H. K., Baum, L., Pang, J. C., Chiu, H. F., Woo, J., Tang, N. L., and Lam, L. C. (2002). Low-density lipoprotein receptor-related protein 8 (apolipoprotein E receptor 2) gene polymorphisms in Alzheimer's disease. *Neurosci. Lett*. 332:216–218.

Mahley, R. W. (1988). Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 240:622–630.

Mahley, R. W., and Rall, S. C., Jr. (2000). Apolipoprotein E: far more than a lipid transport protein. *Annu. Rev. Genomics Hum. Genet*. 1:507–537.

Mahley, R. W., Innerarity, T. L., Rall, S. C., Jr., and Weisgraber, K. H. (1984). Plasma lipoproteins: apolipoprotein structure and function. *J. Lipid Res*. 25:1277–1294.

Mahley, R. W., Weisgraber, K. H., and Huang, Y. (2006). Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* 103:5644–5651.

Mauch, D. H., Nagler, K., Schumacher, S., Goritz, C., Muller, E. C., Otto, A., and Pfrieger, F. W. (2001). CNS synaptogenesis promoted by glia-derived cholesterol. *Science* 294:1354–1357.

May, P., and Herz, J. (2003). LDL receptor-related proteins in neurodevelopment. *Traffic* 4:291–301.

May, P., Reddy, Y. K., and Herz, J. (2002). Proteolytic processing of low density lipoprotein receptor-related protein mediates regulated release of its intracellular domain. *J. Biol. Chem*. 277:18736–18743.

May, P., Bock, H. H., Nimpf, J., and Herz, J. (2003). Differential glycosylation regulates processing of lipoprotein receptors by gamma-secretase. *J. Biol. Chem*. 278:37386–37392.

May, P., Rohlmann, A., Bock, H. H., Zurhove, K., Marth, J. D., Schomburg, E. D., Noebels, J. L., Beffert, U., Sweatt, J. D., Weeber, E. J., and Herz, J. (2004). Neuronal LRP1 functionally associates with postsynaptic proteins and is required for normal motor function in mice. *Mol. Cell Biol*. 24:8872–8883.

May, P., Herz, J., and Bock, H. H. (2005). Molecular mechanisms of lipoprotein receptor signalling. *Cell. Mol. Life Sci*. 62:2325–2338.

Mayer, H., Duit, S., Hauser, C., Schneider, W. J., and Nimpf, J. (2006). Reconstitution of the reelin signaling pathway in fibroblasts demonstrates that Dab1 phosphorylation is independent of receptor localization in lipid rafts. *Mol. Cell Biol*. 26:19–27.

Merdes, G., Soba, P., Loewer, A., Bilic, M. V., Beyreuther, K., and Paro, R. (2004). Interference of human and Drosophila APP and APP-like proteins with PNS development in Drosophila. *EMBO J*. 23:4082–4095.

Morimura, T., Hattori, M., Ogawa, M., and Mikoshiba, K. (2005). Disabled1 regulates the intracellular trafficking of reelin receptors. *J. Biol. Chem*. 280:16901–16908.

Niethammer, M., Smith, D. S., Ayala, R., Peng, J., Ko, J., Lee, M. S., Morabito, M., and Tsai, L. H. (2000). NUDEL is a novel Cdk5 substrate that associates with LIS1 and cytoplasmic dynein. *Neuron* 28:697–711.

Nykjaer, A., and Willnow, T. E. (2002). The low-density lipoprotein receptor gene family: a cellular Swiss army knife? *Trends Cell Biol*. 12:273–280.

Ohshima, T., Ogawa, M., Veeranna, Hirasawa, M., Longenecker, G., Ishiguro, K., Pant, H. C., Brady, R. O., Kulkarni, A. B., and Mikoshiba, K. (2001). Synergistic contributions of cyclin-dependent kinase 5/p35 and reelin/Dab1 to the positioning of cortical neurons in the developing mouse brain. *Proc. Natl. Acad. Sci. USA* 98:2764–2769.

Ohshima, T., Suzuki, H., Morimura, T., Ogawa, M., and Mikoshiba, K. (2007). Modulation of reelin signaling by cyclin-dependent kinase 5. *Brain Res*. 1140:84–95.

Okuizumi, K., Onodera, O., Namba, Y., Ikeda, K., Yamamoto, T., Seki, K., Ueki, A., Nanko, S., Tanaka, H., Takahashi, H., Oyanagi, K., Mizusawa, H., Kanazawa, I., and Tsuji, S. (1995). Genetic association of the very low density lipoprotein (VLDL) receptor gene with sporadic Alzheimer's disease. *Nature Genet*. 11:207–209.

Pitas, R. E., Boyles, J. K., Lee, S. H., Hui, D., and Weisgraber, K. H. (1987). Lipoproteins and their receptors in the central nervous system. Characterization of the lipoproteins in cerebrospinal fluid and identification of apolipoprotein B,E(LDL) receptors in the brain. *J. Biol. Chem*. 262:14352–14360.

Pramatarova, A., Ochalski, P. G., Chen, K., Gropman, A., Myers, S., Min, K. T., and Howell, B. W. (2003). Nck beta interacts with tyrosine-phosphorylated disabled 1 and redistributes in reelin-stimulated neurons. *Mol. Cell Biol*. 23:7210–7221.

Pramatarova, A., Ochalski, P. G., Lee, C. H., and Howell, B. W. (2006). Mouse disabled 1 regulates the nuclear position of neurons in a Drosophila eye model. *Mol. Cell Biol*. 26:1510–1517.

Qiu, S., Zhao, L. F., Korwek, K. M., and Weeber, E. J. (2006). Differential reelin-induced enhancement of NMDA and AMPA receptor activity in the adult hippocampus. *J. Neurosci*. 26:12943–12955.

Rice, D. S., Sheldon, M., D'Arcangelo, G., Nakajima, K., Goldowitz, D., and Curran, T. (1998). Disabled-1 acts downstream of reelin in a signaling pathway that controls laminar organization in the mammalian brain. *Development* 125:3719–3729.

Roses, A. D. (1996). Apolipoprotein E alleles as risk factors in Alzheimer's disease. *Annu. Rev. Med*. 47:387–400.

Rossel, M., Loulier, K., Feuillet, C., Alonso, S., and Carroll, P. (2005). Reelin signaling is necessary for a specific step in the migration of hindbrain efferent neurons. *Development* 132:1175–1185.

Sanada, K., Gupta, A., and Tsai, L. H. (2004). Disabled-1-regulated adhesion of migrating neurons to radial glial fiber contributes to neuronal positioning during early corticogenesis. *Neuron* 42:197–211.

Sasaki, S., Shionoya, A., Ishida, M., Gambello, M.J., Yingling, J., Wynshaw-Boris, A., and Hirotsune, S. (2000). A LIS1/NUDEL/cytoplasmic dynein heavy chain complex in the developing and adult nervous system. *Neuron* 28:681–696.

Sato, N., Fukushima, N., Chang, R., Matsubayashi, H., and Goggins, M. (2006). Differential and epigenetic gene expression profiling identifies frequent disruption of the RELN pathway in pancreatic cancers. *Gastroenterology* 130:548–565.

Schneider, W. J., and Nimpf, J. (2003). LDL receptor relatives at the crossroad of endocytosis and signaling. *Cell. Mol. Life Sci*. 60:892–903.

Schneider, W. J., Nimpf, J., and Bujo, H. (1997). Novel members of the low density lipoprotein receptor superfamily and their potential roles in lipid metabolism. *Curr. Opin. Lipidol*. 8:315–319.

Sheldon, M., Rice, D. S., D'Arcangelo, G., Yoneshima, H., Nakajima, K., Mikoshiba, K., Howell, B. W., Cooper, J. A., Goldowitz, D., and Curran, T. (1997). Scrambler and yotari disrupt the disabled gene and produce a reeler-like phenotype in mice. *Nature* 389:730–733.

Sinha, S., and Lieberburg, I. (1999). Cellular mechanisms of beta-amyloid production and secretion. *Proc. Natl. Acad. Sci. USA* 96:11049–11053.

Snyder, E. M., Nong, Y., Almeida, C. G., Paul, S., Moran, T., Choi, E. Y., Nairn, A. C., Salter, M. W., Lombroso, P. J., Gouras, G. K., and Greengard, P. (2005). Regulation of NMDA receptor trafficking by amyloid-beta. *Nature Neurosci*. 8:1051–1058.

Stockinger, W., Brandes, C., Fasching, D., Hermann, M., Gotthardt, M., Herz, J., Schneider, W. J., and Nimpf, J. (2000). The reelin receptor ApoER2 recruits JNK-interacting proteins-1 and -2. *J. Biol. Chem*. 275:25625–25632.

Stolt, P. C., and Bock, H. H. (2006). Modulation of lipoprotein receptor functions by intracellular adaptor proteins. *Cell Signal* 18:1560–1571.

Stolt, P. C., Jeon, H., Song, H. K., Herz, J., Eck, M. J., and Blacklow, S. C. (2003). Origins of peptide selectivity and phosphoinositide binding revealed by structures of disabled-1 PTB domain complexes. *Structure (Cambridge)* 11:569–579.

Stolt, P. C., Vardar, D., and Blacklow, S. C. (2004). The dual-function disabled-1 PTB domain exhibits site independence in binding phosphoinositide and peptide ligands. *Biochemistry* 43:10979–10987.

Stolt, P. C., Chen, Y., Liu, P., Bock, H. H., Blacklow, S. C., and Herz, J. (2005). Phosphoinositide binding by the disabled-1 PTB domain is necessary for membrane localization and reelin signal transduction. *J. Biol. Chem*. 280:9671–9677.

Strasser, V., Fasching, D., Hauser, C., Mayer, H., Bock, H. H., Hiesberger, T., Herz, J., Weeber, E. J., Sweatt, J. D., Pramatarova, A., Howell, B., Schneider, W. J., and Nimpf, J. (2004). Receptor clustering is involved in reelin signaling. *Mol. Cell Biol*. 24:1378–1386.

Strittmatter, W. J., and Bova Hill, C. (2002). Molecular biology of apolipoprotein E. *Curr. Opin. Lipidol*. 13:119–123.

Tacken, P. J., Teusink, B., Jong, M. C., Harats, D., Havekes, L. M., van Dijk, K. W., and Hofker, M. H. (2000). LDL receptor deficiency unmasks altered VLDL triglyceride metabolism in VLDL receptor transgenic and knockout mice. *J. Lipid Res*. 41:2055–2062.

Takada, H., Imoto, I., Tsuda, H., Nakanishi, Y., Sakakura, C., Mitsufuji, S., Hirohashi, S., and Inazawa, J. (2006). Genomic loss and epigenetic silencing of very-low-density lipoprotein receptor involved in gastric carcinogenesis. *Oncogene* 25:6554–6562.

Takahashi, S., Sakai, J., Fujino, T., Hattori, H., Zenimaru, Y., Suzuki, J., Miyamori, I., and Yamamoto, T. T. (2004). The very low-density lipoprotein (VLDL) receptor: characterization and functions as a peripheral lipoprotein receptor. *J. Atheroscler. Thromb*. 11:200–208.

Taru, H., Iijima, K., Hase, M., Kirino, Y., Yagi, Y., and Suzuki, T. (2002). Interaction of Alzheimer's beta-amyloid precursor family proteins with scaffold proteins of the JNK signaling cascade. *J. Biol. Chem*. 277:20070–20078.

Trommsdorff, M., Borg, J. P., Margolis, B., and Herz, J. (1998). Interaction of cytosolic adaptor proteins with neuronal apolipoprotein E receptors and the amyloid precursor protein. *J. Biol. Chem*. 273:33556–33560.

Trommsdorff, M., Gotthardt, M., Hiesberger, T., Shelton, J., Stockinger, W., Nimpf, J., Hammer, R. E., Richardson, J. A., and Herz, J. (1999). Reeler/disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* 97:689–701.

Tsai, L. H., and Gleeson, J. G. (2005). Nucleokinesis in neuronal migration. *Neuron* 46:383–388.

Verhey, K. J., Meyer, D., Deehan, R., Blenis, J., Schnapp, B. J., Rapoport, T. A., and Margolis, B. (2001). Cargo of kinesin identified as JIP scaffolding proteins and associated signaling molecules. *J. Cell Biol*. 152:959–970.

Wang, L., Wang, X., Laird, N., Zuckerman, B., Stubblefield, P., and Xu, X. (2006). Polymorphism in maternal LRP8 gene is associated with fetal growth. *Am. J. Hum. Genet*. 78:770–777.

Ware, M. L., Fox, J. W., Gonzalez, J. L., Davis, N. M., Lambert de Rouvroit, C., Russo, C. J., Chua, S. C., Jr., Goffinet, A. M., and Walsh, C. A. (1997). Aberrant splicing of a mouse disabled homolog, mdab1, in the scrambler mouse. *Neuron* 19:239–249.

Weeber, E. J., Beffert, U., Jones, C., Christian, J. M., Forster, E., Sweatt, J. D., and Herz, J. (2002). Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. *J. Biol. Chem*. 277:39944–39952.

Willnow, T. E., Nykjaer, A., and Herz, J. (1999). Lipoprotein receptors: new roles for ancient proteins. *Nature Cell Biol*. 1:E157–162.

Wolfe, M. S. (2006). The gamma-secretase complex: membrane-embedded proteolytic ensemble. *Biochemistry* 45:7931–7939.

Xu, M., Arnaud, L., and Cooper, J. A. (2005). Both the phosphoinositide and receptor binding activities of Dab1 are required for reelin-stimulated Dab1 tyrosine phosphorylation. *Brain Res. Mol. Brain Res*. 139:300–305.

Yagi, T., Aizawa, S., Tokunaga, T., Shigetani, Y., Takeda, N., and Ikawa, Y. (1993). A role for Fyn tyrosine kinase in the suckling behaviour of neonatal mice. *Nature* 366:742–745.

Yamamoto, T., and Bujo, H. (1996). Close encounters with apolipoprotein E receptors. *Curr. Opin. Lipidol*. 7:298–302.

Yasuda, J., Whitmarsh, A. J., Cavanagh, J., Sharma, M., and Davis, R. J. (1999). The JIP group of mitogen-activated protein kinase scaffold proteins. *Mol. Cell Biol*. 19:7245–7254.

Yuasa, S., Hattori, K., and Yagi, T. (2004). Defective neocortical development in Fyn-tyrosine-kinase-deficient mice. *Neuroreport* 15:819–822.

Yun, M., Keshvara, L., Park, C. G., Zhang, Y. M., Dickerson, J. B., Zheng, J., Rock, C. O., Curran, T., and Park, H. W. (2003). Crystal structures of the Dab homology domains of mouse disabled 1 and 2. *J. Biol. Chem*. 278:36572–36581.

Zhuo, M., Holtzman, D. M., Li, Y., Osaka, H., DeMaro, J., Jacquin, M., and Bu, G. (2000). Role of tissue plasminogen activator receptor LRP in hippocampal long-term potentiation. *J. Neurosci*. 20:542–549.

Zou, Z., Chung, B., Nguyen, T., Mentone, S., Thomson, B., and Biemesderfer, D. (2004). Linking receptor-mediated endocytosis and cell signaling: evidence for regulated intramembrane proteolysis of megalin in proximal tubule. *J. Biol. Chem*. 279:34302–34310.

Chapter 3
Chemistry of Reelin

Yves Jossin

Contents

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1 Introduction

Reelin is a large extracellular protein involved in several aspects of brain development, such as cell positioning, dendrite growth, synaptic plasticity, and memory, and may be implicated as a susceptibility factor in psychoses (Caviness and Rakic, 1978; Impagnatiello *et al*., 1998; Liu *et al*., 2001; Weeber *et al*., 2002; Jossin, 2004; Beffert *et al*., 2005; Fatemi, 2005). This wide array of functions indicates that Reelin is able to trigger different intracellular signaling pathways depending on the maturation state or the type of target cell that may express different receptors or intracellular signaling modules. In this chapter, I will review the current state of knowledge on the best established and some other putative partners of the Reelin pathway (Fig. 3.1).

2 Reelin

Reelin is a bulky glycoprotein, the structure of which starts with a large N-terminal segment that contains an F-spondin homology domain (163 residues) and a unique region (309 residues). The main body of Reelin consists of 8 repeats of 350–390

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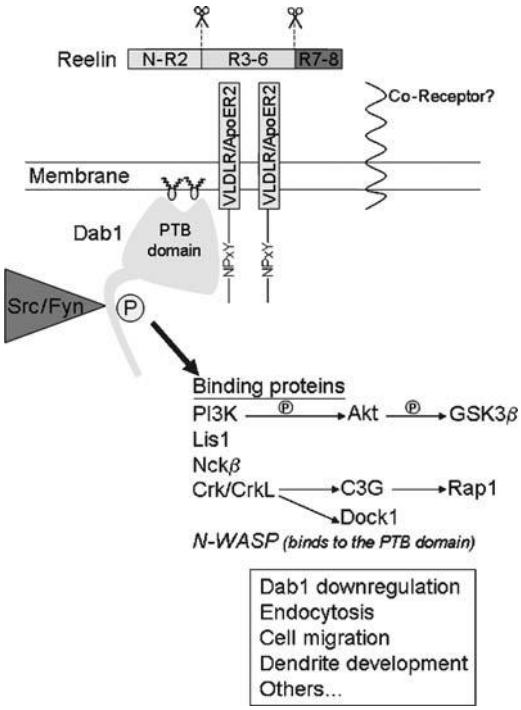

Fig. 3.1 Summary of the Reelin signaling pathway. See text for details (*See Color Plates*)

amino acids. Each repeat contains two related subdomains (A and B) flanking a pattern of conserved cysteine residues that form EGF-like motifs. The protein ends with a basic stretch of 33 amino acids (D'Arcangelo *et al*., 1995) (Fig. 3.2). It is secreted by several different classes of cells, particularly Cajal-Retzius cells (CR) (Schiffmann *et al*., 1997).

Reelin is processed in the extracellular environment at two sites by unidentified, and presumably redundant, metalloproteinases, resulting in the production of five processed fragments (Lambert de Rouvroit *et al*., 1999) (Fig. 3.2). Because of the difficulty in estimating high-molecular-weight proteins in gels, those shown in Fig. 3.2 may slightly differ from previously published sizes. We estimated the sizes in Western blot using C-terminal antibodies that revealed three bands (approx. 450, 370, and 180 kDa), N-terminal antibodies that revealed three fragments (approx. 450, 270, and 80 kDa), and new unpublished antibodies (Jossin *et al*., unpublished data) directed against epitopes in the central fragment that revealed four fragments (approx. 450, 370, 270, and 190 kDa). Processing does not decrease activity, as recombinant proteins made of the predicted central fragments (repeats 3–6) bind to lipoprotein receptors, trigger Disabled-1 (Dab1, see below) phosphorylation, and mimic functions of Reelin during cortical plate development (Jossin *et al*., 2004). Electron micrographs of this active R3–6 fragment exhibit a rodlike shape (Nogi *et al*., 2006). Rather

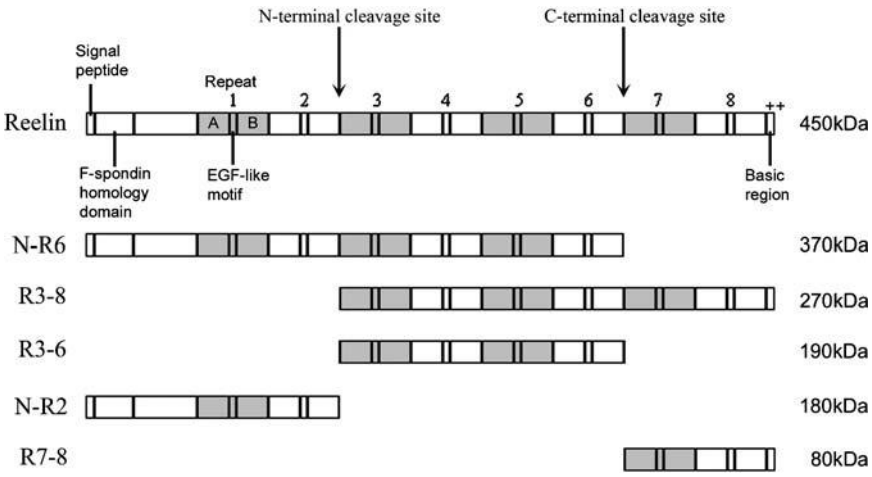

Fig. 3.2 Reelin is cleaved at two sites (N-t and C-t), thus generating five fragments named according to their composition. The corresponding size observed in SDS-PAGE gels is shown on the right-hand side. (Adapted from Jossin, 2004)

surprisingly, although a protein corresponding to the N-terminal fragment of Reelin (N-R2) does not bind to the lipoprotein receptors VLDLR and ApoER2, and a Reelin form amputated of the N-R2 fragment is biologically active (Jossin *et al*., 2004), the CR50 antibody which is directed against an N-terminal epitope is able to block the action of Reelin (Miyata *et al*., 1997). This antibody interferes with the aggregation of Reelin, which may affect its function (Utsunomiya-Tate *et al*., 2000). Clearly, there are still many questions about the role of the different parts of the protein that remain to be studied further.

3 Reelin Receptors: VLDLR and ApoER2

Reelin binds directly to the ectodomains of the lipoprotein receptor Very-Low-Density Lipoprotein Receptor (VLDLR) and Apolipoprotein E Receptor Type 2 (ApoER2) (D'Arcangelo *et al*., 1999; Hiesberger *et al*., 1999), and the repeats 3 to 6 are necessary and sufficient for this binding (Jossin *et al*., 2004). Both receptors are expressed by the neurons, in the cortex and elsewhere, that are Reelin target cells, and mice with double inactivation of the *Vldlr* and *Apoer2* genes have a reelerlike phenotype, whereas single receptor gene mutations generate subtle phenotypes (Trommsdorff *et al*., 1999).

Mice lacking an alternatively spliced exon in the intracellular domain of ApoER2 have normal cortical development but perform poorly in learning and

memory tasks. Reelin enhances LTP through ApoER2 in complex with NMDA receptors in postsynaptic densities of excitatory synapses by inducing the tyrosine phosphorylation of NMDA receptor subunits, which is dependent on the presence of this alternatively spliced exon (Beffert *et al*., 2005).

4 Other Candidate Receptors

The involvement of a Reelin coreceptor in complex or in collaboration with VLDLR and ApoER2 has been proposed but never convincingly demonstrated. Reelin associates with two or more lipoprotein receptor molecules simultaneously to achieve high-affinity interaction (Andersen *et al*., 2003). However, the addition of cross-linking antibodies directed toward the extracellular domain of VLDLR and ApoER2 induces the phosphorylation of Dab1 but is not sufficient to correct the mutant phenotype, whereas a similar induction of Dab1 phosphorylation by recombinant Reelin is able to rescue the phenotype, thus indicating the possible requirement of a coreceptor for full signaling, but not for Dab1 phosphorylation (Jossin *et al*., 2004). Results using a divalent RAP-Fc fusion protein support this view (Strasser *et al*., 2004). However, these negative results could have other explanations and the requirement for a coreceptor still remains unclear.

The proposal that the protocadherin cadherin-related neuronal receptor-1 (CNR1) is a Reelin receptor (Senzaki *et al*., 1999) has been disproved (Jossin *et al*., 2004). The Reelin-alkaline phosphatase fusion protein used in the binding study of Senzaki *et al*. (1999) was extracted from cell lysates and not secreted, suggesting that it may not be properly folded and may bind nonspecifically to CNR1. Our results with identical CNR1 ectodomain fusion proteins, but a secreted and biologically active Reelin, demonstrated no detectable interaction between CNR1 and Reelin, whereas binding of Reelin to VLDLR and ApoER2 was consistently observed in the same conditions (Jossin *et al*., 2004).

$\alpha 3\beta 1$ integrins can bind to the N-terminal region of Reelin, and this has been proposed to inhibit neuronal migration by stimulating the detachment of neurons from radial glia (Dulabon *et al*., 2000; Schmid *et al*., 2005). The relatively high proportion of the N-terminal Reelin fragment and the reduction in Dab1 protein levels in cerebral cortices of $\alpha 3$ integrin-deficient mice (Dulabon *et al*., 2000), and the increased level of $\alpha 3$ integrins in Dab1 mutant cortices (Sanada *et al*., 2004) suggest an interaction with the Reelin pathway. However, the phenotypes of mice with brain-specific inactivation of $\beta 1$ integrins (Graus-Porta *et al*., 2001) or the $\alpha 3$ subunit (Anton *et al*., 1999) are not reeler-like. The N-terminal fragment of Reelin which binds $\beta 1$ integrin is not necessary for the action of Reelin during cortical plate development (Jossin *et al*., 2004). Still, integrins may be involved in other functions of Reelin particularly in synaptic plasticity (Dong *et al*., 2003) or in normal positioning of dentate granule cells (Forster *et al*., 2002).

5 Disabled-1

Mutations in the gene encoding the intracellular adapter protein Dab1, either induced (Howell *et al*., 1997) or spontaneous (in *scrambler* and *yotari* mutant mice), generate a reeler-like phenotype (Sheldon *et al*., 1997). There is no additional cortical defect in mice lacking both Reelin and Dab1, suggesting that the two proteins function in a linear pathway (Howell *et al*., 1999a). Similar to VLDLR and ApoER2, Dab1 is mostly expressed by neurons of the cortex and elsewhere that are targets of Reelin action (Walsh and Goffinet, 2000). Additionally, Dab1 is also expressed together with ApoER2 and VLDLR in the ventricular and subventricular zones by radial glial precursors and newborn neurons (Luque *et al*., 2003).

Dab1 possess a PI/PTB domain (Protein Interaction/Phospho-Tyrosine Binding domain) that docks to the NPxY (Asp-Pro-any amino acid-Tyr) sequence in the intracellular tail of VLDLR and ApoER2, as well as other NPxY-containing transmembrane proteins, such as LDLR, LRP, megalin and amyloid precursor proteins (APP, APLP1, and APLP2) (Trommsdorff *et al*., 1998; Howell *et al*., 1999b; Trommsdorff *et al*., 1999), and integrins (Schmid *et al*., 2005). However, unlike other PI/PTB domains (i.e., Shc, IRS1), the Dab1 PTB motif binds preferentially to unphosphorylated NPxY sites (Howell *et al*., 1999a).

Some phosphatidylinositols, especially phosphatidylinositol-4,5-bisphosphate ($PI4,5P_2$), can bind the PTB domain of Dab1 without affecting its interaction with lipoprotein receptors (Howell *et al*., 1999b; Stolt *et al*., 2003). This binding is required for membrane localization and basal tyrosine phosphorylation of Dab1 independently of VLDLR and ApoER2 and is necessary for effective transduction of the Reelin signal (Huang *et al*., 2005; Stolt *et al*., 2005; Xu *et al*., 2005). These observations suggest that phospholipids recruit Dab1 to membranes but do not play a direct role in relaying the Reelin signal, whereas direct Dab1–receptor interaction is responsible for the signal but not for membrane recruitment.

The NPxY motif is involved in internalization of lipoprotein receptors, but whether receptor internalization plays a role in Reelin signaling remains unclear. One group, however, showed that Dab1 is recruited to the plasma membrane upon Reelin addition, and then the complex is internalized (Morimura *et al*., 2005). Additionally, when the phosphorylation of Dab1 is inhibited, Reelin remains associated with Dab1 near the plasma membrane for a prolonged period (Morimura *et al*., 2005).

Src, Fyn, and Abl are able to phosphorylate Dab1 *in vitro,* but only Fyn, and to a lesser extent Src, are involved in Dab1 phosphorylation *in vivo* (Howell *et al*., 1997; Arnaud *et al*., 2003b; Bock and Herz, 2003; Jossin *et al*., 2003). The combined deficit of Src and Fyn generates a reeler-like phenotype, providing definitive proof that these two kinases play a crucial role (Kuo *et al*., 2005). Dab1 tyrosine residues 198, 220, and 232 are phosphorylated *in vivo* in response to the binding of Reelin to VLDLR and ApoER2 (Keshvara *et al*., 2001) and are indispensable to Reelin signaling (Howell *et al*., 2000). The replacement of the *Dab1* gene by a partial cDNA encoding its PTB domain and the stretch containing five tyrosine residues is able to rescue most (but not all) features of the Dab1 mutant phenotype (Herrick and Cooper, 2002),

suggesting unidentified roles of the C-terminal part of Reelin. Interestingly, the C-terminal segment contains consensus S/T phosphorylation sites, some of which can be phosphorylated by cyclin-dependent kinase 5 (Cdk5) independently of Reelin (Keshvara *et al*., 2002). Following tyrosine phosphorylation, Dab1 is polyubiquitinated and degraded by the proteasome and this may be important to ensure a transient response to Reelin (Arnaud *et al*., 2003a; Bock *et al*., 2004).

6 Downstream of *Dab1*

Mutations in genes coding for the proteins discussed above (single mutation for *Reln* and *Dab1* or double inactivation for *Vldlr* and *Apoer2* or *Src* and *Fyn*) induce a similar phenotype, leaving little doubt about their involvement in the same signaling pathway. Here, I will describe putative downstream partners, identified either as phospho-Dab1 interacting proteins or as enzymes activated in cultured neurons after Reelin stimulation. All of these proteins are known to be able to modulate the cytoskeletal dynamics and thereby may affect neuronal migration or cellular adhesion.

Reelin stimulation of cultured neurons triggers the interaction of Y-phosphorylated Dab1 with the regulatory p85α subunit of phosphatidylinositol 3-kinase (PI3K). This results in increased protein kinase B (PKB) phosphorylation at S473 (known to activate the enzyme) and glycogen synthase kinase-3 beta (GSK3β at S9 (known to inhibit the enzyme) (Beffert *et al*., 2002; Bock *et al*., 2003). GSK3β acts on several targets that include tau and microtubule-associated protein 1b (Map1b). Increased phosphorylation of tau is present in mice deficient in Reelin, Dab1, or both VLDLR and ApoER2, and this may reflect the inhibition of GSK3β (Hiesberger *et al*., 1999). However, strain background has a major influence on this phenomenon, suggesting the presence of modifier genes (Brich *et al*., 2003) and *tau* knockout animals display normal brain organization and neuronal migration (Harada *et al*., 1994). Despite reported inhibition of GSK3β by Reelin, a recent report suggested that Reelin induces Map1b phosphorylation through activation of GSK3 and Cdk5 (Gonzalez-Billault *et al*., 2005). Although *map1b*$^{-/-}$ and *tau*$^{-/-}$ *map1b*$^{-/-}$ mutant mice have reeler-like defective layering of CA1 hippocampal pyramidal cells, they lack neocortical anomalies (Takei *et al*., 2000). However, *map2*$^{-/-}$*map1b*$^{-/-}$ mice exhibit retarded neuronal migration but display normal inside-outside cortical layering (Teng *et al*., 2001). The suggestion that Reelin could activate Cdk5 is not supported by several reports. First, the activity of Cdk5 is not affected by Reelin in primary neuronal culture and is unchanged in mice with mutations of Reelin, Dab1, or VLDLR and ApoER2 (Beffert *et al*., 2004). Second, the phosphorylation of Dab1 by Cdk5 is independent of Reelin (Keshvara *et al*., 2002). And finally, mice lacking both Cdk5/p35 and Reelin/Dab1 exhibit some similar phenotypes, but most defects are additive, suggesting that both pathways work in parallel rather than sequentially (Ohshima *et al*., 2001).

Lissencephaly-1 (Lis1), the gene mutated in the Miller-Dieker syndrome and type 1 lissencephaly, plays a key role in neuronal migration. Interactions between

Lis1 and Reelin signaling were investigated by studies of compound mutant mice with disruptions in the Reelin pathway and heterozygous Lis1 mutations. These animals had a higher incidence of hydrocephalus and enhanced cortical and hippocampal layering defects. Dab1 and Lis1 bound in a Reelin-induced, phosphorylation-dependent manner, clearly points to convergence of genetic and biochemical interactions (Assadi *et al*., 2003).

Nck (noncatalytic region of tyrosine kinase) α and Nckβ adapter proteins are regulators of the actin cytoskeleton implicated in cell movement and axon guidance. Nckβ (but not Nckα) interacts, through its SH2 domain, with Dab1 when Dab1 is phosphorylated on the Reelin-regulated sites Y220 or Y232. Nckβ redistributes from the soma to the leading edge of neuronal processes in about 5% of Reelin-treated primary forebrain neurons, and coexpression of Nckβ with Y-phosphorylated Dab1-RFP (Dab1 fused with a red fluorescent protein) in fibroblasts disrupts the actin cytoskeleton (Pramatarova *et al*., 2003).

The search for proteins that bind to tyrosine-phosphorylated Dab1 in embryonic brain extracts led to the identification of CT10 regulator of kinase (Crk) family proteins (CrkL, CrkI, and CrkII) (Ballif *et al*., 2004; Chen *et al*., 2004; Huang *et al*., 2004). CrkL and CrkII binding to Dab1 involve two critical tyrosine phosphorylation sites, Y220 and Y232. The Crks and CrkL proteins are SH2 and SH3 containing adapter molecules that have been shown to regulate cell migration. Chen *et al*. (2004) showed that CrkII mediates the interaction of dedicator of cytokinesis 1 (Dock1), an exchange factor (GEF) for Ras-related C3 botulinum toxin substrate 1 (Rac1), with phosphorylated Dab1 and proposed a model in which tyrosine phosphorylated Dab1 engages the conserved CrkII-Dock1-Rac signaling cassette, but, when bound to Dab1, this complex would not support neuronal migration. On the other hand, another group showed that Reelin stimulates the tyrosine phosphorylation of C3G, a GEF for Ras-related protein 1 (Rap1), on a site known to be required for activation of Rap1 by C3G, and that this phosphorylation is downstream to Dab1 phosphorylation (Ballif *et al*., 2004). Moreover, Rap1 GTP levels, but not Rac1 GTP levels, in cultured neurons were increased after 15 minutes of stimulation by Reelin, indicating that these two signaling enzymes may be activated in a different way either temporally or spatially in the cell (Ballif *et al*., 2004). Finally, a third group showed that CrkI and CrkII, but not CrkL, induced Dab1 phosphorylation by Src family kinases at Y220, but not Y198 and Y232, when expressed with Dab1 in HEK293T cells (Huang *et al*., 2004).

Dab1 co-immunoprecipitates with N-WASP (neuronal Wiskott-Aldrich syndrome protein) in E14 mouse brain lysates. *In vitro*, an overexpression of the PTB domain of Dab1 in nonneuronal Cos-7 cells causes formation of microspikes and this effect was interpreted to be due to an interaction between Dab1 with an NRFY (Asn-Arg-Phe-Tyr) sequence in N-WASP (Suetsugu *et al*., 2004).

Despite the identification of several candidate partners of Reelin signaling, stronger evidence is clearly necessary to implicate unequivocally any of them in Reelin's functions. The understanding of the mechanism of action of Reelin on target cells remains a formidable experimental challenge.

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References

Andersen, O. M., Benhayon, D., Curran, T., and Willnow, T. E. (2003). Differential binding of ligands to the apolipoprotein E receptor 2. *Biochemistry* 42:9355–9364.

Anton, E. S., Kreidberg, J. A., and Rakic, P. (1999). Distinct functions of alpha3 and alpha(v) integrin receptors in neuronal migration and laminar organization of the cerebral cortex. *Neuron* 22:277–289.

Arnaud, L., Ballif, B. A., and Cooper, J. A. (2003a). Regulation of protein tyrosine kinase signaling by substrate degradation during brain development. *Mol. Cell. Biol*. 23:9293–9302.

Arnaud, L., Ballif, B. A., Forster, E., and Cooper, J. A. (2003b). Fyn tyrosine kinase is a critical regulator of disabled-1 during brain development. *Curr. Biol*. 13:9–17.

Assadi, A. H., Zhang, G., Beffert, U., McNeil, R. S., Renfro, A. L., Niu, S., Quattrocchi, C. C., Antalffy, B. A., Sheldon, M., Armstrong, D. D., Wynshaw-Boris, A., Herz, J., D'Arcangelo, G., and Clark, G. D. (2003). Interaction of reelin signaling and Lis1 in brain development. *Nat. Genet*. 35:270–276.

Ballif, B. A., Arnaud, L., Arthur, W. T., Guris, D., Imamoto, A., and Cooper, J. A. (2004). Activation of a Dab1/CrkL/C3G/Rap1 pathway in Reelin-stimulated neurons. *Curr. Biol*. 14:606–610.

Beffert, U., Morfini, G., Bock, H. H., Reyna, H., Brady, S. T., and Herz, J. (2002). Reelin-mediated signaling locally regulates protein kinase B/Akt and glycogen synthase kinase 3beta. *J. Biol. Chem*. 277:49958–49964.

Beffert, U., Weeber, E. J., Morfini, G., Ko, J., Brady, S. T., Tsai, L. H., Sweatt, J. D., and Herz, J. (2004). Reelin and cyclin-dependent kinase 5-dependent signals cooperate in regulating neuronal migration and synaptic transmission. *J. Neurosci*. 24:1897–1906.

Beffert, U., Weeber, E. J., Durudas, A., Qiu, S., Masiulis, I., Sweatt, J. D., Li, W. P., Adelmann, G., Frotscher, M., Hammer, R. E., and Herz, J. (2005). Modulation of synaptic plasticity and memory by reelin involves differential splicing of the lipoprotein receptor apoer2. *Neuron* 47:567–579.

Bock, H. H., and Herz, J. (2003). Reelin activates SRC family tyrosine kinases in neurons. *Curr. Biol*. 13:18–26.

Bock, H. H., Jossin, Y., Liu, P., Forster, E., May, P., Goffinet, A. M., and Herz, J. (2003). Phosphatidylinositol 3-kinase interacts with the adaptor protein Dab1 in response to Reelin signaling and is required for normal cortical lamination. *J. Biol. Chem*. 278:38772–38779.

Bock, H. H., Jossin, Y., May, P., Bergner, O., and Herz, J. (2004). Apolipoprotein E receptors are required for reelin-induced proteasomal degradation of the neuronal adaptor protein Disabled-1. *J. Biol. Chem*. 279:33471–33479.

Brich, J., Shie, F. S., Howell, B. W., Li, R., Tus, K., Wakeland, E. K., Jin, L. W., Mumby, M., Churchill, G., Herz, J., and Cooper, J. A. (2003). Genetic modulation of tau phosphorylation in the mouse. *J. Neurosci*. 23:187–192.

Caviness, V. S., Jr., and Rakic, P. (1978). Mechanisms of cortical development: a view from mutations in mice. *Annu. Rev. Neurosci*. 1:297–326.

Chen, K., Ochalski, P. G., Tran, T. S., Sahir, N., Schubert, M., Pramatarova, A., and Howell, B. W. (2004). Interaction between Dab1 and CrkII is promoted by Reelin signaling. *J. Cell. Sci*. 117:4527–4536.

D'Arcangelo, G., Miao, G. G., Chen, S. C., Soares, H. D., Morgan, J. I., and Curran, T. (1995). A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* 374:719–723.

D'Arcangelo, G., Homayouni, R., Keshvara, L., Rice, D. S., Sheldon, M., and Curran, T. (1999). Reelin is a ligand for lipoprotein receptors. *Neuron* 24:471–479.

Dong, E., Caruncho, H., Liu, W. S., Smalheiser, N. R., Grayson, D. R., Costa, E., and Guidotti, A. (2003). A reelin–integrin receptor interaction regulates Arc mRNA translation in synaptoneurosomes. *Proc. Natl. Acad. Sci. USA* 100:5479–5484.

Dulabon, L., Olson, E. C., Taglienti, M. G., Eisenhuth, S., McGrath, B., Walsh, C. A., Kreidberg, J. A., and Anton, E. S. (2000). Reelin binds alpha3beta1 integrin and inhibits neuronal migration. *Neuron* 27:33–44.

Fatemi, S. H. (2005). Reelin glycoprotein: structure, biology and roles in health and disease. *Mol. Psychiatry* 10:251–257.

Forster, E., Tielsch, A., Saum, B., Weiss, K. H., Johanssen, C., Graus-Porta, D., Muller, U., and Frotscher, M. (2002). Reelin, disabled 1, and beta 1 integrins are required for the formation of the radial glial scaffold in the hippocampus. *Proc. Natl. Acad. Sci. USA* 99:13178-–3183.

Gonzalez-Billault, C., Del Rio, J. A., Urena, J. M., Jimenez-Mateos, E. M., Barallobre, M. J., Pascual. M., Pujadas, L., Simo, S., Torre, A. L., Gavin, R., Wandosell, F., Soriano, E., and Avila, J. (2005). A role of MAP1B in reelin-dependent neuronal migration. *Cereb. Cortex* 15:1134–1145.

Graus-Porta, D., Blaess, S., Senften, M., Littlewood-Evans, A., Damsky, C., Huang, Z., Orban, P., Klein, R., Schittny, J. C., and Muller, U. (2001). Beta1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. *Neuron* 31:367–379.

Harada, A., Oguchi, K., Okabe, S., Kuno, J., Terada, S., Ohshima, T., Sato-Yoshitake, R., Takei, Y., Noda, T., and Hirokawa, N. (1994). Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature* 369:488–491.

Herrick, T. M., and Cooper, J. A. (2002). A hypomorphic allele of dab1 reveals regional differences in reelin-Dab1 signaling during brain development. *Development* 129:787–796.

Hiesberger, T., Trommsdorff, M., Howell, B. W., Goffinet, A., Mumby, M. C., Cooper, J. A., and Herz, J. (1999). Direct binding of reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron* 24:481–489.

Howell, B. W., Hawkes, R., Soriano, P., and Cooper, J. A. (1997). Neuronal position in the developing brain is regulated by mouse disabled-1. *Nature* 389:733–737.

Howell, B. W., Herrick, T. M., and Cooper, J. A. (1999a). Reelin-induced tryosine phosphorylation of disabled 1 during neuronal positioning. *Genes Dev*. 13:643–648.

Howell, B. W., Lanier, L. M., Frank, R., Gertler, F. B., and Cooper, J. A. (1999b). The disabled 1 phosphotyrosine-binding domain binds to the internalization signals of transmembrane glycoproteins and to phospholipids. *Mol. Cell. Biol*. 19:5179–5188.

Howell, B. W., Herrick, T. M., Hildebrand, J. D., Zhang, Y., and Cooper, J. A. (2000). Dab1 tyrosine phosphorylation sites relay positional signals during mouse brain development. *Curr. Biol*. 10:877–885.

Huang, Y., Magdaleno, S., Hopkins, R., Slaughter, C., Curran, T., and Keshvara, L. (2004). Tyrosine phosphorylated disabled 1 recruits Crk family adapter proteins. *Biochem. Biophys. Res. Commun*. 318:204–212.

Huang, Y., Shah, V., Liu, T., and Keshvara, L. (2005). Signaling through disabled 1 requires phosphoinositide binding. *Biochem. Biophys. Res. Commun*. 331:1460–1468.

Impagnatiello, F., Guidotti, A. R., Pesold, C., Dwivedi, Y., Caruncho, H., Pisu, M. G., Uzunov, D. P., Smalheiser, N. R., Davis, J. M., Pandey, G. N., Pappas, G. D., Tueting, P., Sharma, R. P., and Costa, E. (1998). A decrease of reelin expression as a putative vulnerability factor in schizophrenia. *Proc. Natl. Acad. Sci. USA* 95:15718–15723.

Jossin, Y. (2004). Neuronal migration and the role of reelin during early development of the cerebral cortex. *Mol. Neurobiol*. 30:225–251.

Jossin, Y., Ogawa, M., Metin, C., Tissir, F., and Goffinet, A. M. (2003). Inhibition of SRC family kinases and non-classical protein kinases C induce a reeler-like malformation of cortical plate development. *J. Neurosci*. 23:9953–9959.

Jossin, Y., Ignatova, N., Hiesberger, T., Herz, J., Lambert de Rouvroit, C., and Goffinet, A. M. (2004). The central fragment of reelin, generated by proteolytic processing in vivo, is critical to its function during cortical plate development. *J. Neurosci*. 24:514–521.

Keshvara, L., Benhayon, D., Magdaleno, S., and Curran, T. (2001). Identification of reelin-induced sites of tyrosyl phosphorylation on disabled 1. *J. Biol. Chem*. 276:16008–16014.

Keshvara, L., Magdaleno, S., Benhayon, D., and Curran, T. (2002). Cyclin-dependent kinase 5 phosphorylates disabled 1 independently of reelin signaling. *J. Neurosci*. 22:4869–4877.

Kuo, G., Arnaud, L., Kronstad-O'Brien, P., and Cooper, J. A. (2005). Absence of Fyn and Src causes a reeler-like phenotype. *J. Neurosci*. 25:8578–8586.

Lambert de Rouvroit, C., de Bergeyck, V., Cortvrindt, C., Bar, I., Eeckhout, Y., and Goffinet, A. M. (1999). Reelin, the extracellular matrix protein deficient in reeler mutant mice, is processed by a metalloproteinase. *Exp. Neurol*. 156:214–217.

Liu, W. S., Pesold, C., Rodriguez, M. A., Carboni, G., Auta, J., Lacor, P., Larson, J., Condie, B. G., Guidotti, A., and Costa, E. (2001). Down-regulation of dendritic spine and glutamic acid decarboxylase 67 expressions in the reelin haploinsufficient heterozygous reeler mouse. *Proc. Natl. Acad. Sci. US*. 98:3477–3482.

Luque, J. M., Morante-Oria, J., and Fairen, A. (2003). Localization of ApoER2, VLDLR and Dab1 in radial glia: groundwork for a new model of reelin action during cortical development. *Brain Res. Dev. Brain Res*. 140:195–203.

Miyata, T., Nakajima, K., Mikoshiba, K., and Ogawa, M. (1997). Regulation of Purkinje cell alignment by reelin as revealed with CR-50 antibody. *J. Neurosci*. 17:3599–3609.

Morimura, T., Hattori, M., Ogawa, M., and Mikoshiba, K. (2005). Disabled1 regulates the intracellular trafficking of reelin receptors. *J. Biol. Chem*. 280:16901–16908.

Nogi, T., Yasui, N., Hattori, M., Iwasaki, K., and Takagi, J. (2006). Structure of a signaling-competent reelin fragment revealed by X-ray crystallography and electron tomography. *EMBO J*. 25:3675–3683.

Ohshima, T., Ogawa, M., Veeranna, Hirasawa, M., Longenecker, G., Ishiguro, K., Pant, H. C., Brady, R. O., Kulkarni, A. B., and Mikoshiba, K. (2001). Synergistic contributions of cyclin-dependent kinase 5/p35 and reelin/Dab1 to the positioning of cortical neurons in the developing mouse brain. *Proc. Natl. Acad. Sci. USA* 98:2764–2769.

Pramatarova, A., Ochalski, P. G., Chen, K., Gropman, A., Myers, S., Min, K. T., and Howell, B. W. (2003). Nck beta interacts with tyrosine-phosphorylated disabled 1 and redistributes in reelin-stimulated neurons. *Mol. Cell. Biol*. 23:7210–7221.

Sanada, K., Gupta, A., and Tsai, L. H. (2004). Disabled-1-regulated adhesion of migrating neurons to radial glial fiber contributes to neuronal positioning during early corticogenesis. *Neuron* 42:197–211.

Schiffmann, S. N., Bernier, B., and Goffinet, A. M. (1997). Reelin mRNA expression during mouse brain development. *Eur. J. Neurosci*. 9:1055–1071.

Schmid, R. S., Jo, R., Shelton, S., Kreidberg, J. A., and Anton, E. S. (2005). Reelin, integrin and DAB1 interactions during embryonic cerebral cortical development. *Cereb. Cortex* 15:1632–1636.

Senzaki, K., Ogawa, M., and Yagi, T. (1999). Proteins of the CNR family are multiple receptors for reelin. *Cell* 99:635–647.

Sheldon, M., Rice, D. S., D'Arcangelo, G., Yoneshima, H., Nakajima, K., Mikoshiba, K., Howell, B. W., Cooper, J. A., Goldowitz, D., and Curran, T. (1997). Scrambler and yotari disrupt the disabled gene and produce a reeler-like phenotype in mice. *Nature* 389:730–733.

Stolt, P. C., Jeon, H., Song, H. K., Herz, J., Eck, M. J., and Blacklow, S. C. (2003). Origins of peptide selectivity and phosphoinositide binding revealed by structures of disabled-1 PTB domain complexes. *Structure* 11:569–579.

Stolt, P. C., Chen, Y., Liu, P., Bock, H. H., Blacklow, S. C., and Herz, J. (2005). Phosphoinositide binding by the disabled-1 PTB domain is necessary for membrane localization and reelin signal transduction. *J. Biol. Chem*. 280:9671–9677.

Strasser, V., Fasching, D., Hauser, C., Mayer, H., Bock, H. H., Hiesberger, T., Herz, J., Weeber, E. J., Sweatt, J. D., Pramatarova, A., Howell, B., Schneider, W. J., and Nimpf, J. (2004). Receptor clustering is involved in reelin signaling. *Mol. Cell. Biol*. 24:1378–1386.

Suetsugu, S., Tezuka, T., Morimura, T., Hattori, M., Mikoshiba, K., Yamamoto, T., and Takenawa, T. (2004). Regulation of actin cytoskeleton by mDab1 through N-WASP and ubiquitination of mDab1. *Biochem. J*. 384:1–8.

Takei, Y., Teng, J., Harada, A., and Hirokawa, N. (2000). Defects in axonal elongation and neuronal migration in mice with disrupted tau and map1b genes. *J. Cell. Biol*. 150:989–1000.

Teng, J., Takei, Y., Harada, A., Nakata, T., Chen, J., and Hirokawa, N. (2001). Synergistic effects of MAP2 and MAP1B knockout in neuronal migration, dendritic outgrowth, and microtubule organization. *J. Cell Biol*. 155:65–76.

Trommsdorff, M., Borg, J. P., Margolis, B., and Herz, J. (1998). Interaction of cytosolic adaptor proteins with neuronal apolipoprotein E receptors and the amyloid precursor protein. *J. Biol. Chem*. 273:33556–33560.

Trommsdorff, M., Gotthardt, M., Hiesberger, T., Shelton, J., Stockinger, W., Nimpf, J., Hammer, R. E., Richardson, J. A., and Herz, J. (1999). Reeler/disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* 97:689–701.

Utsunomiya-Tate, N., Kubo, K., Tate, S., Kainosho, M., Katayama, E., Nakajima, K., and Mikoshiba, K. (2000). Reelin molecules assemble together to form a large protein complex, which is inhibited by the function-blocking CR-50 antibody. *Proc. Natl. Acad. Sci. USA* 97:9729–9734.

Walsh, C. A., and Goffinet, A. M. (2000). Potential mechanisms of mutations that affect neuronal migration in man and mouse. *Curr. Opin. Genet. Dev*. 10:270–274.

Weeber, E. J., Beffert, U., Jones, C., Christian, J. M., Forster, E., Sweatt, J. D., and Herz, J. (2002). Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. *J. Biol. Chem*. 277:39944–39952.

Xu, M., Arnaud, L., and Cooper, J. A. (2005). Both the phosphoinositide and receptor binding activities of Dab1 are required for reelin-stimulated Dab1 tyrosine phosphorylation. *Brain Res. Mol. Brain Res*. 139:300–305.

Chapter 4
The C-Terminal Region of Reelin: Structure and Function

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1 The Structure of the C-Terminal Region (CTR) of Reelin

1.1 Definition of CTR

Reelin is a very large secreted glycoprotein of about 3460 amino acids in mammalian species. Reelin is divided into three subdomains from its primary sequence (D'Arcangelo *et al*., 1995): the N-terminal F-spondin-like domain, the eight tandem of Reelin repeat, and the short and highly basic C-terminal region (CTR). The N-terminal F-spondin-like domain is proposed to be necessary and sufficient for multimerization of Reelin (Utsunomiya-Tate *et al*., 2000). The function of each Reelin repeat is not fully understood, but the region between the fifth and sixth repeat is sufficient for binding to the Reelin receptors, very-low-density lipoprotein receptor (VLDLR) and apolipoprotein receptor 2 (ApoER2) (Yasui *et al*., 2007). The CTR, which is about 30 amino acids long, comprises less than 1% of the whole Reelin protein.

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The exact definition of CTR has not been determined and is closely related with the definition of Reelin repeat. In the original work that reported the first identification of mouse Reelin (D'Arcangelo *et al*., 1995), the eighth Reelin repeat was proposed to end at Val^{3427}, making the last 34 amino acid residues CTR. The reason for choosing this boundary was not given. Subsequently, Ichihara *et al*. (2001) found some new repetitive units at its N-terminal region by computerized search and realigned the Reelin repeat. In their alignment, the eighth Reelin repeat ended at Val^{3429} (i.e., CTR = 32 amino acids, Ichihara *et al*., 2001). Deciding the boundary between the eighth Reelin repeat from the alignment of the amino acid sequence has limitations, and it should be determined by functional examinations. Hereafter in this chapter, we adopt the definition by Ichihara *et al*. (2001) solely to avoid confusion, and it should be noted that the exact definition of CTR is an open question. It is also worth mentioning that the results discussed in this chapter are unlikely to be affected by moving the boundary by a few amino acids.

1.2 Characteristics of CTR

CTR is highly positively charged: among 32 amino acid residues of CTR, 12 (38%) are basic (Fig. 4.1), while none are acidic. It does not appear to have any motif that can be a clue to its function, and no posttranslational modification has been reported in this region.

Most of CTR (from Thr^{3431} to the C-terminal end) is encoded in a single exon (exon 65; Royaux *et al*., 1997). Exon 64 is a microexon of 6 nucleotides, coding Val^{3429} and Ser^{3430} (Royaux *et al*., 1997) (Fig. 4.1). Exon 64 may be skipped by alternative splicing (Lambert de Rouvroit *et al*., 1999; Royaux *et al*., 1997), but its biological significance remains unexplored. Importantly, alternative polyadenylation generates two types of exon 63 (Lambert de Rouvroit *et al*., 1999). The "larger" exon 63 (exon 63a) contains termination codons and thus it eventually gives rise to a truncated Reelin protein without the C-terminal 33 amino acids (Lambert de Rouvroit *et al*., 1999). We will discuss this short-form, CTR-less Reelin protein later.

The primary sequence of CTR is completely (100%) conserved in all the mammalian species whose CTR sequences can be found in the available databases, namely, human, chimpanzee, macaque, dog, mouse, rat, cow, and gray short-tailed opossum (Nakano *et al*., 2007). It is also perfectly conserved in chicken and turtle. The sequence of crocodile Reelin found in the database seems to lack the microexon-derived two residues but, apart from that, it is completely conserved with that of mammals. As a matter of course, DNA sequence encoding CTR is very well (>90%) conserved among these organisms (Nakano *et al*., 2007). From the evolutionary point of view, these observations strongly suggest that CTR has an essential physiological function(s) in the land vertebrates. Reelin gene is also found in genome databases of fishes and sea urchins. The sequences of CTR in these species are quite variable and, in some cases, appear to be missing. At present, it remains

Fig. 4.1 The primary sequence of Reelin CTR. The boundary between the eighth Reelin repeat and CTR proposed by Ichihara *et al*. (2001) and by D'Arcangelo *et al*. (1995) are shown by the vertical and the dashed lines, respectively. Basic residues are indicated by "+" symbols. Two residues encoded by the microexon (exon 64) are boxed. The residue numbers counting from the first methionine are indicated below the sequence

obscure whether Reelin is actually transcribed in these species, except in zebrafish (Costagli *et al*., 2002). Function of Reelin in non-mammalian species has not been elucidated.

2 Is CTR Required for Secretion of Reelin?

2.1 Observations from Orleans Reeler Mouse and Earlier Mutational Study

In one of the murine Reelin mutations called Orleans, a frame shift results in the production of aberrant Reelin protein that lacks the C-terminal half of the eighth Reelin repeat as well as CTR, and has 70 unrelated amino acid residues instead (ReelinOrl) (de Bergeyck *et al*., 1997). This protein is not secreted (de Bergeyck *et al*., 1997) and accumulates in the endoplasmic reticulum (ER) (Derer *et al*., 2001). It was also reported that an artificial Reelin mutant protein in which a stop codon was inserted in the middle of the eighth Reelin repeat (lacking the C-terminal 133 residues) was not secreted from transfected COS-7 cells (D'Arcangelo *et al*., 1997). These results suggested that secretion of Reelin was regulated between ER and Golgi apparatus and that this regulation required the C-terminal half of the eighth Reelin repeat and/or the CTR. As the C-terminal half of the eighth Reelin repeat did not seem to have any particular motif and no other role was assigned to CTR at that time, it was not unreasonable to assume that CTR was essential for Reelin secretion.

In 1999, Lambert de Rouvroit *et al*. reported that an alternative polyadenylation can give rise to a truncated Reelin protein without the C-terminal 33 amino acids, and this short-form Reelin is secreted when overexpressed in COS-7 cells (Lambert de Rouvroit *et al*., 1999). From this result, it was proposed that Reelin secretion required residues from 3328 to 3428 (Lambert de Rouvroit *et al*., 1999). However, the amount of secreted CTR-less Reelin was apparently much lower than that of the wild-type Reelin (Lambert de Rouvroit *et al*., 1999), and the expression level of the respective

protein was not provided. Therefore, it was somewhat ambiguous whether CTR is involved in secretion or if it affects expression or stability of Reelin protein.

2.2 Detailed Mutational Study

The regulatory mechanism of Reelin secretion is important not only for understanding its role in the developing brain, but also for elucidation of its significance in adult brain function, especially in synaptic plasticity (Beffert *et al*., 2005), for which the action must be spatiotemporally regulated. Therefore, we set out to analyze the role of CTR in Reelin secretion by making series of mutants and expressing them in various cell lines (Nakano *et al*., 2007). All of the results discussed below are essentially independent of the cell type used.

A mutant Reelin protein that lacked only CTR (ReelinΔC) was secreted, although its efficiency was lower than that of wild-type Reelin, suggesting that CTR is not essential for, but may play a role in, secretion of Reelin. On the other hand, a mutant that lacked the C-terminal 20 residues (including 10 of 12 basic residues) of the CTR was secreted as efficiently as wild-type Reelin. Moreover, replacing CTR with FLAG epitope (DYKDDDDK, ReelinΔC-FLAG), eight arginine (ReelinΔC-Arg8), or glutamate residues had little effect on the secretion efficiency. A mutant in which CTR was replaced with Venus (a variant of yellow fluorescent protein with 239 amino acids (Nagai *et al*., 2002)) was secreted quite efficiently, but the mutants, in which CTR is replaced with eight alanine or histidine, residues were not secreted. These results indicated three important points. First, CTR is not absolutely indispensable for secretion. In particular, basic residues in CTR are totally dispensable. Second, many, but not all, amino acid sequences are able to substitute CTR in facilitating secretion. We presume that a hydrophilic structure is necessary in this region for efficient secretion of Reelin protein. Third, CTR is likely to have another important role, considering that it is highly conserved among species. It should be mentioned that these results did not seem to be cell-type specific. However, we do not exclude the possibility that Reelin secretion *in vivo*, such as by Cajal-Retzius cells, is in fact regulated in a CTR-dependent manner.

We also obtained a clue as to why ReelinOrl is not secreted (Nakano *et al*., 2007). First, a truncated Reelin mutant that terminated just after the seventh Reelin repeat was efficiently secreted, while none of the truncated mutants that terminated in the middle of Reelin repeat were secreted. Thus, it is suggested that abrupt termination in the middle of the eighth Reelin repeat, not the absence of a certain sequence, is the main cause of secretion failure of ReelinOrl. Second, immunocytochemical analysis of overexpressed cells revealed that wild-type Reelin (and all of the mutants that were efficiently secreted) was present mainly in the ER and presumably in secretory vesicles, while ReelinOrl mutant tended to accumulate Reelin around the nucleus. This was consistent with a previous report that ReelinOrl accumulates in the rough ER in Cajal-Retzius cells (Derer *et al*., 2001). Therefore,

it is strongly suggested that ReelinOrl is unable to go beyond the ER in its secretory pathway. On the other hand, ReelinΔC accumulated in ER and dilated it. It was thus indicated that ReelinOrl and ReelinΔC have distinct intracellular fates in the secretory pathway. These observations further support the idea that lack of CTR is not the direct cause of the secretion failure of ReelinOrl.

3 Functions of CTR

3.1 Role of CTR in Activation of Downstream Signaling

The fact that most of the CTR is unnecessary for secretion prompted us to investigate the other physiological function of CTR. We thought that CTR may be involved in activation of downstream signaling and thus stimulated the primary cortical neurons from embryonic mice with conditioned media containing wild-type or mutant Reelin protein (Nakano *et al*., 2007). To our surprise, Reelin mutants that lack CTR were generally much less potent than wild-type Reelin in inducing Dab1 phosphorylation. Importantly, ReelinΔC-Arg8 induced Dab1 phosphorylation more strongly than other mutants that lacked CTR, but not as strongly as wild-type Reelin. Moreover, Reelin mutants with the insertion of an unrelated sequence (FLAG epitope or Venus) between the eighth Reelin repeat and CTR were no more potent than other mutants without CTR. These results highlight three important points. First, CTR is necessary for efficient activation of downstream signaling. Second, the highly basic nature of CTR contributes to activation of downstream signaling. Third, CTR must be located just after the eighth Reelin repeat to have full activity. In other words, CTR functions in concert with the eighth Reelin repeat for activation of downstream signaling.

Our results seem to contradict the previous reports (Jossin *et al*., 2004, 2007), because they argue that the region between the third and sixth Reelin repeats is as potent as full-length Reelin in Dab1 phosphorylation. The reason for the discrepancy is unknown at present, but there are some possibilities. First, concentrations of Reelin and its mutants/fragments may be different between the two groups. Both groups adjusted the concentration of the samples in their own assays, but it is difficult to compare them between the two distant groups. Second, small differences in the methodologies used to make the recombinant samples (e.g., culturing conditions, transfection reagents, serum, and so on) may affect their signaling capacity, for example, by affecting posttranslational modifications. We recently performed fairly quantitative analysis by collaboration with Junichi Takagi's group and found that the full-length Reelin is approximately 100 times as potent as the recombinant protein consisting of the third and sixth Reelin repeats (Nogi *et al*., 2006). This estimation is, however, based on the assumption that all the recombinant proteins in the solutions are potent, but they might be partially misfolded or lack modifications necessary for full potency. Third, both groups use "full-length Reelin" that contained various amounts of proteolytic fragments. The fragments, particularly the

N- and C-terminal fragments whose functions are not well defined, may affect the assay. Fourth, as preparation methods for primary cortical neurons are not completely the same between two groups, expression levels of the receptors, Dab1, kinases, and other factors may be different. Finally, and perhaps most importantly, Dab1 phosphorylation and activation of Src family protein kinase form a positive feedback loop (Bock and Herz, 2003). Therefore, a small fluctuation could easily result in the all-or-none responses. Elucidation of all the molecular mechanisms will solve the discrepancy, and phenotypic analysis of the ReelinΔC-FLAG knockin mice (see Section 4) will reveal the physiological importance of CTR *in vivo*.

3.2 How Is CTR Involved in Activation of Downstream Signaling?

Why does deletion of CTR or insertion of unrelated residues just prior to CTR reduce the Dab1-phosphorylating ability? We first checked whether deletion of CTR affects the dimer formation or oligomerization of Reelin, as oligomerization of Reelin is a prerequisite for Dab1 phosphorylation. However, the results obtained indicated that CTR is not involved in oligomerization of Reelin (Nakano *et al*., 2007).

We next investigated whether deletion of CTR affects interaction between Reelin and its receptors. For this purpose, we first employed the recombinant, soluble ligand-binding domain of ApoER2 fused to human growth hormone (GH-ApoER2LBD) to pull down Reelin protein. Conditioned media containing either wild-type Reelin or ReelinΔC-FLAG were mixed with GH-ApoER2LBD, anti-GH, and Protein-G Sepharose, and the precipitates were analyzed by Western blotting. No difference in binding was observed between wild-type Reelin and ReelinΔC-FLAG, indicating that CTR is not directly involved in the interaction between Reelin and the extracellular domain of ApoER2.

Negatively charged proteoglycans and extracellular matrices are known to play important roles in signaling machinery of many secreted molecules including Wnt (Lin, 2004) and Semaphorin 5A (Kantor *et al*., 2004). As CTR of Reelin is positively charged, we investigated whether it is involved in the interaction between Reelin and its receptors on the cell surface. We found that wild-type Reelin bound much more strongly (or more stably) to the receptor-bearing cell membrane than did Reelin mutants that lacked CTR (Nakano *et al*., 2007). Among the mutants, ReelinΔC-Arg8 bound to the receptor-bearing cell membrane slightly more strongly than other mutants, indicating that Reelin–receptor interaction on the cell membrane is partly, but not solely, mediated by the positive charges of the CTR.

We also investigated binding of Reelin to COS-7 cells expressing ApoER2 by immunostaining without permeabilization (in order to detect cell-surface, but not internalized, Reelin) (Nakano *et al*., 2007). Wild-type Reelin bound strongly to the surface of ApoER2-expressing COS-7 cells, while most mutants lacking CTR did so only weakly. Consistent with the pull-down experiments using isolated cell membrane, ReelinΔC-Arg8 bound to ApoER2-expressing cells more strongly than

mutants lacking the CTR but more weakly than wild-type Reelin. The same phenomena were observed when primary cortical neurons that endogenously express Reelin receptors were used. These results demonstrated that CTR is necessary for a stable association between Reelin and its receptors on the plasma membranes of live cells and positive charges of CTR are partly involved in it.

4 Concluding Remarks

The CTR of Reelin is not necessary for its secretion in any of the cell lines tested. In addition, at least in cerebellar granule cells in culture, Reelin secretion does not appear to be regulated (Lacor *et al*., 2000). Whether secretion of Reelin is regulated or not in other systems remains unknown, however, and requires further investigation.

Our results from *in vitro* assays indicated that CTR is important for efficient induction of Dab1 phosphorylation. This effect of CTR is likely due to its interaction with a co-receptor molecule on the plasma membrane, but the identity of this co-receptor is unknown presently. Jossin *et al*. (2007) proposed that it is the central fragment of Reelin that carries the signaling capacity and that the C-terminal part may contribute to Reelin's binding to extracellular matrix (Jossin *et al*., 2007). This is a quite reasonable model, and we also agree that the CTR may impact the diffusion of Reelin by binding to certain molecules on the cell membrane (Nakano *et al*., 2007). More detailed examinations, ideally using an *in vivo* system, are necessary to understand the role of CTR. In this regard, we recently established ReelinΔC-FLAG knockin mice in which the genome sequence coding CTR (exon 65) is replaced with that coding FLAG epitope. By analyzing them, we will be able to answer the questions such as: (1) Is CTR dispensable for secretion *in vivo*? (2) Is CTR involved in Reelin's action during correct development of cerebral cortex and cerebellum? (3) Is CTR important for localization and/or diffusion of Reelin? It is also important to understand the molecular mechanism by which CTR augments the activation of downstream signaling. Clarification of all of these issues will help in understanding the physiological and pathological roles of Reelin.

References

Beffert, U., Weeber, E. J., Durudas, A., Qiu, S., Masiulis, I., Sweatt, J. D., Li, W. P., Adelmann, G., Frotscher, M., Hammer, R. E., and Herz, J. (2005). Modulation of synaptic plasticity and memory by reelin involves differential splicing of the lipoprotein receptor ApoER2. *Neuron* 47:567–579.

Bock, H. H., and Herz, J. (2003). Reelin activates Src family tyrosine kinases in neurons. *Curr. Biol*. 13:18–26.

Costagli, A., Kapsimali, M., Wilson, S. W., and Mione, M. (2002). Conserved and divergent patterns of reelin expression in the zebrafish central nervous system. *J. Comp. Neurol*. 450:73–93.

D'Arcangelo, G., Miao, G. G., Chen, S. C., Soares, H. D., Morgan, J. I., and Curran, T. (1995). A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* 374:719–723.

D'Arcangelo, G., Nakajima, K., Miyata, T., Ogawa, M., Mikoshiba, K., and Curran, T. (1997). Reelin is a secreted glycoprotein recognized by the CR-50 monoclonal antibody. *J. Neurosci.* 17: 23–31.

de Bergeyck, V., Nakajima, K., Lambert de Rouvroit, C., Naerhuyzen, B., Goffinet, A. M., Miyata, T., Ogawa, M., and Mikoshiba, K. (1997). A truncated reelin protein is produced but not secreted in the 'Orleans' reeler mutation (Reln[rl-Orl]). *Brain Res. Mol. Brain Res*. 50:85–90.

Derer, P., Derer, M., and Goffinet, A. (2001). Axonal secretion of reelin by Cajal-Retzius cells: evidence from comparison of normal and Reln(Orl) mutant mice. *J. Comp. Neurol*. 440:136–143.

Ichihara, H., Jingami, H., and Toh, H. (2001). Three novel repetitive units of reelin. *Brain Res. Mol. Brain Res*. 97:190–193.

Jossin, Y., Ignatova, N., Hiesberger, T., Herz, J., Lambert de Rouvroit, C., and Goffinet, A. M. (2004). The central fragment of reelin, generated by proteolytic processing in vivo, is critical to its function during cortical plate development. *J. Neurosci*. 24:514–521.

Jossin, Y., Gui, L., and Goffinet, A. M. (2007). Processing of reelin by embryonic neurons is important for function in tissue but not in dissociated cultured neurons. *J. Neurosci*. 27:4243–4252.

Kantor, D. B., Chivatakarn, O., Peer, K. L., Oster, S. F., Inatani, M., Hansen, M. J., Flanagan, J. G., Yamaguchi, Y., Sretavan, D. W., Giger, R. J., and Kolodkin, A. L. (2004). Semaphorin 5A is a bifunctional axon guidance cue regulated by heparan and chondroitin sulfate proteoglycans. *Neuron* 44:961–975.

Lacor, P. N., Grayson, D. R., Auta, J., Sugaya, I., Costa, E., and Guidotti, A. (2000). Reelin secretion from glutamatergic neurons in culture is independent from neurotransmitter regulation. *Proc. Natl. Acad. Sci. USA* 97:3556–3561.

Lambert de Rouvroit, C., Bernier, B., Royaux, I., de Bergeyck, V., and Goffinet, A. M. (1999). Evolutionarily conserved, alternative splicing of reelin during brain development. *Exp. Neurol*. 156:229–238.

Lin, X. (2004). Functions of heparan sulfate proteoglycans in cell signaling during development. *Development* 131:6009–6021.

Nagai, T., Ibata, K., Park, E. S., Kubota, M., Mikoshiba, K., and Miyawaki, A. (2002). A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. *Nature Biotechnol*. 20:87–90.

Nakano, Y., Kohno, T., Hibi, T., Kohno, S., Baba, A., Mikoshiba, K., Nakajima, K., and Hattori, M. (2007). The extremely conserved C-terminal region of reelin is not necessary for secretion but is required for efficient activation of downstream signaling. *J. Biol. Chem*. 282:20544–20552.

Nogi, T., Yasui, N., Hattori, M., Iwasaki, K., and Takagi, J. (2006). Structure of a signaling-competent reelin fragment revealed by X-ray crystallography and electron tomography. *EMBO J*. 25:3675–3683.

Royaux, I., Lambert de Rouvroit, C., D'Arcangelo, G., Demirov, D., and Goffinet, A. M. (1997). Genomic organization of the mouse reelin gene. *Genomics* 46:240–250.

Utsunomiya-Tate, N., Kubo, K., Tate, S., Kainosho, M., Katayama, E., Nakajima, K., and Mikoshiba, K. (2000). Reelin molecules assemble together to form a large protein complex, which is inhibited by the function-blocking CR-50 antibody. *Proc. Natl. Acad. Sci. USA* 97:9729–9734.

Yasui, N., Nogi, T., Kitao, T., Nakano, Y., Hattori, M., and Takagi, J. (2007). Structure of a receptor-binding fragment of reelin and mutational analysis reveal a recognition mechanism similar to endocytic receptors. *Proc. Natl. Acad. Sci. USA* 104:9988–9993.