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Reelin Promotes Hippocampal Dendrite Development through the VLDLR/ApoER2-Dab1 Pathway

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Summary

Reelin is a secreted glycoprotein that regulates neuronal positioning in cortical brain structures through the VLDLR and ApoER2 receptors and the adaptor protein Dab1. In addition to cellular disorganization, dendrite abnormalities are present in the brain of *reeler* mice lacking Reelin. It is unclear whether these defects are due primarily to cellular ectopia or the absence of Reelin. Here we examined dendrite development in the hippocampus of normal and mutant mice and in dissociated cultures. We found that dendrite complexity is severely reduced in homozygous mice deficient in Reelin signaling both in vivo and in vitro, and it is also reduced in heterozygous mice in the absence of cellular ectopia. Addition of Reelin interfering antibodies, receptor antagonists, and Dab1 phosphorylation inhibitors prevented dendrite outgrowth from normal neurons, whereas addition of recombinant Reelin rescued the deficit in *reeler* cultures. Thus, the same signaling pathway controls both neuronal migration and dendrite maturation.

Introduction

Dendrites are key morphological features of neurons that define their physiological properties and connectivity. These processes begin to form during embryogenesis but continue to extend and branch profusely long after neuronal migration is complete, contributing significantly to postnatal brain growth. In some areas of the brain, such as the hippocampus, process growth and remodeling continues throughout adult life and is profoundly affected by electrical activity. Disruption of dendrite development is the most consistent anatomical finding in mental retardation (reviewed by Kaufmann and Moser, 2000). Abnormalities consisting of either branching or spine defects are found in many diseases of genetic origin, such as Down's, Fragile X, and Rett syndromes, and in nongenetic disorders associated with mental retardation. Despite their essential role in brain function, much less is known about dendrite growth than other aspects of nervous system development such as axon guidance. In vertebrates, a few proteins such as neurotrophins, Notch, and small GTPases are known to affect dendritic growth and branching (see Whitford et al., 2002, for a recent review). The study of mutant mice in which dendrites are abnormal may lead to the discovery of other molecules that can influence dendrite development.

The morphology and the orientation of dendrites is severely impaired in cortical structures of *reeler* (Stanfield and Cowan, 1979a, 1979b), a mutant mouse carrying null mutation in the *reelin* (*reln*) gene (D'Arcangelo et al., 1995). Homozygous *reeler* mutants are characterized by cellular disorganization in cortical structures of the brain (reviewed by D'Arcangelo and Curran, 1998; Rice and Curran, 2001; Tissir and Goffinet, 2003). Cellular ectopia correlates with the extent of dendrite disorientation, but it is not known whether the absence of Reelin per se adversely affects dendrite development. Heterozygous *reeler* mice do not have an overt phenotype, but a reduction in the number of dendritic spines has been noted (Liu et al., 2001), raising the possibility that Reelin may influence neuronal maturation.

In the embryonic forebrain, Reelin is predominantly expressed by Cajal-Retzius cells in superficial layers of the neocortex and hippocampus (Alcantara et al., 1998; D'Arcangelo et al., 1995, 1997; Ogawa et al., 1995). Since Reelin is a secreted protein (D'Arcangelo et al., 1997), it functions as an extracellular signal that provides a positional cue to radially migrating neurons, thereby directing the formation of cortical layers. This activity is mediated by a signal transduction pathway that includes two high-affinity receptors, the very low-density lipoprotein receptor (VLDLR), the Apolipoprotein E Receptor 2 (ApoER2) (D'Arcangelo et al., 1999; Hiesberger et al., 1999; Trommsdorff et al., 1999), and the intracellular adaptor protein Disabled1 (Dab1) (Howell et al., 1997; Sheldon et al., 1997; Ware et al., 1997). Phosphorylation of Dab1 on specific tyrosine residues (Howell et al., 1999; Keshvara et al., 2001) is upregulated by Reelin binding to VLDLR and ApoER2 and is required for layer formation (Howell et al., 2000). This phosphorylation event is carried out by src-family kinases (Arnaud et al., 2003; Bock and Herz, 2003). In addition to lipoprotein receptors, Reelin has been shown to interact with Integrin $\alpha3\beta1$ (Dulabon et al., 2000). Even though this putative receptor does not appear to be crucial for layer formation, it is involved in the development of the radial glia end feet and the radial scaffold (Forster et al., 2002; Graus-Porta et al., 2001). After birth, Reelin and its signaling molecules continue to be expressed in the hippocampus where they control the migration of dentate granule cells (Drakew et al., 2002; Frotscher et al., 2003). In the adult brain, Reelin is expressed in all cortical layers by GABAergic interneurons (Alcantara et al., 1998; Pesold et al., 1998) and accumulates on dendritic spines (Rodri-

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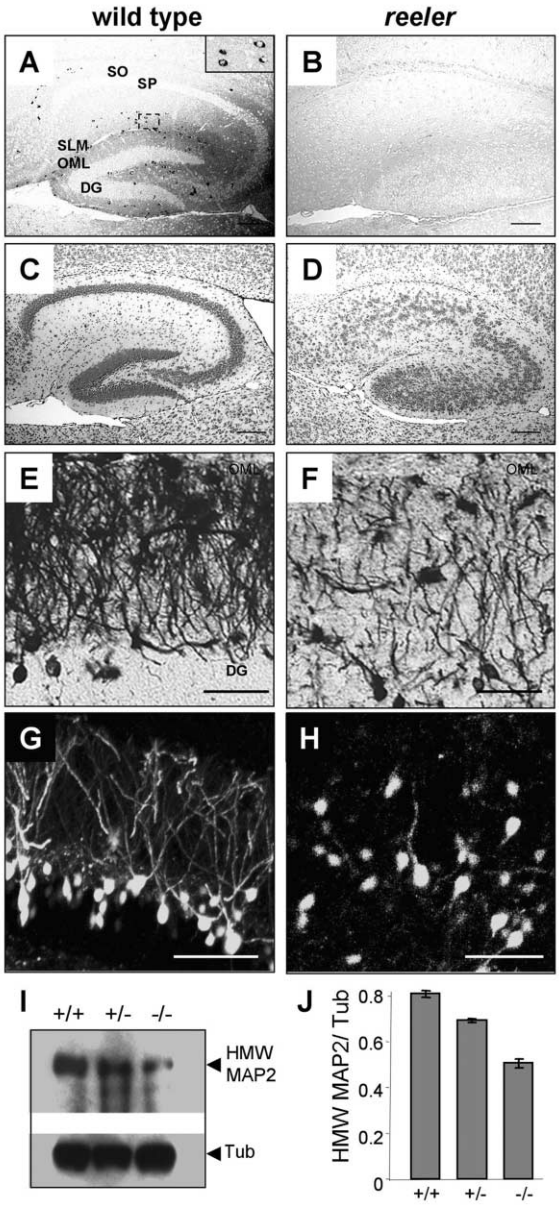

Figure 1. Dendrite Abnormalities in the *reeler* Hippocampus

(A–D) Sagittal sections of postnatal day 14 wild-type and *reeler* hippocampus were processed for immunohistochemistry using Reelin monoclonal antibodies (A and B) or stained with cresyl violet (C and D). Many Reelin-positive cells corresponding to Cajal-Retzius cells (inset) can be seen in the stratum lacunosum moleculare (SLM) and outer marginal layer (OML) in the wild-type but not in the *reeler* section. The cresyl violet staining reveals the cellular disorganization of the hippocampus proper and dentate gyrus in *reeler*.

(E and F) Golgi staining of dentate granule cells in similar hippocampal sections from wild-type and *reeler* mice.

(G and H) Confocal images of YFP-expressing neurons in the dentate gyrus of wild-type and *reeler* mice at postnatal day 14. Note the reduced dendritic arborization of *reeler* neurons.

(I) Western blot analysis of high molecular weight (HMW) MAP2 in hippocampal extracts of wild-type (+/+), heterozygous (+/−), and homozygous *reeler* mice (−/−). The same blot was reprobed with antibodies against β-tubulin (Tub) to ensure equal protein loading in each lane.

(J) Quantitative analysis of MAP2 expression in the hippocampus of wild-type, heterozygous, and homozygous *reeler* mice. The mean ratio between densitometry measurements of HMW MAP2 and β-tubulin bands in three experiments is plotted. Bars represent the standard error of the mean (SEM). The decrease in MAP2 expression in *reeler* homozygous compared to wild-type samples is significant ($p < 0.001$).

Abbreviations: SO, stratum oriens; SP, stratum pyramidale; DG, dentate gyrus. Scale bars equal 200 μm in (A)–(D) and 50 μm in (E)–(H).

guez et al., 2000). Recent data demonstrated that Reelin promotes synapse maturation and the refinement of connectivity in the postnatal peripheral nervous system (Quattrocchi et al., 2003). Together these findings suggested that Reelin plays important roles beyond neuronal migration in postnatal brain development and function.

A previously identified function of Reelin in postnatal hippocampus not related to migration is to promote the branching of entorhinohippocampal projections (Del Rio et al., 1997). However, the Reelin-rich marginal layers also represent the projection territory of apical dendrites of hippocampal pyramidal cells and dentate granule cells. Since these dendrites are stunted in the *reeler* mutant (Stanfield and Cowan, 1979a), we hypothesized that Reelin may be important for their growth. Here we describe the novel activity of Reelin in directly promoting the development of hippocampal dendrites and identify its molecular mechanism of action.

Results

Reduced Dendrite Growth in *reeler* Hippocampus

Analysis of the postnatal day 14 wild-type hippocampus showed that high levels of Reelin protein are present in marginal layer cells of the hippocampus proper and dentate gyrus (Figure 1A). These cells correspond to Cajal-Retzius cells born early during corticogenesis that are still abundant during the first two postnatal weeks but decrease in number in the adult forebrain (Alcantara et al., 1998). In the stratum oriens and radiatum, GABAergic interneurons express lower levels of Reelin. No signal was present in the hippocampus of homozygous *reeler* mice (Figure 1B). In this mutant, pyramidal cells form a split layer in the hippocampus proper, and granule cells are scattered throughout the dentate gyrus (compare Figure 1D to 1C). To examine the dendrite arborization of hippocampal neurons in normal and *reeler* mice, we first used the classic Golgi impregnation technique. As noted previously with this technique (Stanfield and Cowan, 1979a), cellular orientation was abnormal in *reeler*, especially when neurons were located in ectopic positions. Dendritic arbors also appeared noticeably less developed than normal in mutant dentate granule and pyramidal cells (Figures 1E and 1F, and see Supplemental Figures S1A and S1B at http://www.neuron.org/cgi/content/full/41/1/71/DC1). To overcome the staining variability inherent to the Golgi technique, we crossed heterozygous *reeler* mice with transgenic *thy1*-YFP (line H) mice (Feng et al., 2000) and obtained in the second generation both wild-type and *reeler* littermates that express yellow fluorescent protein (YFP) in selected neuronal populations, including hippocampal pyramidal and dentate granule cells. Confocal analysis of YFP-expressing neurons in postnatal day 14 hippocampus

allowed us to visualize dendritic trees of normal and mutant hippocampal neurons and further illustrated the *reeler* branching defect in vivo (Figures 1G and 1H, and Supplemental Figure S2). To quantify the observed difference in dendrite growth, we performed Western blot analysis of hippocampal protein extracts using antibodies directed against the Microtubule-Associated Protein 2 (MAP2). This is a well-known neuronal marker that recognizes all isoforms of MAP2, including the high molecular weight (HMW) dendrite-specific isoforms MAP2a and MAP2b. To normalize for the protein content in each lane, the blot was reprobed with β-tubulin antibodies, and the ratio of the intensity of HMW MAP2 over β-tubulin was measured in triplicate experiments. The results show that the levels of HMW MAP2 are significantly decreased in the hippocampus of homozygous mutants and also, to a lesser extent, in heterozygous *reeler* mice (Figures 1I and 1J). A similar decrease was noted in other areas of the brain such as the cerebral cortex and cerebellum (not shown).

Dendrite Growth in Culture Is Dependent on Reelin

To study the effect of the *reeler* mutation on dendrite development independently of abnormal cell position or altered neuronal connectivity, we established dissociated hippocampal cultures from embryonic day 18 homozygous or heterozygous *reeler* mice and their wild-type littermates. Hippocampal neurons obtained from each genotype were cultured on glass coverslips over a feeder layer consisting of cells derived from the rest of the brain in the same embryos. These homotypic conditions were chosen to ensure that *reeler* hippocampal neurons were cultured in the complete absence of Reelin. After 5 days in culture, hippocampal neurons were fixed and analyzed by phase-contrast microscopy. The data revealed a dramatic reduction of neuritic processes in mutant *reeler* compared to wild-type and heterozygous littermate cultures (Figures 2A–2C).

To examine the development of dendrites specifically, we analyzed the cultures by immunofluorescence using the MAP2 antibody. Microscopic examination revealed that dendritic processes of mutant *reeler* cells were drastically reduced compared to those of wild-type or heterozygous neurons after 5 days in vitro (Figures 2D–2I). To further characterize the defect, we examined the time course of dendrite development in MAP2-labeled cultures derived from each genotype. The data were analyzed quantitatively by measuring the average total length of the dendrites and the total number of branches after 2–6 days in culture (Figures 2J and 2K). This analysis confirmed that dendrite growth and complexity is severely reduced in homozygous *reeler* cultures. The defect was noticeable in the mutant cultures as early as 2 days in vitro, but became more pronounced after 6 days, and it was significant compared to both wild-type or heterozygous values ($p < 0.001$). A smaller but significant reduction of dendrite length and complexity was also detected in heterozygous *reeler* cells after 5 or 6 days in culture, respectively ($p < 0.001$). The fact that dendrites grew less extensively in cultures derived from mutant mice could be due to the absence of Reelin. Alternatively, reduced growth of *reeler* dendrites in culture could be a secondary consequence of a deficit acquired in vivo due to cellular ectopia or abnormal connectivity. To distinguish between these possibilities, we first ascertained that Reelin was present in wild-type and heterozygous *reeler* cultures. Using the monoclonal antibody CR50, Reelin-expressing cells were readily identified in normal cultures (Figure 3A). Secretion of Reelin into the medium of wild-type and heterozygous cultures was confirmed by dot blot analysis using the monoclonal antibody E4 (Figure 3B). Next, we incubated heterozygous *reeler* cultures for 5 days with the Reelin interfering antibody CR50 (D'Arcangelo et al., 1997; Ogawa et al., 1995) or a control mouse immunoglobulin Fc fragment. The CR50 antibody has been previously reported to block the biological function of Reelin in vitro (Del Rio et al., 1997; Ogawa et al., 1995) and in vivo (Nakajima et al., 1997). Biochemical studies demonstrated that this antibody inhibits Reelin binding to the VLDLR and ApoER2 receptors (D'Arcangelo et al., 1999). Dendrites appeared shorter in CR50-treated cultures compared to control (Figures 3C and 3D). Quantitative analysis (Figure 3E) demonstrated that the CR50 treatment significantly reduced dendrite length and complexity, suggesting that dendrite maturation in our experimental assay was strictly dependent upon the presence of Reelin in the culture medium.

Reelin Promotes the Development of MAP2-Positive Processes in Hippocampal Cultures

To determine whether Reelin directly regulates dendrite growth, we attempted to rescue the *reeler* deficit in hippocampal neurons cultured from embryonic day 18 mice using native or recombinant Reelin. Coverslips containing *reeler* hippocampal cells were placed either into homologous *reeler* (Figure 4A) or heterologous wild-type (Figure 4B) feeder dishes for 5 days. Exposure to wild-type cells secreting native Reelin promoted extensive dendrite outgrowth from mutant *reeler* cultures. Quantitative analysis showed that native Reelin caused a 3.5-fold increase in dendrite length and a 2.9-fold increase in the number of branches (Figures 4G and 4H). Next, we incubated *reeler* hippocampal cells in the presence of recombinant Reelin secreted by a stable cell line (CER). The crude serum-free conditioned medium of CER cells or a control line transfected with empty vector (CEP4) was concentrated approximately 50-fold and added to the culture medium. Alternatively, recombinant Reelin was partially purified from CER-conditioned medium by differential ammonium sulfate precipitation, as previously described (Keshvara et al., 2001). Western blot analysis revealed the presence of several isoforms of Reelin in our preparations, including the ~400 kDa full-length and the major proteolytic N-terminal fragments of ~300 and ~180 kDa (Figure 4I; D'Arcangelo et al., 1999). This latter protein was the most abundant isoform, particularly in the concentrated conditioned medium. Treatment of mutant *reeler* cultures with either Reelin-containing medium (Figure 4C) or partially purified protein (Figure 4E) resulted in extensive dendrite outgrowth, whereas control medium (Figure 4D) had no effect. Similar results were obtained when Reelin was partially purified by gel filtration chromatography (data not shown). The effect of recombinant Reelin on dendrite length and complexity was similar to that of the native

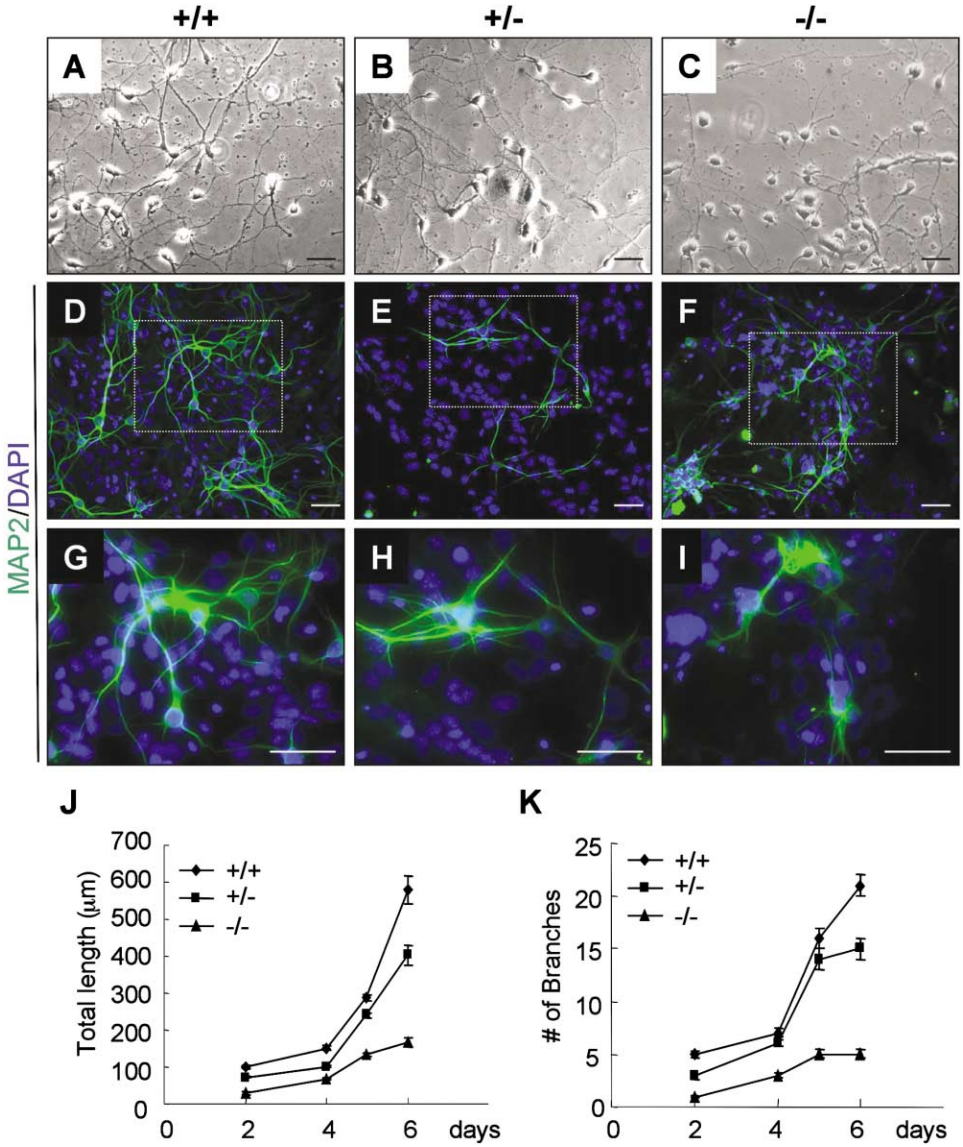

Figure 2. Defective Dendrite Outgrowth in *reeler* Cultures

Dissociated hippocampal neurons from embryonic day 18 wild-type (+/+), heterozygous (+/−), or homozygous (−/−) *reeler* mice were cultured for 5 days in vitro.

(A–C) Phase contrast images.

(D–I) MAP2 immunofluorescence overlaid with DAPI nuclear counterstain. Images in (G)–(I) represent enlargements of boxed areas in (D)–(F).

(J and K) Quantitative analysis of MAP2-labeled dendrites was performed by measuring total dendrite length (J) or the number of dendritic branches (K) in wild-type, heterozygous, or homozygous *reeler* neurons at the indicated days in culture. Bars represent SEM from 25 neurons in three experiments. The difference between dendrite length and number of branches in *reeler* homozygous versus wild-type samples is significant beginning at day 4 in vitro ($p < 0.001$). Significant values for heterozygous samples are obtained after 6 days in culture ($p < 0.001$). Scale bars equal 50 μm.

protein at the concentrations used in these experiments (Figures 4G and 4H). To further characterize the Reelin effect, a dose-response curve was generated using increasing amounts of concentrated CER-conditioned medium (Figure 4J). Analysis of this medium by SDS-PAGE and Coomassie brilliant blue staining indicated that the amount of Reelin was approximately 100 ng/ml. Thus, the total concentration of Reelin that produced half-maximal response (IC_{50}) on dendrite outgrowth in our assay was estimated to be approximately 130 ng/ml. Given that total Reelin is mostly composed of a mixture of 400–180 kDa isoforms, this value corresponds to a concentration of 0.325–0.722 nM, similar to the estimated Kd of Reelin binding to lipoprotein receptors (0.5 nM) (D'Arcangelo et al., 1999; Hiesberger et al., 1999). Finally, we compared the effect of Reelin treatment to that of BDNF, a neurotrophin known to stimulate dendrite growth in hippocampal neurons. Mutant *reeler* hippocampal neurons extended many complex dendrites in the presence of BDNF (Figure 4F). In comparison, Reelin-induced dendrites appeared longer but bore relatively fewer branches (Figures 4G and 4H).

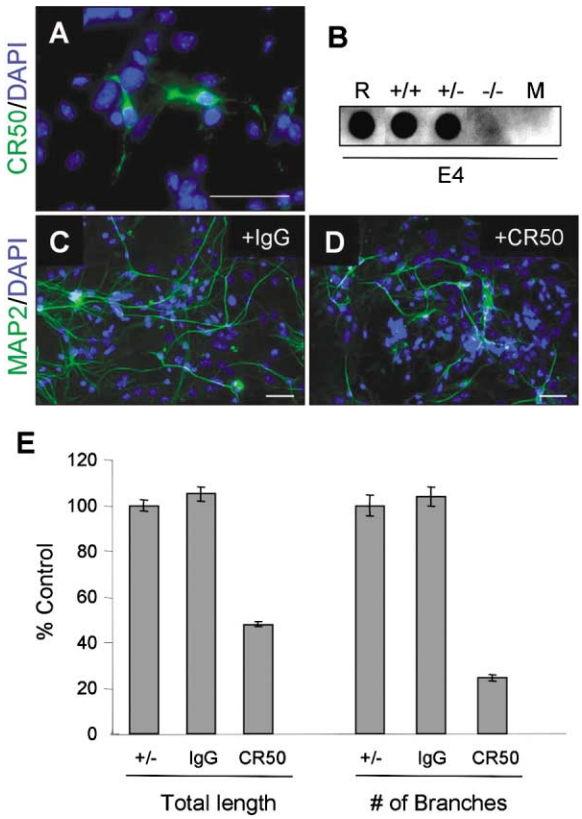

Figure 3. Dendrite Growth Is Dependent on Secreted Native Reelin

(A) Reelin-producing cells in wild-type neuronal cultures were identified by immunofluorescence using the CR50 antibody.

(B) Dot blot analysis of secreted Reelin in the medium of wild-type (+/+), heterozygous (+/−), and homozygous (−/−) *reeler* cultures using the E4 monoclonal antibody. No Reelin was present in the mutant culture medium. Abbreviations: R, recombinant Reelin (positive control); M, medium not exposed to cultures (negative control).

(C and D) MAP2 immunofluorescence of +/− cultures incubated in the presence of control IgG or interfering antibody CR50 (2 μg/ml).

(E) Quantitative analysis of dendrite length and number of branches in cultures that were untreated (+/−) or treated with IgG and CR50. Values were expressed as percentage of untreated control. Bars represent SEM from 25 neurons in three experiments. The differences between CR50 samples and controls are statistically significant ($p < 0.001$).

Scale bars equal 50 μm.

Reelin Promotes Neuronal Dendrite Development Independently of Glial or Progenitor Cells

In this study, we used a mixture of cell types to generate hippocampal cultures and brain feeder layers. Because glial and progenitor cells can be affected by exposure to Reelin (Forster et al., 2002; Frotscher et al., 2003; Weiss et al., 2003), we investigated their possible contribution to neuronal dendrite development. The cellular composition of our culture system was examined in detail by immunofluorescence using cell type-specific markers. Under the standard conditions utilized throughout this study, almost half the cells dissociated from embryonic day 18 brain (hippocampus cultures or feeder layer) and cultured for 5 days consisted of MAP2-positive neurons (45%) (Figures 5A and 5C). Other cells consisted mainly of progenitors (either Nestin+GFAP− or Nestin+GFAP+) and a small proportion of GFAP-positive glial cells (3%). To inhibit the proliferation of glia and progenitor cells, 200 nM Cytosine-β-D-arabinofuranoside (AraC) was added to wild-type culture dishes. This treatment resulted in a noticeable shift in cellular composition. Neurons represented the great majority of the cells after 5 days in vitro (86%) (Figures 5B and 5C) and progenitor cells represented a minority of the cells (14% total Nestin+ cells), whereas glial cells were almost undetectable (<0.5%). When dendrite length and complexity was analyzed in cultures treated with or without AraC, no difference was observed despite the alteration in cellular composition (Figure 5D). Furthermore, AraC treatment did not alter the ability of recombinant Reelin to rescue dendrite defects in *reeler* cultures (Figure 5E). To ensure that Reelin promotes process outgrowth in true neurons and not in radial progenitor cells that are beginning to express neuronal markers, we established hippocampal cultures from postnatal day 1. Double labeling with MAP2 and Nestin antibodies indicated that essentially all MAP2-positive cells in these cultures no longer express the Nestin progenitor marker (Figure 5F). As observed above for embryonic cultures (Figure 4), dendrite elongation was reduced in cultures derived from *reeler* mice compared to normal littermates, and it was stimulated in mutant cells exposed to wild-type medium containing Reelin (Figure 5G). Together these data suggest that the effects of Reelin on dendrite development are not mediated by glial or progenitor cells but may be exerted directly on neuronal cells.

Reelin Promotes Dendrite Development through Lipoprotein Receptor Binding

Cortical layer formation requires the binding of Reelin to VLDLR and ApoER2 receptors expressed by migrating neurons (D'Arcangelo et al., 1999; Hiesberger et al., 1999; Trommsdorff et al., 1999). In addition, Reelin can bind Integrin α3β1 in vitro (Dulabon et al., 2000), but this interaction does not mediate cortical layer formation (Graus-Porta et al., 2001). To determine whether lipoprotein receptors mediate Reelin-dependent dendrite outgrowth in our culture assay system, we first analyzed their expression by immunofluorescence. We found that ApoER2 was expressed at high levels in most hippocampal neurons, but it was seldom seen in glial or progenitor cells (Figures 6A–6C). A faint VLDLR signal was also observed in our cultures. As for ApoER2, VLDLR expression was mostly found in neuronal but rarely in nonneuronal cells (Figures 6D–6F). To investigate the role of lipoprotein receptors in Reelin-induced dendrite outgrowth, we cultured homozygous and heterozygous *reeler* hippocampal cells in the presence or absence of RAP, an inhibitor of ligand binding to this family of receptors. RAP was fused to Glutathione S-Transferase (GST) and purified by affinity chromatography. The addition of 50 μg/ml of GST-RAP to homozygous *reeler* cultures (Figures 6G and 6H) had no effect alone but strongly inhibited dendrite outgrowth when added together with recombinant Reelin. Similar concentrations of GST-RAP were previously shown to inhibit Reelin-induced Dab1 phosphorylation (Hiesberger et al., 1999). The addition of control GST or Integrin α3 interfering

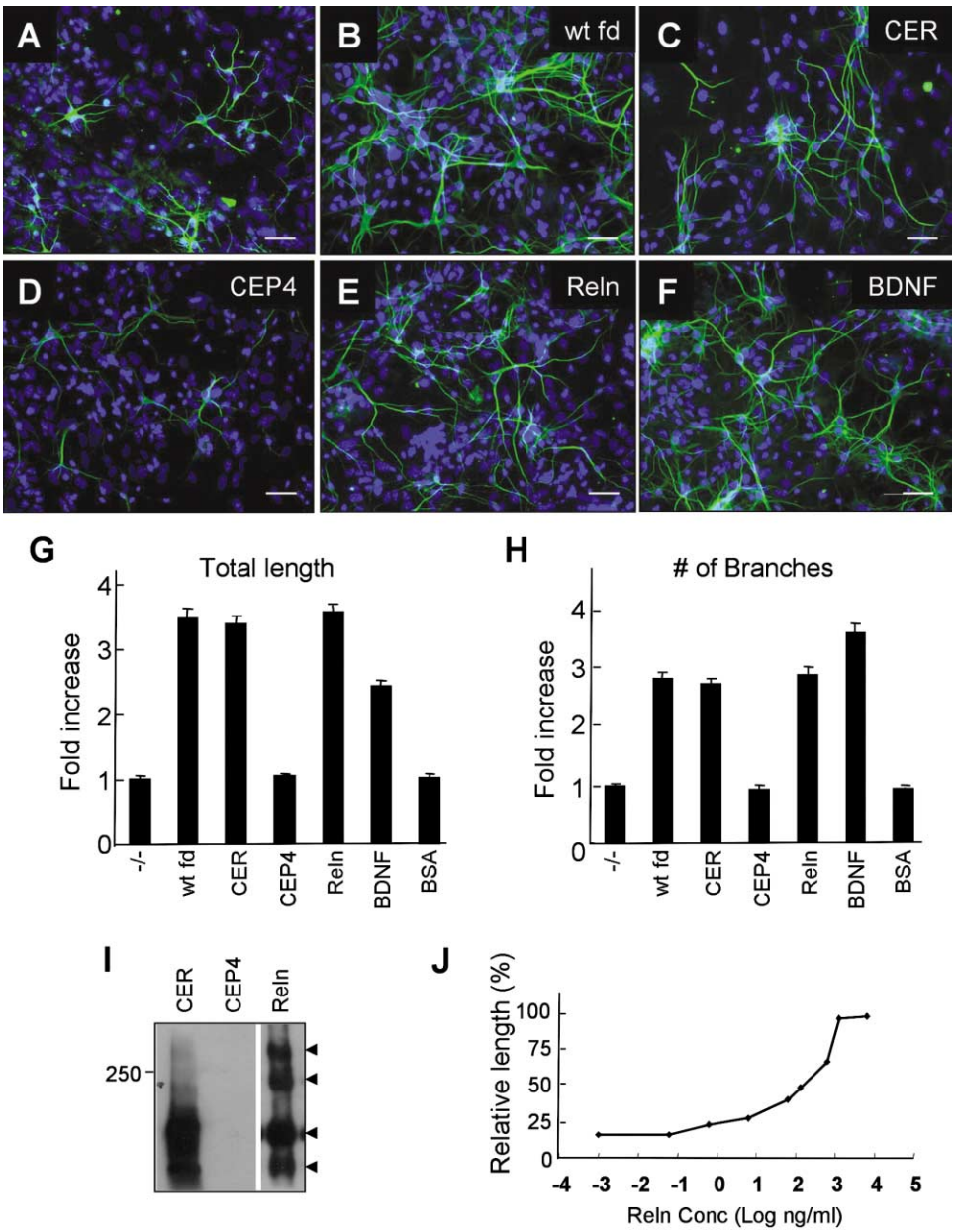

Figure 4. Reelin Rescues Dendrite Abnormalities in *reeler* Cultures

(A–F) MAP2 immunofluorescence of homozygous *reeler* hippocampal neurons cultured over homologous *reeler* feeder cells, except for (B) where hippocampal neurons were cultured over wild-type feeder cells (wt fd). Mutant neurons were untreated (A) or incubated with conditioned medium containing recombinant Reelin (CER) (C), mock conditioned medium (CEP4) (D), partially purified recombinant Reelin (E), or BDNF (F).

(G and H) Total dendrite length (G) and the number of branches (H) were measured and compared to values obtained from untreated cultures (−/−). BSA, bovine serum albumin. Bars represent SEM from 25 neurons in three experiments. The difference between all samples exposed to Reelin versus control is significant ($p < 0.001$).

(I) Western blot analysis of concentrated conditioned medium and the partially purified Reelin preparation used in the experiments above. Bands corresponding to full-length, and several proteolytic products of Reelin (arrowheads) were detected using the E4 antibody.

(J) Dose-response curve of Reelin dendrite-promoting activity. Concentrated CER conditioned medium containing the indicated estimated amounts of Reelin was used to induce dendrite outgrowth from *reeler* hippocampal cultures. Data points represent the mean total length from 25 neurons expressed as a percentage of the maximum value.

Scale bars equal 50 μm.

antibodies had no effect on the ability of Reelin to rescue the *reeler* dendrite deficit. When heterozygous *reeler* cultures (Figures 6I and 6J) were treated with GST-RAP alone, dendrite growth was significantly reduced compared to untreated or control cultures. Addition of recombinant Reelin alone to these heterozygous *reeler* cultures slightly promoted dendrite growth, whereas addition of Reelin and GST-RAP together resulted in an intermediate outgrowth of dendrites, consistent with the competitive nature of Reelin and RAP binding to lipopro-

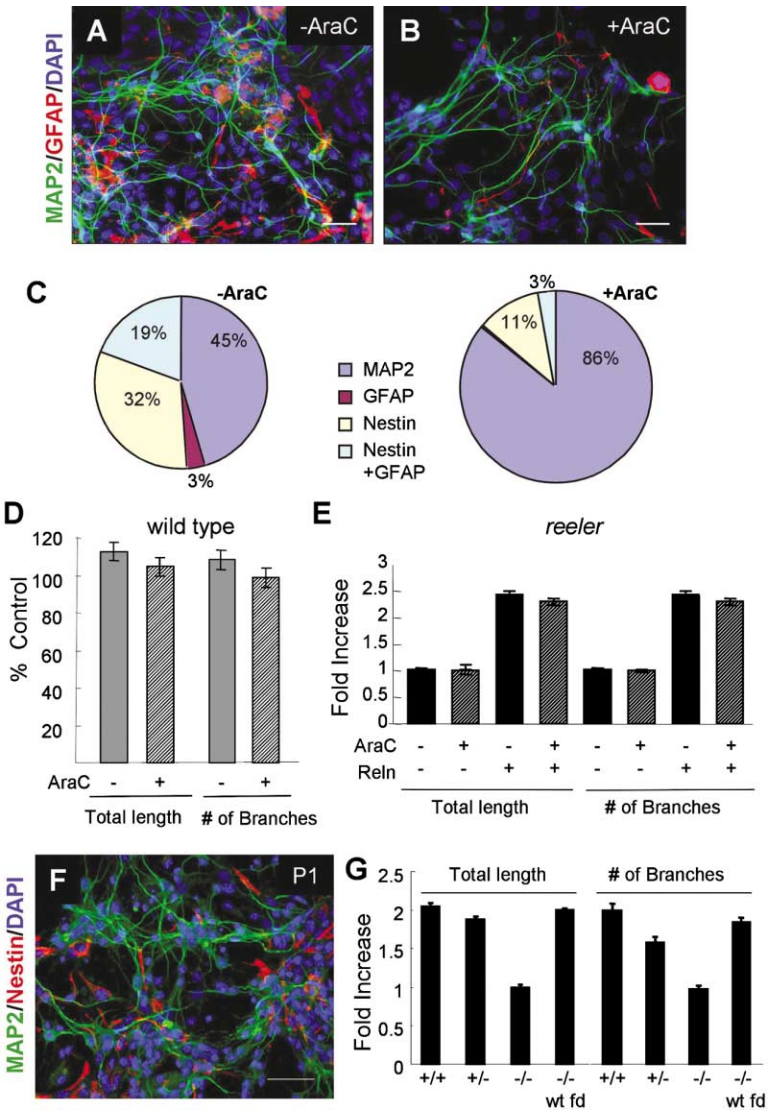

Figure 5. Reelin Promotes Neuronal Dendrite Outgrowth Independently of Progenitor or Glial Cells

Wild-type hippocampal cells were cultured over a homologous feeder layer in the presence or absence of 200 nM AraC to inhibit glial cell proliferation.

(A and B) Overlay images of cells stained with a neuronal marker (MAP2), a glial marker (GFAP), and nuclear stain (DAPI). Note the reduced number of glial cells in (B).

(C) Pie charts illustrate the cellular composition of cultures with or without AraC added to the medium. MAP2-positive neurons represent the majority of cells in the AraC-containing cultures.

(D) Quantitative analysis of dendrite length and complexity in wild-type cells under the indicated culture conditions. The presence of AraC does not influence dendrite outgrowth despite the drastic reduction of glial cell number.

(E) Quantitative analysis of dendrite length and complexity in *reeler* cells under the indicated culture conditions. Reelin stimulates dendrite outgrowth irrespective of the abundance of glial cells.

(F) Overlay image of a postnatal day 1 (P1) culture stained with MAP2 and Nestin antibodies. The two markers do not overlap.

(G) Dendrite complexity in P1 cultures from wild-type (+/+), heterozygous (+/−), and homozygous (−/−) *reeler* mice. Exposure to wild-type feeder layer (wt fd) rescues the *reeler* defects. Plots represent mean values from 25 neurons ± SEM.

Scale bars equal 50 μm.

tein receptors. As for homozygous cultures, the addition of Integrin α3 interfering antibodies had no effect. Taken together, these data suggested that lipoprotein receptors mediate Reelin-induced dendrite development in hippocampal neurons.

Dab1 Is Required for Hippocampal Dendrite Development

Since Dab1 mediates Reelin function in the formation of cortical layers (Howell et al., 1997, 1999; Sheldon et al., 1997), we investigated whether this adaptor protein is also crucial for dendrite outgrowth. In situ hybridization analysis with an antisense probe revealed that *Dab1* mRNA is expressed in the pyramidal layer and in dentate gyrus of normal postnatal hippocampus (Figure 7A). No signal was present in wild-type sections incubated with a sense probe or in Dab1 knockout sections incubated with an antisense probe (Figures 7B and 7C). As previously reported, extensive cellular ectopia was observed in the homozygous mutant hippocampus (Figure 7C). To determine whether dendrites were also defective in the Dab1 knockout hippocampus, we processed sections of wild-type, heterozygous, and homozygous mutant mice with Golgi stain. As noted above for *reeler* (Figure 1), dendrites of dentate granule and pyramidal cells appeared progressively less developed in heterozygous and homozygous mice (Figures 7D–7F and Supplemental Figure S1C–S1F at http://www.neuron.org/cgi/content/full/41/1/71/DC1). When HMW MAP2 levels were analyzed by Western blot, we found that the expression of this dendrite marker was reduced in Dab1 mutants and, to a lesser extent, also in heterozygous mice in the absence of cellular ectopia (Figures 7G and 7H). These data indicated that *dab1* gene dosage profoundly affects hippocampal dendrite maturation in vivo.

Dab1 Mediates Reelin-Induced Dendrite Outgrowth

To investigate the role of Dab1 in Reelin-induced dendrite growth, we cultured hippocampal cells from Dab1 knockout mice and their wild-type and heterozygous littermates at embryonic day 18, under the same homotypic conditions used for *reeler*. When MAP2-positive

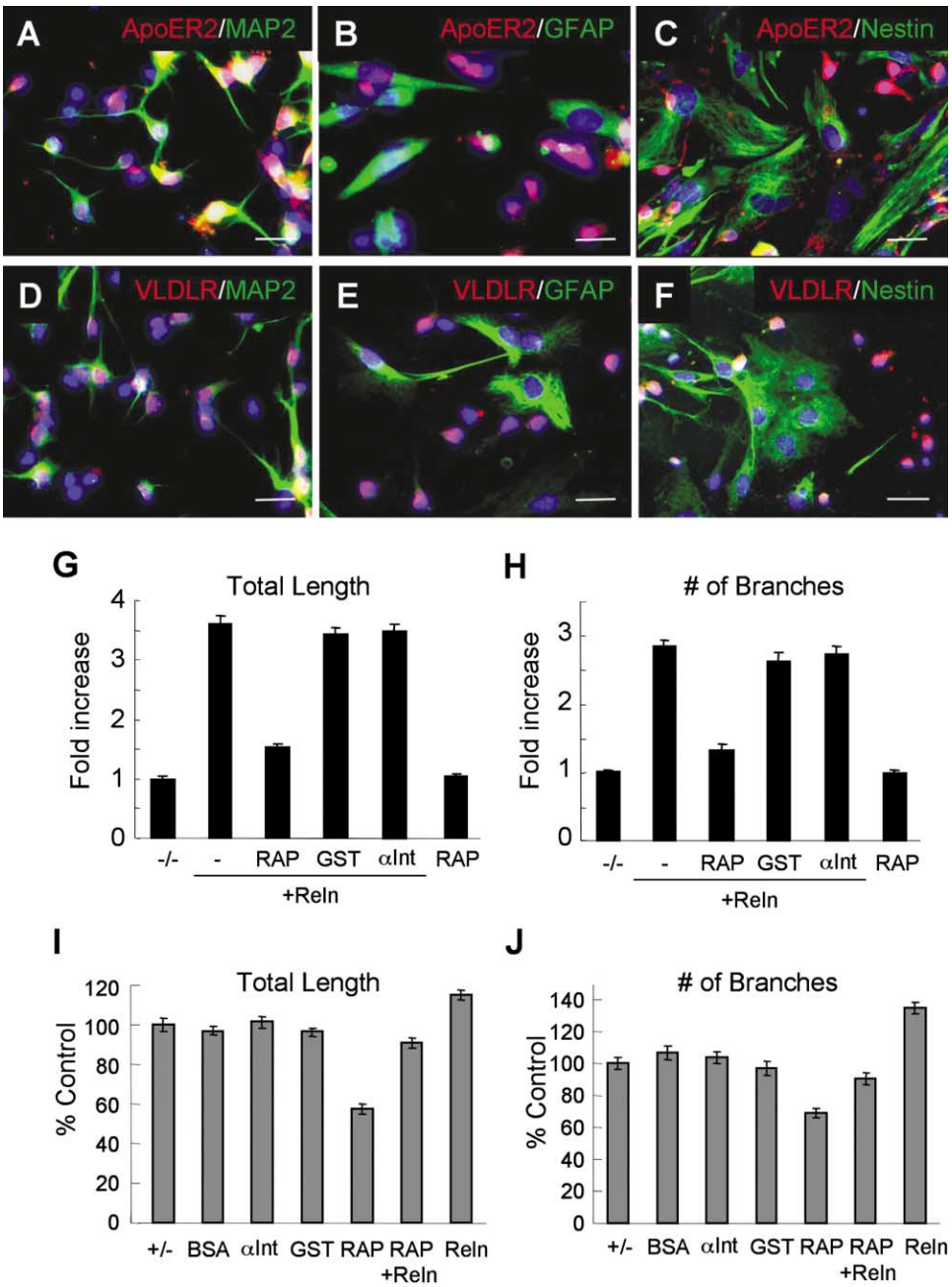

Figure 6. Lipoprotein Receptors Mediate Reelin-Induced Dendrite Growth

(A–F) Identification of cell types expressing ApoER2 or VLDLR in wild-type hippocampal cultures. Both receptors are expressed in most MAP2-positive neurons (A and D) but rarely in glial cells (B and E) or progenitors cells (C and F). Scale bars equal 50 μm.

(G and H) Quantitative analysis of dendrite length and complexity in homozygous *reeler* cultures that were untreated (−/−); treated with Reelin (Reln) in the presence or absence of GST-RAP (RAP), control GST, or Integrin α3 interfering antibodies (α INT); or treated with RAP alone. RAP cotreatment prevented Reelin-induced dendrite outgrowth.

(I and J) Quantitative analysis of dendrite length and complexity in heterozygous *reeler* cultures untreated (+/−) or treated as indicated. Plots were generated as in Figures 3 and 4. RAP inhibits dendrite outgrowth in heterozygous cultures and the effect is reversed by the addition of exogenous Reelin.

processes were analyzed, their development appeared drastically impaired in homozygous mutant Dab1 compared to wild-type cultures, and it was also reduced in heterozygous cultures (Figures 8A–8C, 8G, and 8H), similar to in vivo observations. Since Reelin expression and secretion is normal in Dab1 knockout mice (Goldowitz et al., 1997), and dendrite outgrowth is dependent on Reelin in our assay system (see above), this finding suggested that Dab1 is necessary for Reelin-induced dendrite extension. If this is the case, we predicted that addition of recombinant Reelin would not rescue the Dab1 knockout dendrite deficit. Indeed, we found that

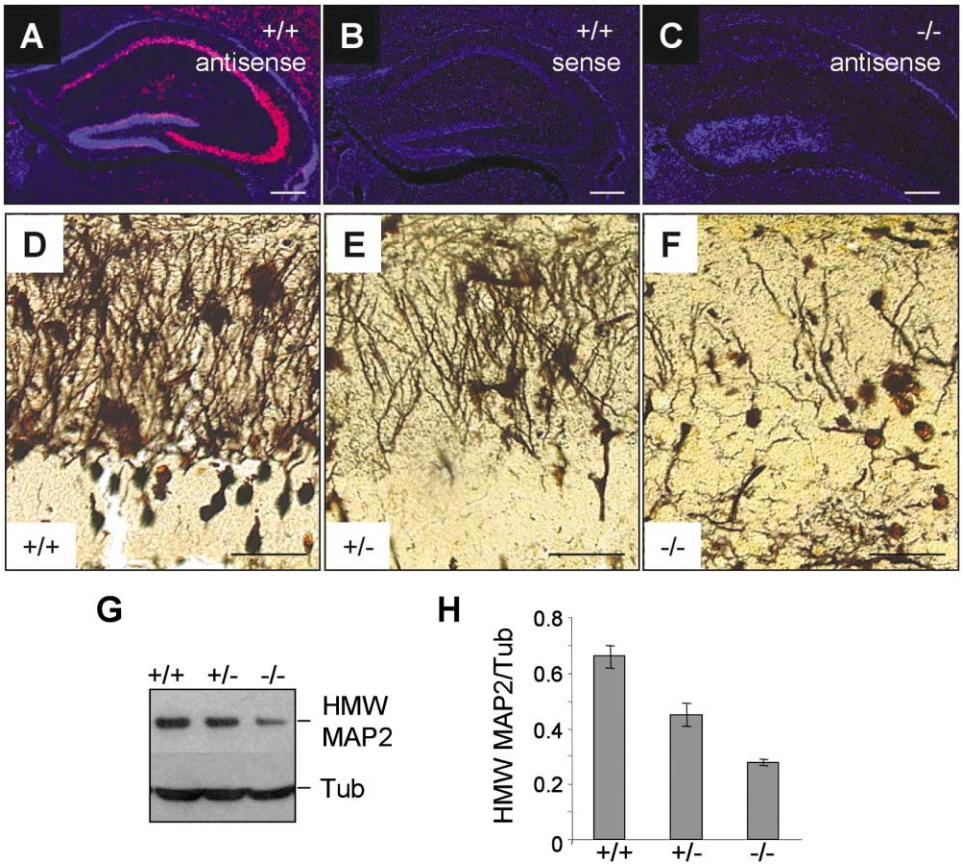

Figure 7. Reduced Dendrite Growth in the Dab1 Knockout Hippocampus

(A–C) In situ hybridization of Dab1 antisense or sense riboprobes in coronal hippocampal sections from wild-type (+/+) or Dab1 knockout mice (−/−). Dab1 signal (red) is detected in the pyramidal cells layer and in the dentate gyrus counterstained with DAPI (blue).

(D–F) Golgi staining of dentate granule cells in wild-type, heterozygous (+/−), and homozygous Dab1 knockout mice. Note the progressive reduced dendrite arborization of heterozygous and mutant mice.

(G) Western blot analysis of HMW MAP2 in hippocampal extracts of the indicated genotypes. The same blot was reprobed with antibodies against β-tubulin (Tub).

(H) The ratio of HMW MAP2 and β-tubulin bands in the indicate genotypes was calculated as in Figure 1. The difference between heterozygous or homozygous Dab1 mutants and wild-type is significant ($p < 0.001$).

Scale bars equal 200 μm in (A)–(C) and 50 μm in (D)–(F).

the addition of similar amounts of recombinant Reelin that rescued the *reeler* defect (Figure 4) did not promote dendrite extension in Dab1 mutant cultures (Figures 8D, 8G, and 8H). Dendrite outgrowth in these cultures, however, was stimulated by BDNF treatment (Figures 8F–8H), indicating that Dab1 is not required for dendrite growth per se but that it specifically mediates Reelin-induced process extension.

Immunofluorescence analysis confirmed the expression of Dab1 in many cultured hippocampal neurons obtained from wild-type (Figure 8I) but not mutant mice (Figure 8J). Since Dab1 phosphorylation in target neurons is crucial for Reelin signal transduction (Howell et al., 1999, 2000), we studied whether this event is also required for dendrite development. Wild-type cultures were incubated with the src-family kinase inhibitor PP2 (10 μM) in the presence or absence of Reelin. This compound was previously shown to block Reelin-induced Dab1 phosphorylation at similar concentrations (Arnaud et al., 2003; Bock and Herz, 2003). As controls, we used the inactive homolog PP3 or vehicle DMSO. PP2 treatment resulted in a significant reduction of dendrite extension that could not be rescued by the addition of Reelin (Figures 8K and 8L), indicating that Dab1 phosphorylation is essential for Reelin-dependent dendrite outgrowth.

Discussion

Dendrite growth is a dynamic process that continues long after migration is complete and enables neurons to receive, integrate, and process information. Here we demonstrated that Reelin stimulates dendrite outgrowth of hippocampal neurons through the activation of a tyrosine kinase-dependent signaling pathway that was previously involved in the control of neuronal migration. Thus, the binding of Reelin to VLDLR and ApoER2 receptors and the activation of Dab1 promote an important aspect of neuronal maturation in addition to layer formation and contribute to the early postnatal development of the hippocampus.

Abnormal dendrite development had been previously

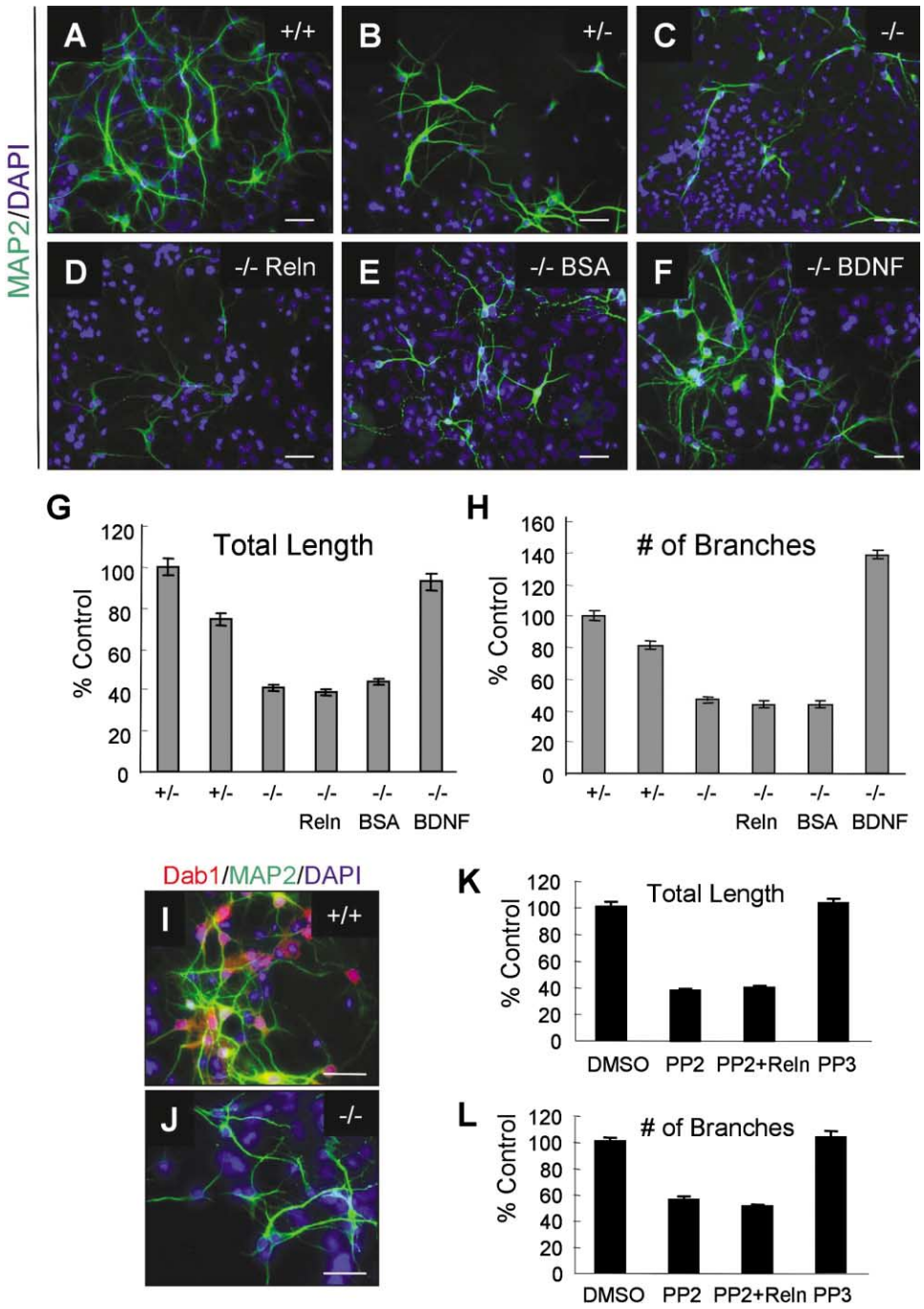

Figure 8. Dendrite Growth Is Dependent on Dab1 Phosphorylation

(A–F) MAP2 immunofluorescence in wild-type (+/+), heterozygous (+/−), or homozygous (−/−) Dab1 knockout cultures. Homozygous cultures were also treated with purified Reelin (Reln), bovine serum albumin (BSA), or BDNF. This latter treatment, but not Reelin, stimulated dendrite growth.

(G and H) Quantitative analysis of dendrites was performed as in Figure 3. The difference between dendrite length and number of branches in heterozygous or homozygous Dab1 mutants compared to wild-type samples is significant ($p < 0.001$).

(I and J) Dab1 immunofluorescence in wild-type or Dab1 homozygous cultures incubated with a polyclonal antibody directed against the C terminus. Dab1 is expressed mainly by MAP2-positive neurons.

(K and L) Quantitative analysis of dendrite length and complexity in wild-type cultures treated with the inhibitor of Dab1 phosphorylation PP2 with or without added Reelin or its inactive homolog PP3. Control cultures were treated with the vehicle DMSO. The PP2 treatment inhibited dendrite outgrowth and could not be overcome by Reelin addition. Plots were generated as in Figure 5.

Scale bars equal 50 μm.

observed in the hippocampus of homozygous *reeler* mice (Stanfield and Cowan, 1979a, 1979b) and in double VLDLR/ApoER2 mutant mice (Drakew et al., 2002) in vivo. The extent of disorientation of dendritic arborization correlated well with the degree of cellular ectopia, suggesting that the orientation of dendrite growth is influenced by positional cues. In this study, we show that the Reelin signaling pathway regulates the length and the complexity of dendrites. Thus, dendrite abnormalities observed in *reeler* and *reeler*-like mutant mice result from a combination of primary (lack of Reelin signal) and secondary (cellular ectopia) defects. Even though the same Reelin signaling machinery mediates both dendrite extension and cellular positioning, we demonstrated that the two functions are not necessarily interdependent. Dendrite outgrowth was reduced in heterozygous *reeler* and Dab1 knockout mice in the absence of cellular ectopia (Figures 1 and 7) and in dissociated cultures deprived of Reelin activity (Figures 2, 3, 6, and 8). On the other hand, Reelin added to the culture medium promoted dendrite extension in the absence of positional information (Figure 4). However, the two functions are linked in vivo during normal cortical development.

The establishment of contacts between pyramidal cortical neurons and Reelin-rich marginal layers is one of the earliest events in cortical development and leads to the formation of an early scaffold that is maintained throughout corticogenesis (Marin-Padilla, 1992). We speculate that Reelin may promote the extension of these projections. This activity may favor the translocation of pyramidal cell bodies to a deeper layer, thus enabling the split of the preplate. A similar mechanism may be exerted on each cohort of cortical neurons that come in contact with Reelin-rich marginal layer, leading to the inside-out mode of corticogenesis. After layer formation is complete and Reelin expression is no longer layer restricted, the Reelin signal may continue to promote dendrite development in the postnatal brain and thus affect neuronal connectivity and the formation of functional circuits.

We observed reduced dendrite growth in Dab1 mutant hippocampus and cultures. Addition of Reelin could not rescue Dab1 knockout dendrite abnormalities, suggesting that the defect is cell autonomous, as expected based on the intracellular localization of the protein. This interpretation is consistent with chimera studies in which Dab1 mutant cells were shown to be unable to migrate properly in the cortex and in the cerebellum despite the presence of normal surrounding cells (Hammond et al., 2001; Yang et al., 2002). Our findings that glia and progenitor cells do not affect dendrite development (Figure 5) and that ApoER2, VLDLR, and Dab1 are mostly expressed by MAP2-positive neurons (Figures 6 and 8) strengthen this view. The present findings are not inconsistent with the observation that Reelin promotes branching of radial progenitor cells (Forster et al., 2002) and further demonstrate its ability to promote maturation in committed, postmitotic neurons. It seems likely that Reelin affects cytoskeletal dynamics and thereby induces morphological alterations in both cell types, which share similar intracellular machineries since radial progenitor cells are known to produce neurons as well as glia.

In the prenatal and early postnatal hippocampus, VLDLR, ApoER2, and Dab1 are expressed by pyramidal and dentate neurons that extend profuse dendrite arborizations in the Reelin-rich marginal layers. Previous reports showed that Reelin accumulates on dendritic spines of cortical and hippocampal neurons (Rodriguez et al., 2000) and that spine density is reduced in heterozygous mice in the absence of cellular ectopia (Liu et al., 2001). Other findings demonstrated that Reelin induces hippocampal long-term potentiation in a lipoprotein receptor-dependent fashion (Weeber et al., 2002). Our present findings are consistent with these studies and provide direct evidence that Reelin is important for hippocampal neuronal maturation and synaptic connectivity. Further studies are required to elucidate whether Reelin stimulates dendrite outgrowth in other regions of the brain such as the cerebral cortex or the cerebellum. In vivo electroporation studies (Tabata and Nakajima, 2002) demonstrated that dendrite development in *reeler* cortical neurons is delayed, suggesting a role for Reelin in neuronal maturation in the neocortex. In the cerebellum, the reduced growth and disorientation of *reeler* Purkinje cell dendrites was previously attributed to their ectopia, causing a depletion of granule cells and the consequent alteration of afferent connectivity (Mariani, 1982). Since Dab1 and VLDLR are expressed in Purkinje cells and dendritic arborization of these cells is reduced in VLDLR knockout mice in the absence of layer defects (Trommsdorff et al., 1999), we propose that Reelin may also directly regulate the growth of Purkinje cell dendrites.

Dendrite development is dependent on the expression of cytoskeletal genes such as MAP2. Isoforms of MAP2 form a family of heat-stable microtubule-associated proteins very abundant in the mammalian central nervous system, where they exert important functions in neurite outgrowth and neuronal plasticity (Craig and Banker, 1994). Here we showed that levels of HMW MAP2 proteins are decreased in *reeler* and Dab1 knockout mice. It is possible that the Reelin signaling pathway influences dendrite development directly through regulation of MAP2 RNA or protein expression. Alternatively, the reduction of MAP2 expression in mutant mice may represent the end result of other molecular events that disrupt dendrite growth.

In addition to cytoskeletal proteins, several neurotrophins have been implicated in dendrite outgrowth. These factors can either inhibit or promote dendrite outgrowth in cortical slices, depending on the neuronal subtype examined. Several lines of evidence indicate that Reelin and neurotrophins affect dendrite development through distinct mechanisms. First, treatment of *reeler* cultures with Reelin or BDNF differentially affected dendrite growth. Reelin preferentially stimulated the length, whereas BDNF increased the number of dendritic branches (Figure 4). Second, Reelin was unable to stimulate dendrite growth in Dab1 knockout culture, whereas BDNF treatment caused extensive outgrowth (Figure 8). Finally, we showed here that Reelin induces dendrite outgrowth through a lipoprotein receptor-Dab1 signaling pathway, which is known to activate a nonreceptor tyrosine kinase of the src family (Arnaud et al., 2003; Bock and Herz, 2003), whereas neurotrophins are known to function through the activation of Trk receptor

tyrosine kinases (Kaplan and Miller, 2000). Since Akt is activated by both Reelin (Beffert et al., 2002) and neurotrophin (Kaplan and Miller, 2000) stimuli, it is possible that the two pathways converge on this kinase to promote some common aspects of dendrite development.

Homozygous mutations in the *Reelin* gene in humans result in lissencephaly with cerebellar hypoplasia (LCH) (Hong et al., 2000), a severe disorder characterized by ataxia, seizures, and cognitive delay. In addition to this *reeler*-like phenotype, subtle alterations of *Reelin* expression have been associated with other mental diseases, such as schizophrenia (reviewed by Costa et al., 2001) and autism (reviewed by Fatemi, 2002). Since dendrite and spine abnormalities are also common anatomical correlates of both schizophrenia (McGlashan and Hoffman, 2000) and autism (Raymond et al., 1996), our present demonstration of a direct role of Reelin in dendrite development suggests a possible general mechanism for the involvement of this protein in cognitive disorders.

Experimental Procedures

Materials

Cell culture medium and reagents were purchased from Invitrogen (Carlsbad, CA). Trypsin, DNase (DN-50), and poly-D-lysine were purchased from Sigma (St. Louis, MO). Glass cover slips were obtained from Carolina Biological (Burlington, NC). The anti-Reelin mouse monoclonal antibodies E4 and CR-50 were purified from hybridoma cell culture supernatants using Hi-Trap protein G columns (Amersham Biosciences, Piscataway, NJ). Mouse monoclonal anti-MAP2 (clone HM-2), anti-β-tubulin (clone TUB-2.1), and rabbit anti-GFAP antibodies were obtained from Sigma (St. Louis, MO). VLDLR monoclonal antibodies (clone 6A6) were from Santa Cruz Biotechnologies (Santa Cruz, CA). ApoER2 rabbit polyclonal antibodies were from J. Herz. Dab1 antibodies CT-38 were from T. Curran. Monoclonal anti-Nestin (clone MAB353), anti-α3 integrin interfering (clone MAB1952Z), and rabbit polyclonal anti-MAP2 antibodies were from Chemicon (Temecula, CA). Receptor associate protein (RAP) was expressed from a GST-fusion construct (a gift of J. Herz). Secondary anti-IgG antibodies used for immunofluorescence were conjugated with Alexa 488 or Alexa 594 (Molecular Probes, Eugene, OR). Secondary antibodies used for Western and blot analyses were HRP-conjugated anti-mouse IgG (Amersham Biosciences, Piscataway, NJ).

Animals

reeler mice (B6C3Fe-*a/a-Relnrl/*+) were obtained from The Jackson Laboratory (Bar Harbor, ME) and genotyped by PCR using the following primers: forward, TAATCTGTCCTCACTCTGCC; reverse wild-type, ACAGTTGACATACCTTAATC; and reverse *reeler*, TGCAT TAATGTGCAGTGTTG. PCR conditions were as follows: 1 cycle 5 min at 94°C; 30 cycles 1 min at 94°C, 2 min at 55°C, 3 min at 72°C; and 1 cycle 10 min at 72°C. Thy1-YFP transgenic mice (B6.Cg-TgN(Thy1-YFPH)2Jrs) were obtained from The Jackson Laboratory (Bar Harbor, ME) and genotyped by PCR as suggested by the distributor. Dab1 knockout mice were obtained from J.A. Cooper and genotyped as described (Howell et al., 1997). Heterozygous *reeler* or *Dab1* knockout mice were intercrossed to obtain hippocampal and brain feeder cultures of all genotypes (+/+, +/−, and −/−).

Histology and Immunohistochemistry

To obtain hippocampal tissue sections, postnatal day 14 mice were perfused transcardially with 0.1 M Phosphate Buffer Saline (PBS) containing 4% paraformaldehyde. Brains were removed and placed in the same fixative overnight at 4°C. Immunostaining for Reelin was performed using paraffin sections with the CR-50 antibody and the Vectastain Elite ABC kit in accordance with the manufacturer's instructions (Vector Laboratories, Burlingame, CA). Cresyl violet and Golgi staining were done according to standard techniques.

In Situ Hybridization

Fresh cryostat brain sections from wild-type or mutant mice were processed for fluorescent nonradioactive in situ hybridization using digoxygenin-labeled sense or antisense riboprobes. *dab1* mRNA was detected using HRP-conjugated anti-digoxygenin antibodies followed by TSA Plus Direct-Cyanine 3 deposition for fluorescent tyramide signal amplification according to the manufacturer's protocol (Molecular Probes, Eugene, OR).

Western Blot Analysis

Hippocampi were dissected from embryonic days 18 mice and homogenized in a lysis buffer (137 mM NaCl, 20 mM Tris, 1% NP40, 10% glycerol, 1 mM PMSF, 10 μg/ml aprotinin, 1 μg/ml pepstatinA, and 1 μg/ml leupeptin at pH 8.0). Protein concentration was determined using the Bradford assay (Bio-Rad, Hercules, CA). 15 μg/lane of each homogenate were separated by SDS-PAGE using 4%–12% gels and transferred to nitrocellulose membranes. Blots were blocked in 5% nonfat milk in TBST (10 mM Tris-HCl [pH 7.6], 150 mM NaCl, and 0.05% Tween 20) and incubated with anti-MAP2 antibodies diluted 1:1000 in blocking solution for 16 hr at 4°C. Antigens were visualized using a chemiluminescence detection system (ECL Plus, Amersham Biosciences, Piscataway, NJ) after incubation with HRP-conjugated anti-mouse IgG secondary antibodies (Amersham Biosciences, Piscataway, NJ). To normalize for the protein content in each lane, blots were stripped and reprobed with β-tubulin antibodies diluted 1:1000 in TBST containing 4% horse serum.

Cell Culture

Primary cultures of hippocampal neurons and brain feeder cultures were prepared from embryonic day 18 mice, except for the experiments in Figures 5F and 5G in which postnatal day 1 mice were used. Pregnant mice were anesthetized with Isoflurane (Abbott Laboratories, North Chicago, IL) and the embryos dissected in HBSS. The entire hippocampus, the cortex, and brain stem were dissected from the individual embryos brain and placed into separated tubes containing Leibovitz's L-15 medium on ice. Slices of approximately 2 mm thickness were cut perpendicular to the long axes of the cortex using a sterile scalpel and digested using 0.25% trypsin and 0.1% DNase at 37°C for 20 min. 1 ml serum was added to stop the digestion, and the cells were collected by a brief centrifugation. Brain cells were transferred to 1.0 ml Leibovitz's L-15 medium containing 0.1% DNase in a 1.5 ml tube. Slices were triturated with a siliconized Pasteur pipette and centrifuged again, and the sediment resuspended in 1.5 ml B27/NeurobasalA medium with low serum (2% horse serum and 2% fetal calf serum). The above step was repeated one time and the cell pellets were resuspended in 1 ml B27/NeurobasalA medium. Hippocampal cells were counted using a hemocytometer and suspended at a density of 7000–8000 cells per plate in 0.2 ml aliquots on glass coverslips (12 mm diameter), which had been coated overnight with a solution of 0.1 mg/ml poly-D-lysine in PBS. After 2 hr incubation in a humidified incubator at 37°C and 5% CO to allow cell attachment, coverslips were transferred to a 35 mm diameter dish containing a monolayer of cells obtained from other parts of the brain of the same embryo. Coverslips were placed so that neurons were facing the feeder monolayer. Cells were rinsed twice with B27/NeurobasalA medium and further cultured for several days. For culture periods greater than 2 days, half of the medium was replaced with B27/NeurobasalA every 2 days. Addition of treatment agents was made as soon as the genotype of the cultures was determined (after approximately 15 hr) and the medium was replenished every 2 days for the length of the culture. Cytosine-β-D-arabinofuranoside (AraC) was added at the 200 nM concentration to reduce cell proliferation. At chosen time points, cells were rinsed with PBS and fixed in 4% paraformaldehyde at 37°C for 30 min. Cells were rinsed in PBS and stored in 70% ethanol at −20°C until further processing.

Immunofluorescence and Confocal Imaging

Fixed cells were rinsed with PBS and incubated in a blocking solution containing 0.5 M Tris-HCl (pH 7.6), 1% BSA, 5% normal goat

serum, and 0.05% sodium azide in the presence of 0.25% Triton X-100 for 10 min at room temperature. Cells were then incubated overnight at 4°C with primary antibodies diluted in blocking buffer. After washing, cells were incubated with secondary antibodies and DAPI for 1.5 hr at ambient temperature. Microscopic analysis was performed with a Nikon TE200 inverted fluorescence microscope equipped with a digital camera (Coolmax Fx, RS Photometrics, Tucson, AZ). Quantitative analysis of dendrite complexity was performed by measuring the total length and the number of MAP2-labeled processes in $n = 25$ individual neurons from three experiments using the MetaView software (Universal Imaging, West Chester, PA). Statistical analysis was conducted using standard error of the mean (SEM) and multivariance ANOVA test. YFP expression in hippocampal sections was analyzed by confocal microscopy using a Fluoview FV300 laser-scanning microscope (Olympus America Inc.) equipped with an argon laser. Kallman average was used for noise reduction and stacks of 0.5 μm optical sections in the z plane were assembled using the Fluoview software.

Production of Recombinant Reelin

Reelin was generated from a stable mammalian cell line (CER) derived from 293-EBNA cells (Invitrogen, Carlsbad, CA). CER cells express and secrete the full-length mouse *reelin* cDNA from the pCEP4 episomal vector (Invitrogen, Carlsbad, CA). The conditioned medium of CER and a control cell line (CEP4) was collected and concentrated 50-fold using Amicon Ultra 30,000 MWCO filters (Millipore, Billerica, MA). Alternatively, Reelin was partially purified from CER cells conditioned medium by ammonium sulfate precipitation (Keshvara et al., 2001) or gel filtration chromatography using a Superdex 200 column (Amersham Biosciences, Piscataway, NJ). Fractions containing Reelin were identified by dot blot and further analyzed by Western blot using the monoclonal antibody E4. For this study we used fractions containing a mixture of Reelin isoforms, including the full-length 400 kDa protein and the major 180 kDa cleaved product.

Dot Blot Assay

Cell medium from dissociated cultures were cleared by centrifugation at 4000 $\times$ g for 20 min at 4°C. The medium was slowly added to the wells (8 ml per well) of a dot blot apparatus and the proteins transferred onto a PVDF membrane. Membrane strips were subsequently blocked in 5% nonfat milk in TBST for 1 hr and then incubated in anti-Reelin E4 monoclonal antibody for 2 hr at room temperature. After washing (3 $\times$ 5 min), blots were incubated in HRP-conjugated anti-mouse secondary antibodies and visualized as described for Western blot analysis.

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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.

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

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.

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

Costa, E., Davis, J., Grayson, D.R., Guidotti, A., Pappas, G.D., and Pesold, C. (2001). Dendritic spine hypoplasticity and downregulation of reelin and GABAergic tone in schizophrenia vulnerability. Neurobiol. Dis. *8*, 723–742.

Craig, A.M., and Banker, G. (1994). Neuronal polarity. Annu. Rev. Neurosci. *17*, 267–310.

D'Arcangelo, G., and Curran, T. (1998). Reeler: new tales on an old mutant mouse. Bioessays *20*, 235–244.

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.

Del Rio, J.A., Heimrich, B., Borrell, V., Forster, E., Drakew, A., Alcantara, S., Nakajima, K., Miyata, T., Ogawa, M., Mikoshiba, K., et al. (1997). A role for Cajal-Retzius cells and reelin in the development of hippocampal connections. Nature *385*, 70–74.

Drakew, A., Deller, T., Heimrich, B., Gebhardt, C., Del Turco, D., Tielsch, A., Forster, E., Herz, J., and Frotscher, M. (2002). Dentate granule cells in reeler mutants and VLDLR and ApoER2 knockout mice. Exp. Neurol. *176*, 12–24.

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. (2002). The role of Reelin in pathology of autism. Mol. Psychiatry *7*, 919–920.

Feng, G., Mellor, R.H., Bernstein, M., Keller-Peck, C., Nguyen, Q.T., Wallace, M., Nerbonne, J.M., Lichtman, J.W., and Sanes, J.R. (2000). Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron *28*, 41–51.

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.

Frotscher, M., Haas, C.A., and Forster, E. (2003). Reelin controls granule cell migration in the dentate gyrus by acting on the radial glial scaffold. Cereb. Cortex *13*, 634–640.

Goldowitz, D., Cushing, R.C., Laywell, E., D'Arcangelo, G., Sheldon, M., Sweet, H.O., Davisson, M., Steindler, D., and Curran, T. (1997). Cerebellar disorganization characteristic of *reeler* in *scrambler* mutant mice despite presence of Reelin. J. Neurosci. *17*, 8767–8777.

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.

Hammond, V., Howell, B., Godinho, L., and Tan, S.S. (2001). disabled-1 functions cell autonomously during radial migration and cortical layering of pyramidal neurons. J. Neurosci. *21*, 8798–8808.

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.

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. Nat. 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.

Kaplan, D.R., and Miller, F.D. (2000). Neurotrophin signal transduction in the nervous system. Curr. Opin. Neurobiol. *10*, 381–391.

Kaufmann, W.E., and Moser, H.W. (2000). Dendritic anomalies in disorders associated with mental retardation. Cereb. Cortex *10*, 981–991.

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.

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.

Mariani, J. (1982). Extent of multiple innervation of Purkinje cells by climbing fibers in the olivocerebellar system of weaver, reeler and staggerer mutant mice. J. Neurosci. *13*, 119–126.

Marin-Padilla, M. (1992). Ontogenesis of the pyramidal cell of the mammalian neocortex and developmental cytoarchitectonics: a unifying theory. J. Comp. Neurol. *321*, 223–240.

McGlashan, T.H., and Hoffman, R.E. (2000). Schizophrenia as a disorder of developmentally reduced synaptic connectivity. Arch. Gen. Psychiatry *57*, 637–648.

Nakajima, K., Mikoshiba, K., Miyata, T., Kudo, C., and Ogawa, M. (1997). Disruption of hippocampal development *in vivo* by CR-50 mAb against Reelin. Proc. Natl. Acad. Sci. USA *94*, 8196–8201.

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.

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 γ-aminobutyric acid in cortex and hippocampus of adult rats. Proc. Natl. Acad. Sci. USA *95*, 3221–3226.

Quattrocchi, C.C., Huang, C., Niu, S., Sheldon, M., Benhayon, D., Cartwright, J., Jr., Mosier, D.R., Keller, F., and D'Arcangelo, G. (2003). Reelin promotes peripheral synapse elimination and maturation. Science *301*, 649–653.

Raymond, G.V., Bauman, M.L., and Kemper, T.L. (1996). Hippocampus in autism: a Golgi analysis. Acta Neuropathol. *91*, 117–119.

Rice, D.S., and Curran, T. (2001). Role of the Reelin signaling pathway in central nervous system development. Annu. Rev. Neurosci. *24*, 1005–1039.

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.

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.

Stanfield, B.B., and Cowan, W.M. (1979a). The development of the hippocampus and dentate gyrus in normal and reeler mice. J. Comp. Neurol. *185*, 461–484.

Stanfield, B.B., and Cowan, W.M. (1979b). The morphology of the hippocampus and dentate gyrus in normal and reeler mice. J. Comp. Neurol. *185*, 393–422.

Tabata, H., and Nakajima, K. (2002). Neurons tend to stop migration and differentiate along the cortical internal plexiform zones in the Reelin signal-deficient mice. J. Neurosci. Res. *69*, 723–730.

Tissir, F., and Goffinet, A.M. (2003). Reelin and brain development. Nat. Rev. Neurosci. *4*, 496–505.

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.

Ware, M.L., 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.

Weiss, K.H., Johanssen, C., Tielsch, A., Herz, J., Deller, T., Frotscher, M., and Forster, E. (2003). Malformation of the radial glial scaffold in the dentate gyrus of reeler mice, scrambler mice, and ApoER2/VLDLR-deficient mice. J. Comp. Neurol. *460*, 56–65.

Whitford, K.L., Dijkhuizen, P., Polleux, F., and Ghosh, A. (2002). Molecular control of cortical dendrite development. Annu. Rev. Neurosci. *25*, 127–149.

Yang, H., Jensen, P., and Goldowitz, D. (2002). The community effect and Purkinje cell migration in the cerebellar cortex: analysis of scrambler chimeric mice. J. Neurosci. *22*, 464–470.