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Disabled-1 acts downstream of *Reelin* in a signaling pathway that controls laminar organization in the mammalian brain

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SUMMARY

Mutation of either *reelin* (*Reln*) or *disabled-1* (*Dab1*) results in widespread abnormalities in laminar structures throughout the brain and ataxia in *reeler* and *scrambler* mice. Both exhibit the same neuroanatomical defects, including cerebellar hypoplasia with Purkinje cell ectopia and disruption of neuronal layers in the cerebral cortex and hippocampus. Despite these phenotypic similarities, Reln and Dab1 have distinct molecular properties. Reln is a large extracellular protein secreted by Cajal-Retzius cells in the forebrain and by granule neurons in the cerebellum. In contrast, Dab1 is a cytoplasmic protein which has properties of an adapter protein that functions in phosphorylation-dependent intracellular signal transduction. Here, we show that *Dab1* participates in the same developmental process as *Reln*. In *scrambler* mice, neuronal precursors are unable to invade the preplate of the cerebral cortex and consequently, they do not align within the cortical plate. During development, cells expressing *Dab1* are located next to those secreting *Reln* at critical stages of formation of the cerebral cortex, cerebellum and hippocampus, before the first abnormalities in cell position become apparent in either *reeler* or *scrambler*. In *reeler*, the major populations of displaced neurons contain elevated levels of Dab1 protein, although they express normal levels of *Dab1* mRNA. This suggests that Dab1 accumulates in the absence of a Reln-evoked signal. Taken together, these results indicate that Dab1 functions downstream of Reln in a signaling pathway that controls cell positioning in the developing brain.

Key words: Neuronal migration, *scrambler*, *reeler*, Mutation, Cerebral cortex, Preplate, Mouse

INTRODUCTION

The characterization of spontaneous and targeted mutations in mice has recently identified several genes that are required for correct cell positioning in the developing brain (D'Arcangelo and Curran, 1998). One of the most significant of these is *reelin* (*Reln*), the gene disrupted in *reeler* mice (D'Arcangelo et al., 1995). The ataxic phenotype of *reeler* mice was first described in 1951 (Falconer, 1951). Subsequent histopathological studies revealed that the *reeler* cerebellum is dramatically decreased in size and the normal laminar organization found in several brain regions is disrupted (Hamburgh, 1960). Reln is a large extracellular protein that is secreted from distinct neuronal populations (D'Arcangelo et al., 1995; Ogawa et al., 1995; Miyata et al., 1996; D'Arcangelo et al., 1997; Nakajima et al., 1997). Although cells that express Reln are positioned normally in *reeler* mice, the topography of neighboring regions is severely disrupted. This observation led to the hypothesis that Reln provides a local signal that controls cell-cell interactions critical for directing cell positioning in the developing brain.

Two novel mouse mutations, *scrambler* and *yotari*, were described recently that exhibit remarkable behavioral and histopathological similarities to *reeler* (Sweet et al., 1996; Goldowitz et al., 1997; González et al., 1997; Yoneshima et al., 1997). Molecular genetic studies revealed that *scrambler* and *yotari* arose from independent mutations in the *disabled-1* (*Dab1*) gene (Sheldon et al., 1997; Ware et al., 1997). These mutations cause aberrant splicing of *Dab1* mRNA that result in the synthesis of little or no *Dab1* protein (Dab1). Furthermore, targeted disruption of *Dab1* has also been shown to cause a *reeler*-like phenotype (Howell et al., 1997b). These findings demonstrate that Dab1, like Reln, is necessary for the formation of laminar structures in the developing brain. Dab1 contains a phosphotyrosine-interacting/phosphotyrosine-binding domain (PI/PTB) and it exhibits some degree of similarity to the *Drosophila disabled* gene. It is an intracellular protein that becomes phosphorylated on tyrosine residues during embryonic development and it can bind to the protein tyrosine kinases Src, Abl and Fyn (Howell et al., 1997a). Based on its biochemical properties, Dab1 is thought to function as an adapter molecule in the transduction of protein kinase signals.

The close phenotypic similarities among *reeler*, *scrambler*, *yotari* and *disabled*-1 null mice, suggest that *Reln* and *Dab1* function in the same signaling pathway that influences neuronal positioning in the developing brain. Although initial studies reported that *Dab1* is expressed during development (Howell et al., 1997a,b; Sheldon et al., 1997), no biochemical link between *Reln* and *Dab1* was established. The simplest relationship between these two molecules would be that either *Dab1* is required for the expression of *Reln* or, conversely, that *Reln* is required for *Dab1* expression. However, *Reln* is expressed normally in *Dab1*-deficient mice and *Dab1* is expressed in *reeler* (Goldowitz et al., 1997; González et al., 1997; Howell et al., 1997b; Sheldon et al., 1997; Yoneshima et al., 1997). Thus, the question remains open as to whether *Dab1* functions independently from *Reln* in a signaling pathway required for normal neuronal positioning or whether its product functions as a downstream effector of *Reln*. Here we demonstrate that *Dab1*, like *Reln*, plays a role in the initial stages of lamination in the cerebral cortex, because the preplate fails to split in *scrambler* mice. A comparative analysis of the temporal and spatial patterns of *Reln* and *Dab1* expression reveals that both genes are expressed very early during development of the cerebral cortex, cerebellum and hippocampus in adjacent cell populations, prior to the time when histological abnormalities appear in either *reeler* or *scrambler*. Furthermore, although the levels of *Dab1* mRNA are comparable in *reeler* and normal mice, ectopic neurons in *reeler* contain approximately 10-fold more *Dab1* protein than their normal counterparts. This finding suggests that Reln affects the turnover of Dab1 and it provides the first evidence for a biochemical link between these proteins. Taken together, our data strongly suggest that Dab1 functions downstream of Reln as a component of a signal transduction pathway that directs cell positioning in the developing brain.

MATERIALS AND METHODS

Mice and tissue collection

Scrambler (*Dab1*^{scm}/*Dab1*^{scm}) and *reeler* (*Reln*^{rl}/*Reln*^{rl}) mice were obtained from breeding colonies at the University of Tennessee and at St. Jude Children's Research Hospital, respectively. *Scrambler* arose on the dancer (DC/Le) strain of mice, and mutants were mated once to the C3HeB/FeJLe strain at The Jackson Laboratory (Bar Harbor, ME). The *scrambler* colony was maintained by sibling matings. The founder colony of B6C3Fe/J-*reeler* mice at St. Jude was purchased from The Jackson Laboratory. Mutant mice were obtained by mating heterozygous males to heterozygous or homozygous females. Both mouse colonies were maintained in a pathogen free environment with a light/dark cycle of 12 hours. In the text, control mice refers to wild-type and heterozygous *reeler* or *scrambler* mice, which are phenotypically normal. Females were examined each morning and if a plug was observed, we designated the stage of development as embryonic day 0.5 (E0.5). Embryos used for in situ hybridization and immunohistochemistry were harvested from females that were first anesthetized with Avertin (0.2 ml/10 g body weight) and killed by cervical dislocation. Embryos younger than E16.5 were immersion-fixed in 4% paraformaldeyhde in 0.1 M sodium phosphate buffer (PB; pH 7.2) overnight at 4˚C. Older embryos, postnatal, and adult mice were perfused transcardially with the same fixative. Tissue was incubated overnight in a series of sucrose solutions (20-30%) and embedded in tissue freezing medium (Triangle Biomedical Sciences, Durham, NC) for cryostat sectioning. Sections, 10-16 μm thick, were mounted on Fisher Superfrost/Plus slides and stored at −20˚C until use for in situ hybridization or immunohistochemistry.

In situ hybridization

The protocol for in situ hybridization was essentially as described previously by Simmons et al. (1989). Hybridization analysis was performed in situ using [^{33}P]UTP riboprobes that were generated by in vitro transcription of amplified DNA products containing the T7 polymerase promoter sequence flanking the desired nucleotide primer sequence. Antisense and sense riboprobes were generated that correspond to either the 3′ untranslated region (UTR) of *Dab1* (nucleotides 1935-2116) or the open reading frame (nucleotides 618-821). For the localization of *Reln*, we used a riboprobe corresponding to nucleotides 5818-5973. Slides were incubated for 10 minutes at room temperature in Proteinase K (10 μg/ml) in a buffer containing 100 mM Tris and 50 mM EDTA (pH 8.0). Slides were then acetylated with acetic anhydride, rinsed in 2× SSC, dehydrated, and exposed to either sense or antisense denatured probe in the following hybridization buffer: 50% formamide, 10% dextran sulfate, 5× Denhardt's solution, 620 mM NaCl, 10 mM EDTA (pH 8), 20 mM PIPES-Na (pH 6.8), 0.2% SDS, 50 mM DTT, 250 μg/ml denatured salmon sperm DNA and yeast tRNA. Hybridization occurred at 60°C overnight in a humid chamber containing 4× SSC and 50% formamide. Hybridized slides were exposed to 0.004 mg/ml RNase A for 30 minutes at 37°C in the appropriate buffer. Slides were washed in 2× SSC for 1 hour at 62°C followed by 0.2× SSC at 65°C for 2 hours, dehydrated in a graded series of ethanol in 0.1 M ammonium acetate, and exposed to Biomax MR film overnight (Kodak). The following morning, slides were dipped in autoradiography emulsion (Type NTB2; Kodak) and placed at 4°C in a light proof box for several days. Following development, slides were counterstained with 0.1% toluidine blue.

Cell birthdating and immunohistochemistry

Early stages of cerebral cortical development were analyzed in *scrambler* mice by labeling cells in S-phase using the thymidine analogue, 5-bromo-2-deoxyuridine (BrdU). Timed-pregnant *scrambler* females were injected intraperitoneally with BrdU (5 mg/ml in saline solution; Sigma), at 50 μg/gm body weight. To label Cajal-Retzius cells and subplate neurons in the preplate, three females were injected twice, once at E10.5 and again 3 hours later, and two other females received a single injection of BrdU at E11.5 (Crandall et al., 1986; Wood et al., 1992; del Río et al., 1995; Ogawa et al., 1995; Sheppard and Pearlman, 1997). Because Cajal-Retzius and subplate cells are transient populations that disappear after birth, we analyzed the distribution of BrdU-labeled cells at E16.5. In addition, we examined the distribution of chondroitin sulfate proteoglycans (CSPGs), which are associated with the preplate and its derivatives in the marginal zone and subplate (Sheppard and Pearlman, 1997), in control and *scrambler* mice. Females were killed as described above and embryos were immersion fixed in a 3:1 solution of 95% ethanol and acetic acid. Brains were dissected, dehydrated in ethanol, cleared in xylene, and embedded in paraffin for sectioning. Sections were prepared at a thickness of 6 μm and incubated with either a mouse monoclonal anti-BrdU antibody diluted 1:100 (Becton-Dickinson, San Jose, CA) or anti-chondroitin sulfate at 1:600 (mouse monoclonal IgM, clone CS-56 from Sigma). The primary antibody was detected using the ABC peroxidase kit (Vector Laboratories, Burlingame, CA) followed by diaminobenzidine (DAB) as described previously (Hamre and Goldowitz, 1996). Sections were counterstained with Cresyl violet, dehydrated, and coverslips applied with Permount (Fisher Scientific).

Cryostat sections used in immunohistochemistry were blocked for 1 hour with 5% normal goat serum (NGS; Vector) diluted in 0.1 M sodium phosphate buffer (pH 7.2) containing 0.01% Triton X-100 (PB-X), then incubated in primary antibody diluted in 2.5% NGS in

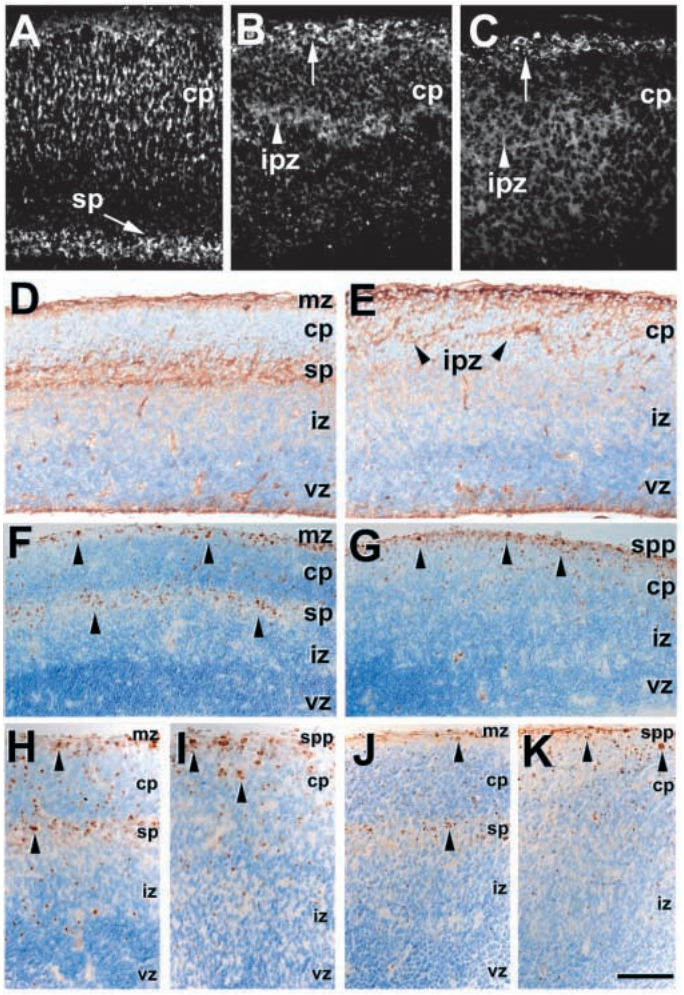

Fig. 1. Subplate neurons are found in ectopic locations in *scrambler* mice. (A-C) Microtubule associated protein (MAP2) immunohistochemistry on E16.5 sagittal sections from control (A), *reeler* (B), and *scrambler* (C) cerebral cortex. In the control, MAP2-positive neurons are assembled into a discrete layer, the subplate (sp, arrow), that is located beneath the MAP2-positive cortical plate (cp) cells. In *reeler* and *scrambler* mice, the subplate is absent and cells that morphologically resemble subplate cells are found near the pial surface (arrows in B,C). The intermediate plexiform zone (ipz), which contains anomalous fibers, is present in both mutants. (D,E) Localization of chondroitin sulfate proteoglycans (CSPGs, brown) in E16.5 control (D) and *scrambler* (E) cerebral cortex in sagittal view. (D) Immunolabeling for CSPGs is intense in the marginal zone (mz) and subplate in the control section. However, most of the labeling in *scrambler* (E) is found near the surface of the cortical plate and in the intermediate plexiform zone (compare with *reeler* in Sheppard and Pearlman, 1997). (F) The majority of BrdU-positive cells (brown/black, arrowheads) labeled on E10.5 is found in the subplate and marginal zone in the E16.5 control cerebral cortex. A few labeled cells are also found in the cortical plate. (G) In contrast, the majority of BrdU-positive cells in *scrambler* is located above the cortical plate in a region that is similar to the superplate (arrowheads, spp) in *reeler*. (H) In this higher magnification view of a different control case, many BrdU-positive cells (arrowheads) labeled at E10.5 are found in the subplate and marginal zone, whereas in *scrambler* (I), they are mostly in the superplate. A few heavily labeled cells are also in the cortical plate. (J) Many BrdU-positive cells (arrowheads) labeled on E11.5 are located in the subplate and marginal zone in the E16.5 control. (K) In contrast, most of the positive cells are confined to the superplate and cortical plate in *scrambler*. The scale bar is approximately 60 μm in A-C, 200 μm in D-G and 150 μm in H-K. Abbreviations not defined above are: iz, intermediate zone; vz, ventricular zone.

PB-X for 1 hour or overnight at 4°C. Primary antibodies and their dilutions used in this study were: anti-Reln CR-50 at 1:200 (Ogawa et al., 1995) and anti-MAP2 (2a + 2b) at 1:200 (mouse monoclonal IgG, clone AP-20 from Sigma). For the localization of Dab1 protein, we used a rabbit polyclonal antibody obtained from B. Howell and J. Cooper. This antibody (B3) was raised against residues 107-243 which overlaps the PI/PTB domain of Dab1 (Howell et al., 1997a). A dilution of 1:200 was adequate to distinguish control and *scrambler* brains (Fig. 4). For double staining, primary antibodies were incubated together as were secondary antibodies. Sections were washed in PB and then incubated with either fluorescein conjugated anti-rabbit or Texas red conjugated anti-mouse antibodies (Vector) diluted at 1:200 in 5% NGS in PB-X for 1 hour at room temperature. Slides were washed for several hours in PB and coverslips applied with Vectashield mounting medium (Vector). Slides were observed on an Olympus BX60 microscope. Images were acquired with a Hamamatsu C5810 video camera and directly imported into Adobe Photoshop (*v*.3.0). Contrast and brightness enhancements were applied equally to each figure. Figures were printed using a Fujix Pictrography 3000.

Immunoblot and mRNA analysis

Brain tissue was removed from E16.5 *reeler* homozygous, heterozygous and wild-type embryos and the neocortex was dissected. Protein extracts from the neocortex and the remainder of the brain were prepared by Dounce-homogenizing snap-frozen tissue in 500 μl of cold lysis buffer containing 0.1% NP-40, 250 mM NaCl, 50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 2 mM PMSF, 20 μM leupeptin, 50 mM NaF per 100 mg tissue. Extracts were clarified by microcentrifugation at 14,000 rpm for 30 minutes. A total of 100 μg of protein extract was loaded per lane onto a 4-12% polyacrylamide gradient gel (Novagen, Inc.), electrotransferred onto nitrocellulose membranes, and incubated with two different rabbit polyclonal antibodies specific for Dab1. The first antibody is the same as that used for immunohistochemistry studies (B3) and the second antibody was raised against a C-terminal peptide of Dab1 (Howell et al., 1997a). As a control, we used an anti-cdk5 antibody (Santa Cruz). Immunoblots were visualized by enhanced chemiluminescence (Boehringer Mannheim). Quantification of Dab1 protein levels was performed by densitometry. For analysis of *Dab1* mRNA, total RNA was isolated from E16.5 *reeler* homozygous, heterozygous and wild-type brains, loaded at 10 μg per lane onto a 1.0% agarose-formaldehyde gel, and hybridized with a full length *Dab1* DNA probe as described previously (Sheldon et al., 1997).

RESULTS

Early formation of the cerebral cortex in *scrambler* mice

The laminar organization of the mouse cerebral cortex begins with the appearance of Cajal-Retzius and subplate neurons in the preplate between embryonic days 10 to 12 (E10-E12). The preplate is located near the pial surface, superficial to the ventricular zone (Marín-Padilla, 1978; Wood et al., 1992; Sheppard and Pearlman, 1997). The first wave of cortical plate neurons exits the cell cycle near the ventricular surface and migrates radially before invading the preplate, thus splitting this structure into the marginal zone, containing the Cajal-

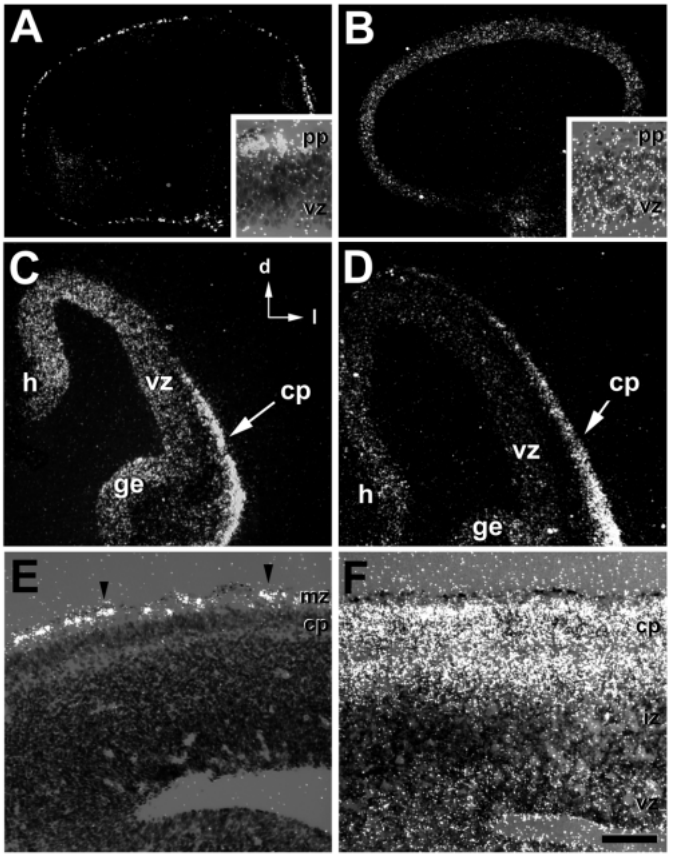

Fig. 2. Distribution of *Reln* and *Dab1* in the developing telencephalon. (A) Sagittal section of the telencephalon at E11.5 probed with antisense *Reln* before the formation of the cortical plate. The inset shows that *Reln* is located in the preplate (pp), which is above the ventricular zone (vz). (B) A neighboring section hybridized with antisense *Dab1*, which is expressed uniformly throughout the ventricular zone. (C) A coronal section of the telencephalon at E12.5 probed with antisense *Dab1*. Although *Dab1* continues to be expressed in the ventricular zone, neurons in the cortical plate (cp, arrow), which is forming in the lateral cerebral cortex, express high levels of *Dab1*. The ventricular zone in the hippocampal region (h) and in the ganglionic eminence (ge), which gives rise to the striatum, is also positive. (D) About 24 hours later, *Dab1*-positive neurons in the cortical plate (arrow) extend more medially in the telencephalon compared to E12.5. (E) In this sagittal section of the cerebral cortex at E14.5, Cajal-Retzius cells in the marginal zone (mz) express *Reln* (arrowheads) directly above the cortical plate. (F) A neighboring section probed with antisense *Dab1*. Neurons in the cortical plate and either migrating cells or cortical fibers in the intermediate zone (iz) contain *Dab1*. In addition, cells in the ventricular zone express *Dab1*. The scale bar is approximately 100 μm in A-D, and 200 μm in E and F. In C and D, d, dorsal and l, lateral.

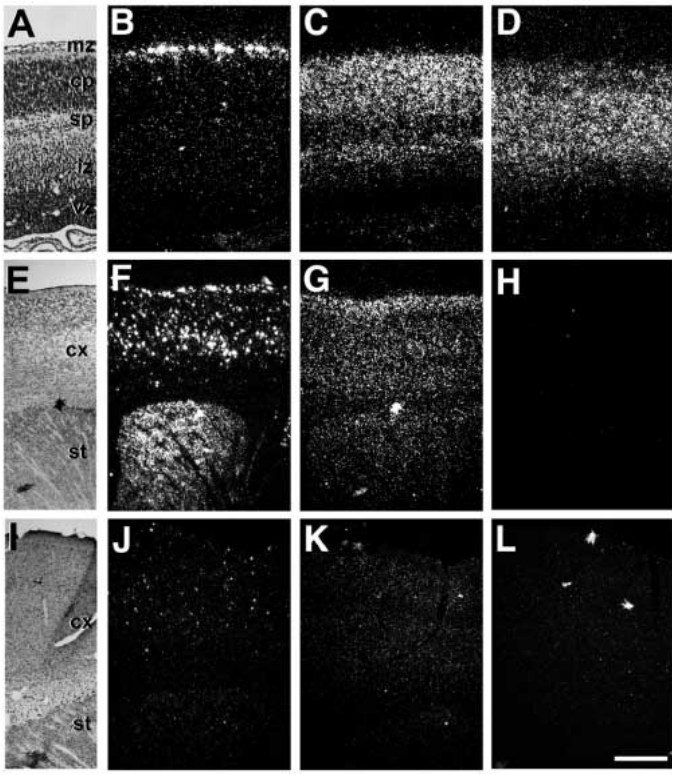

Fig. 3. Distribution of *Reln* and *Dab1* in the cerebral cortex at E16.5 (A-D), P5 (E-H), and in the adult (I-L). (A) Brightfield image of E16.5 cerebral cortex. (B) A neighboring section probed with antisense *Reln*. Cajal-Retzius cells in the marginal zone (mz in A) express *Reln*. (C) An adjacent section probed with antisense *Dab1*. Cortical plate (cp in A) neurons express high levels of *Dab1*. In addition, either migrating cells in the upper portion of the intermediate zone (iz in A) or fibers from cortical neurons contain *Dab1*. The ventricular zone (vz in A) contains very low levels of *Dab1*. (D) Although the distribution of positive cells is abnormal, *Dab1* is expressed in *reeler* cerebral cortex. (E) Brightfield image of P5 section shown in G in darkfield. (F) An adjacent section probed with antisense *Reln*. Although expression of *Reln* persists in Layer I of the cortex (cx), other cortical neurons and cells in the striatum (st) also express *Reln*. (G) Most cells in the cortex express *Dab1*. (H) An adjacent section to that shown in G probed with a *Dab1* sense probe. (I) Brightfield image of the adult cortical cells shown in K in darkfield. (J) In a neighboring section, *Reln* is expressed in a few cells scattered throughout the cortex (cx). (K) Adult cortical cells also express *Dab1*. (L) An adjacent section to that in J probed with a sense *Reln* probe. The scale bar is approximately 200 μm in A-D and 450 μm in E-L.

Retzius cells, and the subplate. Thereafter, newly generated neurons migrate past older neurons and insert into the developing cortical plate directly beneath the marginal zone. Corticogenesis proceeds in this inside-out manner ultimately generating a six-layered structure in mammals (Allendoerfer and Shatz, 1994; Marín-Padilla, 1998). This classic pattern of lamination is disrupted in adult *reeler* mice, which exhibit an inversion of cortical layers (Rakic and Caviness, 1995). The ectopic position of *reeler* cortical neurons is a consequence of the failure of migrating cells to bypass older cells, as the first cohort of neuronal precursors destined for the cortical plate fails to split the preplate (Caviness, 1982; Hoffarth et al., 1995; Ogawa et al., 1995; Sheppard and Pearlman, 1997). Superficially, the apparent inversion of cortical layers observed in adult *scrambler* mice (Sweet et al., 1996; González et al., 1997) resembles that of *reeler*, suggesting that a similar developmental process has been disrupted.

Disruptions in the cytoarchitecture of the cerebral cortex were apparent in *scrambler* mice by E16.5 and the normal radial organization of cell bodies was disorganized in a manner

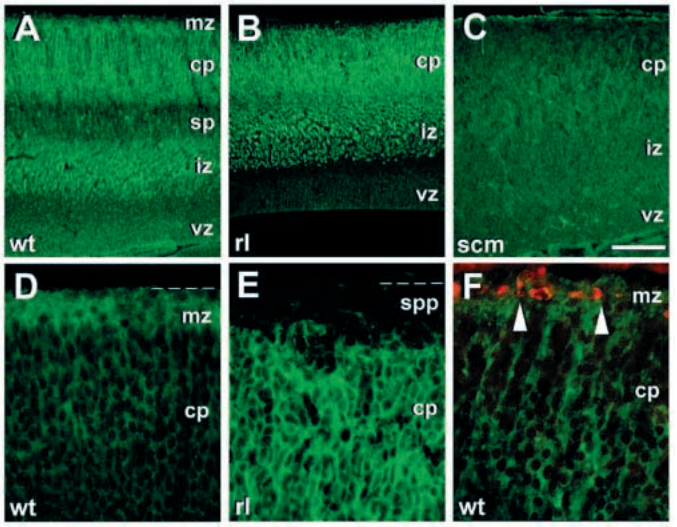

Fig. 4. Distribution of Dab1 protein in the cerebral cortex at E16.5. (A) In this sagittal section, cortical plate (cp) neurons and their fibers in the intermediate zone (iz) contain Dab1. In addition, cells in the region of the subplate (sp) and cells in the ventricular zone (vz) contain Dab1. (B) A section from a *reeler* mouse mounted on the same slide as the section in A and incubated with the same antibody. Although similar cells in the cerebral cortex express Dab1, there is more protein in the mutant compared to the control. The micrograph shown in B was obtained with about one-half of the exposure time as that in A. (C) The section from a *scrambler* brain incubated with the same dilution of Dab1 antibody and exposed for the same time as that in A, shows less immunofluorescence. (D) A higher magnification view of a normal cortex incubated with Dab1 antibody. Fibers in the marginal zone (mz) and cells in the cortical plate contain Dab1. The pial surface, which is missing in this section, is indicated by the broken line. (E) A similar view to that shown in D, but from a *reeler* brain. Beneath the pial membrane (broken line), the superplate (spp), which contains Cajal-Retzius and subplate cells, shows less immunostaining compared to the underlying cortical plate. (F) Cajal-Retzius cells in the marginal zone, identified with the CR-50 antibody (arrowheads, red), express either very low or negligible amounts of Dab1 protein (green). The scale bar in C, is approximately 100 μm in A-C and 25 μm in D-F.

similar to that observed in *reeler*. The tight packing of neuronal cell bodies seen in normal mice was less obvious in *scrambler* and cell-free zones were present within the cortical plate. In *reeler*, these areas are referred to as the intermediate plexiform zone (IPZ), which has been shown to contain fibers innervating ectopic subplate and cortical plate neurons (Caviness, 1976; Pinto-Lord and Caviness, 1979; Yuasa et al., 1994; Sheppard and Pearlman, 1997). The borders of the cortical plate were less distinct in *scrambler*, suggesting that cell topography was already abnormal at E16.5. To address this possibility, we compared the distribution of microtubule associated protein (MAP2), a neuron-specific marker (Crandall et al., 1986), in control, *scrambler* and *reeler* mice. In control mice, the subplate was apparent as a dense array of MAP2-positive cells located beneath the MAP2-positive cortical plate cells (Fig. 1A). Although neuronal cell bodies in the *reeler* cortical plate expressed MAP2, the subplate layer was absent and MAP2-positive neurons, which resemble subplate cells morphologically, were located near the pial surface (Fig. 1B). In the *scrambler* cerebral cortex, the distribution of MAP2-positive neurons resembling subplate cells (Fig. 1C) was remarkably similar to that in *reeler*, suggesting that subplate neurons were displaced like those in *reeler*.

The superficial position of subplate neurons in *reeler* arises from the failure of migrating cortical plate cells to invade the preplate. Thus, the preplate is not split into two layers, one containing Cajal-Retzius neurons and the other containing subplate neurons, during the initial stages of laminar organization. Instead, in the *reeler* cortex the majority of subplate cells is located above the cortical plate with Cajal-Retzius cells in a structure known as the superplate (Caviness, 1982; Ogawa et al., 1995). Although subplate cells are ectopically positioned in the *reeler* neocortex, chondroitin sulfate proteoglycans (CSPGs) continue to be associated with these cells as well as those in the marginal zone (Sheppard and Pearlman, 1997).

To determine whether the preplate fails to split in *scrambler* mice, we analyzed the distribution of CSPGs at E16.5 using a mouse monoclonal antibody. In addition, we labeled Cajal-Retzius cells and subplate neurons by injecting BrdU at E10.5 and E11.5 and we examined their distribution after formation of the cortical plate at E16.5. In control mice, the formation of the cortical plate splits the CSPG-positive preplate into two distinct layers, one associated with the marginal zone and the other with the subplate layer (Fig. 1D). In contrast, CSPG staining of the *scrambler* cerebral cortex was more broadly distributed near the pial surface and staining was also apparent in the IPZ (arrowheads in Fig. 1E), indicating that the preplate had failed to split properly in the mutant. In control mice, the majority of BrdU-positive cells labeled on E10.5 was located in both the marginal zone above the cortical plate and in the subplate layer between the cortical plate and the intermediate zone (Fig. 1F,H). In contrast, most of the BrdU-positive cells in the *scrambler* cerebral cortex were located in the superficial aspect of the cortical plate (Fig. 1G,I). The subplate layer, which was clearly discernible in control mice, was absent in the cerebral cortex of *scrambler* mice. When BrdU was injected approximately 24 hours later, at E11.5, the majority of labeled cells was located in the subplate, in the marginal zone, and in deep regions of the cortical plate in control mice (Fig. 1J). In contrast, the majority of labeled cells in *scrambler* was located near the pial surface (Fig. 1K), indicating that many cortical plate neurons had failed to intercalate between the subplate and the marginal zone. The ectopic location of these early-generated neurons, together with the immunohistochemical and morphological appearance of the embryonic cerebral cortex, demonstrate that the preplate fails to split properly in *scrambler*.

Expression of *Reln* and *Dab1* in the cerebral cortex

To compare the expression patterns of *Reln* and *Dab1* in the cerebral cortex, in situ hybridization analysis was performed before formation of the cortical plate and throughout development of the cerebral cortex. At the preplate stage of development (E11.5), *Reln* was expressed in Cajal-Retzius cells located near the pial surface (Fig. 2A). In contrast, *Dab1* was distributed homogeneously throughout the proliferative zone (Fig. 2B) underneath the cells expressing *Reln*. Approximately 24 hours later (E12.5), cortical plate formation begins in the lateral aspect of the cerebral cortex. In coronal

sections of the cerebral cortex hybridized with a *Dab1* probe, it was apparent that *Dab1* expression reflected the lateral to medial gradient of cortical plate formation. Cells in the cortical plate, which is located at the most lateral aspect of the cerebral cortex at E12.5, appeared to express high levels of *Dab1* compared to cells in the ventricular zone (Fig. 2C). One day later, cells expressing high levels of *Dab1* were observed in more medial aspects of the cerebral cortex, where the cortical plate has formed (Fig. 2D). At E14.5, the cortical plate is several cell diameters thick and the intermediate zone is well developed. At this time, *Reln* expression was highest in the marginal zone (Fig. 2E), whereas *Dab1* was expressed directly beneath this layer in cortical plate cells and in upper regions of the intermediate zone (Fig. 2F). Reduced levels of *Dab1* were also present in the ventricular zone. Thus, *Dab1* and *Reln* are expressed in adjacent cell populations of normal mice before the first morphological abnormalities become apparent in *scrambler* and *reeler* mice.

As corticogenesis proceeds, cells continue to migrate into the cortical plate to form the various layers of the cerebral cortex (Fig. 3A). At E16.5, *Reln* mRNA (Fig. 3B) and protein (Fig. 4F) persisted in cells occupying the marginal zone and, underlying this structure, cells in the cortical plate continued to express *Dab1* mRNA (Fig. 3C) and protein (Fig. 4A). High levels of *Dab1* protein were also detected in fibers in the intermediate zone, whereas lower levels of Dab1 were observed in the ventricular zone (Fig. 4A). Double staining with anti-Dab1 and anti-MAP2 antibodies demonstrated that Dab1 was present in neurons and their apical processes within the cortical plate and the marginal zone (not shown). In *reeler*, Dab1 expression was detected in cortical plate neurons, in cells within the ventricular zone, and in fibers of the intermediate zone (Fig. 4B). However, between the Dab1-positive cortical plate neurons and the pial membrane, there was a region of reduced Dab1 immunostaining (Fig. 4E). This area corresponds to the superplate, which contains Cajal-Retzius neurons and ectopically located subplate neurons.

In the cerebral cortex of postnatal day 5 (P5) mice, *Reln* and *Dab1* were expressed in adjacent layers near the superficial aspect of the cerebral cortex (Fig. 3F,G). In addition to the persistence of *Reln* in the marginal zone, other cortical neurons in deeper layers expressed *Reln* (Fig. 3F). The expression of *Dab1* appeared more intense near the surface of the cortex compared to that in deeper layers (Fig. 3G). In adult mice, both genes were expressed in the cerebral cortex, although the levels were lower compared to those in the developing brain (Fig. 3J,K).

Expression of *Reln* and *Dab1* in the cerebellum

Adult *reeler* and *scrambler* mice exhibit a dramatic reduction in the size of the cerebellum (Mariani et al., 1977; Goffinet, 1983; Sweet et al., 1996; Goldowitz et al., 1997). Granule cells, which arise from a proliferative population known as the external germinal layer (EGL), are reduced in number and Purkinje cells, which arise from a proliferative population near the fourth ventricle, are positioned ectopically in both *reeler* and *scrambler*. Although Purkinje cells are generated at the correct time, between E11 and E14, they fail to migrate radially in the direction of the EGL (Sidman and Rakic, 1973; Goffinet, 1983, 1984; Yuasa et al., 1991, 1993; Goldowitz et al., 1997). Thus, in embryonic *reeler* and *scrambler* mice, Purkinje cells fail to align into a layer beneath the EGL within the cortex of the cerebellum. The EGL is discernible on the dorsal surface of the cerebellum at E13.5 (Fig. 5A). At this time, *Reln* expression was observed both in the EGL (Fig. 5B) and in the nuclear transitory zone (NTZ). The NTZ represents a transient pathway through which neurons destined for the cerebellar nuclei migrate (Miyata et al., 1996). At E13.5, cells expressing *Dab1* were located beneath the *Reln*-positive EGL (Fig. 5C) in

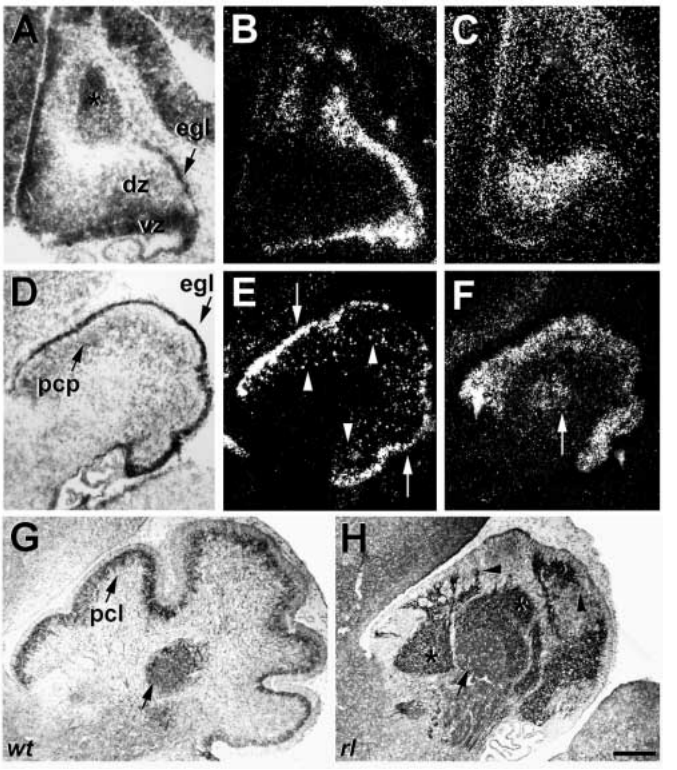

Fig. 5. Distribution of *Reln* and *Dab1* mRNA in the cerebellum at E13.5 (A-C) and E18.5 (D-F), and Dab1 protein in wild type (G) and *reeler* (H) mice at P3. (A) Brightfield image of the cerebellum, shown in darkfield in B, which was probed with antisense *Reln*. (B) Cells in external germinal epithelium (egl in A), which gives rise to granule cells, and in the nuclear transitory zone, which is associated with cerebellar nuclei (asterisk in A), express *Reln*. Neuroepithelial cells in the ventricular zone (vz in A) also contain *Reln*. (C) An adjacent section incubated with a *Dab1* antisense probe. Cells expressing *Dab1* are located in the differentiating zone (dz in A), which contains Purkinje cell precursors. (D) Brightfield image of the cerebellum at E18.5 shown in darkfield in F. (E) An adjacent section hybridized with a *Reln* antisense probe. *Reln* is expressed in the external germinal epithelium (arrows) and in cells in the cerebellar cortex (arrowheads). (F) Darkfield image of a neighboring section hybridized with antisense *Dab1*. Cells of the Purkinje cell plate (pcp in D), which is located beneath the external germinal epithelium, and some cerebellar nuclei cells (arrow) express *Dab1*. (G) Immunohistochemical analysis of a sagittal section from a normal P3 cerebellum using Dab1-specific antibodies, visualized with DAB, demonstrates the presence of Dab1 in the Purkinje cell layer (pcl). In addition, Dab1 immunoreactivity is observed in cerebellar nuclei (arrow). (H) In *reeler* cerebellum, masses of Purkinje cells express Dab1 (asterisks) as well as a few Purkinje cells in the cortex (arrowheads). Deep cerebellar cells are indicated by the arrow. The scale bar is about 200 μm in A-C, G,H, and 500 μm in D-F.

an area known as the differentiating zone (Altman and Bayer, 1985). At E18.5, the Purkinje cell plate is well-formed and the EGL has expanded to cover the surface of the cerebellum (Fig. 5D). At this time, *Reln* was expressed in granule neurons in the EGL and in a few cells located deep in the cortex (Fig. 5E). Cells in the Purkinje cell plate continued to express *Dab1*, which appeared as a broad band beneath the EGL (Fig. 5F). An additional population of cells expressed *Dab1* deep in the cerebellum (Fig. 5F, arrow).

The formation of the Purkinje cell layer in the cerebellum has been proposed to be regulated by Reln although it is not expressed in Purkinje cells themselves (D'Arcangelo et al., 1995; Miyata et al., 1996, 1997). If Dab1 functions in a Reln-dependent pathway in the cerebellum, it would be predicted to be expressed in Purkinje cells. Immunohistochemical analysis of the cerebellum at P3 demonstrated that Dab1-positive cells were located in the Purkinje cell layer, beneath the EGL (Fig. 5G). In the *reeler* cerebellum many of the Purkinje cells that failed to migrate radially formed heterotopic clusters that expressed high levels of Dab1 (Fig. 5H, asterisks). Although some Dab1-positive cells reached the cortex in the *reeler* cerebellum, they failed to align in a layer beneath the EGL (Fig 5H, arrowhead). In normal and *reeler* cerebellum, cells that contained Dab1 were also located in cerebellar nuclei (Fig. 5G,H, arrow).

Expression of *Reln* and *Dab1* in the hippocampus

The formation of the pyramidal layer of the hippocampus proper involves similar processes to those that occur during development of the cerebral cortex. For example, pyramidal neurons are generated in a proliferative layer near the ventricle and they migrate along radial fibers to form the pyramidal cell plate. Subsequently, pyramidal neurons align in an inside-out pattern reminiscent of that in the cerebral cortex. In contrast, there are two phases of cell migration during formation of the dentate gyrus that resemble processes involved in the generation of granule cells in the cerebellum. First, granule cell precursors originate from a restricted population of neuroepithelial cells near the ventricle. These cells migrate to establish a proliferative zone beneath the pyramidal layer. Second, postmitotic granule neurons that arise from these precursor cells migrate radially to form the dentate gyrus in an outside-in pattern (Angevine, 1965; Caviness, 1973; Schlessinger et al., 1978; Nowakowski and Rakic, 1979; Stanfield and Cowan, 1979a; Cowan et al., 1980).

The hippocampus in adult *reeler* and *scrambler* mice is characterized by malpositioning of both pyramidal neurons and granule cells (Caviness and Sidman, 1973; Stanfield and Cowan, 1979b; Sweet et al., 1996; Goldowitz et al., 1997; González et al., 1997). Recently, the organization of cells in the hippocampus proper has been shown to depend on the presence of Reln in the molecular layer (Nakajima et al., 1997). The first abnormality in the *reeler* hippocampus becomes obvious at approximately E16, when the orderly alignment of pyramidal cells, typical of the normal hippocampal plate is not discernible. Instead, pyramidal neurons remain scattered throughout the intermediate zone in *reeler* mice (Stanfield and Cowan, 1979a). In the *scrambler* hippocampus at E16.5, the hippocampal plate is also not apparent suggesting that, as in the *reeler* hippocampus, pyramidal neurons fail to align in a precise layer.

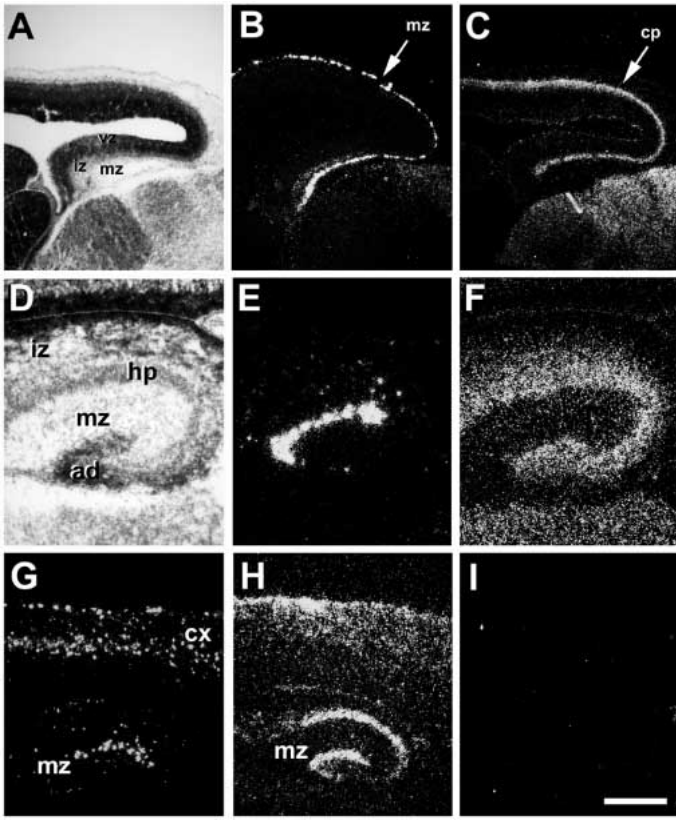

Fig. 6. Distribution of *Reln* and *Dab1* in the hippocampus at E14.5 (A-C), E18.5 (D-F), and P3 (G-I). (A) Brightfield image of the hippocampal primordium in sagittal view showing the ventricular zone (vz), intermediate zone (iz), and marginal zone (mz). (B) Darkfield image of a neighboring section shown in A hybridized with antisense *Reln*. The layer of *Reln*-positive cells in the marginal zone (mz) of the hippocampus is continuous with the marginal zone of the cerebral cortex (mz, arrow). (C) Darkfield of the section shown in A which was probed with antisense *Dab1*. Directly beneath the *Reln*-positive mz shown in B, is a layer of *Dab1*-positive cells in the intermediate zone. These cells are continuous with *Dab1*-positive cells in the cortical plate (cp, arrow) of the cerebral cortex and are likely to represent the first cohort of pyramidal cells in the hippocampus. (D) Brightfield image of the hippocampus at E18.5. (E) An adjacent section probed with antisense *Reln*. The Cajal-Retzius-like cells in the marginal zone contain *Reln*. (F) Darkfield image of the section shown in D probed with antisense *Dab1*. Pyramidal cells in the hippocampal plate (hp in D), granule cells in the area dentata (ad), and cells in intermediate zone (iz in D) contain *Dab1*. (G) Darkfield image of a sagittal section of P3 brain, probed with antisense *Reln*. Although *Reln* persists in the Cajal-Retzius-like cells in the marginal zone of the hippocampus, a few scattered cells outside the marginal zone also express *Reln*. This distribution is similar to that in the overlying cerebral cortex (cx), where *Reln* is present in multiple layers. (H) A neighboring section probed with antisense *Dab1*. High levels of *Dab1* are detected in pyramidal cells in the hippocampus proper and in granule cells in the dentate gyrus. The overlying cortex is also positive for *Dab1*. (I) A neighboring section probed with sense *Dab1* demonstrates no hybridization. The scale bar is approximately 400 μm in A-C, G-H, and 200 μm in D-F.

In the hippocampal primordium at E12.5 the patterns of *Reln* and *Dab1* expression resembled those in the cerebral cortex at E11.5, in which *Reln* expression was localized to the marginal

zone (not shown) and *Dab1* was expressed throughout the ventricular neuroepithelium (Fig. 2C). By E14.5, three layers are apparent in the hippocampus. The densely packed ventricular zone contains the majority of cells in the hippocampal anlage, an intermediate zone, containing the first cells destined for the pyramidal cell layer, lies above the ventricular zone, and the marginal zone is located superficial to this layer (Fig. 6A). In addition, cells destined for the dentate gyrus are apparent as an extension of the ventricular epithelium near the medial extent of the hippocampus. At this time, most cells expressing *Reln* were located in the marginal zone (Fig. 6B), although a few cells in the intermediate zone were also positive. The *Reln*-expressing cells in the marginal zone correspond to Cajal-Retzius-like cells (Soriano et al., 1994). A thin layer of *Dab1*-positive cells first appeared around E14.5, directly beneath the *Reln*-positive marginal zone (Fig. 6C). This layer, which is continuous with *Dab1*-positive cortical plate cells in the cerebral cortex, likely represents the first cohort of pyramidal cells that form the hippocampal plate (Stanfield and Cowan, 1979a). Thus, *Dab1* and *Reln* are expressed in the normal hippocampus in adjacent cell populations before morphological abnormalities are detected in either *reeler* or *scrambler*.

At E18.5 the hippocampal plate is well-formed and granule cells, which have a protracted period of genesis compared to pyramidal cells (Angevine, 1965), can only be seen in the suprapyramidal blade of the dentate gyrus (Fig. 6D). At this time, Cajal-Retzius-like cells in the marginal zone expressed *Reln* (Fig. 6E) adjacent to a region of cells that expressed *Dab1* in the dentate gyrus (Fig. 6F). In the hippocampus proper, cells expressing *Dab1* are located in the pyramidal cell layer and in the intermediate zone (Fig. 6F). Several days later, at P3, the full complement of pyramidal cells is present and the infrapyramidal blade of the dentate gyrus is becoming populated with granule cells. During this period, *Reln* expression persisted in Cajal-Retzius-like cells and, similar to the situation in the cerebral cortex (Fig. 6G), an additional population of cells outside of the marginal zone was also positive. At this time, there was robust expression of *Dab1* in hippocampal pyramidal cells and in granule cells of the supra- and infrapyramidal blades of the dentate gyrus (Fig. 6H).

Dab1 accumulates in ectopic neurons in *reeler* mice

During the course of our immunohistochemical studies we noticed that *reeler* neurons consistently exhibited much stronger staining with anti-Dab1 antibodies compared to those in normal mice. For example, in Fig. 4A,B, the level of Dab1 staining in the cortical plate and intermediate zone is much higher in *reeler* than in the control cerebral cortex. In this figure, panel A was exposed about twice as long as panel B to obtain an equivalent image. In contrast, cells in the ventricular zone of both *reeler* and control cortex expressed similar levels of Dab1. Furthermore, ectopic Purkinje cells, but not cells in the cerebellar nuclei, of *reeler* mice exhibited more intense immunostaining with Dab1 antibodies (Fig. 5G,H). No difference was found in the level of MAP2 staining between normal and mutant mice. These results suggest that Dab1 expression is elevated in *reeler* mice. However, in situ hybridization experiments (Fig. 3C,D) and northern hybridization analysis indicated that *reeler* mice expressed the same level of *Dab1* mRNA as that found in control mice (Fig. 7A). To resolve this conundrum, we compared the levels of Dab1 in *reeler* and normal mice by immunoblot analysis. As shown in Fig. 7B, *reeler* extracts prepared from E16.5 embryos contained approximately 5- to 10-fold more Dab1 protein than brain extracts from normal mice or heterozygous littermates. Similar results were obtained using antibodies that were raised against either the PI/PTB domain or the C-terminal region of Dab1. In some heterozygous brain extracts, more Dab1 was present than that found in wild-type extracts, but the difference in levels was variable. Thus *Dab1* protein, but not mRNA, is elevated in ectopic neurons in *reeler* mice. This finding suggests that Reln exerts a post-transcriptional control on Dab1 expression, either at the level of translational efficiency or protein turnover.

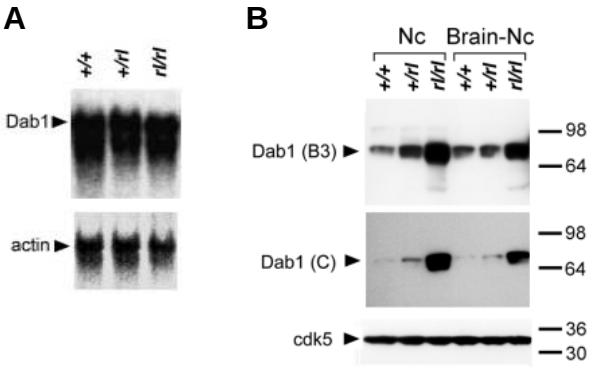

Fig. 7. *Dab1* protein expression is elevated in *reeler* mice. (A) Northern analysis of total RNA isolated from E16.5 brain tissue. Full length *Dab1* cDNA was hybridized with RNA from wild type (+/+), heterozygous *reeler (+/rl*), and homozygous *reeler* (*rl/rl*). The same blot was rehybridized with a control actin probe. (B) Western analysis of protein extracts from E16.5 neocortex (Nc) and the remainder of the brain (Brain-Nc) using antibodies directed against either the N-terminal portion of Dab1 or against a peptide from the C terminus of Dab1. The same blot was stripped and reprobed with an antibody directed against Cdk5 as a control for protein loading and protein transfer.

DISCUSSION

The *reeler* mutation has attracted a significant degree of attention because of the widespread neuroanatomical defects apparent in the central nervous system (CNS) of the mutant mice. Although neurogenesis occurs normally in *reeler*, dramatic defects in cell positioning are apparent in several laminar structures of the CNS. Mouse chimera analysis suggested that the product of the *reeler* gene functions as an extrinsic signal on Purkinje cells as they migrate from their site of origin near the fourth ventricle to their final destination in the cerebellar cortex (Mullen, 1978; Terashima et al., 1986). This prediction was largely supported by the identification and characterization of *Reln*, the gene mutated in *reeler* mice (D'Arcangelo et al., 1995). The product of *Reln* was shown to be a large protein secreted by distinct cell types, such as Cajal-Retzius cells and cerebellar granule cells, during CNS development (Ogawa et al., 1995; Miyata et al., 1996; D'Arcangelo et al., 1997). However, the finding that Reln expression is largely confined to the marginal zone of the developing cerebral cortex, next to the cortical plate which is

disrupted in *reeler* mice, implies that Reln acts through a molecular signaling pathway that ultimately impinges on the neurons that go astray in the mutant mice. The identification of *reeler*-like defects in *scrambler*, *yotari* and *Dab1*-null mice (Sweet et al., 1996; Goldowitz et al., 1997; González et al., 1997; Howell et al., 1997b; Sheldon et al., 1997; Yoneshima et al., 1997) provides a unique opportunity to investigate the respective roles of two distinct genes in the complex biological events that underlie formation of the mammalian cerebral cortex.

Dab1 functions downstream of *Reln*

Reln has several hallmarks of proteins that function as components of the extracellular matrix. It contains a cleavable signal peptide at the N terminus and a series of eight EGF-like repeats similar to those of the tenascin and integrin families (D'Arcangelo et al., 1995). It is secreted by COS cells transfected with a *Reln*-expression clone and from primary cultures of cerebellar cells (D'Arcangelo et al., 1997; Goldowitz et al., 1997). Furthermore, immunohistochemical studies with the anti-Reln antibody, CR-50, suggest that Reln accumulates on Purkinje cell processes (Miyata et al., 1996), even though these cells do not express *Reln* mRNA. Taken together, these observations indicate that Reln acts as an extracellular signal that activates receptors present on the surface of cell populations that go astray in *reeler* and *scrambler* mice.

Dab1 is a cytoplasmic molecule that probably does not interact with Reln directly, however, it has several features that make it a likely candidate to be involved in a Reln-initiated signaling pathway. Dab1 is known to bind protein tyrosine kinases such as Src, Fyn and Abl via its PI/PTB. The observation that Dab1 becomes phosphorylated on tyrosine residues during neuro development demonstrates that it is also a substrate for protein tyrosine kinases (Howell et al., 1997a). These features suggest that Dab1 acts as an adapter protein that links components of a signal transduction pathway such as cell surface receptors and cytoplasmic protein kinases.

Although *Dab1* mRNA is expressed normally during neuro development in *reeler* mice, Dab1 protein levels accumulate approximately 5- to 10-fold in embryonic brain tissue. This dramatic difference in Dab1 protein levels between *reeler* and wild-type mice is present as early as E12.5, and it is maximal at E16.5. In adult mice this difference in Dab1 levels is less pronounced (data not shown). The time of peak overexpression of Dab1 corresponds to the period in which neuronal migration is underway and when Reln is required for the normal positioning of neurons in CNS. This increase could arise if under normal circumstances Dab1 is degraded after fulfilling a signaling function evoked by Reln as part of a switch mechanism that controls cell positioning. Alternatively, it is possible that the absence of Reln causes an increase in the translation rate of *Dab1* mRNA, leading to increased levels of Dab1. Regardless of the mechanism responsible for the increased levels of Dab1 protein in *reeler*, this finding establishes the first biochemical link between these proteins and suggests that Dab1 functions downstream of Reln.

The preplate fails to split properly in the *scrambler* neocortex

Mammalian cortical plate formation occurs subsequent to the appearance of the preplate, a stratum of neurons and fibers that arises early in development. Postmitotic neuronal precursors migrate from the ventricular zone along the surface of radial glia (Rakic, 1972) towards the pial surface where they insert into the preplate, splitting this structure into the superficial marginal zone (layer I) and a deep layer of neurons known as the subplate (Marín-Padilla, 1978, 1998). Previous studies in *reeler* mice demonstrated that many cortical plate neurons accumulate beneath, instead of above, the subplate in a structure known as the superplate (Caviness, 1982; Ogawa et al., 1995; Sheppard and Pearlman, 1997). These observations suggested that Reln plays a direct role in the cell-cell interactions that govern cell positioning within the preplate.

In the *scrambler* cerebral cortex, most of the subplate neurons, which were identified immunohistochemically at E16.5 with anti-MAP2 antibodies and by labeling with BrdU, are located above the cortical plate (Fig. 1). Therefore, cortical plate neurons failed to bypass the subplate despite the presence of Reln in *scrambler* mice (Goldowitz et al., 1997; González et al., 1997; Sheldon et al., 1997). Furthermore, in the *scrambler* cerebral cortex the distribution of CSPGs, which are associated with the preplate and its derivatives, is remarkably similar to that in *reeler* (Sheppard and Pearlman, 1997). Both mutants display an intense staining of CSPGs near the pial surface and in the IPZ, which may indicate the presence of a few subplate neurons intermixed with cortical plate neurons. Thus, the preplate fails to split properly in the *scrambler* cerebral cortex. The fact that the same developmental process is disrupted in *scrambler* and *reeler* mice suggests that *Reln* and *Dab1* participate in a common signaling pathway that controls cell positioning within the cortical plate.

Reln and *Dab1* are expressed in adjacent cells during development

In the *reeler* and *scrambler* cerebral cortex, the first wave of cortical plate neurons fails to insert into the preplate resulting in the accumulation of cortical plate cells beneath the superplate (Caviness, 1982; Ogawa et al., 1995; Sheppard and Pearlman, 1997; present study). *Reln* is produced by Cajal-Retzius cells located in the preplate before the arrival of cortical plate neurons. In contrast, at this time (E11.5) *Dab1* is expressed in neuroepithelial cells in the ventricular zone at low and relatively uniform levels. Approximately 24 hours later, *Dab1* expression levels are more pronounced in the lateral extent of the neocortex where the cortical plate is emerging directly beneath the *Reln*-positive cells. As more neurons arrive in the cortical plate, the overall level of *Dab1* increases (Fig. 2). Although the importance of *Dab1* expression in the ventricular zone is unclear, *Dab1*, like *Reln*, is required for the migration of cortical plate cells into the preplate.

In the cerebellum, adjacent populations of cells express *Dab1* and *Reln* before anatomical abnormalities in *scrambler* and *reeler* are apparent. For example, Purkinje cells in the differentiating zone (dz) of the E13.5 cerebellum express *Dab1* in a region underlying cells in the external germinal layer (EGL) that express *Reln* (Fig. 5). Therefore, it seems likely that the initial signaling events between the *Reln*-positive EGL and the *Dab1*-positive Purkinje cells occur when these cells are in close proximity. In the absence of *Reln* or *Dab1*, this signaling event does not occur and Purkinje cells remain clustered beneath the cortex of the cerebellum. Although granule cells are dramatically decreased in number in adult *scrambler* mice,

their migration occurs normally (Goldowitz et al., 1997). The reduction in granule cell number may be a secondary event caused by a lack of trophic support that would be provided by correctly-positioned Purkinje cells. Thus, the cerebellar defects in adult *scrambler* and *reeler* mice may be a consequence of an initial failure of the interactions between granule cell precursors and Purkinje cells in which the Purkinje cells fail to migrate radially towards a Reln signal secreted by EGL cells.

In the hippocampus of *Reln* and *Dab1* deficient mice, pyramidal cells are not aligned in a monolayer and granule cells are widely distributed in the dentate gyrus (Stanfield and Cowan, 1979b; Sweet et al., 1996; Howell et al., 1997b; Yoneshima et al., 1997). Neither of these cells express *Reln* during development, which is secreted by the Cajal-Retzius-like cells in the marginal zone. However, *Dab1* is expressed in the first cohort of pyramidal cells as they arrive in the intermediate zone underneath the marginal zone at E14.5 (Fig. 6), and later, at E18.5, granule cells express *Dab1* when they migrate radially to form the dentate gyrus. Therefore, *Reln* may provide an instructive signal for both of these populations of migrating cells.

Mechanism of action of *Reln* and *Dab1*

In the three brain regions analyzed here, the migration of neuronal precursors away from their site of origin appears to be unimpeded in the absence of *Dab1*. However, *Dab1*-deficient neurons fail to coalesce into discrete layers. This is most likely a consequence of the inability of the first cohort of cells expressing *Dab1* to respond to a *Reln*-evoked signal, which is present at the terminus of the migratory route of these cells. In the *scrambler* cerebral cortex, the first wave of cortical plate neurons is most likely unable to respond to Reln in the preplate and, as a result, they fail to enter this region. This contrasts with the situation in *Cdk5* and *p35* null mice in which, although the laminar organization of the cortex is disrupted, the first cohort of cortical neurons is able to invade the preplate and form a cortical plate between the marginal zone and subplate (Gilmore et al., 1997; Kwon and Tsai, 1998). In these mice, lamination fails at the next stage in which later-generated cohorts of neurons fail to migrate past the first population which has taken up residence in the cortical plate. This may either be a consequence of a failure in migration per se or it is possible that *Cdk5/p35* is required to alter the properties of the first cortical plate neurons, after they insert into the preplate, permitting them to be bypassed by later generated neurons. In either scenario, *Cdk5/p35* would be envisioned to function downstream of *Dab1* during corticogenesis. Thus, the mechanism of cortical plate formation involves at least two distinct processes, an early event dependent on Reln and Dab1, and a later event that also requires Cdk5/p35, in addition to Reln and Dab1.

The nature of the molecular interactions between Reln and cells that express Dab1 are not yet clear. One possibility is that Reln, which is produced and secreted into the extracellular environment, acts as a guidepost by binding to receptors on adjacent cells resulting in modulation of tyrosine kinases. For example, the majority of cortical neurons ascends to the preplate along radial fibers and presumably contacts Reln in the marginal zone during the formation of the mammalian cortical plate. Reln could conceivably bind to the leading process of these migrating cells instructing them to translocate to the preplate. Subsequently, cells would detach from the radial fiber and differentiate into mature neurons. Consistent with this idea, we found that Dab1 is expressed at high levels in cortical plate neurons at the time when they invade the preplate. In addition, Dab1 is present in neuronal processes in the marginal zone. These observations imply that Dab1 may mediate the functional interaction of Reln with cortical plate neurons. Alternatively, Reln could conceivably interact indirectly with cells containing Dab1 through effects on radial glia, which are known to express molecules required for neuronal migration and laminar organization in the cortex (Cameron and Rakic, 1994; Anton et al., 1996a,b). Finally, we cannot rule out the possibility that Reln binds to other molecules, for example diffusible factors, in the extracellular matrix (Sheppard and Pearlman, 1997) that affect migration. Hopefully, these alternatives will be resolved by the identification and characterization of proteins that interact with both Reln and Dab1 and mediate their functions.

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