Corrigendum

Reelin signaling directly affects radial glia morphology and biochemical maturation

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We apologise to readers for this omission.

Reelin signaling directly affects radial glia morphology and biochemical maturation

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Summary

Radial glial cells are characterized, besides their astroglial properties, by long radial processes extending from the ventricular zone to the pial surface, a crucial feature for the radial migration of neurons. The molecular signals that regulate this characteristic morphology, however, are largely unknown. We show an important role of the secreted molecule reelin for the establishment of radial glia processes. We describe a significant reduction in ventricular zone cells with long radial processes in the absence of reelin in the cortex of reeler mutant mice. These defects were correlated to a decrease in the content of brain lipid-binding protein (Blbp) and were detected exclusively in the cerebral cortex, but not in the basal ganglia of reeler mice. Conversely, reelin addition in vitro increased the Blbp content and process extension of radial glia from the cortex, but not the basal ganglia. Isolation of radial glia by fluorescent-activated cell sorting showed that these effects are due to direct signaling of reelin to radial glial cells. We could further demonstrate that this signaling requires Dab1, as the increase in Blbp upon reelin addition failed to occur in *Dab1*$^{-/-}$ mice. Taken together, these results unravel a novel role of reelin signaling to radial glial cells that is crucial for the regulation of their Blbp content and characteristic morphology in a region-specific manner.

Key words: Neurogenesis, reeler mutant, Dab1, Apoer2, Vldlr, Precursor morphology

Introduction

Radial glial cells are a pivotal cell type in the developing CNS involved in key developmental processes, ranging from patterning and neuronal migration to their newly described role as precursors during neurogenesis (for a review, see Campbell and Götz, 2002). The term 'radial glial cell' refers to their two major characteristics, their long radial processes extending from the ventricular zone (VZ) to the pial surface and their glial properties, such as the content of glycogen granules or the expression of the astrocyte-specific glutamate transporter (Glast; Slc1a3 – Mouse Genome Informatics) (Shibata et al., 1997) or the glial fibrillary acidic protein (GFAP) (Bignami and Dahl, 1974) (for a review, see Kriegstein and Götz, 2003). Notably, recent evidence demonstrates that radial glial cells characterized by long radial processes and astroglial properties constitute the majority of precursors during neurogenesis (Malatesta et al., 2000; Malatesta et al., 2003; Heins et al., 2002; Noctor et al., 2002). Indeed, all radial glial cells divide throughout neurogenesis (Misson et al., 1988; Hartfuss et al., 2001) and give rise to the majority of projection neurons in the cerebral cortex (Malatesta et al., 2003; Miyata et al., 2001; Noctor et al., 2001; Tamamaki et al., 2001). These observations raise the question of how radial glial cells perform these diverse functions and to what extent their molecular and morphological specification is relevant for these diverse roles.

We have previously identified the transcription factor Pax6 as a necessary and sufficient determinant for the neurogenic lineage of cortical radial glia (Götz et al., 1998; Heins et al., 2002). In correlation to an almost complete loss of the neurogenic radial glia, Glast is strongly reduced and the morphology of radial glia is affected in the Pax6-mutant cortex (Götz et al., 1998; Heins et al., 2002). However, Pax6-deficient radial glia are still attached to the pial surface, they still contain the brain lipid-binding protein (Blbp) and neuronal migration is only affected at late developmental stages (Caric et al., 1997; Götz et al., 1998) (N. Haubst and M.G., unpublished). Blbp had been suggested to promote the bipolar morphology of radial glia when neurons attach in vitro (Feng et al., 1994; Kurtz et al., 1994; Anton et al., 1997). ErbB receptors should be involved in mediating this neuron-glia signaling, but the respective mouse mutants show only a minor phenotype in branching of radial glia endfeet and still possess long radial glia processes (Anton et al., 1997). Thus, the proposed role of Blbp in influencing the morphology of radial glia has so far not been substantiated in vivo and the signals that regulate radial glia process extension remain elusive.

We examined reelin as a candidate molecule for influences

on radial glia that are in close contact with reelin-secreting cells in the marginal zone (MZ). Reelin is a large secreted glycoprotein, the absence of which has profound effects on neuronal migration in the cerebral cortex and cerebellum, but not the basal ganglia (D'Arcangelo et al., 1995) (for reviews, see Caviness et al., 1988; Curran and D'Arcangelo, 1998; Lambert de Rouvroit and Goffinet, 1998). Reelin binds to the lipoprotein receptors apolipoprotein receptor 2 (Apoer2; Lrp8 – Mouse Genome Informatics) and the very low-density lipoprotein receptor (Vldlr) (D'Arcangelo et al., 1999; Hiesberger et al., 1999), and their role as receptors for reelin has been confirmed in vivo by the identical phenotype of mice lacking both Apoer2 and Vldlr, and reeler mice (Trommsdorff et al., 1999) (for a review, see Herz and Bock, 2002). Upon reelin binding to Apoer2/Vldlr receptors the cytosolic adapter protein mouse disabled 1 (Dab1) is phosphorylated by Src family kinases (D'Arcangelo et al., 1999; Howell et al., 1999; Hiesberger et al., 1999; Bock and Herz, 2003; Arnaud et al., 2003) and downstream signaling is thought to affect neuronal migration via cytoskeletal changes (Howell et al., 1997; Rice et al., 1998; Hiesberger et al., 1999; Hammond et al., 2001; Beffert et al., 2002). This signaling pathway is supported in vivo since the phenotype of the Dab1-deficient mice scrambler and yotari (Sweet et al., 1996; Goldowitz et al., 1997; Gonzalez et al., 1997; Sheldon et al., 1997; Ware et al., 1997; Yoneshima et al., 1997) and the mice carrying a targeted deletion of the *Dab1* gene (Howell et al., 1997) corresponds to the reeler phenotype.

Importantly, however, VZ cells that are mostly composed of radial glia as described above express Vldlr, Apoer2 and Dab1 (Sheldon et al., 1997; Trommsdorff et al., 1999; Magdaleno et al., 2002; Benhayon et al., 2003; Luque et al., 2003) and phosphorylate Dab1 when stimulated with reelin (Magdaleno et al., 2002; Benhayon et al., 2003). Thus, there is strong evidence that precursor cells, including radial glia, have the prerequisite to directly perceive reelin signals. Indeed, misexpression of reelin under control of the CNS-specific nestin-enhancer in VZ cells of reeler mice leads to a partial rescue of the reeler phenotype (Magdaleno et al., 2002), consistent with a potentially direct effect of reelin onto VZ cells. Moreover, radial glial cells preferentially adhere to reelin-containing substrates in vitro, an effect that also requires Dab1 (Förster et al., 2002; Frotscher et al., 2003). These results prompt the suggestion that reelin might also directly act on radial glial cells. Here we tested this suggestion directly by isolating radial glial cells as described previously (Malatesta et al., 2000; Malatesta et al., 2003; Heins et al., 2002). Moreover, we address the specific role of reelin signaling to radial glial cells in vivo in reeler mice and combine this analysis with complementary gain-of-function experiments by addition of reelin to radial glial cells in vitro.

Materials and methods

Animals and genotyping

We have used the inbred mouse strain C57BL6/J (Charles River Laboratories); reeler mice (B6C3Fe *a/a-Reln* ^{rl}/+, stock number 000235, The Jackson Laboratory; crossed with C57BL6/J); the transgenic mouse lines hGFAP-GFP (94-4) (Zhuo et al., 1997; Malatesta et al., 2000) and hGFAP-EGFP (Nolte et al., 2001; Malatesta et al., 2003); the Tau::EGFP mice where one allele of the tau-locus was replaced by EGFP (Tucker et al., 2001; Heins et al., 2002); and mice carrying a targeted deletion of the *Dab1* gene (Howell et al., 1997) (kept on a mixed Balb/c/Sv129Sv/J background; stock number 003581, Jackson Laboratory). The day of vaginal plug was considered as embryonic day 0 (E0), the day of birth as postnatal day 0 (P0). The hGFAP-(E)GFP- and Tau::EGFP-embryos were identified by fluorescent microscopy and fluorescent cells were sorted using a FACSVantage or FACSort (Becton Dickinson) as described (Malatesta et al., 2000; Malatesta et al., 2003). Wild type and homozygous reeler or *Dab1*$^{-/-}$ littermates were obtained by heterozygous crossings genotyped by PCR on tail DNA (D'Arcangelo et al., 1996; Howell et al., 1997).

Cell dissociation and reelin conditioned medium

Acutely dissociated cells were prepared as described previously (Hartfuss et al., 2001). Conditioned medium was collected from 293 cells expressing either reelin or a control plasmid containing GFP (clone pCrl) (see D'Arcangelo et al., 1997; Förster et al., 2002) (kindly provided by T. Curran, St. Jude Childrens Hospital, Memphis Tennessee, USA) 2 days after culturing in medium containing 10% fetal calf serum (FCS; Sigma) or in chemically defined medium (see Malatesta et al., 2000). The difference in reelin content was confirmed by western blotting using the mABs E4 or G10 directed against reelin (De Bergeyck et al., 1998) (Fig. 5E).

Immunohistochemistry

Immunohistochemistry was performed on vibratome or cryostat sections as described previously (Hartfuss et al., 2001). Primary antibodies were the monoclonal mouse antibody (mAb) Rc2 (IgM; 1:500), the polyclonal Ab (pAb) against Blbp (rabbit, 1:1500; kindly provided by N. Heintz, Rockefeller University, New York, NY), the pAb directed against Glast (guinea pig, 1:8000; Chemicon), the pAb against Apoer2 (rabbit, 1:2000) (Stockinger et al., 1998) (kindly provided by J. Nimpf, Biocentre University of Vienna, Austria), the pAb against Vldlr (goat, 1:100; Q15, Santa Cruz Biotech); the mAb E4 (De Bergeyck et al., 1998) against reelin (IgG1, 1:500; kindly provided by A. Goffinet, University Louvain Medical School, Brussels, Belgium), the rat mAB TEC-3 against Ki67 (IgG, 1:25; Dianova Immundiagnostics), the mAb anti-nestin (IgG1, 1:4; Developmental Studies Hybridoma Bank), the pAb against phosphohistone3 (rabbit, 1:500; Biomol), the mAb against β-tubulin-III (IgG2b, 1:100; Sigma), the mAb against NeuN (IgG1, 1:50; Chemicon) and the mAb directed against GFAP (IgG1, 1:200; Sigma). Secondary antibodies (Dianova) were used at standard conditions.

BrdU labelling

Pregnant mice were injected intraperitoneally 1 hour prior to hysterectomy with 5-bromo-2-deoxyuridin (BrdU, 5 mg in PBS per 100g body weight). In vitro, BrdU was added at a final concentration of 10 μM either for the whole culture period to label all dividing cells, or, to detect cells in S phase, for only 1 hour (after 10 hours culturing of cells without BrdU, BrdU was added for 1 hour, then BrdU was removed by several washes and cultures were further incubated in BrdU-free medium for 11 hours).

DiI-labelling and 3D-reconstruction

The lipophilic dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI, Molecular Probes) was injected as ethanol/sucrose suspension in the lateral ventricle of embryonic brains or the lumen of the spinal cord (C57BL6/J, E12-16) ex vivo and stored in 2% paraformaldehyde in PBS for 10-20 days at room temperature resulting in a complete labelling of cell membranes. Frontal vibratome sections (150 μm) were cut and analysed by confocal laser scanning microscopy (CLSM). Series of single optical section images (≤1 μm) were used to assemble a 3D-reconstruction of the brain slice using Imaris™-program (Bitplane AG, Switzerland) that were used for the morphological analysis of DiI-labeled cells as described in Fig. 3.

LightCycler real-time RT-PCR

Total RNA was extracted from sorted cells using the RNeasy-kit (Qiagen) and 1 μg of total RNA was used to synthesize cDNA (Superscript, Invitrogen). RT-PCR was performed with a Roche LightCycler instrument. cDNA was amplified at the following conditions (95°C for 5 minutes; 45 cycles of 15 seconds at 95°C, 8 seconds at 55°C, 25 seconds at 72°C). Quantitative analysis of the LightCycler data was performed employing LightCycler analysis software. The crossing points (CP) are the intersections between the best-fit lines of the log-linear region and the noise band. The CPs were normalized to that of GAPDH to compensate for variability in RNA amount. We calculated the relative expression level as $2^{(CP1-CP2)}$. CP1 indicates the crossing point of the mRNA level of the housekeeper GAPDH; CP2 indicates the crossing point of the mRNA level of the gene of interest. The mRNA level of GAPDH in each tissue sample was set to 1. The following primers were used: for Blbp (5′-gATgCTTTCTgTgCCACCTg-3′; reverse 5′-CTgCCTCCACACCAAAgACA-3′); for GAPDH (5′-ATTC-AACggCACAgTCAAgg-3′; reverse 5′-TggATgCAgggATg-ATgTTC-3′); for Vldlr (5′-TCCAAgTTgCACATgCTCTC-3′; reverse 5′-CCAgCTCTgACCCAgTgAAT-3′) and for Apoer2 (5′-gCAACCACTCCCAgCATTAT-3′; reverse 5′-TACCAC-TATgggCACgATgA-3′).

Results

Reduction of Blbp-positive radial glia in the reeler cortex, but not GE

To examine the differentiation of radial glia in the absence of reelin-signaling, Glast, Blbp and the antigen of Rc2 were detected immunohistochemically in vibratome sections of wild-type and reeler mutant littermate cortex during the peak of neurogenesis (embryonic day (E) 14 and 16). Whereas the Rc2-immunoreactivity was similar in radial glia of wild type and reeler (Fig. 1A′,B′), the Blbp-immunoreactivity in radial glia appeared reduced in reeler mice (Fig. 1A″,B″). Interestingly, this decrease in Blbp-immunoreactivity in the reeler mutant telencephalon was restricted to the cortex and not observed in the ventral telencephalon, the ganglionic eminence (GE; Fig. 1C-D″). Notably, few reelin-immunoreactive cells are in contact with endfeet of radial glia arising from this region, in contrast to the cortex (Fig. 1E-G), suggesting that reelin-signaling plays no role for radial glia in the GE, but is required for Blbp content in radial glia of the cortex.

In sections, it is difficult to discriminate whether targeting of Blbp might be altered in the reeler mutant cortex such that Blbp is reduced only in the radial processes but still present in the soma or whether the subpopulation of radial glia containing Blbp (see Hartfuss et al., 2001) is truly reduced. To address this, we used acutely dissociated cell preparations where co-localization at the single cell level can be clearly assessed. The quantitative analysis of Rc2-, Glast- and Blbp-immunoreactive precursor cells [double-stained with anti-Ki67, for details see Materials and methods, and Hartfuss et al. (Hartfuss et al., 2001)] revealed that in the early developing cortex, around the

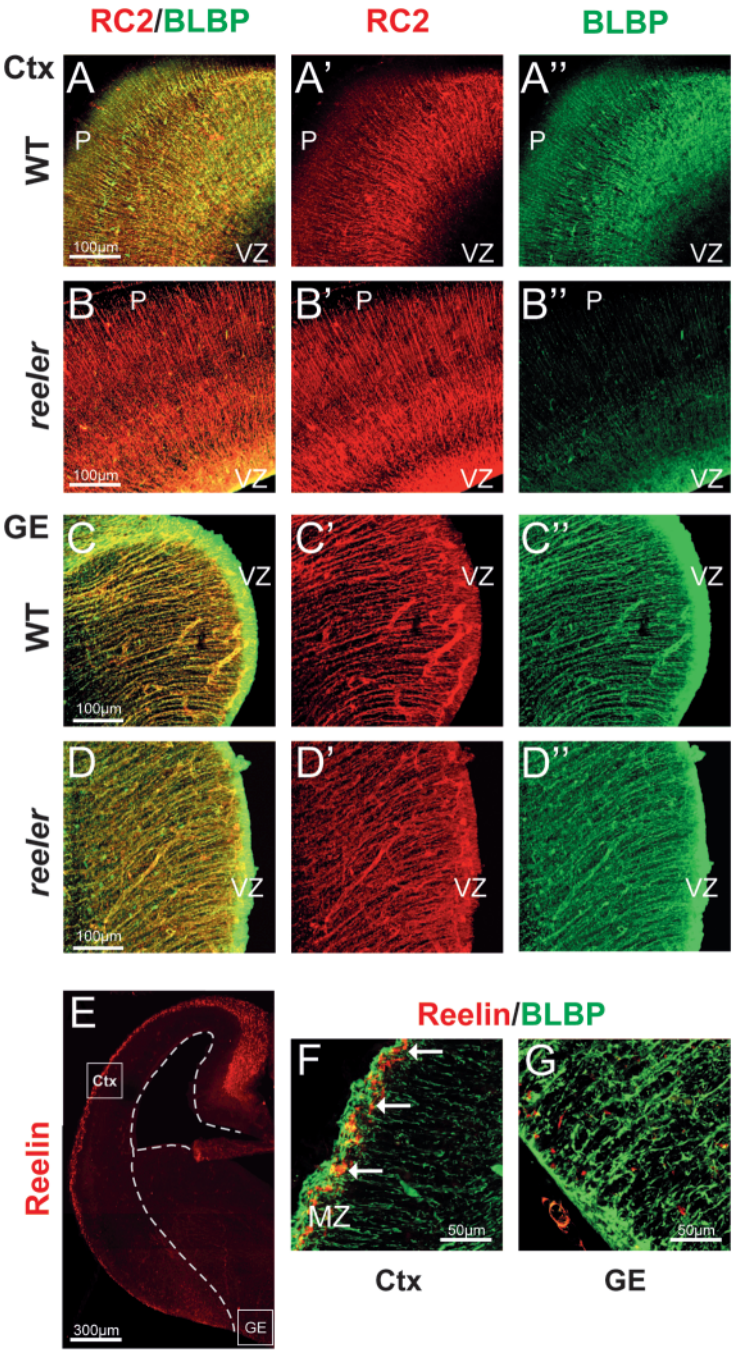

Fig. 1. Neurochemically identified subpopulations of radial glia in wild-type and reeler telencephalon. Frontal vibratome sections of wild-type (A,C,E-G) and reeler (B,D) telencephalon stained for Blbp (A-D,F,G), Rc2 (A-D) or reelin (E-G). A-D show sections of embryonic day (E) 16 wild-type (A,C) and reeler (B,D) cortex stained for Rc2 (red) and Blbp (green) as indicated in the micrographs. A-D show maximum intensity pictures (~50 μm), A′,A″ and B′,B″ depict single optical sections (~5 μm). Note that Rc2-immunoreactivity is similar, but the Blbp-immunoreactivity is strongly reduced in the reeler cortex, whereas no difference is seen in the GE. E depicts a low power view of reelin-immunoreactive cells in the telencephalon at E14. The broken line indicates the outline of the ventricle and border between cerebral cortex (Ctx) and ganglionic eminence (GE). (F,G) High power views of the cortex (F) and ventral telencephalon (G), as indicated by the white boxes in E. Note the close vicinity of reelin-immunoreactive neurons (red, examples indicated by arrows) and the Blbp-immunopositive endfeet of radial glial cells (green) from the cerebral cortex (F), but not from the GE (G).

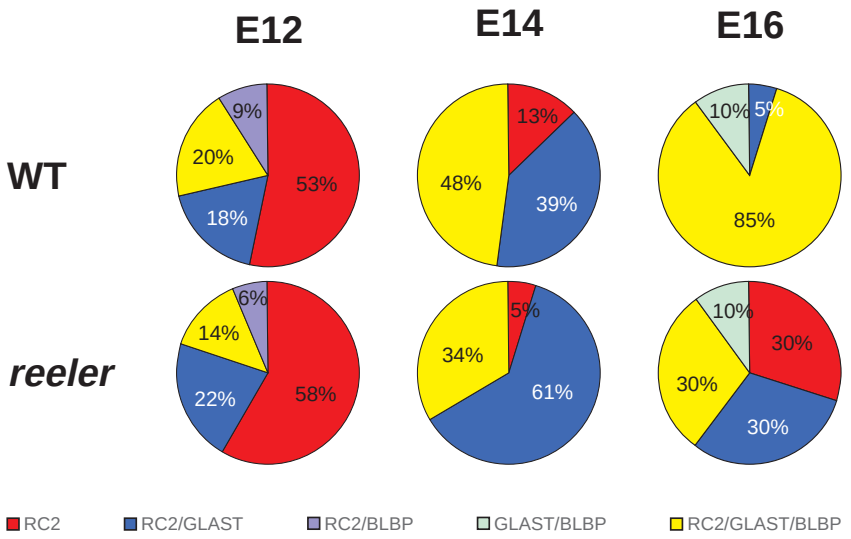

Fig. 2. Quantitative analysis of precursor subtypes in wild-type and reeler mice throughout neurogenesis. The pie charts depict the quantitative co-localization analysis of precursor cells immunoreactive for Rc2, Blbp and Glast in acutely dissociated cells of wild-type and reeler cortex at E12, E14 and E16. The analysis was performed as described in detail in Hartfuss et al. (Hartfuss et al., 2001). Briefly, triple immunostainings were performed in different combinations of Rc2, anti-Glast, anti-Blbp and anti-Ki67 to detect all dividing cells as described in the above reference. Note that the number of Blbp-positive precursors is severely reduced in the reeler compared with wild-type cortex. [Number of cells analysed: wild-type, *n*(E12)=311, *n*(E14)=1821, *n*(E16)=302; reeler, *n*(E12)=307, *n*(E14)=1853, *n*(E16)=295.]

onset of neurogenesis (E12), the number of Blbp-immunoreactive cells was still comparable between wild-type and reeler cortex even though a slight decrease was detectable already (Fig. 2, Blbp-positive cells; wild type, 29%; reeler, 20%). Indeed, reelin-immunopositive cells are still very few and some are still being generated at this stage (Hevner et al., 2003; Stoykova et al., 2003). However, at E14, when radial glia processes find a continuous layer of reelin-immunoreactive cells in the MZ and when Blbp-immunoreactivity was seen reduced in sections, the number of Blbp-immunoreactive cortical cells was also reduced in acutely dissociated cells (Fig. 2; wild type, 48%; reeler, 34%; $P<0.01$). During development, their number further decreased in the reeler cortex compared with wild type (Fig. 2; E16; wild type, 95%; reeler, 40%; $P<0.01$) and then also the number of Glast-immunoreactive cells was reduced at E16 (by 30%; $P<0.05$, Fig. 2). Note that the number of Glast-positive cells first increases in wild-type and reeler mutant cortex, and only later (between E14 and E16) decreases again (Fig. 2). By contrast, Blbp-positive cells remained almost at the same level from E12-16 in the reeler cortex, whereas there is a constant increase in Blbp-positive cells during this period in wild-type cortex until almost all precursors contain Blbp at E16. Taken together, these results suggest that the maturation of Blbp-positive radial glial cells depends from the onset on the presence of reelin in the cortex, but not the GE.

Process extension of radial glia is impaired in the reeler cortex, but not the GE

As Blbp had been suggested to regulate the radial morphology of radial glia, we examined the length of radial glia processes in wild-type and reeler mutant embryos that had not been addressed in previous analyses. To achieve this, we used the lipophilic dye DiI to trace cells from the ventricular surface (VS) as depicted in Fig. 3. Cells located in the VZ are attached by an apical process to the VS throughout the cell cycle where only the nucleus moves up and down (Sauer, 1935). Application of DiI onto the VS therefore allows labeling of VZ cells with their complete morphology including the radial processes (Fig. 3A,B). It proved to be necessary to perform a 3D-reconstruction of the cell morphology in 150 μm vibratome sections (for details see Materials and methods) to discriminate processes that are cut and leave the section from those that are truly short as well as to reliably separate superimposed cells as exemplified in Fig. 3A,A′. The labeled cells were classified according to the length of their basal processes. Cells with a long basal process reaching into the cortical plate were classified as ‘long’ cells (see example in Fig. 3B). The second class comprises cells with a shorter basal process ending underneath the cortical plate (‘short’ cells) and the third cell type had a clear growth-cone at the tip of its basal process that appeared to extend towards the pial surface (‘growth-cone’ cells, see example in Fig. 3B). Finally, we detected also cells with a very short (<10 μm) or no basal process at all (‘club shaped’ cells, Fig. 3B). When quantifying the proportion of these morphologically distinct cell types, it became apparent that the majority of cells labeled from the ventricular surface possess long radial processes extending into the cortical plate in wild-type cortex (80-52%; Fig. 3D). The predominance of cells with long radial processes was also observed in other regions of the developing CNS such as the GE and the spinal cord (SC; Fig. 3D), and is consistent with the predominance of radial glia among VZ precursors (Malatesta et al., 2000; Malatesta et al., 2003; Hartfuss et al., 2001; Noctor et al., 2002). Note that only about 10% of VZ cells have a short process, the morphology previously assigned to ‘neuronal’ precursors. Taken together, the majority of VZ cells in wild-type cortex extend radial processes into the close vicinity of reelin-secreting cells in the MZ.

Notably, the proportion of VZ cells with a long radial process was significantly decreased in the reeler cortex (to 57% of the wild-type value; $P<0.01$; Fig. 3C,D) and the ‘club shaped’ population, characterized by a complete absence of a basal radial process, constituted the largest subpopulation of VZ cells in the reeler cortex (Fig. 3C,D). Even the population with ‘short’ basal processes ending underneath the cortical plate was reduced in the reeler cortex to less than half of their proportion in wild-type cortex (Fig. 3D), suggesting severe problems in radial fiber extension or maintenance in the absence of reelin. Interestingly, radial glial cells of the GE were not altered in their morphology in the reeler mutant (Fig. 3D), consistent with their normal Blbp-immunoreactivity (Fig. 1C-

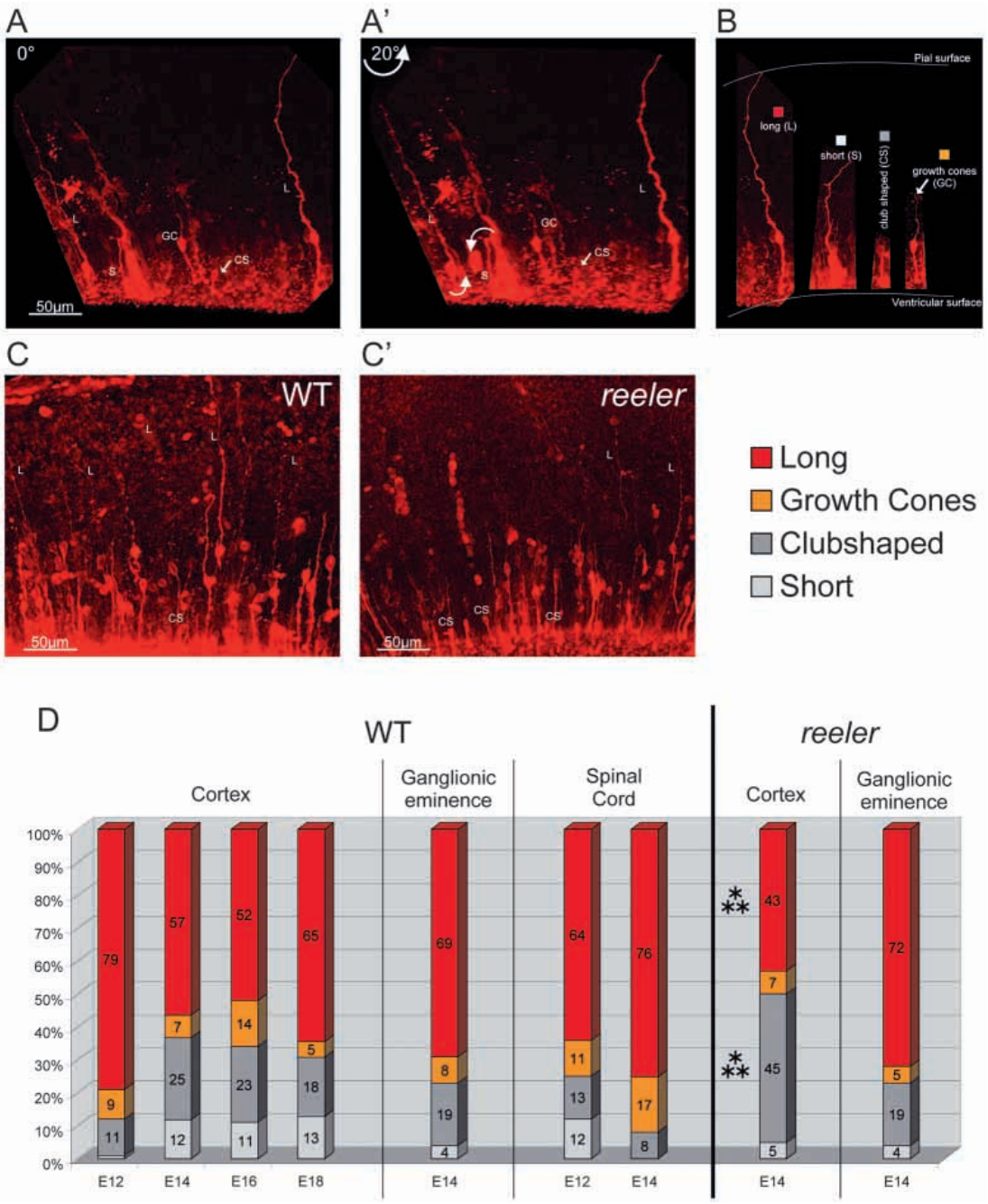

Fig. 3. Morphologically identified subpopulations of ventricular zone cells in wild-type and reeler CNS. (A-A′) Single frames of a 3D-reconstruction of precursors traced from the ventricular surface by DiI in E14 mouse cortex. Frame A′ represents a rotation of 20° (*y*-axis) with regard to frame A. Note the two cells indicated by the curved arrows previously hidden behind cells with long radial processes. For the quantification depicted in D, cells labeled from the ventricular surface were classified as: L, long precursor (the radial process reaching the pial surface, red in D); S, short precursor (with the process ending below the cortical plate, light-gray in D); CS, club-shaped precursors without a basally oriented process (dark-gray in D); GC, precursors with a radial process terminating with a growth cone-like structure (orange in D) as exemplified in A,A′,B. (C,C′) Maximum intensity of entire stacks used for 3D reconstructions of DiI-labeled VZ cells in E14 cortices of wild-type (C) and reeler (C′) littermates. Note that the majority of DiI-labeled ventricular zone cells has long radial processes in all regions and at all stages analysed in the wild-type CNS (left side), while their number is significantly reduced in the reeler cortex, but not the GE. Student's *t*-test was used for significance analysis and three asterisks in D indicate $P<0.01$. [Number of cells analysed: Ctx, *n*(E12)=90, *n*(E14)=250, *n*(E16)=116, *n*(E18)=40; GE, *n*=267; SC *n*=130; reeler Ctx, *n*=294; GE, *n*=61.]

D″). Taken together, these results show a cortex-specific decrease in Blbp and radial processes in reeler mutant radial glia.

Proliferation and neuronal differentiation in the reeler telencephalon

As the contact to the basement membrane is thought to influence cell polarity and thereby cell proliferation and fate (Huttner and Brand, 1997), we examined these aspects in the reeler mutant cortex where fewer radial glial cells are in contact with the basement membrane and also contain reduced levels of Blbp that might affect these aspects (Hartfuss et al., 2001). We first analyzed the number of mitotic cells in the VZ and the subventricular zone (SVZ) by PH3-immunohistochemistry, but no significant difference was detected between E14 wild-type and reeler littermates [PH3+cells/section, wild-type cortex VZ 54±2 (*n*=804), SVZ 31±2 (*n*=463); reeler cortex VZ 49±2 (*n*=985), SVZ 23±2 (*n*=456)]. Regarding to the orientation of cell division, no differences could be observed between wild-type and reeler mutant littermates [wild-type cortex: angle to the ventricular surface is 90-60°, 71±14%; 60-30°, 6±6%; and 30-0°, 23±12% (*n*=111)] [reeler cortex: 90-60°, 70±8%; 60-

30°, 7±9%; and 30-0°, 24±9% (n=97)]. Thus, no changes in the total number of precursors or the orientation of their cell division could be detected in the reeler mutant cortex. In addition the progeny of radial glial cells seemed not affected by the absence of reelin as the immunoreactivity of the neuronal marker β-tubulin-III was closely comparable between wild-type and reeler mice (Fig. 4A), suggesting that neurogenesis also occurs normally in the absence of reelin. Because, however, the precursors with long radial processes were reduced by 14% in the reeler cerebral cortex, one might expect only a small reduction in the number of neurons generated. To detect such small alterations in the number of neurons we used FACS analysis of crosses between reeler and Tau::EGFP-mice that contain the EGFP gene in the Tau locus and hence all neurons are green fluorescent (Tucker et al., 2001; Heins et al., 2002). However, the number of neurons is identical in wild-type and reeler mutant cortex at E14 (Fig. 4B). Thus, neither the lack of reelin nor the decrease in Blbp and the decrease in pial attachment seems to exert a detectable effect on cell proliferation or the generation of neuronal progeny by radial glial cells.

Reelin signaling affects Blbp content of radial glial cells in vitro

To examine whether the observed reduction of Blbp in the reeler cortex is a direct consequence of the loss of the reelin protein, wild-type and reeler cells from E14 cortex were cultured in reelin-conditioned or control medium (Fig. 5A-D) collected from 293 cells stably transfected with a control or reelin-expression plasmid (see Materials and methods; Fig. 5E) (D'Arcangelo et al., 1997; Beffert et al., 2002; Förster et al., 2002). Note that reelin-antibodies detect the three characteristic bands of 400, 250-300 and 180 kDa on SDS-PAGE of the supernatant collected from reelin-expressing 293 cells (Fig. 5E) as has been described for partially purified reelin (Benhayon et al., 2003). Moreover, previous work showed no differences in Dab1 phosphorylation after application of purified reelin or 293 supernatant (Benhayon et al., 2003) (J.H., unpublished). When cells were cultured only for 2 hours in control- or reelin-conditioned medium no differences were detected in the number of Rc2- and Blbp-immunopositive cells (Fig. 5B). However, when cells were fixed after 24 hours in vitro, the number of Blbp-immunoreactive cells cultured in reelin-conditioned medium was significantly increased (P<0.01 compared with control medium; Fig. 5A-D). The same results were observed in chemically defined medium conditioned from control or reelin-expressing cells (Rc2/Blbp-immunoreactivity: control medium: 39±1%, n=299; reelin-conditioned medium 61±1%, n=312; P<0.01). Moreover, the number of Blbp-positive cells from reeler cortex remained significantly lower compared to wild-type cortex when cultured in control medium (P<0.01; Fig. 5B,C), but this difference disappeared after incubation in reelin-conditioned medium (Fig. 5B,C). These results demonstrate that reeler mutant cells that have never been in contact with reelin protein are still able to respond (see Howell et al., 1999). Indeed, the addition of reelin leads to a complete rescue of the decrease in Blbp-positive cells from reeler mutant cortex in vitro. These results further show that the amount of reelin released by MZ cells in cultures from wild-type cortex is limiting and that the higher content of reelin in the reelin-conditioned medium further increases the number of Blbp-immunoreactive cells even in wild-type cortex. Interestingly, however, cells from the GE and spinal cord, do not react to reelin in vitro (Fig. 5D), further supporting the specificity of the observed effects. To examine whether this upregulation occurs at the transcriptional level, we performed real-time RT-PCR (for details see Materials and methods). RNA was prepared from E14 cells

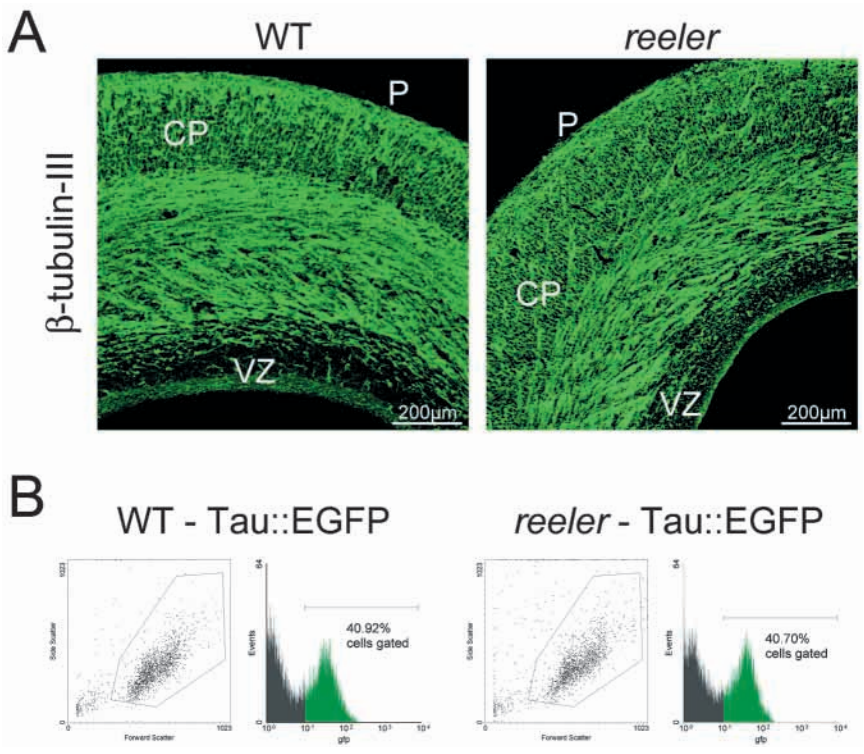

Fig. 4. Neuronal differentiation in reeler cortex. A shows confocal pictures (maximum intensity) of E16 frontal sections of wild-type and reeler cortex stained for the neuron-specific antigen β-tubulin-III. Note that the thickness of the cortical plate is comparable in wild-type and reeler cortex, while alterations in the organization of the cortical plate and fiber tracts are already visible. To detect small quantitative changes in the number of neurons, reeler mutant cells were crossed with Tau::EGFP mice that express GFP in neurons (Tucker et al., 2001). GFP-positive neurons were quantified at the fluorescent-activated cell sorter (FACS) as depicted in B in examples of sort profiles of a wild-type-Tau::GFP and a reeler-Tau::GFP cortex at E14. Left histograms show the dot plot of cells in forward scatter (FSC; x-axis) and side scatter (SSC; y-axis). The polygonal area is indicating the gated, i.e. analysed, healthy cells. The histograms on the right side show the number of events (x-axis) and the GFP-intensity (y-axis) and cells with green fluorescence above background are depicted in green. Note the identical number of green fluorescent neurons in wild-type and reeler cortex.

cultured in reelin-conditioned or control medium and reverse transcribed into cDNA. Blbp mRNA was highly increased (6.26×) in cortical cells cultured for 24 hours in reelin-conditioned compared with cells cultured in control medium.

However, the increase in Blbp-positive cells and mRNA could also result from a selective increase in the proliferation of the Blbp-expressing subpopulation of precursors. To test this possibility, cells were cultured in the presence of the DNA-base analogue BrdU in reelin-conditioned or control medium (see Materials and methods for details). However, the analysis of the labelling index (LI=BrdU+cells/Ki67+ precursors) (Hartfuss et al., 2001) of Rc2-single- and Rc2/Blbp-double-immunoreactive cells showed no increase in the proliferation of Blbp-positive cells in reelin-conditioned

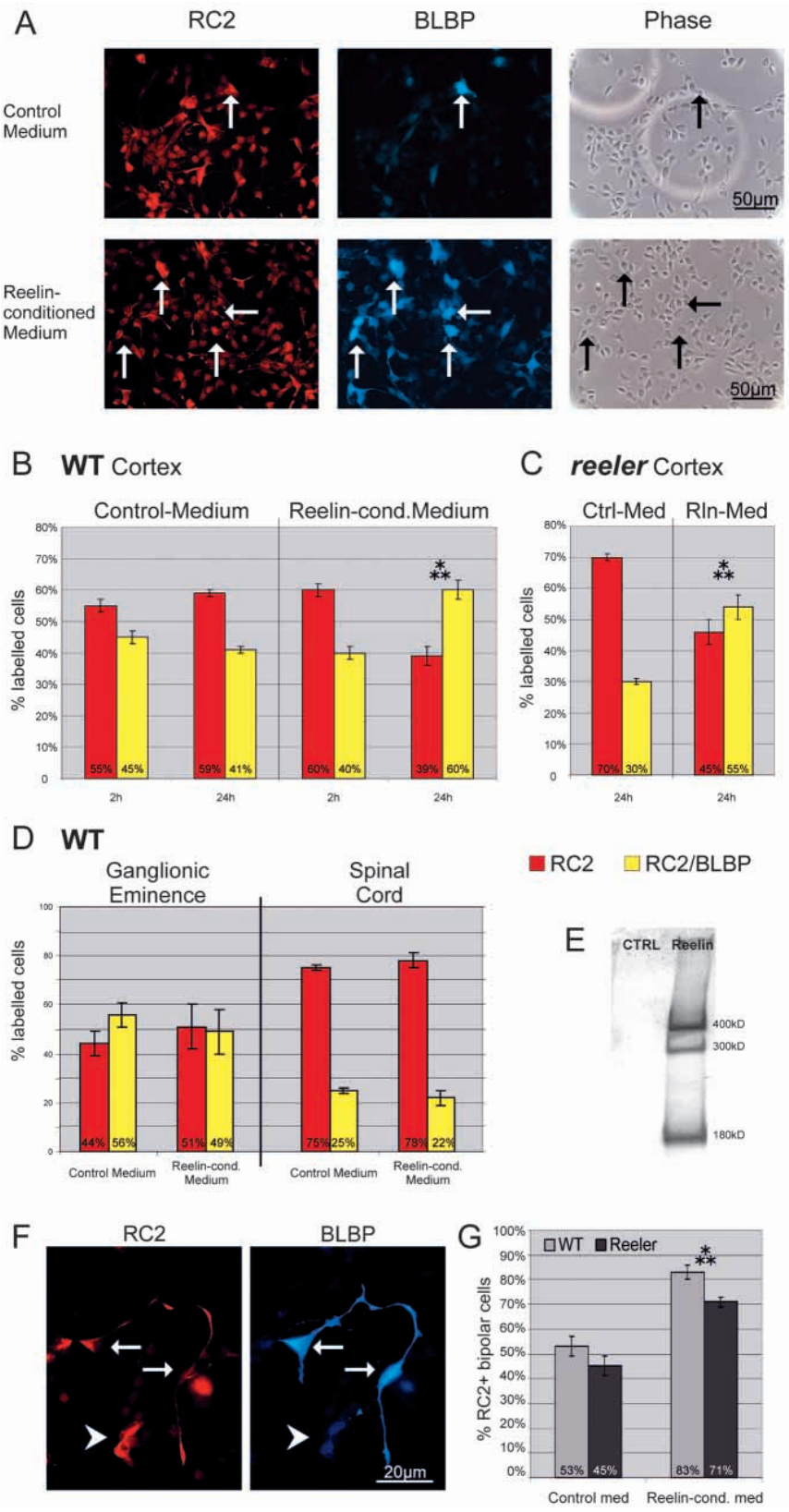

Fig. 5. Reelin signaling affects Blbp content and morphology of radial glial cells in vitro. (A) Fluorescent micrographs of Rc2- (red) and Blbp- (blue) immunoreactive cells dissociated from E14 cortex and cultured for 24 hours in control (top) or reelin-conditioned (bottom) medium. Note the increase in the number of Blbp-immunoreactive cells cultured in reelin-conditioned medium (middle bottom panel). Arrows indicate double-labelled cells. (B-D) Histograms of the percent of Rc2-only (red bars) or Rc2- and Blbp-immunoreactive cells (yellow bars) dissociated from different brain regions of E14 embryos cultured for 2 or 24 hours in control or reelin-conditioned medium. Note that the proportion of Blbp-immunoreactive cells significantly increases (Student's *t*-test, ***P<0.01) after a 24 hour exposure to reelin-conditioned medium. This increase also occurred in cells from reeler cortex to the same number of Blbp-positive cells as induced in wild-type cortex, suggesting that the decrease in Blbp-positive cells of the reeler cortex can be fully rescued by the addition of reelin. [Number of cells analysed: (B) 2 hours, *n*(Ctrl-med)=1652, *n*(Rln-med)=1523; (B) 24 hours *n*(Ctrl-med)=3478, *n*(Rln-med)=3457; (C) *n*(Ctrl-med)=1263; *n*(Rln-med)=1274; (D) *n*(GE)=973, *n*(SC)=886.] (E) Western blot of chemically defined culture medium conditioned for 48 hours by cells stably transfected with a control (CTRL) or reelin-expression plasmid (see D'Arcangelo et al., 1997; Beffert et al., 2002; Förster et al., 2002) showing a high amount of reelin in the reelin-conditioned, but not the control medium. (F,G) Morphological analysis of radial glial cells after exposure to reelin in vitro. (F) Two corresponding micrographs depicting examples of an Rc2-only positive precursor without processes (arrowheads) and two Rc2/Blbp-double-immunoreactive bipolar cells with processes longer than their cell soma after culturing in reelin-conditioned medium. The histograms in G depict the proportion of Rc2-positive cells from E14 wild-type (light gray) or reeler (dark gray) cortex with a bipolar morphology. Note that cells with bipolar morphology increase significantly (Student's *t*-test, ***P<0.01) after exposure to reelin-conditioned medium for 24 hours. [Number of cells analysed: Ctrl-med, *n*(wild type)=429, *n*(Rln)=204; Rln-med, *n*(wild type)=396, *n*(Rln)=232.]

medium, either when BrdU was present in the medium for 24 hours [control LI Rc2 0.89±0.05, LI Rc2/Blbp 0.96±0.03 (n=607); Rln-medium LI Rc2 0.93±0.1, LI Rc2/Blbp 0.83±0.04 (n=617)] or when BrdU was added for only 1 hour [S-phase labelling control LI Rc2 0.37±0.17, LI Rc2/Blbp 0.45±0.09 (n=595); Rln-medium LI Rc2 0.47±0.12, LI Rc2/Blbp 0.53±0.17 (n=603)]. Moreover, we could not detect any significant effect of reelin on neuronal differentiation and the overall population of precursors [β-tubulin-III-positive neurons after 24 hours in control medium: 53±5% (n=411); in reelin-conditioned medium: 61±1% (n=425); P>0.2]. Taken together, these data show that the increase in Blbp-positive cells after addition of reelin is not due to alterations in cell division or differentiation but is caused by specific upregulation of Blbp protein and mRNA in radial glial cells that were formerly Blbp negative.

Reelin increases the length of radial glia processes in vitro

As we found a correlation between the decrease of Blbp and radial processes in radial glia of the reeler cerebral cortex, we next tested whether the addition of reelin in vitro is also able to influence the process extension of radial glial cells. When cells were cultured in control medium, about 50% of precursor cells had extended processes of more than a cell diameter length 24 hours after dissociation (Fig. 5F,G). The vast majority of these cells had a bipolar morphology and virtually no precursors acquired a multipolar morphology. Interestingly, the proportion of precursors with a bipolar morphology in vitro (53%) closely resembles the proportion of VZ cells with a long radial morphology labeled in vivo (57%). In reelin-conditioned medium, however, the number of cells with processes increased significantly to 80-90% (P<0.01) and no significant difference was detectable between precursors from wild-type or reeler mutant cortex (Fig. 5F,G), consistent with a full rescue of the deficits in process length of reeler mutant radial glia in vitro. Taken together, these results show that reelin addition affects Blbp-immunoreactivity and the extension of radial processes from radial glial cells.

Direct signaling of reelin to radial glial cells

To examine whether the upregulation of Blbp by reelin is mediated by direct signaling to radial glia or mediated indirectly via the neurons present in the cultures, we used two approaches to reduce the number of neurons. First we isolated the non-fluorescent cells from the E14 cortex of the Tau::EGFP mouse line that contains GFP specifically in all neurons (see above) (see Tucker et al., 2001; Heins et al., 2002). Selection of GFP-negative cells reduced the proportion of neurons from 57%±3 (n=767) in unselected cells to 25% β-tubulin-III-immunoreactive cells analyzed 2 hours after sorting and plating (n=123). Interestingly, the increase in Blbp-immunoreactive cells after reelin addition was not reduced but even higher in these cultures despite the reduced number of neurons (Fig. 6A′, compare to Fig. 5B). However, because a quarter of all cells were still neurons in these cultures, we further decreased their proportion by positively sorting radial glial cells from the hGFAP-GFP-mouse line that contains GFP in radial glial cells as described previously (Malatesta et al., 2000; Heins et al., 2002). When GFP-positive cells were isolated by FACS (Fig. 6B), sorted cells were almost all Rc2-positive and only 13%±7 (n=197) were β-tubulin-III-immunoreactive 2 hours after sorting. However, despite the strong reduction of neurons in these cultures Blbp-immunoreactivity could still be significantly (P<0.04) increased by reelin-conditioned medium (Fig. 6B′), strongly suggesting that Blbp upregulation is mediated by direct signaling to radial glial cells.

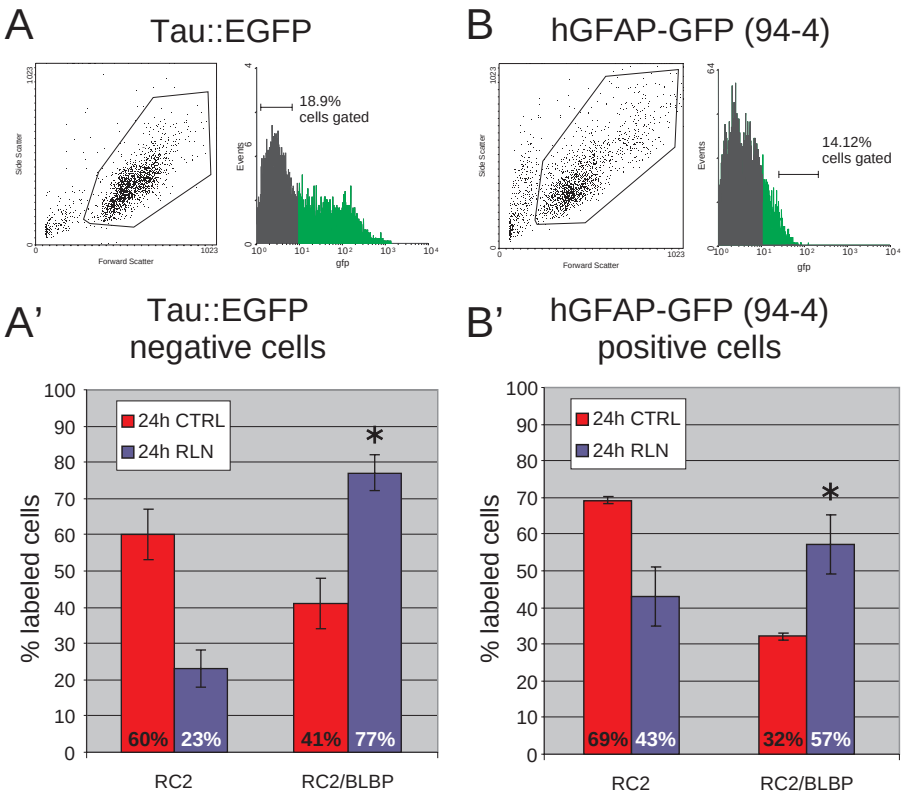

Fig. 6. Reelin signals directly to radial glial cells. (A,B) FACS profiles as described in Fig. 4 of two independent sorts selectively enriching for radial glial cells. In A, GFP-negative (black fraction in the histogram) precursor cells were isolated from the Tau::EGFP-mouse mouse line containing GFP in postmitotic neurons (Tucker et al., 2001); in B, GFP-positive cells (green fraction in the histogram) were isolated from the hGFAP-GFP mouse line with GFP in radial glia (Zhuo et al., 1997; Malatesta et al., 2000). Histograms in A′ and B′ depict the proportion of Rc2-only or Rc2- and Blbp-immunopositive cells cultured after the sorting in control (red bars) or reelin-conditioned (blue bars) medium for 24 hours. Note the significant (Student's t-test,*P<0.04) increase in Blbp-immunoreactive radial glia after exposure to reelin-conditioned medium, indicating that the presence of neurons is not required to mediate this effect. [Number of cell analysed: A, n(Ctrl)=237, n(Rln-med.)=354; B, n(Ctrl)=215, n(Rln-med.)=367.]

Signaling of reelin to radial glia depends on Dab1

Most of the studies examining reelin signaling did not discriminate between signaling to neurons or precursors/radial glia. This raises the question of whether the same or a different signaling cascade mediates the effect of reelin onto radial glia. Previous work has shown that ventricular zone cells and radial glia of the embryonic cortex contain Apoer2 and Vldlr (Benhayon et al., 2003; Luque et al., 2003). However, it is conceivable that cells lose these receptors after trypsinisation and the cells cultured in vitro might not express these receptors. This is not the case, as Apoer2 and Vldlr immunoreactivity were detected in cells isolated from the E14 cortex after 2 (data not shown) or 24 hours (Fig. 7A; data not shown). Reelin receptors were colocalized with radial glial markers (Fig. 7A) and neuronal markers (data not shown). Recent experiments have shown that reelin binds more strongly to Apoer2 than to Vldlr, and (consistent with this) *Apoer2*-deficient mice exhibit stronger defects in the cerebral cortex (Benhayon et al., 2003; Weiss et al., 2003). To detect whether there are also differences in expression levels of these receptors on radial glia or neurons, we performed real time RT-PCR with RNA isolated from sorted radial glia (EGFP-positive cells in the E14 cortex of hGFAP-EGFP mice) (Malatesta et al., 2003) or sorted neurons (EGFP-positive cells from E14 cortex of Tau::EGFP mice) (Heins et al., 2002). Interestingly, we found similar levels of Apoer2 mRNA in radial glia and in neurons, while Vldlr was expressed at 10-fold higher levels in neurons than in radial glia (Fig. 7B). Taken together, these data further add to the recent evidence that radial glial cells contain Apoer2 and Vldlr receptors as well as the adaptor protein Dab1 (Förster et al., 2002; Magdaleno et al., 2002; Benhayon et al., 2003; Luque et al., 2003).

We therefore examined next whether this signaling pathway is really crucial for the effects observed here (the reelin-mediated increase of Blbp in radial glia). To achieve this, we compared the number of Blbp-immunoreactive cells from *Dab1*$^{-/-}$ mice (Howell et al., 1997) and their wild-type littermates in control and reelin-conditioned medium (Fig. 7C). Consistently, the addition of reelin to wild-type cortical cells resulted in a significant increase in the number of Blbp-positive cells (P<0.01), while this effect was absent in *Dab1*$^{-/-}$ cells isolated from littermates and cultured simultaneously (Fig. 7C). Thus, Dab1 is necessary to mediate the increase of Blbp upon reelin signaling to radial glial cells (see also Förster et al., 2002).

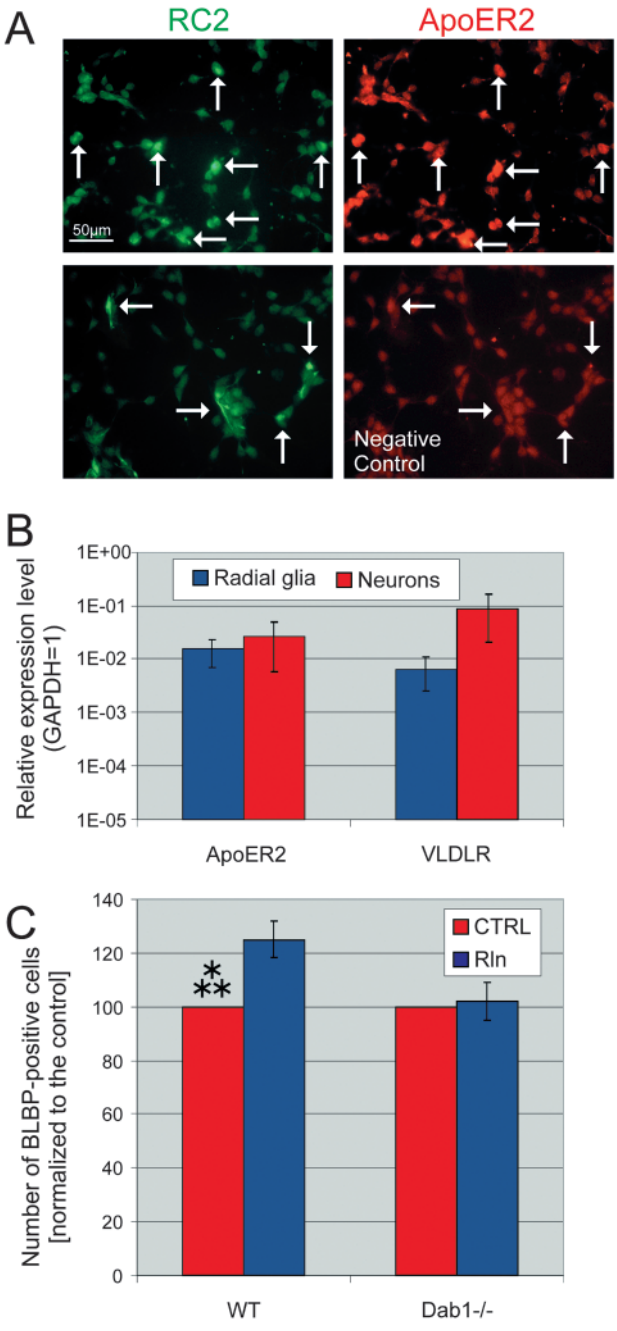

Fig. 7. Radial glial cells possess reelin receptors and require Dab1 for reelin-mediated signaling. (A) Corresponding fluorescent micrographs of cells isolated from E14 cortex cultured for 24 hours and stained with Rc2 (green) and anti-Apoer2 (red) as indicated in the panels. The lower panels show the absence of unspecific staining after omitting the primary Apoer2-antibody. Arrows indicate double-positive cells in the upper panels and single-positive (RC2-immunoreactive) cells in the lower panels. (B) The quantitative analysis of Apoer2 and Vldlr mRNA using light cycler quantitative RT-PCR with mRNA isolated from sorted radial glial cells [GFP-positive cells from E14 hGFAP-EGFP mice (Malatesta et al., 2003) and sorted neurons (EGFP-positive cells from E14 Tau::EGFP mice) (see Fig. 6A) (Heins et al., 2002)]. Note that radial glia and neurons contain comparable levels of *Apoer2* mRNA, while *Vldlr* mRNA is found at higher levels in neurons. (C) Quantitative analysis of Blbp-immunoreactive cells among E14 cortical cells from wild-type or *Dab1*$^{-/-}$ littermates cultured for 24 hours either in control or reelin-conditioned medium (compare Fig. 5). Note that the number of Blbp-positive cells increased upon reelin addition only in wild type but not in cells lacking Dab1, suggesting that Dab1 is required to mediate reelin signaling to radial glial cells. [Number of cells analysed: Ctrl, n(wild type or *Dab1*$^{+/-}$)=750, n(*Dab1*$^{-/-}$)=400; Rln-medium, n(wild type or *Dab1*$^{+/-}$)=700, n(*Dab1*$^{-/-}$)=300 from 12 different embryos.]

Discussion

Radial glial defects in the reeler mutant mouse

Our 3D-reconstruction of precursor morphology revealed that the majority of precursors in the VZ of the cortex possess long radial processes reaching towards the pial surface. These data

further support the view that radial glial cells represent the majority of VZ precursors, consistent with previous immunohistochemical (Hartfuss et al., 2001), FACS (Heins et al., 2002) and retroviral analyses (Noctor et al., 2002) (for a review, see Kriegstein and Götz, 2003). The number of cells with long radial processes is significantly decreased in the absence of reelin in the reeler mutant cortex, leading to a converse increase in precursors devoid of any basal process. These data are consistent and further extent earlier morphological analyses showing that the pial endfeet are less arborized and the glia limitans formed by these endfeet is partially disrupted in the reeler mutant cortex (Pinto-Lord et al., 1982; Hunter-Schaedle, 1997). A possible scenario might be that the decrease in adhesion and anchorage of radial glial endfeet to the basement membrane eventually leads to the retraction of radial glial processes. However, radial glial cells still have long radial processes ending about 50 μm below the pial surface in mouse mutants with defects in radial glia endfeet attachment (Graus-Porta et al., 2001; Halfter et al., 2002), suggesting that the complete process retraction is not a necessary consequence of pial endfeet detachment. Thus, process extension seems to occur independent from process attachment at the pial surface. Our data therefore imply reelin as a necessary factor for radial process elongation or maintenance, besides its apparent role in anchoring the radial glial endfeet. These data are also in close agreement with observations in the postnatal dentate gyrus where radial process formation is almost abolished in the reeler mice (Förster et al., 2002; Weiss et al., 2003).

Interestingly, the lack of reelin seems to affect only a subpopulation of radial glial cells, as some radial glial cells still manage to extend long radial processes also in the reeler mutant and establish contacts with the basement membrane forming a glia limitans that is only partially disrupted (N. Haubst and M.G., unpublished) (Gadisseux and Evrard, 1985). From the tight correlation between Blbp regulation and radial process extension that we observed in this work, we would hypothesize that the radial glial cells with normal process outgrowth and anchorage were those that still contain Blbp even in the absence of reelin, suggesting that reelin signaling is dispensable for some radial glia to express Blbp and elongate a radial process. Indeed, Blbp-positive radial glial cells in other CNS regions, such as the GE and the spinal cord, neither require reelin signaling, as their morphology and Blbp content were not affected in the reeler mice, nor react to reelin addition in vitro. This is in pronounced contrast to the significant Blbp upregulation and radial process extension evoked by reelin in cortical radial glia in vitro. These results therefore suggest that several pathways exist that regulate radial glia process extension and Blbp content. Indeed, a variety of mostly uncharacterized factors have been proposed to act on process extension of radial glial cells that are released either by neurons (Hunter and Hatten, 1995; Anton et al., 1997; Rio et al., 1997; Hasling et al., 2003) or by cells located in the MZ (Soriano et al., 1997; Super et al., 2000) in different brain regions. Because several of these factors also seem to act in the cerebral cortex, several signaling pathways apparently converge to regulate radial glia process extension, consistent with the loss of some, but not all, long radial processes in the reeler cortex. However, the clear defects of radial glia morphology in the reeler cerebral cortex and hippocampus (Förster et al., 2002; Weiss et al., 2003) underline the importance of this signaling pathway. These defects had previously been interpreted as premature transformation of radial glial cells in the reeler mutants (Hunter-Schaedle, 1997). Our data do not support this interpretation. First, the normal increase in Blbp during development in wild-type cortex is decreased from its onset in the reeler mutant cortex rather than occurring prematurely. Second, we did not observe the decrease in Rc2-immunoreactivity (see Fig. 1) or the premature increase in GFAP-immunoreactivity (data not shown) in reeler mutant radial glial cells described previously (Hunter-Schaedle, 1997). Moreover, the increase in precursors without any basally oriented radial process in the reeler mutant cortex described here is notably different from the bi- or multipolar glial precursors leaving the VZ as observed in mouse mutants with premature glial differentiation (Nieto et al., 2001). Similarly, the normal proliferation and neurogenesis as examined here in detail for the reeler cortex also does not support the notion of premature gliogenesis. Finally, reeler mutant mice exhibit also in the cerebellum several defects in glial differentiation, including the reduction of Glast (Ghandour et al., 1981; Benjelloun-Touimi et al., 1985; Fukaya et al., 1999). Taken together, these data suggest that reelin signaling is required for some aspects of radial glia and astrocyte differentiation.

Radial glia defects and their role as precursors

Importantly, the radial glia defects of reeler mutants differ from those of other mouse mutants, such as the *Pax6* mutant where Glast, but not Blbp, is downregulated (Götz et al., 1998; Heins et al., 2002). Reelin-secreting cells are even increased in number in the MZ of the *Pax6* mutant cortex (Stoykova et al., 2003). In contrast to the profound defects in the precursor function of radial glial cells in this cortex (Heins et al., 2002; Estivill-Torrus et al., 2002), no such defects were detected in the cortex of reeler mutant mice. The neurochemical and morphological defects of reelin-deficient radial glial cells occur in 50-15% of the precursor pool (Figs 1-3), but not even small changes in neurogenesis or cell proliferation could be detected implying that neither Blbp nor the long radial process is necessary for the precursor function of radial glial cells.

Radial glia defects and neuronal migration

In contrast to the normal cell fate and cell proliferation, radial glia defects are likely to contribute to the defects in radial cell migration observed in the reeler cortex. In line with this suggestion is the region-specific correlation of radial glial defects and aberrant radial migration in the reeler mutant mice. Indeed, radial glia morphology or Blbp content is normal in the spinal cord of reeler mice (data not shown) as is radial cell migration. Interestingly, only the tangential migration of preganglionic neurons is affected in the spinal cord of reelin-deficient mice (Yip et al., 2000; Phelps et al., 2002). Thus, the effect of reelin on tangential cell migration seems to be mediated in a radial glia independent manner, potentially by signaling directly to the migrating neurons. With regard to radial cell migration, however, the radial glia process seems essential both for glia-guided neuronal migration (Rakic, 1972), as well as the somal translocation of neurons generated from radial glia maintaining their basal processes (Morest, 1970; Miyata et al., 2001; Nadarajah et al., 2001). As the cortex constantly increases its thickness during development, both

modes of radial cell migration depend crucially on radial process extension. Notably, a recent model explains the defects in cortical layering of reeler mutants solely by the defects in radial process of neurons migrating by somal translocation (Luque et al., 2003).

Direct reelin signaling to radial glia

Our data suggest that these defects in radial glial cells are mediated by direct signaling of reelin to radial glial cells. We show that radial glial cells purified from the cerebral cortex react to reelin addition by upregulation of Blbp. The almost complete absence of neurons in vitro even enhanced this effect compared with cultures with many neurons. These data also argue against an additional indirect effect on Blbp mediated via neuronal signaling. Taken together with the early onset of the defect in Blbp in reeler mutant cortex, these data suggest that the radial glia phenotype in reeler mice is mostly due to the lack of direct signaling to radial glia, rather than some indirect effects, e.g. aberrant neuronal migration. It has been suggested previously that neuronal attachment to radial glia positively influences their Blbp content (Feng et al., 1994). However, the attachment of neurons to radial glia is rather increased in the reeler cortex (e.g. Caviness et al., 1988) and can thus not explain the decrease in Blbp-positive cells. Taken together, these results therefore favor a direct regulation of Blbp by reelin signaling to radial glia in vivo as demonstrated directly in vitro.

In vivo, reelin most probably acts on radial glia in the MZ where their processes are in close contact to the reelin-secreting cells (see Fig. 1). Because reelin-secreting cells are only a small subpopulation of cortical neurons, their secretion of reelin into the culture medium in vitro is negligible and hardly detectable in western blot analysis of the culture medium (data not shown). Accordingly, the increase of Blbp-positive cells during development in vivo (Hartfuss et al., 2001) is halted in vitro where no change in Blbp-positive cells can be observed during 24 hours. However, the high reelin content in medium conditioned by a reelin-secreting cell line results in Blbp upregulation and the extension of radial processes. This in vitro model might therefore mimic the situation in the MZ, where reelin concentrations are high, and might influence process extension or maintenance possibly via the regulation of Blbp. Consistent with this suggestion is the close correlation of the appearance of reelin- (Stoykova et al., 2003) and Blbp-immunoreactive cells in wild-type cortex (Hartfuss et al., 2001) at a time when the cortex starts to thicken and radial glia need to extend their processes. At postnatal stages, the transformation of radial glia into astrocytes coincides with the degeneration of reelin-secreting cells in layer 1 (Super and Uylings, 2001).

Experiments in which reelin is misexpressed in nestin-positive cells (most of which are radial glia as described above) further support the direct signaling of reelin to radial glial cells (Magdaleno et al., 2002). Reelin synthesis in VZ precursors is sufficient to rescue some, but not all, migrational defects of the reeler mutant cortex (Magdaleno et al., 2002). These data therefore suggest either that direct signaling to neurons is responsible for the effects not rescued in these transgenic mice, or that reelin has to be deposited in the MZ, either to be highly concentrated in the extracellular milieu present there or to provide a directional signal to radial glia, e.g. to stabilize their contact to the basement membrane. As we have shown that reelin signaling acts via the same pathway (via the adaptor protein Dab1) in radial glia as it does in neurons, one may think of experimentally addressing the relative contribution of reelin signaling to radial glia and neurons by, for example, a neuron-specific Cre-mediated knock-out of Dab1. However, when most neurons migrate by somal translocation (see Luque et al., 2003), this issue becomes semantic. In the cases when the basally generated neuron inherits the radial process from its mother radial glia (Miyata et al., 2001) and then migrates by somal translocation, reelin always signals to the same radial process, first the one of the radial glia mother cell, and later to the process of the differentiating and migrating neuron. However, recent experiments suggest that somal translocation might dominate only during early stages of cortical development (Nadarajah et al., 2001) and become less frequent at later stages (Weissman et al., 2003). The most crucial next experiment would therefore be to examine the mode of cell migration in the reeler mutant cortex, whether it is really predominantly somal translocation that is stalled in the absence of reelin (or Dab1). Taken together, these results shed new light on the migrational defects of the reeler mutation and suggest a crucial role of a direct signaling of reelin to radial glial cells.

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