Physiological Notch signaling promotes gliogenesis in the developing peripheral and central nervous systems

Merritt K. Taylor*, Kelly Yeager and Sean J. Morrison†

Constitutive activation of the Notch pathway can promote gliogenesis by peripheral (PNS) and central (CNS) nervous system progenitors. This raises the question of whether physiological Notch signaling regulates gliogenesis in vivo. To test this, we conditionally deleted Rbpsuh (Rbpj) from mouse PNS or CNS progenitors using Wnt1-Cre or Nestin-Cre. Rbpsuh encodes a DNA-binding protein (RBP/J) that is required for canonical signaling by all Notch receptors. In most regions of the developing PNS and spinal cord, Rbpsuh deletion caused only mild defects in neurogenesis, but severe defects in gliogenesis. These resulted from defects in glial specification or differentiation, not premature depletion of neural progenitors, because we were able to culture undifferentiated progenitors from the PNS and spinal cord despite their failure to form glia in vivo. In spinal cord progenitors, Rbpsuh was required to maintain Sox9 expression during gliogenesis, demonstrating that Notch signaling promotes the expression of a glial-specific gene. These results demonstrate that physiological Notch signaling is required for gliogenesis in vivo, independent of the role of Notch in the maintenance of undifferentiated neural progenitors.

KEY WORDS: Notch, Gliogenesis, Neural stem cells, Neural crest, Central nervous system, Spinal cord, Mouse

INTRODUCTION

A fundamental question in developmental neurobiology concerns the mechanisms that regulate the transition from neurogenesis to gliogenesis in vertebrates. Notch signaling regulates binary fate decisions during neural development (Artavanis-Tsakonas et al., 1999; Harris, 1997; Morrison, 2001). However, genetic analysis of the necessity of Notch signaling during neural development in Drosophila suggests a complex and context-dependent role (Umesono et al., 2002). In some lineages, Notch signaling promotes gliogenesis, including promoting the expression of gliogenic genes such as gcm (Udolph et al., 2001). In other lineages, Notch signaling promotes neurogenesis by inhibiting the expression of gcm (Van De Bor and Gianandrè, 2001).

Notch signaling in vertebrates also has complex and context-dependent effects, although overexpression of Notch pathway components either promotes gliogenesis or the maintenance of undifferentiated progenitors. Multiple Notch receptors and ligands are expressed throughout the developing peripheral (PNS) and central (CNS) nervous systems (Lindsell et al., 1996; Williams et al., 1995). Overexpression of activated Notch1, or its downstream transcriptional effectors Hes1 or Hes5, promotes gliogenesis in the retina (Furukawa et al., 2000; Hojo et al., 2000; Scheer et al., 2001). Overexpression of activated Notch1 promotes the formation of radial glia in the telencephalon (Gaiano et al., 2000; Yoon et al., 2004), and instructs adult hippocampus progenitors to become astrocytes in culture (Tanigaki et al., 2001). In the developing PNS, the Notch ligand delta-like 1 (Dll1) instructs neural crest stem cells (NCSCs) in culture to undergo gliogenesis (Kubu et al., 2002; Morrison et al., 2000). In this case, Notch signaling does not simply inhibit neurogenesis, because even transient exposure to Dll1 causes an irreversible commitment to glial differentiation (Morrison et al., 2000).

Consistent with the idea that Notch can send a positive signal that promotes glial lineage determination in vertebrates, RBP/J (a DNA-binding protein that interacts with the intracellular domain of activated Notch to regulate transcription) can directly bind the promoters of glial genes and activate transcription (Anthony et al., 2005; Ge et al., 2002; Tanigaki et al., 2001). Although these studies indicate that increased Notch signaling can promote gliogenesis, virtually all of these data were obtained in gain-of-function and/or in vitro analyses, raising the question of whether physiological Notch signaling also regulates gliogenesis in vivo.

Overexpression of Notch pathway components can sometimes overestimate the physiological role of Notch signaling. Overexpression of Notch3 promotes astrocyte differentiation from adult hippocampus progenitors (Tanigaki et al., 2001) despite the lack of obvious neural phenotypes in Notch3-deficient mice (Kreb's et al., 2003). A number of studies have reported effects of Notch1 (Bigas et al., 1998; Carlesso et al., 1999; Milner et al., 1996; Stier et al., 2002; Varnum-Finney et al., 2000) or jagged 1 (Jag1 – Mouse Genome Informatics) (Jones et al., 1998; Karanu et al., 2000; Varnum-Finney et al., 1998) overexpression on hematopoietic cell self-renewal and differentiation, but when these genes were conditionally deleted from mice there was no effect on hematopoietic stem cell frequency or function (Mancini et al., 2005). Furthermore, when the Notch pathway is activated in cultured progenitors, it is possible that unphysiological aspects of the culture environment might lead to outcomes that would not be observed in vivo. For all of these reasons, it is crucial to determine whether Notch signaling is necessary for gliogenesis in vivo in order to understand its physiological role in neural development.

The presence of four Notch receptors and at least five Notch ligands in mammals has made it difficult to test what aspects of neural development are regulated by Notch in vivo. Deletion of Notch1 (Swiatek et al., 1994), Notch2 (Hamada et al., 1999), Jag1 (Xue et al., 1999), Dll1 (Hrabe de Angelis et al., 1997) or delta-like 4 (Dll4 – Mouse Genome Informatics) (Kreb's et al., 2004) leads to
severe developmental defects and the death of mouse embryos prior to embryonic day (E) 11.5, before there is an opportunity to study the effects of these mutations on gliogenesis. By contrast, deletion of Notch3 (Krebs et al., 2003), Notch4 (Krebs et al., 2000), delta-like 3 (Dll3 – Mouse Genome Informatics) (Dunwoodie et al., 2002), or jagged 2 (Jag2 – Mouse Genome Informatics) (Jiang et al., 1998) leads to milder phenotypes. Some receptors/ligands may have little physiological function in vivo (Krebs et al., 2003). In other cases, the overlapping expression of multiple receptors and ligands may lead to functional redundancy in vivo (Krebs et al., 2000). As a result, it has been difficult to assess whether Notch signaling plays a physiological role in many aspects of neural development.

Loss-of-function experiments indicate that physiological Notch signaling regulates CNS progenitor maintenance. Premature neuronal differentiation occurs in embryos deficient for Notch1 or Rbpsuh (Rbp – Mouse Genome Informatics), the gene that encodes RBP/J (de la Pompa et al., 1997). RBP/J interacts with the intracellular domains of all four Notch receptors and is required to mediate their transcriptional effects (Kato et al., 1996; Kato et al., 1997). Deletion of Rbpsuh thus abolishes canonical Notch signaling. Notch1- or Rbpsuh-deficient embryos also have many fewer CNS stem cells (Hitoshi et al., 2002). Deletion of Hes1 and Hes5, Notch target genes that act downstream of RBP/J (Ohtsuka et al., 1999), leads to a loss of neuroepithelial cells and premature neuronal differentiation in the spinal cord (Hatakeyama et al., 2004), as well as to anatomical defects in cranial nerves and sensory ganglia (Hatakeyama et al., 2006). Conditional deletion of Notch1 in the cerebellum leads to premature neuronal differentiation and a subsequent reduction in gliogenesis (Lutolf et al., 2002). Neural progenitors cultured from Deltal-deficient embryos also exhibit increased neurogenesis and defects in gliogenesis (Grandbarbe et al., 2003). Deletion of Notch1 and Notch3 from forebrain progenitors reduced brain fatty acid-binding protein (BFABP; Fabp7 – Mouse Genome Informatics) expression in vivo, but it was uncertain whether this reflected reduced gliogenesis or just reduced levels of FABP expression in progenitors and differentiated cells (Anthony et al., 2005). It is not clear from these observations whether Notch acts at multiple stages of neural development, first to maintain undifferentiated progenitors and subsequently to promote gliogenesis, or whether the defects in gliogenesis are secondary to a premature depletion of undifferentiated progenitors.

To examine this we generated Wnt1-CreRbpsuh[fl/fl] mice to conditionally delete Rbpsuh from neural crest cells. Wnt1-Cre induces efficient recombination throughout cephalic and trunk neural crest cells (Chai et al., 2000; Hari et al., 2002; Jiang et al., 2000; Joseph et al., 2004; Zirlinger et al., 2002). The Rbpsuh[fl/fl] mice were previously generated and shown to permit conditional deletion and loss of Rbpsuh function (Han et al., 2002; Tanigaki et al., 2002; Tanigaki et al., 2004). We observed that conditional deletion of Rbpsuh from neural crest cells had only minor effects on neurogenesis, but severely reduced gliogenesis throughout most of the PNS. We observed a reduction in the number of NCSCs in some regions of the PNS in the absence of Rbpsuh, indicating that Notch signaling does play a role in the maintenance of NCSCs in at least some locations. However, at least some NCSCs remained present throughout all regions of the late gestation PNS. The severe reduction in gliogenesis despite the ongoing presence of undifferentiated NCSCs suggests that physiological Notch signaling is required to promote gliogenesis beyond simply maintaining undifferentiated neural progenitors. We also examined the neural tubes of Nestin-CreRbpsuh[fl/fl] mice. Nestin-Cre conditionally deletes genes in neuroectodermal progenitors in the developing CNS, including within the neural tube (Tronche et al., 1999; Yang et al., 2006). We again detected little effect of Rbpsuh deletion on the numbers of neurons that formed, but profound effects on gliogenesis including significantly fewer astrocytes and significantly more oligodendrocytes. These data demonstrate that physiological Notch signaling promotes gliogenesis in the developing CNS.

MATERIALS AND METHODS

Mice

Mice were housed in the Unit for Laboratory Animal Medicine at the University of Michigan according to University Committee on the Use and Care of Animals guidelines. For genotyping, the following primers were used on genomic DNA isolated from mouse tissue. For detection of the Cre gene in crosses involving Wnt1-Cre or Nestin-Cre mice: Cre F, 5′-ATTGCTGTACCTGGTCGTGG-3′ and Cre R, 5′-GAAAATGCT-TCTGTCCGGTGC-3′, yielding a 210 bp product. For detection of the wild-type Rbpsuh allele: Rbpsuh wt F, 5′-GTTCTAAAGCTTG- GTCCCCGAG-3′ and Rbpsuh wt R, 5′-GCTGAGGTTGTACCTTGCAG-3′, yielding a 487 bp PCR product. For detection of the floxed Rbpsuh allele: Rbpsuh fl F, 5′-GCAATCCATCTTGCCATCGC-3′ and Rbpsuh fl R, 5′-GAAGGTCCTTGGTACACAGATAGC-3′, yielding a 598 bp PCR product.

Immunohistochemistry and tissue preparation

To examine BrdU incorporation, pregnant dams were injected with 50 μg/g of BrdU (Sigma, St Louis, MO) and sacrificed 30 minutes later. Embryos were immediately dissected and fixed in 4% paraformaldehyde overnight at 4°C. For some markers (antibodies against Pdgf and Sox10), it was necessary to fix the embryos for 2.5 hours at 4°C. The embryos were then washed in PBS, cryoprotected in 15% sucrose and mounted in Tissue-Tek OCT (VWR, West Chester, PA) prior to snap-freezing and sectioning. Tissue sections (12 μm) were collected using a Leica cryostat.

For immunohistochemistry, tissue sections were blocked in modified GSS (PBS containing 5% goat serum and 0.5% Triton X-100). Primary antibodies were diluted in modified GSS and incubated with the sections overnight at 4°C, followed by washing then secondary antibody incubation for 1 hour at room temperature. Antibodies included those against TuJ1 (Tubb3 – Mouse Genome Informatics) (Covance, Berkeley, CA, MMS-435P, 1:1000), BFABP (gift from T. Muller, Max-Delbrück-Center, Berlin, Germany; 1:2000), activated caspase 3 (BD Pharmingen, San Diego, CA, 559565, 1:1000), Brdu (gift from B. Novitch, University of Michigan, Ann Arbor, MI; 1:2000), p75 (p75NTR – Mouse Genome Informatics) (Chemicon, Temecula, CA, MAB377, 1:1000), Olig2 (gift from B. Novitch, University of Michigan, Ann Arbor, MI; 1:2000), and Sox10 (gift from D. Anderson, California Institute of Technology, Pasadena, CA; 1:50), NeuN (NeuN60 – Mouse Genome Informatics) (Chemicon, Temecula, CA, MAB377, 1:1000), Olig2 (gift from B. Novitch, University of Michigan, Ann Arbor, MI; 1:2000), p75 (p75NTR – Mouse Genome Informatics) (Chemicon, AB1544, 1:5000) and nestin (BD Pharmingen, 611658, 1:1000). Slides were counterstained in 2.5 μg/ml DAPI for 10 minutes at room temperature, then mounted using ProLong antifade solution (Molecular Probes, Eugene, OR).

The in situ method for detection of Mhp (probe was a gift from A. Gowan, Wayne State University, Detroit, MI) was adapted from that of White and Anderson (White and Anderson, 1999).

Whole-mount immunohistochemistry

E9.5 embryos were fixed overnight in 4% paraformaldehyde, then bleached with 5:1 PBS:30% H2O2 at room temperature for 3-5 hours. Embryos were washed in PBS before blocking in two washes of PBS block (PBS containing 5% goat serum, 0.2% Triton X-100, 1% DMSO and 0.5% BSA) for 1 hour each wash. Embryos were incubated overnight with 1:50 dilution of anti-Sox10 antibody (Chemicon AB5804, 1:200), Sox10 (gift from D. Anderson, California Institute of Technology, Pasadena, CA; 1:50), NeuN (NeuN60 – Mouse Genome Informatics) (Chemicon, Temecula, CA, MAB377, 1:1000), Olig2 (gift from B. Novitch, University of Michigan, Ann Arbor, MI; 1:2000), and Sox10 (gift from D. Anderson, California Institute of Technology, Pasadena, CA; 1:50). Embryos were incubated overnight with 1:100 dilution of anti-Sox10 antibody overnight at 4°C. Embryos were washed five times in PBS block for 1 hour per wash and incubated with goat anti-rabbit peroxidase (Vector laboratories, Burlingame, CA, PI-1000, 1:200) overnight at 4°C, and then washed again five times in PBS block for 1 hour each wash. The embryos were washed in acetate imidazole buffer (175 mM sodium acetate, 10 mM imidazole, pH 7.2 with 30% glacial acetic acid) three times for 1 hour each wash and then incubated in Ni-DAB (125 mM sodium acetate, 10 mM imidazole, 100 mM NiSO4, 0.3 mg/ml DAB) for 20 minutes.
H$_2$O$_2$ (0.0003%) was added and the embryo incubated at room temperature for 5-10 minutes to form the deposition product. The embryos were then washed, dehydrated in a reverse series of methanol dehydration steps and stored in 100% methanol until photographs could be taken.

**Isolation of neural tissue**

PNS tissues (DRG, sympathetic chain and gut) were dissected from E13.5 mouse embryos and collected in ice-cold D-PBS buffer. The cells were then dissociated in 0.025% trypsin/EDTA (Invitrogen 25300-054) plus 1 mg/ml type-4 collagenase ( Worthington, Lakewood, NJ, #4186) in Ca and Mg-free HBSS (Invitrogen, #14175-095) at 37°C for 4 minutes. The dissociation was quenched with staining medium (L15 containing 1 mg/ml BSA (Sigma, A-3912), 10 mM HEPES at pH 7.4, 1% pen/strep (BioWhittaker, Rockland, ME)) that contained 25 mg/ml deoxyribonuclease type 1 (Sigma, D-4527).

Cells were filtered through a nylon screen (45 μm, Sefar America, Depew, NY) with the exception of sympathetic chain, for which filtration was not necessary. Before adding to culture, cells were resuspended in staining medium and counted using a hemocytometer to determine cell viability, density and to ensure complete dissociation.

CNS tissue was dissected from the E19.5 embryonic upper thoracic spinal cord into ice-cold D-PBS buffer. The cells were then dissociated with 0.025% trypsin/EDTA in Ca and Mg-free HBSS at 37°C for 2 minutes. Dissociation was terminated with staining medium and the tissue was lightly triturated before being filtered through a nylon mesh and resuspended in fresh staining medium. Cell density and viability were determined by counting cells in Trypan Blue with a hemocytometer before culturing.

**Tissue culture**

The culture medium was a 5:3 mixture of DMEM-low glucose/neurobasal medium (Invitrogen, Carlsbad, CA) supplemented with 20 ng/ml human basic FGF (R&D Systems, Minneapolis, MN; #233-FB), 1% N2 (Invitrogen), 2% B27 (Invitrogen), 50 μM 2-mercaptoethanol, and 1% pen/strep (Biowhitaker). CNS cultures also contained 20 ng/ml human EGF (R&D Systems, #236-EG)) and 10% chick embryo extract (preparred as described by Stemple and Anderson (Stemple and Anderson, 1992)). PNS culture medium also contained 15% chick embryo extract, 35 ng/ml (110 nM) retinoic acid (Sigma) and 20 ng/ml human IGF1 (R&D Systems, #291-G1). All cultures were maintained at 37°C in 6% CO2/balance air. For differentiation, adherent neurospheres were fixed and stained as described subsequently for lineage determination.

In an attempt to distinguish between these possibilities, we cultured gut NCSCs from Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ mice and littermate controls to examine the differentiation of these cells. However, the infrequent NCSC colonies that arose in culture from Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ mice consistently retained at least one uncombined Rbpsuh allele. This supported the idea that Notch signaling was required for the maintenance of gut NCSCs, but prevented us from examining the consequences of Rbpsuh deficiency on gut NCSC differentiation in culture.

**RESULTS**

Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ mice formed normal numbers of neural crest cells that appeared to migrate normally compared with littermate controls (see Fig. S1 in the supplementary material). Although germline deletion of Dll1 leads to defects in neural crest formation and migration (De Bellard et al., 2002; Hrabe de Angelis et al., 1997), this appears to be at least partially attributable to defects in somite formation. Since Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ mice appear to have normal somites (see Fig. S1D in the supplementary material), defects in migration might not be expected in these mice.

To begin to examine the effect of Rbpsuh deletion on PNS development, we examined the numbers of migrating neural crest cells (p75$^+$) that colonized the gut, as well as the numbers of neurons (TuJ1$^+$) and glia (BFABP$^+$) in the foregut, midgut and hindgut that arose from these migrating neural crest cells. We did not detect any difference between Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ mice and littermate controls in the number of p75$^+$ neural crest progenitors that migrated through the gut at E10.5 (Fig. 1C). At E10.5, we also did not detect any statistically significant differences in neurogenesis (gliogenesis was not detected at this point) (Fig. 1D). By E14.5, however, clear differences had emerged throughout the gut, with Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ embryos exhibiting significantly fewer neurons and glia per section, relative to littermate controls (Fig. 1E). The difference in glia at E14.5 was particularly profound, with virtually no glia observed in Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ guts. The significant reduction in the numbers of neurons and glia per section in Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ guts persisted through E18.5 (Fig. 1F). We were not able to determine the effect of Rbpsuh deletion on postnatal gut development because the mice died within hours of birth.

We did not detect any difference in the rate of proliferation of p75$^+$ cells (Fig. 1G) or the number of activated caspase-3$^+$ cells undergoing cell death (not shown) in Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ guts as compared with littermate controls. However, Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ guts did have an approximately threefold reduction in the frequency of NCSCs that formed multilineage colonies in culture as compared with control embryos (Fig. 1H). This difference was not rescued by addition of the giogianic factor neuregulin-1-β1 (Nrg) to the cultures (Fig. 1H). These data demonstrate that Rbpsuh is required to maintain normal numbers of NCSCs and to generate normal numbers of neurons and glia throughout the gut, although these data do not distinguish whether Rbpsuh is only required for the maintenance of undifferentiated progenitors or is also required subsequently for lineage determination.

To begin to examine the effect of Rbpsuh deletion on neural crest migration, we examined the numbers of migrating neural crest cells (p75$^+$) that colonized the gut, as well as the numbers of neurons (TuJ1$^+$) and glia (BFABP$^+$) in the foregut, midgut and hindgut that arose from these migrating neural crest cells. We did not detect any difference between Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ mice and littermate controls in the number of p75$^+$ neural crest progenitors that migrated through the gut at E10.5 (Fig. 1C). At E10.5, we also detected no evidence of premature neuronal differentiation in Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ guts as compared with control embryos. However, Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ guts did have an approximately threefold reduction in the frequency of NCSCs that formed multilineage colonies in culture as compared with control embryos (Fig. 1H). This difference was not rescued by addition of the giogianic factor neuregulin-1-β1 (Nrg) to the cultures (Fig. 1H). These data demonstrate that Rbpsuh is required to maintain normal numbers of NCSCs and to generate normal numbers of neurons and glia throughout the gut, although these data do not distinguish whether Rbpsuh is only required for the maintenance of undifferentiated progenitors or is also required subsequently for lineage determination.

In an attempt to distinguish between these possibilities, we cultured gut NCSCs from Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ mice and littermate controls to examine the differentiation of these cells. However, the infrequent NCSC colonies that arose in culture from Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ mice consistently retained at least one uncombined Rbpsuh allele. This supported the idea that Notch signaling was required for the maintenance of gut NCSCs, but prevented us from examining the consequences of Rbpsuh deficiency on gut NCSC differentiation in culture.

**Rbpsuh deletion leads to profound defects in gliogenesis in sensory ganglia**

To gain further insight into the role of canonical Notch signaling in PNS development, we examined the consequences of Rbpsuh deletion in developing sensory (dorsal root) ganglia. At E10.5, there was no difference between Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ embryos and littermate controls in the number of p75$^+$ neural crest cells per section (Fig. 2E) or TuJ1$^+$ neurons per section (Fig. 2F). We thus detected no evidence of premature neuronal differentiation in sensory ganglia of Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ embryos based on either TuJ1 (Fig. 2F) or peripherin (data not shown) staining. Significantly less neurogenesis was observed at later stages of development in Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ ganglia, which had approximately half as many neurons per section as control ganglia at E14.5 and one third as many neurons per section at E18.5 (Fig. 2F). In contrast to this modest reduction in neurogenesis, there was a profound reduction in gliogenesis, with almost no BFABP$^+$ glia in Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ sensory ganglia between E13.5 and E18.5 (Fig. 2A-F), demonstrating that virtually no gliogenesis occurred in Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$ embryos. This reflected a difference in gliogenesis, not just a difference in BFABP expression, as Wnt1-Cre$^\text{Rbpsuh}^{fl/fl}$
Development 134 (13)

Fig. 1. The numbers of NCSCs, neurons and glia are reduced in the gut after Rbpsuh deletion. (A, B) Transverse sections of the guts from E13.5 control (A) or Wnt1-Cre<sup>+</sup> Rbpsuh<sup>fl/fl</sup> (B) mouse embryos were stained for neurons (TuJ1) and glia (BFABP). Scalebar: 10 μm. (C) The numbers of p75<sup>+</sup> neural crest cells that initially colonized the gut did not differ between E10.5 control and Wnt1-Cre<sup>+</sup> Rbpsuh<sup>fl/fl</sup> embryos (the number of p75<sup>+</sup> cells per section; three sections per gut region per mouse). (D-F) At representative levels of the developing gut (foregut, midgut and hindgut), the numbers of neurons (TuJ1<sup>+</sup>) and glia (BFABP<sup>+</sup>) per section were similar to wild type in Wnt1-Cre<sup>+</sup> Rbpsuh<sup>fl/fl</sup> embryos at E10.5 (D) but significantly (P<0.05) lower at E14.5 (E) and E18.5 (F). (G) There was no difference in the rate of proliferation of p75<sup>+</sup> cells at E14.5 between control and mutant embryos (BrdU was administered for 30 minutes; three to seven sections per gut region per mouse, three to four mice per genotype). Additionally, there was no significant difference in the rate of cell death between control and mutant mice at any stage of development (E10.5, E14.5 and E18.5) or at any level of the gut (foregut, midgut and hindgut) based on staining for activated caspase 3 (data not shown). (H) However, a significantly (P<0.05) lower percentage of cells from E13.5 Wnt1-Cre<sup>+</sup> Rbpsuh<sup>fl/fl</sup> guts gave rise to multilineage NCSC colonies in clonal-density cultures in both standard medium and medium supplemented with neuregulin (+Nrg) (three to seven independent experiments). All error bars indicate s.d.

sensory ganglia also exhibited a similar reduction in S100β<sup>+</sup> cells (see Fig. S2 in the supplementary material). This difference in gliogenesis did not reflect differences in proliferation or cell death, as we did not observe any differences in the rate of proliferation (Fig. 2G) or the frequency of apoptotic cells (Fig. 2H) in sensory ganglia from Wnt1-Cre<sup>+</sup> Rbpsuh<sup>fl/fl</sup> and control embryos. These data suggest that neural crest progenitors were either prematurely depleted prior to the onset of gliogenesis or that they were unable to undergo gliogenesis in vivo.

We cultured dissociated E13.5 dorsal root ganglion cells at clonal density to test whether NCSCs or other neural crest progenitors persisted after Rbpsuh deletion. Under standard conditions, we observed significantly (P<0.05) fewer multilineage colonies and significantly fewer colonies of all types that contained Gfap<sup>+</sup> glia (G-containing; these included multilineage colonies, glia-only colonies and other colonies) from Wnt1-Cre<sup>+</sup> Rbpsuh<sup>fl/fl</sup> embryos (Fig. 3A). In contrast to this reduction in gliogenesis, Wnt1-Cre<sup>+</sup> Rbpsuh<sup>fl/fl</sup> progenitors formed normal frequencies of myofibroblast-containing colonies and neuron-containing colonies (Fig. 3A).

To test whether the paucity of glia-containing colonies from Wnt1-Cre<sup>+</sup> Rbpsuh<sup>fl/fl</sup> embryos reflected a defect in gliogenesis or a depletion of progenitors, we added the gliogenic factor Nrg to the culture medium to ascertain whether stimulation by Nrg might rescue gliogenesis by Rbpsuh-deficient progenitors. In the presence of Nrg, we observed normal numbers of glia-containing colonies (Fig. 3B). The increase in glial colonies and the decreases in neuron-containing and myofibroblast-containing colonies in the presence of Nrg is likely to reflect increased survival by glial progenitors, as well as increased gliogenesis at the expense of neurogenesis and myogenesis by uncommitted progenitors; Nrg promotes both survival and glial lineage determination by neural crest progenitors (Dong et al., 1995; Morrison et al., 1999; Shah et al., 1994). Upon genotyping individual colonies, Rbpsuh excision was extensive but variable. In some experiments, all colonies exhibited complete Rbpsuh excision, but on average, 65% of colonies exhibited a complete loss of Rbpsuh. Rbpsuh expression levels in freshly dissected sensory ganglia from Wnt1-Cre<sup>+</sup> Rbpsuh<sup>fl/fl</sup> embryos were only 23±12% of wild-type levels by qRT-PCR (data not shown).

We thus found no evidence for a depletion of neural crest progenitors with glial potential in sensory ganglia after Rbpsuh deletion, although it is possible that the late-onset reduction in neurogenesis reflects a reduction in the second wave of neurogenic progenitors that form nociceptive neurons in sensory ganglia. Nonetheless, these data demonstrate that neural crest progenitors with glial potential persist at least through E13.5 in the sensory ganglia of Wnt1-Cre<sup>+</sup> Rbpsuh<sup>fl/fl</sup> embryos. The failure of these progenitors to undergo gliogenesis in vivo, or to exhibit normal gliogenesis in standard medium, demonstrates that canonical Notch signaling is required for gliogenesis beyond simply promoting the maintenance of stem/progenitor cells. Our results further suggest that Nrg was able to bypass the block in gliogenesis in Rbpsuh-deficient neural crest progenitors in culture.
**Rbpsuh deletion leads to profound defects in gliogenesis despite normal neurogenesis in sympathetic ganglia**

Neural crest migration into sympathetic ganglia appeared normal as similar numbers of p75+ cells were observed in the sympathetic ganglia of Wnt1-Cre° Rbpsuh°/° mice and control littermates at E10.5 (Fig. 4C). Neurogenesis was also normal at all stages of development from E10.5 to E18.5 in the sympathetic chain of Wnt1-Cre° Rbpsuh°/° mice (Fig. 4A,D). By contrast, gliogenesis was grossly reduced in Wnt1-Cre° Rbpsuh°/° mice, with no BFABP+ glia observed at E14.5 and eightfold fewer glia observed at E18.5 (Fig. 4D). This difference in gliogenesis did not reflect differences in cell proliferation or cell death, as BrdU incorporation (Fig. 4E) and the frequency of activated caspase-3+ cells (Fig. 4F) appeared normal in Wnt1-Cre° Rbpsuh°/° embryos. This also did not simply reflect a difference in BFABP expression after Rbpsuh deletion, as the Wnt1-Cre° Rbpsuh°/° mice also had fewer S100β+ cells per section in the sympathetic chain (see Fig. S3A versus E in the supplementary material). Moreover, in contrast to control mice, the S100β+ cells that were present in sympathetic ganglia from Wnt1-Cre° Rbpsuh°/° mice also expressed p75 (see Fig. S3B versus F in the supplementary material). Since NCSCs express p75 and S100β (Morrison et al., 1999), these data suggest that undifferentiated progenitors persist in the sympathetic chain in the absence of Rbpsuh, but fail to undergo gliogenesis.

To test this proposition, we cultured dissociated sympathetic ganglion cells at clonal density from E13.5 Wnt1-Cre° Rbpsuh°/° embryos and littermate controls. In standard medium, cells from Wnt1-Cre° Rbpsuh°/° embryos rarely formed multilineage colonies or any Gfap+ glia-containing colonies, in contrast to cells from control embryos (Fig. 5A). However, when the medium was supplemented with Nrg, sympathetic chain cells from Wnt1-Cre° Rbpsuh°/° embryos formed multilineage colonies and other colonies that contained glia in numbers that were similar to control embryos (Fig. 5B). In four independent experiments, 89±21% of colonies cultured from Wnt1-Cre° Rbpsuh°/° sympathetic chain exhibited complete Rbpsuh excision (data not shown). These data demonstrate that undifferentiated neural crest progenitors persist in the sympathetic chain of Wnt1-Cre° Rbpsuh°/° mice, but fail to undergo gliogenesis in vivo or in the absence of Nrg in culture. Thus, the defect in gliogenesis observed in vivo cannot be caused by a depletion of neural crest progenitors and must instead be caused by a requirement for Rbpsuh in glial lineage determination or glial differentiation in the sympathetic chain.

**Rbpsuh is also required for normal gliogenesis in the CNS**

To examine the role of Rbpsuh in the CNS, we studied spinal cord development in Nestin-Cre° Rbpsuh°/° embryos and littermate controls. At E11.5, we did not detect any difference between Nestin-Cre° Rbpsuh°/° embryos and littermate controls in the numbers or locations of Chx10+ cells, Olig2+ cells, HB9+ (Hlx9 – Mouse Genome Informatics) cells or GABA2+ (Neurog2 – Mouse Genome Informatics) cells (see Fig. S4 in the supplementary material). This suggested that overall patterning within the spinal cord was grossly normal. We did however observe a small but statistically significant reduction in the number of Gata2+ cells per section in the Nestin-Cre° Rbpsuh°/° spinal cord (see Fig. S4C versus D,K in the supplementary material). These data raised the possibility that Rbpsuh is required for the generation of normal numbers of at least certain p2-domain progenitors, which normally give rise to Gata2+ interneurons, Chx10+ interneurons, and BFABP+ astrocytes in the developing spinal cord (Jessell, 2000; Muroyama et al., 2005).

By E14.5, Nestin-Cre° Rbpsuh°/° embryos exhibited clear differences to control embryos in glial fate determination within the pMN and p2 domains of the developing spinal cord. The total number of neurons per cross-section through the spinal cord based on TuJ1 staining or NeuN staining was not affected by Rbpsuh.
The total number of HB9⁺ motor neurons (that arise from the pMN domain) was also not affected by Rbpsuh deletion (see Fig. S5E-G in the supplementary material). However, neuronal identity within the p2 domain was affected, as Nestin-Cre⁺ Rbpsuh±/fl embryos had a modest but significant (P<0.05) increase in the number of Chx10⁺ V2a interneurons, and a modest but significant (P<0.05) reduction in the number of Gata2⁺ V2b interneurons (see Fig. S5 in the supplementary material). Glial fates were much more strikingly affected in Nestin-Cre⁺ Rbpsuh±/fl embryos, with a significant reduction in BFABP⁺ astrocyte progenitors (Fig. 6A,B,G-I) and a significant increase in Olig2⁺ oligodendrocyte progenitors (Fig. 6C,D,G-I). This suggested that, in the absence of Rbpsuh, p2-domain glial progenitors that would normally acquire an astrocyte fate instead became oligodendrocytes, a fate normally associated with the pMN domain. Nonetheless, we cannot rule out the possibility that oligodendrocyte lineage cells preferentially expanded and astrocyte lineage cells failed to expand in the absence of Rbpsuh. Indeed, BFABP⁺ astrocyte progenitors exhibited greater BrdU labeling in control mice (Fig. 6L versus M), whereas Olig2⁺ oligodendrocyte progenitors exhibited greater BrdU labeling in Nestin-Cre⁺ Rbpsuh±/fl mice (Fig. 6N versus O).

To test whether these differences observed at E14.5 translated into decreased numbers of astrocytes and increased numbers of oligodendrocytes in the absence of Rbpsuh, we examined the E19.5 spinal cord. We again observed decreased numbers of Gfap⁺ and BFABP⁺ astrocytes (Fig. 7A versus B,G,H) and increased numbers of Mbp⁺ and Sox10⁺Pdgfr⁺ oligodendrocytes (Fig. 7C versus D,E and F,G) in the Nestin-Cre⁺ Rbpsuh±/fl spinal cord as compared with littermate controls. We observed no effect of Rbpsuh deletion on the numbers of NeuN⁺ neurons (Fig. 7G). These data indicate that Notch signaling is necessary to regulate gliogenesis in the developing spinal cord by promoting the generation of astrocytes and inhibiting the generation of oligodendrocytes during the window of development that we studied.

Olig2⁺ progenitors in the spinal cord generate multilineage colonies in culture under the influence of basic FGF, which causes a subset of cells in these colonies to lose Olig2 expression and to form astrocytes (Gabay et al., 2003). To test whether spinal cord progenitors from Nestin-Cre⁺ Rbpsuh±/fl mice retained the ability to form multilineage colonies in culture, we cultured dissociated E19.5 thoracic spinal cord cells at clonal density in non-adherent cultures, then transferred the resulting neurospheres to adherent cultures (C-N). Representative photos of single fields of view from within multilineage colonies cultured from control cells in standard medium (C-E), Nestin-Cre⁺ Rbpsuh±/fl cells in standard medium (F-H), control cells in the presence of Nrg (I-K) and Nestin-Cre⁺ Rbpsuh±/fl cells in Nrg (L-N) show that Rbpsuh-deficient NCSCs rarely formed glia (H, Gfap, red), except in the presence of Nrg (N) (three to six independent experiments).
the in vivo results in indicating that Rbpsuh is required for the generation of normal numbers of astrocytes in the developing spinal cord, without being required (at least during this window of development) for the maintenance of undifferentiated progenitors.

**Notch signaling is required to maintain Sox9 expression during gliogenesis**

The foregoing data raised the question of whether Notch signaling is required for the expression of genes that regulate glial lineage determination. To address this, we examined the effect of Rbpsuh deletion on Sox9 expression. Sox9 is expressed by undifferentiated neuroepithelial progenitors within the ventricular zone of the developing spinal cord as well as by glial-restricted progenitors and differentiated glia (Stolt et al., 2003). Conditional deletion of Sox9 from the developing spinal cord using Nestin-Cre leads to severe reductions in astrocyte formation (Stolt et al., 2003). Through E12.5, Nestin-Cre+ Rbpsuhfl/fl mice had normal numbers of Sox9+ cells in spinal cord sections (Fig. 8A,B,F). However, from E13.5 on, Nestin-Cre+ Rbpsuhfl/fl mice had increasingly severe reductions in the numbers of Sox9+ cells relative to littermate controls (Fig. 8C-F). The loss of Sox9 expression at E13.5 and E14.5 did not appear to be explained by the death of Sox9-expressing cells because we detected only rare Sox9+ cells that stained for activated caspase 3 in either treatment at these time points. However, by E19.5, Sox9+ cells underwent increased cell death in the absence of Rbpsuh (Fig. 8G).

Whereas Rbpsuh was required for Sox9 expression, we did not detect an effect of Rbpsuh deletion on the expression of another regulator of glial specification, Scl (Tal1 – Mouse Genome Informatics) (Muroyama et al., 2005) (Fig. 8H). These data suggest that Notch signaling is required for glial lineage determination in the spinal cord partly because it is required to maintain Sox9 expression by glial progenitors.

This requirement for canonical Notch signaling in CNS gliogenesis is not limited to the spinal cord as we also observed significantly reduced numbers of astrocytes and significantly increased numbers of oligodendrocytes after Rbpsuh deletion in the E19.5 diencephalon. The diencephalon from Nestin-Cre+ Rbpsuhfl/fl mice had significantly reduced numbers of astrocytes, whether we stained for Glast (Slc1a3 – Mouse Genome Informatics), S100/H9252 or Sox9 (see Fig. S6 in the supplementary material). The diencephalon from the same mice had significantly increased numbers of Olig2+ oligodendrocytes (see Fig. S6 in the supplementary material). This demonstrates that Notch signaling also regulates gliogenesis in at least certain regions of the brain. However, it was difficult to precisely compare the frequency of glia throughout the brain because Rbpsuh deletion led to an expansion of the ventricles as well as some hemorrhaging (data not shown). As a result, the morphology of the mutant brains was somewhat different from that of control littermates, making it difficult to compare homologous brain regions.

**DISCUSSION**

Our data demonstrate that canonical Notch signaling plays a physiological role in gliogenesis in vivo. We observed defects in gliogenesis throughout the PNS and CNS in the absence of Rbpsuh.
Yet defects in neurogenesis were modest or undetectable, and progenitors capable of undergoing gliogenesis in culture persisted even after the defects in gliogenesis were evident in vivo. Rbpsuh-deficient sensory ganglia exhibited a modest reduction in neurogenesis but an almost complete lack of gliogenesis, despite the persistence of normal numbers of NCSCs and other progenitors that could form glia in cultures supplemented with Nrg (Figs 2, 3). Rbpsuh-deficient sympathetic chain exhibited normal neurogenesis despite an almost complete lack of gliogenesis (Fig. 4). Again, NCSCs and other progenitors that could form glia in cultures supplemented with Nrg persisted in normal numbers into late gestation (Fig. 5). In the Rbpsuh-deficient spinal cord, levels of neurogenesis were normal, but there was a significant decrease in the number of astrocytes and a significant increase in the number of oligodendrocytes (Figs 6, 7). Consistent with the increase in Olig2+ progenitors, Rbpsuh-deficient spinal cord cells formed an increased frequency of multilineage colonies in culture (Fig. 7). These data indicate that physiological Notch signaling is required for normal gliogenesis in vivo independent of its effects on progenitor maintenance.

The mechanism by which Notch signaling promotes gliogenesis appears to involve a role in the expression of at least certain glial specification genes. Notch signaling is required for the maintenance of Sox9 expression during the gliogenic phase of spinal cord development (Fig. 8). Since Sox9 is required for the formation of astrocytes in the developing spinal cord (Stolt et al., 2003), the loss of Sox9 expression after Rbpsuh deletion is consistent with the dramatic reduction in astrocytogenesis that we observed. We did not detect reduced expression of other glial specification genes, such as Scl (Fig. 8) (Muroyama et al., 2005), suggesting that Notch is not globally required for the expression of such genes. Additional work will be required to determine whether Rbpsuh directly or indirectly regulates Sox9 expression. Moreover, the loss of Sox9 expression cannot completely explain...
the gliogenic defects in the absence of Rbpsuh, because loss of Sox9 by itself is associated with a transient reduction in oligodendrocyte formation (Stolt et al., 2003), rather than an expansion as we observed. There must therefore be additional mechanisms by which Notch signaling regulates gliogenesis. Nonetheless, these data demonstrate that just as Notch signaling is required for the expression of the glial-specification gene gcm in some Drosophila glia (Udolph et al., 2001), Notch signaling is also required in mammals to maintain the expression of at least certain glial-specification genes.

These data do not rule out a role for Notch signaling in the maintenance of undifferentiated neural progenitors in vivo. We observed a significant reduction in the frequency of gut cells that formed multilineage NCSC colonies in culture. In contrast to what we observed in other regions of the PNS, this deficit was not seen in other regions of the spinal cord. We observed a significant reduction in the frequency of gut cells that formed multilineage NCSC colonies in culture. In contrast to what was observed in other regions of the PNS, this deficit was not seen in other regions of the nervous system, it is likely to vary over developmental time. Notch signaling might promote progenitor maintenance early in neural development and promote gliogenesis later in neural development. Our failure to observe a depletion of neural progenitors in most locations could have been caused by a relatively late conditional deletion of Rbpsuh, after the onset of neurogenesis.

A recent study conditionally deleted Notch1 in the developing spinal cord using Nestin-Cre and observed an increase in neuronal differentiation and a decrease in the number of Olig2+ progenitors in the E11.5 neural tube (Yang et al., 2006). However, this study did not test whether Notch1 deletion led to a loss of cells that could form multilineage colonies in culture or changes in astrocyte or oligodendrocyte differentiation at later stages of spinal cord development. As Yang et al. observed after Notch1 deletion, we also observed a loss of the central canal in the spinal cord after Rbpsuh deletion, although this occurred later in our study. However, unlike Yang et al., we did not observe any difference in the frequency of Olig2+ cells or Ngn2+ cells between Nes-Cre+/+ Rbpsuhfl/fl mice and littermate controls at E11.5 (see Fig. S4 in the supplementary material). Moreover, we observed a substantial increase in oligodendrocyte differentiation at later stages of spinal cord development (Figs 6, 7). Although these results would appear to contrast with those of Wang et al., our results are consistent with a prior study that also observed increased spinal cord oligodendroglialgenesis during late fetal development after conditional Notch1 deletion (Genoud et al., 2002). Our results are also consistent with other studies that found an inhibitory effect of Notch signaling on oligodendrocyte differentiation (Wang et al., 1998). Different results might be obtained depending on precisely when conditional deletion occurs, and Rbpsuh deletion might have different effects than Notch1 deletion, particularly if progenitors in different domains of the spinal cord express different Notch receptors.

The near complete absence of gliogenesis in Rbpsuh-deficient sensory and sympathetic ganglia in vivo, despite the ability of progenitors from these ganglia to form glia in culture in the presence
of Nrg, suggests that gliogenic mechanisms in culture can differ from those employed under physiological conditions, consistent with some prior reports (Gabay et al., 2003). Our data suggest that Notch signaling is required for gliogenesis in these ganglia in vivo but that Nrg can bypass this requirement in culture. Nrg instructs NCSCs in culture to acquire a glial fate (Morrison et al., 1999; Shah and Anderson, 1997) and is necessary for gliogenesis in the PNS in vivo (Dong et al., 1995; Meyer and Birchmeier, 1995; Riethmacher et al., 1997). However, Nrg was expressed around Rbpsuh-deficient sympathetic ganglia in vivo (data not shown) and yet these cells still failed to form glia in vivo. This indicates that Notch signaling is required for gliogenesis in vivo in a way that is not recapitulated when Nrg is added to the culture medium. One possibility is that Notch signaling is required in vivo to promote the transition from neurogenesis to gliogenesis by overcoming the neurogenic influence of ongoing bone morphogenic protein signaling (Morrison et al., 1999).

The original evidence indicating that Notch ligands can instruct NCSCs to acquire a glial fate in culture came from experiments performed on NCSCs isolated from the developing sciatic nerve (Morrison et al., 2000). In the current study, we did not observe any defects in peripheral nerve gliogenesis in Wnt1-Cre+ Rbpsuhfl/fl mice [these nerves appeared normal by electron microscopy and Krox20 (Egr2 – Mouse Genome Informatics) staining; data not shown]. We also observed only a small (but statistically significant; \(P<0.05\) by a paired \(t\)-test) reduction in the frequency at which

**Fig. 7.** Rbpsuh deletion reduces the generation of astrocytes and increases the generation of oligodendrocytes in the E19.5 mouse spinal cord without depleting progenitors that could form multilineage colonies in culture. (A-F) The differences observed at E14.5 persisted in the E19.5 spinal cord, including decreased numbers of Gfap+ astrocytes (A,B) and increased numbers of Mbp+ oligodendrocytes (C,D) in the Nestin-Cre+ Rbpsuhfl/fl spinal cord as compared with littermate controls. We also observed more Sox10+ Pdgfrα+ oligodendrocyte lineage cells in the Nestin-Cre+ Rbpsuhfl/fl spinal cord (E,F, arrows). C,D represent montages of multiple non-overlapping images of the same section. (G) Quantification revealed a significant (\(*, \ P<0.05\)) reduction in the number of BFABP+ astrocytes and significant increases in the numbers of Olig2+, Sox10+ and Mbp+ oligodendrocyte lineage cells in the Nestin-Cre+ Rbpsuhfl/fl spinal cord (three to seven sections per mouse, three to five mice per genotype). Because glia are difficult to count in sections based on filamentous Gfap staining, we acutely dissociated cells from E19.5 spinal cord and plated them in culture at low density for 6 hours then stained for Gfap expression. (H) We observed a significantly lower percentage of Nestin-Cre+ Rbpsuhfl/fl cells in culture that were Gfap+ (\(*, \ P<0.05\); 1000 cells counted per mouse, three mice per genotype). There was no significant difference in the number of cells undergoing cell death (activated caspase 3+) in control and Nestin-Cre+ Rbpsuhfl/fl mice (data not shown). (I-P) Dissociated E19.5 thoracic spinal cord cells were cultured at clonal density then stained for neurons (TuJ1+), astrocytes (Gfap+) and oligodendrocytes (O4+). Representative multilineage colonies from control (I-L) and Nestin-Cre+ Rbpsuhfl/fl (M-P) spinal cord cells show more Gfap+ cells and stronger Gfap staining in control colonies. (Q) The percentage of spinal cord cells that formed each type of colony. Nestin-Cre+ Rbpsuhfl/fl spinal cord cells were significantly more likely than control cells to form multilineage colonies (N+A+O) and oligodendrocyte-containing colonies (O-containing), but significantly less likely to form astrocyte-only colonies (A). \(P<0.05\). The increase in neuron-containing colonies reflected the increase in multilineage colonies, not an increase in neuronal-restricted progenitors. Scale bars: 50 \(\mu\)m in A-F.
Physiological Notch signals promote gliogenesis

Together, the results from this and prior studies indicate that Notch signaling plays reiterated roles in neural development, initially promoting the generation or maintenance of neural progenitors and later promoting gliogenesis.

This work was supported by the Howard Hughes Medical Institute and the National Institutes of Health (R01 NS40750). M.K.T. was supported by a National Research Service Award from the National Institute of Neurological Disorders and Stroke (F32 NS046202). We thank Tasuku Honjo for generously

Supplementary material
Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/134/13/2435/DC1

References


Carlesso, N., Aster, J. C., Sklar, J. and Scadden, D. T. (1999). Notch1-induced multilineage NCSC colonies formed in culture from Wnt1-Cre+ Rbpsuhfl/fl nerve cells (0.6±0.5%), as compared with control littermate cells (1.1±0.7%). However, Rbpsuh was not efficiently deleted in these NCSCs: only 7±10% of neurospheres showed excision of both Rbpsuh alleles (n=6 independent experiments). This raises the possibility that Rbpsuh was not efficiently deleted from neural crest progenitors that participated in sciatic nerve development. As a result, it remains uncertain whether Notch signaling plays a physiological role in peripheral nerve gliogenesis.

This is the first study to examine the consequences of a complete loss of canonical Notch signaling on gliogenesis in vivo and to distinguish between effects on glial lineage determination/differentiation versus progenitor maintenance. We observed an almost complete loss of gliogenesis in sensory and sympathetic differentiation versus progenitor maintenance. We observed an
distinguish between effects on glial lineage determination/loss of canonical Notch signaling on gliogenesis in vivo and to
gliogenesis.

We observed an
distinguish between effects on glial lineage determination/loss of canonical Notch signaling on gliogenesis in vivo and to
gliogenesis.

This is the first study to examine the consequences of a complete loss of canonical Notch signaling on gliogenesis in vivo and to distinguish between effects on glial lineage determination/differentiation versus progenitor maintenance. We observed an almost complete loss of gliogenesis in sensory and sympathetic ganglia in the absence of Rbpsuh, despite normal or nearly normal neurogenesis, and despite the persistence of normal frequencies of progenitors that could form glia in cultures supplemented with Nrg. This demonstrates that physiological Notch signaling plays a crucial role in the promotion of gliogenesis in vivo, independent of its effects on progenitor maintenance. Notch signaling was similarly necessary for the regulation of gliogenesis in the CNS, as Rbpsuh deletion reduced Sox9 expression and astrocyte differentiation in the developing spinal cord. These results are consistent with our prior demonstration that Notch activation can instruct NCSCs to acquire a glial fate (Morrisson et al., 1999) and with the observation that Notch activation can promote gliogenesis in the CNS (Furukawa et al., 2000; Gaiano et al., 2000; Hojo et al., 2000; Scheer et al., 2001; Tunigaki et al., 2001; Yoon et al., 2004).
delay of human hematopoietic progenitor cell differentiation is associated with altered cell cycle kinetics. Blood 93, 838-848.


Physiological Notch signals promote gliogenesis

RESEARCH ARTICLE 2447


