Notch1 Signaling Regulates Radial Glia Differentiation through Multiple Transcriptional Mechanisms

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Signaling by the Notch1 receptor is critical for the formation of radial glia in the developing nervous system. We have shown previously that Notch1 regulates the molecular and morphological differentiation of radial glia through the transcriptional activation of at least two genes, brain lipid binding protein (BLBP) and the erbB2 receptor tyrosine kinase. However, the mechanisms by which this occurs remained undefined. Here we demonstrate that Notch1 effects on radial glia gene expression are mediated by two downstream mechanisms, one that depends on Suppressor of Hairless [Su(H)] and the other on Deltex1 (DTX1). These two Notch1-binding proteins contribute to the regulation of BLBP and erbB2 expression, respectively. Importantly, our results suggest that, although these events can occur simultaneously, a hierarchical relationship might exist between DTX1 and Su(H), because overexpression of DTX1 or a dominant-negative form of this protein inhibits Su(H)-mediated events but not vice versa. In contrast to the effects of DTX1 overexpression, interference RNA-mediated knock-down of DTX1 blocks Notch1-induced erbB2 promoter activation and radial glia formation selectively, without affecting Su(H)-dependent pathways, indicating that loss of DTX1 expression and expression of dominant-negative DTX1 result in different alterations in cell differentiation and gene expression. Together, these results show that Notch1 regulates radial glia formation through two distinct transcriptional mechanisms and that the outcomes of Notch1 signaling may depend on the relative expression levels of its coregulators.

Key words: Notch1; Su(H); erbB; cerebellum; glia; BLBP; DTX

Introduction

Neuronal migration is a critical step in brain development. Neurons are generated in germinal layers located in the walls of the neural tube and then migrate to the position they will occupy in the mature brain. Newborn excitatory cortical neurons and most cerebellar neurons migrate along specialized cells called radial glia (Rakic 1971a,b). These cells extend radial processes from the germinal layers through the developing anlage and thus provide a scaffold for migrating neurons. In the cerebellum, Bergmann glia cells are the radial glia that guide and support the migration of granule neurons (Goldowitz and Hamre, 1998).

Bergmann glia have been used as a model to study the mechanisms that regulate radial glia formation and function in vivo and in vitro (Sotelo and Changeux, 1974; Hatten, 1985; Rio et al., 1997; Borghesani et al., 2002; Patten et al., 2003; Yue et al., 2005). Hatten (1985) originally demonstrated that contact with granule neurons induces cerebellar astroglia to adopt a radial glia phenotype. This transformation includes the formation of radial processes (Hatten, 1985) and the expression of the radial glia marker brain lipid binding protein (BLBP) (Feng et al., 1994). More recently, we showed that this radial glia differentiation depends on activation of the Notch1 receptor and subsequent downstream transcriptional events (Patten et al., 2003). When Notch1 receptors expressed by cerebellar astroglia are activated by contact with granule neurons, which express Jagged1, Notch1 signaling induces the transcription of BLBP and the receptor tyrosine kinase erbB2. Upregulation of BLBP is important for adhesion between migrating neurons and radial glia (Feng et al., 1994), although the increase in erbB receptor expression makes the glia responsive to the erbB ligand neuregulin1 (NRG1), which is also expressed by the granule neurons (Rio et al., 1997). NRG1-erbB receptor signaling then induces the morphological transformation of the astroglia into radial glia (Patten et al., 2003).

The studies described above also suggested that multiple intracellular pathways mediate the effects of Notch1 signaling in radial glia. In canonical Notch signaling, ligand binding induces receptor proteolysis, releasing the Notch1 intracellular domain (NICD). NICD then translocates to the nucleus and binds to Suppressor of Hairless [Su(H)] [also known as C-promotor binding factor 1 (CBF1), RBP-J, and LAG1], converting it from transcriptional repressor to transcriptional activator (for review, see Lai, 2002). We found that one effect of Notch1 on radial glia formation, the activation of BLBP expression, depends on this canonical Su(H) pathway. However, we were surprised by the
observation that the Notch1-mediated erbB2 expression and glial morphological differentiation were independent of Su(H) (Patten et al., 2003), indicating that noncanonical Notch1 signaling is involved in the formation of radial glia.

Deltex1 (DTX) has been implicated in noncanonical Notch1 signaling (Kishi et al., 2001) and was originally identified in Droso-

philida as a positive regulator of Notch1 function (Xu and Artavanis-Tsokhas, 1990; Matsuno et al., 1995; Ramain et al., 2001). There are four mammalian DTX proteins named DTX1 through DTX4 (Kishi et al., 2001; Storck et al., 2005). These proteins form homodimers and heterodimers with each other and bind to the ankyrin repeats of NICD (Kishi et al., 2001; Yamamoto et al., 2001; Takeyama et al., 2003). Although in mammalian cells DTX1 is seen mostly as a positive regulator of Notch1, it has been suggested that it could also act as a negative regulator (Matsuno et al., 1998; Sestan et al., 1999). However, the mechanisms by which DTX contributes to Notch1 signaling, its potential gene targets, and the ways in which DTX and Su(H) interact in mammalian cells remain undefined.

Here we show that DTX1 function is necessary for the Notch1-mediated regulation of radial gli differentiation, with DTX1 activity being necessary for the activation of erbB2 transcription and radial glia morphological differentiation. Furthermore, our experiments indicate that a hierarchical relationship might exist between DTX1 and Su(H), the first being dominant over the latter. These results provide new insights into mechanisms of brain development, in particular how Notch1 signals to potential gene targets, and the ways in which DTX and Su(H) interact in mammalian cells remain undefined.

Materials and Methods

In situ hybridization. The DTX1 probe was generated by PCR with two primers: forward, 5'-GCAAGACAGCAGGATAG-3' and reverse, 5'-GGATAAGACAGGTCACAAGG-3'. The 514 bp PCR product was then cloned into pCRII-TOPO expression vector (Invitrogen, Carlsbad, CA) to generate the sense and antisense probes. The preparation of digox- inin (DIG)-labeled riboprobe for DTX1 and the in situ analysis was performed as described by Messersmith et al. (2000). Briefly, FVB/N wild-type mice (Charles River Laboratories, Wilmington, MA) were fixed by intracardial perfusion with 4% paraformaldehyde in PBS, and the cere- bellum was dissected, cryoprotected, and cut in 15 μm parasagittal sec- tions. DIG-labeled riboprobes were hybridized to cryosections, detected using an alkaline phosphatase-conjugated anti-DIG antibody (Roche Products, Welwyn Garden City, UK), and developed with a nitroblue- tetrazolium-chloride/5-bromo-4-chlor-indolyl-phosphate substrate (Roche Products). When immunostaining and in situ analysis were combined, the in situ protocol was followed as described, and then sections were fixed in 4% paraformaldehyde for 10 min, rinsed with PBS, blocked in 4% horse serum with 0.1% Triton X-100, and probed with a rabbit anti-calbindin antibody (1:50; Abcam, Cambridge, MA), followed by an anti-mouse HRP-conjugated secondary antibody (Jackson Immuno-Research, West Grove, PA).

Immunostaining. Cells in culture were fixed in 4% paraformaldehyde for 10 min, rinsed with PBS, blocked in 4% horse serum with 0.1% Triton X-100, and probed with a rabbit anti-GAP (1:50; DakoCytomation, High Wycombe, UK) or a mouse anti-green fluorescent protein (GFP) (1:200; Santa Cruz Biotechnology, Santa Cruz, CA) antibody, followed by anti-rabbit cyanine 3 or anti-mouse Alexa Fluor 488-conjugated sec- ondary antibody (Jackson ImmunoResearch). Nuclei were stained with Hoechst 33342 (Invitrogen).

Glial and neuron cell culture. Primary astroglial and granule neurons were purified from postnatal day 6 (P6) Long–Evans rat or FVB mouse cerebellum (Charles River Laboratories) by selective preplating as de- scribed by Patten et al. (2003). Briefly, cerebella were excised in PBS and dissociated in astrocyte media [DMEM, 10% fetal bovine serum (FBS), and 2 mM l-glutamine (Invitrogen)] with 0.02% DNase. Cells were plated onto uncoated dishes to remove fibroblasts, and then the supernatant was plated onto three 10 cm poly-1-hysine (0.5 μg/ml; BD Bio- sciences, Franklin Lakes, NJ) coated dishes to attach glia. Plates were washed with PBS to remove and collect neurons. Glia were passaged 3–4 d later to remove any remaining neurons, producing a glial culture >95% pure. All cells were incubated at 37°C with 5% CO2. Cell lines. The oligodendrocyte cell line (OLN-93) and HEK 293T and NIH3T3 cell lines were grown in DMEM with high glucose, 10% FBS, and 2 mM l-glutamine (Invitrogen).

Generation of lentiviruses for interference RNA expression. The vector pLl3.7 (from Luc Van Parijs, Massachusetts Institutes of Technology, Cambridge, MA) was used to generate small hairpin RNAs as described by Rubinson et al. (2003). The following oligonucleotides were subcloned into the Hypla/Xhol sites (nucleotides targeting DTX4 are underlined): interference RNA (RNAi) DTX1-A, forward, 5'-TGGCGCCAGAGTTGCTAGATTCAAGAGATCTCAGACCCTTGGCGCTTTTTTC-3'; RNAi DTX1-A reverse, 5'-TCGAGAAAAAGGCCGAAGGTGCTAGATCTTGAATTCGAGCTTGCCTGCGAACA-3'; RNAi DTX1-B, forward, 5' -TGGCGCTTACGACATGAGGTTCACAGAAGGCTCTGAGCTGATGACGCTTTTTC-3'; RNAi DTX1-B reverse, 5'-TCGAGAAAAAGCGCCGAAGATCCATCTTGTAGTTCGAGCAGCTTTTC-3'.

Contactin purification. Two 10 cm dishes of 70% confluent HEK 293T cells were transfected with a rat Ig-contactin plasmid (Peles et al., 1995). Two days later, cells were passed onto three 15 cm dishes and incubated with OptiMem media (Invitrogen) and penicillin/streptomycin (Invitrogen) for 24 h. Medium was collected and replaced for another 24 h. The conditioned media was added to 1 ml of protein A/G beads, washed with PBS, and rotated overnight at 4°C. The beads were then washed twice with PBS, eluted twice with 3 ml of 0.1 M glycine, pH 2.5, for 15 min each, and eluted once with PBS for 5 min. Eluates were neutralized with 1 M Tris, pH 8, and centrifuged at 3000 × g for 5 min in a 30 kDa Centrifugal Filtration Device (Amicon, Beverly, MA).

Plasmids. The 1.7 kb BLBP promoter (Feng and Heintz, 1995), the 4.5 kb erbB2 promoter (White and Hung, 1992), and the CBF1-dependent reporter (Hsieh et al., 1996) were subcloned into a pGL3basic luciferase reporter vector (Promega, Madison, WI) as described by Patten et al. (2003). The wild-type Su(H) construct encodes a full-length Xenopus Su(H), and the dominant-negative (DN)-Su(H) construct encodes a Xe-

notus Su(H) with mutated DNA binding domains (Wettstein et al., 1997). The NICD construct encodes the entire intracellular domain of the Notch1 receptor [formerly referred to as FCDN1 by Patten et al. (2003)] (Shawber et al., 1996). The wild-type DTX1 construct encodes the full-length human Deltex (amino acids 1–620), and the DN-DTX1 construct encodes a truncated human Deltex (amino acids 1–412) (Yamamoto et al., 2001). To generate the flag-tagged mouse DTX1 con- struct (flag-DTX1), mouse DTX1 (B0530505, clone 8170690; American Type Culture Collection, Manassas, VA) was cloned into the Noti-Xb plasmid sites of pA1-fg using the following primers: 5'-GAAGGGGCCGCATCAGTTGCCGAGACAGACAG-3'; and 5'-CGATTCCTAGA TCCAGCCTTGCCCATG GCCCT-3'.

Transfections. Primary astrocytes at 70% confluence were transfected with Fugene 6 reagent (Boehringer Mannheim, Indianapolis, IN), ac- cording to the recommended protocol. To detect the transfected cells, cells were cotransfected with a plasmid encoding GFP at a molar ratio of 3:1 (target plasmid/GFP plasmid). Previously, the level of coexpression was per-

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Astroglial morphology assay. Glia were plated at 10,000 cells/200 mm². The next day, cells were transfected, and, 24 h later, freshly dissociated neurons were added at a ratio of 10:1 (neurons/glial). When glia infected with RNAi viruses were used, they were infected 10 d before transfection. More than 98% of the cells were infected as determined by GFP expression. The radial glia morphology was determined as described by Patten et al. (2003). Briefly, cells were fixed with 4% paraformaldehyde and labeled with a rabbit anti-GFAP antibody (Dako-Cytomation) and the appropriate secondary antibody. The morphology of cells in random fields was assessed in cells that expressed both GFAP and GFP, as determined by immunostaining. Glia were considered to be radial when they had at least one thin process longer than 50 μm. This criterion was based on previous studies (Hatten, 1985; Rio et al., 1997). The results presented represent the mean ± SEM of at least three independent experiments, and 200–400 cells were examined for each assay.

Western blot. To test for RNAi knock-down of DTX1, HEK 293T cells were cotransfected with plasmids encoding flag-DTX1 and control, DTX1-A, or DTX1-B RNAi constructs in a 1:6 molar ratio. After 24 h, samples were then lysed in SDS sample buffer, separated by SDS-PAGE on a 12% gel, and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA). Blots were probed with a mouse anti-flag antibody (Sigma, St. Louis, MO), stripped, and then probed with a mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (Chemicon, Temecula, CA) for loading control. To test for the effectiveness of contactin, OLN-93 cells were lysed in SDS sample buffer, separated by SDS-PAGE on a 4–20% gradient gel (Invitrogen), and transferred to PVDF membranes (Millipore). Blots were probed with a mouse anti-MAG antibody (Chemicon), stripped, and then probed with a rabbit anti-GAPDH antibody (Chemicon). Detection was performed using the appropriate HRP-conjugated secondary antibodies (Jackson ImmunoResearch) and developed using the appropriate HRP-conjugated secondary antibodies (Jackson ImmunoResearch) and developed using the appropriate HRP-conjugated secondary antibodies (Jackson ImmunoResearch). The detection was performed using the appropriate HRP-conjugated secondary antibodies (Jackson ImmunoResearch). The detection was performed using the appropriate HRP-conjugated secondary antibodies (Jackson ImmunoResearch). The detection was performed using the appropriate HRP-conjugated secondary antibodies (Jackson ImmunoResearch).

Statistical analysis. Statistical significance for the astroglial morphological assays was determined by χ² test using the Statview program. Statistical significance for the luciferase assays was determined by Wilcoxon’s signed-rank test.

Results

DTX1 expression is developmentally regulated in the cerebellum

To determine whether DTX1 might play a role in Bergmann radial glia during cerebellar development, we first analyzed its pattern of expression by in situ hybridization (ISH). At P6, a time when the primary role of Bergmann glia is to support the migration of cerebellar granule cells, strong DTX1 expression could be detected in cells of the Purkinje cell layer, in which Bergmann glia cell bodies reside. No signal could be detected in the adult or when the probe was used. Scale bar, 250 μm. B, DTX1 ISH (blue) was combined with immunostaining for calbindin (brown) in parasagittal sections of the P6 cerebellum. DTX1 ISH signal did not overlap with calbindin staining of Purkinje cells, indicating that DTX1 expression is specific to Bergmann glia (top). A sense strand control for the ISH did not detect any specific signal (bottom). Scale bar, 100 μm. C, D, Purified P6 cerebellar glia and P6 whole cerebella were subjected to RT-PCR analysis with primers specific to DTX1. D, Purified cerebellar glia and NIH3T3 cells were lysed and subjected to Western blot analysis with an anti-DTX1 antibody that detected a band at 70 kDa. E, G, External granule cell layer.
Neurons regulate radial glia morphological differentiation through Notch1-DTX1 signaling

Using this in vitro assay, we showed previously that some aspects of the Notch1-induced differentiation of cerebellar astroglia into radial glia, i.e., the acquisition of radial morphology, occur through a pathway independent of Su(H) (Patten et al., 2003). To begin to determine whether DTX1 is critical for these Su(H)-independent events, we made use of a dominant-negative form of DTX1 (DN-DTX1). This truncated DTX1, which lacks the C-terminus domain III, can bind to NICD and has been shown to block Notch1-DTX signaling (Yamamoto et al., 2001). We first tested whether DN-DTX1 expression inhibits the ability of neurons to induce the morphological differentiation of radial glia. Expression of DN-DTX1, but not wild-type DTX1, blocked neuronal-induced morphological differentiation (Fig. 2A, B), indicating that DTX function is necessary for this event. To examine whether the effects of DN-DTX1 reflect alterations in Notch signaling, we tested whether DN-DTX1 blocks the glial morphological change induced by expression of the constitutively active NICD. Cerebellar astroglia were transfected with plasmids encoding NICD, DTX1, and DN-DTX1 alone or in combination. Similar to what we observed in neuron–glia cocultures, expression of DN-DTX1 blocked the ability of NICD to induce radial glia formation (Fig. 2C, D). Interestingly, DN-DTX1 expression also lowered the number of radial glia in unstimulated cultures (Fig. 2C).

Neurons mediate erbB2 expression via Notch1-DTX1 signaling

Previously, we showed that the induction of radial glia morphological differentiation mediated by neuronal contact and by Notch1 signaling depends on the transcriptional activation of the erbB2 gene, also a Su(H)-independent process (Patten et al., 2003). Therefore, we tested whether the induction of erbB2 transcription by Notch1 signaling and neuronal contact is mediated by DTX. An erbB2–luciferase reporter construct (Patten et al., 2003) was cotransfected into cerebellar astrocytes with plasmids encoding NICD, DN-DTX1, DTX1, or a control plasmid. NICD expression activated the erbB2 promoter, as we showed previously (Patten et al., 2003), and this effect was blocked by DN-DTX1 (Fig. 2E). Similar to what we observed for glial morphology (Fig. 2C), expression of DN-DTX1 reduced the basal level of erbB2 promoter activity (Fig. 2E). In contrast, expression of wild-type DTX1 alone had no effect on basal erbB2 activity and did not affect the ability of NICD to regulate transcription. Consistent with these observations, expression of DN-DTX1 also blocked the induction of erbB2 transcription by neuronal contact (Fig. 2F). Together, these results suggest that Notch1-DTX1-mediated erbB2 transcription is part of the mechanism by which neurons regulate radial glia morphological differentiation.

Expression levels of Su(H) and DTX1 determine Notch1 downstream transcriptional events

The results described above indicate that Notch1 signaling regulates radial glia differentiation through two different pathways. On one hand, Notch1-Su(H) signaling mediates the acquisition of radial glia identity, defined by the expression of the radial glia gene BLBP (Patten et al., 2003; Anthony et al., 2005). On the other hand, Notch1-DTX1 signaling contributes to the morphological differentiation of radial glia, which depends on the transcriptional activation of the erbB2 gene. Because Su(H) and
DTX1 bind to overlapping regions of NICD (Wettstein et al., 1997; Yamamoto et al., 2001) and it has been reported that overexpression of DTX1 can prevent the binding of Su(H) to NICD (Yamamoto et al., 2001), it was important to explore whether the Su(H)- and DTX-dependent pathways interact during the process of radial glia formation. To this end, we tested whether overexpression of one of these Notch1 mediators would alter signaling through the other in cerebellar astroglia.

To study the effects of DTX1 on Su(H)-dependent signaling we tested whether expression of DTX1 or DN-DTX1 alters the NICD-induced activation of a CBF1 and a BLBP reporter construct. The CBF1 reporter contains four tandem arrayed CBF1 binding sites upstream of the firefly luciferase gene (Hsieh and Heintz, 1995) and is commonly used to test Notch-1-Su(H) signaling. The BLBP reporter contains the 1.7 kb BLBP promoter (Feng and Heintz, 1995), which we and others have shown to be regulated by Notch-1-Su(H) signaling (Patten et al., 2003; Anthony et al., 2005). To study the effects of Su(H) on DTX-dependent signaling, we tested whether expression of Su(H) alters the induction of erbB2 reporter activation and radial glia morphology by NICD or neuronal contact. Surprisingly, expression of either DTX1 or DN-DTX1 reduced the ability of NICD to induce CBF1 reporter activity (Fig. 3A) and completely eliminated the NICD-induced BLBP reporter activity (Fig. 3B). In contrast, Su(H) did not alter the DTX-mediated events, i.e., the activation of erbB2 transcription (Fig. 3C) and the morphological differentiation of the glia (Fig. 3D). Similar results were obtained previously with DN-Su(H) (Patten et al., 2003). These results suggest that there is hierarchical relationship between DTX1 and Su(H) and indicate that the endogenous expression levels of DTX1 and Su(H) could be critical for determining the biological outcome of Notch1 activation during radial glia differentiation.

DTX1 knock-down by RNAi prevents radial glia morphological differentiation and induction of erbB2 expression without altering Su(H)-dependent events

The observation that DN-DTX1 not only blocks DTX-dependent events but also Su(H)-dependent ones indicated that the interpretation of experiments using DN-DTX1 could be confounded by the promiscuity of DN-DTX1. Thus, to test the roles of DTX1 in radial glia differentiation and transcription specifically, we used RNAi knock-down of DTX1. Two lentiviruses expressing different RNAi constructs targeted toward mouse DTX1; DTX1-A and DTX1-B were generated. The ability of these RNAi constructs to knock down DTX1 expression was proven measuring the levels of an epitope-tagged DTX1 (Fig. 4A). Similarly, DTX1 RNAi also knocked down expression of endogenous DTX1 in NIH3T3 cells (Fig. 4B). We then tested whether DTX1 knock-down inhibits the ability of neurons to induce radial glia differentiation.

Discussion

Our experiments show that Notch1 signaling controls the formation of radial glia through at least two distinct intracellular pathways, each regulating the transcription of different genes necessary for radial glia function, BLBP and an erbB receptor. Transcriptional activation of these genes requires the Notch1 co-regulators Su(H) and DTX1, respectively. Importantly, we found that the biological outcomes of knocking down DTX1 by RNAi and expressing DN-DTX1 are different. Whereas knock-down of DTX1 blocks DTX-mediated events without affecting Su(H) sig-
naling, overexpression of DN-DTX1 blocks both DTX- and Su(H)-mediated events. This apparent nonspecific effect of DN-DTX1, which likely reflects a competition between DN-DTX and Su(H) for binding to NICD (Yamamoto et al., 2001), indicates that some studies in which the biological roles of DTX-mediated Notch1 signaling were studied using DN-DTX1 could now be interpreted differently. Interestingly, overexpression of wild-type DTX1 also blocked Su(H) signaling, although the reverse was not the case. This suggests that differences in the expression levels of Notch1 coregulators, in particular DTX1, may be critical for determining the outcomes of Notch1 activation. That is, Su(H)-mediated events could be blocked in cells expressing high levels of DTX1.

 Whereas we found that Notch1-induced erbB2 expression in cerebellar astroglia occurs via DTX1, it has been reported that Notch1-induced erbB2 expression in cultures of cortical radial glia is blocked by a DN-Su(H) construct (Schmid et al., 2003). These results suggest that the ways in which Notch1 signaling regulates radial glia formation may differ between regions of the CNS. This difference between cortical and cerebellar radial glia could reflect distinct temporal and spatial expression levels of DTX1 and Su(H). Additional studies examining the pattern and levels of expression of DTX1 and Su(H) should provide more insight into how these pathways function throughout development.

 It has been speculated that DTX-dependent Notch1 signaling may be driven by ligands that are different from those that activate Su(H) events (Kadesh, 2004). However, we have shown that Jagged1, a Notch1 ligand expressed by granule cells in the developing cerebellum, induces the expression of both BLBP and erbB2 in astroglia (Patten et al., 2003), indicating that it is capable of inducing both Su(H)- and DTX-mediated events and could be the Notch1 ligand responsible for regulating these aspects of Bergmann glia function. Nevertheless, the possibility that in vivo DTX signaling may be activated by alternate ligands should not be overlooked (Hu et al., 2003; Cui et al., 2004; Eiraku et al., 2005). One molecule we have considered is contactin, a neural cell adhesion molecule reported to activate Notch1-DTX1 signaling in oligodendrocytes (Hu et al., 2003). Granule neurons express contactin, and contactin knock-out mice have abnormal cerebellar development and are ataxic (Berglund et al., 1999), making this molecule a strong candidate for a Notch1 ligand that regulates Bergmann glia differentiation. However, we tested the effects of contactin on cerebellar astroglia and found it does not activate Notch1 signaling in cerebellar astroglia, alter glia morphology, or activate the BLBP and erbB2 promoters (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). Therefore, contactin is unlikely to play a role in the morphological differentiation of radial glia. It remains to be tested whether this molecule plays a role earlier in development in cell fate choices that drive precursor cells to become radial glia. Another molecule that has been implicated recently in Notch1 signaling in the cerebellum is Delta/Notch-like epidermal growth factor–related receptor (DNER). Eiraku et al. (2005) proposed that this putative Notch1 ligand, which is expressed by Purkinje cells, regulates Bergmann glia radial process formation through the activation of DTX1 signaling. However, because these conclusions were based on the use of DN-DTX1, which also blocks Su(H) function, it remains to be determined whether elimination of DTX1 is sufficient to eliminate the biological effects of DNER-mediated Notch1 signaling.

 Similar to what we observed in cerebellar astroglia, the DTX family members appear to act as positive regulators of Notch1 signaling in other cell types. For example, activation of the Notch1 receptor in myoblasts regulates their differentiation through pathways that are both dependent and independent of Su(H) (Shawber et al., 1996; Noiziger et al., 1999), the Su(H)-independent pathway being positively mediated by DTX signaling (Kishi et al., 2001). Similarly, DTX1 has been shown to act as a positive regulator of Notch1 in Xenopus embryos, in which misexpression of DTX1 results in expansion of the neuroepithelium in a way similar to that induced by misexpression of activated Notch1 (Kishi et al., 2001). DTX1 also mimics Notch1 activation in a neural precursor cell line (MSN-70), in which it inhibits their differentiation, a process that is independent of Su(H) (Yamamoto et al., 2001). Interestingly, in contrast to our glia experiments in which overexpression of DTX1 has no effect on erbB2 expression and radial glia morphology, in some of these studies (Kishi et al., 2001; Yamamoto et al., 2001), overexpression of DTX1 alone was sufficient to mimic Notch1 activation, in some cases even activating processes previously considered to depend on Su(H) (Hori et al., 2004). Therefore, the effects of DTX in different cell types can vary, but the reasons for these differences remain to be determined.

 Some reports showed that DTX1 can also negatively regulate Notch1 signaling. Sestan et al. (1999) demonstrated that Notch1 activation inhibits neurite outgrowth in embryonic day 15–16 cortical neurons and that overexpression of DTX1 relieves this inhibition. However, based on our results, it is remains to be determined whether these observations reflected transcriptional activation of genes important for neurite outgrowth or whether they resulted from DTX1 overexpression blocking a Su(H)-independent inhibition of process outgrowth. DTX1 also has ubiquitin ligase activity (Takeyama et al., 2003; Liu and Lai, 2005), which has lead to the speculation that it could act as a negative regulator of Notch1 by mediating its ubiquitination and degradation (Kanwar and Fortini, 2004) or as a positive regulator by promoting internalization and stabilization of NICD (Hori et al., 2004). However, because in our experiments DN-DTX1, which lacks the ubiquitin ligase domain (Takeyama et al., 2003), inhibits Notch1-induced erbB expression and radial glia formation, it is unlikely that the negative effects of DN-DTX1 were mediated by ubiquitination of the Notch1 receptor.

 In summary, we examined how activation of the Notch1 receptor leads to multiple downstream transcriptional events, involving Su(H) and DTX1, that are critical for the formation of radial glia. It will be interesting to explore further the biological significance of the interactions between the DTX1 and Su(H) pathways suggested by our experiments in glia and to test whether they are relevant for other cell types that are regulated by Notch1 (Noiziger et al., 1999; Sestan et al., 1999; Kishi et al., 2001). Importantly, unlike Su(H), DTX1 has no known DNA binding domains. Identification of DNA-binding proteins that form part of the Notch1-DTX1 complex could further elucidate how this molecule mediates Notch1 effects on transcription.

References


