Development 129, 2391-2399 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
DEV7932

Context-dependent utilization of Notch activity in *Drosophila* glial determination

Yoshihiko Umesono[1,4,*], Yasushi Hiromi[1,3] and Yoshiki Hotta[2,3,4]

[1]Division of Developmental Genetics, [2]National Institute of Genetics, [3]Department of Genetics, Graduate University for Advanced Studies, Mishima, 411-8540, Japan
[4]CREST, Japan Science and Technology Corporation, Kawaguchi, 332-0012, Japan
*Author of correspondence at present address: Laboratory for Evolutionary Regeneration Biology, RIKEN Center for Developmental Biology, Kobe, 650-0047, Japan (e-mail: yumesono@lab.nig.ac.jp)

Accepted 11 February 2002

SUMMARY

During *Drosophila* neurogenesis, glial differentiation depends on the expression of *glial cells missing* (*gcm*). Understanding how glial fate is achieved thus requires knowledge of the temporal and spatial control mechanisms directing *gcm* expression. A recent report showed that in the adult bristle lineage, *gcm* expression is negatively regulated by Notch signaling (Van De Bor, V. and Giangrande, A. (2001). *Development* 128, 1381-1390). Here we show that the effect of Notch activation on gliogenesis is context-dependent. In the dorsal bipolar dendritic (dbd) sensory lineage in the embryonic peripheral nervous system (PNS), asymmetric cell division of the dbd precursor produces a neuron and a glial cell, where *gcm* expression is activated in the glial daughter. Within the dbd lineage, Notch is specifically activated in one of the daughter cells and is required for *gcm* expression and a glial fate. Thus Notch activity has opposite consequences on *gcm* expression in two PNS lineages. Ectopic Notch activation can direct gliogenesis in a subset of embryonic PNS lineages, suggesting that Notch-dependent gliogenesis is supported in certain developmental contexts. We present evidence that POU-domain protein Nubbin/PDM-1 is one of the factors that provide such context.

Key words: Notch, *gcm*, Nubbin, Gliogenesis, PNS, Asymmetric cell division, *Drosophila melanogaster*

INTRODUCTION

The two major cell types that compose the nervous system, neurons and glia, are in close contact with each other in all animals. Glial cells provide neurons with survival and axonal guidance cues, electrically shield axons by ensheathing them, and function as macrophages upon neuronal death. Such intimate functional relationships suggest a logical link of gliogenesis with neurogenesis through induction or by lineage. Indeed neurons and glia are known to arise from common multipotent precursors in both vertebrates and invertebrates (Turner and Cepko, 1987; Luskin et al., 1988; Udolph et al., 1993; Condron and Zinn, 1994). Signals that separate glial and neuronal lineages are largely unknown.

In *Drosophila*, development of CNS as well as PNS glial cells is dependent on the expression of *glial cells missing* (*gcm*) (also called *glide*) (reviewed by Anderson, 1995). *gcm* encodes a transcription factor that is sufficient to activate the glial fate through regulation of the expression of its downstream target genes (Hosoya et al., 1995; Jones et al., 1995; Schreiber et al., 1997). *reversed polarity* (*repo*), a glia-specific homeobox gene (Campbell et al., 1994; Xiong et al., 1994; Halter et al., 1995), is a good candidate for a direct target of *gcm*, as it contains multiple GCM-binding sites in the 5′ upstream region (Akiyama et al., 1996). In *gcm* mutants, presumptive glial cells fail to differentiate, and are often transformed toward neurons. Thus, *gcm* acts as a binary switch between glial and neuronal cell fates and its transcriptional regulation plays a crucial role in their binary decisions.

Within the glial determination pathway, the *gcm* gene currently occupies the most upstream position (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996; Schreiber et al., 1997). This suggests that *gcm* transcription is regulated by a combination of factors that themselves are not specific to glia. The *gcm* promoter may integrate a set of developmental signals that are identical in all *gcm*-positive glia. Alternatively, each glial subtype may have its own regulatory system, using various developmental cues differently depending on their context. Distinguishing these possibilities requires a comparative analysis of *gcm* regulation in multiple glial subtypes.

The transmembrane receptor Notch is used in many developmental contexts for determination of binary cell fates, such as asymmetric cell divisions (reviewed by Artavanis-Tsakonas et al., 1999). Dividing cells can receive a cell-intrinsic cue by Numb, a membrane protein containing a phosphotyrosine-binding domain, that binds Notch and represses Notch signaling in one of the daughter cells (Uemura et al., 1989; Posakony, 1994; Guo et al., 1995; Jan and Jan, 1995). Recently, a role for Notch signaling in glial

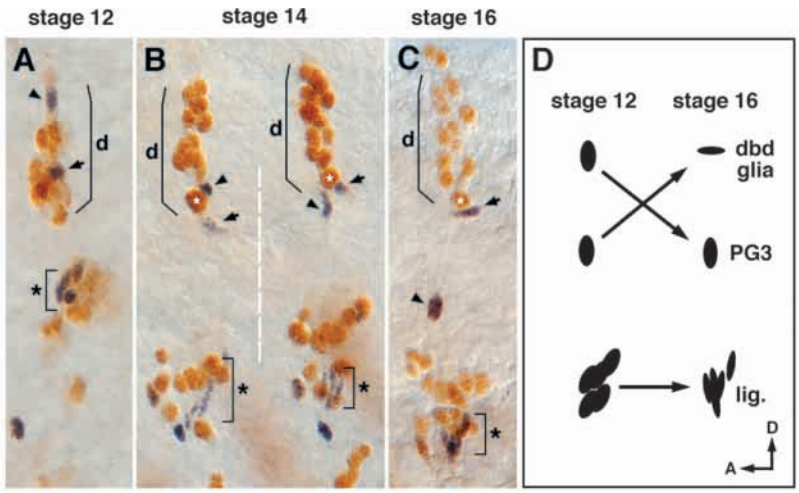

Fig. 1. Developmental dynamics of glial cells in normal embryonic PNS. (A-C) Double labeling using a glial marker REPO (black) and a neuronal marker ELAV (orange) in the PNS (single abdominal segment; dorsal up, anterior to left). Three types of REPO-positive glial cells are present in an abdominal hemisegment of the embryonic PNS; one dbd support glial cell (DBDG), one PG3 cell (arrowhead) and five ligament cells of the lateral chordotonal organ (black asterisk). (A) Stage 12 embryo. (B) Stage 14 embryo. (C) Stage 16 embryo. The dbd neuron (white asterisk) can be identified at the dorsal side of the DBDG in late stage embryos. d, dorsal cluster of sensory neurons. (D) Summary of developmental dynamics of the REPO-positive glial cells (black) (single abdominal segment; dorsal up, anterior to left). PG3 undergoes extensive ventral migration between stage 12 and stage 16. The dorsoventral positions of the PG3 and DBDG are reversed between these two stages. lig., ligament cells.

differentiation was found in the adult PNS; Notch signaling negatively regulates *gcm* expression and glial cell differentiation during asymmetric division in the bristle lineage (Van De Bor and Giangrande, 2001). Given the ability of Notch to function in many developmental decisions, Notch activation may be an obligatory signal in repressing *gcm* transcription in asymmetric divisions that generate glia. However, a previous report suggested an opposite role for Notch signaling in glial differentiation in the embryonic PNS. The dorsal bipolar dendritic (dbd) lineage consists of one neuron and a glial cell, where glial differentiation depends on *gcm* activity (Bodmer et al., 1989; Brewster and Bodmer, 1995; Jones et al., 1995). In this lineage the *numb* mutation shows a double-glia phenotype at the expense of the neuron (Brewster and Bodmer, 1995). If Numb acts by repressing Notch activity in the dbd lineage, this result would imply that Notch promotes, rather than represses, glial development in this lineage.

In this study, we present direct evidence that Notch positively regulates glial differentiation in the dbd lineage. Our data indicate that Notch signaling activates *gcm* expression in one of the two sibling cells and acts postmitotically to specify the glial fate. Thus, the effect of Notch signaling on *gcm* transcription is reversed in the bristle lineage compared to the dbd lineage. We identified an additional lineage where Notch promotes glial fate, and have shown that a molecular context similar to that of the dbd lineage works during asymmetric cell division. Our data indicates that one of the factors that provides Notch-dependent gliogenic context is likely the POU-domain protein Nubbin/PDM-1.

MATERIALS AND METHODS

Fly stocks

Canton-S was used as the normal strain. The following mutant stocks were used in this study: *Notch*^{55e11} (Simpson, 1994), *sanpodo*^{C55} (Dye et al., 1998), *numb*^{1} (Uemura et al., 1989), *sc*^{B57} (Lindsley and Zimm, 1992) and *gcm*^{e1} (Hosoya et al., 1995). *UAS-N*$^{\Delta B2a2}$ (=*UAS-Notch*^{act}) (Doherty et al., 1996), *UAS-gcm* (Hosoya et al., 1995) and *UAS-nub* (=*UAS-pdm-1*) (Neumann and Cohen, 1998) strains were used for ectopic expression by crossing these lines with the *C155-GAL4* effector line that directs expression in all embryonic neurons (Lin and Goodman, 1994). *UAS-nub UAS-N*$^{\Delta B2a2}$ strain was produced by chromosome recombination. *p12xSu(H)bs-lacZ* strain (Go et al., 1998) was used to visualize the Su(H)-dependent Notch activity, and is referred to as Su(H)-reporter in the text.

Embryo staining

Antibody staining was carried out as described previously (Ito et al., 1995). The following primary antibodies were used: rabbit anti-REPO (Halter et al., 1995) (a gift from G. M. Technau) at 1:500; rat anti-REPO (Yuasa et al., personal communication) (a gift from H. Okano) at 1:500; rabbit anti-Nubbin/PDM-1 (Yeo et al., 1995) (a gift from W. Chia) at 1:500; mouse anti-ELAV (9F8A9; Developmental Studies Hybridoma Bank) at 1:100; mouse mAb 22C10 (Fujita et al., 1982) at 1:100-200 and mouse mAb anti-β-galactosidase (40-1a; Developmental Studies Hybridoma Bank) at 1:100-200. Secondary antibodies used were biotinylated goat anti-rat, anti-rabbit, anti-mouse (Vector Laboratories), FITC-conjugated goat anti-rat and Cy3-conjugated goat anti-rabbit (Jackson) antibodies, all at 1:200. Biotinylated secondary antibodies were detected using the ABC elite kit (Vector Laboratories). Double labeling involving horseradish peroxidase (HRP) histochemistry was performed using diaminobenzidine as a substrate, with NiCl for the first staining, and without NiCl for the second staining. Immunofluorescence was viewed with a BioRad MRC 1024 confocal microscope. Mutant embryos were identified by the lack of anti-β-galactosidase staining from the balancer chromosome or by their typical phenotypes reported previously. Whole-mount in situ hybridization was performed essentially as described previously (Lehmann and Tautz, 1994). Digoxigenin-labeled RNA probes were generated from full-length *gcm* cDNA (Hosoya et al., 1995) and from full-length *repo* cDNA (Xiong et al., 1994) (a gift from H. Okano). Fluorescence-labeled RNA probe was generated from 1.2 kb PCR fragment derived from *nubbin*/*pdm-1* cDNA. TSA system (NEN Life Science Product) was used for the fluorescence-labeled RNA detection. Images were processed using Photoshop software (Adobe).

RESULTS

Postmitotic activation of *gcm* determines glial fate in the dbd lineage

Three types of REPO-positive glial cells are present in each abdominal hemisegment of the embryonic PNS; one dbd support glial cell (DBDG), one PG3 glial cell and five ligament cells of the lateral chordotonal organ (Campbell et al., 1994; Xiong et al., 1994; Halter et al., 1995) (Fig. 1). DBDG and the dbd neuron can be reliably identified by their location and characteristic cell morphologies at stage 16 (Bodmer et al.,

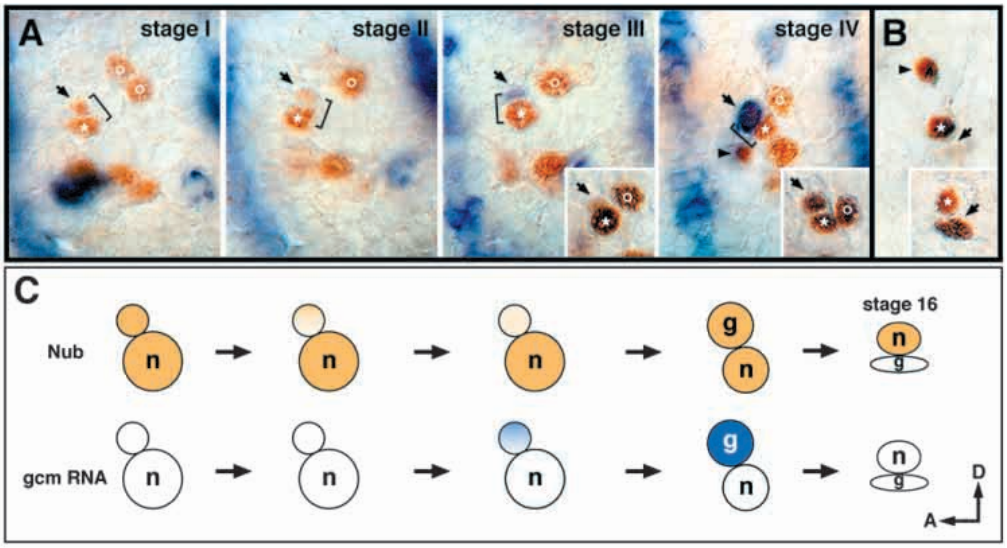

Fig. 2. Developmental sequence of gene expression patterns in the dbd lineage. (A,B) Double labeling of *gcm* mRNA (purple) and Nubbin (orange) in the embryonic PNS (single abdominal segment; dorsal up, anterior to left). The dbd lineage is bracketed. The behavior of Nubbin-positive cells located dorsal to the dbd lineage (white circle) acts as a temporal indicator of embryogenesis. The Nubbin expression in this cell is absent by stage 13. We tentatively refer to the processes of the glial fate induction as stages I-IV. (stage I) Nubbin expression is initially detected in both daughter cells at the beginning of stage 12. The smaller daughter cell (arrow), located apicodorsally to the larger daughter cell differentiates as DBDG. The presumptive dbd neuron is indicated by an asterisk. (stage II) Nubbin becomes down-regulated in the presumptive glial cell prior to the onset of the *gcm* expression. (stage III) The expression of *gcm* is initiated in the smaller daughter cell where Nubbin expression is low (arrow). (stage IV) After *gcm* becomes highly activated, Nubbin is re-expressed in the glial daughter cell (arrow). Single labeling of Nubbin in the dbd lineage is shown in inset for stages III and IV. The dda neuron (out of focus) is indicated by an arrowhead. (B) At stage 16, Nubbin expression is again restricted to the dbd neuron. Double labeling of REPO (black) and Nubbin (orange) in the dbd lineage at stage 16 is shown in the inset. Strong REPO expression is detected in DBDG, in which *gcm* expression has already disappeared (arrow). The dda neuron is located dorsal to the dbd lineage (arrowhead). In this stage, Nubbin expression is detected in the dbd neuron, dda neuron and ligament cells within the PNS. (C) Summary of the expression dynamics of *gcm* mRNA and Nubbin in the dbd lineage. g, glial cell; n, neuron.

1989; Brewster and Bodmer, 1995) (Fig. 1C). However, the pattern of cell division in the dbd lineage remained unclear because of the lack of useful markers. We found that the expression of a POU domain protein Nubbin (also called PDM-1) enables a developmental analysis of the dbd lineage (Fig. 2A). The sensory organ precursor (SOP) of the dbd lineage could be identified at the beginning of stage 12 as a large, weakly Nubbin-expressing cell in the anterior-dorsal region of the abdominal segments (data not shown). This cell soon divided asymmetrically, producing two Nubbin-positive daughters, the larger one located basal to the smaller one. The larger cell expressed neuronal marker protein ELAV and differentiated as the dbd neuron, whereas the smaller daughter expressed REPO, migrated dorsally and became DBDG. No further cell division was observed in this lineage.

While the dbd neuron continued to express Nubbin, its sibling glial cell underwent a rapid change in Nubbin expression during gliogenesis (Fig. 2A,C). Shortly after mitosis, Nubbin was temporally down-regulated in the presumptive glial cell, and *gcm* mRNA expression was initiated. Upon establishment of high *gcm* activation, Nubbin was re-expressed in the glial daughter cell. *gcm* expression remained at high levels during stage 12 and then disappeared rapidly. Subsequently, Nubbin was again down-regulated in DBDG, resulting in expression specific to the dbd neuron at stage 16 (Dick et al., 1991; Lloyd and Sakonju, 1991) (Fig. 2B). The final down-regulation of Nubbin in DBDG was dependent on *gcm* function, whereas the preceding modulation occurred normally in *gcm* mutant embryos (data not shown).

Notch activity is restricted to the glia in the dbd lineage

To assess the role of Notch in the binary fate decision of the dbd lineage, we first analyzed Notch activity within this lineage. It is known that activation of Notch results in the nuclear translocation of an intracellular domain of Notch together with a transcription factor Suppressor of Hairless [Su(H)] (reviewed by Honjo, 1996; Weinmaster, 1997; Bray, 1998). We used transgenic lines containing a *lacZ* reporter construct driven by the *E(spl)mγ* promoter fused to multimerized Su(H)-binding sites, which we refer to as the Su(H)-reporter. This line allows effective visualization of a direct response to the Su(H)-dependent Notch activity in vivo (Go et al., 1998); in the *sanpodo* mutant, the phenotype of which mimicks the loss of Notch activity (Park et al., 1998; Skeath and Doe, 1998), the Su(H)-reporter activity was dramatically reduced throughout the embryo, as it was in *Notch* mutant embryos (Fig. 3A-C). During normal development of the dbd lineage, strong Su(H)-reporter activity was observed in the glial cell but not in the dbd neuron (Fig. 3D). When we expressed a constitutively active form of Notch (*UAS-Notch^{act}*) in all neurons, the Su(H)-reporter became activated in the presumptive dbd neuron (Fig. 3E). Conversely, in the *sanpodo* mutation, the reporter expression was undetectable in the presumptive DBDG (Fig. 3F). Since the *sanpodo* mutation produces a double-neuron phenotype at the expense of the glial cell (Dye et al., 1998), the reporter expression correlates with the glial fate. In contrast, forced *gcm* expression in the presumptive neuron failed to activate the Su(H)-reporter even though neuron-to-glia transformation took place (Jones et al., 1995) (Fig. 3G). Thus Notch activity is likely to be upstream of *gcm* expression and glial differentiation.

dbd glial differentiation fails in *Notch* mutant embryos

To test the requirement of Notch in embryonic PNS glial development, we examined REPO expression in *Notch* mutant

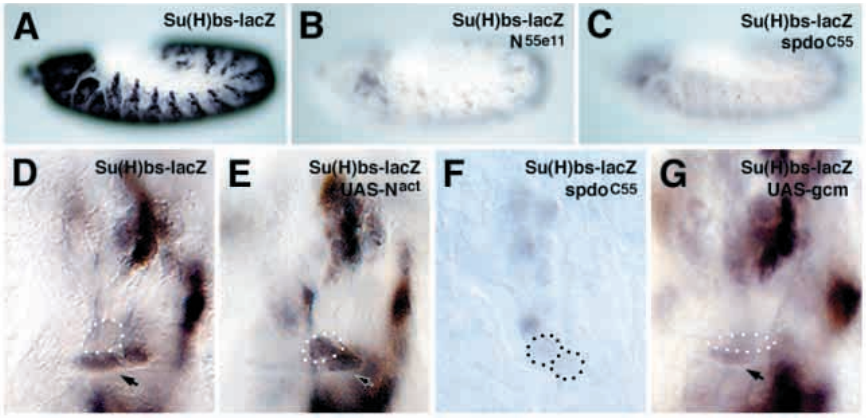

Fig. 3. Visualization of Notch-dependent Su(H)-reporter activity (*p12xSu(H)bs-lacZ*) in embryos (single abdominal segment; dorsal up, anterior to left). (A-C) The reporter *lacZ* expression in stage 12 embryos. (A) A normal control embryo. (B) *Notch*^{55e11} mutant embryo. (C) *sanpodo*^{C55} mutant embryo. The reporter gene activity was dramatically reduced in B and C. (D-G) The *lacZ* reporter expression in the dbd lineage of stage 16 embryos. Arrows in D, E and G indicate DBDG. (D) In normal embryos strong nuclear staining is observed in DBDG but not in the neuron (encircled by dots). (E) When a constitutively active form of Notch was expressed in neurons (*UAS- Notch*^{act}/*C155-GAL4*), ectopic Su(H)-reporter activity was observed in the dbd neuron (dotted circle). (F) In the *sanpodo* mutation, which produces a double-neuron phenotype at the expense of the glial cell, the reporter activity is undetectable in cells of the dbd lineage cells (dotted circle). (G) Misexpression of *gcm* in neurons (*UAS-gcm*/*C155-GAL4*) does not activate the reporter in the dbd neuron that is transformed to a glial cell (dotted circle).

embryos. Since Notch is required for lateral inhibition, its removal leads to production of excess neuronal precursors in the PNS as well as in the CNS (reviewed by Artavanis-Tsakonas et al., 1995; Campos-Ortega, 1995). Indeed, at the earliest stage of the dbd SOP division, excess Nubbin-positive cells were located at the correct position, suggesting that supernumerary dbd SOPs were produced (data not shown). However, at stage 12 no REPO-positive cells were associated with Nubbin-positive neurons, indicating the absence of DBDG (Fig. 4B). This requirement for *Notch* in gliogenesis was specific to the dbd lineage, as supernumerary REPO-positive PG3 and ligament cells were present in *Notch* mutant embryos (Fig. 4B).

To test whether the defects in glial development within the dbd lineage arose through the misregulation of *gcm*, we analyzed *gcm* mRNA expression during stage 12, when its expression level is highest. No *gcm* expression was found within the dbd lineage of *Notch* mutant embryos, whereas PG3 and ligament cells were *gcm* positive (Fig. 4D). These results indicate that there is a specific requirement of Notch activity for *gcm* expression in the dbd lineage.

Notch pathway activates glial fate

If Notch activity plays an instructive role in gliogenesis, artificial activation of Notch in the presumptive neuron may cause a neuron-to-glia transformation. When constitutively active Notch was expressed in all neurons, the dbd neuron was replaced by an extra REPO-positive cell that was associated with and resembled a glial cell (83% of hemisegments; n=42, Fig. 5B). In contrast, expression levels of neuronal markers ELAV (data not shown) and Nubbin were dramatically reduced in the dbd lineage (Fig. 5F). Accompanying glial transformation of the presumptive dbd neuron to a glial cell, *gcm* was ectopically expressed in this cell. *gcm* responded quickly to Notch activation; at stage 12, *gcm* mRNA was already detectable in the presumptive neuron, coinciding with the expression of the driver construct (Fig. 5J). Although normal expression of *gcm* in the glial cell was transient, ectopic *gcm* that was induced by constitutively active Notch continued to at least stage 16 (Fig. 5L). This is unlikely to be due to

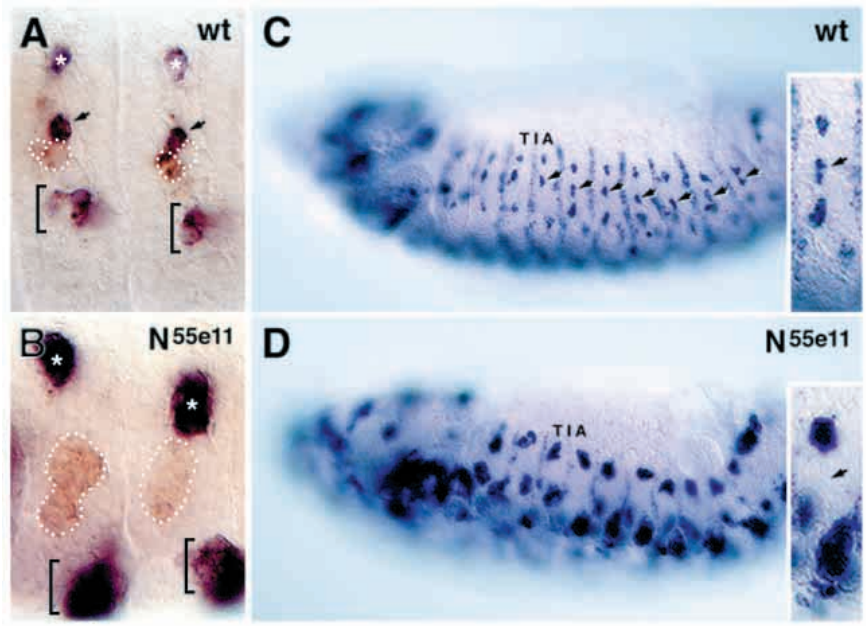

Fig. 4. Loss of DBDG in *Notch* mutants. (A,B) Double labeling of *repo* mRNA (purple) and *nubbin* mRNA (orange) in stage 12 embryos. (A) Two *nubbin*-positive cells (dotted circle; one is the dbd neuron and the other is the dda neuron) are associated with a glial cell (arrow) in normal embryos. (B) In *Notch*^{55e11} mutant embryos, increased numbers of *nubbin*-positive neurons (encircled by dotted line) are present, but no DBDG is generated. The *Notch* mutant shows a neurogenic phenotype, resulting in an excess of *repo*-positive cells such as PG3 glial cell (asterisk) and ligament cells (bracket) in the PNS. *gcm* mRNA expression in a normal embryo (C) and a *Notch*^{55e11} mutant (D) at the same stage shown in A and B. *gcm* expression in the dbd lineage (C, arrows) is absent in the *Notch* embryo (D, arrow). Insets in C and D are higher magnification views of one parasegment. Dorsal is up and anterior to left.

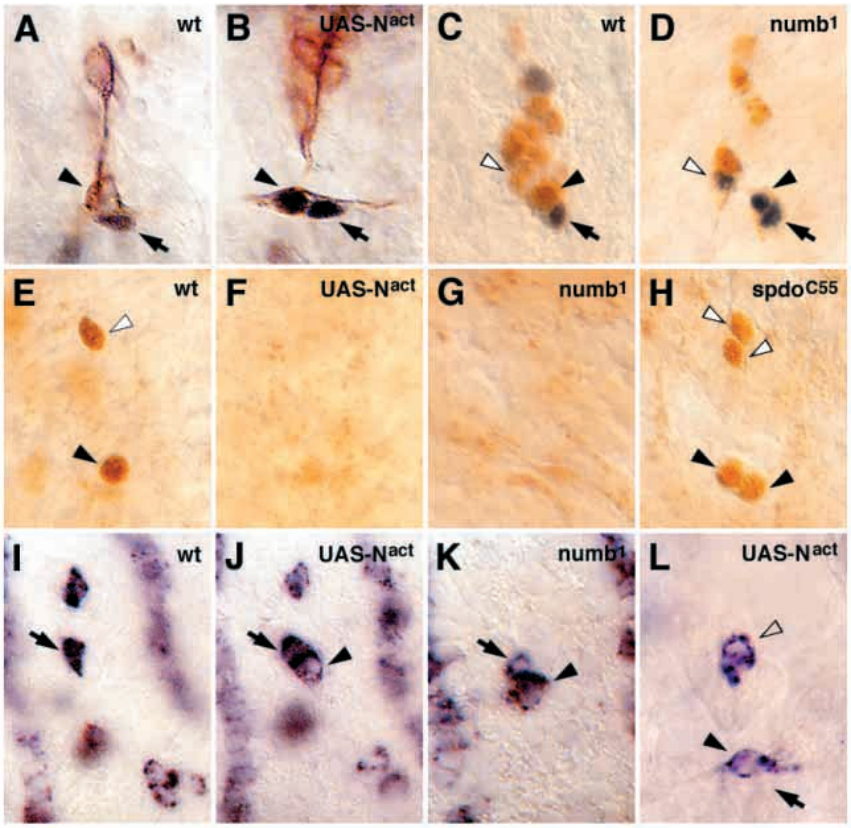

Fig. 5. Roles of the Notch signaling pathway in the dbd and dda lineages. (A,B) The dbd lineage in stage 16 embryos (single abdominal segment; dorsal up, anterior to left). The dorsal cluster of sensory neurons are stained brown (cytoplasm) with mAb 22C10. The dbd neuron is indicated by an arrowhead. DBDG (arrow) is stained black with anti-REPO (nucleus). (A) Normal embryo. (B) Ectopic REPO expression is observed in the nucleus of the dbd neuron when a constitutively active form of Notch is expressed in neurons (*UAS-Notch^{act}/C155-GAL4*). Note that the morphology of the neuron is also transformed towards that of a glial cell. (C,D) Double labeling of REPO expression (black) and ELAV expression (orange) in the dorsal cluster of PNS. (C) Stage 13 normal embryo. (D) Stage 13 *numb^{1}* embryo. The dda neuron (white arrowhead) and the dbd neuron (black arrowhead) ectopically express REPO. ELAV expression is still observed in the cytoplasm of the dda neuron as well as in the dbd neuron, but not in DBDG (arrow). (E-H) Nubbin expression in the dorsal cluster of sensory neurons at stage 16. In normal embryos (E), Nubbin expression is restricted to the dbd neuron (black arrowhead) and the dda neuron (white arrowhead). Both neurons are absent in *C155-GAL4/UAS-Notch^{act}* embryos (F), and in *numb^{1}* embryos (G). Both neurons are duplicated in *sanpodo^{C55}* embryos (H). (I-K) *gcm* mRNA expression in the dbd lineage at stage 12 (single abdominal segment. dorsal up, anterior to left). (I) *gcm* expression is observed in the presumptive DBDG (arrow) of normal embryos. (J) *C155-GAL4/UAS-Notch^{act}* gain-of-function embryo. Ectopic *gcm* expression is observed in the presumptive dbd neuron (arrowhead). (K) *numb^{1}* mutant embryo. Ectopic *gcm* expression is observed in the presumptive dbd neuron (arrowhead). In contrast, *gcm* expression is absent in the presumptive PG3 and the ligament cells. (L) *C155-GAL4/UAS- Notch^{act}* embryo at stage 16 (single abdominal segment; dorsal up, anterior to left). Ectopic *gcm* expression is maintained in the dbd neuron that is transformed into a glial cell (black arrowhead). *gcm* expression in endogenous DBDG has already disappeared by this stage (arrow). An extra *gcm*-positive cell (open arrowhead) is also observed in the dorsal cluster of sensory neurons.

autoregulation, because forced expression of *gcm* in the dbd neuron did not activate transcription of a *lacZ* reporter gene inserted into the *gcm* locus (data not shown). These data indicate that within the dbd lineage Notch activation is sufficient for inducing *gcm* expression.

Our misexpression experiment also offers an explanation for the double-glia phenotype of *numb* mutants within the dbd lineage (Brewster and Bodmer, 1995) (Fig. 5D). We found that in *numb* mutant embryos, *gcm* was misexpressed in the presumptive dbd neuron (Fig. 5K). In contrast, Nubbin expression was absent in the presumptive dbd neuron (84% of hemisegments; n=49, Fig. 5G). Since this phenotype is identical to the result of an artificial activation of Notch in neurons, it is likely that Numb represses Notch activity in the neuronal daughter upon asymmetric division of the dbd SOP. Thus both in the adult bristle lineage (Van De Bor and Giangrande, 2001) and in the dbd lineage, Numb causes asymmetric activation of Notch in two daughter cells. However, the effect of Notch activation is context dependent; in the bristle lineage Notch activity results in the repression of *gcm* expression, whereas in the dbd lineage Notch induces *gcm* transcription.

The Notch pathway can activate *gcm* transcription outside the dbd lineage

While the dbd lineage is the only place in the embryonic PNS where Notch activity is necessary for glial development, we discovered that artificial activation of Notch can initiate gliogenesis outside this lineage. When constitutively active Notch was expressed in all neurons, often two REPO-positive cells formed in the dorsal cluster of sensory organs (Fig. 6A). One of these cells is the transformed dbd neuron, as discussed above. We identified that another cell was derived from the dorsal dendritic arbor (dda) organ (Lloyd and Sakonju, 1991; Brewster et al., 2001) (Fig. 6A), and focused our analysis on this dda lineage.

The dda lineage has many similarities with the dbd lineage. While most embryonic sensory organs form as a result of the proneural activities of the bHLH proteins encoded by the *ASC* (*Achaete-scute Complex*) genes, both dbd and dda organs form independently of *ASC* genes, and require the proneural activity of *absent md neurons and olfactory sensilla* (*amos*) (Huang et al., 2000) (Fig. 6B). In addition, both dbd and dda SOPs express Nubbin. Just as in the dbd lineage, the dda SOP was seen to divide asymmetrically, and both daughters initially had high levels of Nubbin (Fig. 6B). One of the sibling cells continued to express Nubbin and differentiated into a dda neuron (Fig. 5E). In the other sibling cell, *gcm* and REPO expression was induced and Nubbin expression was down-regulated (Fig. 6B). Unlike the dbd glial cell, however, REPO expression was not maintained in the dda lineage and became

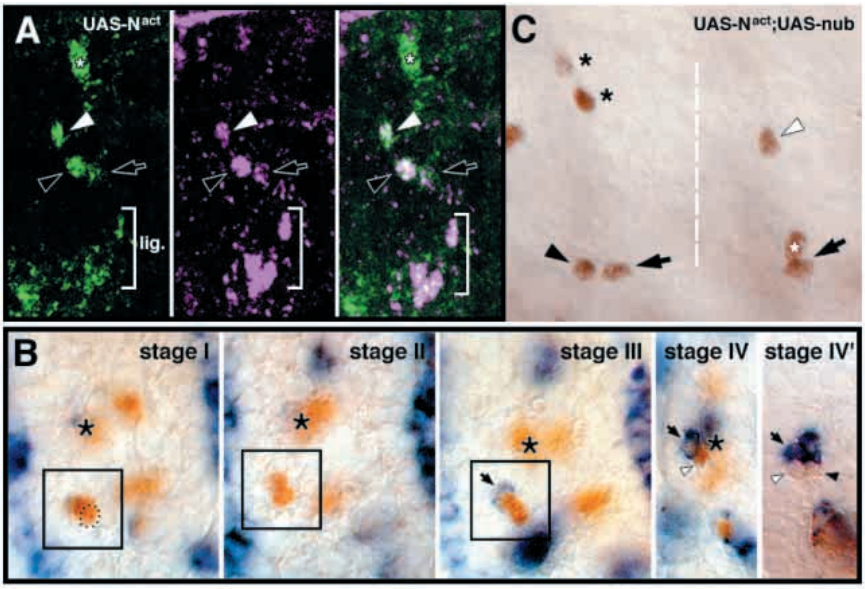

Fig. 6. The dda lineage displays similarities with the dbd lineage. (A) Effects of Notch activation in the PNS at stage 14. Confocal image of *C155-GAL4*/*UAS-Notch*^{act} embryo. (Left) REPO (green), (middle) Nubbin (purple), (right) merged images. Ectopic REPO expression is observed in the dbd neuron (black arrowhead) and the dda neuron (white arrowhead). Nubbin expression is observed in DBDG (arrow) and in the ligament cells (Dick et al., 1991; Lloyd and Sakonju, 1991), but not in the PG3 (asterisk). lig., ligament cells. (B) Developmental sequence of asymmetric cell division in the dda lineage. Double labeling of *gcm* mRNA (purple) and Nubbin (orange) in stage 12 PNS (single abdominal segment; dorsal up, anterior to left). The dda lineage is boxed. The position of the dbd lineage (out of focus) is indicated by an asterisk. (stage I) Both daughter cells of the dda SOP express Nubbin. The smaller daughter cell (dotted circle) is located apical to the large daughter cell. (stage II) Two symmetrical daughter cells expressing Nubbin are observed. At this stage there is no detectable expression of *gcm* in the dda lineage. (stage III) One of the daughter cells transiently accumulates *gcm* mRNA (arrow). (stage IV) Two cells constituting the dda lineage migrate dorsally and are situated anterior to the dbd lineage. Nubbin expression in the presumptive glial cell becomes significantly down-regulated (arrow). Nubbin is never re-expressed in this cell, unlike the presumptive glial cell in the dbd lineage. (stage IV′) *repo* mRNA (purple) and ELAV (orange) expression in *ASC* mutant at stage IV. In this genetic background the dorsal cluster contains only dda and dbd lineages (Brewster et al., 2001). Weak expression of ELAV is detected in the presumptive dda neuron (white arrowhead) and the dbd neuron (black arrowhead) at this stage. (C) REPO expression in *C155-GAL4*/*UAS- Notch*^{act}, *UAS-nub* embryo at stage 16. Ectopic REPO expression is observed in the presumptive dbd neuron (black arrowhead), as well as in two cells (black asterisk) that occupy the position dorsal to the glial-transformed dda neuron (white arrowhead). White asterisk indicates PG3 cell; arrow, DBDG. PG3 of the left parasegment is out of focus.

undetectable by stage 16 (Fig. 1). The final fate of the sibling of the dda neuron is not known.

To analyze the role of Notch signaling within the dda lineage, we examined the effects of removing *sanpodo* and *numb* activities. In normal embryos a single dda neuron can be found dorsal to the dbd organ in each abdominal hemisegment at stage 16. In *sanpodo* mutant embryos, duplication of the dda neuron was frequently observed (71% of hemisegments; *n*=49, Fig. 5H), whereas in *numb* mutants they were absent (63% of hemisegments; *n*=49, Fig. 5G). Thus, as in the dbd lineage, Notch activity represses neuronal development in the dda lineage. In *numb* mutant embryos, we detected ectopic *gcm* and REPO expression in the presumptive dda neurons (34% of hemisegments; *n*=41, Fig. 5D and data not shown), a phenotype mimicking the artificial activation of Notch in neurons (Fig. 5L, Fig. 6A). We conclude that Notch signaling can activate *gcm* transcription not only in the dbd lineage, but also in the dda lineage.

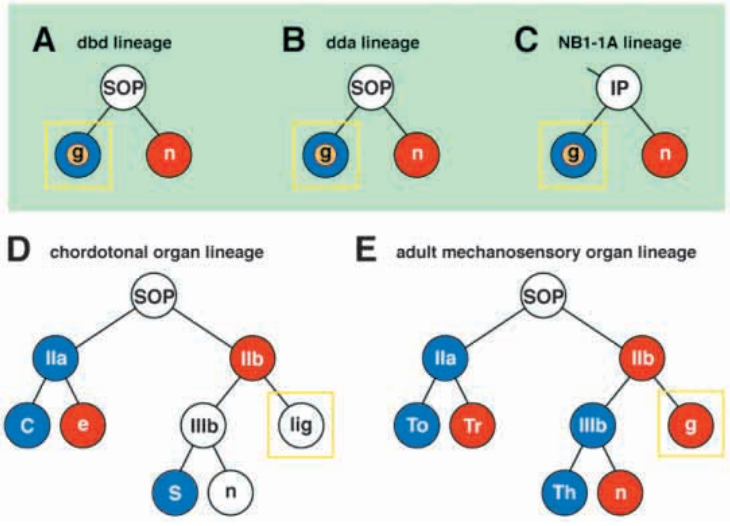

Fig. 7. Comparison of Notch-dependent and Notch-independent gliogenic lineages. Schematic representations of five gliogenic lineages in the CNS (C) and PNS (A,B,D,E). The cells in which Notch is likely to be active are shown in blue. This assignment is based on the expression of the Su(H)-reporter gene (this work), and the mutant phenotypes of *Notch* and *sanpodo* embryos. The cells that receive Numb protein upon division, or those whose fate requires *numb* function, are shown in red. The *gcm/repo*-positive cell is boxed in yellow. Nubbin expression is shown in orange. (A-C) Three independent lineages in which Notch activates glial fate (this work) (Udolph et al., 2001). In all three lineages Nubbin is expressed in the presumptive glial cell before *gcm* expression initiates in that cell.
(D) Chordotonal organ lineage (Orgogozo et al., 2001). In the chordotonal lineage, the ligament cell neither expresses Su(H)-reporter nor requires Notch activity for its fate specification. It expresses Nubbin only after REPO expression is observed in this cell, contrasting with the situation in three lineages shown in A-C. Whether there is a requirement or localization of Numb in IIIb, the ligament cell and the neuron is not known.
(E) Lineage in which Notch represses glial fate (Van De Bor and Giangrande, 2001). Whether Nubbin is expressed in this lineage is unknown. SOP, sensory organ precursor cell; IP, intermediate precursor; IIa, IIb, secondary precursor cells; IIIb, tertiary precursor cell; g, glial cell; n, neuron; C, cap cell; e, ectodermal cell; S, scolopale cell; lig, ligament cell; To, tormogen cell; Tr, trichogen cell; Th, thecogen cell.

The specific response of dbd and dda lineages to Notch activation suggests that factors specifically expressed in these lineages may provide the developmental contexts that allow Notch activation to be interpreted as a gliogenic signal. Although the proneural gene *amos* is specifically expressed in these two lineages, its expression is transient and is absent by the time of the SOP division (Huang et al., 2000). Furthermore, coexpression of AMOS and constitutively active Notch in all neurons did not generate any additional glial cells compared to Notch activation alone (data not shown). Another candidate is the POU-domain protein Nubbin. In both dbd and dda lineages, Nubbin expression is initiated in the SOP and high levels of Nubbin protein are found in both daughter cells after the SOP division (Fig. 2, Fig. 6B). To test whether Nubbin can modify the effect of Notch activation, we coexpressed Nubbin and constitutively active Notch in all neurons. Upon such treatment, a few extra REPO-positive glial cells appeared dorsally to the dda lineage (Fig. 6C). These cells likely correspond to the extra glial cells that have been reported to form upon ectopic expression of GCM in neurons (Jones et al., 1995). This suggests that some of the presumptive neurons in the dorsal cluster were redirected to the glial differentiation pathway upon Notch activation. Such a phenotype was never observed when constitutively active Notch alone or Nubbin alone was expressed (data not shown). We propose that Nubbin is one of the factors that provide a developmental context for Notch-dependent *gcm* expression and glial differentiation.

DISCUSSION

Notch plays context-dependent roles in gliogenesis

We demonstrated that Notch signaling promotes glial fate during asymmetric division in the embryonic dbd lineage. Notch is specifically activated in the presumptive DBDG owing to the negative regulation by Numb in the sibling cell, and provides instructive information to induce *gcm* transcription and glial development. Expression of *gcm* occurs quickly after the artificial activation of Notch, even in cells that have initiated neuronal development. In *gcm* mutants, DBDG are transformed into neurons, although the activation of Notch, visualized by the Su(H)-reporter, is normal in the presumptive glia (data not shown). Likewise, ectopic expression of *gcm* in presumptive dbd neurons caused neuron-to-glia transformation without affecting Notch activity. These findings suggest that *gcm* expression appears to be the sole target of Notch activation in establishing glial fate in the dbd lineage. Within the 3.5 kb region upstream of the *gcm* gene, we identified two sequences perfectly matching the consensus core sites for Su(H) (Bailey and Posakony, 1995; Lecourtois and Schweisguth, 1995). Thus, *gcm* could be a direct target of Su(H), downstream of the Notch signaling pathway.

While our present data demonstrate a positive role for Notch in gliogenesis in the dbd lineage, other embryonic PNS glial cells do not require Notch activity for their formation. For example, in the adult bristle lineage Notch has an opposite function on gliogenesis; that of repressing *gcm* expression and glial development (Van De Bor and Giangrande, 2001). Thus the role of Notch in the regulation of *gcm* expression is context-dependent (Fig. 7). Notch has recently been shown to be a component of combinatorial signaling in cell fate determination in the *Drosophila* eye (Flores et al., 2000). It is possible that Notch signaling has different consequences depending on other factors that act on the same regulatory element.

A common developmental context allows Notch to promote gliogenesis

The context-dependent effect of Notch suggests that the *gcm* promoter may have a modular structure where each unit integrates different developmental signals. However, given the large diversity of glial subtypes in the nervous system, it is unlikely that each glial subtype has its own regulatory sequences and a unique mode of regulation. We favor a model in which *gcm* has a limited number of regulatory elements that respond to developmental signals that are present in multiple environments. Indeed we have shown that a subset of glial subtypes respond in a similar way to Notch signals: in addition to the dbd lineage, the dda lineage can also induce *gcm* transcription upon Notch activation. Comparison of these two lineages offers hints on the nature of the developmental context in which Notch activation causes *gcm* transcription.

One common feature that distinguishes dbd and dda lineages from other PNS lineages is the cell division pattern of their SOP. In dbd and dda lineages, SOPs divide to generate a neuron and a glial cell through an asymmetric division. In other gliogenic PNS lineages, the sibling cells of glial cells are not postmitotic neurons, but tertiary precursors that undergo further division to generate neurons and associated cells (Fig. 7). These observations suggest that an interaction with the neuronal sibling may play a crucial part in promoting the Notch-dependent *gcm* activation during asymmetric cell division. Recently, Notch was shown to positively regulate *gcm* expression in the Neuroblast 1-1A lineage of the CNS, where the sibling pattern is identical to that of the dbd lineage (Udolph et al., 2001) (Fig. 7C). This also supports the idea that the cell division pattern provides a context that determines the effect of Notch activity.

We showed that coexpression of constitutively active Notch with Nubbin also generates ectopic glia outside dbd and dda lineages. This raises the possibility that Nubbin may be a part of the developmental context that allows Notch to promote gliogenesis. Within the embryonic PNS, dbd and dda neurons are the only two neurons that express Nubbin. In both lineages, Nubbin is present in both SOP daughter cells, at the time of glia versus neuron cell fate choice. Furthermore, we detected temporal activation of Nubbin in presumptive glial cells derived from the NB1-1A lineage (data not shown). Nubbin thus might create a permissive environment for the activation of *gcm* expression by the Notch signal (Fig. 7A-C). Since coexpression of Nubbin and constitutively active Notch does not cause glial transformation of all neurons, additional factors must exist that create a Notch-dependent gliogenic context.

Nubbin is a POU-domain transcription factor with sequence-specific DNA-binding activity (Neumann and Cohen, 1998). The contextual role of Nubbin in Notch-dependent expression of *gcm* could employ a similar mechanism to the modulation of Notch activity in wing development, where Nubbin and Su(H) bind on the same enhancer element of Notch target genes (Neumann and

Cohen, 1998). It will be interesting to further analyze the role of Nubbin in gliogenic lineages.

We thank Y. Akiyama-Oda, S. M. Cohen, D. Eastman, K. Matsuno and M. Okabe for fly stocks, and W. Chia, G. M. Technau, Y. Yuasa, and H. Okano for cDNAs and antibodies. The 40-1a monoclonal antibody generated by J. R. Sanes was obtained from the Developmental Studies Hybridoma Bank at The University of Iowa. We also thank K. Agata, L. Tsuda, M. Okabe and the members of our laboratory for helpful discussions, and Ms. M. Sakai, Ms. C. Asaka and Ms. M. Aono for technical assistance. This work was supported by the CREST program of Japan Science and Technology Corporation (Y. Hotta) and grants from the Ministry of Education, Culture, Sports, Science and Technology (Y. Hiromi). Y. Hotta is a CREST researcher and Y. U. is a CREST fellow of Japan Science and Technology Corporation.

REFERENCES

Akiyama, Y., Hosoya, T., Poole, A. M. and Hotta, Y. (1996). The gcm-motif: a novel DNA-binding motif conserved in *Drosophila* and mammals. *Proc. Natl. Acad. Sci. USA* **93**, 14912-14916.

Anderson, D. J. (1995). A molecular switch for the neuron-glia developmental decision. *Neuron* **15**, 1219-1222.

Artavanis-Tsakonas, S., Matsuno, K. and Fortini, M. E. (1995). Notch signaling. *Science* **268**, 225-232.

Artavanis-Tsakonas, S., Rand, M. D. and Lake, R. J. (1999). Notch signaling: cell fate control and signal integration in development. *Science* **284**, 770-776.

Bailey, A. M. and Posakony, J. W. (1995). Suppressor of Hairless directly activates transcription of *Enhancer of split complex* genes in response to Notch receptor activity. *Genes Dev.* **9**, 2609-2622.

Bier, E., Ackerman, L., Baebel, S., Jan, L. and Jan, Y. N. (1988). Identification and characterization of a neuron-specific nuclear antigen in *Drosophila*. *Science* **240**, 913-915.

Bodmer, R., Carretto, R. and Jan, Y. N. (1989). Neurogenesis of the peripheral nervous system in *Drosophila* embryos: DNA replication patterns and cell lineages. *Neuron* **3**, 21-32.

Bray, S. (1998). A Notch Affair. *Cell* **93**, 499-503.

Brewster, R. and Bodmer, R. (1995). Origin and specification of type II sensory neurons in *Drosophila*. *Development* **121**, 2923-2936.

Brewster, R., Hardiman, K., Deo, M., Khan, S. and Bodmer, R. (2001). The selector gene *cut* represses a neural cell fate that is specified independently of the *Achaete-Scute-Complex* and *atonal*. *Mech. Dev.* **105**, 57-68.

Campbell, G., Göring, H., Lin, T., Spana, E., Andersson, S., Doe, C. Q. and Tomlinson, A. (1994). RK2, a glial-specific homeodomain protein required for embryonic nerve cord condensation and viability in *Drosophila*. *Development* **120**, 2957-2966.

Campos-Ortega, J. A. (1995). Genetic mechanisms of early neurogenesis in *Drosophila melanogaster*. *Mol. Neurobiol.* **10**, 75-89.

Campuzano, S. and Modolell, J. (1992). Patterning of the *Drosophila* nervous system: the *achaete-scute* gene complex. *Trends Genet.* **8**, 202-208.

Condron, B. G. and Zinn, K. (1994). The grasshopper median neuroblast is a multipotent progenitor cell that generates glia and neurons in distinct temporal phases. *J. Neurosci.* **14**. 5766-5777.

Dick, T., Yang, X., Yeo, S and Chia, W. (1991). Two closely linked *Drosophila* POU domain genes are expressed in neuroblasts and sensory elements. *Proc. Natl. Acad. Sci. USA* **88**, 7645-7649.

Doherty, D., Feger, G., Younger-Shepherd, S., Jan, L. Y. and Jan, Y. N. (1996). Delta is a ventral to dorsal signal complementary to Serrate, another Notch ligand, in *Drosophila* wing formation. *Genes Dev.* **10**, 421-434.

Dye, C. A., Lee, J., Atkinson, R. C. Brewster, R., Han, P. and Bellen, H. J. (1998). The *Drosophila sanpodo* gene controls sibling cell fate and encodes a tropomodulin homolog, an actin/tropomyosin-associated protein. *Development* **125**, 1845-1856.

Flores, G. V., Duan, H., Yan, H., Nagaraj, R., Fu, W., Zou, Y., Noll, M. and Banerjee, U. (2000). Combinatorial signaling in the specification of unique cell fates. *Cell* **103**, 75-85.

Fujita, S. C., Zipursky, S. L., Benzer, S., Ferrus, A. and Shotwell, S. L. (1982). Monoclonal antibodies against the *Drosophila* nervous system. *Proc. Natl. Acad. Sci. USA* **79**, 7929-7933.

Go, J. M., Eastman, S. D. and Artavanis-Tsakonas, S. (1998). Cell proliferation control by Notch signaling in *Drosophila* development. *Development* **125**, 2031-2040.

Guo, M., Bier, E., Jan, L. Y. and Jan, Y. N. (1995). *tramtrack* acts downstream of *numb* to specify distinct daughter cell fates during asymmetric cell divisions in the *Drosophila* PNS. *Neuron* **14**, 913-925.

Halter, D. A., Urban, J., Rickert, C., Ner, S. S., Ito, K., Travers, A. A. and Technau, G. M. (1995). The homeobox gene *repo* is required for the differentiation and maintenance of glia function in the embryonic nervous system of *Drosophila melanogaster*. *Development* **121**, 317-332.

Honjo, T. (1996). The shortest path from the surface to the nucleus: RBP-Jk/Su(H) transcription factor. *Genes Cells* **1**, 1-9.

Hosoya, T., Takizawa, K., Nitta, K. and Hotta, Y. (1995). *glial cells missing*: a binary switch between neuronal and glial determination in *Drosophila*. *Cell* **82**, 1025-1036.

Huang, M., Hsu, C. and Chien, C. (2000). The proneural gene *amos* promotes multiple dendritic neuron formation in the *Drosophila* peripheral nervous system. *Neuron* **25**, 57-67.

Ito, K., Urban, J. and Technau, G. M. (1995). Distribution, classification, and development of *Drosophila* glial cells in the late embryonic and early larval ventral nerve cord. *Roux's Arch. Dev. Biol.* **204**, 284-307.

Jan, Y. N. and Jan, L. Y. (1995). Maggot's hair and bug's eye: role of cell interactions and intrinsic factors in cell fate specification. *Neuron* **14**, 1-5.

Jones, B. W., Fetter, R. D., Tear, G. and Goodman, C. S. (1995). *glial cells missing*: a genetic switch that controls glial versus neuronal fate. *Cell* **82**, 1013-1023.

Lecourtois, M. and Schweisguth, F. (1995). The neurogenic Suppressor of Hairless DNA-binding protein mediates the transcriptional activation of the *Enhancer of split* complex genes triggered by Notch signaling. *Genes Dev.* **9**, 2598-2608.

Lehmann, R. and Tautz, D. (1994). *In situ* hybridization to RNA. In *Methods in Cell Biology* vol. 44: *Drosophila melanogaster* (eds Goldstein and Fyrberg), pp. 575-598. San Diego: Academic Press.

Lin, D. M. and Goodman, C. S. (1994). Ectopic and increased expression of fasciclin II alters motoneuron growth cone guidance. *Neuron* **13**, 507-523.

Lindsley, D. L. and Zimm, G. G. (1992). The genome of *Drosophila melanogaster*. San Diego: Academic Press.

Lloyd, A. and Sakonju, S. (1991). Characterization of two *Drosophila* POU domains genes, related to *oct-1* and *oct-2*, and regulation of their expression patterns. *Mech. Dev.* **36**, 87-102.

Luskin, M. B., Pearlman, A. L. and Sanes, J. R. (1988). Cell lineage in the cerebral cortex of the mouse studied in vivo and in vitro with a recombinant retrovirus. *Neuron* **1**, 635-647.

Neumann, C. J. and Cohen, S. M. (1998). Boundary formation in *Drosophila* wing: Notch activity attenuated by the POU protein Nubbin. *Science* **281**, 409-413.

Park, M., Yaich, L. E. and Bodmer, R. (1998). Mesodermal cell fate decisions in *Drosophila* are under the control of the lineage genes *numb*, *Notch*, and *sanpodo*. *Mech. Dev.* **75**, 117-126.

Posakony, J. W. (1994). Nature versus nurture: asymmetric cell divisions in *Drosophila* bristle development. *Cell* **76**, 415-418.

Schreiber, J., Sock, E. and Wegner, M. (1997). The regulator of early gliogenesis *glial cells missing* is a transcription factor with a novel type of DNA-binding domain. *Proc. Natl. Acad. Sci. USA* **94**, 4739-4744.

Simpson, P. (1994). *The Notch Receptors.* Austin, Texas: R. G. Landes Company.

Skeath, J. B. and Doe, C. Q. (1998). Sanpodo and Notch act in opposition to Numb to distinguish sibling neuron fates in the *Drosophila* CNS. *Development* **125**, 1857-1865.

Turner, D. L. and Cepko, C. L. (1987). A common progenitor for neurons and glia persists in rat retina late in development. *Nature* **328**, 131-136.

Udolph, G., Prokop, A., Bossing, T. and Technau, G. M. (1993). A common precursor for glia and neurons in the embryonic CNS of *Drosophila* gives rise to segment-specific lineage variants. *Development* **118**, 765-775.

Udolph, G., Rath, P. and Chia, W. (2001). A requirement for *Notch* in the genesis of a subset of glial cells in the *Drosophila* embryonic central nervous system which arise through asymmetric cell divisions. *Development* **128**, 1457-1466.

Uemura, T., Sheperd, S., Ackerman, L., Jan, L. Y. and Jan, Y. N. (1989). *numb*, a gene required in determination of cell fate during sensory organ formation in *Drosophila* embryos. *Cell* **58**, 349-360.

Van De Bor, V. and Giangrande, A. (2001). Notch signaling represses the glial fate in fly PNS. *Development* **128**, 1381-1390.

Vincent, S., Vonesch, J.-L. and Giangrande, A. (1996). *glide* directs glial fate commitment and cell fate switch between neurones and glia. *Development* **122**, 131-139.

Weinmaster, G. (1997). The ins and outs of Notch signaling. *Mol. Cell. Neurosci.* **9**, 91-102.

Xiong, W. C., Okano, H., Patel, N. H., Blendy, J. A. and Montell, C. (1994). *repo* encodes a glial-specific homeo domain protein required in the *Drosophila* nervous system. *Genes Dev.* **8**, 981-994.

Yeo, S. L., Lloyd, A., Kozak, K., Dinh, A., Dick, T., Yang, X., Sakonju, S. and Chia, W. (1995). On the functional overlap between two *Drosophila* POU homeo domain genes and the cell fate specification of a CNS neural precursor. *Genes Dev.* **9**, 1223-1236.