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Asymmetric Division of Neuroglioblasts

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Some neurons and glial cells originate from neuroblasts and glioblasts, stem cells that delaminate from the ectoderm of developing fly embryos. A second class of glial cells and neurons differentiates from multipotent precursors, the neuroglioblasts. The differentiation of both glial cell types depends on glial cell deficient/glial cell missing (glide/gcm). Although it has been shown that this transcription factor promotes gliogenesis at the expense of neurogenesis, the cellular mechanisms underlying this fate choice are poorly understood. Using loss and gain of function *glide/gcm* mutations here we show that the cell fate choice takes place in the neuroglioblast, which divides and produces a glioblast and a neuroblast. Such choice requires the asymmetric distribution of *glide/gcm* RNA, which accumulates preferentially on one side of the neuroglioblast and is inherited by one cell, the presumptive glioblast. Interestingly, glial cells can differentiate from cells that delaminate as neuroglioblasts or they can arise from cells that start expressing *glide/gcm* several hours after delamination of a neuroblast. Altogether, these findings identify a novel type of asymmetric cell division and disclose the lineage relationships between glia and neurons. They also reveal the mode of action of the glide/gcm promoting factor. **© 1999 Academic Press**

Key Words: **glide/gcm; cell fate determinant; gliogenesis; asymmetric division; nervous system.**

INTRODUCTION

One of the most important issues in developmental biology is understanding how cell identity is established. Indeed, although it is known that vertebrate and invertebrate neural stem cells (also called neuroglioblasts) give rise to glial cells and neurons (Leber *et al.*, 1990; Williams *et al.*, 1991; Davis and Temple, 1994; Condron and Zinn, 1994; Bossing *et al.*, 1996; Schmidt *et al.*, 1997), the precise mechanisms by which the glial fate is determined still await to be elucidated.

The fly gene *glial cell deficient/glial cell missing* (*glide/gcm*) encodes a transcription factor that promotes glial differentiation (Hosoya *et al.*, 1995; Jones *et al.*, 1995; Akiyama *et al.*, 1996; Vincent *et al.*, 1996; Bernardoni *et al.*, 1997, 1998; Schreiber *et al.*, 1997; Miller *et al.*, 1998). Lack of glide/gcm results in the transformation of glial cells into neurons while *glide/gcm* ectopic expression in the nervous system leads to the reciprocal phenotype, indicating that this gene directs the fate choice between neurons and glial cells (Hosoya *et al.*, 1995; Jones *et al.*, 1995; Vincent *et al.*, 1996; Bernardoni *et al.*, 1998). *glide/gcm* may act either in differentiated cells by inducing neuronal repressors such as *tramtrack* (*ttk*) (Giesen *et al.*, 1997) or in the stem cell, the neuroglioblast. In order to identify the step at which *glide/gcm* is required, we have analyzed the mutant phenotype and the profile of *glide/gcm* expression at early developmental stages.

Here we show that *glide/gcm* loss and gain of function mutations affect the expression of neuroblast-specific genes. This result clearly indicates that *glide/gcm* represses the neuroblast fate. Strikingly, the glide/gcm product is already present in the neuroglioblast. Furthermore, *glide/gcm* RNA is asymmetrically localized in this cell and preferentially inherited by one of the two daughter cells, the glioblast. Finally, glial cells can differentiate from cells that delaminate as neuroglioblasts or they can arise from cells that start expressing *glide/gcm* several hours after delamination of a neuroblast. Thus, our data

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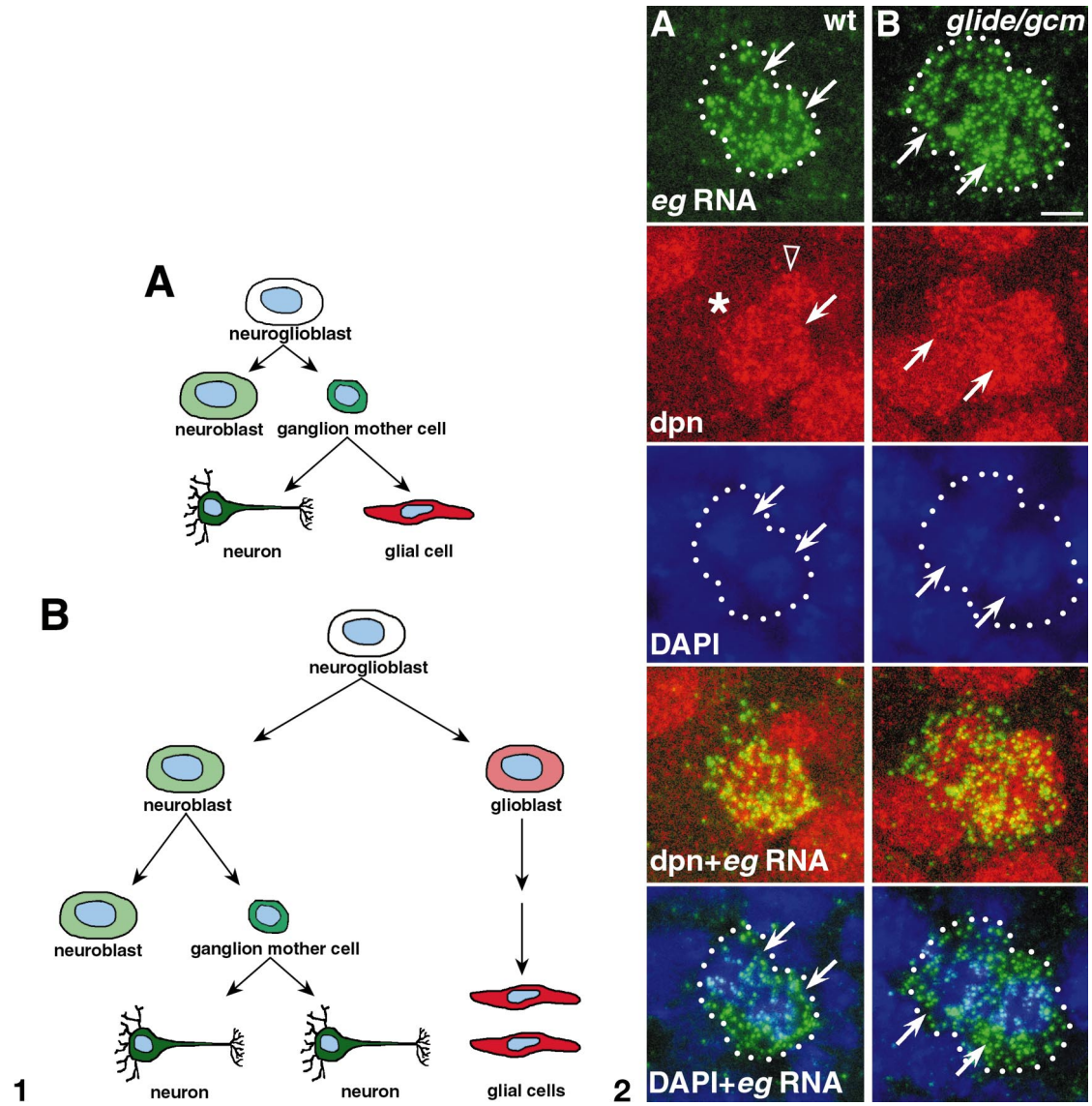

FIG. 1. Models of glial cell differentiation. (A) Asymmetric division of the stem cell (neuroglioblast) produces a neuroblast and a ganglion mother cell, which in turns divides and gives rise to a neuron and a glial cell. (B) Asymmetric division of the neuroglioblast produces a neuroblast and a glioblast, committed precursors of neurons and glial cells, respectively.

FIG. 2. The number of neuroblasts increases in *glide/gcm* embryos. (A, B) Triple labeling of the 6-4 lineage in a thoracic segment: (A) wild-type (wt); (B) *glide/gcm*$^{N7\text{-}4}$ (*glide/gcm*) embryo at early stage 11. Green and red labelings show the distribution of the *eagle* RNA and that of the deadpan protein, respectively. Chromatin is revealed by DAPI blue labeling. Arrows indicate the two daughter cells derived from the first mitosis of the 6-4 stem cell. Note that, in the wild type, one of the two eagle-positive cells does not express *dpn* (asterisk in A), while in the mutant both cells express it (see the two arrows in B). The dotted lines indicate the two eagle-positive cells. An unrelated neuroblast is adjacent to the dpn- and eagle-positive cell (empty arrowhead). In this and in Figs. 3–5, anterior is to the left. Bars (A, B), 3.9 μm.

demonstrate that the decision between glial and neuronal fates takes place at the level of the stem cell, which divides asymmetrically to produce glio- and neuroblasts and that this novel type of cell division is triggered by an intrinsic mechanism.

METHODS

Stocks

Wild-type *Sevelen* stock or the enhancer trap line *rA87* (Jones *et al.*, 1995; Vincent *et al.*, 1996) were used as control. *glide/gcm*$^{N7\text{-}4}$

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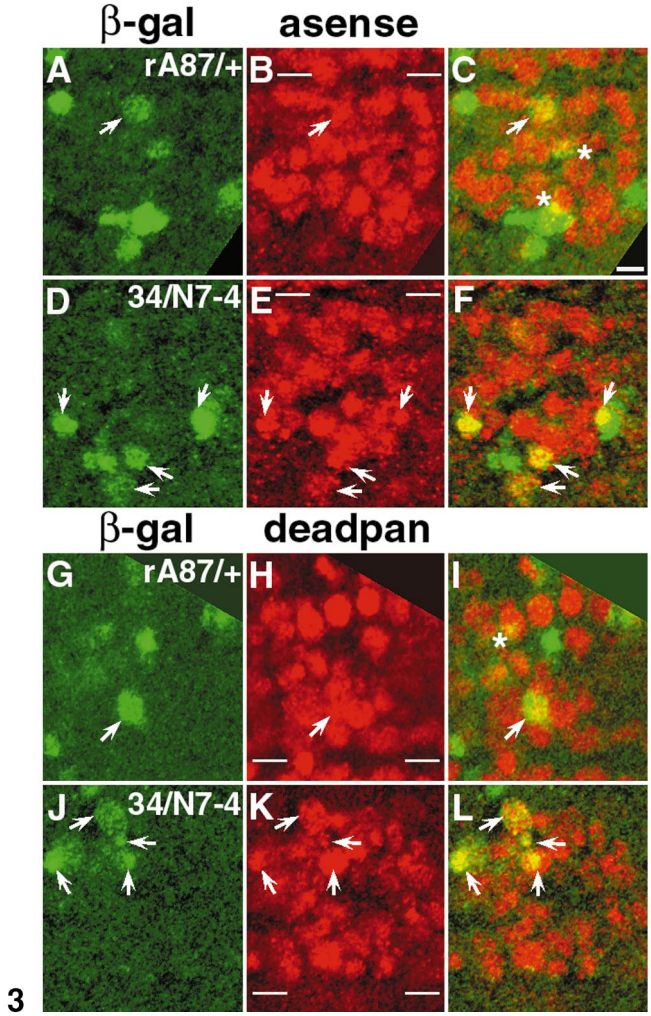

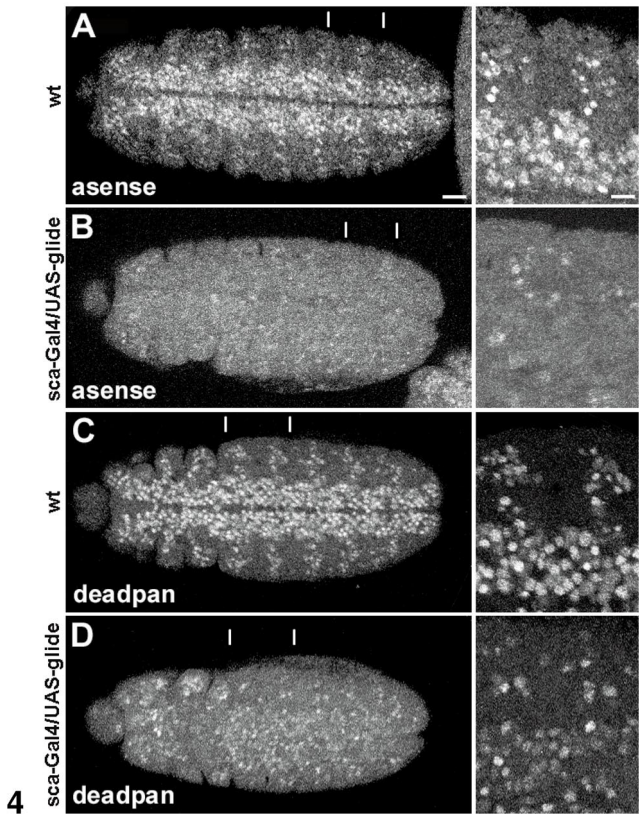

FIG. 3. Glioblasts are transformed into neuroblasts in *glide/gcm* embryos. Ventral views of early stage 12 embryos (first abdominal segment) double labeled with anti-β-gal and anti-ase (A–F) or anti-β-gal and anti-dpn (G–L). Each panel shows the juxtaposition of several optical sections. (A–C, G–I) *rA87/+;* (D–F, J–L) *glide/gcm*$^{N7\text{-}4}$*/glide/gcm*^{34} embryos. Panels C, F, I, and L indicate the double labelings. Thin lines show the position of the midline. Arrows indicate the cells coexpressing the β-gal and one of the two neuroblast-specific markers. The coexpression was verified by analyzing each optical section. Signals that do not really colocalize, i.e., signals that are on different levels, are indicated by asterisks. In the wild type, colocalization also occurs in a second cell that cannot be seen because located at another focal level. Note that the number of coexpressing cells is strongly increased in the mutant (D–F, J–L) compared to the wild type (A–C, G–I), even though the total number of β-gal-expressing cells is reduced in the mutant compared to *rA87/+* embryos. Bar, 7 μm.

FIG. 4. The neuroblast fate is repressed in embryos expressing *glide/gcm* ectopically. Ventral views of early stage 12 (A, C), wild-type (wt), or (B, D) sca-glide (sca-Gal4/UAS-glide) embryos labeled with anti-ase (A, B) or anti-dpn (C, D). Insets show higher magnifications of the regions indicated by the thin vertical bars. Note in B and D the decreased number of ase- and dpn-positive cells. Bars: (A–D) 35.5 μm; (insets) 14.2 μm.

and *glide/gcm*^{34} are null and hypomorphic mutations, respectively, described in Vincent *et al.* (1996). The *UAS-glide/gcm* transgenic line *M24A* (Bernardoni *et al.*, 1998) was crossed with a *scabrous–Gal4* line (*sca-Gal4*) (gift from M. Mlodzik). The genotype of the progeny was recognized using blue balancer chromosomes.

Immunohistochemistry and in Situ Hybridization

Embryos from staged collections (0–10 h after egg laying) were immunolabeled as described in Vincent *et al.* (1996). Antibodies against the following proteins were used at the indicated concentrations: glide/gcm (1:300); deadpan (1:50); asense (1:500); eagle (1:1000); and β-galactosidase (β-gal) (Cappel, Sigma; 1:1000). Secondary antibodies conjugated with Oregon green (Molecular Probes), FITC, Cy3, and Cy5 (Jackson) were used at 1:400.

In situ hybridization on staged embryos was performed according to Hughes and Krause (1998) except for the permeabilization step which was carried out as in Robinow *et al.* (1997). Digoxigenin-labeled riboprobes were prepared using the *glide/gcm* cDNA and a fragment containing the *eagle* third exon. The

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hybridization signal was detected using a sheep anti-digoxigenin primary antibody (Boehringer Mannheim; 1:1000) and a Cy3-conjugated anti-sheep secondary antibody (Jackson; 1:400). The chromatin fluorescent labeling was performed using DAPI at 50 ng/ml in 1× PBS–0.3% Triton X-100. Preparations were analyzed using a confocal (DMRE, Leica) microscope.

RESULTS

The Neuroblast Fate Is Ectopically Activated in glide/gcm Embryos

About 30 neural stem cells per hemisegment delaminate from the embryonic neuroectoderm in five discrete waves (Campos-Ortega and Hartenstein, 1985; Doe, 1992). Seven of these cells have been called neuroglioblasts (NGB) because they give rise to neurons and glial cells (1-1 abdominal, 2-2 thoracic, 1-3, 2-5, 5-6, 6-4 thoracic, 7-4), whereas the others are pure neuronal (neuroblast or NB) or glial (glioblast or GB) precursors (Doe, 1992; Broadus *et al.*, 1995; Bossing *et al.*, 1996; Schmidt *et al.*, 1997). Although the name given to the mixed precursors indicates that they generate both cell types, their lineage is still poorly understood. Glial cells arising from NGBs could originate from the ganglion mother cell (GMC), the classical product of the neuroblast division (Fig. 1A), or they could originate through a novel type of asymmetric division that gives rise to a neuroblast and a glioblast (Fig. 1B). To identify the division products of a NGB we took advantage of the *glide/gcm* mutation, which affects the fate choice between glial cells and neurons. If the supernumerary neurons observed in *glide/gcm* embryos arise from the transformation of glial cells into neurons, the profile of neuroblasts should remain unmodified. On the other side, if the switch takes place at the level of the glioblast, we expect an increase of cells labeled with neuroblast-specific markers. This would imply that glial cells arise from NGBs that divide and produce glioblasts and neuroblasts.

The markers used for this analysis are the classical neural stem cell markers *asense* (*ase*) (Brand *et al.*, 1993; Jarman *et al.*, 1993; Dominguez and Campuzano, 1993) and *deadpan* (*dpn*) (Bier *et al.*, 1992) also called panneural genes because expressed in all stem cells, irrespective of whether these cells are pure (NB) or mixed neural precursors (NGB). Interestingly, we did observe a discrete increase of anti-dpn and anti-ase labeling in mutant compared to wild-type embryos, suggesting a defect in early phases of the glial lineage (data not shown). Given the complex and dynamic pattern of panneural gene expression, we used a neuroblast-specific marker, the anti-eagle antibody, which allowed us to identify the 6-4 lineage (Higashijima *et al.*, 1996; Dittrich *et al.*, 1997; Schmidt *et al.*, 1997). In the thorax of wild-type embryos, the eagle-positive 6-4 stem cell divides and gives rise to four to six neurons and to three glial cells (Schmidt *et al.*, 1997). When the eagle-positive cell divides, it produces a cell expressing *eagle* strongly and a cell expressing it weakly (Fig. 2A). The first produces neurons while the second produces glial cells, as assessed by double labeling with anti-eagle and neuronal or glial markers (data not shown). Strikingly, only the cell expressing *eagle* strongly also expresses the neuroblast-specific marker *dpn* (Fig. 2A). In *glide/gcm* embryos, however, both daughter cells express *dpn* at high level (Fig. 2B), which eventually results in an excess of eagle-positive neurons (data not shown). These results altogether reveal that the fate switch takes place at the level of the stem cell (dpn-positive cell) which divides and produces a neuroblast (dpn-positive cell) and a glioblast (dpn-negative cell). They also clarify the nature of the mutant phenotype and the role of *glide/gcm* in glial differentiation.

Lack of glide/gcm Transforms Glioblasts into Neuroblasts

To confirm that the ectopic NB labeling observed in *glide/gcm* embryos occurs in cells that should have taken the glial fate, we used the $glide/gcm^{34}$ allele (Vincent *et al.*, 1996) obtained by P element mutagenesis on the *rA87* enhancer trap line (Giangrande *et al.*, 1993; Jones *et al.*, 1995; Vincent *et al.*, 1996). The β-gal profile of expression in *rA87*, which carries an insert in the *glide/gcm* locus, is identical to that of *reverse polarity* (*repo*), which labels differentiated glial cells as well as glial precursors (Campbell *et al.*, 1994; Xiong *et al.*, 1994; Halter *et al.*, 1995). The $glide/gcm^{34}$ mutation is an imprecise excision event in which the β-gal expression is retained, which allows the identification of the cells that should have become glial cells. Although $glide/gcm^{34}$ is a lethal mutation in which most glial cells do not differentiate, some glide/gcm product is still active (Vincent *et al.*, 1996). To render the phenotype stronger than in homozygous embryos, we crossed this hypomorphic allele with the null $glide/gcm^{N7\text{-}4}$ mutation and double labeled $glide/gcm^{N7\text{-}4}/glide/gcm^{34}$ embryos with anti-β-gal and anti-ase. The wild-type control was obtained by labeling *rA87*/+ embryos.

In wild-type stage 12 embryos, all the neural stem cells have delaminated, some of them having already given rise to neurons (Campos-Ortega and Hartenstein, 1985; Doe, 1992). Most glial precursors are detectable, even though they have not undergone extensive migration yet. Interestingly, four to six cells expressing the β-gal also express *ase* in mutant hemisegments (Figs. 3D–3F) whereas only two cells coexpress the antigens in wild-type embryos (Figs. 3A–3C and data not shown). Similarly, we found additional cells expressing both the β-gal and *dpn* in transheterozygous *glide/gcm* embryos (Figs. 3G–3L), which confirms that the neuroblast fate fails to be repressed in the glial lineage. The position of these cells along the anteroposterior and dorsoventral axes as well as their number were comparable to those observed with anti-ase, although the total number of β-gal-positive cells expressing *ase* is slightly higher than that of the cells expressing *dpn* (data not shown). This is in agreement with the fact that *ase* is also expressed in the GMC.

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The observation that only half of the β-gal-positive cells express panneural markers has at least two possible explanations. First, *ase* is only expressed in the NB and in the GMC; therefore, lineages in which the GMCs that have already divided and given rise to neurons would not be detected. Second, the transheterozygous combination produces a hypomorphic phenotype. It is very likely that, in null embryos, more cells would coexpress the antigens.

glide/gcm Represses the Neuroblast Fate

Ectopic expression of *glide/gcm* results in ectopic glial cell differentiation within and outside the nervous system (Hosoya *et al.*, 1995; Jones *et al.*, 1995; Bernardoni *et al.*, 1998). Using the *sca-Gal4* driver, which leads to the expression of *UAS-glide/gcm* throughout the neurogenic region, we tested whether ectopic glide/gcm induces gliogenesis by repressing the neuroblast fate. Indeed, we observed a consistent decrease in the number of ase-positive cells (Fig. 4). This decrease is more evident as development proceeds, in agreement with the observation that ectopic glial differentiation induced by *sca-glide* expression starts in few cells at stages 9–10 and increases at later stages (Miller *et al.*, 1998; Bernardoni, Miller, and Giangrande, unpublished results). The decrease of the neuroblast number was also observed using anti-dpn, confirming that the role of glide/gcm is to induce the glial fate by actively suppressing the neuroblast fate (Fig. 4 and data not shown).

glide/gcm Is Expressed in the Neuroglioblast

The transformation of glioblasts into neuroblasts in *glide/gcm* embryos prompted us to study the cellular mechanism underlying this cell fate choice and to analyze the lineage that gives rise to glial descendants. Although the loss and gain of function phenotypes suggest that *glide/gcm* plays a role in the neuroglioblasts, the enhancer trap line *rA87*, which carries a P element in the *glide/gcm* locus, is not expressed in these cells (Fig. 3). To solve this conundrum, we analyzed the profile of expression of *glide/gcm* at early stages of development and performed a double immunolabeling with anti-glide/gcm and anti-eagle to recognize the 6-4 thoracic stem cell. Interestingly, we found that this cell expresses *glide/gcm* before any cell division (Fig. 5A). Thus, the neural stem cell already contains the glial promoting factor, while the enhancer trap line only reflects the late pattern of expression, which is restricted to the glial lineage (glioblasts and glial cells).

Surprisingly, we observed that the glide/gcm product is localized in the cytoplasm of the NGB (Fig. 5). Upon NGB division, glide/gcm is mostly detected in the glioblast, although in some cases it is also present at low levels in the NB (data not shown). Since we did not detect asymmetric distribution of glide/gcm in the NGB, the preferential accumulation of the product in one of the daughter cells most likely depends on *de novo* synthesis. This is in agreement with the observation that glide/gcm contains a PEST sequence, typical of proteins characterized by a rapid turnover (Hosoya *et al.*, 1995). Just after the division of the NGB, glide/gcm is localized in the cytoplasm and in the nucleus of the GB. Later on, by the time when the NB issued from the first division has already divided, the glide/gcm product is detectable in the GB, which has not divided yet, and is mostly localized in the nucleus (Fig. 5). We also confirmed the expression of *glide/gcm* in stem cells by using anti-glide/gcm and anti-dpn (data not shown). Finally, the protein data were corroborated using simultaneously anti-dpn and a *glide/gcm* riboprobe (Fig. 5). These results altogether show that *dpn* is expressed in mixed precursors (NGBs) before cell division and in the precursors of pure lineages, NBs and GBs such as the longitudinal glioblast (LGB) (Fig. 5D and data not shown). In the progeny of the mixed precursors (NGBs), *dpn* expression is limited to one cell, the presumptive NB (Fig. 2A), most likely because its expression is repressed in the presumptive GB by the glide/gcm product.

Our data also suggest that transport of the glide/gcm protein from the cytoplasm to the nucleus takes place in the glial lineage.

Akiyama-Oda *et al.* (1999) recently reported that the glide/gcm product is detectable only upon division of NGB 6-4 (which they call NB 6-4T), in the nucleus of the presumptive glioblast. The fact that we found the glide/gcm product already in the neuroglioblast most likely reflects a difference in the sensitivity between the anti-glide/gcm antibodies. This is also in agreement with the observation that the antibody used by Akiyama-Oda *et al.* (1999) does not detect any cytoplasmic labeling, which is less compact (thus less intense) than the labeling observed in the nucleus.

glide/gcm RNA Is Asymmetrically Distributed in the NGB and Preferentially Inherited by the GB

One strategy used during development to establish cell diversity takes place at cell division and involves the asymmetric RNA distribution and inheritance of cell fate determinants (see for reviews, Hawkins and Garriga, 1998; Jan and Jan, 1998). For example, the fly *prospero* (*pros*) RNA specifically accumulates at the pole of the neuroblast from which the GMC will bud off (Li *et al.*, 1997; Broadus *et al.*, 1998). To elucidate the process that controls the glioblast vs neuroblast choice, we determined whether *glide/gcm* RNA is asymmetrically distributed in the stem cell. To this aim, we analyzed a glide/gcm-positive cell located at the position of neuroglioblast 5-6, which gives rise to three to five neurons and to two to five glial cells (Schmidt *et al.*, 1997). The identity of this cell was confirmed using the *sevenup-lacZ* transgenic line as a lineage-specific marker (Doe, 1992; and data not shown). Figure 6A shows that, indeed, the *glide/gcm* transcript is unevenly distributed.

We confirmed that this is not specific to the NGB 5-6 lineage by analyzing a second neuroglioblast, the thoracic 6-4 (Fig. 6B). It is worth noting that the 6-4 and the 5-6

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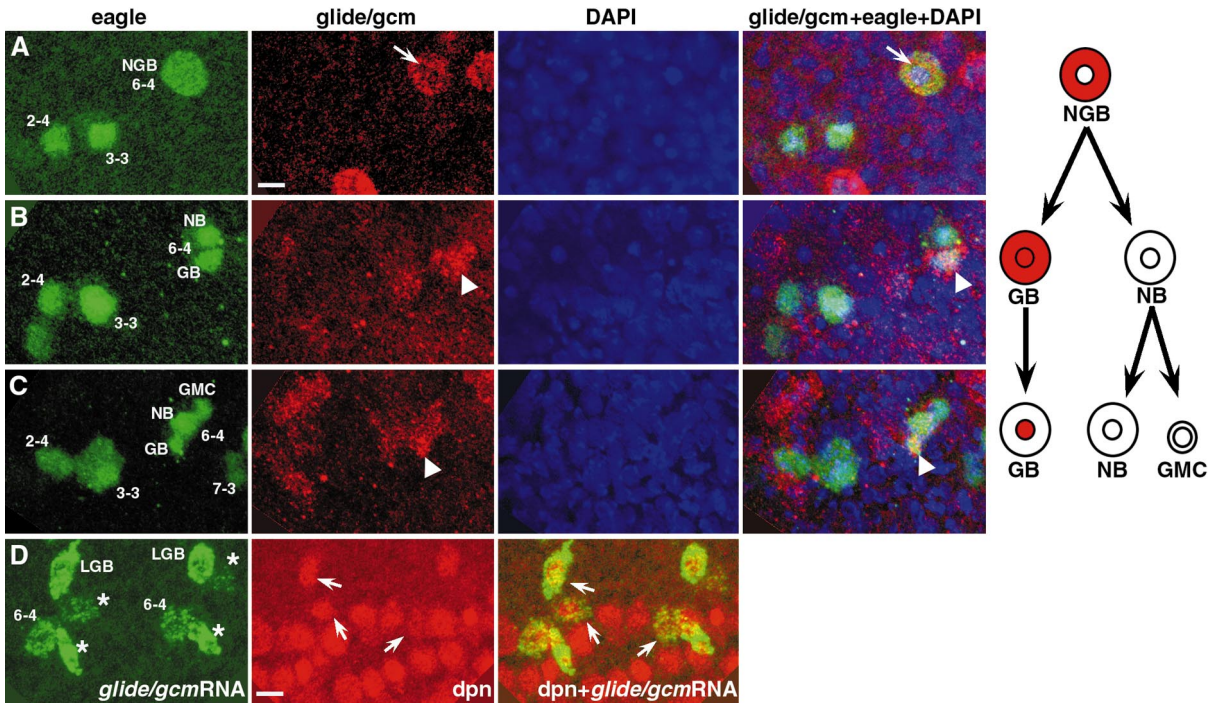

FIG. 5. *glide/gcm* is expressed in neural stem cells. (A–C) Triple labeling with anti-glide/gcm (red), anti-eagle (green), and DAPI (blue). 2-4, 3-3, 6-4, and 7-3 indicate the stem cells labeled by anti-eagle (thoracic segment). All but 6-4 are pure neuroblasts. Arrow shows the exclusion of glide/gcm labeling from the nucleus. Arrowhead indicates the glioblast issued from NGB 6-4. NGB and GMC indicate the neuroglioblast and the ganglion mother cell, respectively. Stages: (A) early 11; (B) 11–12; (C) early 12. (C) Note that the accumulation of glide/gcm in the nucleus can only be detected in the glioblast at the stage of three eagle-positive cells. (D) Two hemineuromeres of a stage early 11 embryo double labeled with a *glide/gcm* riboprobe (green) and anti-dpn (red). By their position, the *glide/gcm*-expressing cells adjacent to 6-4 and LGB are the NGBs 2-5 and 7-4, indicated by asterisks. Arrows indicate colocalization. In all panels, the midline is to the bottom. LGB indicates the longitudinal glioblast. The right part of the panel describes the dynamic of cell division in the 6-4 lineage. NGB, NB, and GB indicate the neuroglioblast, the glioblast, and the neuroblast, respectively. glide/gcm labeling is indicated in red. Bars: (A–C) 7.8 μm; (D) 9.7 μm.

lineages display rather different behaviors. Indeed, while the 6-4 stem cell delaminates at stage 10 and expresses *glide/gcm* before any cell division, neuroglioblast 5-6 is one of the earliest delaminating stem cells (first wave, stage 8) (Doe, 1992; Schmidt *et al.*, 1997) but only starts expressing *glide/gcm* almost 4 h later, at the end of stage 11, after the production of neuronal progeny (Rickert and Technau, personal communication). Strikingly, we found no asymmetric distribution in two pure glioblasts (Figs. 6C and 6D), the longitudinal glioblast, which produces seven to nine

FIG. 6. *glide/gcm* RNA asymmetric distribution in neural stem cells. Double labeling using a *glide/gcm* riboprobe (green) and DAPI (blue). Stages: (A) 11; (B) early 11; (C) 10–11; (D) 11. (A, B) Two examples of mixed lineages; (C, D) two examples of pure lineages. 5-6 and LGB, NGB 5-6 and the longitudinal glioblast, respectively. 6-4T and 6-4A, the thoracic and the abdominal 6-4 stem cell, respectively. Note the difference between A and B and C and D with respect to *glide/gcm* RNA distribution, asymmetric in mixed precursors, symmetric in pure precursors. Five 5-6, 10 6-4T, 4 LGB, and 4 6-4A were analyzed. The right-most panels show quantification data relative to the accumulation of *glide/gcm* RNA. Dotted lines indicate the nuclei of *glide/gcm*-positive cells. Color coding is used to represent different levels of accumulation, the lowest (0) being indicated in blue and the highest (250) in red. Arrows indicate the pole at which *glide/gcm* RNA preferentially accumulates and glioblasts will form. In this figure and in Fig. 7, anterior is to the top. Bar, 2.7 μm.

FIG. 7. *glide/gcm* RNA expression in the glial lineage. (A) Late stage 11 embryo double labeled using a *glide/gcm* riboprobe (green) and DAPI (blue). Two cell stage in the 5-6 lineage (5-6). NB and GB, the neuroblast and the glioblast, respectively. Once the neuroglioblast has divided, one of the two daughter cells, the GB, accumulates much more transcript than the other, the NB. (B) Early stage 11 embryo double labeled using a *glide/gcm* riboprobe (green) and the anti-eagle antibody (red). Two cell stage in the 6-4T lineage. Same analysis as above. Bar: (A) 2.7 μm; (B) 3.2 μm.

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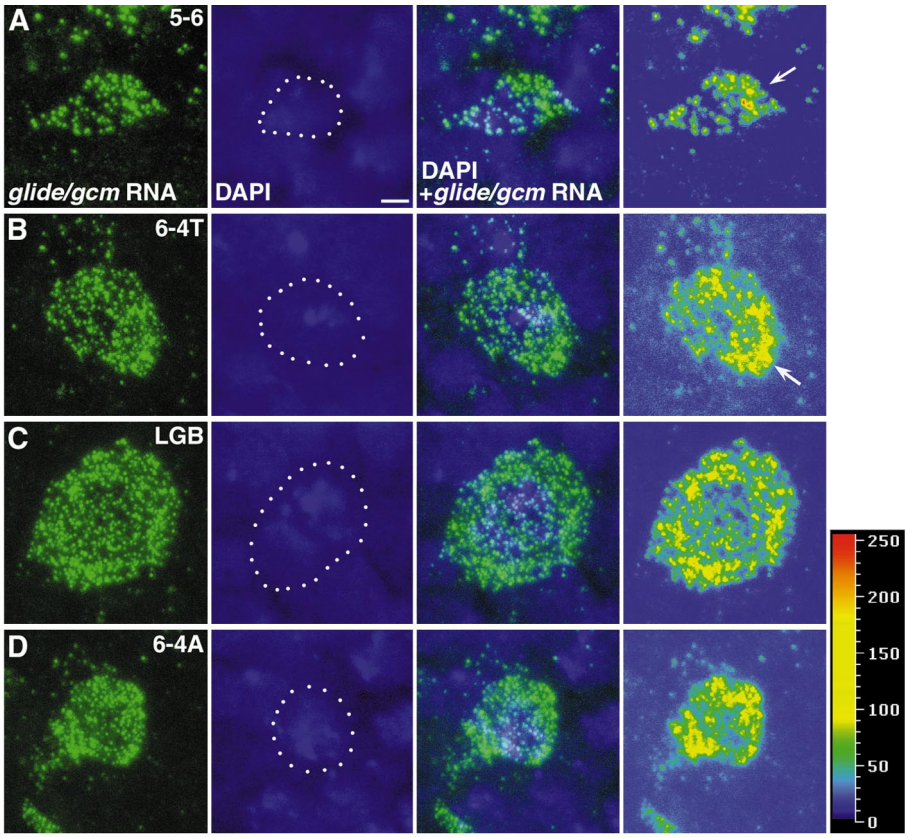

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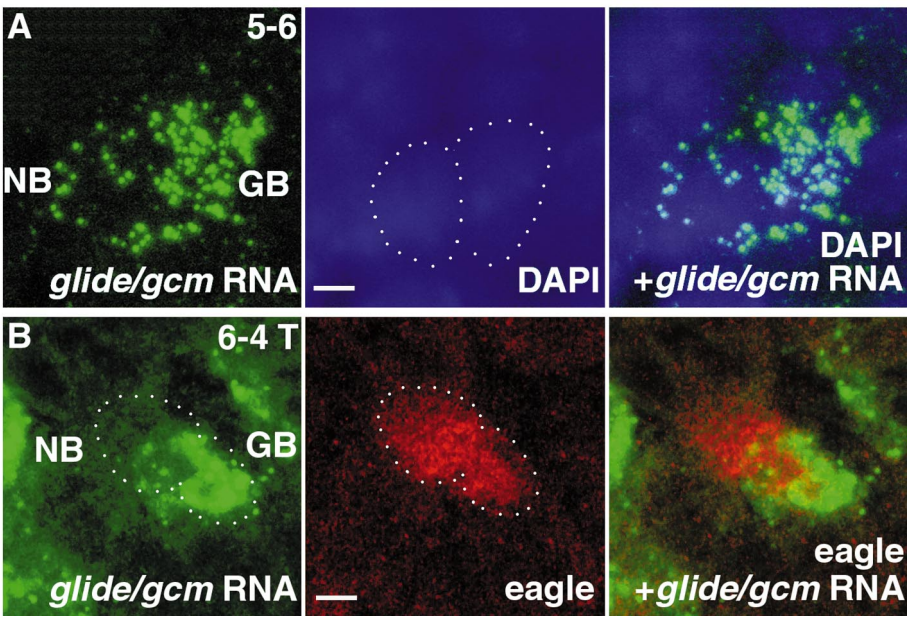

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glial cells, and the abdominal 6-4, which gives rise to two glial cells (Schmidt *et al.*, 1997). This indicates that this mechanism is restricted to lineages in which a choice between neurons and glial cell must take place.

The *in situ* data obtained at the confocal microscope were submitted to a quantification analysis that takes into consideration the signals coming from each optical section (right-most panels in Fig. 6). This analysis is more sensitive and reflects the *in vivo* situation more accurately than a simple projection, in which signals accumulating in several sections at a given point along the XY axes are considered only once. Such analysis has revealed an asymmetry that is consistent and reproducible in the lineages that produce neurons and glial cells. No asymmetry has been detected in the pure lineages that give rise only to glial cells. In details, the quantification of the signal has been performed on the whole stack of confocal images. Color coding has been used to reveal the total signal detected over several Z sections at each point along the XY axes, so that regions of intense labeling are mostly yellow and regions of weak labeling are mostly green. The glioblast takes origin from the yellow side of the cell (arrow) and the neuroblast from the green side.

Similar results on the asymmetric *glide/gcm* RNA distribution in the first cell of the 6-4 lineage were also obtained by Akiyama-Oda *et al.* (1999). As already shown for the 6-4 lineage (Akiyama-Oda *et al.*, 1999), the division of NGB 5-6 is parallel to the epithelial plane and asymmetric RNA distribution is detectable only after prophase (data not shown). To confirm the asymmetric distribution, we followed the profile of *glide/gcm* RNA in the 5-6 lineage after neuroglioblast division and found that the transcript preferentially accumulates in one of the two daughter cells, the glioblast (Fig. 7).

DISCUSSION

glide/gcm and the Establishment of the Glial Fate

Although the existence of mixed precursors in the fly central nervous system has been documented (Bossing *et al.*, 1996; Schmidt *et al.*, 1997), the cellular mechanisms of the fate choice between neurons and glia are unknown. Neuroglioblasts may divide into a NB and a GMC which will eventually produce glial cells and neurons or they may produce a NB and a GB, a type of asymmetric division that has never been documented. The mutant phenotype and the early pattern of *glide/gcm* expression described in this paper allow us to infer the origin of glial cells (Fig. 1B). Since *glide/gcm* is expressed in the neuroglioblast and the number of neuroblasts increases in the absence of glide/gcm, we can conclude that glial cells originate from a glioblast. Common precursors for neurons and glial cells have also been found in vertebrates (Leber *et al.*, 1990; Williams *et al.*, 1991; Davis and Temple, 1994). Since *glide/gcm* is conserved throughout evolution it is tempting to speculate that similar mechanisms take place in the vertebrate nervous system.

Interestingly, we have observed that the choice between the neuronal and the glial fate induced by *glide/gcm* expression does not take place in the first division of all stem cells. This indicates that, in NGBs like the 5-6, the potential to produce glial cells appears late in the lineage as it has been observed in the grasshopper median neuroblast (Condron and Zinn, 1994). In the future, it will be important to define the *glide/gcm* promoter in order to identify the genes inducing *glide/gcm* expression in the different lineages.

Finally, while some glial cells arise from NGBs, others, such as the longitudinal glial cells, arise from dedicated precursors, the pure GBs. Interestingly, *dpn* is expressed in both NGBs and pure GBs. We speculate that panneural gene activity establishes a neural stem cell potential. Some stem cells, NGBs and pure GBs, but not NBs, start expressing *glide/gcm* and acquire a gliogenic potential. The difference between NGBs and GBs relies on the activity of genes that dictate *glide/gcm* RNA localization. Cells in which these genes are active (NGBs) produce neurons and glial cells, while cells in which they are not active (pure GBs) only produce glial cells. Activation of the glial fate eventually results in the repression of the neuroblast fate. Thus, cell identity is conferred through a multistep mechanism involving transcriptional and posttranscriptional gene regulation.

Repression of the Neural Fate and Activation of the Glial Fate

It has been proposed that the role of the glide/gcm transcription factor is to activate two independent pathways, both necessary for gliogenesis. On one side, glide/gcm induces the expression of genes that execute and maintain the glial differentiation program. Among them are *glide/gcm* itself (Miller *et al.*, 1998) as well as the putative targets *repo* (Campbell *et al.*, 1994; Xiong *et al.*, 1994; Halter *et al.*, 1995; Akiyama *et al.*, 1996), *pros* (Doe *et al.*, 1991; Vaessin *et al.*, 1991), and *pointed* (*pnt*) (Klambt, 1993; Klaes *et al.*, 1994). On the other side, glide/gcm is thought to activate genes that repress the neuronal program such as *ttk* (Harrison and Travers, 1990; Giesen *et al.*, 1997). Strikingly, while ectopic *ttk* expression results in neuronal loss, *ttk* embryos do not display an increase of neuronal markers in the central nervous system nor does *ttk* ectopic expression result in the decrease of neuroblast number (Giesen *et al.*, 1997). Therefore, our data reveal that although *ttk* may participate in the repression of neuronal differentiation, yet to be identified factors must be regulated by *glide/gcm* in order to repress the neuroblast fate in the neuroglioblast. In the future, it will be important to identify the direct targets of *glide/gcm* responsible for this repression.

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glide/gcm and prospero: Differences and Similarities

glide/gcm and *pros* encode nuclear proteins that behave as cell fate determinants in the developing nervous system. *glide/gcm* is expressed in the neuroglioblast. Its role is to promote the glioblast fate and repress the neuroblast fate. *pros* is expressed in the neuroblast and is necessary to induce the ganglion mother cell fate and to repress the neuroblast fate (Chu-Lagraff *et al.*, 1991; Doe *et al.*, 1991; Vaessin *et al.*, 1991; Matsuzaki *et al.*, 1992). In both cases, the RNA is asymmetrically distributed and preferentially inherited by one of the daughter cells (Li *et al.*, 1997; Broadus *et al.*, 1998; Akiyama-Oda *et al.*, 1999; the present article). *glide/gcm* RNA accumulates at the side from which the glioblast will originate, *pros* RNA accumulates at the basal side of the neuroblasts, from which the ganglion mother cell will bud off. In the case of *pros*, RNA distribution depends on the staufen RNA-binding protein (Li *et al.*, 1997; Broadus *et al.*, 1998); it will be interesting to determine whether *glide/gcm* RNA distribution is regulated in a similar way.

The prospero protein also localizes to the basal side of the neuroblast and is inherited by the ganglion mother cell (Hirata *et al.*, 1995; Knoblich *et al.*, 1995; Spana and Doe, 1995). It has been suggested that *pros* RNA and protein may act redundantly to establish ganglion mother cell-specific patterns of gene expression (Broadus *et al.*, 1998). Strikingly, while *glide/gcm* RNA is asymmetrically distributed, the protein is present in the NGB but is not asymmetrically distributed. It is most likely that the protein is inherited by both cells, glioblast and neuroblast, but is rapidly degraded due to the presence of the PEST motif (Hosoya *et al.*, 1995). In the glioblast, RNA accumulation allows *glide/gcm*-positive autoregulation (Miller *et al.*, 1998) to take place. This reinforces the initial asymmetry and specifically promotes the establishment of the glial fate in this cell. Thus we speculate that, in the case of *glide/gcm*, rapid protein degradation in both daughter cells and positive autoregulation in one of the two cells functionally replace the protein asymmetric distribution observed for *pros*.

Interestingly, although glide/gcm contains a nuclear localization signal and behaves as a transcription factor (Akiyama *et al.*, 1996; Schreiber *et al.*, 1997; Miller *et al.*, 1998), it does not accumulate in the NGB nucleus. Just after division, glide/gcm is detected in the cytoplasm and in the nucleus of the glioblast. Later on, most labeling is detected in the nucleus of this cell. Hence, a further regulatory step in the *glide/gcm* pathway is the cytoplasm to nucleus transport. It is worth noticing that, in the case of *pros*, the protein is nuclear in the ganglion mother cell, where it activates ganglion mother cell-specific genes and represses NB-specific genes, but not in the NB itself (Vaessin *et al.*, 1991; Matsuzaki *et al.*, 1992). Indeed, a signal inducing cell diversity, such as RNA asymmetric distribution of a cell fate determinant, must be brought about in one cell (the NB for *prospero;* the NGB for *glide/gcm*), but must only be effective in the progeny of that cell (the GMC and the GB, respectively). Thus, cytoplasmic localization of glide/gcm (prospero) in the NGB (NB) prevents this cell from taking the glial (GMC) fate. In the future, it will be important to determine the factors that are responsible for the regulatory step that induces cytoplasm to nucleus transfer and renders the glial promoting signal effective.

Autonomous and Nonautonomous Decisions in the Development of the Nervous System

The development of vertebrate and invertebrate nervous systems requires the activity of proneural and neurogenic genes (reviewed in Campuzano and Modolell, 1992; Ghysen *et al.*, 1993). The interplay of these two classes of genes involves cell interactions and autonomous decisions that define the number of neural stem cells. In addition, genes involved in pattern formation define the identity of these cells. Thus, the establishment of a neuroglioblast is the result of a complex developmental cascade.

Both the profile of expression of *glide/gcm* and its mutant phenotype suggest that glial differentiation takes place once the neuroblast identity has been established. In addition, *glide/gcm* RNA is first detected at stage 10, after the activity of proneural and neurogenic genes. This implies that glial differentiation relies on several pathways acting in concert and converging on the regulation of a single gene, the glial promoting factor *glide/gcm*.

One of the most challenging issues will be to understand how the novel type of cell division described in this study is integrated in the cell autonomous and nonautonomous cascade of events regulating the development of the nervous system. From the molecular point of view, it will be interesting to determine whether both autonomous and nonautonomous mechanisms act in the same cell to stabilize the initial fate choice, as has been observed for *Notch* and *numb* in the sensory organ precursor cell (Guo *et al.*, 1996). A mutagenesis screen is currently under way in order to identify the genes that participate to the first steps of glial cell determination by regulating *glide/gcm* expression and/or activity.

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