A Novel Mode of Asymmetric Division Identifies the Fly Neuroglioblast 6-4T

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Asymmetric cell divisions and segregation of fate determinants are crucial events in the generation of cell diversity. Fly neuroblasts, the precursors that self-reproduce and generate neurons, represent a clear example of asymmetrically dividing cells. Less is known about how neurons and glial cells are generated by multipotent precursors. Flies provide the ideal model system to study this process. Indeed, neuroglioblasts (NGBs) can be specifically identified and have been shown to require the glide/gcm fate determinant to produce glial cells, which otherwise would become neurons. Here, we follow the division of a specific NGB (NGB6-4T), which produces a neuroblast (NB) and a glioblast (GB). We show that, to generate the glioblast, glide/gcm RNA becomes progressively unequally distributed during NGB division and preferentially segregates. Subsequently, a GB-specific factor is required to maintain glide/gcm expression. Both processes are necessary for gliogenesis, showing that the glial vs. neuronal fate choice is a two-step process. This feature, together with glide/gcm subcellular RNA distribution and the behavior of the NGB mitotic apparatus identify a novel type of division generating cell diversity. © 2001 Academic Press

Key Words: stem cells; neural precursors; neuroblast; neuroglioblast; glide/gcm; fly; gliogenesis; nervous system; differentiation; asymmetric division.

INTRODUCTION

Neural stem cells are self-reproducing cells that generate the different types of neurons and glial cells populating the nervous system. The establishment of different fates relies on asymmetric determinant distribution and specific orientation of division. Fly neuroblasts (NBs) produce a neuroblast and a small cell, the ganglion mother cell (GMC), which divides once and produces two neurons (Doe, 1992; Broadus et al., 1995; Bossing et al., 1996; Schmidt et al., 1997; Schmid et al., 1999). NBs divide apico-basally and require the prospero (pros) gene, whose RNA and product segregate to one daughter to activate the GMC fate (Chu-Lagraff et al., 1991; Doe et al., 1991; Vaessen et al., 1991; Matsuzaki et al., 1992; Hirata et al., 1995; Knoblich et al., 1995; Spana and Doe, 1995; Ikeshima-Kataoka et al., 1997; Li et al., 1997; Broadus et al., 1998). Asymmetric cortical Pros distribution requires the localization of Miranda (Mira) and Staufen (Stau), which responds to cell polarity cues (Li et al., 1997; Broadus et al., 1998; Kuchinke et al., 1998; Shen et al., 1998; Schuld et al., 1998; Wodarz et al., 1999; Schober et al., 1999; Hawkins et al., 1998; Jan and Jan, 1998).

A second type of precursor, the neuroglioblast (NGB), also divides asymmetrically but produces two cell types, neurons and glial cells (Bossing et al., 1996; Schmidt et al., 1997; Schmid et al., 1999; Bernardoni et al., 1999). The choice between the two cell fates in the NGB requires the Glial cell deficient/glial cell missing (Glide/gcm) transcription factor (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996; Akiyama et al., 1996; Bernardoni et al., 1997, 1998; Schreiber et al., 1997; Miller et al., 1998). glide/gcm RNA is unequally distributed in the dividing neuroglioblast: one daughter inherits most transcripts and adopts the glial fate, the other takes the default neuronal fate (Bernardoni et al., 1999; Akiyama-Oda et al., 1999).

To understand how different fates are produced by stem cells, we need to know how the neuroglioblast divides and how the RNA asymmetry is generated. For this reason, we have carefully followed the distribution of glide/gcm RNA during the division of a neuroglioblast (NGB), NGB6-4T, and have analyzed glide/gcm mutants to understand how...
neurons and glia are generated from a common precursor. These results indicate that the neuronal vs. glial fate choice requires two steps, asymmetric distribution of glide/gcm RNA in the neuroglioblast and maintenance of glide/gcm expression in the cell that inherits most transcripts, the glioblast. The latter step requires the Prospero transcription factor. We also show that glide/gcm RNA progressively accumulates at the pole of the cell from which the glioblast will arise, the asymmetry becoming evident at metaphase. In addition, we have found that glide/gcm RNA displays a different subcellular localization from that of other fate determinants of the nervous system and forms globular particles. Finally, the overall mode of division of the NGB is different from other asymmetric divisions with respect to mitotic apparatus and orientation of division.

In conclusion, the analysis of NGBT6-4 allows us to identify a novel type of division in which the asymmetry must be established in the mother and sustained in the daughter cell.

MATERIALS AND METHODS

Stocks

Wild-type sevenless stock was used as a control. The following null alleles were also used: glide/gcm17–4 (Vincent et al., 1996); pros17 (Broadus et al., 1998); Df(3R)ora19 (Shen et al., 1997); and staufen123 (St. Johnston et al., 1991). Ectopic pros expression was obtained by using the eagle-GAL4 (Dittrich et al., 1997) and scabrous-GAL4 (gift from M. Mlodzik) (Miller et al., 1998) drivers, the UAS-pros (generated by F. Matsuzaki, Tokyo), and UAS-glide/gcm reporters (Bernardoni et al., 1997).

Immunohistochemistry and in Situ Hybridization

Staged embryos (0–9 h after egg laying) were immunolabeled as described (Vincent et al., 1996). For anti-α-tubulin labeling, embryos were processed as described (Gonzalez and Glover, 1993). Antibodies against the following proteins were used at the indicated concentrations: Eagle (1:1000); Prospero (MR1A hybridoma; 1:10); Miranda (1:1000); PH3 (Upstate Biotechnology; 1:50,000); RK2 (1:500); γ-tub (clone GTU-88, Sigma; 1:1000); α-tub (clone DM1A, Sigma; 1:1000), and β-galactosidase (β-gal) (Cappel; 1:1000). Secondary antibodies conjugated with Oregon Green (Molecular Probes), FITC, Cy3, and Cy5 (Jackson) were used at 1:500. In situ hybridizations were performed as described (Bernardoni et al., 1999). Digoxigenin-labeled probes were prepared by using the glide/gcm, eagle, and miranda cDNAs. Hybridization signals were detected by using a mouse anti-digoxigenin primary antibody (Boehringer Mannheim, 1:100) and an FITC-conjugated anti-mouse secondary antibody (Jackson, 1:500). Chromatin labeling was obtained by using DAPI at 100 ng/ml in 1× PBS-0.3% Triton X-100.

Confocal analyses were performed with a DMRE microscope (Leica).

RESULTS

The Asymmetric Division of a Neuroglioblast Displays Novel Features

In fly embryos, neuroblasts divide along the apico-basal axis. Their duplicated centrosomes are randomly located.

FIG. 1. Profile of NGB6-4T division and glide/gcm RNA distribution. (A–C) α-Tubulin (green) and glide/gcm RNA (red) distribution during NGB division; α-tubulin allows to follow spindle organization during mitosis (arrowheads). Chromatin is revealed by DAPI labeling in blue. (D) NGB6-4T daughter cells soon after division. glide/gcm RNA is detected in red, eagle RNA in green. (A) At prophase, newly duplicated centrosomes located at the apical pole of the cell start migrating basally. glide/gcm RNA is not yet asymmetrically distributed. Posterior is to the right. (B) By metaphase, centrosomes reach the equator of the cell, the mitotic spindle becomes parallel to the epithelium plane, and chromatin displays the highest level of compaction. glide/gcm RNA starts being asymmetrically distributed. Asterisks indicate regions devoid of glide/gcm transcripts. Latero-anterior is to the left. (C) Early anaphase. The mitotic spindle has rotated by almost 90° along the apico-basal axis. glide/gcm RNA further accumulates at one pole of the cell. Lines are located at the middle of chromatin labeling. (D) Dots show the apico-lateral-postero-neuroblast and the baso-medio-anterior glioblast. Note that glide/gcm RNA is almost entirely inherited by the glioblast and that daughter cells are oriented at 45° with respect to the epithelium plane. (E) Triple labeling of a stau123 embryo. glide/gcm RNA is in red, γ-tubulin (centromeral marker) in green, DAPI in blue. (F) Triple labeling of a mira embryo. glide/gcm is in red, eagle RNA in green, DAPI in blue. Note that, in the next figures, eagle expression is always detected by using an anti-Eagle antibody. All samples were analyzed by confocal microscopy (X-Y mode). Panels show 90° rotations so that apical is to the top. Merge panels show the multiple labeling. Right-most panels show schematic representations of the dividing cells. (ap), (bas), (ant), (pos), (lat), and (med) indicate apical, basal, anterior, posterior, lateral, and medial, respectively. (lat-ant) and (med-pos) indicate latero-anterior and medio-posterior, respectively. Bar: 4 μm.

FIG. 2. Pros is asymmetrically localized by the glioblast. Multiple labeling of the 6-4T lineage with anti-Prospero (red), anti-Eagle (green), and DAPI (blue). Dotted lines indicate the nuclei of the Eagle-positive cells, arrows indicate Pros localization. Right-most panels show the multiple labeling. (A, B) Wild-type NGB at single cell stage. Samples were scanned at the confocal microscope (X-Y mode) and analyzed upon rotation by 90° so that apical is to the top. (A) Prospero is initially localized to the apical side of the delaminating NGB. (B) By late metaphase/early anaphase, Prospero is localized basally. (C, D) Projections of confocal images (X-Y mode). Medial to the top, anterior to the right. Two cell stage in (C) wild-type and (D) glide/gcm17–4 embryos. (C) Soon after NGB division, Prospero is localized to the nucleus of the medial-most daughter cell (the glioblast in the wild-type). Asterisk in (D) indicates an unrelated nucleus. NB and GB indicate the neuroblast and the glioblast, respectively. Bar: 4 μm.
apically or basally (whereas, in epidermoblasts, they are always basally located) (Kaltschmidt et al., 2000). At division, both centrosomes migrate to the equator and rotate, so that, by metaphase, they orient themselves along the apico-basal axis. pros RNA is localized cortically, apically at interphase and basally at metaphase (Li et al., 1997; Broadus et al., 1998).

To understand the differences between precursors producing neurons and glial cells and those producing only neurons, we followed the division of an identified precursor of neurons and glial cells, the neuroglioblast 6-4 in the thorax (NGB6-4T), which divides and produces a NB and a GB. To do so, we used mitotic and DNA markers together with glide/gcm RNA or the eagle lineage-specific marker (Schmidt et al., 1997; Dittrich et al., 1997; Higashijima et al., 1996) (Fig. 1, and data not shown). This allowed us to simultaneously analyze the mode of division and RNA distribution during the different phases of cell division. Optical sections were taken from the apical to the basal side of the cell. The whole stack of images was subsequently rotated by 90° to produce Z-sections.

Mode of NGB Division

Interphase NGB6-4T displays apically localized centrosomes (data not shown). By prophase, the chromatin has become compact, as revealed by DAPI labeling (Fig. 1A). At this stage, the mitotic spindle starts being organized at the apical pole (20/20 NGBs analyzed) and migrates to reach the equator of the cell, as revealed by anti-α-tubulin labeling (Fig. 1A). It is not until metaphase that the spindle acquires its typical cone-shaped morphology. By metaphase, the spindle has oriented itself parallel to the plane of the epithelium, like in dividing epidermoblasts (Fig. 1B). At late metaphase/early anaphase, the spindle rotates and becomes almost apico-basal. This is seen in Fig. 1C, in which chromosomes start being separated. By the end of the division, however, the spindle is always oriented at 45° as

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FIG. 3. Pros is necessary but not sufficient to induce the glial fate, whereas Glide/gcm is necessary and sufficient. Early stage 13 embryos simultaneously labeled with anti-repo (RK2) (red) and anti-Eagle (green). Ventral views, anterior to the right. Two thoracic segments are shown: T2 and T3. (A) wild-type (wt); (B) prospero (pros); (C) e.g., GAL4; UASpros; (D) e.g., -GAL4/UASglide/gcm; (E) scabrous-GAL4/UASpros; (F) scabrous-GAL4; UASglide/gcm embryos. Arrows indicate neurons deriving from NGB6-4T. Arrows indicate the lateral Eagle labeling, which in the wild-type corresponds to the neuronal component of the lineage. Arrowheads indicate the four MM-CBGs and the two M-CBGs originating from the two NGB6-4Ts present in each segment. Asterisks show the absence of the glial component. Horizontal lines indicate the midline. Note in (D) and in (F) the presence of Repo labeling at lateral position, due to the expression of glide/gcm. Bar: 25 μm.
compared to the typical apico-basal orientation of the neuroblast (n = 13) (Fig. 1D). This is most likely due to spindle wobbling, which also takes place in the division of neuroblasts (Kalthschmidt et al., 2000). Thus, NGB6-4T divides in an oblique fashion. Akiyama-Oda et al. (1999) reported that the NGB6-4T divides orthogonal to NBs. The discrepancy between our and previous data most likely stems from the fact that markers for centrosomes and mitotic spindle define the orientation of division more accurately as compared with DNA markers.

With respect to the other axes (antero-posterior; medio-lateral), duplicated centrosomes and mitotic spindles of NGB6-4T initially form along the antero-posterior axis, as in epidermoblasts (Kalthschmidt et al., 2000) (Fig. 1A). During division, however, the spindle rotates so that the nascent glioblast (GB) is more basal, anterior, and medial than the neuroblast (Fig. 1D). The more medial and anterior position of the GB is in agreement with findings from Akiyama-Oda et al. (2000). Thus, the overall orientation of NGB division is intermediate to those of epithelial cells and NBs. Three-dimensional animations of Figs. 1B and 1C have been enclosed as MPEG files.

The follow-up of the division has also enabled us to clarify other aspects of NGB asymmetry. First, to our surprise, the asymmetry of the mitotic spindle is detected from very early to the end of division (prophase: Fig. 1A; metaphase: Fig. 1B; anaphase: Fig. 1C), being much larger on the side that will produce the NB than on the GB-producing side. This was documented by using markers specific for the spindle (α-tubulin) (Fig. 1) and for the centrosomes (γ-tubulin) throughout the division of NGB6-4T (Fig. 1E, and data not shown). In the case of the NB, the mitotic spindle becomes asymmetric after metaphase (Kalthschmidt et al., 2000; Bonaccorsi et al., 2000). Second, the nuclear and cytoplasmic size of the two daughters display minor differences (Fig. 1D). To determine the nuclear surface, we analyzed the DAPI labeling of daughter cells and determined the number of pixels contained in each nucleus. In average, the nuclear surface of the GB is 84.8% that of the NB (n = 11; SD 0.087). GMCs, on the other side, are almost three times smaller than neuroblasts (Doe et al., 1998; Demerec, 1994). The analysis was performed by taking into account the fact that the two nuclei lay in different planes and may not display maximum perimeter in the same focal plane.

Distribution of glide/gcm RNA

glide/gcm transcripts seem evenly distributed at prophase (n = 11) and start being asymmetric by metaphase (n = 4) (Figs. 1A and 1B). The process of RNA localization takes place progressively from metaphase on. By the end of NGB division, most glide/gcm RNA accumulates at the medio-basal-anterior side of the cell, from which the GB will arise (number of cells analyzed at anaphase: 10; number of cells at telophase: 4) (Figs. 1B–1D). Double in situ experiments were also performed to show that the RNA of glide/gcm but not that of eagle, another gene expressed in the 6-4T lineage, is asymmetrically distributed (Fig. 1D). Finally, in contrast with what has been shown for pros (Hirata et al., 1995; Knoblich et al., 1995; Spana and Doe, 1995), glide/gcm RNA accumulates at one pole of the cell rather than being restricted to the cortex.

Thus, several features distinguish the neuroblast from neuroglioblast 6-4T: (1) the type of fate determinant; (2) its subcellular localization during division; (3) the organization of the mitotic apparatus; (4) the polarity of division; and (5) the size of the daughter cells. These data strongly suggest that the mode of cell division reflects the differentiative potential of each precursor.

Prospero Segregates with glide/gcm and Is Required for Glial Differentiation

Since Pros acts as a cell fate determinant in the NB, we asked whether it also plays a role in the NGB6-4T. By labeling wild-type embryos with anti-Pros, anti-Eagle, and DAPI, we found that pros is expressed in the delaminating NGB6-4T, where it is initially localized to the apical cortex (Fig. 2A). At division, however, it accumulates cortically at the side of the cell from which the GB will arise (Fig. 2B, and data not shown) and later translocates to the GB nucleus (Fig. 2C). Thus, Pros is present in the NGB and segregates with glide/gcm RNA.

We therefore determined whether Pros is required for the differentiation of the NGB6-4T-derived glial cells. In the wild-type, the GB issued from this NGB divides and produces three glial cells, the M-CBG and two MM-CBGs (Akiyama et al., 1996), which express the Reverse polarity (Repo) protein (Campbell et al., 1994; Halter et al., 1995) (Fig. 3A). The three cells are absent in pros embryos as shown by double labeling with repo and eagle (Fig. 3B). In addition, the number of lateral cells expressing eagle at high levels, is increased, as compared to wild-type embryos. Since, in the wild-type, NGB6-4T glial cells are located more medially and express eagle at lower levels than neurons (Bernardoni et al., 1999; Akiyama-Oda et al., 1999; Dittrich et al., 1997; Higashijima et al., 1996) (see also Fig. 3A), it is likely that glial cells in pros embryos do not differentiate because they are completely or partially transformed into neurons.

To ask whether pros is sufficient to induce the glial fate, we overexpressed pros in the 6-4T lineage by using the eagle-GAL4 driver (Dittrich et al., 1997). We never detected an increase of NGB6-4T-derived glial cells, indicating that, although required for glial differentiation, Prospero is not sufficient to promote gliogenesis (Fig. 3C). These results were further confirmed by using the scabrous-GAL4 (sca-GAL4) driver (Fig. 3E) (Miller et al., 1997), which induces expression throughout the neurogenic region, much earlier than eagle-GAL4. The use of sca-GAL4 eliminates the possibility that the eagle-GAL4-dependent Pros expression
takes place too late during the lineage to promote gliogenesis. Inspection of the NGB6-4T lineage with an anti-Pros antibody confirmed that pros expression had been induced by the two drivers (data not shown).

In contrast with what observed with Pros, expression of glide/gcm under the control of the same drivers, does induce ectopic Repo labeling within the 6-4T lineage (Figs. 3D and 3F). This labeling is located at a more lateral position as compared to wild-type NGB6-4T glial cells, suggesting that ectopic cells have not migrated correctly. Thus, Pros is necessary but not sufficient, whereas Glide/gcm is both necessary and sufficient to induce the expression of glial markers. Indeed, Repo labeling induced by glide/gcm ectopic expression was already observed just after the first division, a stage at which Repo is not yet detected the wild-type NGB6-4T (data not shown).

**Pros Maintains glide/gcm Transcription in the GB**

To understand the mode of action of Pros, we determined its precise role in gliogenesis. Since pros and glide/gcm code for transcription factors (Schreiber et al., 1997; Miller et al., 1998; Hassan et al., 1997), we asked whether any of these genes regulates the other. glide/gcm transcription is indeed affected in pros mutants (Fig. 4B). Interestingly, the amount of glide/gcm RNA present in pros embryos is even lower than that observed in embryos that lack glide/gcm auto-regulation (Fig. 4C). On the contrary, pros transcription is not regulated by glide/gcm (Fig. 2D). Thus, pros regulates glide/gcm expression.

Gain-of-function experiments clarified the epistatic relationships. pros expression in the two daughters of the NGB6-4T results in the increase of glide/gcm RNA levels. This increase, however, specifically takes place in the 6-4T lineage (Doe, 1992) (Fig. 5B, and data not shown). Thus, Mira but not Stau, seem to affect glial differentiation. The fact that we never observed a complete neuron-to-glia transformation (12 glial cells) indicates that Mira participates with other factors to the establishment of the glial fate.

Multiple labeling of stau and mira embryos revealed defects in glide/gcm RNA localization, due to the presence of transcripts in the NB-generating side (Figs. 1D–1F). However, the phenotype is not present in all lineages: three out of four NGB6-4T telophases were affected in stau embryos, four of five in mira embryos. Mira is expressed in the NGB and segregates to the GB (Fig. 6).

Interestingly, we observed that the glide-specific RNA labeling appears as globular in shape (Fig. 1). To provide evidence that this is not an artifact that misrepresents the distribution of glide/gcm transcripts, we run a double in situ hybridization with riboprobe specific for mira and for glide/gcm and compared transcript distribution. mira transcripts are sharply localized and form an homogeneous cortical crescent, in agreement with previous data from Schuldt et al. (1998). In contrast, the glide/gcm-specific RNA distribution...
signal shows the typical globular shape and is not restricted to the cortex (Fig. 7). These findings also confirm that the methodology used to detect glide/gcm RNA does allow the observation of cortical RNA anchoring. In this experiment, no attempt was made to recognize the precise stage of the NGB6-4T, due to incompatibility with probes specific for tubulin. However, rotation of the confocal sections revealed that mira transcripts are localized apically (data not shown), suggesting that the cell has not entered metaphase yet, as also confirmed by the widespread distribution of glide/gcm transcripts.

**DISCUSSION**

**Fate Decisions and Modes of Divisions of Neural Precursors**

The study of NGB6-4T has allowed us to identify a novel type of asymmetric division with respect to several features (Fig. 8). Neuroblasts produce cells with different size and fate. In this paper, we show that NGB daughter cells show minor differences in size. Thus, the two features can be uncoupled. In neuroblasts, the mitotic spindle shows signs of asymmetry by anaphase (Kaltschmidt et al., 2000; Bonaccorsi et al., 2000), while in the NGB6-4T it is already asymmetric by prophase and stays asymmetric until the end of division, as shown by mitotic spindle and centrosome labeling. More importantly, asymmetry of the mitotic spindle is always associated with different fates, irrespective of daughter cells’ size. In the future, it will be important to determine whether differences in mitotic spindle size are the cause rather than the consequence of the asymmetry.

Fate choices autonomously regulated require the acquisition of an appropriate cell polarity. It is generally thought that delamination from the epithelial layer provides neuroblasts with an apico-basal polarity that insures the generation of different fates. However, we here show that NGB6-4T, which also delaminates from the epithelium and produces different cell types, does not divide along the apico-basal axis. It will be interesting to determine whether the apically localized Bazooka protein (Kuchinke et al.,...
1998; Wodarz et al., 1999; Schober et al., 1999), which controls epithelial polarity, directs the orientation of neuroglioblast 6-4T, possibly in combination with specific positional cues, or whether different mechanisms control orientation of division according to the differentiative potentials.

NGB division also differs from other asymmetric divisions described in the fly nervous system. In the sensory organ lineage, the PI precursor divides along a planar polarity and shows no asymmetry in the mitotic spindle, the PIIb precursor divides like a classic neuroblast (Rogiers et al., 2001; Bellaïche et al., 2001). Interestingly, the PIIb division, which produces the glial precursor of peripheral nervous system, is at an oblique angle from the apico-basal axis, much like that of the NGB. It will be important to clarify whether the oblique angle is an important distinction.

It is interesting to notice that some features make the NGB multipotent precursor an intermediate between pure neuroblasts and epidermoblasts. Not only is the orientation of its division oblique, but also the newly duplicated centrosomes are always apical, whereas epidermoblast centrosomes are initially located basally and those of neuroblasts are apical or basal (Kaltschmidt et al., 2000).

The simultaneous utilization of different types of markers labeling the mitotic apparatus, the cell fate determinants, and the NGB6–4T lineage has revealed a new mode of division in the nervous system. This type of division was overlooked in the past due to the lack of adequate tools. The NGB6-4T is one of the simplest neuroglioblasts to analyze, because its lineage tree is already known and because it produces neurons and glial cells just after delamination. Other lineages, such as the 5-6, initially produce neurons and only after some divisions give rise to glial cells (Bernardoni et al., 1999). In the future, it will be necessary to determine the precise lineage tree of such lineages and to determine whether yet other modes of division can account for the differentiation of different types neurons and/or glial cells. This will allow us to understand whether the overall mode of division of neural precursors reflects the potential to produce one (neuroblasts) or two cell types (neuroglioblasts).

The Establishment of the Glial Fate Is a Two-Step Process

The establishment of different fates upon asymmetric division relies on the unequal distribution of fate determinant(s). Prospero is expressed in the neuroblast and inherited in the GMC, where there is no de novo pros transcription (Broadus et al., 1998). Thus, all the information is provided by the mother, i.e., the neuroblast. Here, we present a case in which the initial asymmetry established in the mother must be sustained and amplified in the progeny. glide/gcm RNA asymmetric distribution does result in the preferential segregation to the GB (Bernardoni et al., 1999; Akiyama-Oda et al., 1999). glide/gcm expression, however, is maintained and amplified in this cell through direct autoregulation and the expression of cell-specific cofactors (Miller et al., 1998). Glide/gcm accumulation eventually activates the glial fate and represses the neuronal fate (Bernardoni et al., 1999; Akiyama-Oda et al., 1999; Van de Bor et al., 2000). The progressive activation of the glial fate is also confirmed by the finding that Repo, which contains several binding sites for Glide/gcm, is expressed in the GB progeny but is almost undetectable in the GB itself (data not shown).

The present study shows that Pros transcription factor is necessary to maintain glide/gcm expression and thereby activate the glial program in the glioblast. Indeed, in the absence of Pros, glide/gcm RNA progressively disappears from the GB. The gain-of-function phenotype also demonstrates that Pros is not sufficient to initiate glide/gcm expression nor to induce the glial fate on its own. Pros protein and RNA most likely form a complex with Stau and Miranda. In the absence of Miranda, which is necessary to localize them (Shen et al., 1997, 1998; Schuldt et al., 1998), both daughter cells inherit the RNA and the protein. In addition, they both inherit glide RNA. As a consequence, the two daughter cells adopt the GB fate (Fig. 9). We speculate that Pros is required for both glide/gcm-independent and glide/gcm-dependent maintenance, since in its absence the levels of glide/gcm expression are even lower than in the absence of autoregulation. The glide/gcm promoter contains, indeed, several binding sites for the Pros protein (data not shown). Finally, as shown in Fig. 3, Pros does not affect all glial cells, therefore it is likely that specific factors will be required in other lineages.

**glide/gcm Presents Novel Features of RNA Localization and Asymmetric Segregation**

Asymmetric RNA distribution as a means to establish cell diversity is a strategy that has been conserved throughout evolution (Hazelrigg, 1998). In the budding yeast, the mating type switching is controlled by asymmetric localization of ASH1 mRNA by a process that is dependent on actin and myosin (Long et al., 1997; Takizawa et al., 1997). In the neuroblast, Mira directs Stau localization to apical and then basal cortex. Stau in turn physically interacts with the pros 3'UTR (Li et al., 1997; Shen et al., 1998; Schuldt et al., 1998). Cortical crescents depend on microfilaments, which anchor Stau and Mira asymmetrically during division (Broadus et al., 1998; Shen et al., 1998; St. Johnston et al., 1991; Kraut et al., 1996; Broadus and Doe, 1997; Knoblich et al., 1997).

glide/gcm displays several differences with respect to pros. First, asymmetric distribution is not evident before metaphase (see Fig. 8). Second, asymmetry occurs progressively during cell division rather than being sharply apical at interphase and basal at metaphase. Third, glide/gcm transcripts are present at the cortex and in the cytoplasm.
All these data suggest the existence of different RNA localization pathways in asymmetrically dividing cells. That Stau and Mira may participate to the process is suggested by the mislocalization of glide/gcm RNA in stau and mira. In addition, the glide/gcm 3'UTR displays a stem-loop secondary structure (G.R. and A.G., data not shown), a conformation that is necessary for the interaction of Staufen with bicoid 3'UTR (Ferrandon et al., 1994, 1997; Macdonald and Struhl, 1998). However, this mechanism is not sufficient to ensure a correct fate choice. Moreover, mira and stau are not fully penetrant with respect to glide/gcm RNA distribution. Finally, and more importantly, the cytoplasmic localization of some glide/gcm transcripts as well as the kinetics of asymmetry calls for a cortical microfilament independent mechanism. Thus, the same RNA may be the target of two localization pathways, which complements the observation that the same RNA binding protein may localize transcripts using pathways with different cytoskeletal requirements (Kaltschmidt et al., 2000).

Due to its features, we speculate that glide/gcm RNA localization arises by differential degradation/stabilization, as has been shown for Hsp83 mRNA (Hazelrigg 1998). In the future, it will be important to analyze RNA asymmetric distribution in vivo by using transgenic flies that allow us to simultaneously follow the NGB6-4T lineage as well as the glide/gcm RNA. It will also be interesting to analyze other lineages in order to determine whether the same RNA localizing pathway is required one RNA localizing pathway, whereas all mixed precursors require two. The presence of two pathways may act as a backup system, as suggested by the absence of a glial phenotype in stau embryos, or may allow a fine-tuning of RNA distribution. We know that Glide/gcm dosage is important to trigger the glial fate (Bernardoni et al., 1999).

**FIG. 8.** Schematic representation of NB and NGB6-4T divisions. Drawings showing a NB (top) and NGB6-4T (bottom) to compare the different modes of division. Glide/gcm and glide/gcm RNA are represented in different colors. Black dots indicate the centrosomes, which are asymmetric starting from interphase in the case of NGB6-4T, after metaphase in the case of the NB. Notice the different location of centrosomes in the two types of cells: 100% apical in NGB6-4T; 36% basal and 64% apical in the NB (Kaltschmidt et al., 2000). Metaphase orientation as well as the final orientation of division is also different between neuroblasts and NGB6-4T. NB division produces daughter cells with different size, whereas that of NGB6-4T produces cells of similar size.

**FIG. 9.** Role of Stau, Mira, and Pros in the NGB6-4T lineage. Schematic representation of wild-type (WT) and mutant lineages. On the left is the NGB at telophase, on the right the two daughter cells: NB and GB. Miranda, Prospero, Glide/gcm, and glide/gcm RNA are represented in different colors. Black dots indicate the centrosomes. In the wild-type, the Glide/gcm protein is localized to the cytoplasm, glide/gcm RNA, Mira, and Pros all segregate to the side from which the GB is generated; while Mira and Pros are localized to the cortex, glide/gcm RNA has a broader distribution. After division, most RNA is inherited by the GB, where Prospero segregates. Glide/gcm is inherited by both cells, but rapidly degraded. In the GB, however, maintenance of glide/gcm expression leads to accumulation of the protein, which progressively localizes to the nucleus (Bernardoni et al., 1999). In stau embryos, glide/gcm RNA is symmetrically distributed in the NGB and is inherited by both cells. Even segregation, however, is not enough to trigger glide/gcm maintenance in both daughters. As a consequence, the glial program is only implemented in the presumptive GB, which contains Pros. In mira embryos, on the other side, both glide/gcm RNA and Pros are evenly localized. This allows the two daughters to adopt the glial fate. In pros embryos, glide/gcm cannot be maintained in the GB, which results in both cells adopting the NB fate.
The identification of the molecules acting in glide/gcm RNA localization will be necessary to better understand the glia versus neuron fate choice. Finally, it will be important to define how the activity of these molecules is connected with cell cycle and positional cues in the developing nervous system.

ACKNOWLEDGMENTS

We thank W. Pak, the Tübingen Stock Center, V. Rodriguez, M. Mlodzik, and J. Urban for stocks. We thank J. Urban, C. Mollinari, C. Gonzalez, C. Doe, A. Wodarz, A. Brand, H. Vaessen, A. Travers, and A. Tomlison for antibodies. We thank C. Plessy for quantification of the data on the mutant embryos as well as Rachel Walther for excellent technical help. We are indebted to J.-L. Vonesch for developing imaging programs. Thanks to Y. N. Jan, M. Gatti, J. Bellaiche, Y., Gho, M., Kaltschmidt, J. A., Brand, A., and Schweisguth, F., (2001). Frizzled regulates localization of cell fate determinant: a novel DNA-binding motif conserved in Drosophila. Development 128, 50–57.

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Asymmetric Division and the Fly Neuroglioblast 6-4T


Received for publication March 2, 2001
Revised April 13, 2001
Accepted April 16, 2001
Published online May 30, 2001