CHAPTER 4

Dissection of the Embryonic Brain Using Photoactivated Gene Expression

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Abstract

The *Drosophila* brain is generated by a complex series of morphogenetic movements. To better understand brain development and to provide a guide for experimental manipulation of brain progenitors, we created a fate map using photoactivated gene expression to mark cells originating within specific mitotic domains and time-lapse microscopy to dynamically monitor their progeny. We show that mitotic domains 1, 5, 9, 20 and B give rise to discrete cell populations within specific regions of the brain. Mitotic domains 1, 5 , 9 and 20 give rise to brain neurons; mitotic domain B produced glial cells. Mitotic domains 5 and 9 produce the antennal and visual sensory systems, respectively, where each sensory system is composed of several disparate cell clusters. Time-lapse analysis of marked cells showed complex mitotic and migratory patterns for cells derived from these mitotic domains.

Introduction

Fate maps serve as critical tools for developmental biologists to chart tissue morphogenesis and as guides for experimental manipulation. The ideal fate map should contain information about cell movements, mitotic patterns, morphology, cell-cell contacts and cell death as well as specific patterns of gene expression and the consequence of altered gene expression and cellular interactions. *Drosophila* fate maps start at the cellular blastoderm stage, which is composed of about 5,000 cells.[1] Prior to this stage there are no lineage-restricted fates, aside from the pole cells.[2] The only physical landmarks at cellular blastoderm are the anterior-posterior and dorsoventral axes. To fate map the embryo, a Cartesian coordinate system relative to percent position along these axes was used to mark the initial position of cells in the blastoderm.[3,4] Mapping was originally done by ablation[4,5] and more recently by dye marking of cells.[6,7] Ablation studies required the removal of rather large numbers of cells since embryos were able to compensate for small losses of cells.[5] The dye marking approaches have been very successful, but are limited in that they do not provide a means to alter the behavior of the marked cells.

Alternative fate mapping methods are: gynandomorph analysis[8] and the generation of mitotic clones.[9] The latter method is useful for producing marked clones of cells. These methods produce genetically perturbed clones of cells, but there is little control over their location.

To develop a more reliable and precise coordinate system than the Cartesian coordinate system, we took advantage of the mitotic domain map. Mitotic domains are bilaterally symmetric groups of cells that divide in a stereotypic sequence that are indicators of cell fate.[10,11] Cells within a mitotic domain are restricted to a limited set of fates that are distinct from the sets of cellular fates observed in neighboring mitotic domains.[12,13]

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To enable the marking of cells in a spatially and temporally restricted manner, we developed a method for activating gene expression using a micro-beam of light.[12] This method, which is referred to as photoactivated gene expression, is based on the GAL4-expression method.[14] Instead of supplying GAL4 genetically, chemically "caged" GAL4VP16 is injected into syncytial stage embryos that carry a UAS-transgene. Expression of the UAS-transgene is activated by briefly irradiating the cell, or cells, of choice with a long-wavelength UV microbeam, thus un-caging the GAL4VP16 protein. This method has been used to activate the expression of benign markers, such as LacZ and GFP, and to alter cell behavior. Time-lapse microscopy and whole-mount embryo preparations are used to track the behavior of marked cells.

This chapter focuses on the origin of the embryonic brain. We show that the brain is derived from five separate mitotic domains, each of which undergo distinct morphogenetic behaviors to generate discrete, non-overlapping regions of the brain. Several different mechanisms are used to internalize blastoderm cells.

Procephalic Blastoderm Fate Map

The procephalic region of the embryo is made up of thirteen mitotic domains (individual mitotic domains will be abbreviated as δ*N*). We have fate mapped eleven procephalic mitotic domains (for δ2, δ8, δ10, δ15 see ref. 12; for δ3, δ18, δ20 see ref. 13; for δ1, δ5, δ9, δB see ref. 15). All of these mitotic domains produced non-overlapping sets of distinctly fated cells. Of these mitotic domains, δ1, δ5, δ9, δ20 and δB form the embryonic brain. We were interested in determining the morphogenetic movements of brain-forming cells. How are these cells internalized? Do they form discrete brain regions? Do they differentiate into neurons and glia? What other cell-types are generated by these mitotic domains? To map the fates of cells within selected mitotic domains, we used photoactivated gene expression to initially mark cells and monitored their development either by three-dimensional, time-lapse microscopy or post-fixation immunohistochemical staining.

Brain-Forming Mitotic Domains Populate Distinct Brain Regions

Mitotic domains 1, 5, 9 and B occupy a large area that roughly corresponds to the procephalic neuroectoderm (Fig. 1A). The strategy for mapping how these mitotic domains contribute to the brain, was to photoactivate patches of cells within a chosen mitotic domain in *UAS-lacZ* or *UAS-nGFP* embryos during stage 8. Photoactivated embryos were aged to stages 14-16 and immuno-stained or live-imaged to detect the expression of the UAS-transgene product. Mitotic domains 1, 5 and 9 generated cells that occupied discrete regions of the brain, suggesting that they may be neurons rather than glial cells, which are scattered.[16] Mitotic domain B produced a dispersed population of cells that will be discussed later. A compendium of many mapping experiments was prepared (Figs. 1B,C and 2B). Each colored line in Figure 1B,C outlines the region of marked cells observed in a single embryo mapped onto a dorsal or lateral view of the embryonic brain. These data show that all three mitotic domains give rise to three distinct, non-overlapping regions of the embryonic brain, demonstrating their early regional specification. The axons emanating from these mitotic domains follow very different paths, indicating their distinct character.

Time-lapse recordings of photoactivated *UAS-nGFP* embryos revealed the complex morphogenetic movements made by each of these mitotic domains to form part of the brain (Fig. 2). The schematic shown in Figure 2B starts at stage 9 when GFP fluorescence is clearly visible, 60-90 minutes following photoactivation. A significant amount of cell movement takes place in the head between stage 7, when cells were photoactivated, and stage 9, placing the cells from each mitotic domain some distance from the site of photoactivation (compare Fig. 1A and Fig 2B, stage 9). The migration pattern is also distinct for each mitotic domain. The following sections will highlight unique features of these mitotic domains.

Mitotic Domain 1 Generates Anterior Protocerebrum Neurons

Mitotic domain 1 is a large, two-lobed region. Photoactivation of different regions of δ1 generated clones of different cell-types. Photoactivation of the anterior-ventral region of δ1 revealed that

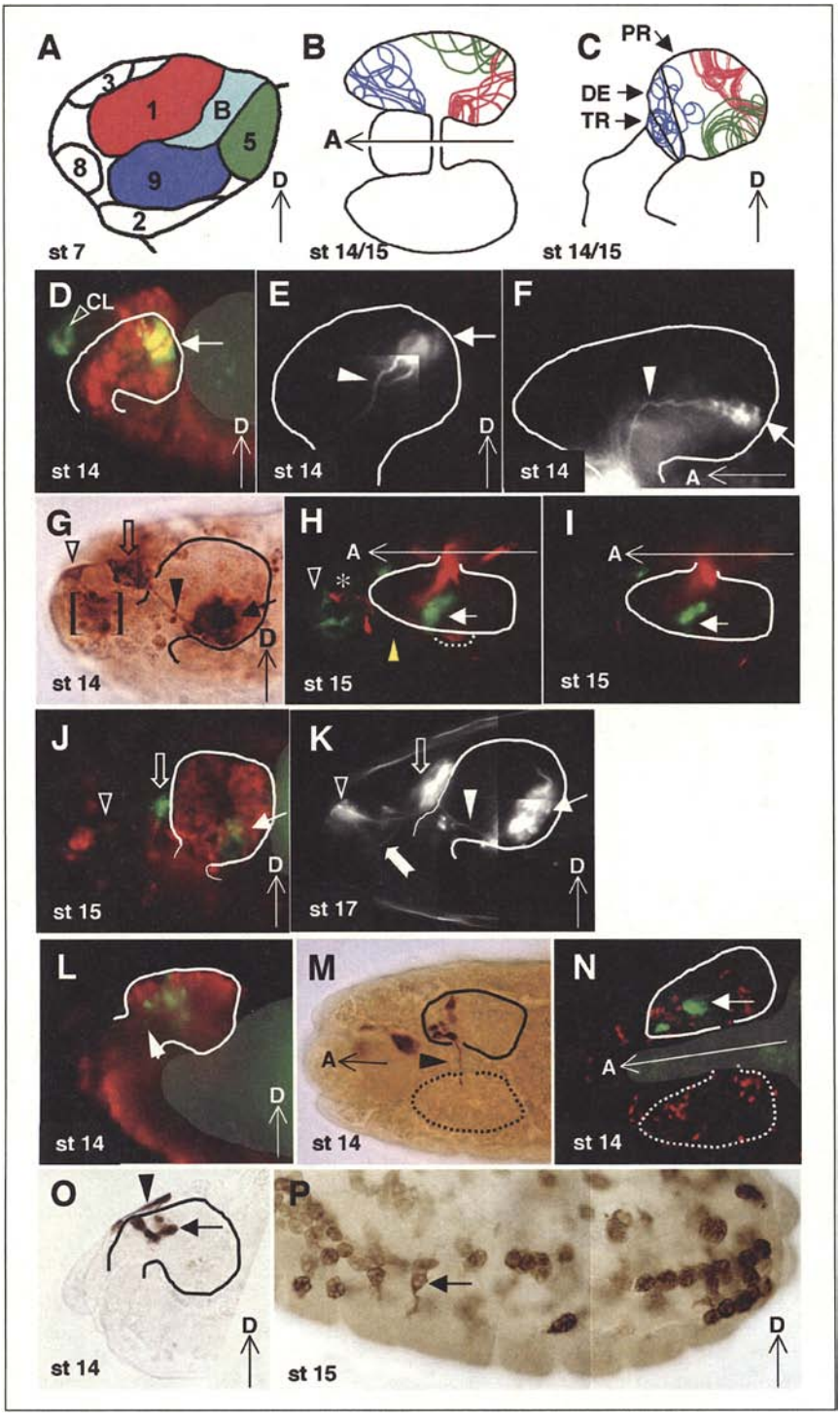

Figure 1, legend viewed on following page.

Figure 1, viewed on previous page. Fate mapping of δ1, δ5, and δ9 cells. For all figures, the embryo anterior is to the left. Dorsally mounted embryos are indicated by a horizontal arrow pointing to the anterior, marked A. Laterally mounted embryos are indicated by a vertical arrow pointing dorsally, marked D. The stage of the embryo is indicated in the bottom left corner of each panel. A) Schematic representation of the head mitotic domains at stage 7, which was used to guide photoactivation experiments. B,C) Cartoon of the regions within the embryonic brain that are populated by each mitotic domain. Each line represents results from an individual embryo (δ1-red n = 31, δ5-green n = 21, δ9-blue n = 31). The remaining panels show micrographs of photoactivated embryos. The affected brain hemisphere is bounded by a solid line. D-F) Photoactivation of δ1. D) 2-4 cell photoactivation of a *UAS-nGFP* embryo stained with anti-GFP antibody (green) and anti-ELAV (red). The δ1 derived cells are visible in both the brain (solid arrow) and the clypeolabrum (CL). E,F) 2-4 cell photoactivation of *UAS-tauGFP* embryos. The arrowheads indicate the pioneer axons of the embryonic peduncle. G-K) Photoactivation of δ5. All images are of 5-8 cell photoactivations. G) A photoactivated *UAS-lacZ* embryo showing the four different δ5 structures: the posterior group within the brain (solid arrow), the middle group just anterior to the brain (open arrow), the anterior group (open arrowhead) and the epithelial group (bracketed). The axon connecting the posterior and middle groups is indicated by the closed arrowhead. H,I) A *UAS-lacZ* embryo stained with antibodies against β-galactosidase (green) and FasII (red). H) Composite image of 3 adjacent optical sections showing FasII-positive: optic lobe (outlined with dashed line), Bolwig's organ (asterisk) and Bolwig's nerve (yellow arrowhead). GFP-expressing, δ5 brain cells are in a different focal plane (solid arrow) that is adjacent to optic lobe. The δ5 anterior group (open arrowhead) is adjacent to Bolwig's organ. I) An in-focus optical section of the GFP-positive δ5 cells within the brain (solid arrow). J) A δ5 photoactivated *UAS-nGFP* embryo stained with anti-GFP (green) and anti-ELAV (red; using the same arrow scheme as panel G). K) Composite of projected images of a δ5-photoactivated, *UAS-tauGFP* embryo (using the same arrow scheme as panel G). The bifurcated axon tract projecting to the maxillary complex is indicated by a notched arrow. L-P) Photoactivation of δ9. L) 2-4 cell photoactivation of a *UAS-lacZ* embryo stained with anti-β–galactosidase (green) and anti-ELAV (red). The closed arrowhead indicates an axon extending toward the ventral nerve cord. M) Single cell photoactivation of a *UAS-lacZ* embryo histochemically stained with anti-β–galactosidase. The closed arrowhead indicates an axon extending to contralateral brain hemisphere. N) 2-4 cell photoactivation of a *UAS-lacZ* embryo stained with anti-β–galactosidase (green) and anti-Repo (red). The arrow indicates the patch of β-galactosidase-positive cells that were not expressing Repo. O) Single cell photoactivation of δ9 in a *UAS-lacZ* embryo histochemically stained with anti-β–galactosidase showing marked epidermal (closed arrowhead) and brain cells (closed arrow). P) 5-8 cell photoactivation of δ9 in a *UAS-lacZ* embryo histochemically stained with anti-β–galactosidase showing marked migratory cells (arrow) throughout the entire embryo. Yolk auto-fluorescence which appears in the green fluorescence channel is masked gray in D, J, L and N. Reprinted from: Robertson K et al. Dev Biol 2003; 260:124-137; ©2003 with permission from Elsevier.[15]

this region contributed mostly to the clypeolabrum (Fig. 1D, see the green fluorescent cells outside of the brain as indicated by the letters CL). The posterior-dorsal region gave rise to cells located predominantly in the anterior-medial part of the protocerebrum (when referring to brain location, we use the neuroaxis as the frame of reference) (Fig. 1D-F and cartooned in Fig. 1B,C, see the areas bounded by the red lines). These results indicate that δ1 is divided into two sub-regions.

In the protocerebrum, marked δ1 cells populated two adjacent clusters of cells. These cells co-labeled with the pan-neuronal marker, ELAV (Fig. 1D, arrow).[17] In contrast, very few δ1-derived cells expressed the glial cell marker, Repo.[18] Less than 2% of the marked δ1 cells were glia, indicating that δ1 cells gave rise to neurons rather than bipotential progenitors. Time-lapse analysis of photoactivated δ1 cells showed that these cells were internalized *en mass*. The mass then moved posteriorly along the midline to their final position in the protocerebrum (Fig. 2B).

The location and double cluster appearance of δ1 neurons suggested that they may form the embryonic mushroom bodies. To further test this possibility, δ1 axons were marked by photoactivation using *UAS-tauGFP* embryos. These TauGFP marked axons had the typical morphology of the embryonic mushroom bodies (Fig. 1E,F, arrowhead).[19,20]

Mitotic Domain 5 Produces the Embryonic Antennal System

Mitotic domain 5, which is initially located just anterior to the cephalic furrow near the dorsal midline (Fig. 1A), produces four distinct cell populations; one epidermal and three neuronal (Fig. 1G, 2A,B). Time-lapse analysis of photoactivated *UAS-nGFP* embryos revealed the complex migration pattern of this mitotic domain (Fig. 2A). The photoactivated patch of cells first elongated along the edge of the cephalic furrow adjacent to the maxillary segment (Fig. 2A, frame 1, stage 9). The most anterior-ventral cells remained in the epidermis and moved to the anterior tip of the embryo. As head involution began, the non-epidermal δ5 progeny became internalized at the boundary between the mandibulary and maxillary segments and separated into two populations (Fig. 2A, frames 5-6, stages 13-14). The inward movement of these cells appeared to be via invagination. One population, the posterior group, which ultimately forms the antennal lobe of the brain, remained stationary at the mid-anterior region of the embryonic brain, while the second group migrated over the ventral surface of the developing brain (Fig. 2A, frames 6-7, stage 14-15). This was followed by another splitting of cells from the second group, which migrated into the position of the antennal sensory organ (Fig. 2A, frames 7, stage 15). This culminated in populations of ~20 posterior group, ~5-6 middle group and 2-4 anterior group cells; the number of epidermal cells was not determined.

Immuno-chemical staining of δ5 photoactivated *UAS-lacZ* embryos showed that the three internalized populations were connected by axonal fibers (Fig. 1G, solid arrowhead). All four groups of cells arising from δ5 are shown in Fig. 1G. The neuronal character of cells within these groups was revealed by counter-staining with anti-ELAV antibody; about half of the photoactivated cells within the anterior and posterior groups expressed ELAV (Fig. 1J, arrow, open arrowhead). We further confirmed the neuronal nature of the δ5 derived brain cells, as well as those of the anterior group, by photoactivating δ5 cells in *UAS-tauGFP* embryos. Tau-GFP highlighted the axons of the posterior group within the brain, the axon tracts between the groups and the structure of the most anterior group (Fig. 1K). The axons of the δ5-derived brain cells can also be seen extending into other parts of the brain (Fig. 1K). Many of these processes appear to terminate in the region of the brain populated by δ1 mushroom body precursors (compare Fig. 1K and E, which correspond to δ5 and δ1, respectively).

The pattern of δ5 cell types was reminiscent of the cell types produced by δ20, which will be described later.[13] The morphogenetic movements of δ5 and δ20 were also similar; but not identical, δ20 cells form a more elongated pattern prior to internalization. We confirmed that mitotic domains 5 and 20 yielded different structures by photoactivating δ5 cells in *UAS-lacZ* embryos and immuno-stained for FasII and β-galactosidase expression. FasII is expressed in the optic lobe, Bolwig's nerve and Bolwig's organ, but not antennal cells.[21] FasII was not expressed in any of the photoactivated δ5 cells (Fig. 1H,I). δ5-derived brain cells (Fig. 1H,I, closed arrow) were adjacent to the optic lobe (Fig. 1H and I, broken line); there was no overlap. Likewise, δ5 cells in the anterior group (Fig. 1H, open arrowhead) were adjacent to Bolwig's organ, not overlapping (Fig. 1H, asterisk). Thus, δ5-derived cells do not contribute to any part of the visual system.

The morphology and position of the δ5-derived cells indicate that this mitotic domain gives rise to the antennal sensory system, where the anterior group corresponds to the antennal sensory organ and the posterior group, which is in the brain, corresponds to the antennal lobe.

Mitotic Domain 9 Produces Three Apparently Unrelated Cell Types

A unique feature of δ9 is that the entire cell population divides perpendicularly to the embryonic surface during the 14th mitosis, creating two populations of cells, predicted to be epidermal and brain.[10] To ensure that both layers of progeny were marked, δ9 cells were irradiated prior to, or during, the 14th mitosis. Three distinct cell types were derived from δ9: posterior brain (Fig. 1L-N), dorsal midline epidermis (Fig. 1O, closed arrowhead) and an unidentified population of migratory cells (Fig. 1P).

To determine the lineage relationship of these three cell populations, different-sized patches of cells within δ9 were photoactivated in *UAS-lacZ* and *UAS-GFP* embryos (Table 1). Time-lapse

Table 1. Distribution of different cell types arising from δ9

Cell Type(s)*	Single Cell Photoactivation (Percent, n = 29)	2-4 Cell Photoactivation (Percent, n = 72)	5-8 Cell Photoactivation (Percent, n = 24)
E	10	4	0
B	31	15	0
M	7	21	12
E + B	45	13	13
E + M	0	0	0
B + M	0	28	29
E + B + M	7	19	46

*E indicates epithelial cells; B indicates brain cells; M indicates migratory cells. Different sized patches of δ9 cells were photoactivated in *UAS-lacZ* embryos just prior to, or during, the 14th mitosis. The embryos were aged to stage 14 through 16 and stained with anti-β-galactosidase antibody. Only embryos with multiple marked cells were scored. Reprinted from: Robertson K et al. Dev Biol 2003; 260:124-137; ©2003 with permission from Elsevier.[15]

recordings showed that all three cell types experienced significant amounts of cell death, making it extremely difficult to draw firm conclusions about lineage relationships. The origin of the migratory cells is not clear. None of the clones were composed of both epithelial and migratory cells, indicating that epithelial cells do not give rise to migratory cells directly. Thus, the migratory cells are either derived from brain progenitors or delaminated directly from the blastoderm. A significant fraction of embryos had marked migratory-only clones, particularly with 2-4 cell photoactivation, supporting the delamination hypothesis. The brain- and migratory-cell progenitors appear to be evenly distributed across δ9.

Time-lapse analysis revealed that initially the brain and epidermal progenitors moved in unison anterior and dorsally, before separating, leaving the epidermal cells at the dorsal midline (Fig. 2B, stage 12, blue hatching), while the brain progenitors continue to move posteriorly to their final location in the brain (Fig. 2B). All photoactivated δ9 brain cells expressed ELAV (Fig. 1L); none expressed Repo (Fig. 1N), indicating δ9-derived brain cells are neurons, not glia. These neurons occupied the deutero-, proto- and tritocerebrum (Fig. 1C,L), thus, the formation of the three cerebral neuromeres does not appear to be specified by separate mitotic domains. In many embryos an axon could be seen to project either through the tritocerebrum toward the ventral nerve cord (Fig. 1L, closed arrowhead) or toward the contralateral hemisphere through the tritocerebral commissure (Fig. 1M, closed arrowhead). These structures are similar to those described by Therianos et al.[22]

Mitotic Domain 20 Generates the Entire Visual System

Mitotic domain 20 is the most posterior of the three dorsal head mitotic domains (Fig. 3A). A small number of cells within δ20 was marked by photoactivating *UAS-lacZ* expression. Since the cells in δ20 divide much later than surrounding mitotic domains and most of the cells divide inside the cephalic furrow,[10] it was difficult to distinguish δ20 cells as they divide. Therefore, δ20 cells were identified as those cells surrounded by the amnioserosa and mitotic domains 5, 18 and B (Fig. 3A, green circle).

Photoactivating cells in the center of δ20 gave rise to a set of bilaterally symmetrical structures spanning from the anterior tip to the brain (Fig. 3B), including many head sensory organs and nerves of the peripheral nervous system (PNS), the posterior part of the brain, and the dorsal pouch epithelium above the clypeolabrum. Activation of δ20 cells also gave rise to a significant amount of cellular debris, indicating that some cells were dying. Photoactivation procedure does not affect cell death patterns.[12]

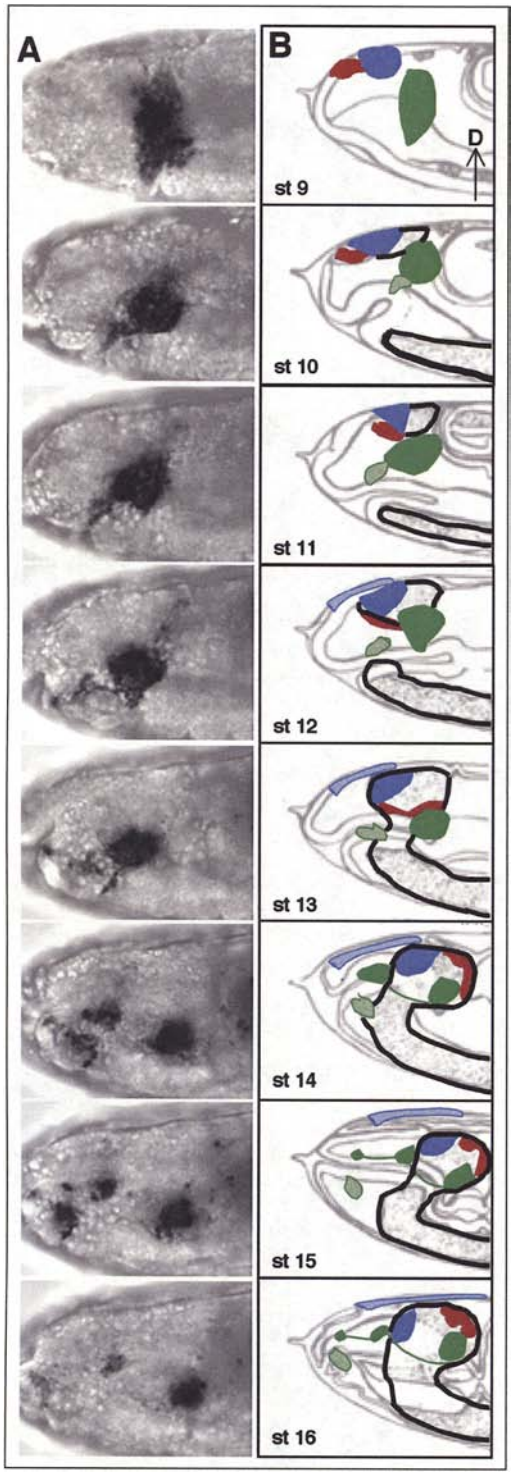

Figure 2. Time-lapse images and cartoon of *UAS-nGFP* embryo following photoactivation. Column A) A series of images from a time-lapse recording of a δ5 photoactivated embryo. The GFP fluorescence is shown in negative so that marked cells appear black overlaying transmitted light images. Lateral view of stages 9-16 as a projection of seven 5 μm optical sections. Column B) Diagrammatic representation of the position of the progeny from mitotic domains 1, 5, and 9 from stage 9 to 16 shown as a lateral view. This series was constructed from multiple time-lapse experiments (δ1-red, δ5-green, δ9-blue, the brain is outlined in black). Reprinted from: Robertson K et al. Dev Biol 2003; 260:124-137; ©2003 with permission from Elsevier.[15]

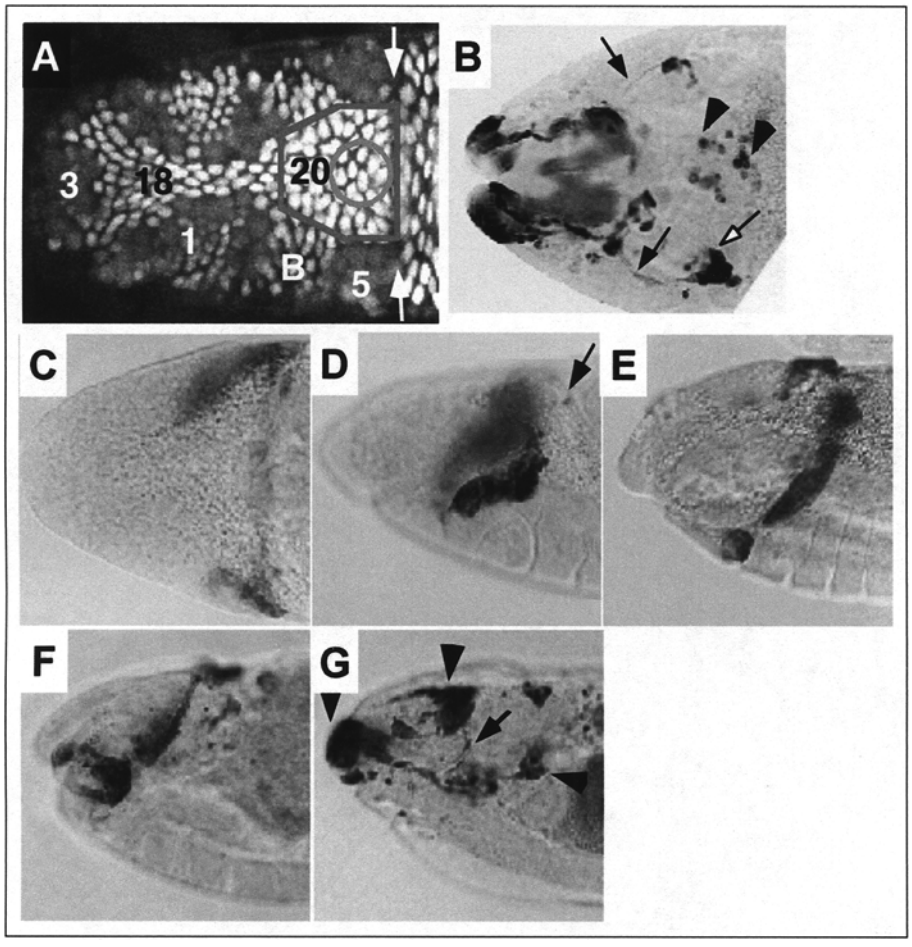

Figure 3. Fate mapping of δ20. A) Individual cells of the dorsal head mitotic domains were visualized by the expression of nuclear GFP (*Ubi-GFPnls*) by confocal microscopy. δ20 is highlighted with a gray border. The numbers indicate mitotic domains. Interphase nuclei appear bright and have sharp edges while mitotic cells are large and appear diffuse. The white arrows point to the cephalic furrow. The gray circle indicates a typical size and location of the UV photoactivation beam. All embryos are shown with anterior to the left. B) Fates of mitotic domains were visualized by GAL4 dependent activation of *lacZ* using the photoactivated gene expression system. Shown here is a dorsal view of a stage 17 embryo with photoactivated δ20 cells. Cells in the posterior part of the brain (white arrow) and head PNS (including axons projecting to the brain; arrows) were marked as well as cellular debris (arrow heads). C-G) Developmental time-course of δ20. Dorsal (C) and lateral (D to G) images of δ20-photoactivated embryos. The marked cells were visualized with an anti-β-galactosidase antibody. C) The cells in δ20 moved away from the dorsal midline during germband extension. D) At late stage 11, the cells reached the dorsal border of the gnathal segments. The first sign of cell death was apparent as a small spot moving away from the group of marked cells (arrow). E) At stage 13, the marked δ20 cells extended along the lateral surface. F) During stage 14, the ventral cells continued to move anteriorly into the stomodeal invagination. G) At the end of embryogenesis, cells were distributed into three clusters (arrowheads); the anterior tip, dorsal pouch and the brain, connected via nerve-like projections (arrow). Reprinted from: Namba R, Minden JS. Dev Biol 1999; 212:465-476; ©1999 with permission from Elsevier.[13]

δ20 starts as a single domain on the dorsal midline. During germband extension, the cells of δ20 moved bilaterally away from the dorsal midline (Fig. 3C). By two hours after photoactivation at stage 7, most δ20 cells had migrated laterally away from the dorsal midline where they formed the dorsal border of the gnathal segments (Fig. 3D). By the end of stage 13, the cells formed a narrow strip spanning from the ventral to the dorsal surface (Fig. 3E). At the end of embryogenesis, cells in this narrow strip were distributed into three clusters spanning the entire length of the head (Fig. 3F). The ventral δ20 cells moved anteriorly with the gnathal segments during stomodeal invagination (Fig. 3G) and eventually reached the anterior tip. The more dorsal δ20 cells formed the dorsal ridge and became a part of the dorsal pouch, while some cells delaminated and occupied the ventral posterior part of the brain lobe. The cells in the brain lobe were usually connected to a cell cluster in the anterior tip of the embryo by long nerve-like projections (Fig. 3G arrow).

Photoactivation of δ20 marked a pair of lateral clusters of cells in the dorsal pouch adjacent to the pharynx in the stage 16 embryo (Fig. 4A). This cluster projected a nerve to the posterior part of the brain and the entire projection path was marked by the *lacZ* expression. The location and morphology of this structure suggested that it was the larval photoreceptor organ, Bolwig's organ, which was confirmed by staining with a PNS-specific antibody, mAb 22C10.[23,24] Double staining δ20 activated embryos for β-galactosidase expression and with mAb22C10 showed that the photoactivated δ20 cells coincided with the Bolwig's organ, fasciculated axons of the Bolwig's organ (Bolwig's nerve), and cells at the termini of Bolwig's nerve presumably in the optic lobe (Fig. 4B).

In addition to producing the larval visual system, some δ20 cells were observed to form an epithelium on top of the Bolwig's nerve projection path (Fig. 4, white arrows). The Bolwig's nerve

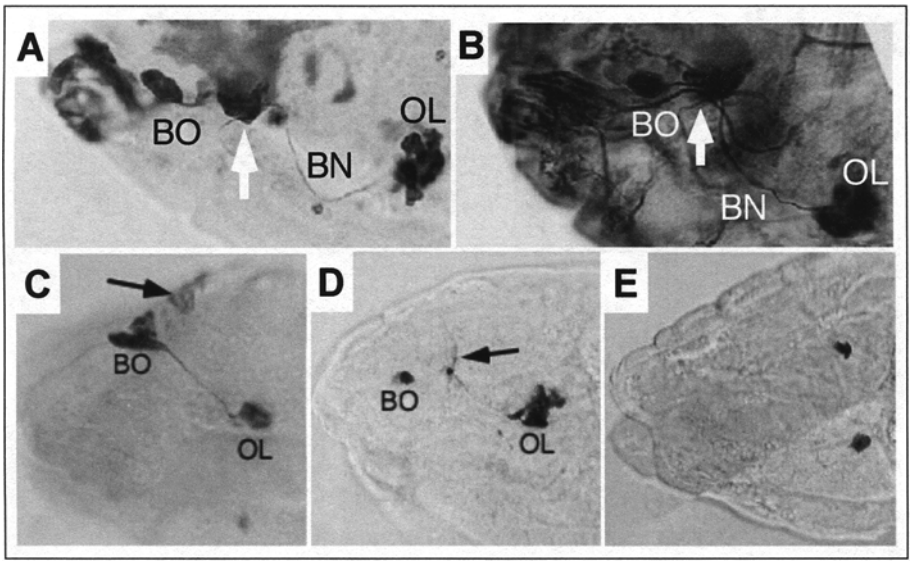

Figure 4. δ20 generates the larval visual system. A,B) Dorsal view of δ20 marked embryos. A) β-galactosidase was expressed in the Bolwig's organ (BO), Bolwig's nerve (BN) and the optic lobe (OL). White arrows point to cells in the dorsal pouch on top of the Bolwig's nerve path. B) β-galactosidase expression (blue) in the larval visual system overlaps with PNS marker expression as visualized with mAb22C10 (brown). C-E) Single-cell photoactivation of δ20; marked cells were found in the developing larval visual system at stage 14 (C) and at stage 17 (D), both lateral views. They were confined to the Bolwig's organ (BO), the optic lobe (OL) and the dorsal pouch (arrows). E) A dorsal view of an embryo with marked cells in both optic lobes. Reprinted from: Namba R, Minden JS. Dev Biol 1999; 212:465-476; ©1999 with permission from Elsevier.[13] A color version of this figure is available online at www.eurekah.com.

extends posteriorly from the Bolwig's organ located inside the dorsal pouch epithelium and makes a sharp, ventral turn near the posterior edge of the dorsal pouch to follow along the basal surface of the brain into the optic lobe. The δ20 cells that formed the epithelial structure were often found at or near where the Bolwig's nerve made the ventral turn, which corresponds to the location of the eye-antennal disc placode.

Photoactivation of single cells in the center of δ20 gave rise to marked cells in the optic lobe, Bolwig's organ, and a small area of the dorsal pouch, presumably the eye-antennal disc placode, exclusively (Fig. 4C,D). This photoactivation typically marked the larval visual system either on the left- or right-hand side of the embryo, while a small fraction of these embryos had marked, visual system cells on both sides of the embryo midline (Fig. 4E). These results show that all of the cell-types that make up the larval visual system can be derived from a single δ20 cell.

Mitotic Domain B Generates Brain Glia

Progeny of mitotic domains 1, 5 and 9 populated almost all of the brain volume (Fig. 1B,C). None of these mitotic domains generated significant numbers of glial cells. Photoactivation of the remaining mitotic domain, δB, revealed a major source of brain glia. Photoactivation of cells in three locations along the length of this elongated mitotic domain (Fig. 1A) in *UAS-nGFP* embryos revealed that their progeny formed small clusters of cells in the presumptive protocerebrum at stage 14 (Fig. 5A, solid arrow). The distribution of these clusters in the stage 14 embryonic brain is diagrammed in Figure 5E,F. These clusters were located deep within the brain and were variable in size. Each of the clusters of marked δB cells was surrounded by dispersed cells (Fig. 5A and cartooned as dots in

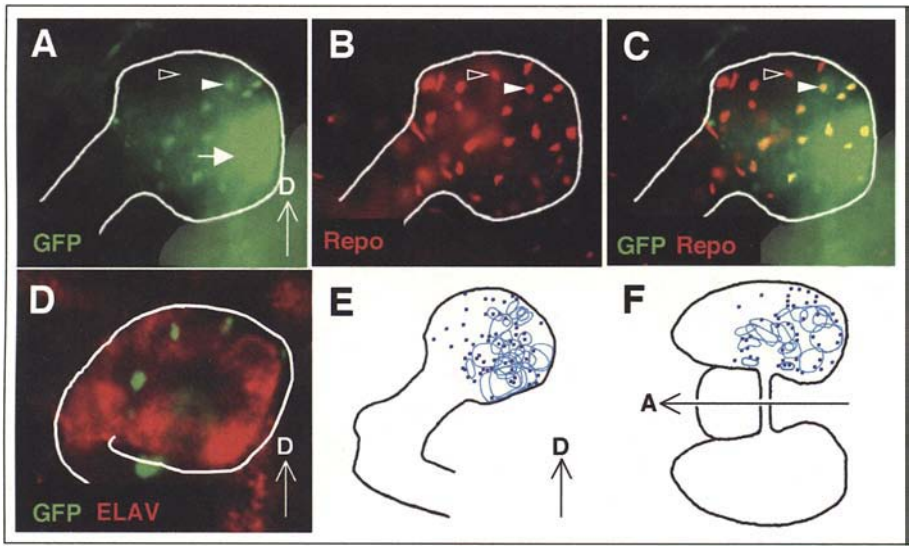

Figure 5. Brain glia originate from δB. A-C) 2-4 cell photoactivation of δB in a *UAS-nGFP* embryo stained with anti-GFP (green) and anti-Repo (red). A) Shows the green fluorescent channel. A cluster of GFP-positive cells below the focal plane (indicated by the solid arrow) that is surrounded by individual cells (solid arrowhead). B) Shows the anti-Repo signal revealing glial cells. C) Shows the superposition of A and B. Notice the double labeled cells (solid arrowhead) and Repo-only glial cells (open arrowhead). D) 2-4 cell photoactivation of δB in a *UAS-nGFP* embryo stained with anti-GFP (green) and anti-ELAV (red). Notice that none of the GFP-positive cells also express the neuronal marker, ELAV. E,F) Schematic representations of marked δB cells within the brain, lateral and dorsal views, respectively. The blue outlined areas represent marked clusters; the blue dots represent isolated cells. Reprinted from: Robertson K et al. Dev Biol 2003; 260:124-137; ©2003 with permission from Elsevier.[15]

Fig. 5E,F). Three-quarters of δB photoactivated embryos had marked, dispersed brain cells that also expressed Repo, indicating that they were glial cells (Fig. 5A-C). None of the marked cells in δB photoactivated embryos expressed ELAV (Fig. 5D), indicating that they are unlikely to be neurons. There are two classes of embryonic brain glia: the subperineural glia that are mostly located in the brain periphery and the neuropil glia.[16] Glial cells arising from δB were identified as subperineural glia by their position. Neuropil glia were never observed, suggesting that this subtype of glial cells may arise from a different source.

Conclusion

The most difficult aspect of fate mapping the head region of the *Drosophila* embryo is its complex morphogenesis. We have fate mapped the majority of mitotic domains within the *Drosophila* procephalic blastoderm using the photoactivated gene expression system and determined that the embryonic brain develops from five mitotic domains: δ1 (posterior-dorsal part), δ5, δ9, δ20 and δB. The final position of the mitotic domain progeny within the brain does not reflect their relative blastoderm positions. Thus, the mitotic domains follow specific morphogenetic trajectories. Several different mechanisms are employed to internalize brain progenitors: the posterior-dorsal part of δ1 and δB invaginate en mass, δ5 and δ20 also invaginate together, and δ9 uses oriented mitosis and possibly delamination. Together, these mitotic domains constitute non-overlapping regions of the brain. This fate map will provide an avenue for performing region-specific experiments. The discrete behavior of the brain-forming mitotic domains raises several interesting questions about the ancestral origin of the brain. One such question is, did the various brain compartments evolve from a common group of cells and later specialize or did the compartments evolve independently and later coalesce to form the brain?

References

1. Foe VE, Odell GM, Edgar BA. Mitosis and morphogenesis in the Drosophila embryo. In: Bate M, Martinez AA, eds. The Development of Drosophila melanogaster. New York: Cold Spring Harbor Laboratory Press, 1993:149-300.
2. St. Johnston D. Pole plasm and the posterior group genes. In: Bate M, Martinez AA, eds. The Development of Drosophila melanogaster. New York: Cold Spring Harbor Laboratory Press, 1993:325-364.
3. Hartenstein V, Technau GM, Campos-Ortega JA. Fate-mapping in wild-type Drosophila melanogaster. III. A fate map of the blastoderm. Roux's Arch Dev Biol 1985; 194:213-216.
4. Jurgens GR, Lehman M, Schardin M et al. Segmental organization of the head in the embryo of Drosophila melanogaster. A blastoderm fate map of the cuticle structures of the larval head. Roux's Arch Dev Biol 1986; 195:359-377.
5. Underwood EM, Turner FR, Mahowald AP. Analysis of cell movements and fate mapping during early embryogenesis in Drosophila melanogaster. Dev Biol 1980; 74(2):286-301.
6. Bossing T, Technau GM. The fate of the CNS midline progenitors in Drosophila as revealed by a new method for single cell labelling. Development 1994; 120(7):1895-1906.
7. Technau GM, Campos-Ortega JA. Fate-mapping in wild-type Drosophila. II. Injections of horseradish peroxidase in cells of the early gastrula stage. Roux's Arch Dev Biol 1985; 194:196-212.
8. Gehring WJ, Wieschaus E, Holliger M. The use of 'normal' and 'transformed' gynandromorphs in mapping the primordial germ cells and the gonadal mesoderm in Drosophila. J Embryol Exp Morphol 1976; 35(3):607-616.
9. Janning W. Aldehyde oxidase as a cell marker for internal organs in Drosophila melanogaster. Naturwissenschaften 1972; 59(11):516-517.
10. Foe VE. Mitotic domains reveal early commitment of cells in Drosophila embryos. Development 1989; 107(1):1-22.
11. Minden JS, Agard DA, Sedat JW, et al. Direct cell lineage analysis in Drosophila melanogaster by time-lapse, three-dimensional optical microscopy of living embryos. J Cell Biol 1989; 109(2):505-516.
12. Cambridge SB, Davis RL, Minden JS. Drosophila mitotic domain boundaries as cell fate boundaries. Science 1997; 277(5327):825-828.
13. Namba R, Minden JS. Fate mapping of Drosophila embryonic mitotic domain 20 reveals that the larval visual system is derived from a subdomain of a few cells. Dev Biol 1999; 212(2):465-476.
14. Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 1993; 118(2):401-415.

15. Robertson K, Mergliano J, Minden JS. Dissecting Drosophila embryonic brain development using photoactivated gene expression. Dev Biol 2003; 260(1):124-137.
16. Hartenstein V, Nassif C, Lekven A. Embryonic development of the Drosophila brain. II. Pattern of glial cells. J Comp Neurol 1998; 402(1):32-47.
17. Koushika SP, Lisbin MJ, White K. ELAV, a Drosophila neuron-specific protein, mediates the generation of an alternatively spliced neural protein isoform. Curr Biol 1996; 6(12):1634-1641.
18. Halter DA, Urban J, Rickert C et al. The homeobox gene repo is required for the differentiation and maintenance of glia function in the embryonic nervous system of Drosophila melanogaster. Development 1995; 121(2):317-332.
19. Tettamanti M, Armstrong, DJ, Endo K et al. Early development of the Drosophila mushroom bodies, brain centers for associative learning and memory. Dev Genes Evol 1997; 207:242-252.
20. Kurusu M, Awasaki T, Masuda-Nakagawa LM et al. Embryonic and larval development of the Drosophila mushroom bodies: concentric layer subdivisions and the role of fasciclin II. Development 2002; 129(2):409-419.
21. Holmes AL, Heilig JS. Fasciclin II and Beaten path modulate intercellular adhesion in Drosophila larval visual organ development. Development 1999; 126(2):261-272.
22. Therianos S, Leuzinger S, Hirth F et al. Embryonic development of the Drosophila brain: formation of commissural and descending pathways. Development 1995; 121(11):3849-3860.
23. Fujita SC, Zipursky SL, Benzer S et al. Monoclonal antibodies against the Drosophila nervous system. Proc Natl Acad Sci USA 1982; 79(24):7929-7933.
24. Goodman CS, Bastiani MJ, Doe CQ et al. Cell recognition during neuronal development. Science 1984; 225(4668):1271-1279.