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Migration and function of glia in the developing *Drosophila* eye

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SUMMARY

Although glial cells have been implicated widely in the formation of axon tracts in both insects and vertebrates, their specific function appears to be context-dependent, ranging from providing essential guidance cues to playing a merely facilitory role. Here we examine the role of the retinal basal glia (RBG) in photoreceptor axon guidance in *Drosophila*. The RBG originate in the optic stalk and have been thought to migrate into the eye disc along photoreceptor axons, thus precluding any role in axon guidance. Here we show the following. (1) The RBG can, in fact, migrate into the eye disc even in the absence of photoreceptor axons in the optic stalk; they also migrate to ectopic patches of differentiating photoreceptors without axons providing a continuous physical substratum. This suggests that glial cells are attracted into the eye disc not through haptotaxis along established axons, but through another mechanism, possibly chemotaxis. (2) If no glial cells are present in the eye disc, photoreceptor axons are able to grow and direct their growth posteriorly as in wild type, but are unable to enter the optic stalk. This indicates that the RBG have a crucial role in axon guidance, but not in axonal outgrowth per se. (3) A few glia close to the entry of the optic stalk suffice to guide the axons into the stalk, suggesting that glia instruct axons by local interaction.

Key words: *Drosophila*, Visual system development, Glia, Migration, Axon guidance

INTRODUCTION

Glia play diverse supportive roles in the adult nervous system, including wrapping and insulating neurons, providing them with nourishment, maintaining their ionic homeostasis, and helping to establish and maintain the blood-brain and blood-nerve barrier. During development, glia help control the patterning of neuronal differentiation and, in particular, guide axons to their targets (Klaes et al., 1994; Klämbt et al., 1996).

The specific role that glia play in the formation of axon pathways appears to depend on the context. In *Drosophila*, the glia along the midline of the central nervous system (CNS) provide complex guidance cues for commissural axons (Klämbt et al., 1991; Tear et al., 1993). Midline glia bear not only attractive cues, such as the Netrins, which attract commissural fibers to the midline, but also repulsive cues, such as Slit, which prevent commissural fibers from recrossing the midline (Seeger et al., 1993; Mitchell et al., 1996; Tear et al., 1996; Kidd et al., 1999). However, the role of glia in the formation of other axonal pathways is less clear. On the one hand, studies of mutants such as *glial cells missing* (*gcm*), *pointed* (*pnt*) and *reversed polarity* (*repo*) in *Drosophila* suggest an ancillary role (Klaes et al., 1994; Halter et al., 1995; Hosoya et al., 1995; Jones et al., 1995). The analysis of *gcm* mutants, which lack all glia except for those in the midline, demonstrates that most axon pathways in the embryo, and specifically the longitudinal axon tracts, can develop without glia, albeit with greater variability, and suggests a merely facilitory role for glia in the formation of non-commissural pathways (Hosoya et al., 1995; Jones et al., 1995). By contrast, toxin-induced ablation of longitudinal glia early in development leads to a complete loss of the longitudinal axon tracts, suggesting that longitudinal glia are strictly required for growth cone guidance in the formation of these axon tracts (Hidalgo et al., 1995). Genetic and toxin ablation studies agree that glia have an essential role in the maintenance of established axon pathways.

In order for glial cells to fulfill their role in axon guidance or even maintenance, they have to be positioned correctly with respect to the neurons. For many glial cell populations, this means migration over many cell diameters within a restricted period of time during development. Examples of migrating glial cells include the midline glia in the *Drosophila* CNS and the oligodendrocytes of the optic nerve in vertebrates (Small et al., 1987; Klämbt et al., 1991). The mechanisms involved in glial migration are not well understood, and only a few cellular and molecular components have so far been identified. In vertebrates, the fibroblast growth factor (FGF) receptor as well as several components involved in cell adhesion have been implicated in oligodendrocyte motility (Milner et al., 1996; Payne et al., 1996; Osterhout et al., 1997). Similarly, in *Drosophila*, the FGF receptor Breathless (Btl) has been implicated in the migration of a subset of midline glia in the embryo, suggesting that activation of the Ras signaling pathway is involved in the migration process (Klämbt et al., 1992; Fried-Reichman et al., 1994).

In this study, we report on the migration and function of glial cells in the developing adult eye of *Drosophila*. A previous

study has shown that the glial cells in the eye, also called the retinal basal glia (RBG), are born in the optic stalk and migrate into the eye disc (Choi and Benzer, 1994). This migration is tightly linked to photoreceptor differentiation, as the number of glial cells filling the eye disc increases with the number of photoreceptors. Conversely, in mutants with no photoreceptors, such as *eyes absent*, glial cells do not enter the eye disc at all and remain in the optic stalk. Based on these results and the observation that glial cells are closely associated with photoreceptor axons, Choi and Benzer (1994) suggested that the RBG migrate into the eye disc along established photoreceptor axons; this would preclude any role for the RBG in the guidance of photoreceptor axons.

Here we show that this model cannot be true. While the RBG indeed require photoreceptor differentiation in the eye disc, we show that they can migrate into the eye disc even in the absence of photoreceptor axons in the optic stalk; this suggests that glial cells do not rely on haptotaxis along established axons to find their way into the eye disc. This notion is supported by our finding that glial cells can migrate to ectopic patches of differentiating photoreceptors without axons providing a continuous physical substratum. These results are compatible with glial cells having a role in axon guidance. In fact, we further show that the RBG, while not necessary for the outgrowth or the posterior orientation of photoreceptor axons within the eye disc, are critically required for guiding the axons into the optic stalk. This guidance function is most likely performed by way of a local interaction between the axons and the glia just before the axons exit the eye disc, and may involve physical contact.

MATERIALS AND METHODS

Drosophila stocks

The *GMRGAL4* line was kindly provided by M. Freeman, *ombGAL4* by S. Cohen, *UASRhoA^{V12}* by M. Mlodzik, *UASdpp* by J. Treisman, *Act>FLPout>GAL4* by S. L. Zipursky, *Act>DRaf>lacZ* by G. Struhl, *UASlacZ* and *UASnuclacZ* by C. Desplan, *UASRas1^{N17}* by D. Montell and *N^{ts1}* by N. Baker.

Lineage-tracing experiments

y,hsFLP122; Adv/Cyo females were crossed to *Act>DRaf>lacZ* males. First instar larvae at 36-48 hours of development were heat shocked at 35° or 38°C for half an hour to induce *lacZ*-positive clones. Larvae were dissected at the third instar larval stage and stained with antibodies against β-galactosidase and the glial marker Repo.

Generation of *dpp*-expressing clones

This was done as described in Pignoni and Zipursky (1997). *y,hsFLP122;UASdpp/TM6B* males were crossed to *Act>CD2>GAL4; UASlacZ* females. The progeny of the cross were heat shocked at 24-36 hours of development for 30 minutes at 35°C. Female larvae were dissected at the late third instar larval stage and stained with 22C10, α-β-galactosidase, α-Repo and α-Dof (Downstream of FGF receptor) antibodies.

Histology

Eye-brain complexes were dissected in PBS and fixed in 4% paraformaldehyde for 30 minutes followed by repeated washes in PBS-0.3% Triton X. Primary and secondary antibodies were diluted in PBS-TX and incubated overnight. Samples were mounted in 80% glycerol. Primary antibodies used were: rabbit α-Repo (gift from G. Technau) at a 1:250 dilution, goat FITC α-HRP (Cappel) at a 1:25 dilution, mouse α-β-galactosidase (Cappel) at a 1:200 dilution, 24B10 antibodies (gift from L. Zipursky) at a 1:100 dilution, 22C10 antibodies (gift from C. Goodman) at a 1:5 dilution, and α-Dof antibodies (gift from M. Affolter) at a 1:100 dilution. Secondary antibodies used were: Cy5 goat α-rabbit (Jackson Labs) at 1:50, Cy3 goat α-mouse (Jackson Labs) at 1:400. Specimens were imaged on a Zeiss 510 LSM or a Biorad MRC 600.

RESULTS

In the developing *Drosophila* visual system, the eye disc is connected to the optic lobe by the optic stalk. Photoreceptor cells are generated in the eye disc in a posterior-to-anterior progression; the progressing wave of differentiation is marked at its front by an indentation of the disc epithelium, called the morphogenetic furrow (Fig. 1A; Bate and Martinez Arias, 1993). A few rows posterior to the morphogenetic furrow, the photoreceptor neurons begin to grow axons into the basal layer of the disc, where they turn and then proceed posteriorly to exit the eye disc through the optic stalk (Fig. 1B,E; Choi and Benzer, 1994). The larval optic nerve, called the Bolwig nerve, runs along the peripodial membrane, through the apical portion of the eye and antennal discs. Glial cells enter the eye disc from the optic stalk and migrate anteriorly on the basal surface of the eye epithelium (Fig. 1C,F; Choi and Benzer, 1994). The anterior border of glial migration is defined roughly by the row of differentiating photoreceptors whose axons have just begun to turn posteriorly (Fig. 1D,G,H). Thus, glia are present only in the axonal portion of the eye disc. The glial cells closely associate with the photoreceptor axons and develop extensive processes that surround the axons and fill the intervening space (Choi and Benzer, 1994). The glial cells in the eye disc and in the optic stalk express Repo, a homeobox protein that is also expressed by most glial cells in the embryo, as well as Downstream of FGF receptor (Dof), a downstream component of the FGF receptor pathway (Halter et al., 1995; Xiong and Montell, 1995; Vincent et al., 1998).

Glial cell migration

Choi and Benzer (1994) have suggested that the RBG migrate into the eye disc along photoreceptor axons. To test this hypothesis, we created eye discs in which photoreceptor cells were able to differentiate but unable to send axons into the optic stalk. We expressed a constitutively active form of *RhoA* (*RhoA^{V12}*) in the photoreceptors using the *UAS/GAL4* system (Brand and Perrimon, 1993; Boutros et al., 1998). Rho is a small GTPase that has been shown to regulate actin cytoskeleton dynamics (Nobes and Hall, 1995; Ridley, 1995; Luo et al., 1997). Mutations in *Rho* family members can also affect axon guidance (Zipkin et al., 1997). We targeted activated *RhoA* expression to the photoreceptors (and not the glia) by using *GMRGAL4* as a driver (Hay et al., 1994; Freeman, 1996). The resulting photoreceptors fail to differentiate correctly and grow very short axons that extend only within the eye disc but fail to project into the optic stalk (Fig. 2A,D); the Bolwig nerve, moreover, is either absent or fails to grow into the optic stalk. We find that, under such conditions, glial cells are still able to populate the eye discs, albeit in smaller numbers (Fig. 2B,C). This result demonstrates

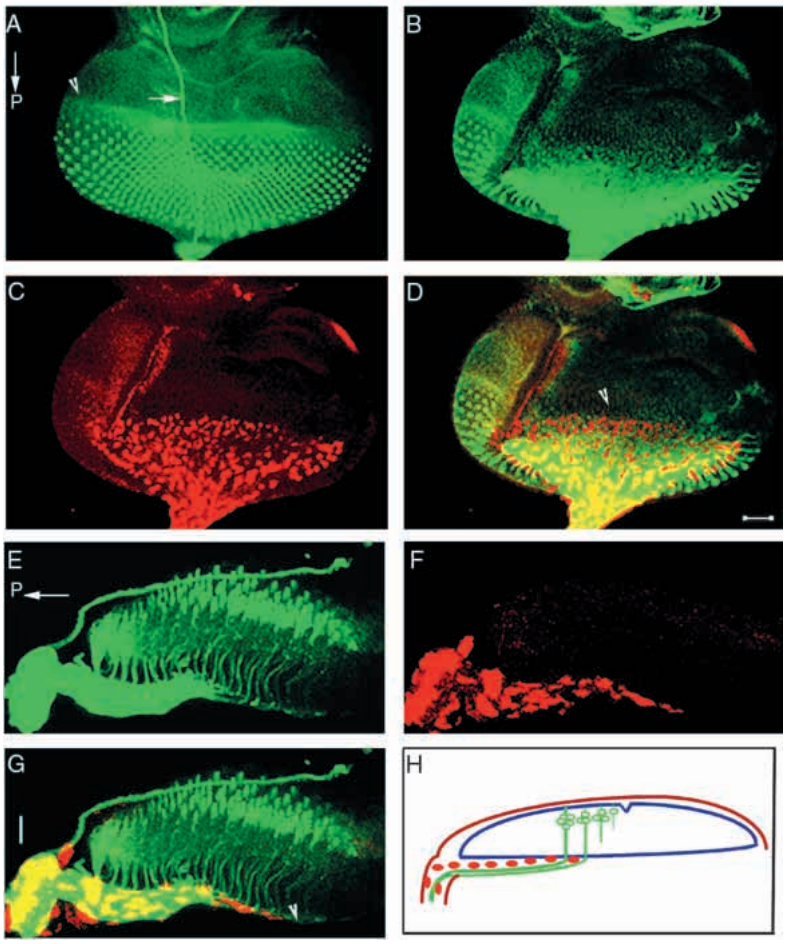

Fig. 1. Organization of photoreceptors and glia in a third instar larval eye disc. (A) Apical surface of an eye disc showing photoreceptor cell bodies labeled with α-HRP antibodies (green). The Bolwig nerve (arrow) is seen bisecting the eye disc as it grows towards the optic stalk. The morphogenetic furrow is marked by an arrowhead. (B) Basal layer of eye disc showing photoreceptor axons growing towards the optic stalk. (C) Same optical section as in B, showing retinal basal glia (RBG) labeled with α-Repo antibodies (red). (D) An overlay of B and C shows that the RBG lie a few rows posterior to the morphogenetic furrow (arrowhead) and closely associate with the axons. (E-G) Sections through the eye disc along the apical-basal axis reveal the relationship of axons to glia. (E) Photoreceptor axons first grow basally in the eye disc and then turn posteriorly and grow towards the optic stalk. (F) RBG cells are seen entering from the optic stalk and filling the eye disc basally from posterior to anterior. (G) An overlay of E and F shows that glial migration trails behind the anteriormost row of photoreceptor differentiation and is confined to the portion of the eye disc where axons have turned towards the optic stalk (arrowhead). (H) Schematic showing the relative positions of the photoreceptor cell bodies, axons and glia along the apical-basal axis. (A-D) Posterior is down, dorsal to the right; (E-H) posterior is to the left. Scale bar in D for A-D, 26 μm; in G for E-G, 10 μm.

that the presence of photoreceptor axons in the optic stalk is not required for RBG migration and thus opens the possibility that the RBG play a role in photoreceptor axon guidance.

To further explore the process of glial migration into the eye disc, we sought to create a patch of differentiating photoreceptors anterior to the morphogenetic furrow and observe whether the glia would now migrate towards such an ectopic source of attraction. To this end, we generated random clones of *decapentaplegic* (*dpp*)-expressing cells, by inducing FLP-out events in *Act>FLPout>GAL4*, which drive expression of *UASdpp* (see Materials and Methods; Staehling-Hampton and Hoffman, 1994; Pignoni and Zipursky, 1997). Clones of ectopic *dpp* expression in the anterior portion of the eye disc initiate ectopic morphogenetic furrows and premature photoreceptor differentiation (Fig. 3A,C,E; Pignoni and Zipursky, 1997). The ectopic morphogenetic furrow forms a circle that grows outward in a concentric progression, creating a growing patch of differentiating photoreceptors within it. Of the 64 discs of this kind that we examined, 6 displayed glial migration directed towards the anterior patch of photoreceptors (Fig. 3D,F,G). In all 6 discs, the patches of photoreceptors were large and located in close proximity to the main body of photoreceptors. The number of glial cells reaching the patches was relatively small, compared with areas of the same size in the posterior of the eye disc (Fig. 3D). In all 6 cases, the photoreceptors in the ectopic patch had grown axonal processes, but these processes were very short and projected

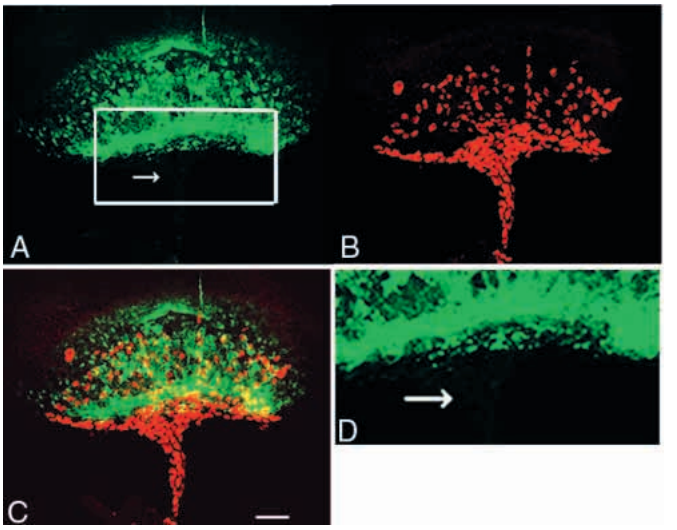

Fig. 2. RBG can migrate into the eye disc without photoreceptor axons in the optic stalk. (A) An eye disc of a *GMRGAL4; UASRhoA^{V12}* animal shows photoreceptor cell bodies and axons labeled with α-HRP antibodies (green). Abnormal differentiation of the photoreceptors results in axons remaining in the eye disc and not entering the optic stalk (arrow). (B) Many glial cells, labeled with α-Repo antibodies (red), enter the eye disc seen in A. (C) An overlay of A and B. (D) High-magnification view of boxed area in A, showing that there are no axons growing into the optic stalk (arrow). Scale bar, 30 μm.

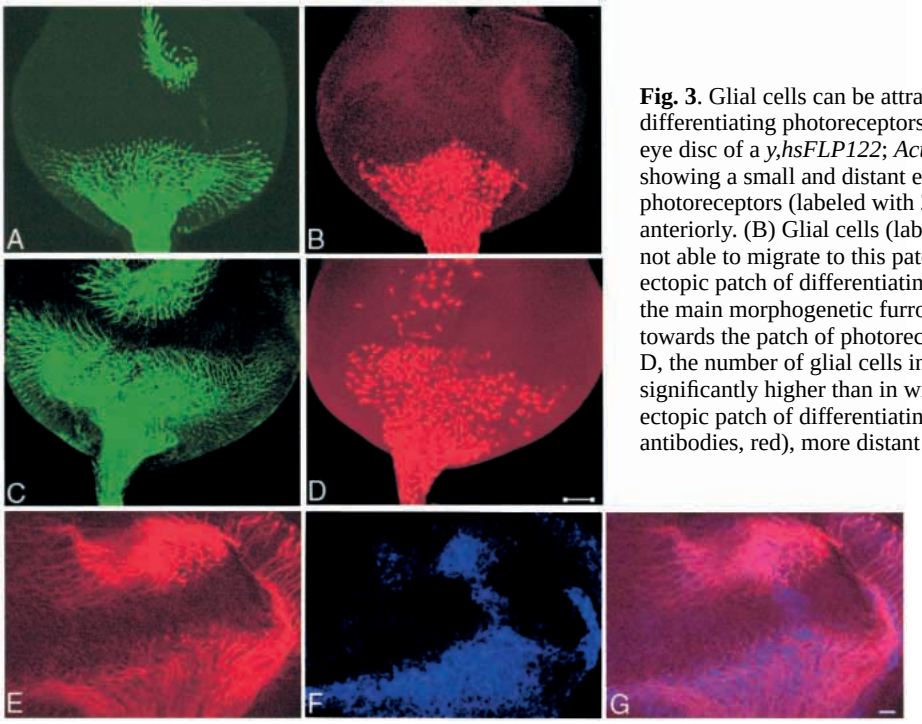

Fig. 3. Glial cells can be attracted to large ectopic patches of differentiating photoreceptors in the anterior of the eye disc. (A) An eye disc of a *y,hsFLP122*; *Act>CD2>GAL4*; *UASdpp* animal showing a small and distant ectopic patch of differentiating photoreceptors (labeled with 22C10 antibodies, green) developing anteriorly. (B) Glial cells (labeled with α-Repo antibodies, red) are not able to migrate to this patch. (C) An eye disc with a large ectopic patch of differentiating photoreceptors developing close to the main morphogenetic furrow. (D) A few glial cells migrate towards the patch of photoreceptors seen in C. Note that, in B and D, the number of glial cells in the posterior portion of the eye disc is significantly higher than in wild type. (E) Another eye disc with an ectopic patch of differentiating photoreceptors (labeled with 22C10 antibodies, red), more distant from the main morphogenetic furrow than in C. (F) Glial cells bodies (labeled with α-Dof (Downstream of FGF receptor, a cytoplasmic protein) antibodies, blue) are seen migrating towards this patch. (G) Overlay of E and F. Note that, in both C and E, no axons are connecting the ectopic patch to the main body of photoreceptors. Scale bar, 30 μm in A-D; 10 μm in E-G.

randomly. Thus, there were no axons providing a physical connection between the ectopic patches and the main body of photoreceptors along which the glia could have travelled. Interestingly, the glial cells were not able to populate small or distant ectopic patches of differentiating photoreceptors (Fig. 3A,B). The largest distance that glial cells traversed from the normal anterior border of glial migration to the ectopic *dpp* patches was 30 μm. Based on these observations, two mechanisms of migration seem possible. Glial cells may respond to a diffusible chemoattractant emanating from differentiating photoreceptors, whose effective range would depend on the strength of the source, and thus on the number of photoreceptors secreting it. Alternatively, glial cells may be guided toward photoreceptors by the stabilization of their filopodial contacts with nascent photoreceptor axons; thus, the distance over which glia could reach without axons as a physical substratum would be determined by the length of the glial filopodia.

Whatever the exact mechanism of migration, it is noteworthy that the boundary of RBG migration appears to be more tightly controlled than the number of cells immigrating into the eye disc. In the wild type, a mid-third instar larval eye disc contains approximately 15 rows of differentiating ommatidial clusters and, in all animals that we examined (n=10), glial cells fill the basal layer of all but the three to four anteriormost rows. Thus, the anterior boundary of RBG migration appears to be rather strictly defined. In contrast, the ratio of glial cells to ommatidia varies from eye disc to eye disc (n=10) and ranges from 1:1.6 to 1:3.6. Given that the number of ommatidia per eye is highly stereotypic among different animals (Ready et al., 1976), this two-fold variation in glial cell number suggests that glial immigration is less stringently controlled than ommatidial differentiation.

We also sought to examine the pattern of migration of the RBG once they enter the eye disc. Do clonally related glial cells disperse randomly within the eye? Do they respect any dorsoventral boundaries? To address these questions, we undertook a lineage-tracing experiment. We created genetically marked clones using the *Act>DRaf>lacZ* FLP-out cassette developed by Struhl and Basler (1993). To induce FLP-out events, we activated FLP recombinase under heat-shock control by heat pulses at various temperatures and different stages of development. We calibrated the heat-shock regimens to ensure a low frequency of recombination. Between 36 and 48 hours of development, a 30 minute heat shock at 38°C resulted in marked glial cells in one out of every four eye discs (n=62), while a heat shock of the same duration at 35°C resulted in marked glial cells in only one out of every thirteen eye discs (n=92). Given the low frequency, it is safe to assume that, in each animal, the marked cells were derived from a single mother cell and thus constitute a clone. Clones generated under either regimen contained cells that remained as a group but did not respect any dorsoventral boundaries (Fig. 4A,B). Based on the irregular shape of the clones, no obvious patterns of migration were apparent.

Glial cell function

Do the RBG have a function in guiding the growth of photoreceptor axons? Photoreceptor axons initially grow towards the basal layer of the disc, then turn 90° and grow posteriorly to exit the eye disc into the optic stalk (see Fig. 1). In order to examine whether RBG are required for the posteriorly directed growth of the axons or for their exit into the stalk, we sought to create eye discs that have no glia.

One way to do this is to forestall glial migration into the eye disc by interfering with signal transduction pathways involved in cell migration. Studies on other populations of migrating cells have demonstrated that Ras signaling can influence the

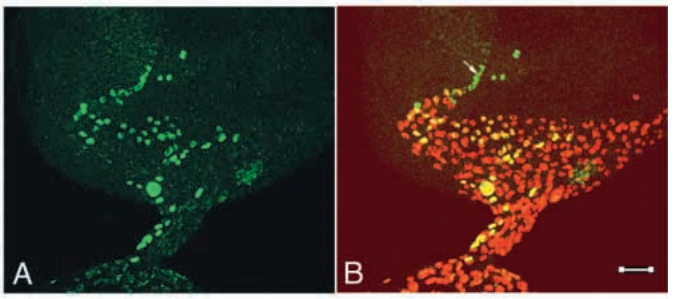

Fig. 4. RBG migration is not restricted with respect to the dorsoventral axis. (A) An example of a glial cell clone created by FLP-out at 36-48 hours of development. Cells within a clone are labeled with α-β-galactosidase antibodies (green). (B) The overlay (yellow) of β-galactosidase-positive cells (green) and Repo-positive cells (red) shows that the clone consists of glial cells. Clonally related cells lie on either side of the dorsoventral midline. The few β-galactosidase-positive cells (green) that are not Repo-positive are epithelial cells at the margins of the eye disc (arrow). Scale bar, 30 μm.

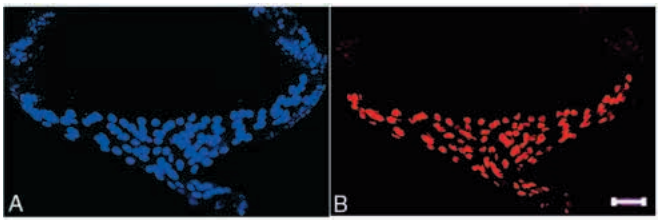

Fig. 5. *ombGAL4* is expressed in the glial cells of the eye disc and of the optic stalk. (A) In *ombGAL4; UASnuclacZ* animals, β-galactosidase-positive nuclei (blue) are seen in the basal layer of the eye disc. Note that *ombGAL4* is also expressed in epithelial cells at the margins of the eye disc. (B) The β-galactosidase-positive cells in the axonal portion of the eye disc are glia, as seen by labeling with α-Repo antibodies (red), whereas the β-galactosidase-positive cells at the margins are not. Scale bar, 30 μm.

locomotion of cells. Dominant negative Ras inhibits migration of a small subset of follicle cells, known as the border cells, when expressed during their migratory period (Lee et al., 1996). In addition, the FGF receptor Breathless (Btl), whose activation leads to stimulation of the Ras signaling pathway, is required in the posterior pair of midline glia to complete its anterior migration in the embryo (Klämbt et al., 1992; Fried-Reichman et al., 1994). We therefore attempted to interfere with glial migration into the eye disc by expressing dominant negative *Ras1* (*Ras1^{N17}*) in glial cells using *ombGAL4* as a driver. In the visual system, *ombGAL4* is expressed in the glial cells and in the margins of the eye disc, but not in the photoreceptors (Fig. 5; Poeck et al., 1993; Lecuit et al., 1996). In about 10% of the *ombGAL4; UASRas1^{N17}* eye discs that we examined (*n*=200), the number of glial cells in the optic stalk is reduced, and these glial cells fail to migrate into the eye disc (Fig. 6A). In such cephalic complexes, photoreceptors are able to grow axons, and these axons orient their growth towards the posterior of the eye disc as in wild type (Figs 6B,C, 7A,B). However, the axons then remain stuck in the eye disc and are unable to exit into the optic stalk (Fig. 6B,C). This result demonstrates that (a) the RBG are not necessary for axonal growth per se or for the orientation of axons towards the

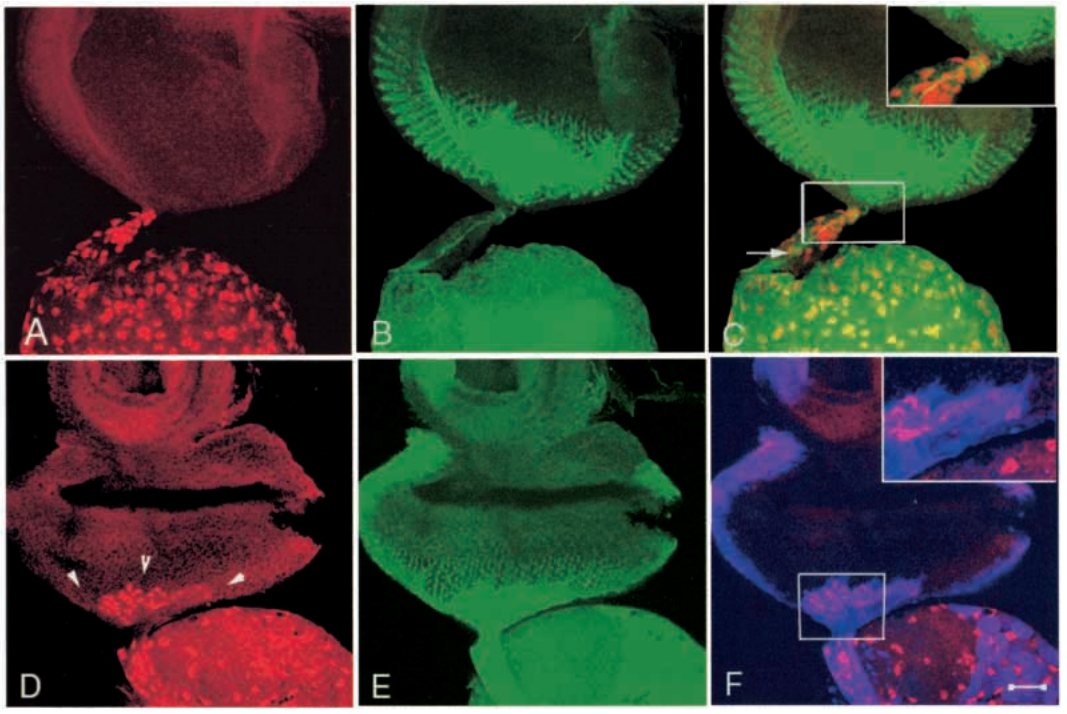

Fig. 6. RBG are critically required for the entry of photoreceptor axons into the optic stalk. (A) In approx. 10% of the *ombGAL4; UASRas1^{N17}* discs examined, Repo-positive glial cells (red) are unable to migrate into the eye disc. (B) As a result of the lack of glia, photoreceptor axons (green) fail to exit from the eye disc and to enter the optic stalk. Photoreceptors are labeled with α-HRP antibodies. (C) An overlay of A and B. The lone axon bundle seen growing through the stalk is the Bolwig nerve (arrow). Inset shows a high magnification view of the optic stalk. (D) In approx. 40% of the *ombGAL4; UASRas1^{N17}* animals, a small number of glia are able to reach the eye disc. These glia remain in a cluster at the posterior end of the eye disc and fail to populate the more anterior, differentiating portion of the eye disc (arrowheads). (E) Axons (green) in such discs succeed in exiting the eye disc and grow into the optic stalk. (F) The cell bodies of the few glial cells in the eye disc, visualized by the expression of cytoplasmic β-galactosidase (blue, overlay looks purple), are small and do not extend much anteriorly. Inset shows a magnified view of the glial cell bodies; we do not observe any cytoplasmic extensions or processes growing far beyond the nuclei. Scale bar, 30 μm.

posterior, and (b) RBG are required for guiding photoreceptor axons into the optic stalk.

Our data allow a further specification of this requirement. In about 40% of the *ombGAL4; UASRas1^{N17}* animals, eye discs have a partial migration phenotype, i.e. they contain a small number of glial cells ranging from 3 to 20 (Fig. 6D). In all cases, these glial cells are found very close to the optic stalk at the very posterior end of the eye disc, where they remain in a cluster. Interestingly, in such discs, axons are able to exit into the optic stalk normally, indicating that the presence of a few glial cells in the posterior portion of the eye disc is sufficient to guide photoreceptor axons into the optic stalk (Fig. 6E). We determined the size of the glial cell bodies by introducing a *UASlacZ* transgene into *ombGAL4; UASRas1^{N17}* animals; this allowed us to visualize glial cell bodies using α-β-galactosidase antibodies. We find that, even when few glial cells are present in the eye disc, their cell bodies are small, with short processes that remain confined to the posterior of the eye disc (Fig. 6F). These findings, in conjunction with the fact that glial cells are not required for axon growth within the eye disc, suggest that the mechanism by which glia guide the axons involves either physical contact or a short-range diffusible molecule.

DISCUSSION

Our results demonstrate that the presence of RBG in the eye disc is critical for the ability of photoreceptor axons to exit the eye disc and enter the optic stalk, but is not required for axonal growth within the disc or for its posterior orientation. In the absence of glial cells in the eye disc, photoreceptor axons grow towards but cannot enter the optic stalk; if even a few glia are present near the entrance to the optic stalk, they can. The number of glial cells that is required for the successful exit of the axons into the optic stalk is small and the requirement occurs at a critical choicepoint. This picture of glial cell function in axon guidance agrees with the one derived from genetic ablation of glial cells in the embryo, but contradicts the one arising from toxin ablation (Hidalgo et al., 1995; Hosoya et al., 1995; Jones et al., 1995). Genetic ablation of midline or longitudinal glia in the embryo does not affect axonal growth per se, but it does affect the direction of axon growth if the glia bear important guidance cues, as is the case with the midline glia (Seeger et al., 1993; Tear et al., 1993; Mitchell et al., 1996).

What might be the nature of the interaction between the photoreceptor axons and RBG in guiding axons into the optic stalk? The fact that the presence of glia in the optic stalk does not suffice to elicit entry of axons into the stalk, whereas the presence of just a few glia with small cell bodies in the eye disc near the optic stalk does, suggests that the glia guide the axons through a local interaction, either a contact-mediated process or a very short-range diffusible signal.

Interestingly, our findings in the insect visual system have a striking parallel in the vertebrate visual system. Embryonic retinal ganglion cell (RGC) axons in the mouse exit the retina through the optic disc into the optic stalk. The glial cells that constitute the optic disc are important for the exit of RGC axons into the optic stalk, but not for growth within the retina (Deiner et al., 1997). The molecular nature of this interaction is known: the optic disc glia secrete Netrins, which act as local chemoattractants for the RGC axons. In the Netrin 1-deficient mouse, most RGC axons do not make it into the optic stalk, resulting in optic nerve hypoplasia (Deiner et al., 1997). In the fly visual system, Netrin transcripts have been detected in the optic stalk (Q. G. and U. G., unpublished observation); however, animals lacking both Netrin A and Netrin B do not show any axon pathfinding phenotypes, making it unlikely that the Netrins are the signals used by the RBG to attract axons (Gong et al., 1999). Thus, the molecular nature of the guidance cue(s) provided by the glial cells in the *Drosophila* eye disc remains to be elucidated.

What causes RBG to migrate into the eye disc? Previous studies have shown that the number of glia increases progressively as more photoreceptors differentiate within the eye disc and that lack of photoreceptor differentiation results in a failure of glial cells to migrate into the eye disc (Choi and Benzer, 1994). Our study demonstrates that the RBG, while depending on some level of photoreceptor differentiation in the eye disc, do not require the presence of photoreceptor axons in the optic stalk to find their way into the eye disc, and that they do not require axons as a continuous substratum to migrate to ectopic patches of differentiating photoreceptors in the anterior portion of the eye disc. The ability of glial cells to migrate without a continuous axonal substratum is a prerequisite for their having any function in axon guidance; thus, our finding that the presence of glial cells in the eye disc is required for the entry of photoreceptor axons into the optic stalk serves as further proof that glial migration into the eye disc does not depend on the presence of axons in the stalk. Our data therefore rule out the model proposed by Choi and Benzer (1994; haptotaxis along established axons); this leaves two possible models for the migration of RBG into the eye disc. One mechanism is that differentiating photoreceptors secrete a diffusible chemoattractant, providing a gradient along which the RBG travel. Alternatively, nascent photoreceptor axons within the eye disc may be contacted by glial filopodia while the glial cells are still in the optic stalk. Such contact might stabilize the filopodia and enable the glia to enter the

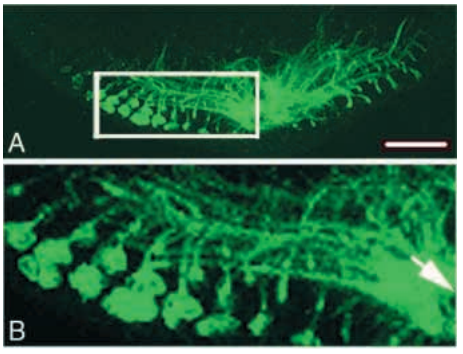

Fig. 7. RBG are not required for axon outgrowth or for posterior orientation of photoreceptor axons. (A) In *ombGAL4; UASRas1^{N17}* eye discs with no glial cells, axons are able to grow within the disc. (B) In a high magnification view of A, these axons are seen growing posteriorly towards the optic stalk (arrow), as in wild type. Photoreceptors are labeled with 24B10 antibodies, which label only mature photoreceptors and axons. Scale bar, 30 μm.

eye disc. This latter model would require glial filopodia to project over a large distance. In the case of the ectopic *dpp* patches, this distance can be as much as 30 μm, which is approximately four times the average diameter of RBG cells.

Whatever the precise mechanism of migration, it appears that the number of RBG entering the eye disc is not very tightly controlled. Even in wild type, the ratio of glia to ommatidia in the developing eye varies by a factor of 2. We also found that an overproliferation of differentiating photoreceptors, achieved through temporary inactivation of a temperature-sensitive allele of *Notch* (N^{ts1}; Cagan and Ready, 1989), does not lead to a consistent and significant increase in the number of glial cells entering the eye (R. R. and U. G., unpublished observation). A possible explanation for this finding is that glial migration into the eye disc depends on additional factors, such as substratum availability, which may be compromised in N^{ts1} mutants due to excessive differentiation and outgrowth of axons.

In contrast to the number of migrating cells, the boundary of RBG migration into the eye disc appears to be controlled rather strictly. Glial cells are found only in the axonal portion of the eye disc and, under the N^{ts1} mutant conditions just described, glial cells continue to respect this boundary. A diffusible chemoattractant could not by itself produce such a sharp anterior boundary of RBG migration; thus, the chemoattraction model has to assume that the RBG preferentially adhere to axonal surfaces or that a chemorepellant is secreted in the anterior, undifferentiated portion of the eye disc. Whatever the mechanism, it can be partially overridden by the presence of an ectopic patch of photoreceptors in the anterior of the eye disc, provided the patch is large and close enough to the main body of photoreceptors.

What might be the function of this restriction on the anterior migration of the RBG? A primary function would seem to be the prevention of anterior misrouting of photoreceptor axons. As we have shown, RBG can, through a local interaction, provide critical cues for the guidance of photoreceptor axons. Thus, glial cells (mis-)migrating anteriorly might, if close and numerous enough, override the mechanism that is responsible for the posterior orientation of the photoreceptor axons in wild type and cause anterior misrouting of axons. We do not observe such a misrouting of axons in the ectopic *dpp*-expression experiment, perhaps because the number of ectopic glia is relatively small.

Our experiments show that RBG migration can be disrupted by modulating the level of Ras activity. This finding accords with similar results that have been obtained for several other types of migrating cells, establishing that Ras levels are critical for regulated motility (Anand-Apte and Zetter, 1997; Porter and Vaillancourt, 1998). However, the factors that lie upstream of Ras in the regulation of cell migration have been identified in only a small number of cases. The FGF receptor Btl, which controls tracheal and glial migration in *Drosophila* embryos, is one such example (Klämbt et al., 1992; Fried-Reichman et al., 1994). To date, we have not found any evidence that FGF receptors play a role in the migration of the RBG. Neither Btl nor Heartless, the other FGF receptor known in *Drosophila* (Gisselbrecht et al., 1996), is expressed in these cells as assayed by RNA in situ hybridization and antibody stainings, respectively (R. R. and U. G., unpublished observation). We cannot, however, rule out the possibility that other FGF receptors may exist in the fly.

Overall, the study of glial migration and function in the developing *Drosophila* eye reveals a high level of interdependence between neurons and glia, a theme that is familiar in the development of both vertebrate and invertebrate nervous systems (Raff et al., 1993). Differentiating photoreceptors trigger glial migration into the eye disc. As we have shown here, glial cells, in turn, provide the crucial cue for photoreceptor axons to exit the eye disc and enter the optic stalk. Again in turn, growing axons may help to determine the final destination of the glial cells, which are then needed to maintain neuronal function in the later stages of development and in adult life. Further genetic studies will be instrumental in dissecting the molecular pathways underlying this complex symbiotic interplay between neurons and glia during the development of the *Drosophila* visual system.

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