Journal Club

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# Carpet Cells Regulate Glial Cell Motility in the Developing *Drosophila* Eye

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Review of Silies et al. (http://www.jneurosci.org/cgi/content/full/27/48/13130)

Glial cells and axons must migrate to reach target cells in the expanding environment of a growing organism. Precise nervous system assembly requires careful regulation of cellular motility. The *Drosophila* visual system has served as a valuable model to study how specific connections are achieved during nervous system development. For example, in the developing eye, photoreceptor (R-) cells are born during midlarval to late larval stages when the morphogenetic furrow, a wave of cellular differentiation, passes across the eye imaginal disc. R-cell axons grow from the eye disc, through a connecting structure called the optic stalk, into the optic lobe of the brain. A number of genes that are required for the proper layer-specific targeting of R-cell axons have been identified (Rao et al., 2000; Kaminker et al., 2002; Cafferty et al., 2004).

The regulatory processes that direct glial cell migration during visual system development are less well understood than that of axons. Glial cells travel from the optic stalk into the eye imaginal disc in the direction opposite that of R-cell axons (Choi and Benzer, 1994). In other developmental contexts, *Drosophila* glial cells require contact with established axon tracts for migration. For example, both midline and longitudinal glia migrate directly on commissural and longitudinal axon tracts in the *Drosophila* CNS (for review, see Cafferty and Auld, 2007). In the visual system, however, glial cells continue to migrate into the eye disc even when the growth of R-cell axon tracts is prevented (Rangarajan et al., 1999). This result suggests that an alternative mechanism for directing glial cell motility exists in the visual system. Such an alternative mechanism is suggested in a recent paper published in *The Journal of Neuroscience*, in which Silies et al. (2007) have demonstrated that the migration of glial cells into the developing *Drosophila* eye is largely regulated by glia–glia interactions.

Silies et al. (2007) initially characterized gliogenesis in the developing eye by counting the number of cells positively stained with a glial nucleus-specific antibody in confocal stacks of eye imaginal discs at different developmental stages [Silies et al. (2007), their Fig. 2*A–D* (http://www.jneurosci.org/cgi/content/full/27/48/13130/F2)]. The number of glia in the eye disc increased throughout larval development until the end of the third instar larval stage, when ~325 cells were counted per eye disc [Silies et al. (2007), their Fig. 2*E* (http://www.jneurosci.org/cgi/content/full/27/48/13130/F2)]. Further staining of glial nuclei with a mitotic marker revealed that glial cell proliferation occurred in both the optic stalk and the basal side of the eye disc [Silies et al. (2007), their Fig. 2*F–H* (http://www.jneurosci.org/cgi/content/full/27/48/13130/F2)]. Thus, the increasing number of glia observed in the eye disc over time resulted from both migration into the eye disc and cell division within the disc.

Glial cell types in the eye disc were labeled and identified using a genetic "flip-out" approach. Silies et al. (2007) performed a series of matings to generate flies that combined an Flp recombinase, under the control of a glial-specific promoter, with a "flip-out" cassette. An Flp recombinase is an enzyme that catalyzes recombination events at specific DNA sequences, called Flp recombinase targets (FRTs). Cells are labeled when the Flp recombinase catalyzes the removal of ("flips out") an inert stuffer sequence, flanked by FRT sites, from a cassette to activate a reporter gene. In this paper, glial-specific Flp recombinase expression triggered GFP (green fluorescence protein) labeling exclusively in glial cells. Confocal microscopy of "flipped-out" imaginal discs in which R-cells and glial cell nuclei were immunostained allowed Silies et al. (2007) to identify six distinct glial cell types [Silies et al. (2007), their Figs. 3 (http://www.jneurosci.org/cgi/content/full/27/48/13130/F3), 4 (http://www.jneurosci.org/cgi/content/full/27/48/13130/F4)]. Among these cell types are glia not previously described in the *Drosophila* visual system, including the following: (1) R-cell

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axon-ensheathing glia in the optic stalk; (2) migratory glia located on the basal side of the eye disc; (3) differentiating glia proximal to R-cell axons in the eye disc; (4) glial cells close to the morphogenetic furrow that extend filopodia; and (5) marginal glia that overlap adjacent glia. A novel glial cell that extended processes across one half of the eye disc was observed. The authors named this cell a "carpet glia" because of its large, thin cell structure.

Examination of glial cells by confocal and electron microscopy (EM) suggested that carpet cells separate outer perineurial glia from inner glia. Septate junctions, which are invertebrate intercellular junctions that provide barriers to solute diffusion, were detected by immunostaining against a septate junction marker and observed in both the optic stalk [Silies et al. (2007), their Fig. 5*A*,*B* (http://www.jneurosci.org/cgi/content/full/27/48/13130/F5)] and eye disc [Silies et al. (2007), their Fig. 5*C*,*D* (http://www.jneurosci.org/cgi/content/full/27/48/13130/F5)] using confocal microscopy. EM of the optic stalk revealed that septate junctions were formed by carpet glia. The carpet glial cells were observed to extend around the stalk, form junctions where the carpet cells contact each other, and thereby separate outer perineurial glia from inner glia [Silies et al. (2007), their Fig. 6 (http://www.jneurosci.org/cgi/content/full/27/48/13130/F6)]. EM of a longitudinal section through the eye disc also revealed a separation of outer and inner glia by the carpet glia [Silies et al. (2007), their Fig. 7*A* (http://www.jneurosci.org/cgi/content/full/27/48/13130/F7)].

Based on the morphological data from confocal microscopy and EM, Silies et al. (2007) proposed that glia–glia interactions regulate glial migration into the eye imaginal disc (Fig. 1). According to this model, outer perineurial glial cells migrate from the optic stalk into the eye disc along carpet glia. At the margin of the carpet glia, perineurial glia contact epithelial and neuronal cells that might trigger glial differentiation along and ensheathment of axon tracts. Thus, carpet glial cells separate immature migrating glia from mature glia that ensheath R-cell axons and are required for proper glial cell movement into the eye disc.

Silies et al. (2007) used three experiments to test the proposed model for glial cell migration into the developing eye. First, Silies et al. (2007) blocked mitosis in either the outer or the inner glial cells of the eye disc [Silies et al. (2007), their Fig. 8*F–H* (http://www.jneurosci.org/cgi/content/full/27/48/13130/F8)]. Prevention of mitosis in the outer but not the inner glia resulted in a reduction of the total number of glial cells in the eye imaginal disc, as revealed by immunostaining. This result indicates that glial cells are separated in the eye disc by age: the immature, mitotically active cells are segregated to the outer region of the eye disc, whereas older, terminally differentiated glia are found in the inner region of the disc. Next, a marker was expressed constitutively in the younger, outer cells [Silies et al. (2007), their Fig. 8*I* (http://www.jneurosci.org/cgi/content/full/27/48/13130/F8)]. Confocal microscopy revealed that glial cells expressing the outer glial cell marker eventually ensheathed R-cell axons, suggesting that the outer glia migrate to inner positions of the eye disc and ensheath axons. In a final experiment, Silies et al. (2007) selectively ablated carpet glia at the end of larval development and visualized the effect on glial migration into the eye disc by staining glial cell nuclei. In the absence of carpet glia, glial cells were found to move further into the eye imaginal disc epithelium when compared with wild-type eye discs, indicating that the carpet glia restrict aberrant glial cell migration into the eye disc [Silies et al. (2007), their Fig. 9*C*,*D* (http://www.jneurosci.org/cgi/content/full/27/48/13130/F9)]. Together, these results support the idea that glial cells move from outer to inner regions of the eye disc in a manner regulated by carpet cells.

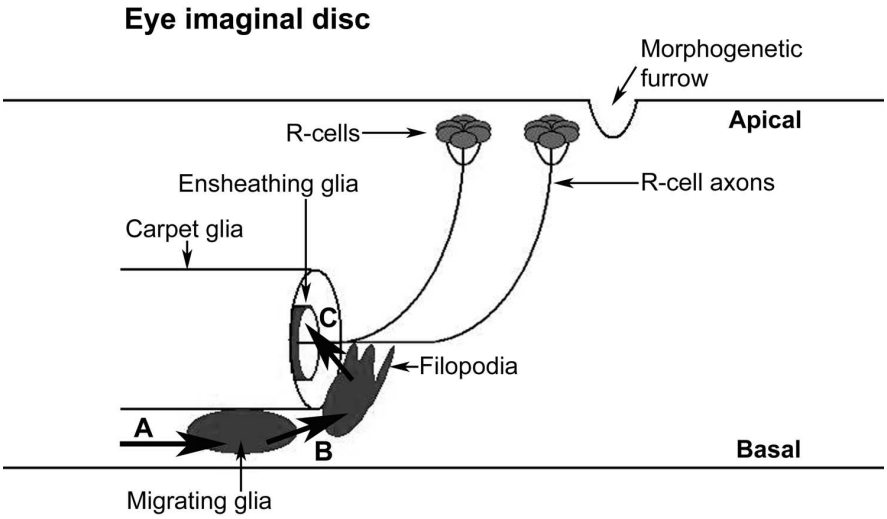


**Figure 1.** Model for glial migration from the optic stalk into the eye imaginal disc. ***A***, Immature, mitotically active glial cells migrate into the eye imaginal disc by following carpet glial cell membranes. ***B***, At the margin of the carpet cell membranes, migrating glia extend filopodia that contact epithelial and neuronal cells. ***C***, Glial cells are triggered to differentiate along and ensheath R-cell axon tracts.

The nervous system is assembled in a carefully orchestrated manner in order for motile cells to locate specific targets. Silies et al. (2007) have identified and demonstrated a requirement for carpet glia to restrict glial migration into the eye disc. Further work is required for a complete understanding of the regulation of glial cell migration in the eye disc. For instance, the mechanisms that control migration of the carpet glia into the eye disc are currently unknown. Also, the molecular requirements that allow outer perineurial glial cells to migrate along and release from carpet glia have not been identified. Presumably, adhesion molecules allow perineurial glia to adhere to carpet glia during migration into the eye disc and release at the margin of the carpet glia. The current study by Silies et al. (2007) provide the first clues to reveal how glia–glia interactions regulate glial cell migration in the developing *Drosophila* eye.

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