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Optomotor-blind expression in glial cells is required for correct axonal projection across the *Drosophila* inner optic chiasm

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Abstract

In the *Drosophila* adult visual system, photoreceptor axons and their connecting interneurons are tied into a retinotopic pattern throughout the consecutive neuropil regions: lamina, medulla and lobula complex. Lamina and medulla are joined by the first or outer optic chiasm (OOC). Medulla, lobula and lobula plate are connected by the second or inner optic chiasm (IOC). In the regulatory mutant *In(1)omb^{H31}* of the T-box gene *optomotor-blind* (*omb*), fibers were found to cross aberrantly through the IOC into the neuropil of the lobula complex. Here, we show that *In(1)omb^{H31}* causes selective loss of OMB expression from glial cells within the IOC previously identified as IOC giant glia (ICg-glia). In the absence of OMB, ICg-glia retain their glial cell identity and survive until the adult stage but tend to be displaced into the lobula complex neuropil leading to a misprojection of axons through the IOC. In addition, adult mutant glia show an aberrant increase in length and frequency of glial cell processes. We narrowed down the onset of the IOC defect to the interval between 48 h and 72 h of pupal development. Within the 40 kb of regulatory DNA lacking in *In(1)omb^{H31}*, we identified an enhancer element (ombC) with activity in the ICg-glia. ombC-driven expression of *omb* in ICg-glia restored proper axonal projection through the IOC in *In(1)omb^{H31}* mutant flies, as well as proper glial cell positioning and morphology. These results indicate that expression of the transcription factor OMB in ICg-glial cells is autonomously required for glial cell migration and morphology and non-autonomously influences axonal pathfinding.

Keywords: *Drosophila*; Optic chiasm; Glia; *Optomotor-blind*; T-box; Visual system development; Cell migration; Axonal pathfinding; Gliopodia

Introduction

In the developing nervous system, axonal growth cones are guided towards their target region by a variety of cues, which can be presented by several different cell types along the way, such as other neurons or glial cells. Presentation and interpretation of attractive and repellent signals are crucial for correct axonal pathfinding. Proper neuronal connectivity also requires the establishment of confined neuronal compartments, which are separated by glial septa (Younossi-Hartenstein et al., 2003). In *Drosophila*, glial cells have been shown to be involved in a number of steps in the development of the nervous system, such as axonal guidance (discussed in more detail below), axonal pruning (Awasaki and Ito, 2004; Watts et al., 2004), synapse function (Allen and Barres, 2005; Auld et al., 2003; Auld and Robitaille, 2003) and neuronal survival (Booth et al., 2000; Buchanan and Benzer, 1993; Xiong and Montell, 1995; for reviews, see Chotard and Salecker, 2004; Edenfeld et al., 2005; Kretzschmar and Pflugfelder, 2002; Tayler and Garrity, 2003).

In the embryonic CNS, midline glia present guidance cues to commissural axons such as the attractive signal Netrin and the repellent Slit (Jacobs, 2000). Longitudinal and segment boundary (SBC) glial cells have been identified as guidepost cells for outgrowing motor axons (Auld, 1999). However, there is no absolute requirement for these glial cells as indicated by the overall normal axonal patterning in *glia cell missing* (*gcm*)

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mutants where glial cells are transformed into neurons (Hosoya et al., 1995; Jones et al., 1995; Sepp et al., 2001). On the other hand, a clear role for glial cells has been established in the development of the adult *Drosophila* visual system. Lamina glia are required as intermediate targets for R1–R6 photoreceptor axons. Two different kinds of processes have been recognized. First, lamina glia need to migrate correctly to their final position within the developing lamina, a process which is affected in *nonstop* and *JAB1/CSN5* mutants (Poeck et al., 2001; Suh et al., 2002). Second, lamina glial cells are likely to display signals to the arriving R1–R6 growth cones instructing them to halt their growth. No such signal has been identified so far. While several genes have been identified as being required neuronally for R1–R6 axon targeting to the lamina (Garrity et al., 1999; Martin et al., 1995; Newsome et al., 2000; Rao et al., 2000; Ruan et al., 1999, 2002; Senti et al., 2000; Suh et al., 2002), *nonstop* remains the only one reported to play a role in the lamina glial cells (Poeck et al., 2001).

Altered axonal projection patterns can also be a secondary result of a disruption of neuronal compartment integrity. The four neuronal compartments in the *Drosophila* optic lobes (lamina, medulla and lobula complex, comprised of lobula and lobula plate) are descendants of two different progenitor regions, the outer and the inner optic anlage (Hofbauer and Campos-Ortega, 1990; Meinertzhagen and Hanson, 1993; Younossi-Hartenstein et al., 1996). Cell populations derived from different anlagen come to lie in close proximity during development without intermingling, such as the lamina neurons and glia derived from the outer optic anlage and the lobula cortex neurons formed by the inner optic anlage (Dearborn and Kunes, 2004; Hofbauer and Campos-Ortega, 1990; Meinertzhagen and Hanson, 1993). In certain mutants, the mixing of cells across the border of these two neuropils results in the disorganization of lamina glia correlated with aberrant projections of R1–R6 to the medulla (Fan et al., 2005; Tayler et al., 2004). The border between lamina and lobula cortex was shown to depend on the presence of secreted Slit around lamina glia and expression of the Slit receptor Robo on distal cells of the lobula cortex (Tayler et al., 2004). Also, the glycosyl transferase Egghead is required in the lobula cortex primordium for compartment border integrity. In *egghead* mutants, defects in boundary formation can be visualized as disruptions in the curtain between lamina and lobula cortex generated by sheath-like glial cell processes (Fan et al., 2005).

In the *Drosophila* optic lobes (OL), photoreceptor axons and their connecting interneurons are organized in a retinotopic pattern, in which the dorso-ventral and anterior–posterior relations between retinal photoreceptor cells are preserved at the interneuron level throughout the proximal neuropil regions. The relative spatial relationships are retained within the individual neuropils even though the bulk orientations of the medulla and lobula complex are distinctly shifted (by rotation and translation) relative to the coordinates of the retina. The four OL neuropil regions are connected by two chiasmata: the first or outer optic chiasm (OOC) connects lamina and medulla, the second or inner optic chiasm (IOC) connects medulla, lobula and lobula plate.

Currently, little is known about structure and development of the *Drosophila* IOC. A model of fiber paths connecting the three neuropils, which surround the IOC was proposed based on studies of larger Dipteran flies (Braitenberg, 1968, 1970). According to this model, which is likely to hold also for *Drosophila*, medulla and lobula plate as well as lobula and lobula plate are connected retinotopically in a horizontal plane by non-inverted connections, whereas medulla and lobula are connected via two types of inverted fiber paths.

Both chiasmata contain glial cells, which can be distinguished from other optic lobe glia by their position, morphology and the expression of specific genetic markers (Tix et al., 1997; for a description of all glial cell types found in the optic lobes, see Dearborn and Kunes, 2004; Eule et al., 1995). Because of their large nuclei and cell bodies the IOC glia were termed "giant" (g), hence ICg-glia (Tix et al., 1997). Along the dorso-ventral axis, ICg-glia and fiber bundles that cross the IOC are arranged in an alternating pattern (Dearborn et al., 2002; Tix et al., 1997). The ICg-glia, like fiber tract glia in other systems, serve to insulate these tracts from one another by wrapping them with thin cytoplasmic extensions (Tix et al., 1997).

In certain combinations of *optomotor-blind* (*omb*) alleles, projection defects are observed in the IOC (Brunner et al., 1992). *omb* encodes a transcription factor of the T-Box family of DNA-binding proteins (Pflugfelder and Heisenberg, 1995; Pflugfelder et al., 1992). *omb* was first identified in a screen for genes required for proper large field optomotor response (Heisenberg et al., 1978). *omb* is expressed in several cell types and tissues during embryonic and larval development (Grimm and Pflugfelder, 1996; Poeck et al., 1993). In the visual system, OMB is found in many types of glial cells including the three types of lamina glia: satellite, epithelial and marginal glia (Dearborn and Kunes, 2004; Huang and Kunes, 1998; Poeck et al., 1993). *omb* transcription is under the control of numerous enhancers located up- and downstream of and within the transcription unit (Sivasankaran et al., 2000). A 45-kb regulatory region downstream of the transcription unit was identified and named "optic lobe regulatory region" (OLR) due to the fact that deletions of parts or of the entire region lead to various neuroanatomical defects within the optic lobes which are associated with defects in visual behavior. The OLR was dissected into three subregions by use of deletion and inversion mutants removing successively larger parts of the regulatory region, with OLR1 lying closest to and OLR3 farthest away from the transcription unit (Brunner et al., 1992). In mutants in which OLR2+3 are deleted, a disruption of the IOC manifests itself with ectopic fibers invading the adult lobula complex which is not seen in mutants in which OLR3 alone is lacking. The severity of the phenotype is increased when the entire OLR is deleted. Other behavioral and neuroanatomical phenotypes, apparently unrelated to the IOC defect, are seen in these mutants, such as a reduced number of fibers in the anterior optic tract, the loss of the lobula plate giant horizontal and vertical cells and a reduction of the large field response (Brunner et al., 1992).

Projection defects through the IOC as a result of mutations in other genes have been described before (see discussion) but a