The Drosophila visual system is composed of the eye, which senses visual information, and the underlying optic lobe (Fig.1, red), which receives and processes this information. Within the optic lobe (Fig.2), there are four neuropils of which the medulla is the largest and mediates motion and colour processing. There are over 100 genetically and morphologically distinct cell types in the medulla.

The Fly Visual System as a Model for Studying the Development of the Vertebrate Retina

Neurons of both the vertebrate retina and Drosophila medulla are stratified into parallel synaptic layers and possess similar classes of neurons including: 1) intrinsic neurons (vertebrate amacrine cells, Drosophila RGCs), and 2) projection neurons (vertebrate RGCs and Drosophila TM1/TM2 neurons) (Fig.3). Furthermore, the neurons of the retina and medulla (and their progenitors) share the expression of homologous genes (Table 1, Fig.6), thus making findings in the fly especially relevant to human visual system development and disease.

Identifying Dorsal-Ventral OPC Patterning Genes

A micro-pipette-based cell extraction technique published by Caygill et al. (2012) was used to collect fluorescently labeled dorsal and ventral Optix+ OPCs (Fig.8). These cells were sent out for bulk RNA-sequencing using the Illumina HiSeq2500 system. Bioinformatic analysis was performed to identify genes upregulated in dorsal or ventral samples.

24 genes were expressed at significantly different levels in the dorsal and ventral OPC cell samples (Fig.9), including 4 transcription factors. Spalt (sal) and spalt-related (sat) were highly enriched in the dorsal samples, while disco-related (disco) and disco-related (disc) were enriched in the ventral samples.

Sal and Disco Form Strict Dorsal and Ventral OPC Compartments

A small population of cells in the Drosophila embryo, termed the optic placode, give rise to the OPC present in the larva (Fig.10). Immunostaining with antibodies against Sal and Disco confirmed that these transcription factors already define strict dorsal-ventral compartments in the optic placode from early embryonic developmental stages (Fig.11).

The OPC is spatially (Fig.6A) and temporally (Fig.6B) patterned by the expression of transcription factors. Thus, each neural stem cell of the OPC possesses a spatio-temporal identity based on the combination of spatial and temporal factors expressed.

Unique Neuronal Populations are Born Across the Dorsal-Ventral Axis

Each medulla neuron type can be assigned a unique birth address based on the spatio-temporal identity of the stem cell from which it was born. Neurons with identical spatial and temporal birth addresses can assume different fates based on whether they are born in the dorsal or ventral half of the OPC (Fig.7). There are no known genes that differentially pattern the dorsal and ventral OPC.

Sal Shares Cross-Regulatory Relationships with the Ventral Patterning Gene, disco, to Maintain Dorsal OPC Identity

Complimentary gain-of-function and loss-of-function experiments reveal that sal shares cross-regulatory relationships with disco. Ectopic expression of sal in the ventral-OPC (v-OPC) is sufficient to repress disco (Fig.13). Similarly, ectopic expression of disco in the dorsal-OPC (d-OPC) is sufficient to repress sal (Fig.14). Sal expression in the d-OPC is not necessary for repression of disco (Fig.15). In contrast, disco expression is necessary for the repression of sal in the v-OPC allowing these ventral stem cells to take on a dorsal, Sal+ identity (Fig.16).

Ectopic sal Expression Affects the Specification of Ventrally-Derived Medulla Neurons

Based on the above results, a dorsal (Disco; Sal+) identity can be conferred to v-OPC progenitors by knocking out disco expression, effectively “dorsalizing” the stem cells of the v-OPC. A genetic driver line used to label ventrally born Dm4 medulla neurons no longer labels these neurons when the v-OPC is “dorsalized” (Fig.18), indicating a loss of this neuron type in the medulla. As expected, a driver line specific to dorsal-born Dm11 neurons maintains its ability to label these neurons when the v-OPC is deficient for disco (Fig.17).

Uncovering the Downstream Targets of Sal and Disco

I have generated driver lines which separately label OPC stem cells and neuronal progeny of the dorsal and ventral, Vsx1 and Optix compartments (Fig.19). After FACS sorting these cells, single-cell multiomic RNA and ATAC-seq will be performed on the samples. The unique chromatin landscapes of dorsal versus ventral cells may uncover the epigenetic mechanisms that underly how Sal and Disco confer dorsal and ventral identities to stem cells respectively. Additionally, RNA-seq analysis will identify the downstream targets of Sal and Disco involved in neural specification.

Role of SALL in the Developing Vertebrate Retina

The SALL family of transcription factors are the vertebrate homologues of sal. Several of the SALL genes are expressed in the developing vertebrate retina (i.e., SALL2, Fig.20), and mutations in these genes have been implicated in developmental defects such as retinal coloboma in humans (Fig.21, arrows).

Interestingly, SALL3-/- mice show a disproportionate loss of NF165, a marker for horizontal cells, in the vertebral retina (Fig.22). Our finding that Sal expression is restricted to dorsal OPC stem cells may help to explain why this asymmetric phenotype in the mouse retina occurs and our work is antecedent to the identification of novel genetic regulators and targets of the SALL genes in the developing vertebrate retina.

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