Characterization of *Spalt*, the *Drosophila* homolog of the vertebrate *SALL* genes, in the developing fly visual system

Priscilla Valentino and Ted Erclik
Department of Cell and Systems Biology, University of Toronto

The *Drosophila* Visual System

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.

Fig.1

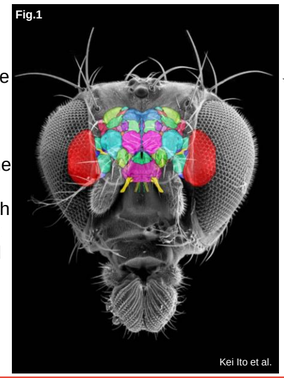
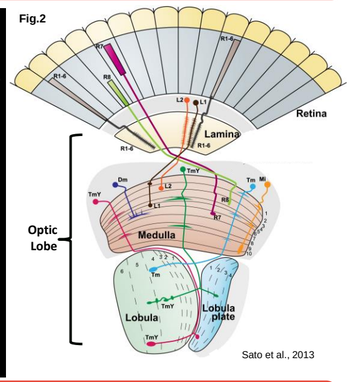
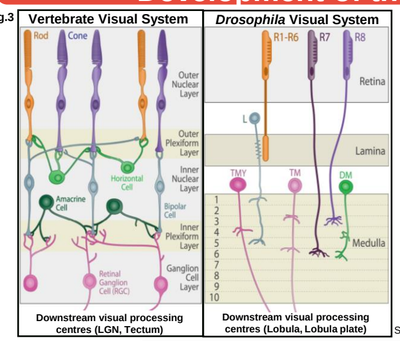


Fig.2



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

Fig.3



Sanes and Zipursky, 2010

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* Dm neurons), and 2) projection neurons (vertebrate RGCs and *Drosophila* Tm/Tmy 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.

Table 1

	Homologous Visual System Development Genes					
Drosophila	Vsx1	Optix	Rx	Hth	Ey	D
Vertebrate	Vsx2	Six3	Rx	Meis1	Pax6	Sox2

Development of the Medulla

Fig.4

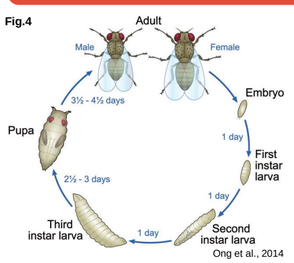
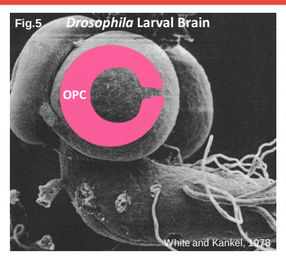


Fig.5 *Drosophila* Larval Brain



Optic lobe neurogenesis commences during larval development (Fig.4). Medulla neurons develop from a crescent-shaped population of neural stem cells in the larval brain termed the outer proliferation centre (OPC) (Fig.5).

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.

Fig. 6A Spatial Patterning

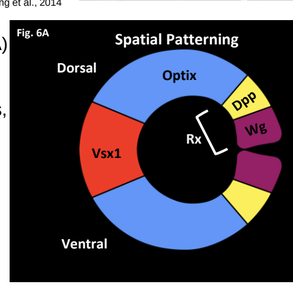
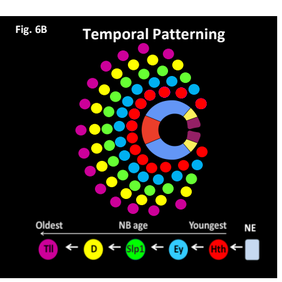
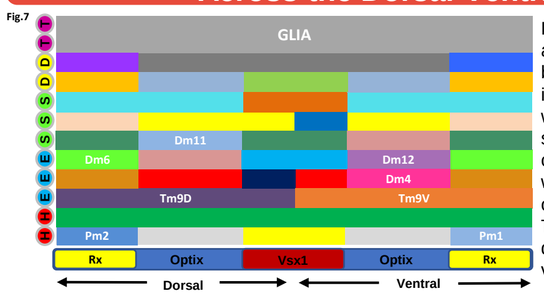


Fig. 6B Temporal Patterning



Unique Neuronal Populations are Born Across the Dorsal-Ventral Axis

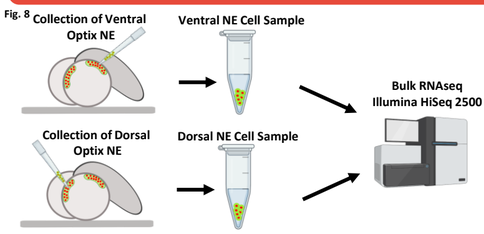
Fig.7



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 ventral or dorsal half of the OPC (Fig.7). There are no known genes that differentially pattern the dorsal and ventral OPC.

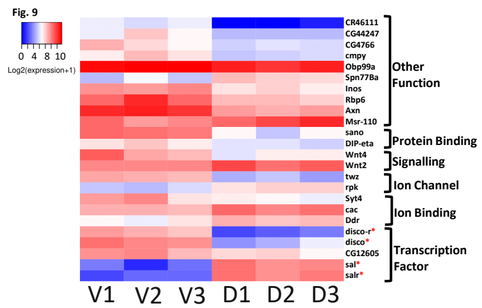
Identifying Dorsal-Ventral OPC Patterning Genes

Fig. 8



A micropipette-based cell extraction technique published by Caygill et al. (2012) was used to collect fluorescently labeled dorsal and ventral Optix⁺ OPC cells (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.

Fig. 9



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* (*salr*) were highly enriched in the dorsal samples, while *disconnected* (*disco*), and *disco-related* (*disco-r*) were enriched in the ventral samples.

Sal and Disco Form Strict Dorsal and Ventral OPC Compartments

Fig.10

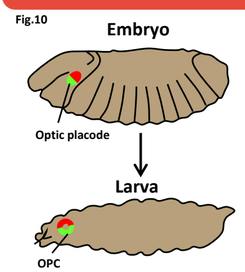
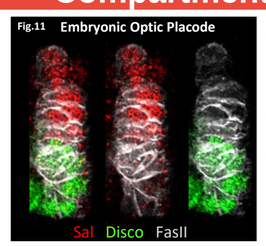


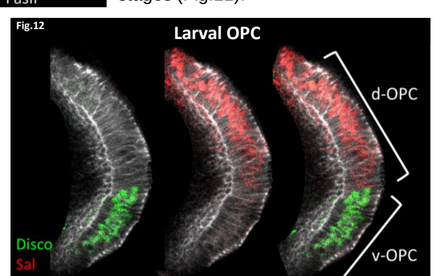
Fig.11 Embryonic Optic Placode



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 compartments in the optic placode from early embryonic developmental stages (Fig.11).

Sal and Disco continue to be expressed in the larval OPC and are exclusively expressed in dorsal and ventral progenitors, respectively (Fig.12). Dorsal-ventral compartmentalization of the OPC by Sal and Disco is maintained throughout all stages of medulla neurogenesis.

Fig.12



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

Fig. 13 *sal* GOF Clones

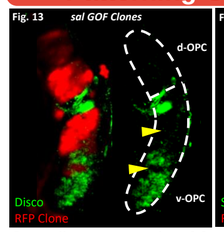


Fig. 14 *disco* GOF Clones

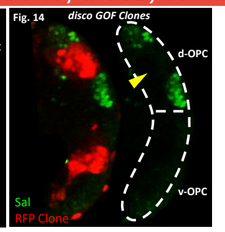


Fig. 15 *sal* LOF Clones

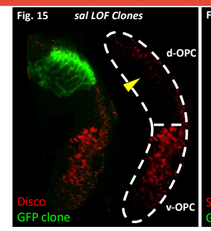
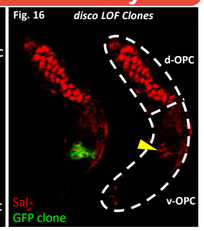


Fig. 16 *disco* LOF Clones



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

Fig.17

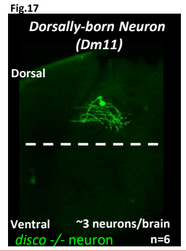
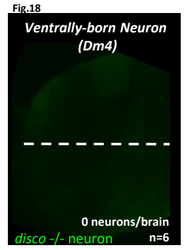


Fig.18

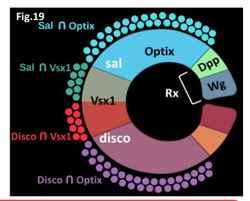


Based on the above results, a dorsal (Disco⁻, Sal⁺) identity can be conferred to v-OPC progenitors by knocking out *disco* expression, effectively "dorsalizing" the stem cells of the v-OPC. A genetic driver line used to label ventrally-born Dm4 medulla neurons no longer labels these neurons when the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

Fig.19



Role of *SALL* in the Developing Vertebrate Retina

Fig.20 *SALL2 in situ*

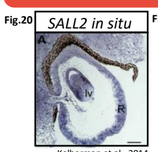
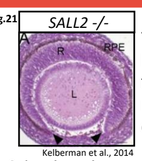


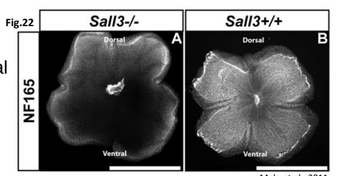
Fig.21 *SALL2 -/-*



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 ventral 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 anticipated to lead to the identification of novel genetic regulators and targets of the *SALL* genes in the developing vertebrate retina.

Fig.22



Melo et al., 2011

Acknowledgements

The authors would like to thank Bryan Stewart and Baohua Liu for their assistance with the cell isolation protocol. Funding for this project was provided by the Vision Science Research Program and the Ontario Graduate Scholarship. mRNA library preparation and RNA-seq was performed by Sickkids: TCAG.