

Utilizing ScRNA-seq to identify axonal guidance cues and receptors in olfactory sensory neuron development



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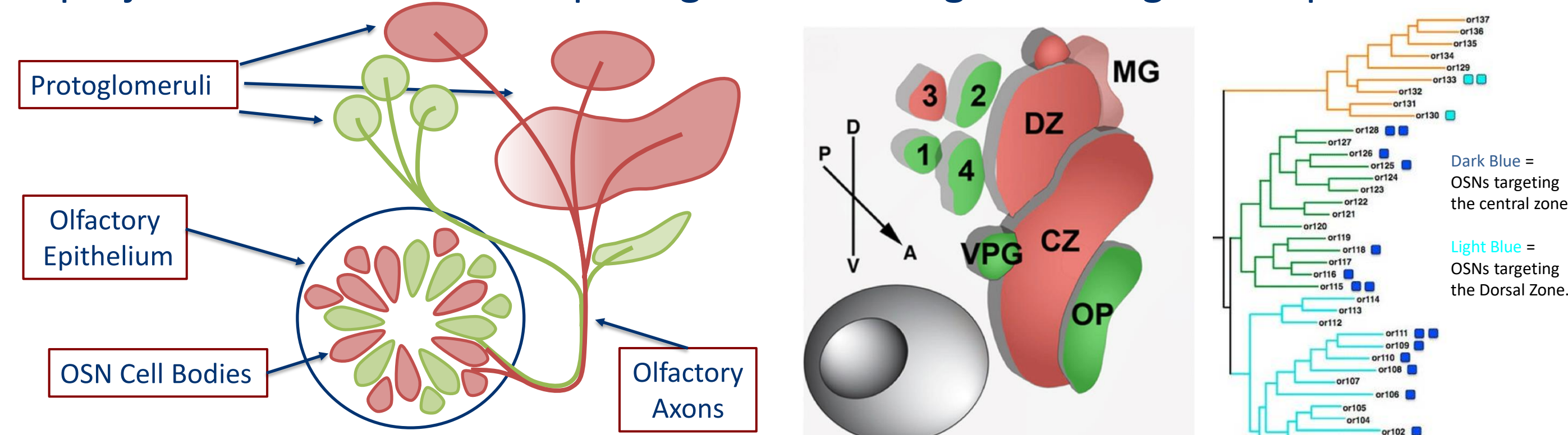


Abstract

This study aimed to identify guidance molecules involved in the development of olfactory sensory neurons (OSN) in zebrafish. Olfactory sensory neurons express a single odorant receptor (OR) from a repertoire of OR genes giving each OSN a unique identity. OSNs expressing the same OR project axons to the same OR-specific target within the olfactory bulb through a guidance process that is not completely understood. We utilize a scRNA-seq approach to identify candidate guidance molecules and other genes involved in this developmental process. OSNs were collected by FACS from transgenic 36hpf, 48hpf and 72hpf embryos in which a promoter element from olfactory marker protein drove a fluorescent marker. Samples were processed by the NGSC at UPenn using the 10X chromium platform 3' V3 and sequenced using the NovaSeq6000 to an average depth of 74,000 reads per cell. The 36hpf sample contained 4,748 cells, the 48hpf sample contained 11,057 cells, and the 72hpf sample contained 5,913 cells. Analysis was performed in R using Seurat version 4.03 and DESeq2. Clustering analysis and Uniform Manifold Approximation and Projection (UMAP) was performed revealing multiple unique clusters in all three time points. Two distinct mature OSN clusters were observed at each timepoint. Additionally, cells were force-clustered by OR homology clade expression. DESeq2 analysis was performed and identified a number of guidance molecules differentially expressed between the clades including ephrins, protocadherins and robo2. Additional transmembrane molecules not previously associated with axonal pathfinding were also differentially expressed between OSNs expressing ORs from different clades.

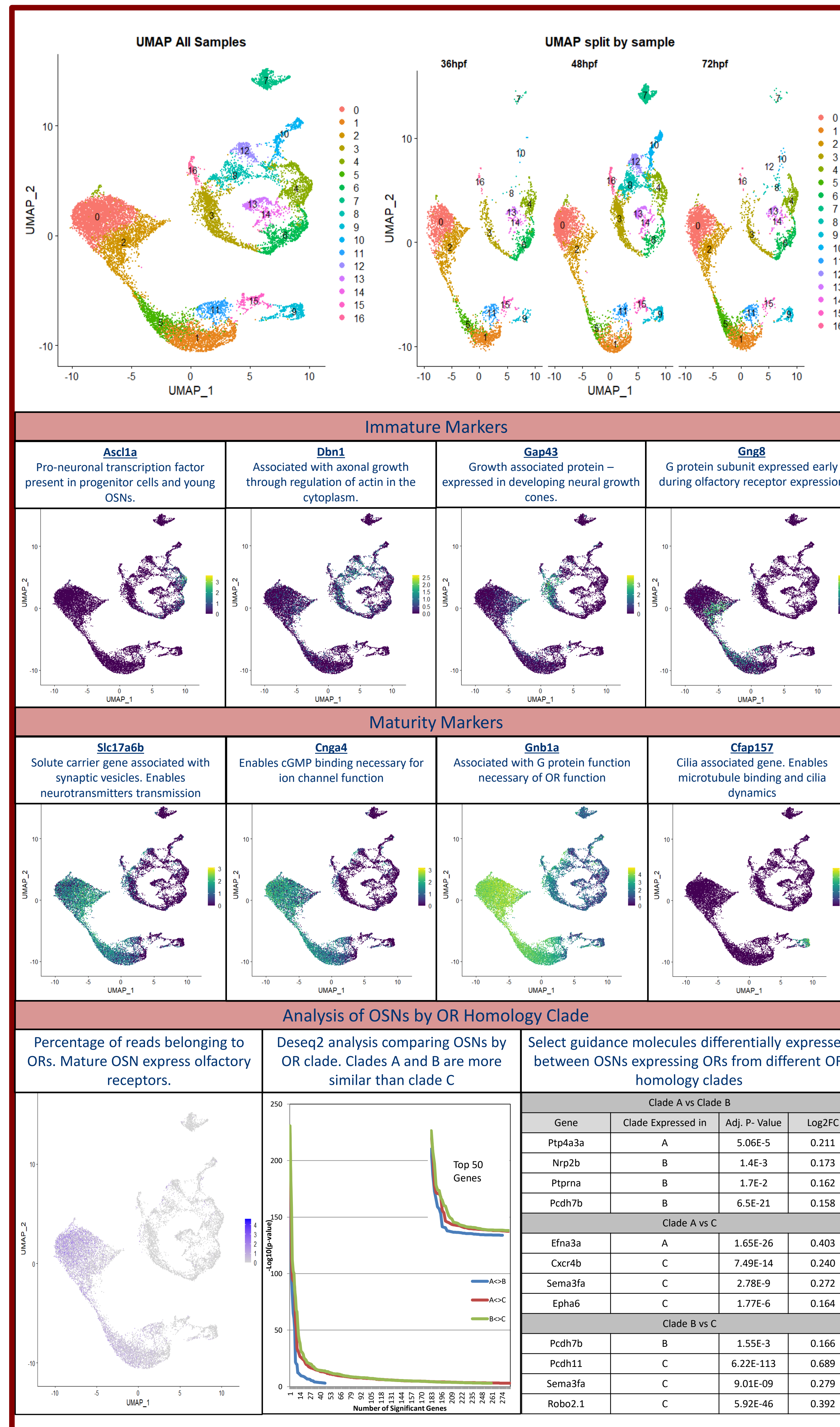
Background

- OSNs are born in the olfactory epithelium and project axons to the olfactory bulb. OSNs express a single OR from a repertoire of OR genes. OSNs expressing the same odorant receptor ultimately coalesce at OR-specific and individually identifiable neuropil called a glomeruli.
- Early in development OSNs target larger protoglomerular regions before individual glomeruli form.
- We hypothesize that OSNs expressing ORs from the same homology clade project axons to the same protoglomerular region during development.



- Using BAC recombineering OSNs expressing ORs from Clades A B or C were individually labeled . ORs from homology clades A and B projected axons to the central zone (CZ) while ORs from homology clade C projected to the dorsal zone (DZ). (Shao et al. 2017)

Results



Methods

- Fish expressing OMP:RFP were collected at 36hpf, 48hpf, and 72hpf. Olfactory tissue was dissected from the fish to isolate individual OSNs.



- Following the 10x Genomics pipeline, the reads were mapped to the zebrafish genome grz11.
- Analysis of the data was performed in R using a combination of Seurat and Deseq2.
- For each cell expressing a single OR an OR identity was established. For cells expressing more than one OR, a dominant OR was assigned if the more highly expressed OR was expressed 5 fold higher than the lesser expressed OR.
- Cells that did not have a clear dominant OR were not included in homology clade analysis.
- For homology clade analysis cells were split into the respective clades
 - Clade A = OR subfamilies 101-114
 - Clade B = OR subfamilies 115-128
 - Clade C = OR subfamilies 129-137

Implications

- UMAP cluster maps looked very similar over several developmental timepoints, consistent with ongoing generation and maturation of Olfactory Sensory Neurons during these early developmental times.
- A clear developmental trajectory was observed. Early neurons expressed the pro-neuronal TF ascl1a through OR associated gene gng8. Two main clusters of mature OSNs are observed expressing synapse associated slc17a6b through cilia associate Cfap157.
- OSNs could be identified by the OR they expressed.
- Deseq2 analysis identified multiple guidance molecules differentially expressed between OSNs expressing ORs from different homology clades.
- Several of the differentially expressed candidate guidance molecules we detected have previously been shown to play a functionally significant role in axonal guidance while many additional candidates remain to be studied.

Citations

- Dang P, Fisher SA, Stefanik DJ, Kim J, Raper JA (2018) Coordination of olfactory receptor choice with guidance receptor expression and function in olfactory sensory neurons. PLoS Genet 14(1): e1007164. <https://doi.org/10.1371/journal.pgen.1007164>
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- Tan L, Li Q, Xie XS. Olfactory sensory neurons transiently express multiple olfactory receptors during development. Mol Syst Biol. 2015 Dec 8;11(12):844. doi: 10.15252/msb.20156639. PMID: 26646940; PMCID: PMC4704490.