Poster

Functional analysis of genes and cis-regulatory elements involved in the specification of optic cup territories identified by scRNAseq and scATACseq

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ABSTRACT

Motivation: In all metazoans, sight depends on the intimate relation and combined function between photoreceptors and pigmented cells, which derive from common precursors through the bifurcation of a single gene regulatory network (GRNs) into two mutually exclusive developmental programs; the neural retina (NR) and retinal-pigmented epithelium (RPE). Studying how GRNs guide the differentiation of these tissues is an essential step to better understand the molecular bases of retinal degenerative diseases and the dynamics of embryonic development. Here we use the development of the optic cup in zebrafish (Danio rerio) as a vertebrate model organism, while continuing a well-established line of work that previously identified active cis-regulatory elements in each NR/RPE domain and captured the transition states of genetic networks using ATAC-seq and scRNA-seq epigenomics techniques respectively from different pools of sorted cells derived out from distinct domains of the optic cup at several stages of development. In this study we plan to functionally analyze a battery of candidate genes that are relevant transcription factor/targets in multiome analysis from ATAC-seq and slightly to strongly overexpressed in pseudobulk differential expression analysis from scRNAseq at 18-somite stage. Among the genes chosen for functional analysis, there are 5 from the retina, 6 from the RPE, 2 from the optic stem, 2 from the diencephalon and another 2 from the telencephalon, together with characterized genes whose expression are well-known to serve as experimental controls: vsx2, bhlhe40, vax1, gbx2 and emx1 respectively for each type of tissue.

Methods: To carry out the functional analysis of the previously identified genes we are following a fluorescent in situ hybridization (FISH) protocol performe on wildtype zebrafish embryos fixed at 18h post-fertilization. This is a powerful and commonly used technique in whole-mounted embryos to assess phenotype, allowing us via super-resolution microscopy to examine temporal and spatial patterns of gene expression by visualizing its mRNA with a fluorescent riboprobe able to specifically recognize a DNA sequence. The first step was the identification of species-specific mRNA sequences and the design of primers for each candidate gene to reduce the risk of non-specific binding of the riboprobes or background signal. The synthesis of riboproteins was carried out after amplifying and purifying the cDNA sequences ordered.

REFERENCES

Buono, L., Corbacho, J., Naranjo, S. et al. Analysis of gene network bifurcation during optic cup morphogenesis in zebrafish. Nat Commun 12, 3866 (2021). https://doi.org/10.1038/s41467-021-24169-7

Martinez-Morales JR, Cavodeassi F, Bovolenta P. Coordinated Morphogenetic Mechanisms Shape the Vertebrate Eye. Front Neurosci. 2017 Dec 20;11:721. doi: 10.3389/fnins.2017.00721. PMID: 29326547; PMCID: PMC5742352.

Martínez-Morales JR, Rodrigo I, Bovolenta P. Eye development: a view from the retina pigmented epithelium. Bioessays. 2004 Jul;26(7):766-77. doi: 10.1002/bies.20064. PMID: 15221858.