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CHROMATIN SIGNATURES OF NEURONAL SUBPOPULATIONS WITH DIVERGENT PROJECTION AT MIDLINE IDENTIFY NEW REGULATORS OF AXON GUIDANCE DECISIONS

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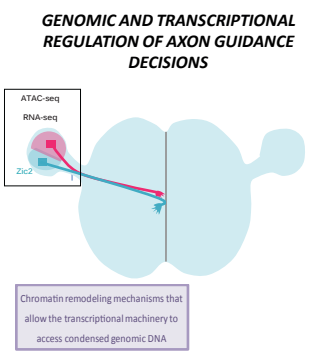
Therapeutic approaches aimed to restore the function of damaged neuronal circuits will benefit from a better understanding of the mechanisms driving and constraining circuit assembly. The binary decision of crossing or avoiding the midline that retinal ganglion cells (RGCs) axons take at the optic chiasm during embryonic development is essential for binocular vision and represents a simple and robust model to identify novel mechanisms controlling axon guidance decisions during circuits formation. By comparing the transcriptome and the profiles for chromatin accessibility and occupancy of crossed and uncrossed RGCs, we identified key differences between these two populations of neurons. Our unbiased screens revealed important differences in the expression of guidance molecules and the binding of transcription factors to regulatory regions, exposing novel transcriptional mechanisms underlying axon guidance decisions. In vivo functional assays with two of these candidate regulatory genes demonstrate the implication of the transcription factor Lhx9 and the chaperone synuclein-g in the navigation of RGCs. Overall, our study retrieved new molecules potentially implicated in axonal pathfinding, thereby contributing to a better understanding of the transcriptional regulatory logic underlying axon guidance decisions

Introduction



Methods

Generation of reporter mouse lines for contra- & ipsilateral RGCs & Isolation of nuclearly tagged RGCs

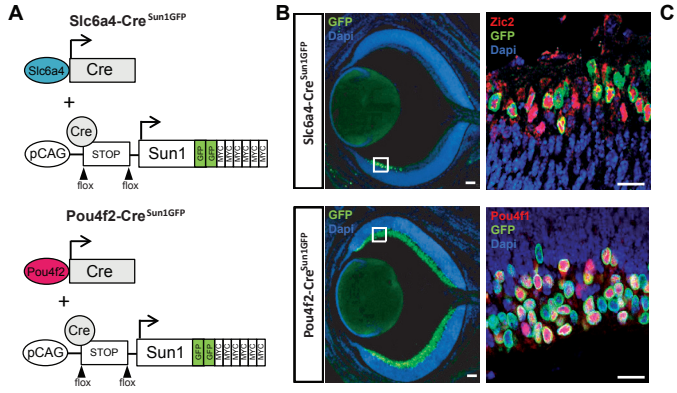


Figure 1. Generation of mouse lines to specifically label ipsilateral or contralateral RGCs and cells isolation for mRNA-seq and ATAC-seq. A. Schematic representation of mouse crossing to generate reporter mouse lines for ipsi and contralateral RGCs. B. Double immunofluorescence with GFP antibody (green) and Pou4f2 or Zic2 antibodies (red) to detect ipsilateral or contralateral RGCS at E16.5. C. Isolation of contralateral and ipsilateral RGCS of retinas from Pou4f2-Cre$^{Sun1\text{-}GFP}$ and Slc6a4-Cre$^{Sun1\text{-}GFP}$ mice at E16.5 by flow cytometry.

Results

Transcriptomic signatures of iRGCs & cRGCs

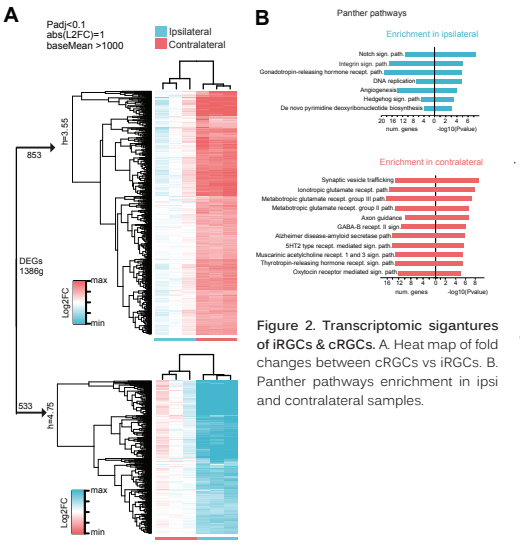


Figure 2. Transcriptomic sigantures of iRGCs & cRGCs. A. Heat map of fold changes between cRGCs vs iRGCs. B. Panther pathways enrichment in ipsi and contralateral samples.

Chromatin accessibility differences in iRGCs & cRGCs

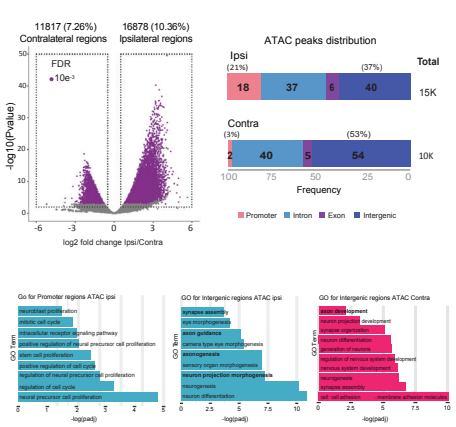


Figure 3. Chromatin accessibility differences in neurons with different phenotypes in axon pathfinding. A. Chromatin accessibility differences between iRGCs and cRGCs and ATAC peak distribution in genomic regions. B. Gene ontology for promoter and intergenic regions for ATAC candidates in iRGCs and cRGCs.

Transcription factor footprints

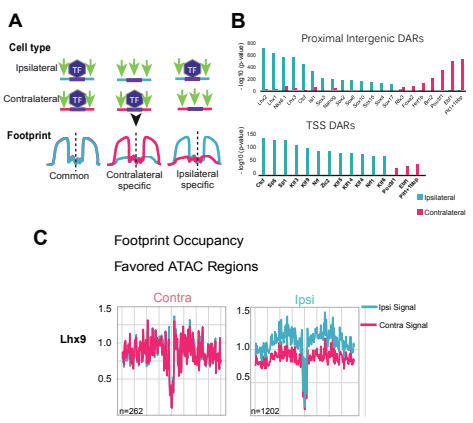


Figure 4. Transcription factor footprints define different regulatory landscape in axonal pathfinding cell-types. A. Schematic representation of footprint occupancy. B. Transcription factor motifs enrichment at footprinted sites at proximal intergenic DARs and TSS DARS. C. Footprint occupancy at Lhx9 gene.

ATAC-seq & RNA-seq combined analysis

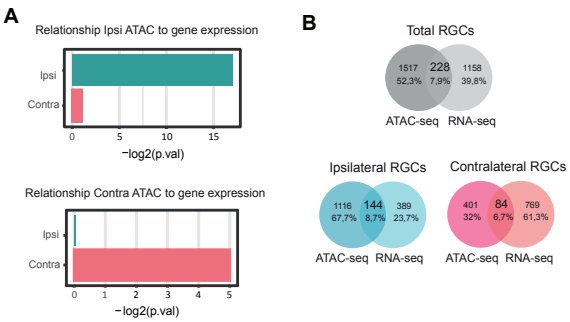


Figure 5. Overlap between chromatin accessibility changes and transcriptional profiles between contra- and ipsilateral RGCs

New players regulating axon pathfinding

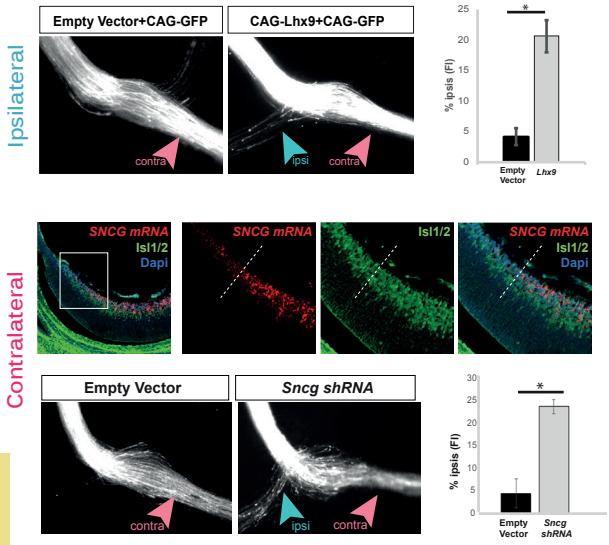


Figure 6. New players regulating axon pathfinding. A. Embryos electroporated with Lhx9-encoding plasmids display an increase in the number of ipsilateral axons compared to age-matched controls. Mean (±SEM) ($p < 0.05$, Student's t test).
B. Sncg is sufficient to guide contralateral RGC axons. In situ hibridization of Sncg coupled with Isl1/2 immunofluorescence at E16.5. Embryos electroporated with shRNA-encoding plasmids display an increase in the number of ipsilateral axons compared to age-matched controls. Mean (±SEM) ($p < 0.01$, Student's t test).

Conclusions

1. Genetic tagging of the nuclear envelope in iRGCs and cRGCs coupled with cell sorting enables significant differences in both chromatin accessibility and transcriptome profiles at the promoters of many genes that are known to participate in midline crossing.
2. The analysis validates this design as a powerful approach to unveil new genomic mechanisms underlying axon guidance decisions at the midline.
3. Our screens have identified several genes as candidate factors to regulate axon guidance decision of contralateral and ipsilateral retinal ganglion cells.

Funding