Characterizing mRNA expression in the retinal ganglion cells of the developing chick retina

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The Eye

Fig. 1.1. A drawing of a section through the human eye with a schematic enlargement of the retina.
Close up of the retina

Rod and cone photoreceptors

INL neurons

Ganglion cells
Why Retinal Ganglion Cells?

• 2nd leading cause of blindness worldwide
Model Organisms

• Mouse

• Chick

• Zebra Fish
Chick Retinal Ganglion Cells
Single Cell Isolation

Isolated Retina → papain digest → Dissociated Cells → Single Cell Isolation → Check Quality
PCR Screens

Are the single cells ganglion cells?

Neurofilament Light

Can we begin to identify different types of cells?

Cath5
Irx1
Slit1
Tach1
Correlated Expression of Retinal Ganglion Cell Genes
In Situs Showing Ebf3 Expression in RGCs

E4

E6

E14
Early Expressed Ganglion Cell Genes (E4)

- Irx1
- Fgf13
- Pou6f2
- EST_294n4
Late Expressed Ganglion Cell Genes (E14)

Syn-gamma

Irx1

EST-382L1

Map6

Scn2a
Adult Chick Ganglion Cell In Situs- Identifying Subsets

E14

NF-L

Merge

Syn-g
Genes Expressed in a Specific Subset of RGCs
Undifferentiated Non-Ganglion Cells

mitochondrial ribosomal protein L12
solute carrier family 25 (mitochondrial carrier; mitochondrial)
centromere protein H
kinesin family member 23
KIAA1524
protein CASC5-like
RNA binding motif protein 38
Rac GTPase activating protein 1
family with sequence similarity 83, member D
hairy and enhancer of split 5-like
replication factor C (activator 1) 4, 37kDa
budding uninhibited by benzoimidazoles 1 homolog (yeast)
cyclin B2
cell division cycle 20 homolog (S. cerevisiae)
spindle and kinetochore associated complex subunit 3
polymerase (RNA) II (DNA directed) polypeptide l, 14.5kDa
DNA replication helicase 2 homolog (yeast)
CTFL, chromosome transmission fidelity factor 8 homolog (S. cerevisiae)
frizzled family receptor 5
chromosome 5 open reading frame, human C1orf23
cyclin A2
chromosome 5 open reading frame, human C1orf23
non-SMC condensin I complex, subunit D2
mitochondrial ribosomal protein S34
glutaminyl-tRNA synthetase
exonuclease 1
Holiday junction recognition protein
Gga.1245.1.S1_at
kinesin family member 20A
kinesin family member 11
TPX2, microtubule-associated, homolog (Xenopus laevis)
discs, large (Drosophila) homolog-associated protein 5
centromere protein F, 350/400kDa (mitosin)
DEAH (Asp-Glu-Ala-His) box polypeptide 36
MIS12, MIND kinetochore complex component, homolog (S. pombe)
CDC28 protein kinase regulatory subunit 2 ||| SECIS binding protein 2
sperm associated antigen 5
asp (abnormal spindle) homolog, microcephaly associated (Drosophila)
chromosome 8 open reading frame, human C1orf52
family with sequence similarity 72, member A
Conclusions

• Using single cell transcriptomics, we can find genes critical to developing and adult RGCs
  • We have also found correlated genes that define other cell types including progenitor cells

• These results can be confirmed using in situ hybridizations on sections of the chick retina

• We have developed a fluorescent in situ to further characterize different subtypes of RGCs
What’s Next?

• Get a better understanding of the precise role played by some of the genes we have identified
  ▫ Isolate factors that determine ganglion cell fate

• Find common themes across species (mouse, chick, zebra fish) that will inform us how to generate ganglion cells from stem cells

• Use these genes to produce RGCs from induced stem cells (iPSCs)
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In Situ Process

mRNA strand

Probe

Anti-DIG antibody

DIG

DIG

DIG

NBT and BCIP

Expressed genes show purple