Exploring distinct retinal ganglion cell types at single cell resolution

TERRY LUND

IOWA STATE UNIVERSITY

BIOLOGY AND PSYCHOLOGY

Glaucoma Overview

- 2nd leading cause of blindness
- Risk factor: elevated pressure in the eye
- High risk groups: people over 60, family members of those already diagnosed and diabetics.
- No cure
- Vision loss is caused by damage to the optic nerve and death of retinal ganglion cells
- Gradual loss of vision
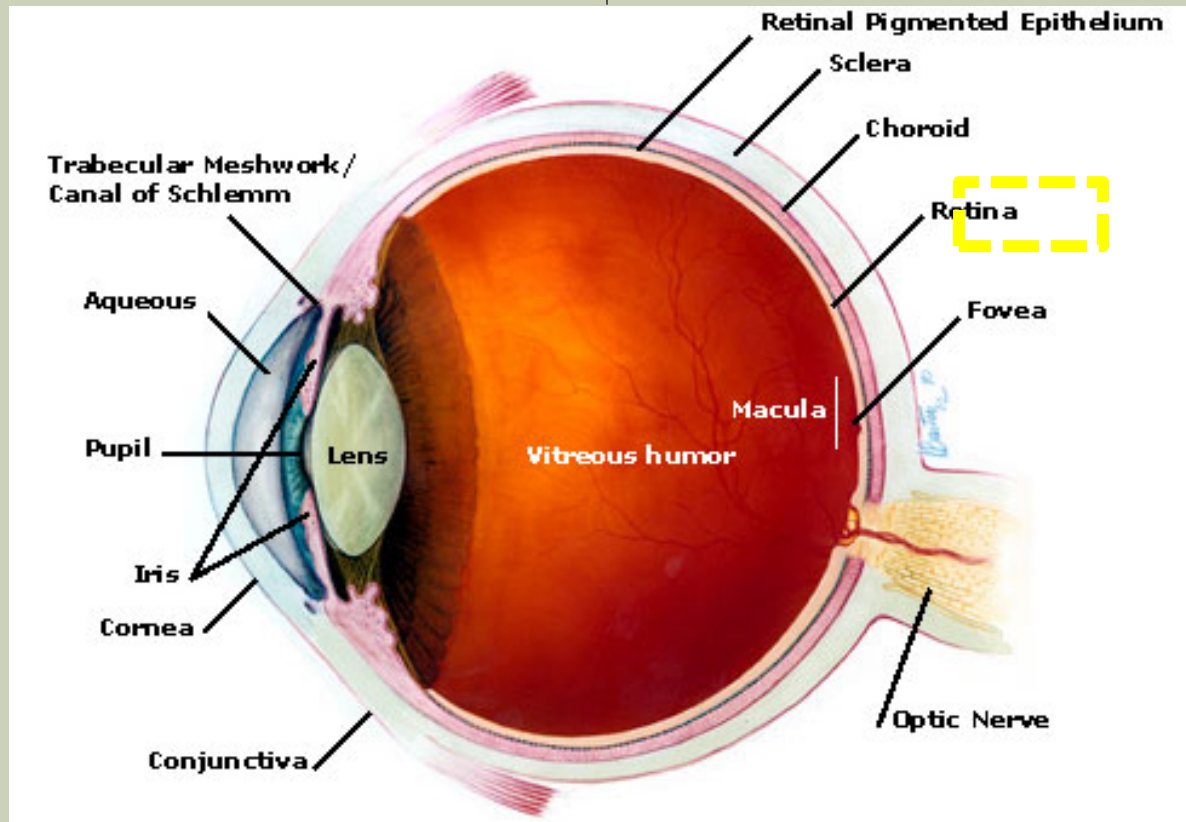
Through Their Eyes
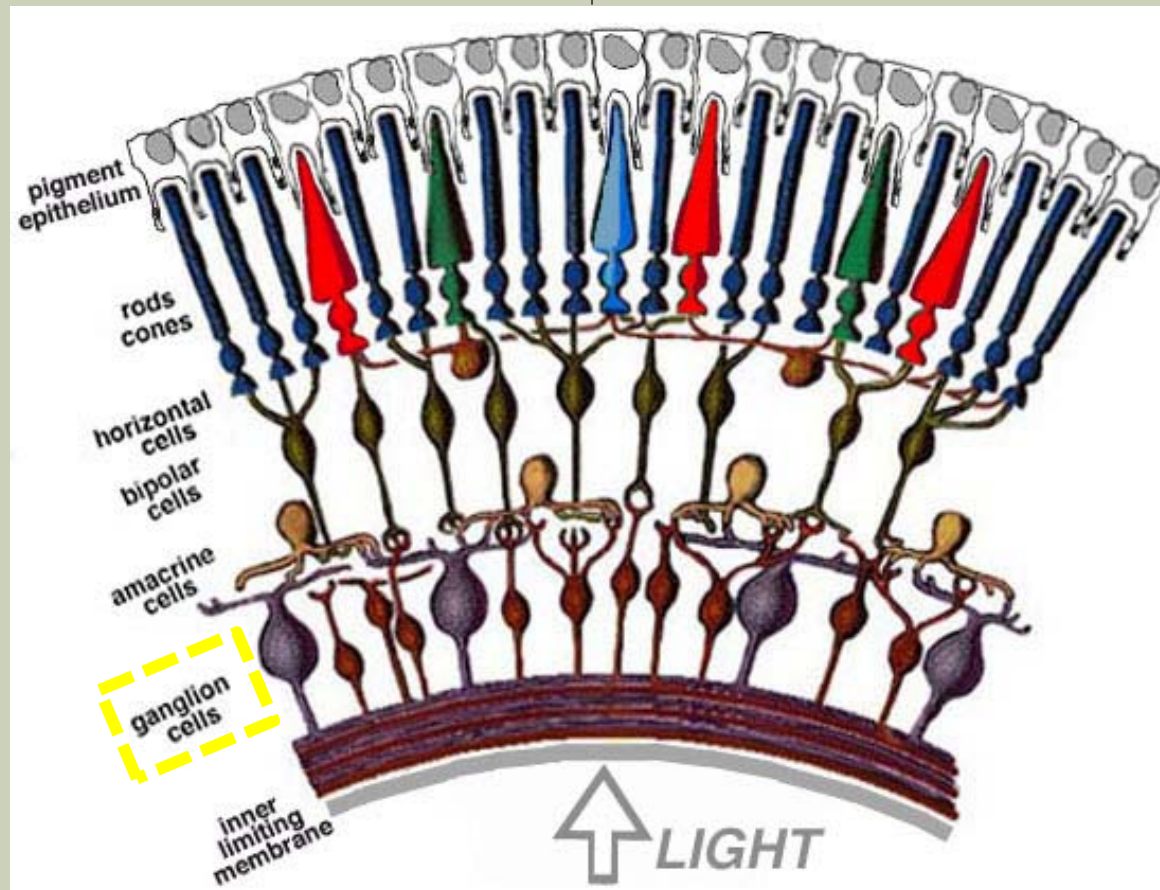
Normal Vision

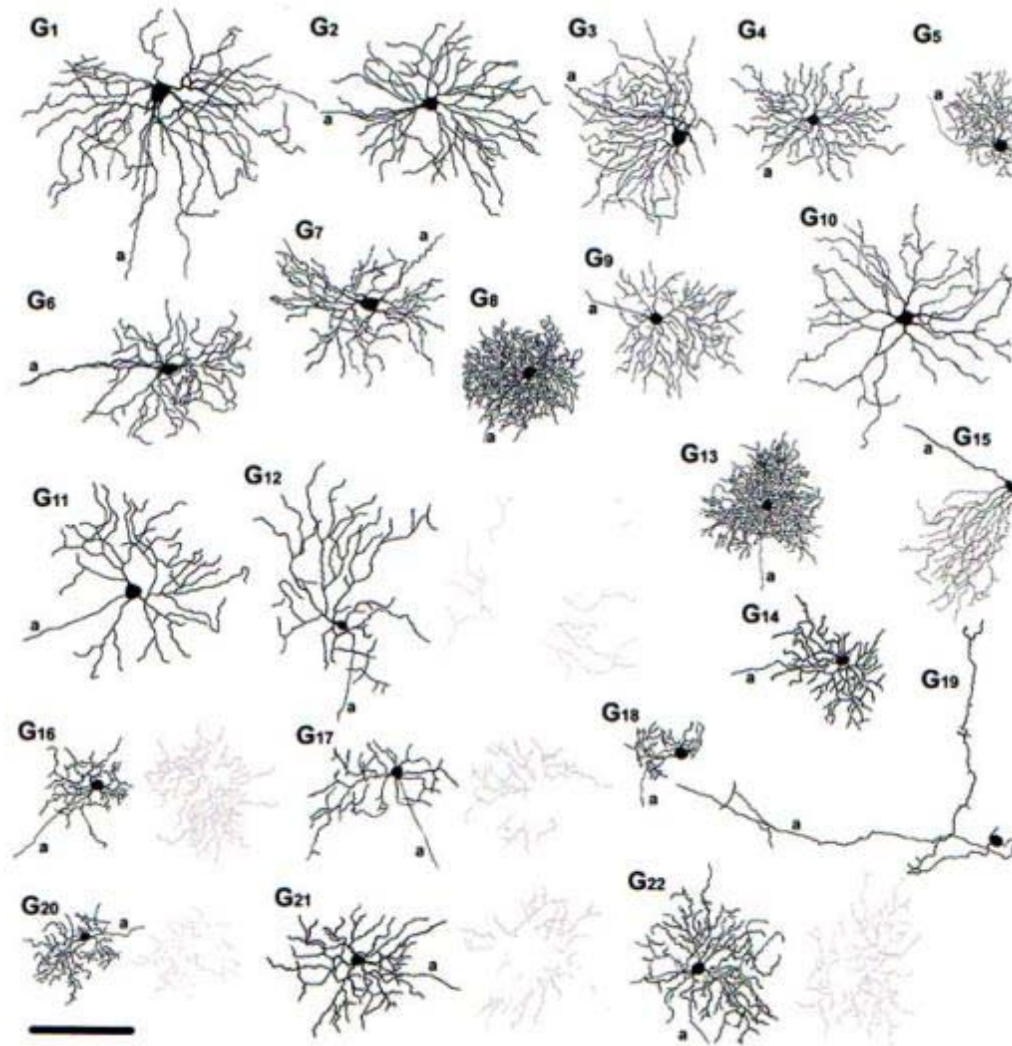## Advanced Glaucoma

HOW DOES THIS HAPPEN?

Anatomy of the Eye

Retina

Not all ganglion cells are the same

How do we understand these cells?
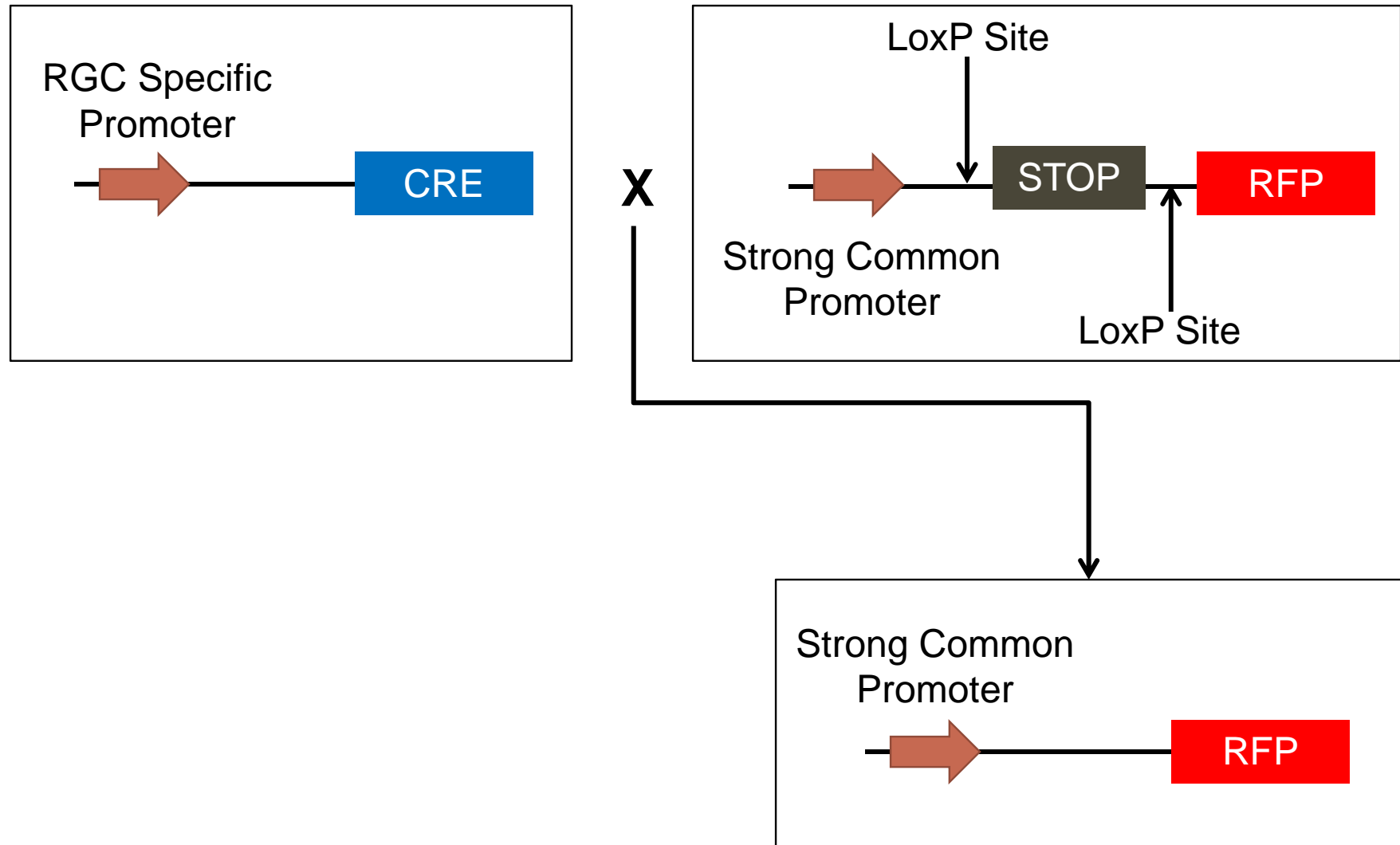
- RGC small and diverse population

- Single cell gene profiling to analyze gene expression networks

- Future Goal: use these networks to generate RGCs from stem cells

HOW CAN WE ACCOMPLISH THIS?

Cre/LoxP to Label Cells

RGC Specific
Promoter

CRE

X

LoxP Site

Strong Common
Promoter

STOP

RFP

LoxP Site

Strong Common
Promoter

RFP

How to isolate single cells?

- Genetically engineered mice with red fluorescent protein to visualize RGCs
- Pick single cells
- PCR-based screening
- Use microarray to determine genes expressed in single cells
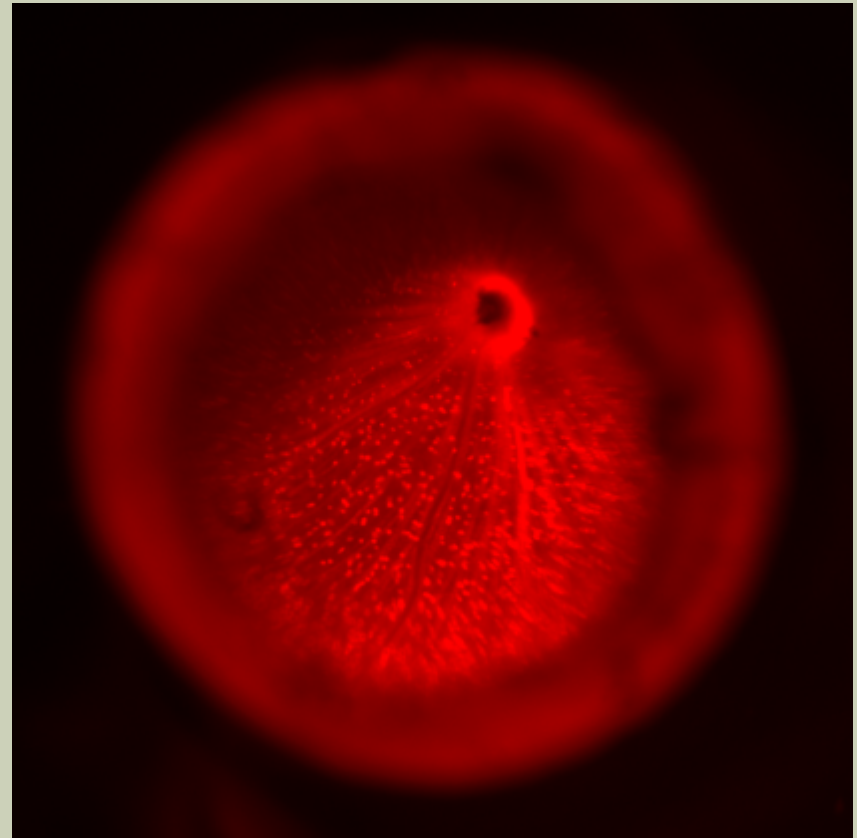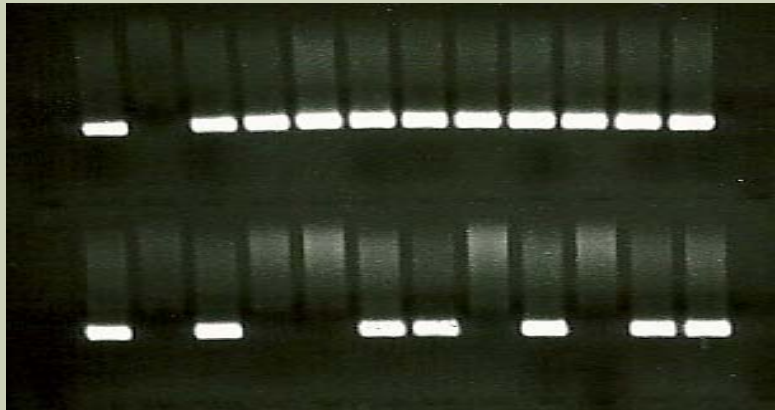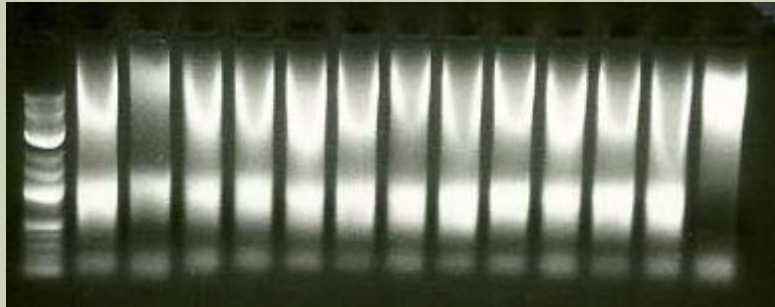
Single-cell Isolation Overview

Isolated Retina

papain digest

Dissociated Cells

Single Cell Isolation

Check Quality
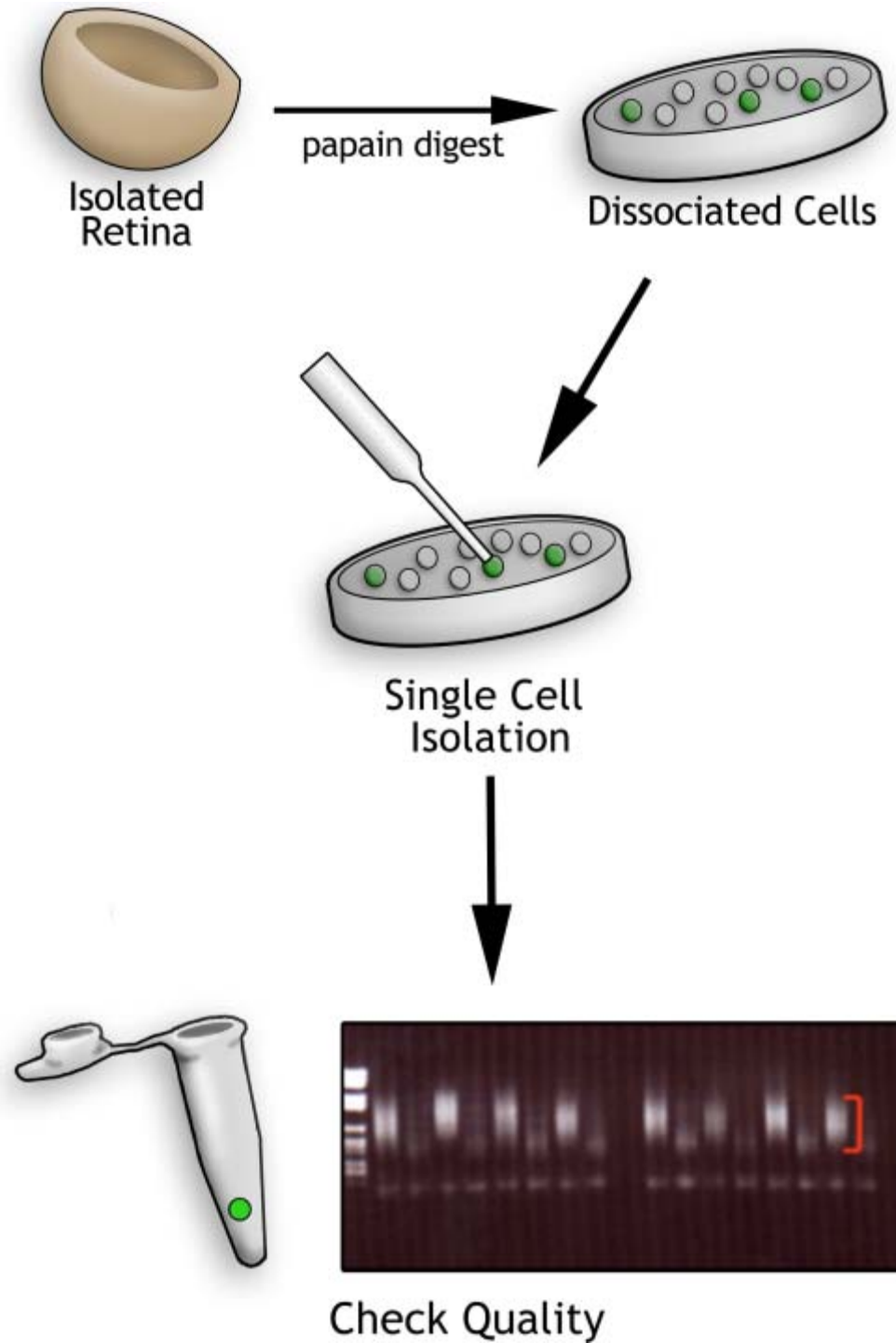
A PCR-based screening strategy for classifying developing RGCs

Markers

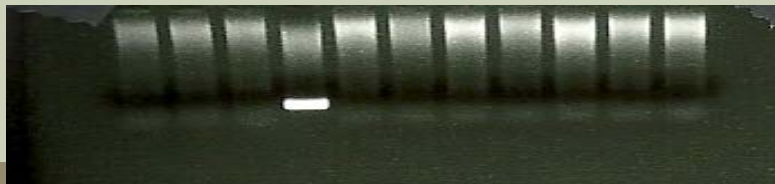**Single cell cDNAs**

No cell control

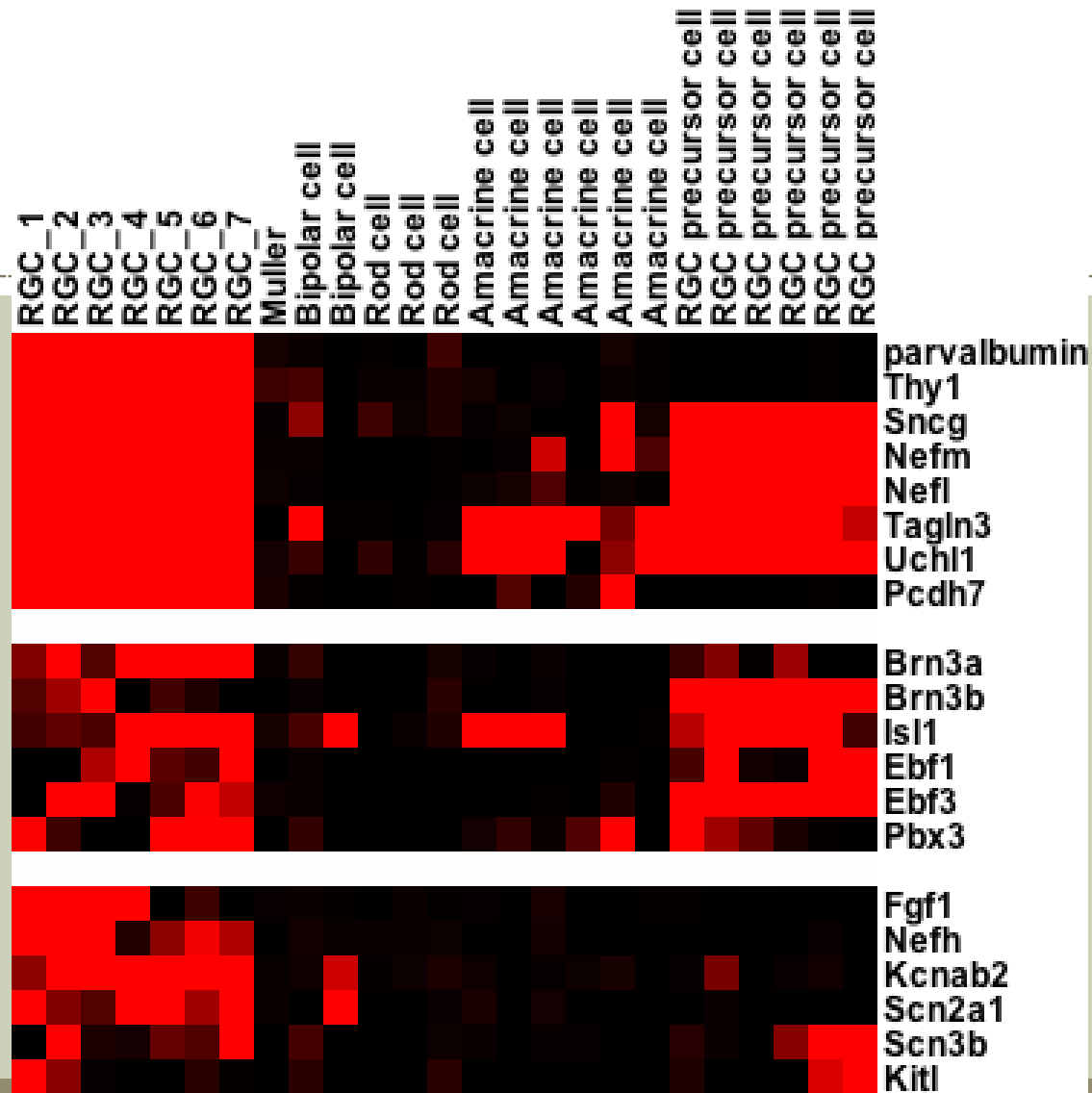**Sncg** — Tells us cells are RGCs

Tac1 — Genes expressed in RGC subsets

Crx — Assesses contamination

Transcriptional profiling of adult RGCs

Finding Genes expressed in just RGCs
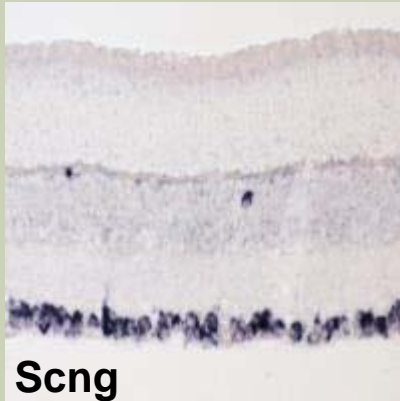
Procedure

Design primers

Clone gene segments for in situ probes

Synthesize in situ probes

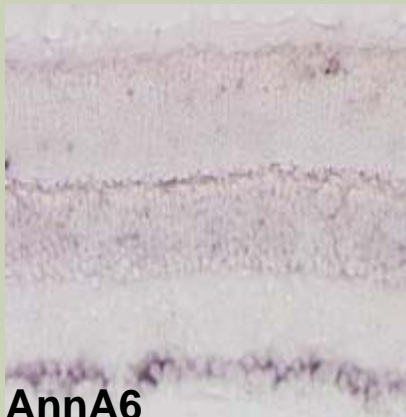Dissect and section retina onto slides

Perform in situ hybridization

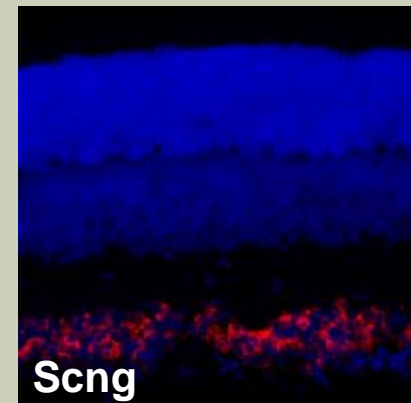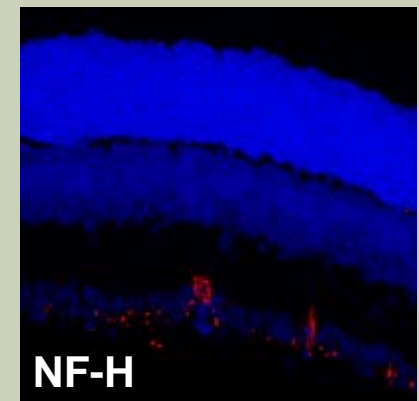# Genes expressed in RGCs

Scng
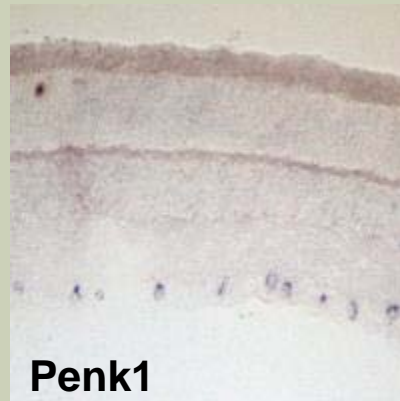
EST 439704

EST 220329

AnnA6

Clecf

Scng

Genes expressed in RGC subsets

Kcng4

Fgf1

NF-H

Scn2a1

Penk1

Tusc5

NF-H
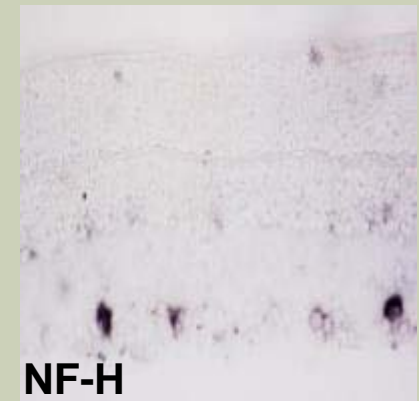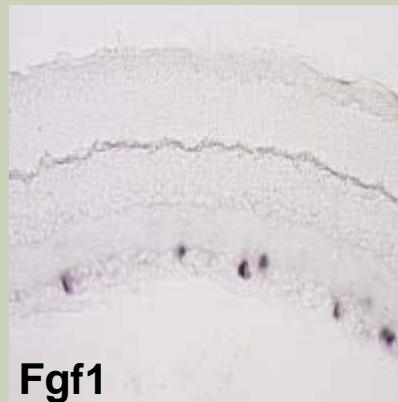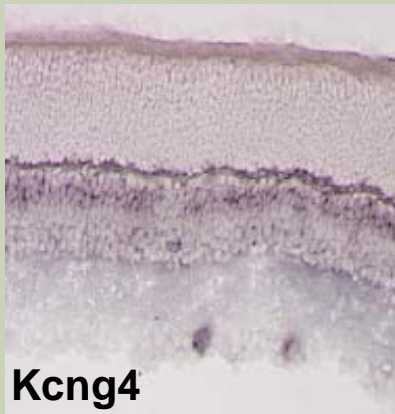
Possible contrast sensitivity RGC genes

WD repeat domain 41
adenylate cyclase 1
potassium channel, subfamily T, member 1
nuclear prelamin A recognition factor-like
ribosomal protein S6 kinase polypeptide 1
adaptor-related protein complex 2, beta 1 subunit
tectonin beta-propeller repeat containing 2
nuclear fragile X mental retardation protein interacting protein 2
HEAT repeat containing 5B
metaxin 2

Summary

- We have begun to characterize adult mouse ganglion cells via single cell analysis and *in situ* hybridization
- We have identified genes expressed only in subsets of ganglion cells
- We have found a cluster of genes that possibly define contrast sensitivity ganglion cells
- Looking to the future:
  - Examine gene function in different models
  - Correlate our adult gene expression with that of developing ganglion cell
  - Better define the subsets we have identified

Acknowledgments

- **Dr. Jeff Trimarchi**
- **Graduate Students and Lab Technicians**
  - Jillian Goetz
  - Greg Martin
- **Undergraduate Students**
  - Miranda Lee
  - Brock Pope
  - Kallie Weinand
  - Diane Fru
  - Brenden Bartels