Encephalopsin (Opn3) is transiently expressed in Opn5+ retinal ganglion cells and regulates retinal clock dynamics

Reena Jacob

Undergraduate Research Spring Semester 2020

Dr. Richard Lang Research Lab: UC Department of Ophthalmology

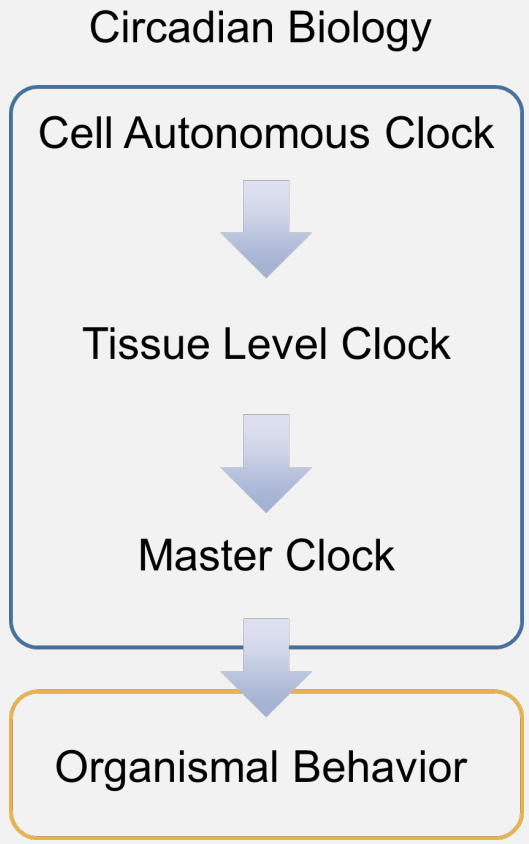
BACKGROUND

The retina is a light-sensitive tissue that not only has an important role for image-forming vision but also retinal signaling which is a major input into the circadian clock system.

- Mammals have a master clock (suprachiasmatic nucleus; SCN)
    - SCN receives input from retina and relays time-of-day information to peripheral tissues aligning local tissue clocks (liver, skin, gut, etc.) with the light-dark cycle.

Approximately 1/3 of the United States population are shift workers and will encounter multiple circadian disruptions in their schedule.

- Shift work has a significant impact on the Circadian clock therefore it is important to understand the components of clock biology in the mammalian system.



HYPOTHESIS & OBJECTIVE

Photoentrainment of the eye has been thought to be controlled by rods, cones, and retinal ganglion cells (RGC's) since they are the main compartments of photoreceptors.

- Recent research has shown that rods, cones and Opn4 retinal ganglion cells are not important for the tissue level clock in the retina.
- Peripheral tissues synchronize their clocks with the light-dark cycle autonomously though expression of atypical opsins.

Clock-phase is dependent on Opn5 and it has been identified that Opn3 is a regulator of clock dynamics in the retina.

<u>Hypothesis</u>
Opn3 and Opn5 photopigments are expressed in the same cell (RGC's) and the combination of both opsins regulate all clock dynamics in the retina.

<u>Objective</u>
To understand if Encephalopsin (Opn3) is a regulator of Opn4+ and Opn5+ retinal ganglion cell physiology and can therefore modulate the retinal clock amplitude.

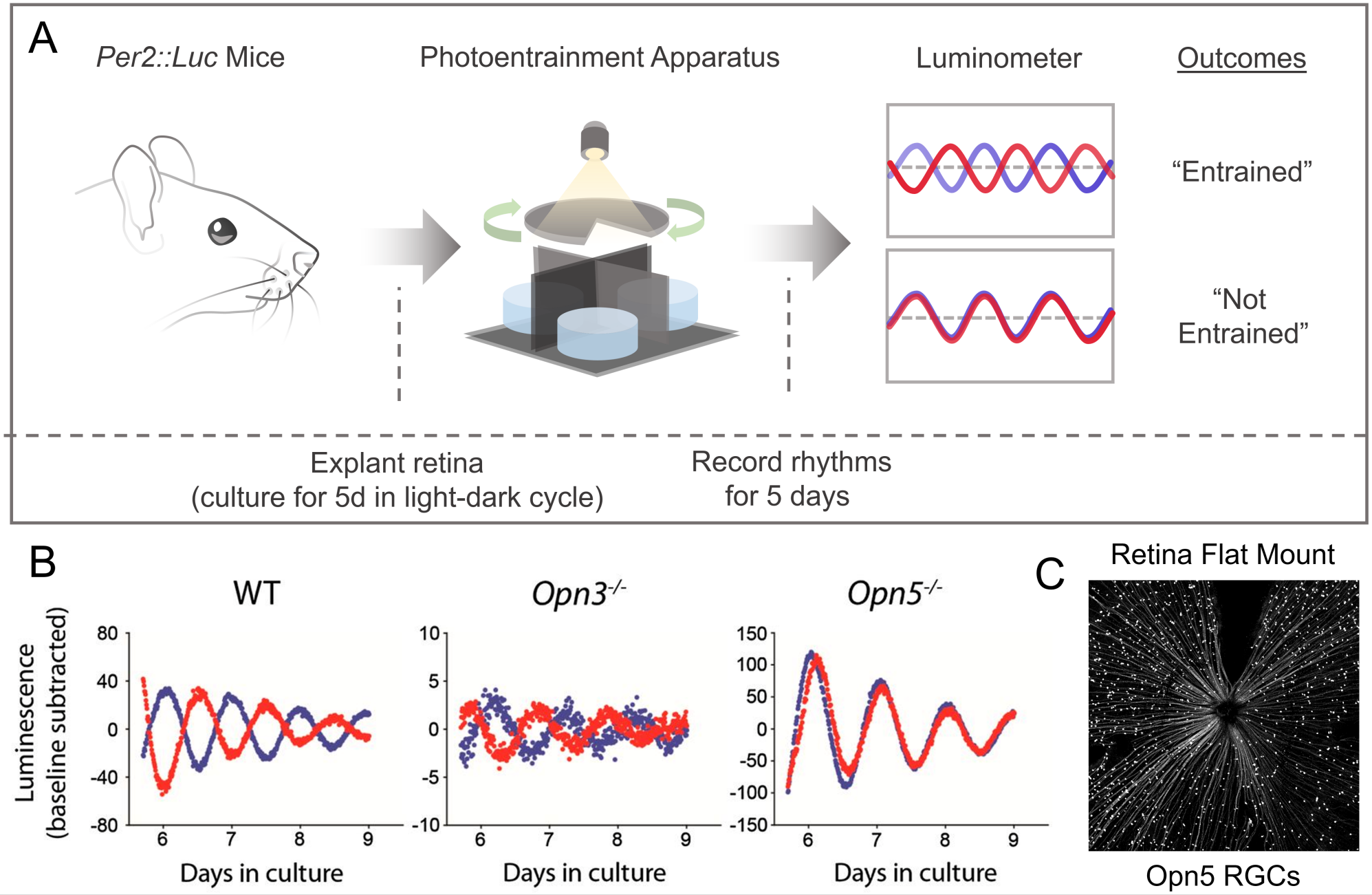


Figure 1. Retina-autonomous circadian rhythms are generated by atypical opsins Opn3 and Opn5. (A) Schematic representation of ex vivo photoentrainment protocol of mouse retina, with predicted outcomes based on luciferase waveforms. (B) Per2 rhythms as assessed by Per2::Luc explants as described in (A). Representative plots from wildtype (WT; Per2::Luc), Opn3-/-; Per2::Luc, and Opn5-/-; Per2::Luc animals. (C) Flat mounted retina indicating Opn5 retinal ganglion cells (RGCs) in the Opn5Cre/+; Ai14 reporter mouse line. [1]

PHOTOENTRAINMENT PROTOCOL AND CORRESPONDING PLOTS

Figure 1

- **A:** Protocol of photoentrainment apparatus to understand determinants for circadian photoentrainment taken from Ethan Buhr's publication.
    - Retina is placed in apparatus with moving mask therefore tissue that is diagonal to each other will experience opposing light/dark cycles.
        - Entrained outcome: retinas were experiencing light and aligning Per2 rhythm to be antiphase (tissue is responding to the light/dark cycle)
        - Not Entrained outcome: no photo response capability
- **B:** Wild type mice have an entrained rhythm. When Opn3 is removed, there is a lowered amplitude in the system. As Opn5 is removed, the amplitude remains high however it is "in-phase" and therefore not entrained.
- **C:** Opn5 is shown to be in retinal ganglion cells

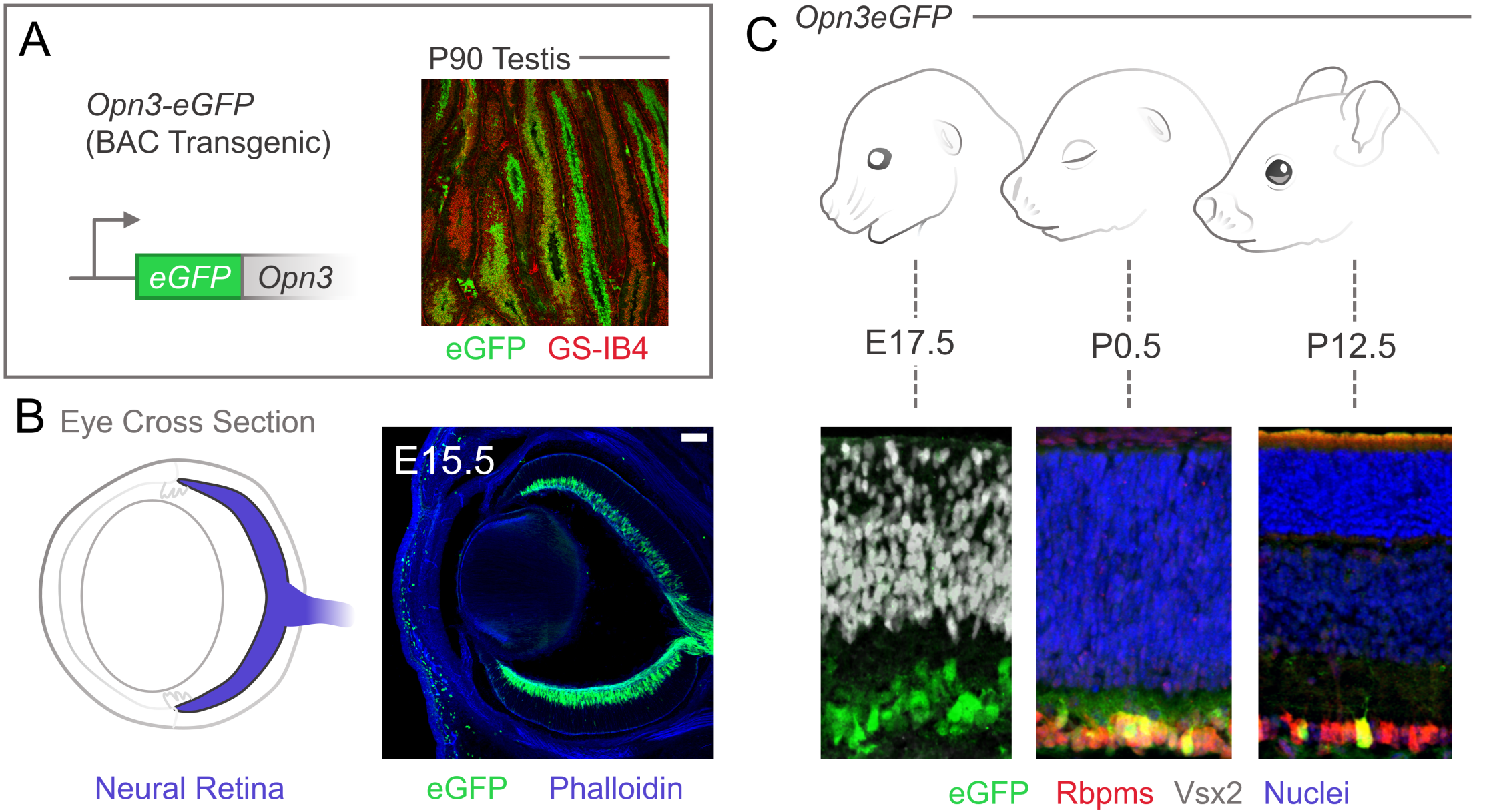


Figure 2. Opn3 is expressed dynamically in retinal ganglion cells (RGCs) over development. (A) Schematic representation of the transgenic Opn3-eGFP reporter mouse line and a positive control tissue (testis)cut at 10μm thickness. (B) Cross section of the E15.5 (embryonic day 15.5) Opn3-eGFP mouse eye, with representative sections stained for eGFP (green) and Phalloidin (blue)cut at 10μm. (C) Developmental time course of eGFP expression over retinal development (E17.5 –P12.5), with representative cross sections of the retina stained with eGFP (green), Vsx2 (grey, progenitor cells), Rbpms (red, retinal ganglion cells), and nuclear stain (blue, Hoechst 33342)cut at 10μm.

OPN3 IN RGC'S THROUGHOUT DEVELOPMENT

Figure 2

- A: Fluorescent protein (eGFP) is inserted into the Opn3 gene. Testis is shown as a positive control since Opn3 is expressed in testis as well.
- **B:** Cross section of the eye at E15.5. There are eGFP signals specifically in the retinal ganglion cell layer of the retina
  - A prerequisite of being in the same cell type is that Opn3 should also be in retinal ganglion cells
- **C:** Retina is harvested at different life stages and stained for various markers. (Rbpms is a marker for ganglion cells and Vsx2 is a marker for retinal progenitor cells).
  - In early eyes, Opn3 is not marking progenitors.
  - At P0.5 and P12.5, there is a red and green combination which suggests that these cells are retinal ganglion cells since there is an overlap.
    - Over developmental time, there is a significant decrease in eGFP positive cells.
    - Opn3 is indeed in ganglion cells but its expression is transient in the retina.

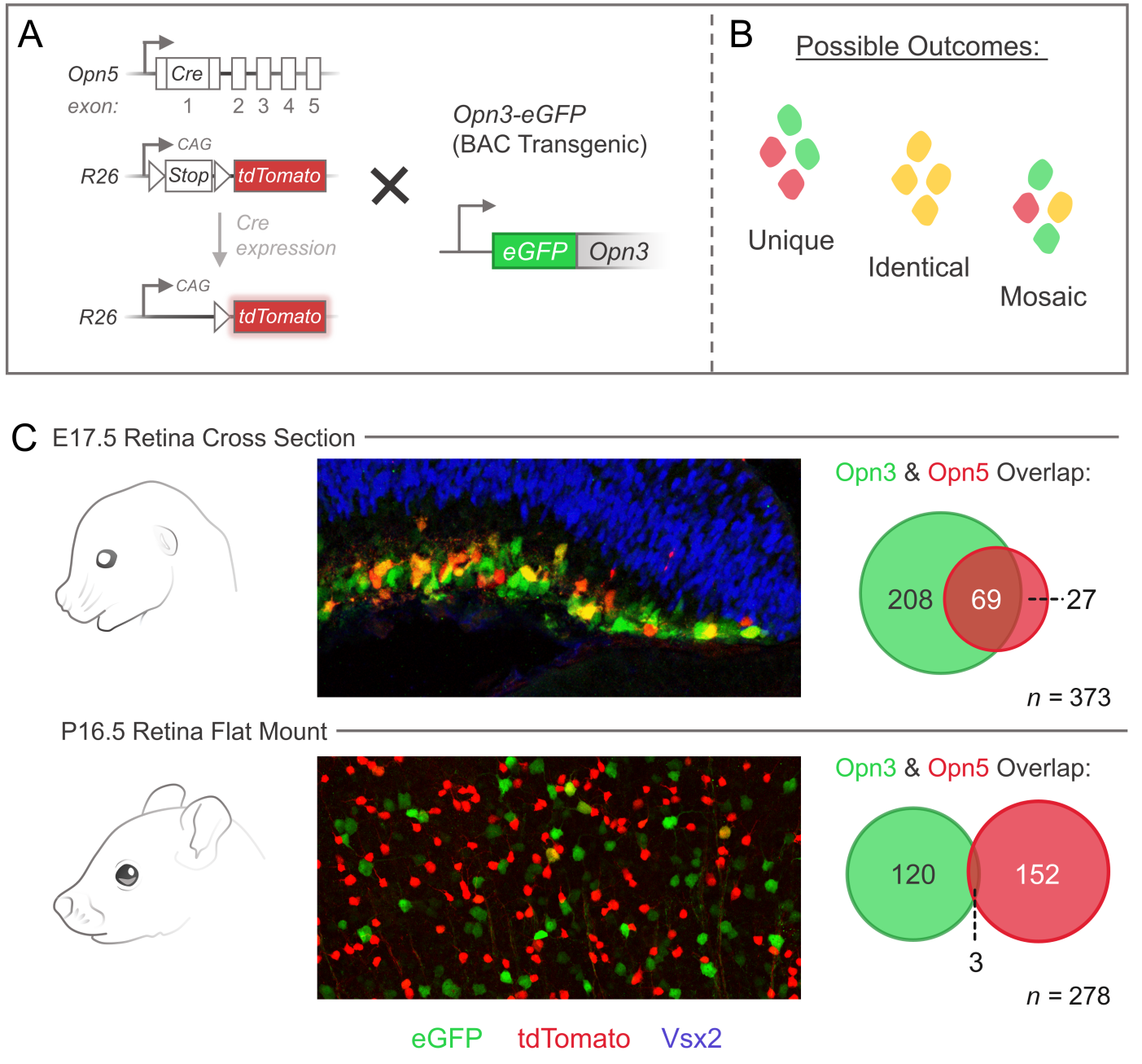


Figure 3. Opn3 and Opn5 expression overlap is transient, with the majority being developmental. (A) Schematic representation of Opn5Cre/+; Ai14 (tdTomato) and Opn3-eGFPmouse lines crossed to generate experimental mice for overlap analysis. Additionally, possible outcomes (unique, identical, or mosaic populations) are highlighted as a schematic. (C) Overlap analysis at E17.5 & P16.5 Opn5Cre/+; Ai14; Opn3-eGFP retinae in cross section (E17.5) and flat mount (P16.5). Venn diagrams showing assessment of overlap from n = 3 animals (373 total cells; E17.5) and n = 3 (278 total cells assessed; P16.5)

OPN3 & OPN5 EXPRESSION OVERLAP

Figure 3

- **A:** tdTomato marks anything expressed with Opn5. Combined with the the previous genetic tool as stated in Figure 2 (using eGFP), there are three possible outcomes.
- **B:** Possible Outcomes:
    - Unique: Although both in ganglion cells, they do not represent the same cell
    - Identical: 100% overlap
    - Mosaic: some populations are independent, and some are overlapped
- **C:** Retina at E17.5 contains mostly yellow cells (more red cells overlapped with green).
    - Opn3 is expressed strongly in early development and Opn5 cells are therefore dual positive for Opn3.
    - Overtime, it is shown that Opn3 signaling regresses to fewer cells and have a more unique and independent outcome as seen in P16.5.

RESULTS

- This study shows that there is a dynamic change in opsin expression over time where early in the developing mammalian retina, there is a population of cells that expresses both opsins.

- Once mice have reached the eye-opening stage of life, the cells seem to become two different populations which can therefore impact the clock. As this point, if Opn3 is effecting the physiology of the clock, it is likely that Opn3 is probably not affecting the clocks activity acutely since it is not in the same cell type in the adult stage.

- Opn3 likely has a role in early retinal development in shaping the clock dynamics and most likely does not play a significant role in adult clock dynamics.

CONCLUSIONS

Through this research, Opn3 is expressed in retinal ganglion cells as early as E17.5, but the overlap between Opn3 and Opn5 is transient. Deletion of Opn3 failed to elicit developmental alterations in cell types associated with the retinal clock, suggesting that the clock amplitude reduction is not a consequence of fewer oscillating cells.

Questions to ask:

- If we delete Opn3 later on in the life stage after retinal development, will there be a significant effect in amplitude on the clock?
- Is Opn3 required for the survival of Opn5 ganglion cells?

Next Steps:

- Additional experiments needed to understand which aspects of the retina play a role in the adult clock dynamics.

SPECIAL THANKS

- Thank you to my amazing and hardworking research mentor, Shane DSouza, who has gone above and beyond to train me in the lab and made sure I had a complete understanding of this project.
- Another thank you to Dr. Richard Lang, my PI, who was more than welcoming in bringing me into the lab.
- Thank you, Dr. Mosley, for putting faith in my career goals and encouraging me to research at Cincinnati Children's,

References

[1] Buhr et al. - https://www.pnas.org/content/112/42/13093