

General Background



Pleurodeles waltl

- ❖ Commonly referred to as the Iberian ribbed newt
- ❖ Performs lens regeneration through re-identification of dorsal iris pigmented epithelial cells (IPE) through adulthood
- ❖ Process differs from development where lens cells originate from the cornea
- ❖ Whole organ regeneration after complete removal is rare

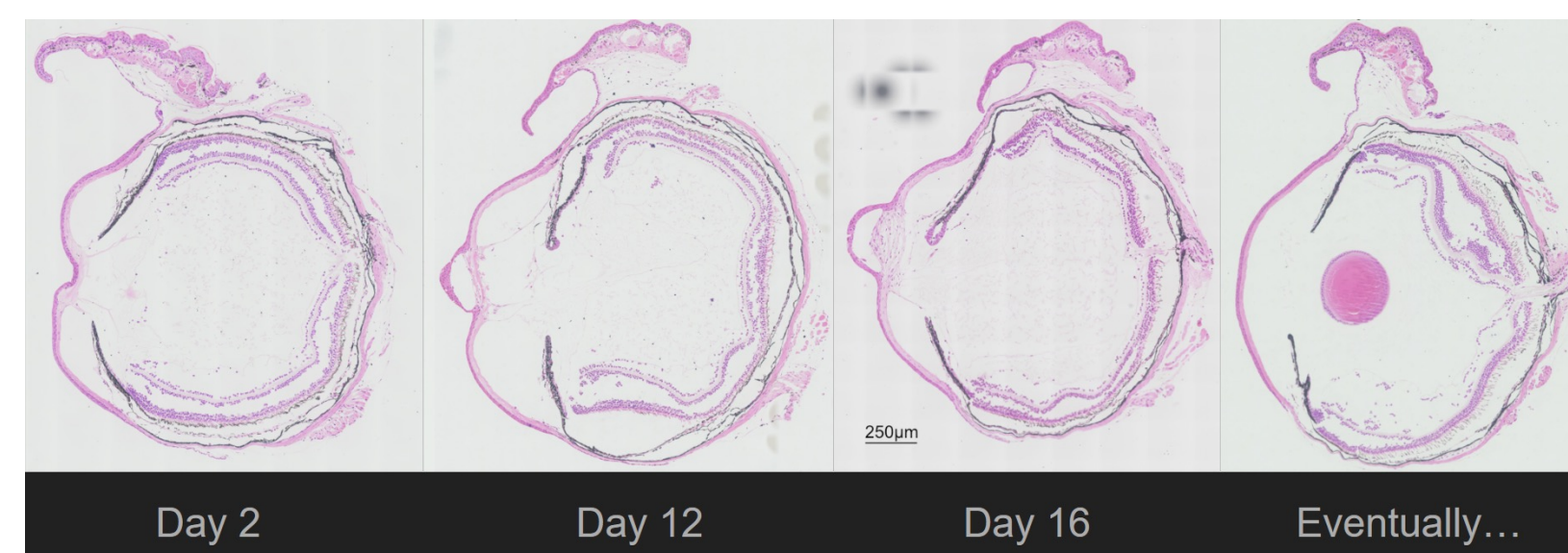


Figure 1. Time course of a three-month-old *P. waltl* regenerating its lens. Day 2 illustrates lack of lens, whereas day 12 and day 16 the formation of a lens vesicle can be seen. Tissue will differentiate until an entire free-floating lens is regenerated.

Importance and Applications of Lens Regeneration

- ❖ Cataracts are caused by clouding of the lens
- ❖ 50% of people over the age of 80 in the United States of America currently have or have previously had cataracts
- ❖ Center for Disease Control (CDC) categorizes "Vision" as one of the top 10 disability categories
- ❖ Current treatments of vision related diseases and degenerations are invasive – find treatment applications which harness the power of regeneration to limit invasive approaches

PDE4 and cAMP

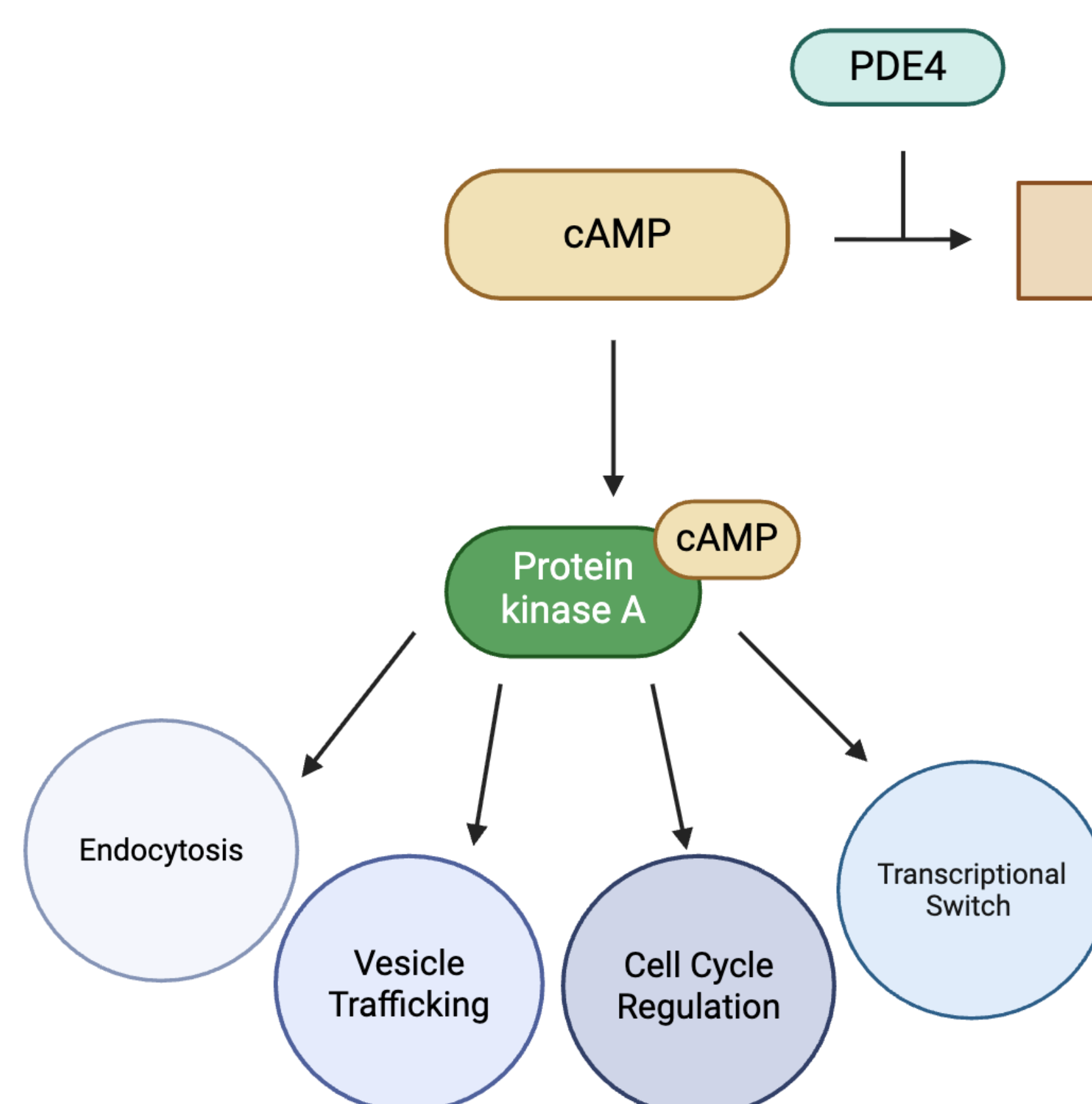


Figure 2. Simplified pathway showing relationship between Protein Kinase A (PKA), cyclic AMP (cAMP), and Phosphodiesterase-4 (PDE4). PDE4 is responsible for the hydrolyzation of cAMP into 5'-AMP and is involved in regulation of cell proliferation and apoptosis. cAMP interacts with PKA which is involved in regulation of cell mechanisms such as endocytosis, vesicle trafficking, cell cycle regulation, and transcriptional switching.

Methods

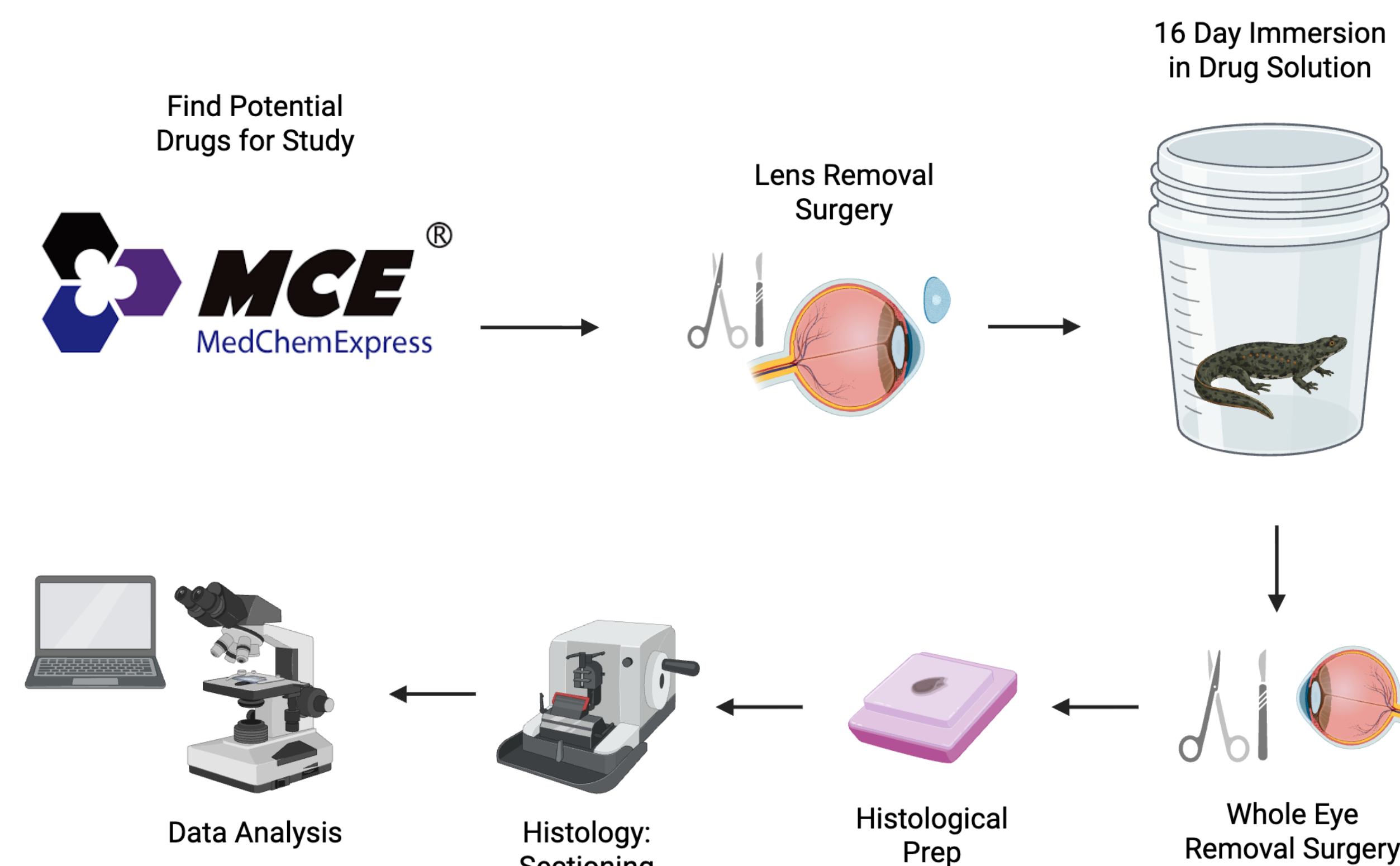


Figure 3. Depiction of methods throughout experimentation. Potential drugs were found on MedChemExpress and validated through literature searches. Organisms undergo lens removals and are placed in a drug solution for 16 days. Whole eye removal follows, eyes are then put through histological prep process, sectioned and data analyzed using Fiji ImageJ.

Results

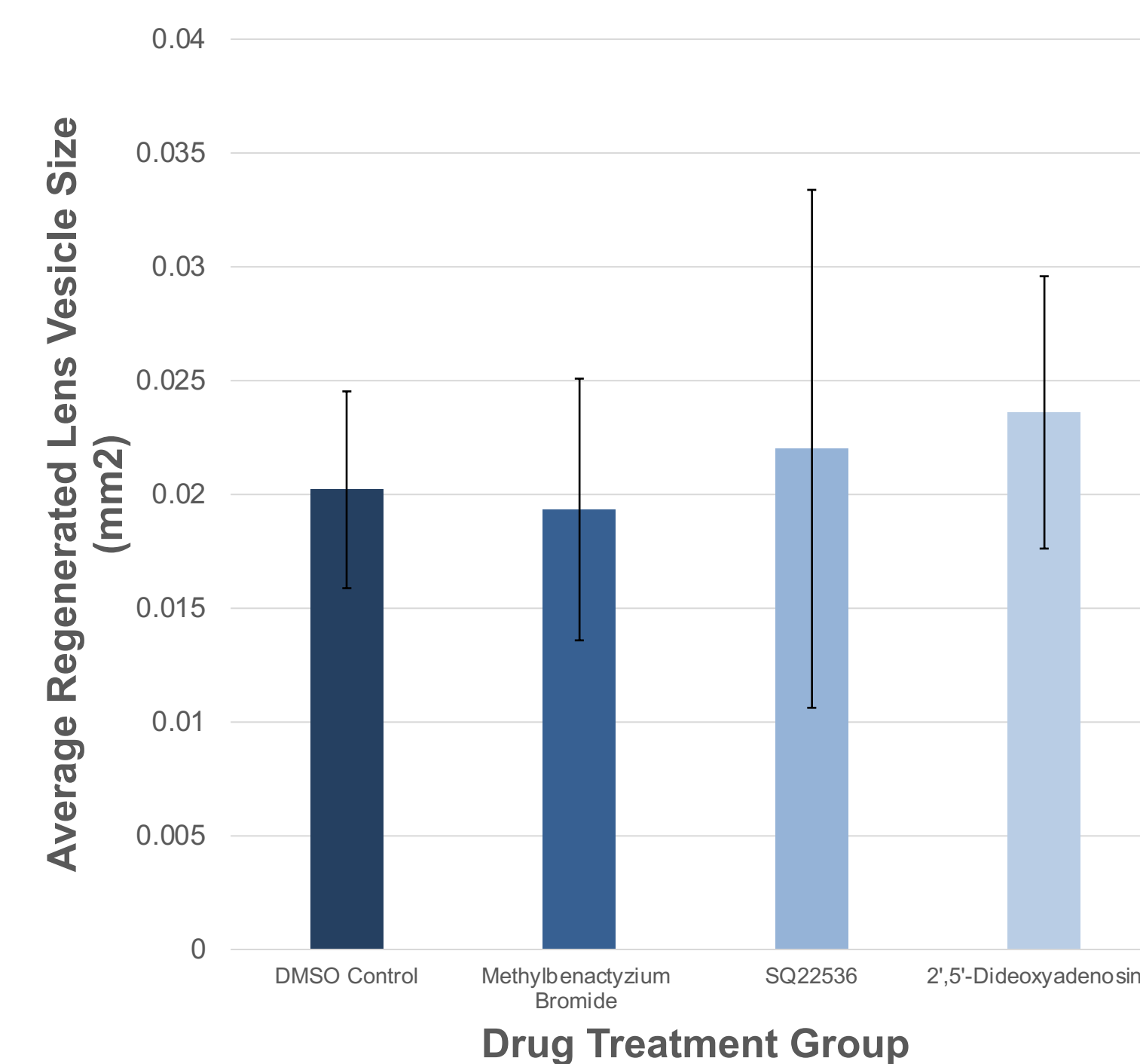


Figure 4. Average regenerated lens vesicle size for treatment groups. Averages taken of areas in square millimeters, error bars represent calculated standard deviation. Data shows no statistically significant difference between the controls and treated groups.

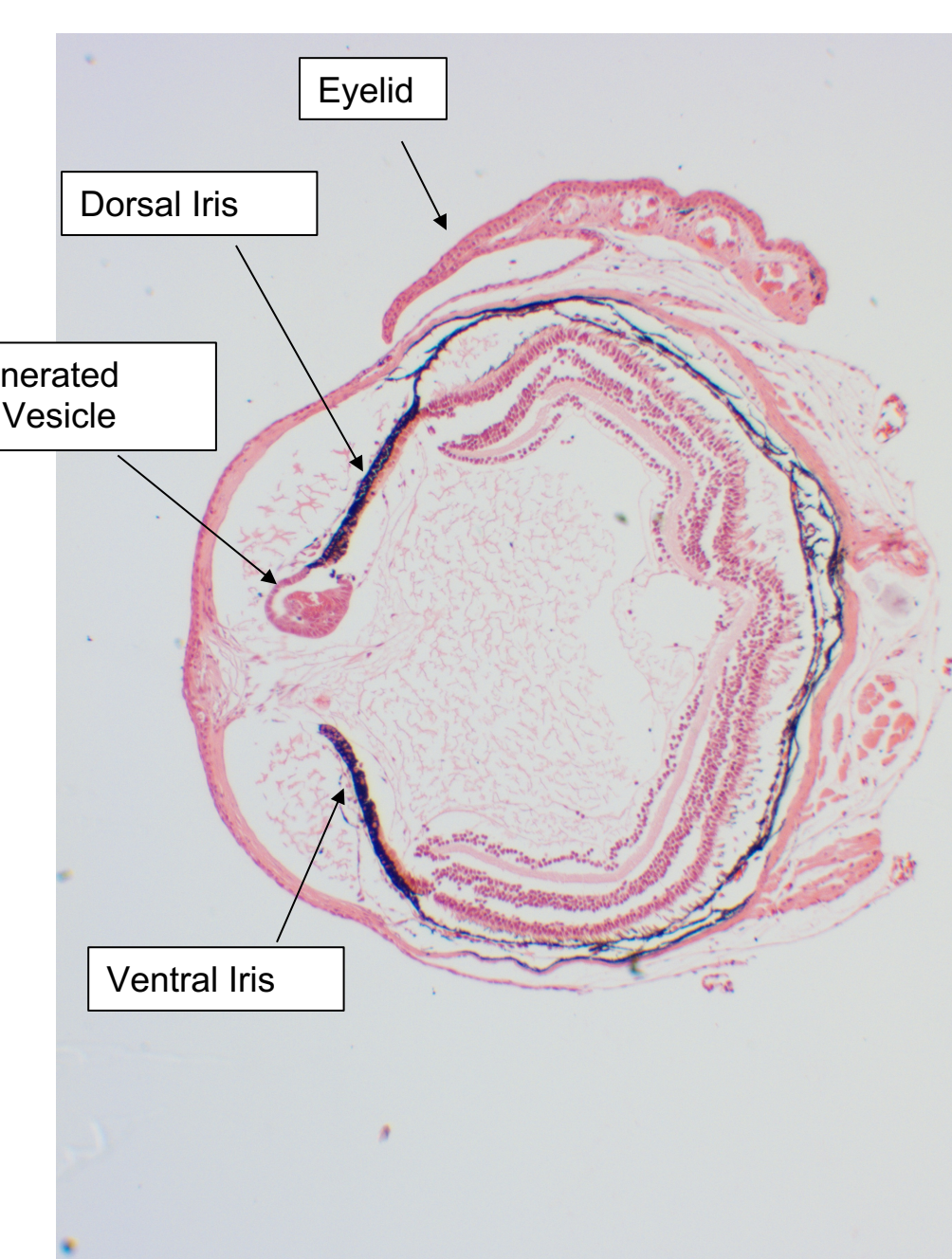


Figure 5. Image of H&E stained cross-section of eye control sample treated with DMSO. Labels and arrows indicate tissue markers of interest which have consistent shape and orientation throughout the samples.

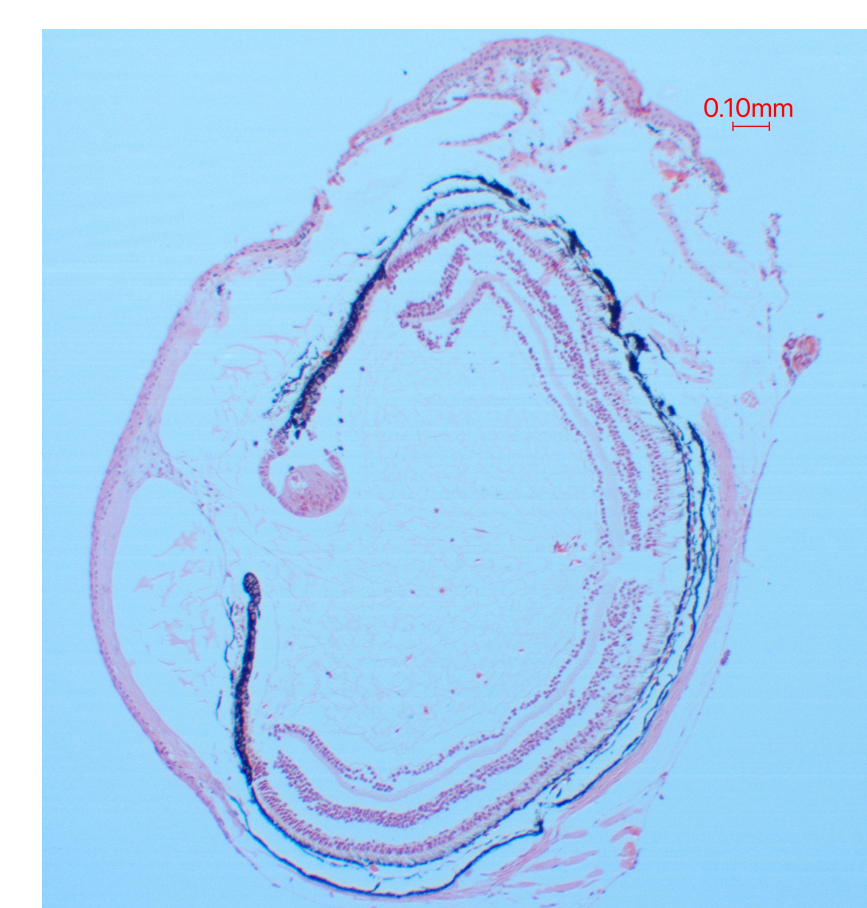


Figure 6. Image of H&E stained cross-section of eye treated with Methylbenactyzium Bromide. Scale bar represents 0.1mm.

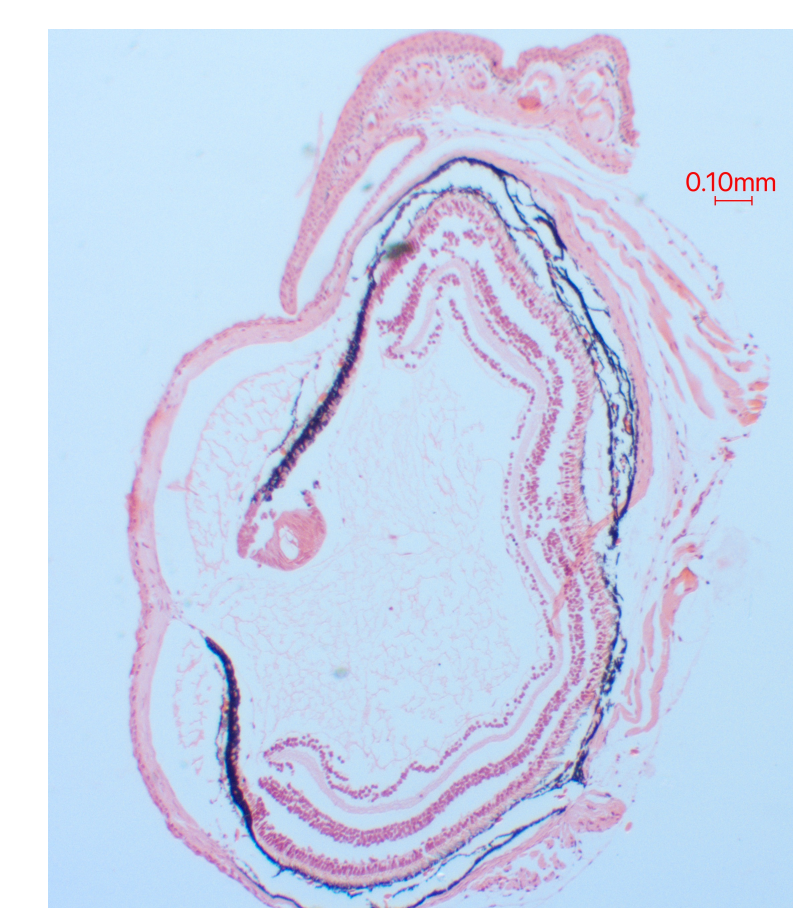


Figure 7. Image of H&E stained cross-section of eye treated with SQ22536. Scale bar represents 0.1mm.

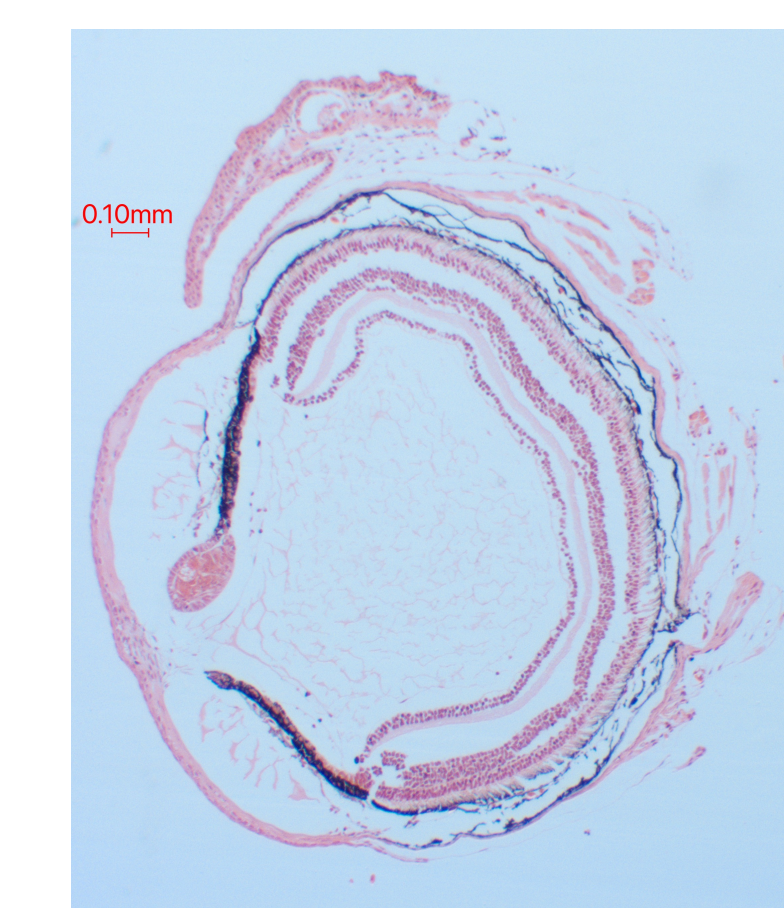


Figure 8. Image of H&E stained cross-section of eye treated with 2',5'-Dideoxyadenosine. Scale bar represents 0.1mm.

Conclusions

- ❖ Lens vesicle sizes of organisms exposed to drugs did not vary significantly across any of the trials when compared to controls.
- ❖ PDE4 does not seem to be directly responsible for the regeneration of the lens in *P. waltl*.
 - Exposure to Methylbenactyzium Bromide had no effect on the rate of regeneration.
- ❖ Inhibition of Adenylate Cyclase to reduce cAMP levels had no effect on ability of *P. waltl* to regenerate a lens.
 - Exposure to SQ22536 and 2',5'-Dideoxyadenosine had no effect on the rate of regeneration.

Future Experimentation and Goals

Pretreatment of Drug

- ❖ Treat with drugs prior to lens removal to observe effects of altered state before injury

PDE4 agonist

- ❖ Look at the other angle for PDE4 to see if agnostic drugs have an altered response

Injection of Drug

- ❖ Drugs are validated through oral or injected administration in mice
- ❖ Molecular size may have affect on absorption ability in *P. waltl*
- ❖ Injection into ocular cavity may ensure adequate administration of calculated dosage

References and Acknowledgements

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