Control of Eye Regeneration in *Xenopus laevis*

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Abstract

Regeneration is a response to injury that results in the functional regrowth of damaged or lost body parts. Uncovering this mechanism is part of the NASA Mission Directorate Strategic Goal 1: Expand Human Knowledge Through New Scientific Discoveries. However, regeneration mechanisms are not well understood. Studying a highly regenerative animal model can further our understanding of the natural regeneration process. The African clawed frog, *Xenopus laevis*, is an excellent model for studying regeneration as it can regenerate multiple organs including the eyes. *Xenopus* embryos regenerate eyes within 5 days (Kha et al., 2018). The vacuolar-ATPase (V-ATPase) is a proton pump that moves hydrogen ions across the cell membranes. This pump is important in regulating the membrane voltage of cells and is known to control limb regeneration. Chemical inhibition of V-ATPase blocked *Xenopus* eye regeneration and resulted in small regrowth-inhibited eyes. Thus, V-ATPase function is required for this process. This project aims to determine the role of V-ATPase during eye regeneration by examining the defects caused by V-ATPase inhibition. My results show that regrowth-inhibited eyes are on average 50% smaller than the contralateral eye. Tissue sections showed that the overall morphology of the regrowth-inhibited eye is the same as a normal eye, suggesting that there is unlikely to be defects in eye formation. Thus, our data indicate that the role of V-ATPase during eye regeneration is to control eye stem cell proliferation. Knowing this process may give us insight into why humans lack this capability and allow for research in medicinal applications. The development of this application may benefit humanity in improving the quality of life for humans that are seriously injured.

Animal Model: *Xenopus laevis*

Figure 1

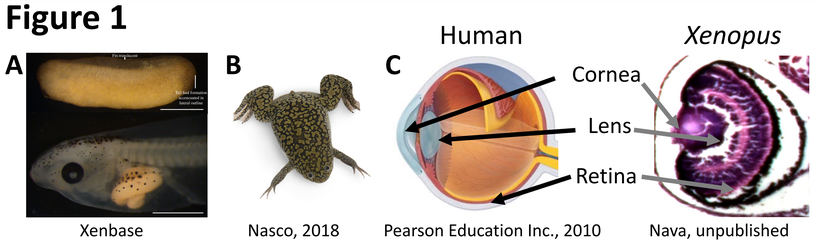


Figure 1. (A) *Xenopus laevis* stage (st.) 27 embryo above and st. 45 tadpole below (not to scale). (B) Adult *Xenopus laevis* female. (C) Comparison of human eye and *Xenopus laevis* eye to show they share a similar structure.

Xenopus Embryos Regenerate Eyes

Figure 2

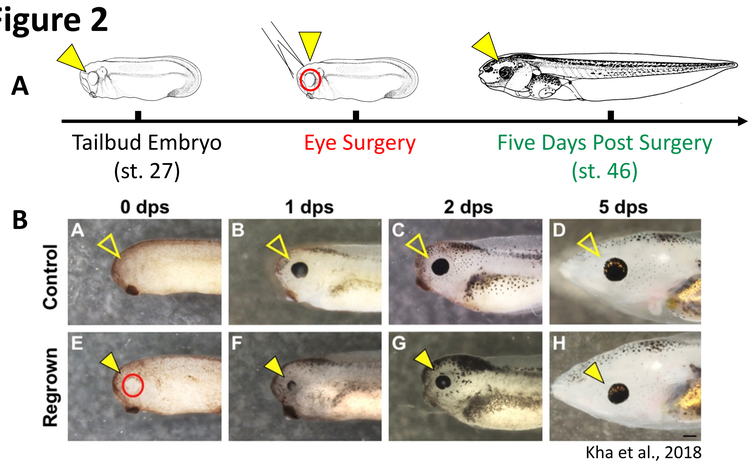


Figure 2. (A) Eye Regeneration Assay. (B) Top row: open arrowheads show control, unoperated eyes of age-matched siblings from st. 27 to st. 46. Bottom row: closed arrowheads show eyes regenerating at same time points. (dps = days post surgery.)

V-ATPase is a Proton (H^+) Pump

Figure 3

- V-ATPase is a multi-subunit H^+ pump
- Concanamycin A is a highly specific V-ATPase inhibitor

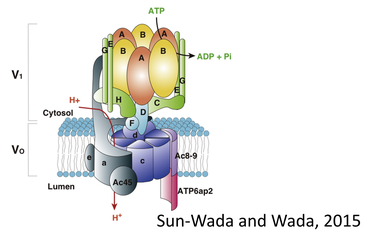


Figure 3. V-ATPase is found on cellular membranes. Concanamycin A interacts with V_0 subunit c of V-ATPase to inhibit V-ATPase function.

V-ATPase is Required for Eye Regeneration

Figure 4

- Average difference in eye size between operated and unoperated eye in a treated tadpole is about 50%

(n=16, p<0.001)

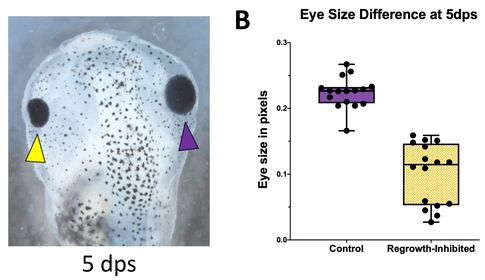


Figure 4. Chemical treatment with concanamycin blocked eye regrowth. (A) 5 dps (days post surgery) tadpole: yellow triangle indicates regrowth-inhibited eye and purple triangle indicates contralateral unoperated eye. (B) Comparison of eye size in pixels, purple is unoperated eye and yellow is regrowth-inhibited eye.

Methods to Determine the Role of V-ATPase

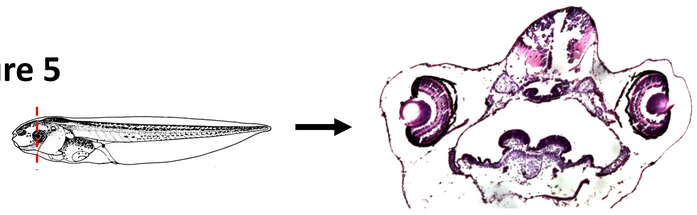
Hypothesis

Concanamycin A, a V-ATPase inhibitor, blocks eye regeneration by preventing proliferation of eye stem cells.

Methods: use histology to examine eye structure

1. Fix and embed 5 dps tadpoles in paraffin wax
2. Section tadpole eyes
3. Stain sections using Hematoxylin and Eosin

Figure 5



- Hematoxylin (purple) stains nucleic acids
- Eosin (pink) stains cytoplasm.

Figure 5. Red line indicates direction and location of tissue section

Regrowth-Inhibited Eyes Show Similar Structure to Normal Eyes

Figure 6

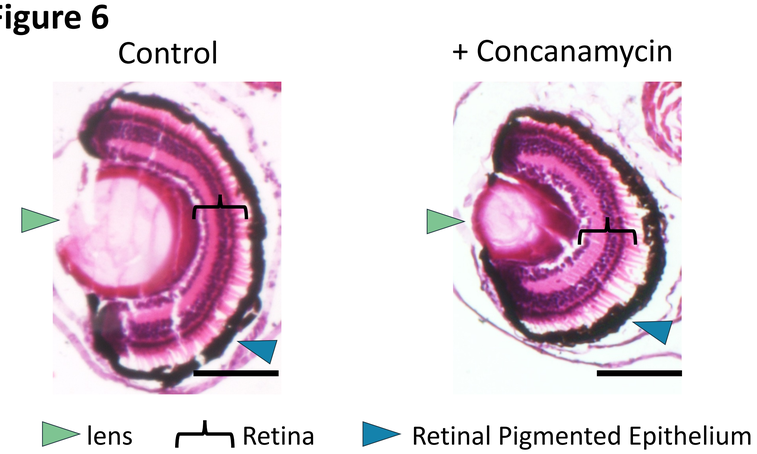


Figure 6. Transverse eye sections at 5dps. Left is contralateral unoperated eye and right is regrowth-inhibited eye. Major eye structures labeled: lens, retina, and retinal pigmented epithelium. Scale bar = 1 µm.

- Treatment with concanamycin resulted in significantly smaller eyes after eye removal surgery.
- Regrowth-inhibited eyes showed a similar eye structure to unoperated eye and contained a lens, retinal pigmented epithelium, and a retina.
- Overall eye development appears unaffected by V-ATPase inhibition.

V-ATPase Regulates Stem Cell Proliferation

- Inhibition of V-ATPase using Concanamycin A resulted in small non-regrowing eyes that retain normal eye structures.
- Eye formation was unaffected by V-ATPase inhibition.
- V-ATPase functions to regulate eye stem cell proliferation.
- Determining the function of V-ATPase can give insight into how stem cells grow and divide

Selected References

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