Bioelectricity Regulates Regeneration in Vertebrate Eyes

Cindy X. Kha and Kelly Ai-Sun Tseng
School of Life Sciences, University of Nevada, Las Vegas

Abstract

Regeneration is a complex process that allow injured tissues to be repaired in some animals. However, animals vary in their regenerative ability so an important goal in regeneration is to define a common mechanism. Recent studies have shown spaceflight causes injury to eye tissues, potentially causing long-term vision problems for astronauts. Therefore, investigating the mechanism to allow injury repair minimizes long-term health risk**.** The clawed frog *Xenopus laevis* displays a remarkable ability to regenerate many injured tissues, including eyes. The vacuolar (V)-ATPase H^+ pump regulates bioelectrical signaling in cells and is expressed during eye development. Its activity is also required for limb regeneration. To investigate if V-ATPase participates in eye regeneration, we use the *Xenopus* eye regeneration model. Previously, we demonstrated that *Xenopus* embryos rapidly regenerated functional eyes within 5 days. Molecular inhibition of V-ATPase blocked eye regeneration, indicating that V-ATPase is required for this process. The block of eye regeneration with V-ATPase inhibition was due to decreased eye stem cell proliferation. Expression of a yeast H^+ pump restored eye regeneration in the absence of V-ATPase activity. This data suggested that the H^+ pump function of V-ATPase is sufficient to initiate regeneration. **Overall, our results showed V-ATPase is required for eye regeneration through regulating stem cell proliferation. Further study of V-ATPase may lead to new strategies for developing regenerative therapies.**

Advantages of Studying the *Xenopus* Frog

Figure 1

Figure 1. (A) Adult *Xenopus* frog. **(B)** *Xenopus* tadpole. **(C)** *Xenopus* eye is comparable to a human eye.

Xenopus Regenerate Eyes After Surgery

Figure 2

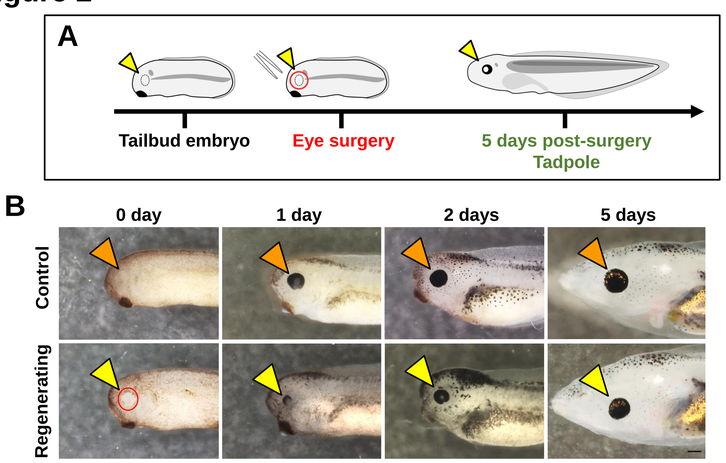

Figure 2. (A) Eye regeneration assay. Forceps removes eye tissues. Eye regeneration at surgical site was observed for 5 days. Yellow arrowheads show site of surgery. **(B)** Orange arrowheads in upper panels show control, unoperated eyes. Yellow arrowheads in bottom panels show regenerating eyes over 5 days. At 5 days, the regenerated eye is comparable to the control eye in size and structure. Scale bar: (**B)**= 200 µm.

V-ATPase is Required for Eye Regeneration

Figure 3

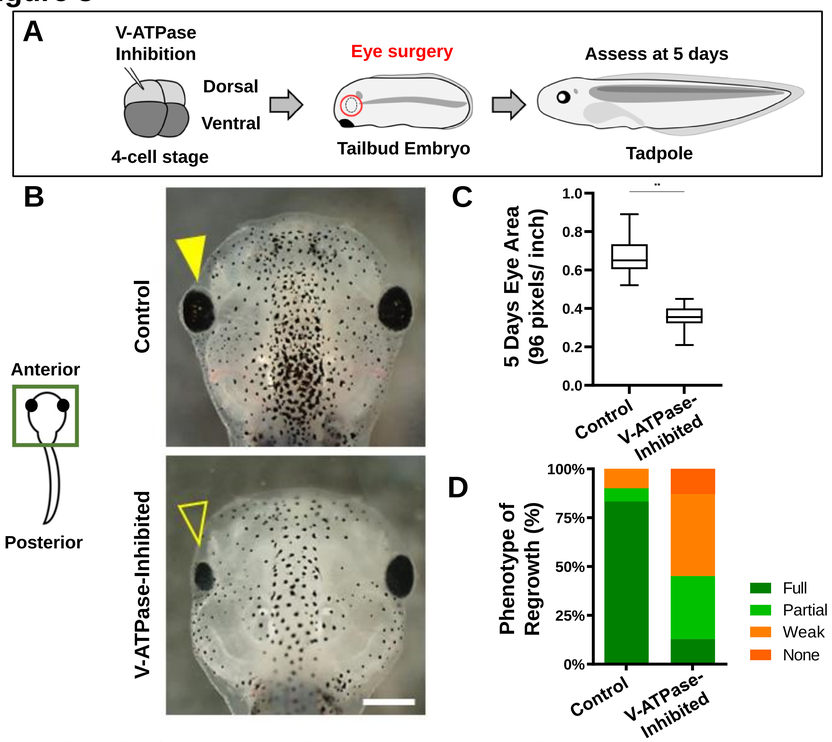

Figure 3. (A) Molecular V-ATPase inhibition assay. mRNA to abolish V-ATPase activity is injected into embryos, eye surgery performed and assessed for eye regeneration at 5 days. **(B)** Closed arrowhead show control, untreated tadpole with a regenerated eye. Open arrowhead show treated tadpole with a small inhibited eye. **(C)** V-ATPase inhibited eyes are smaller. **(D)** V-ATPase inhibited eyes show mostly weak to no regeneration of eye tissues. ** denotes p<0.01. Scale bar**:** (B)= 500 µm.

Increased H^+ Pump Activity Restored Eye Regeneration

Figure 4

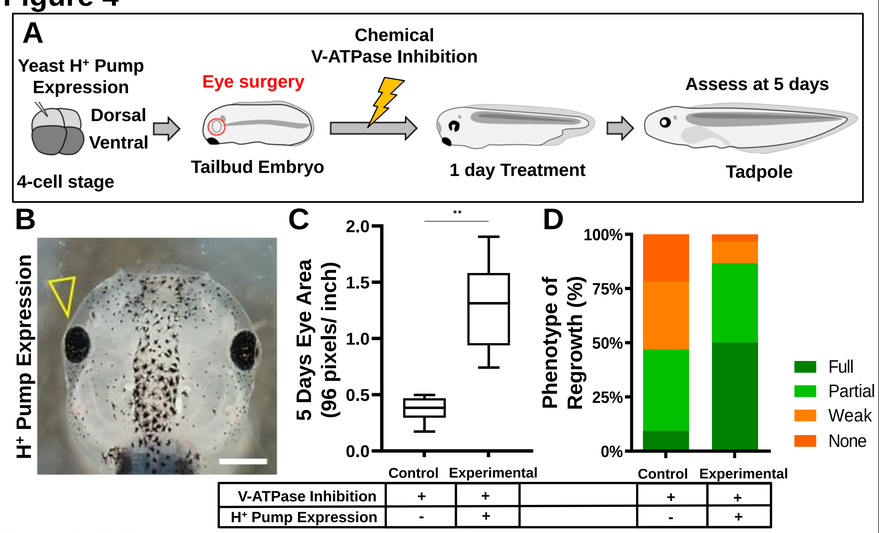

Figure 4. (A) Eye regeneration restoration assay. mRNA to increase H^+ pump activity is injected into embryos, eye surgery performed, treated with a chemical V-ATPase inhibitor, and assessed for eye regeneration at 5 days. **(B)** Open arrowhead show increased H^+ pump activity restored eye size after V-ATPase inhibition.**(C)** Restored eyes are larger compared to V-ATPase inhibited eyes. **(D)** Restored eyes show mostly full and partial regenerated eye tissues.** denotes p<0.01. Scale bar**:** (B)= 500 µm.

V-ATPase Regulates Eye Stem Cell Proliferation

Figure 5

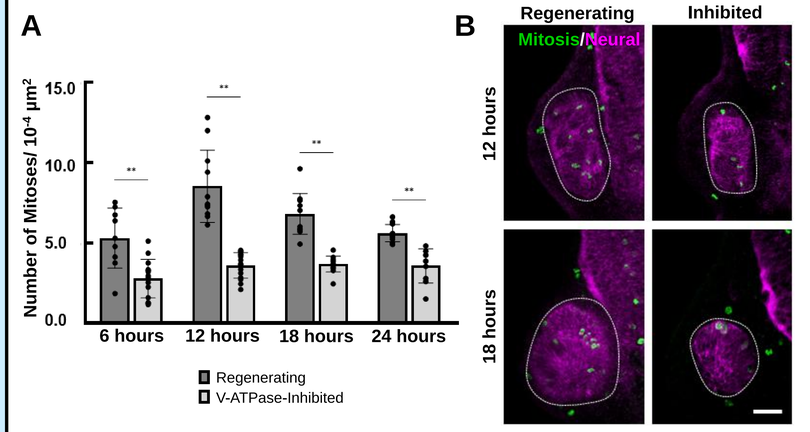

Figure 5. Quantification of eye stem cell proliferation using a marker to identify mitotic cells at 6, 12, 18 and 24 hours after eye surgery and with treatment. **(A)** V-ATPase inhibited eyes show less mitotic activity in area of eye tissues at all timepoints. **(B)** Representative images of V-ATPase inhibited eyes at 12 and 18 hours show less markers for mitotic activity. Dotted white line indicates area of eye tissue. Green= mitotic marker. Magenta= neural tissue marker. ** denotes p<0.01. Scale bar**:** (B)= 50 µm.

Summary

V-ATPase Activity is Required for Eye Regeneration

- V-ATPase inhibition blocks eye regrowth. Thus V-ATPase is required for eye regeneration.

Increased H^+ Activity Restored Eye Regeneration

- The H^+ pump activity of V-ATPase is sufficient to drive regeneration. Overexpression of a yeast H^+ pump restored eye regeneration in the presence of V-ATPase inhibition.

V-ATPase Regulates Eye Stem Cell Proliferation

- The decreased cell proliferation rate in V-ATPase-inhibited non-regrowing eyes demonstrated that V-ATPase acts to promotes stem cell proliferation during eye regeneration.
- **Future research will characterize the V-ATPase pathway to define the components that control eye stem cell proliferation in order to induce regeneration.**

Acknowledgement

This material is based upon work supported in part by the National Aeronautics and Space Administration under Grant No. 80NSSC20M0043

References

- Kha, C. X., Son P.H., Lauper, J., Tseng, K.A. (2018). A model for investigating developmental eye repair in Xenopus laevis. Exp Eye Res. 169: 38-47, doi.:10.1016/j.exer.2018.01.007
- Kha, C. X., Guerin, D. J., Tseng, K. A.-S. (2020). Studying in vivo retinal progenitor cell proliferation in Xenopus laevis. Retinal Development Methods in Mol Biol., 19–33. doi: 10.1007/978-1-0716-0175-4_2
- Adams D. S., Masi A. and Levin M. (2007). H+ pump-dependent changes in membrane voltage are an early mechanism necessary and sufficient to induce Xenopus tail regeneration. Development 134, 1323-1335. 10.1242/dev.02812