May 8th, 11:00 AM - 1:00 PM

Locating Vitamin D Receptors (VDRs) in Annual Killifish, Austrofundulus Limnaeus

Rosalia N. Tanori
Portland State University

Amie L. Romney
Portland State University

Jason E. Podrabsky
Portland State University

Locating Vitamin D Receptors (VDRs) in Annual Killifish, *Austrofundulus Limnaeus*

Rosalia N. Tanori, Dr. Amie L. Romney, Dr. Jason E. Podrabsky
Department of Biology, Center for Life in Extreme Environments
Portland State University, Portland OR 97201

Background

- Cell signaling from environmental cues, biotic or abiotic, plays an integral role in the development of all animals. Abiotic cues such as light and temperature are two pervasive environmental factors present in almost all environments on Earth, yet we know little of how they affect developmental trajectories. Exposure to light and heat profoundly alters the development of annual killifishes through a mechanism thought to be regulated by vitamin D signaling.
- The annual killifish, *Austrofundulus limnaeus*, is native to South America and inhabits temporary ponds caused by pronounced wet and dry seasons. Annual killifish have adapted to their unpredictable environment by producing stress-tolerant embryos able to undergo a period of dormancy termed diapause.
- Previous research explored the role of temperature and vitamin D signaling in regulating the development of *A. limnaeus*. Using RNA sequencing, the expression of genes critical in the synthesis of vitamin D_3 was described in embryos developing along two different developmental trajectories that are induced by differences in light and heat.

Vitamin D_3 signaling → Normal development
Inhibition of vitamin D_3 → Developmental arrest

Research Question

Where is the genomic location of the vitamin D receptor (VDR) within *A. limnaeus*?

Specific Aims

1. Utilize western blot analysis to detect VDR-A and VDR-B within different tissues from *A. limnaeus*.
2. Utilize immunofluorescence imaging to visualize VDR binding to nuclear DNA in cells isolated from *A. limnaeus*.
3. Utilize CUT&RUN analysis of VDR binding sites in the genome of *A. limnaeus*.

Methodology

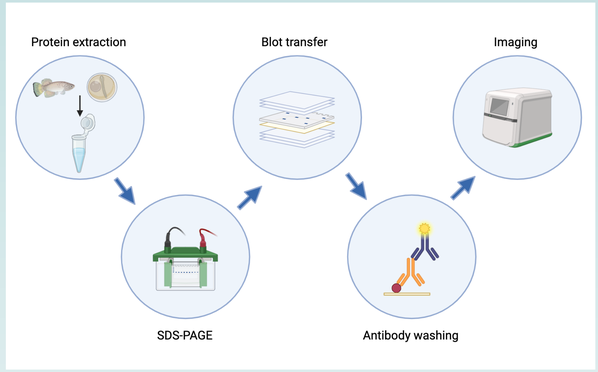

Figure 1. Western blot workflow utilizing *A. limnaeus* tissues and embryos.

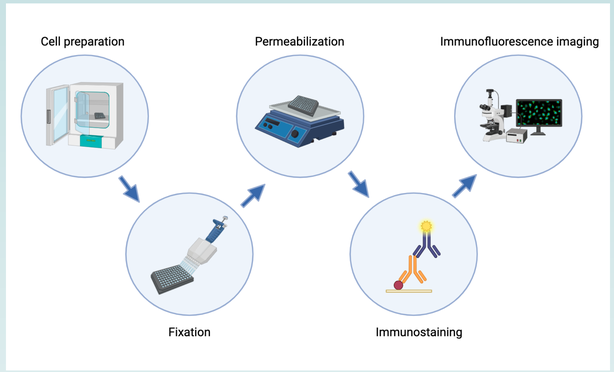

Figure 2. Immunocytochemistry workflow utilizing *A. limnaeus* cells (WS40NE cells).

Results

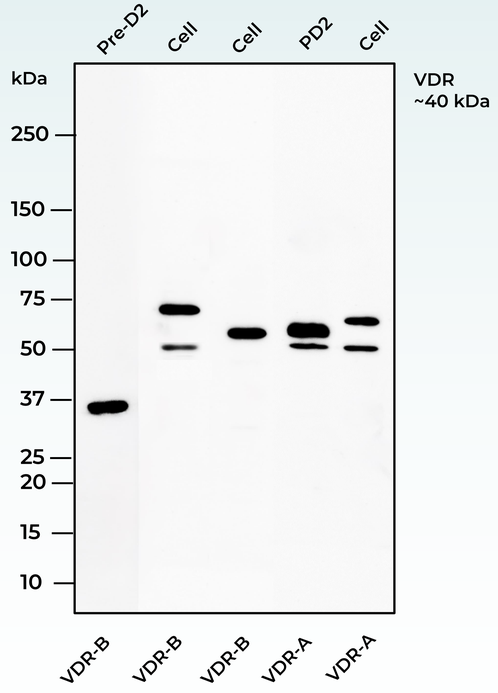

Figure 3. Western blot banding for *A. limnaeus* embryos and cells. One band for pre-diapause II embryos (Pre-D2) using antibody for VDR-B (#4805 56-day bleed). Two bands for cells using antibody for VDR-B (#4806 72-day bleed). One band for cells using antibody for VDR-B (#4805 Terminal). Two bands for post-diapause II embryos (PD2) using antibody for VDR-A (#4808 72-day bleed). Two bands for cells using antibody for VDR-A (#4808 72-day bleed).

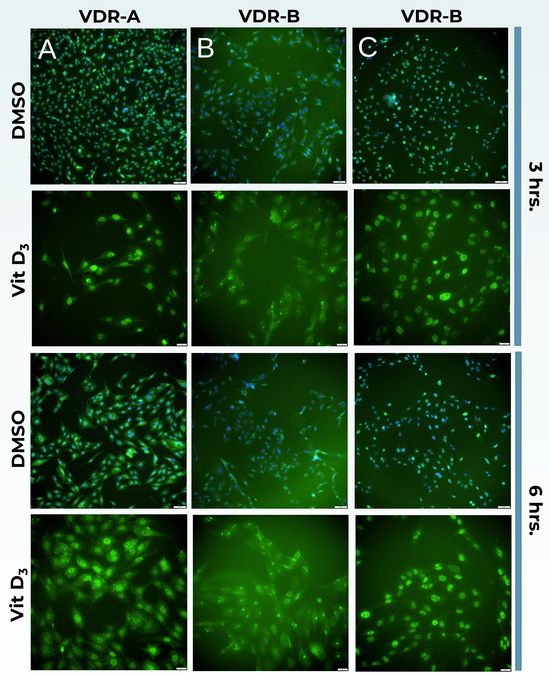

Figure 4. Nuclear staining in WS40NE cells. Grouped by DMSO (control) and vitamin D_3 (experimental) treatments, VDR antibodies, and time. Control rows imaged at 20x, expressing DAPI and Alexa Flour 488. Experimental rows imaged at 40x, expressing Alexa Flour 488. **A)** Column treated with antibody for VDR-A (#4808 72-day bleed). **B)** Column treated with antibody for VDR-B (#4805 56-day bleed). **C)** Column treated with antibody for VDR-B (#4806 72-day bleed).

Discussion

- Positive results appear for four out of seven custom purified antibodies in pre-diapause II embryos, post-diapause II embryos, and in cultured cells.
- Differing sizes and number of bands possibly due to degradation, post-translational modifications, and isoform reactivity.
- The results will give insight into molecular mechanisms *A. limnaeus*, has developed for combating life in an extreme environment.
- The results will allow us to gain insight into the degree of vitamin D's role in the promotion of normal development.

Next Steps

To further investigate and visualize VDR binding to nuclear DNA in cells from *A. limnaeus*:

- Utilize a smearing technique with slides using a cell suspension of specific tissues.

To investigate where the VDR transcription binding sites are in *A. limnaeus'* genome:

- Perform a Cut & Run assay utilizing annual killifish fish cells and purified custom antibodies for VDR-A and VDR-B.
- Sequencing results will be mapped with a reference genome (*A. limnaeus*) to determine the VDR binding sites.
- Use motif analysis in conjunction with peak calling to determine the specific structure of the binding site patterns.

Acknowledgements & References

I would like to thank everyone in the Podrabsky Lab for continuous support, training, and camaraderie. Additionally, I'd like to thank the Singer Lab for generously providing access to imaging equipment.

Funded by NSF ISO Grant 2025832

Scan QR code for references!