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Improved Genome Maintenance and DNA Replication in the Anoxia Tolerant Annual Killifish

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Improved Genome Maintenance In The Anoxia Tolerant Annual Killifish

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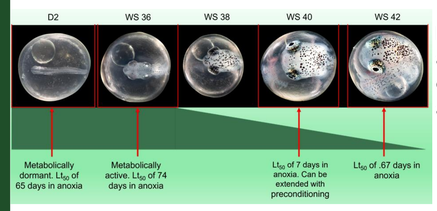
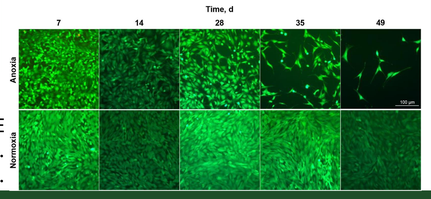
Model System

- *Austrofundulus limnaeus* is found in ephemeral ponds in Venezuela and has evolved the ability to enter into embryonic diapause, a state of developmental dormancy and metabolic arrest that supports survival.
- Dormant and developing embryos can withstand high levels of known genotoxic stressors, such as months of anoxia and high dose irradiation.
- We expect that these embryos have improved genome maintenance in order to withstand these genetic insults, but exactly the type of damage created and the maintenance pathways utilized is unknown.
- To conduct this work we used a cell line derived from killifish embryos (PSU-AL-WS40NE) which has similar levels of tolerance to embryos.



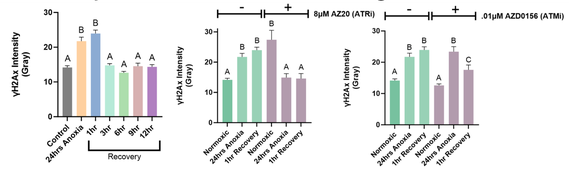
Embryos of the Annual Killifish are able to withstand long exposures to anoxia that varies along development.



Live/dead staining of PSU-AL-WS40NE cell line Derived from a WS40 embryo. Cells can survive for 49 days of anoxia..

DNA Damage During Anoxia

- Exposure to anoxia and recovery in normoxia causes an increases in DNA damage, shown through levels of gamma-Histone H2Ax (a phosphorylated form of the histone)
- Inhibition of the core proteins involved in the activation of gamma-H2Ax, ATM and ATR, supports the idea that the main source of genetic damage is stalled and collapsed replication forks instead of exogenous sources of damage. This suggests that replication may be stalled during anoxia and thus determining the role of the cell cycle in relation to this damage is a vital next step.

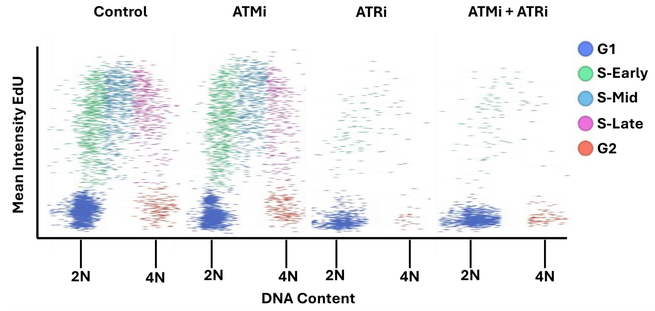


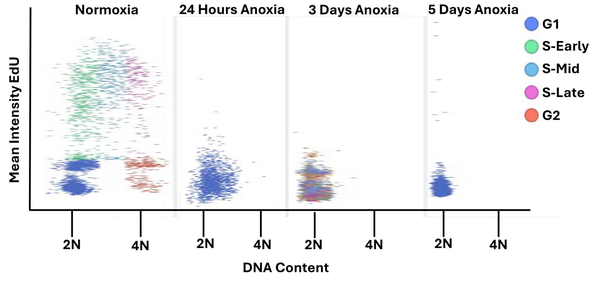
Cells were exposed to 24 hours of anoxia and allowed to recover for up to 12 hours in normoxia. ATM and ATR were inhibited throughout anoxia and recovery. Levels of genomic damage were measured using gamma-H2Ax fluorescence intensity.

Methods and Results

To determine the cell cycle stage we used Quantitative Image Based Cytometry (QIBC). Using an EdU pulse and DAPI staining we were able to identify specific cell cycle stages of thousands of cells and then correlate that with gamma-H2Ax signal.

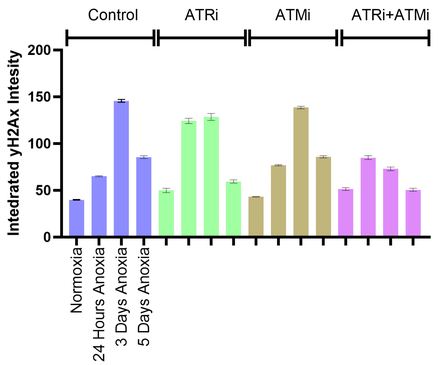
Cells were exposed to inhibitors for either ATM, ATR, or both for 24 hours. A 15-minute EdU pulse was done at the time of sampling and cells were then stained for EdU, DAPI, gamma-H2Ax, and Cleaved Caspase-3





Monitoring cells over 5 days of anoxia indicates a loss of replicating cells and a striking pattern of cells only in the G_1 phase of the cell cycle.

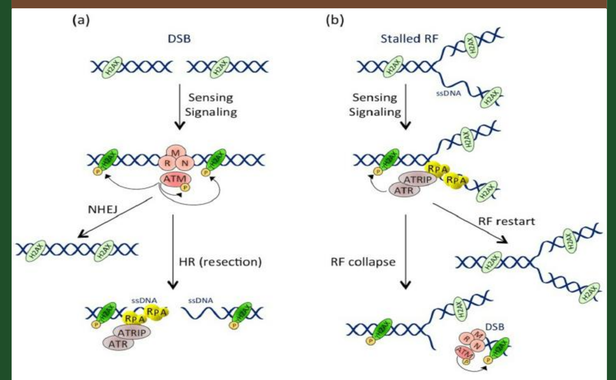
To confirm previous work that showed exposure to anoxia causes an increase in gamma-H2Ax levels that is lost with ATR inhibition we again dosed cells with these inhibitors through 5 days of anoxia. Interestingly we see a steady increase of gamma-H2Ax through 3 days followed by a sharp decrease at the 5th day of anoxia. Inhibition of ATM has no effect while inhibition of ATR causes an increase of gamma-H2Ax signal after 24 hours.



Conclusion

- The cell cycle is severely disrupted due to anoxic exposure, and these data suggests that active replication is not occurring.
- An increase in gamma-H2Ax signal was observed after anoxic exposure. The signal decreases after 5 days and it would be interesting to see if these levels would return to normal after longer exposures.
- Unlike previous work we see that inhibition of ATR does not cause a loss of gamma-H2Ax signal during anoxia. This could point to potential biases inherit in standard immunofluorescence data where hundred of selected cells are analyzed compared to thousands of cells chosen at random throughout a well.
- ATM and ATR inhibition does not completely knock down gamma-H2Ax expression, giving a possibility that there is an alternate pathway for gamma-H2Ax activation.

ATM and ATR



Bezine, Elisabeth (2014)

Acknowledgments

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Citations

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