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Neural Regulation of the Cell Cycle During Limb Regeneration

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Opportunity

Regeneration has captivated the curiosity of scientists for hundreds of years. It occurs seamlessly in salamander limbs and tails, but mammalian regeneration is very limited. One of the most peculiar and mysterious aspects of regeneration is its dependency upon an intact nerve supply. It has long been known that the removal of nerves will inhibit regeneration. The specifics of this relationship, to a large extent, area still unknown.

The Mexican axolotl salamander (**Figure 1)** is a unique model that allows the investigation of the unexplored effects of absence of nerves on cell cycle kinetics during limb regeneration (**Figure 2)**. By severing the three brachial nerves that innervate the left limb during regeneration while keeping the nerves intact in the right limb, we will determine how denervation affected the cell cycle through cell proliferation analysis.

Aim

Our goal is to determine how denervation affects the cell cycle of proliferating cells during limb regeneration.

Figure 1 Three adult axolotls
Picture courtesy of Johanna E. Farkas, Ph.D.

Figure 2 Stages of regenerating salamander limb
Picture courtesy of James R. Monaghan, Ph.D.

Approach

(Illustrated in **Figure 3**)

1. Denervate regenerating left limb.
2. Inject BrdU and EdU (**Figure 4**) intraperitoneally.
3. Collect and section blastema.
4. Stain cells for EdU using Click-it chemistry and BrdU immunohistochemistry.
5. Quantify fluorescent cells.

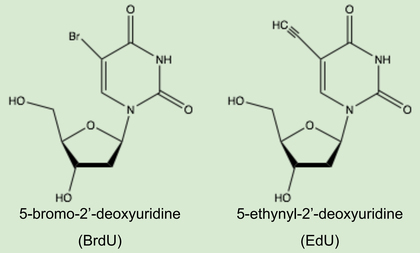

Figure 4 Structural Formula of EdU and BrdU

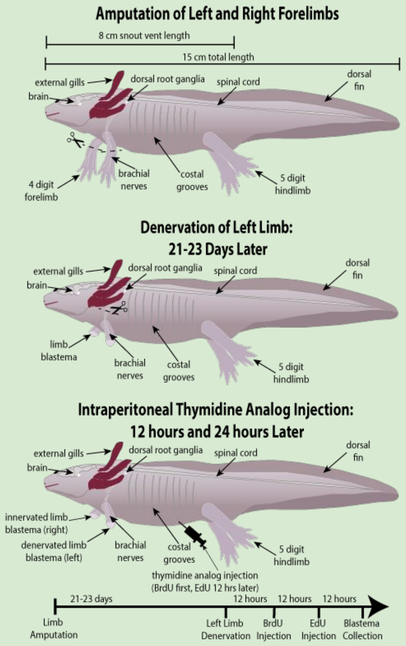

Figure 3 Axolotl limb amputation, denervation, and thymidine analog injection
Axolotl pictures courtesy of James R. Monaghan, Ph.D.

Results and Next Steps

Axolotls were injected with EdU and BrdU, and their blastemas were stained using click chemistry and anti-BrdU antibodies. Fluorescent microscopy of tissue sections collected from regenerating limbs are shown in **Figure 5**. Next, we will quantify cell cycle \cycle kinetics by measuring immunofluorescence of each cell to determine the difference between the denervated limb and the innervated limb based on **Figure 6**. In the future, we will also stain the sections for mitotic and apoptotic markers.

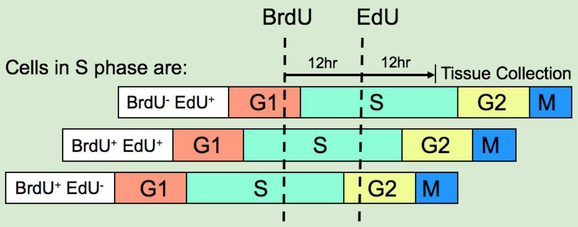

Figure 6 - Thymidine Analog Expression at Cell Cycle Stages
Diagram based on EdU BrdU diagram of James Monaghan, Ph.D.

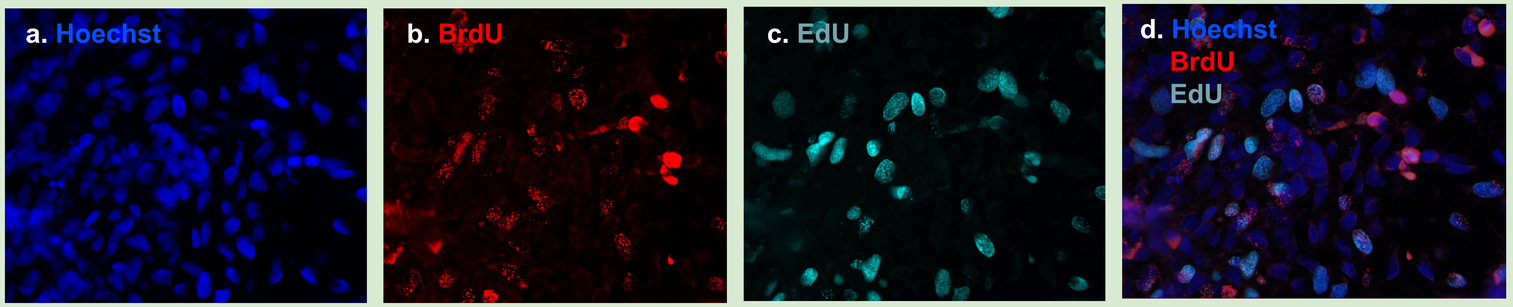

Figure 5 (a-d) Innervated limb blastema labeled with nuclei marker (Hoechst) and proliferation markers (EdU and BrdU)
Pictures courtesy of James R. Monaghan, Ph.D.

Impact

In this project we analyse the cell cycle kinetic of denervated and innervated regenerating limb. Our findings will determine where cells stall in the cell cycle after denervation, which will provide insight into the mechanism of cell cycle control during complex tissue regeneration.

Acknowledgements

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