The Circadian Control of Limb Regeneration in the Axolotl Salamander
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Abstract
Axolotl salamanders are enigmas among vertebrates because they can regenerate many tissues as adults including limbs (Fig. 1). Studying the molecular basis of the axolotl’s regenerative abilities could be informative for future regenerative therapies. Proper tissue regeneration is dependent upon the tight regulation of cellular proliferation, but our understanding of how cell proliferation is regulated in the context of regeneration is lacking. Based upon the importance of circadian rhythms in animal homeostasis and cell proliferation across the animal kingdom, we hypothesized that undisrupted circadian rhythms are necessary for limb regeneration by regulating cell division. Using the adult axolotl arm as a model, we demonstrate that disruption of the circadian clock using modified light/dark cycles has a negative impact on regeneration rate. Axolotls were housed in modified housing units that controlled light/dark cycles throughout the regeneration process and imaged biweekly until control limbs regenerated. We found that housing in constant light as well as constant dark had a significant inhibitory effect on limb regeneration rate compared to normal housing conditions. Surprisingly, housing animals in an alternating 3.5hr light/dark condition did not have a significant impact on regeneration rate. These results suggest that cell proliferation and limb regeneration are inhibited when lighting conditions are disrupted, but transitions between light and dark may be more important than a continuous 12hr light/dark cycle. Future studies are underway to explain this phenomenon at the cellular and molecular level to reveal how circadian rhythms contribute to complex tissue regeneration.

Regenerative Rates are Photoperiod Dependent

Fig. 2. Limb regeneration imaged 39 days post-amputation.

Fig. 3. Graph of limb regeneration measured in millimeters over 29 days.

Specific Aims
• Show when and where genes involved in circadian rhythms and tissue growth are expressed in the regenerating limb

Conclusion
This work suggests that circadian rhythms play a role in complex tissue regeneration. The specific role that it plays is still unclear, but studies are in the works to decipher the underlying mechanisms.

We are currently processing regenerating limb tissue samples for histological and cell cycle (BrdU) analysis. This will attempt to answer the first specific aim.

It is also important to note that, while contradictory to our findings, our recent set of axolotls exhibit the complete opposite effect (Fig.4.). The ones exposed to constant light have also regained pigmentation in their gills and along their spinal columns (Fig.5.).

References