Background

Regeneration, or the repair of lost or damaged tissue, happens in almost every organism at some scale. While most animals are capable of smaller scale regeneration, like regrowth of damaged skin cells, select organisms are also able to perform more complex regeneration, like the regrowth of limbs or the spinal cord. Axolotls and newts are among the most studied of these organisms, though zebrafish are also valuable research models in understanding regeneration (Farkas and Monaghan 2017). However, most mammals are not capable of complex regeneration at the same scale as these organisms. This poses a problem for humans with limb amputations or spinal cord injuries; once gone, the tissue is gone forever. The question we must therefore ask is why can some animals regenerate organs and appendages while others cannot?

In epimorphic regeneration, two major tissue types orchestrate vertebrate regeneration: the blastema and the wound epithelium (Haas and Whited 2017). Though these two tissues are generally though to be the primary effectors of regeneration, other tissues play a role as well. Classic studies in the 1800s, for example, showed that regeneration in amphibians requires the nervous system (Mullen et al. 1996; Kumar et al. 2007). Furthermore, nerves are sufficient to grow ectopic limbs in axolotls; if the nerve is redirected from the true wound to a different 'false wound', the limb will grow from the false wound instead (Torres 2016). Together, these experiments suggest that the nervous system plays a key role in regeneration.

The molecular nature of these nerve-derived signals, however, remains largely unknown. One category of neuronal signals -- neuropeptides -- are promising candidates. Neuropeptides are released by neurons, and elicit responses in both neuronal and non-neuronal tissues (Kohno et al. 2003). The aim of this study was to explore the roles played by neuropeptides in regeneration of zebrafish tailfin tissues. Specifically, I hypothesized that neuropeptide cart3, which is expressed in sensory neurons that innervate the tailfin tissue and is transcriptionally elevated after tailfin amputation, plays a role in enhancing regeneration (Ouyang et al. 2017).

Methods

Cart3 has never been specifically implicated in limb regeneration before. In order to assess the role of cart3 in regeneration, we used two lines of zebrafish, HSCartA, a dominant heatshock-inducible overexpression of cart3, and zfp4, a recessive zinc finger protein disruption of cart3. Each line of fish was crossed and raised to 2 days post fertilization, at which point the caudal fin was cut at the base of the notochord, just before the pigmentation. Fish were then allowed to grow uninterrupted until 2 days post amputation (the middle of the regenerative process), at which point caudal fin regrowth of both over- and under-expression fish was measured. Data was then normalized and compared to their wildtype siblings, after which statistical analysis was performed in order to determine statistical significance.

Results

We saw that there was a statistically significant increase in regenerative capacity with cart3 overexpression (Fig. 1). On average, cart3 overexpression fish were 7.2% longer than their wildtype siblings. Similarly, we saw a statistically significant decrease

in regenerative capacity with cart3 underexpression (Fig. 2). Cart3 underexpression fish were on average 5.7% shorter than their wildtype siblings.

Discussion and Conclusions

These data indicate both that cart3 is sufficient and necessary for normal regenerative capacity. The unknown nature of regenerative mechanisms makes this discovery quite important and allows us a starting point to truly understanding how regeneration works. This could lead to therapies that may help injured humans regrow tissues. However, there is still much work to do in understanding cart3's role in the regenerative process. For example, just how does cart3 facilitate regeneration? We would also like to examine the role of the gene *leptin b* in the regenerative process, since it is known to increase cart3 expression (Kang et al. 2016). Specifically, we want to examine whether *leptin b* over- and under-expression has the same effect as cart3 over- and under-expression. Regardless, these data indicate that there is much to be discovered in the field of regeneration.

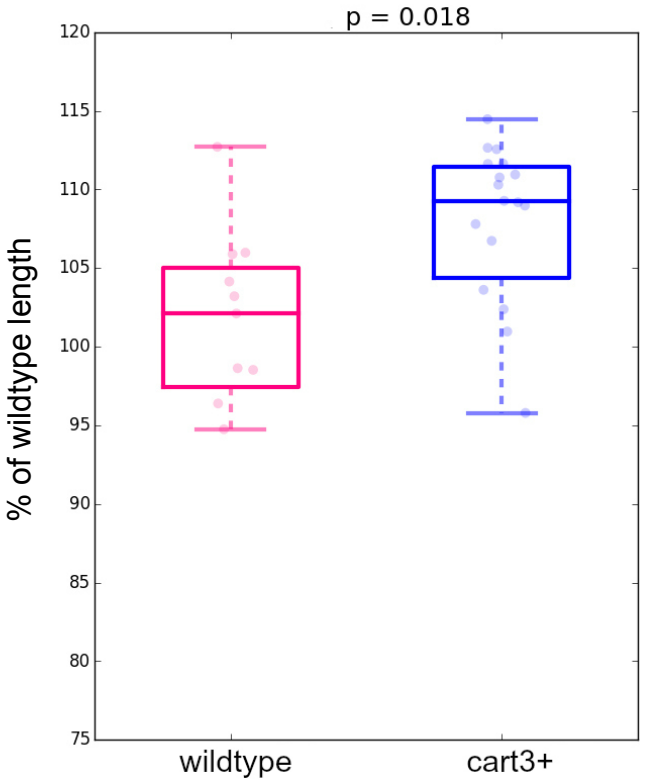

Figure 1. Wildtype fish versus fish with an overexpression of cart3; p=0.018. Our data showed a significant increase in regenerative capacity of cart3 overexpression fish compared to wildtype fish.

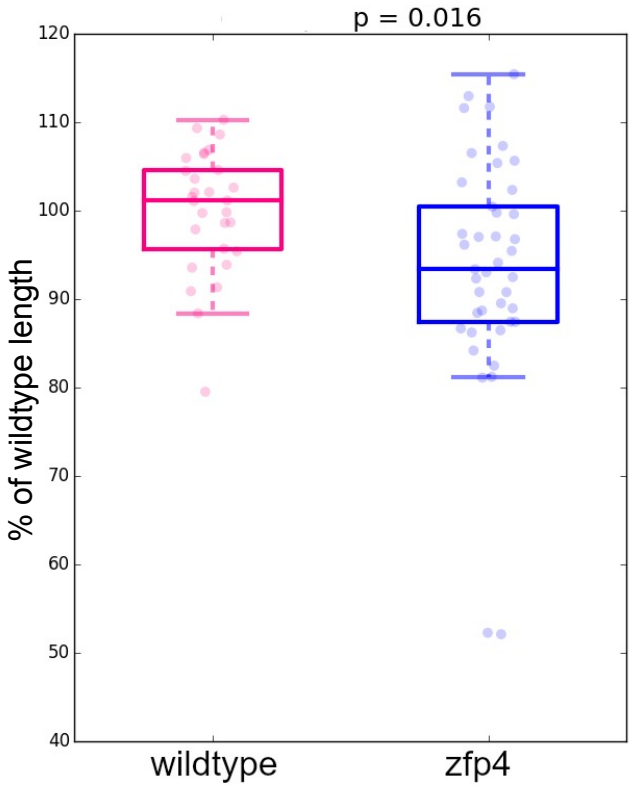

Figure 2. Wildtype fish versus fish with an underexpression of cart3; p=0.016. Our data showed a significant decrease in regenerative capacity of cart3 underexpression fish compared to wildtype fish.

Sources

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