Investigating the contribution of Protein Tyrosine Phosphatases (PTPs) in zebrafish fin regeneration

佐藤　麻莉（筑波大学 生物学類）　　指導教員：中田　和人（筑波大学 生命環境系）

Introduction

Living organisms have a great variety in many aspects, including in their capacity for regeneration. For example, zebrafish and amphibians, including salamanders and frogs, have a high ability to regenerate following injury. Unlike humans these animals are able to fully regenerate their tails and limbs partially de-differentiating cells near the injury site and driving them to re-enter cell cycles and redifferentiate.
Thus, investigating the processes which enable them to recover from injury with high quality, we aim to develop a way to induce higher quality of regeneration in humans.

In order to reach this goal, it is required to understand the mechanisms that permit these animals to have such high ability to regenerate. Recently, it was discovered that elevated reactive oxygen species (ROS) plays an essential role in regeneration. animals with high regenerative capacity[1, 2]. However, how ROS promotes regeneration and what are their downstream targets remain unclear.

The aim of my project was to investigate whether the family of Protein Tyrosine Phosphatases (PTPs) might be critical downstream targets of ROS during regeneration. PTPs are known to be highly sensitive to changes in ROS levels, where increased ROS levels inhibit their activity, via oxidation of their catalytic cysteines. Since PTPs dephosphorylate the target of protein tyrosine kinases, PTPs act as inhibitors of many cellular processes required for regeneration, such as cell proliferation and signalling. Thus we hypothesise that elevated ROS inhibits the PTPs, so that cell signalling and proliferation can proceed unimpeded during regeneration in these organisms.

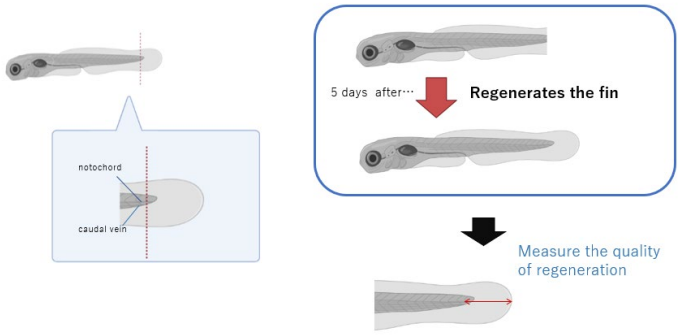
My aim was to test if PTPs are critical downstream targets of ROS during tail and fin regeneration in zebrafish. To address this aim, I investigated whether PTP mutants could rescue regeneration in larval tail regeneration assays.

Materials and Method

Three *ptp* mutants in zebrafish, which others had shown are oxidised during adult caudal fin regeneration[3] were used in this study.

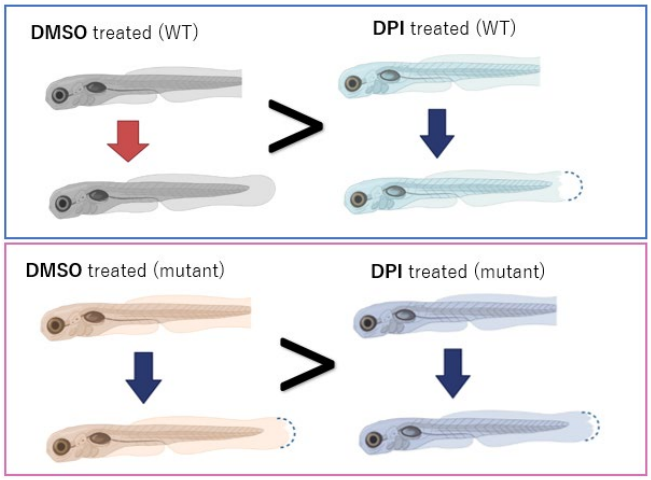
2-day old zebrafish larvae that were either WT, or had mutations in *ptpn1*, *ptpn9a* or *ptprea* had their distal tails amputated. Some of these larvae were treated from 1hr before and after amputation with either DMSO (control) or DPI, which inhibits ROS production.

The larvae where then imaged at 5 days post amputation and regeneration was assessed by measuring the length of the recovering tails.



Result

There was no significant differences in regeneration across the different genotypes, but DPI treated inhibited regeneration. Based on these results, we find that none these PTPs studied is sufficient to rescue regeneration in zebrafish larvae that has attenuated ROS production.



Discussion

My findings suggest that no individual PTP is sufficient to rescue regeneration in zebrafish larvae that has attenuated ROS production. However, it does not exclude the possibility that there are several PTPs that act downstream of ROS during regeneration. This possibility could be addressed in the future by performing similar experiments to the ones described here, but in compound mutant lines (i.e. zebrafish that are double or triple mutant for these *ptp* genes).

References

1. Love et al., (2013) Nat Cell Biol *15*, 222–228.
2. Gauron et al., (2013) Sci Rep *3*, 2084.
3. Wu et al., (2017) Sci Rep *7*, 8460.

This research was conducted under the supervision of Professor Enrique Amaya of the University of Manchester through the exchange student program.