

Manipulation of the Wnt Signaling Pathway and Analysis of Gene Expression in Axolotls

Introduction

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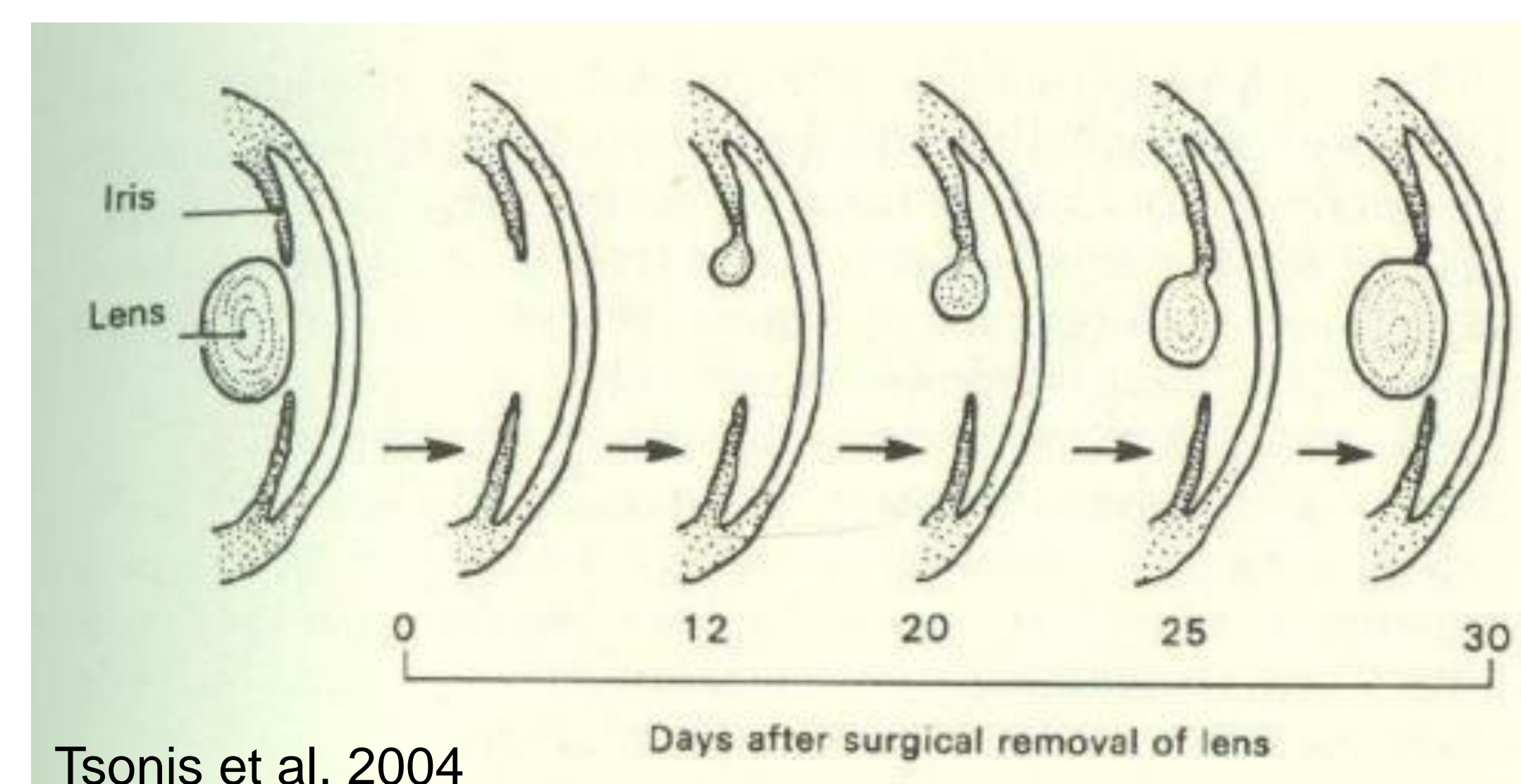
Background: Urodele amphibians have the ability to regenerate many organs. Specifically axolotls, *Ambystoma mexicanum*, regenerate their lens after complete removal, between embryonic stages 44 and 52. Through process of differentiation and transdifferentiation, a lens can regenerate from the dorsal and ventral iris. As the axolotls mature, however, they lose this regeneration capability.



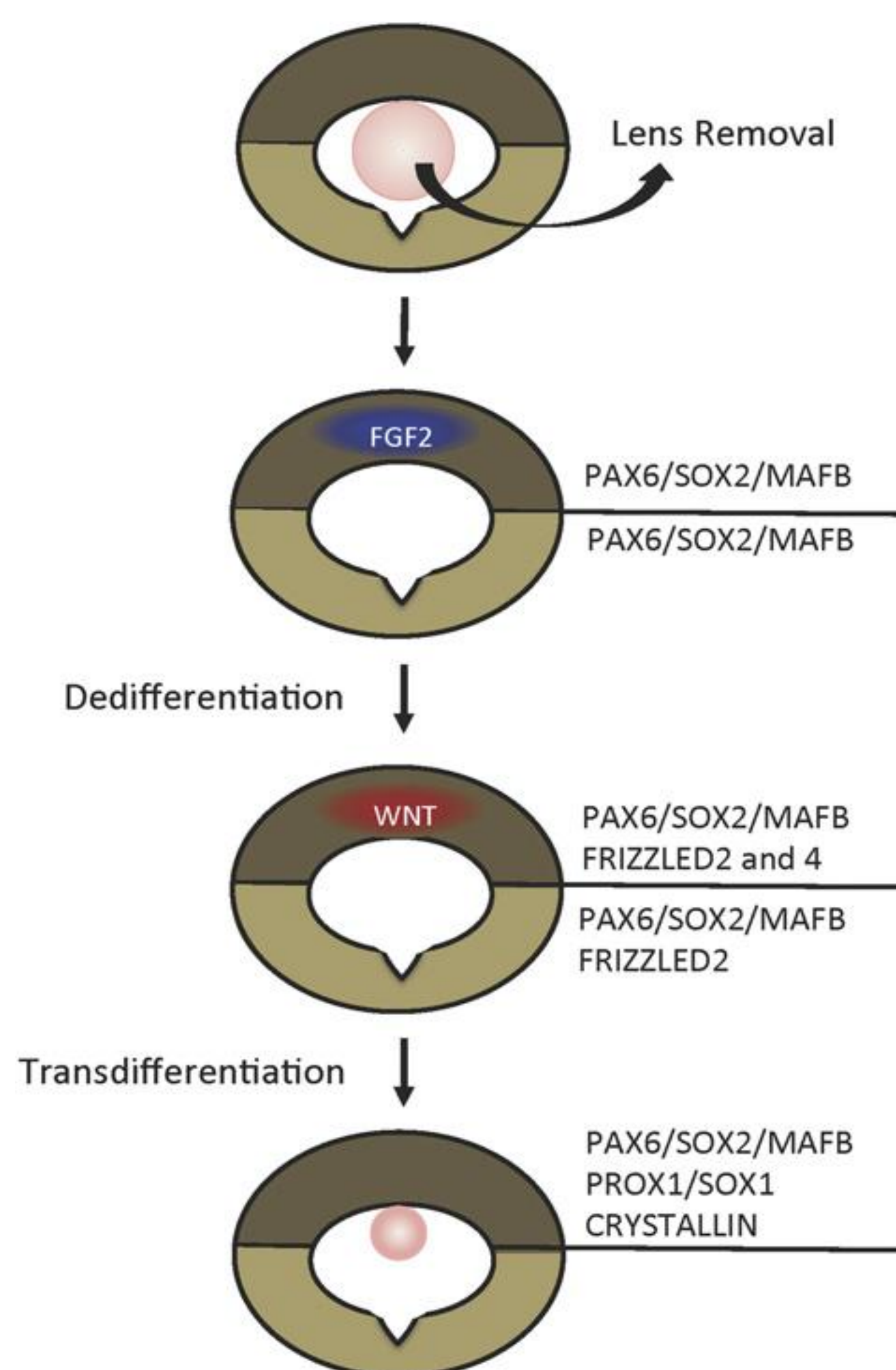
Therefore, an adult axolotl serves as a non-regenerating control in the study of lens regeneration. Studying the differences in gene expression between axolotls before and after stage 52 can provide significant information about the mechanism of regeneration. Prior research has shown that the Wnt signalling pathway is important in the transdifferentiation process, and therefore could play an important role in regeneration.

Objective: The goal of this study was to manipulate gene expression through the application of activators and inhibitors of the Wnt pathway to gain a better understanding for the role of this pathway during the process of regeneration.

Stages of Lens Regeneration

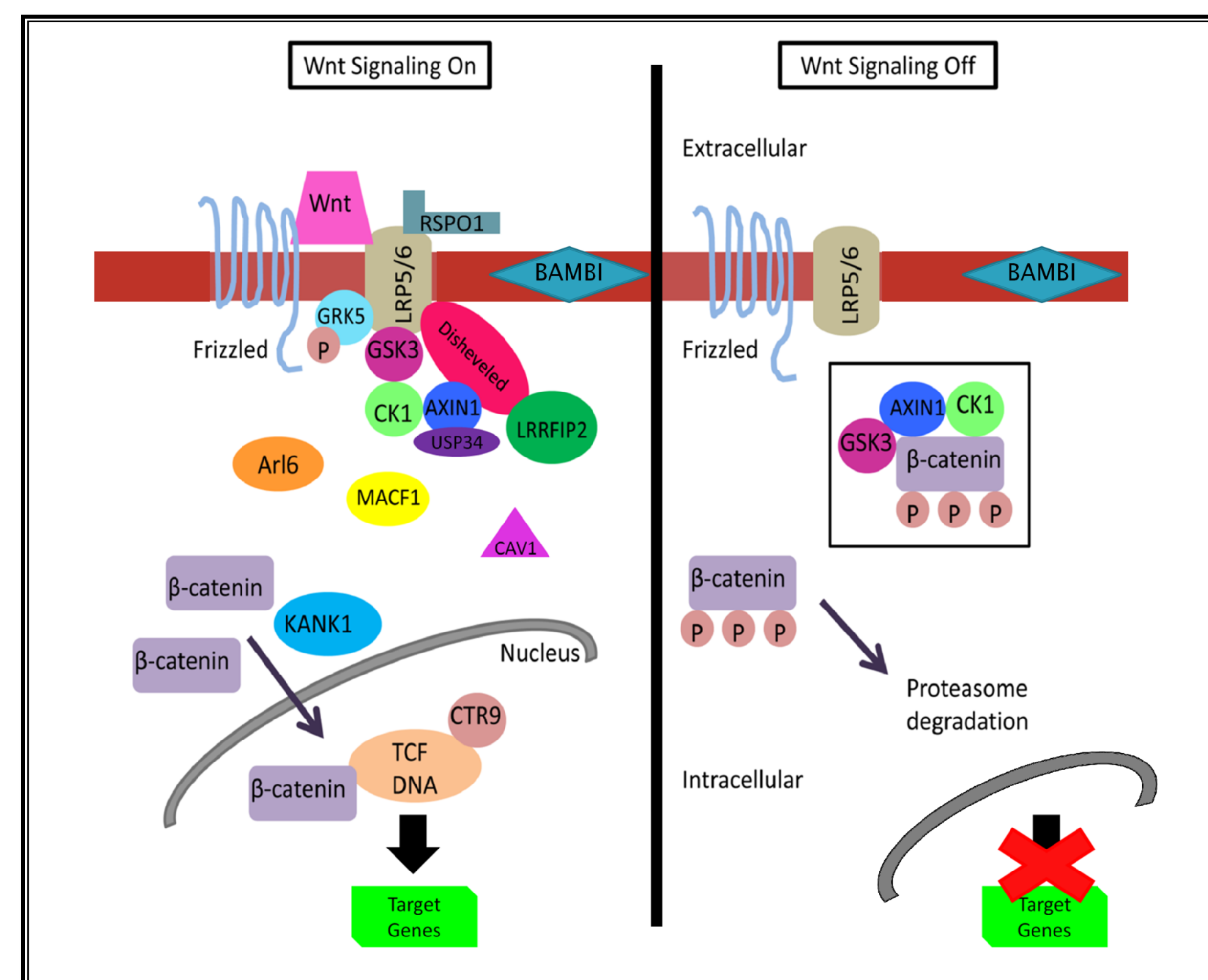


Gene Expression during Lens Regeneration

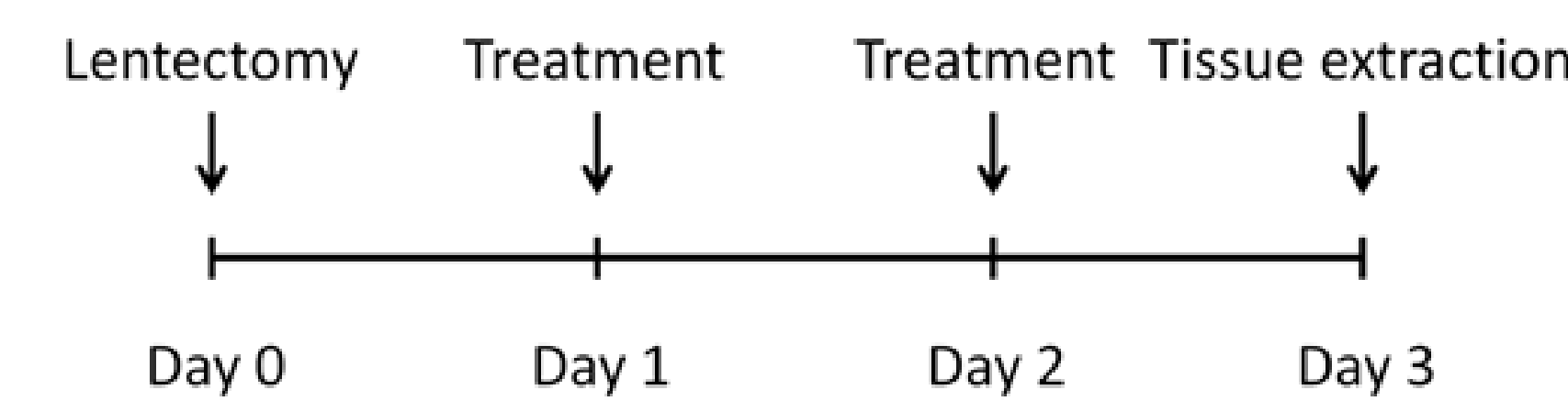


(Barbosa-Sabanero, K. et al, 2012)

The Wnt Signalling Pathway



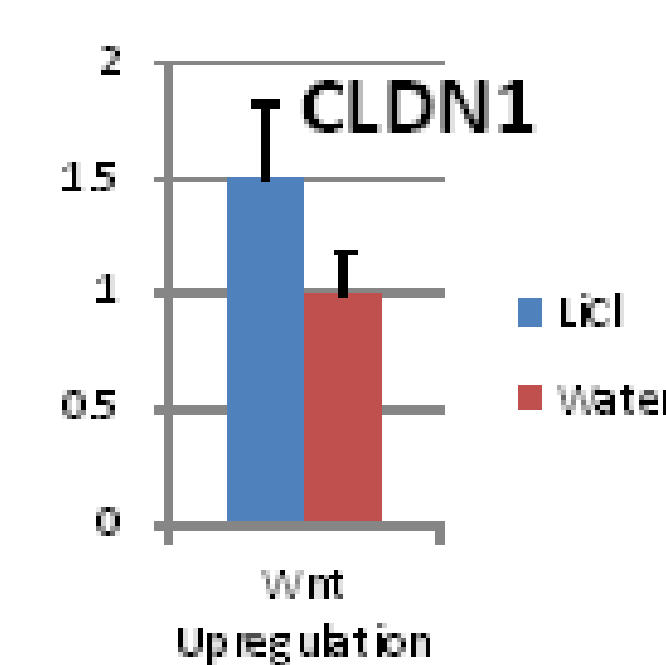
Method 1: qPCR



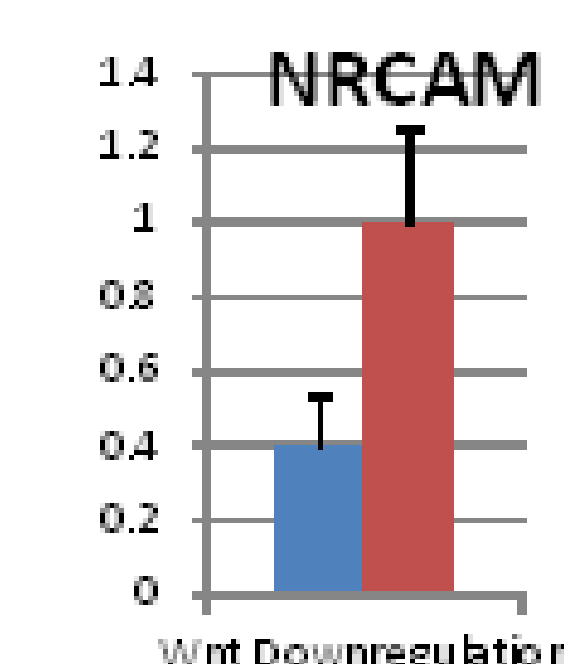
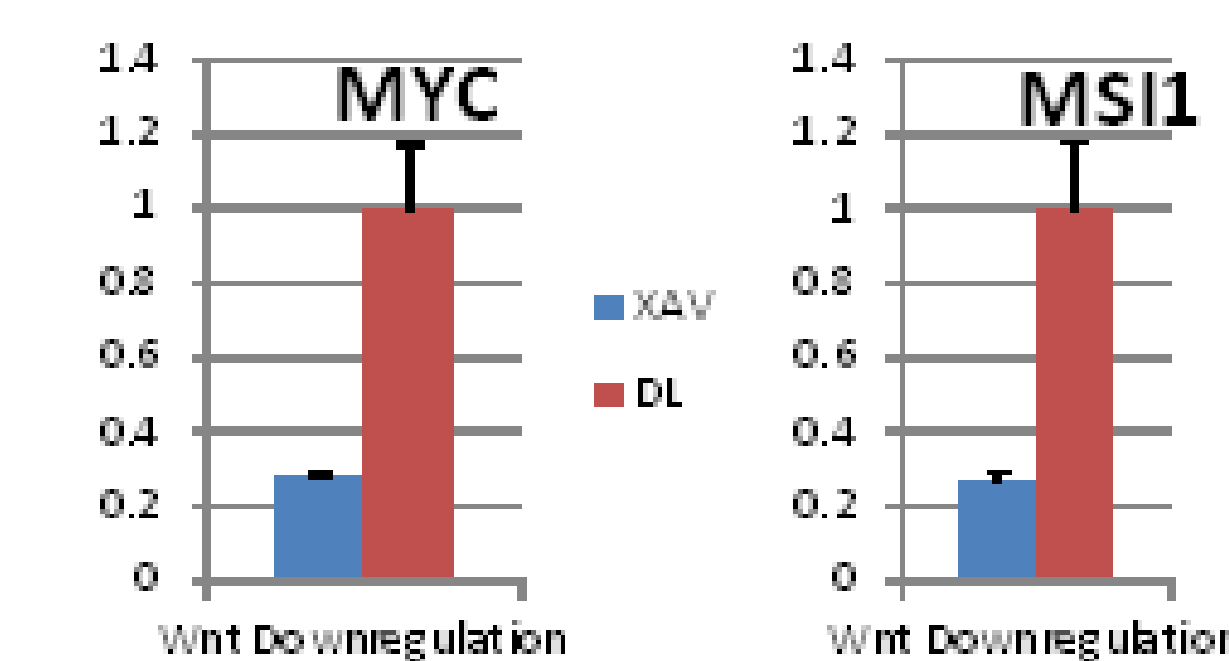
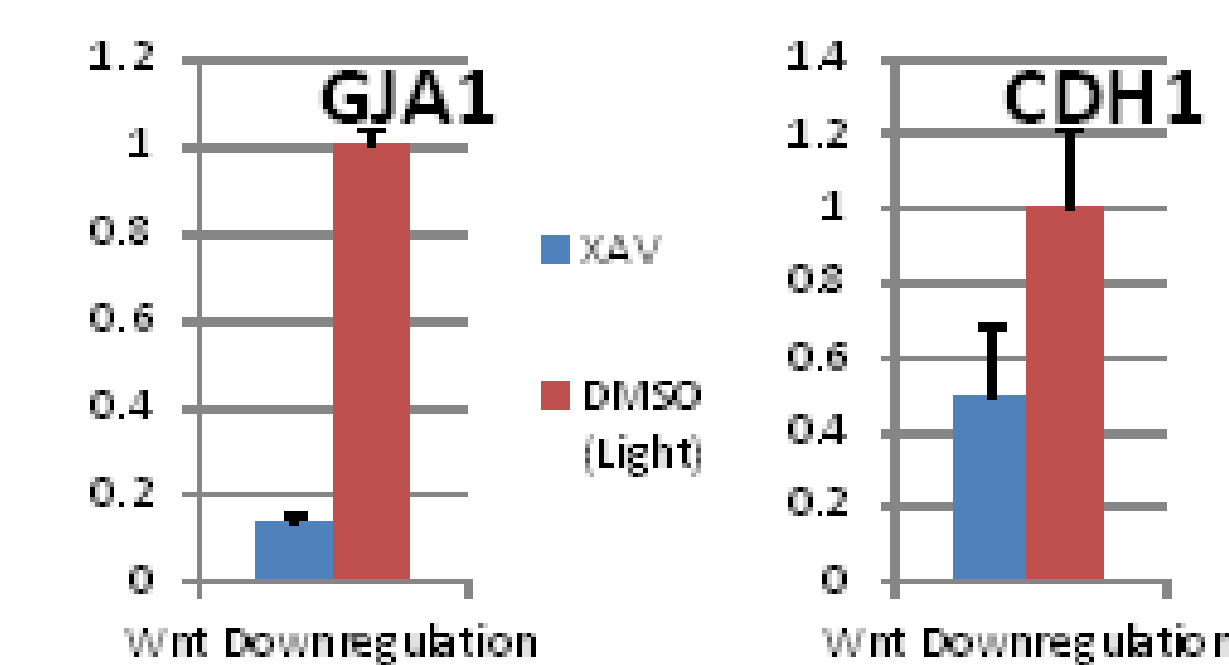
Axolotls per treatment: 4
Volume in box: 100mL

Reagent	Working concentration
LiCl	1mM
XAV939	10uM

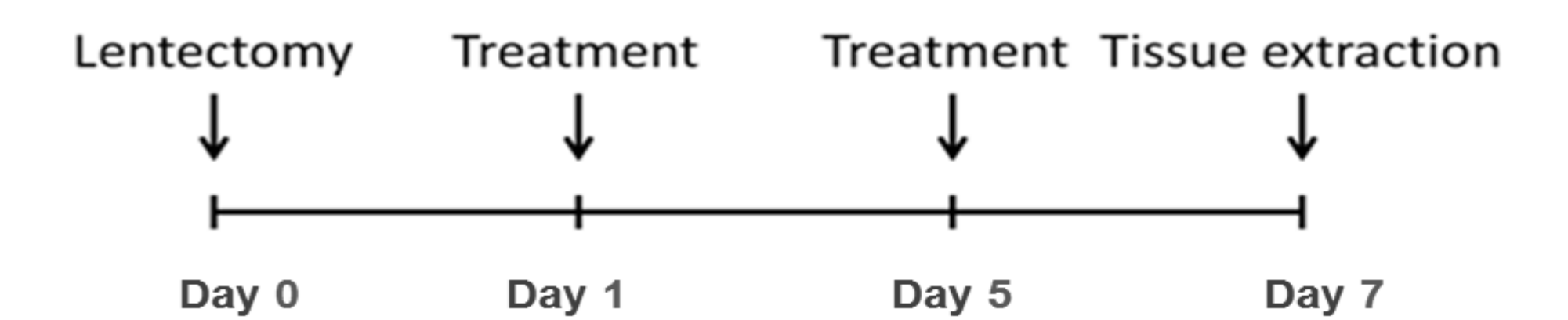
LiCl Activator



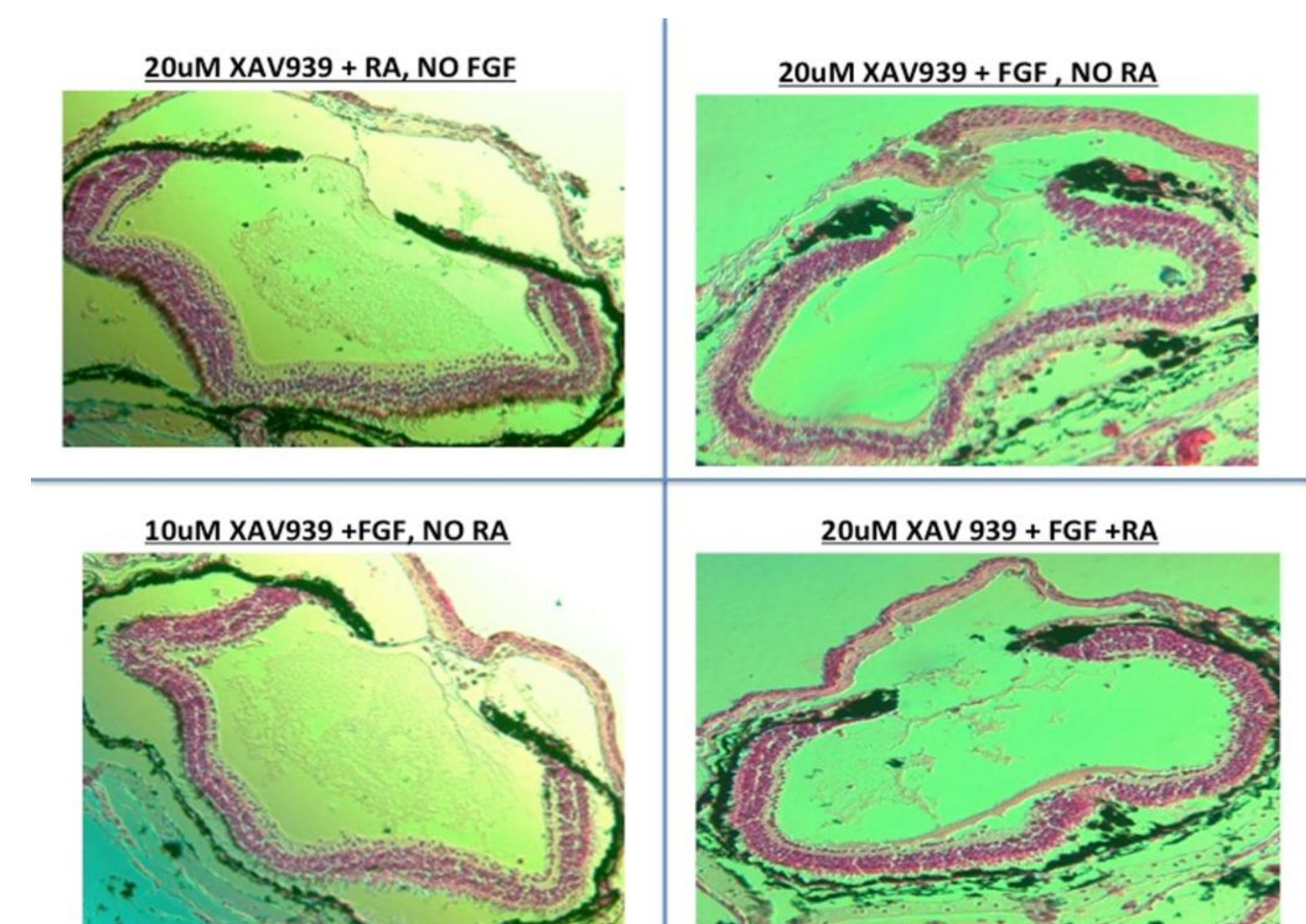
XAV939 Inhibitor



Method 2: Histology



Treatment Description	Axolotls started	Axolotls collected
20uM XAV939 + RA, NO FGF Wnt Inhibitor	5	3
20uM XAV939 + FGF, NO RA Wnt Inhibitor	5	4
10uM XAV939 + FGF, NO RA Wnt Inhibitor	1	1
20uM XAV 939 + FGF + RA Wnt Inhibitor	5	3
Only FGF	4	3
3M LiCl + FGF + RA	3	0
No Drugs, Control	1	1



Conclusions

Through various combinations of drug treatments, no lens was formed. Low doses of LiCl did not activate the Wnt Pathway and high concentrations of LiCl was potentially lethal to the axolotls. XAV939 was a prospective inhibitor, but failed to induce lens regeneration even in combination with FGF and retinoic acid. Therefore, we conclude that the process of regeneration is complex and is not regulated by one pathway alone. The Wnt Pathway may regulate regeneration in conjunction with other pathways. Understanding lens regeneration in the axolotls in relation to the Wnt pathway can provide useful information about the mechanism of regeneration. With this knowledge, potential applications of tissue regeneration could provide therapeutic techniques in higher vertebrates and eventually humans.

Acknowledgements

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