Exploring the function and expression of the *cyp26b1* gene during tooth development
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I studied the role of retinoic acid and Cyp26b-1 in tooth development. Focusing on the function and expression pattern of *cyp26b-1* is important in learning what factors contribute to the size and shape of teeth in different organisms. Retinoic acid is a secreted molecule that regulates the expression of numerous target genes that influence development. Retinoic acid signaling is required for tooth development, but too much retinoic acid can change the shape of teeth and inhibit calcification. The *cyp26b-1* gene codes for the protein Cyp26b1. This protein degrades retinoic acid as a normal part of the signaling process. My research focused on studying possible *cyp26b-1* enhancers, which are sequences of DNA that amplify and drive the transcription of the *cyp26b-1* gene.

The ultimate goal of the summer project I worked on was to find an enhancer for *cyp26b-1* in zebrafish that drives teeth expression and insert it into the white cloud mountain minnow. Zebrafish have shorter and wider teeth, while white cloud mountain minnows have longer and thinner teeth. Our hypothesis was that one reason to explain this difference is the timing of *cyp26b-1* gene expression. My research focused on determining whether three sequences in the zebrafish genome were enhancers for *cyp26b-1* in the teeth.

I collected embryos to study by setting up outcrosses between fish that had a Green Fluorescent protein (GFP) plasmid inserted into its genome and wildtype fish. The first step in determining if one of the sequences was a potential enhancer was looking for GFP expression in the embryos that came from these outcrosses. I found GFP expression in one of the lines I studied, meaning the regulatory sequence associated with this line had the potential to be an enhancer for *cyp26b-1* that drives teeth expression. To see if this enhancer drives gene expression in the teeth, I fixed embryos in formaldehyde and performed an antibody staining procedure.

To visualize the results of my antibody stain, I dissected the fish to remove excess tissue and used a specialized microscope to see cells that surround and make up the teeth. The antibody stains show nuclei of cells in blue, GFP in green, and calcium in red. Seeing GFP in cells provides evidence that these are cells that express *cyp26b-1* and are regulated by the potential enhancer I am studying.

I also performed polymerase chain reactions (PCR) and gel electrophoresis to see if the line of fish with GFP expression had the GFP plasmid inserted correctly. I designed primers, short sequences of nucleotides used in starting DNA synthesis, for my reaction so that they would attach to both sides of the plasmid insert. Because the DNA came from fish that had one copy of wildtype DNA and presumably one copy of DNA with the plasmid insert, I would expect to see two different sizes of DNA amplified. Gel electrophoresis allowed me to visualize these sizes because it uses electrical currents and pores in a gel to allow DNA samples to travel various lengths depending on the number of nucleotides in each sample.

The results of my antibody stain showed that there was no GFP expression in the teeth, providing evidence that this sequence does not drive *cyp26b-1* expression in the teeth. It is likely that this sequence in the genome is an enhancer in other regions since these fish expressed GFP in other places of the body. The results of my PCR and gel provide evidence that the GFP plasmid with this potential regulatory sequence was inserted incorrectly because there were only bands associated with the expected number of nucleotides of wildtype DNA. I further tested this by performing another PCR, using primers from inside the plasmid insert to see if I could detect bands, but found nothing. This could suggest that large deletions happened when the plasmid was inserted into the genome using CRISPR-Cas9.

Moving forward, I would continue to look for GFP expression in embryos from the outcrosses between the other two lines that had different potential enhancer sequences associated with them. I would perform the same set of steps above to decide if either of them could be enhancers. Once an enhancer for *cyp26b-1* gene expression in the teeth is found, we will be able to do more experiments to find out more about the gene's function and role in shaping teeth.

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