Effects of the Runx Gene Family on Tooth and Bone Development in Zebrafish

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Abstract:

The transcription factor Runx2 plays an important role in the skeletal development of vertebrates. Zebrafish ( ) possess two orthologs of the Runx2 gene, and . These two genes present slightly different expression patterns in developing zebrafish (Flores et al., 2003). The role of the Runx2 transcription factor has been studied in odontogenesis in mice (Camilleri et al., 2006), and here I aim to explore its role in zebrafish odontogenesis using green fluorescent protein (GFP) transgenic zebrafish. The CRISPR/Cas9 system mediated the insertion of GFP so that it was controlled by the promoter of the target gene, in my case either creating GFP knock-in transgenic fish I found that the two genes 0.00(o)4Io]T .42 Tm549.92 Tk3ifcThe objective of this project was to create and study stable 'reporting' mutant lines of the and genes. These fish could then be used to create homozygous mutants for each gene in addition to creating double mutants of both genes. It was hoped that studying these fish and their GFP expression patterns could help explain the role of each gene in tooth development.

Methods:

The CRISPR-injected fish were crossed with wildtype fish in crossing tanks. Embryos were observed under a fluorescence microscope to detect GFP expression. To visualize tooth development some embryos were fixed with formaldehyde. A GFP-HRP antibody was applied to the fixed embryos as well as TSA substrate to aid in visualizing the GFP.

sequence at various ages to compare stages of development. The embryos were stained in sequence twice throughout the work to catch various stages of formation of the larval dentition. In the first sequence the embryos were 58, 82, 104, and 122 hpf. In the second sequence the embryos were 61, 73, 104, and 120 hpf. In these sequences the GFP appeared in the dental mesenchyme of teeth early in their development, which was seen from 61 to 104 hpf, and possibly could be observed before and after this window. To know exactly when the gene turns on and off in developing tooth germs, this would have to be studied further. There was no expression observed in the dental mesenchyme by 120 hpf, but some expression was visible in the dental epithelium. Expression was also visible in the dental epithelium at 104 hpf in addition to the mesenchymal expression (Figure 2). The zebrafish tooth develops through an interaction between the dental mesenchyme and epithelium. The mesenchymal and epithelial cells work together in the formation of the tooth but play slightly different roles in the process (Verstraeten et al., 2010). Knowing whether is expressed in the mesenchyme, epithelium, or both is important in understanding its role in tooth development.

The PCR reactions visualized with gels showed that the plasmid had been inserted into the genomic DNA in the reverse orientation. Knowing the orientation of the GFP plasmid allows for the proper modeling of the genetic makeup of the transgenic fish, which in turn allows for the correct design of primers to locate the ends of the transgene. Using a variety of primers that flanked the insertion site, the 5' end was 'located' but th exact implications in tooth development. Creating a double mutant for would be an important next step in this work.

The same goes for the mutant, as the results of the apotome imagine are only significant if the plasmid was inserted to accurately replace the gene. The gels showed both ends of the plasmid insertion could be located, so it is with confidence that the GFP patterning correctly imitates where would be expressed.

Acknowledgments and References:

Works Cited

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