The Role of *pitx2* in Zebrafish Tooth Development  
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In the Jackman lab we study the molecular signals responsible for the early stages of tooth development. This summer I studied the role of the transcription factor *dlx2b* on zebrafish tooth development. Zebrafish teeth are a particularly useful means for examining the effects of molecular signals on development. Teeth, unlike many other organs, develop relatively independently of surrounding tissue, thus experimental manipulations can be observed and quantified. Zebrafish are a useful model organism because it is a vertebrate with a relatively short generation time that produces large clutches of transparent embryos. These characteristics are beneficial because as a vertebrate, findings can be related and applied to other vertebrates, such as humans, and external, quickly developing embryos allow for frequent experimental manipulations, close observation during all aspects of development, and frequent acquisition of results.

This project examines the role of the bicoid-like homeodomain gene *pitx2* in zebrafish tooth development. This gene is the earliest known transcription factor specifically expressed in the oral ectoderm and serves as an activator for the transcription factor *dlx2b* (Green, *et al.*, 2001), which is specifically expressed in zebrafish teeth. The function of *dlx2b* is of interest because loss of function experiments suggest that it plays a role necessary for tooth development. Knockdown of Dlx1 and Dlx2 in mouse hinders molar development (Thomas, 1997) and similar hindrance of tooth development was found in zebrafish. Knockdown of *dlx2b* alone had little effect on teeth, but as progressively more *dlx* genes were eliminated, teeth became smaller and misshapen (Jackman, 2006). These results suggest that *dlx* genes are necessary for tooth development. By illuminating the function of an upstream activator of *dlx2b* further information as to the genes necessary to initiate and continue tooth development can be revealed. Through my project I explored the role of the *dlx2b* activator, *pitx2*, through loss of function experiments.

The function of *pitx2* was inhibited by injection of one-cell staged embryos with a splice blocking morpholino. This chemical binds to *pitx2* mRNA and inhibits protein translation by not allowing modification of the pre-mRNA transcript. Assessment after *pitx2* knockdown through the use of Alizarin red and Alcian blue histological staining of teeth and cartilage and visualization with confocal microscopy revealed the expected number of teeth for the developmental stage but in asymmetrical patterns. Additionally, some fish exhibited both the expected number and orientation of teeth but the left/right plane of the pharyngeal region appeared shifted, as evidenced by the position of the notochord.

These results suggest that *pitx2* functions in early developmental patterning, contributing to the position and symmetry of the teeth and the pharyngeal region. Future experimentation includes co-injection of the *pitx2* morpholino and *pitx2* mRNA, which will attempt to rescue *pitx2* function. Additionally, the *pitx2* binding sites in the *dlx2b* regulatory region will be mutated to evaluate if *pitx2* expression is necessary for the activation of the transcription factor *dlx2b*. Preliminary results suggest that *pitx2* plays a role necessary for the proper development and orientation of zebrafish teeth, and subsequent experiments will seek to further illuminate *pitx2* function.

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References
