Identifying regulators of the zebrafish gene dlx2b during tooth development

Sarah Liu, 2013

Studying tooth development can provide insight into organogenesis and may eventually revolutionize human dentistry. Aspects of tooth development are regulated by genes participating in cell signaling pathways. However, little is known about the molecular signals that drive the earliest stages of tooth formation. To study tooth development, one excellent study species is the zebrafish (Danio rerio), due to the large quantities of optically clear, externally growing embryos it produces. As a vertebrate, its development shares many similarities with human development. Due to these similarities, insights into zebrafish organogenesis may be quite applicable to human development. Therefore, understanding zebrafish tooth morphogenesis may uncover homologous mechanisms in humans.

This summer, I studied the zebrafish gene dlx2b, which acts as a transcription factor and is one of many genes participating in the initiation and regulation of tooth development. Tooth development is initiated with the thickening of epithelial tissue, which forms a bud into the neural crest cell-derived mesenchyme beneath (Thesleff 2003). In zebrafish, this early structure, known as a tooth germ, exhibits discrete expression of the dlx2b gene, making this gene an effective marker of the tooth germ. Examining dlx2b genomic cis-regulation, which is controlled by other transcription factors, could provide greater understanding of the mechanisms of tooth development.

In order to discover more about the genes regulating dlx2b expression, the Jackman lab has been examining the cis-regulatory region of the dlx2b gene. Cis-regulatory regions typically contain putative binding sites for many different transcription factors. The Jackman lab has characterized the 5’ end of a cis-regulatory enhancer sufficient to drive dlx2b expression in developing teeth, but the 3’ extent of this enhancer is unknown. This summer I created GFP reporter constructs containing dlx2b cis-regulatory sequences with shortened 3’ ends. I injected these constructs into single-cell embryos, where the constructs were integrated into the genome. If the dlx2b cis-regulatory segment contained within the construct was sufficient to drive expression, the tooth cells fluoresced green when examined using confocal microscopy.

My results indicate that a construct containing 200 base pairs of the dlx2b cis-regulatory region from the 3’ end was not sufficient to drive GFP expression, but that a construct containing a sequence from 856 – 200 base pairs did drive GFP expression. While both of these constructs acted as controls in my study, I plan to use this data next year in my Honors project to continue trimming the 3’ end of the dlx2b cis-regulatory region to further narrow down possible transcription factor binding sites. Uncovering upstream regulators of dlx2b will provide insight into which genes direct when and where teeth form during development. Ultimately, this knowledge could be used in human dentistry to regrow damaged or diseased teeth.

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