Characterising the genomic regulation of \textit{dlx2b} during zebrafish tooth development

Hana Littleford, 2012

The Jackman lab investigates the molecular signals that control development of zebrafish teeth. Teeth are a convenient subject of study because they develop independently of the surrounding tissues, and the large number and transparency of zebrafish embryos make them an ideal model organism. Zebrafish are also unusual in that they have lost all oral teeth through evolution and retain teeth only in their pharynx, unlike their close relatives. My project focused on \textit{dlx2b}, a transcription factor, or gene which turns on other genes, which is essential for forming proper teeth (Thomas et al 1997). Unlike other genes expressed in the developing zebrafish teeth, \textit{dlx2b} expression is restricted to the pharyngeal region. \textit{dlx2b} is also one of the first genes expressed in the tooth region during development, so elucidation of its regulation could lead to further understanding of the entire tooth development process.

My work investigated two potential sources of \textit{dlx2b} regulation. The first of my two projects involved attempting to locate and characterise the \textit{dlx2b} genomic enhancer, the element responsible for turning on \textit{dlx2b}, which previous work by the Jackman lab has proved is located within 2 kilobases (kb) of the 5’ end of \textit{dlx2b}. I ligated portions of this 2kb region to the green fluorescent protein (GFP) gene and injected this construct into single-celled embryos, where it was integrated into the genome. If the portions of the 2kb region contained the enhancer, the cells expressed GFP and fluoresced green. Further visualisation was done using confocal microscopy, which allowed me to confirm that the cells were expressing GFP in the teeth in a pattern consistent with that of \textit{dlx2b} expression and proving that the enhancer was contained in the specific area of the 2kb region used in my injections. My results from this study indicated that the genomic enhancer is within 1.5kb of \textit{dlx2b}.

Within this 2kb regulatory region are putative binding sites for several transcription factors, including NF-kB, which has been shown to regulate molar cusp development in mice (Ohazama et al 2004). For my second project, I mutated the putative NF-kB binding site, making it impossible for NF-kB to interact with the \textit{dlx2b} regulatory region. During my Honors project next year I am planning to inject single-celled embryos with this mutated DNA to see whether the absence of NF-kB interactions cause any change in the expression of \textit{dlx2b}. I am also planning to use a drug which modifies NF-kB molecules and prevents them from binding to DNA, to investigate if a global lack of NF-kB interactions during development leads to any change in tooth number, shape, or placement, and will continue to locate the \textit{dlx2b} enhancer.

\textbf{Faculty Mentor: William Jackman}

\textbf{Funded by the INBRE summer fellowship}


Thomas, Bethan L; Tucker, Abigail S; Qiu, Mensheng; Ferguson, Christine A; Hardcastle, Zoe; Rubenstein, John LR; Sharpe, Paul T. (1997). Role of \textit{dlx-1} and \textit{dlx-2} genes in patterning of the murine dentition. \textit{Development.} 124, 4811-4818.