Role of *dlx* genes in the development of the zebrafish pituitary gland

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Introduction

In vertebrates, *dlx* genes play an important role in the development of the forebrain. Zebrafish are an excellent model organism in developmental biology in part because of their transparency, small size and rapid growth. Researchers in the Ekker lab are working on characterizing the function of *dlx* genes in zebrafish through lineage tracing analyses to observe the migration and differentiation of cells that express these transcription factors.

Our genetic labeling technique uses double-transgenic zebrafish containing Cre-ER^{T2} recombinase under the control of *dlx* enhancers and a Ubi switch construct. When induced with tamoxifen, the Cre-ER^{T2} recombinase removes the GFP sequence in cells expressing *dlx* genes at the time of the induction. This results in the expression of mCherry, switching fluorescence from green to red (Figure 1).

In these lineage tracing analyses, we observed cells expressing mCherry in the pituitary gland of adult fish that were induced early in embryonic development. Expression of *dlx* genes in the pituitary has never been reported. The purpose of this research project is to characterize the role of *dlx-1a/2a* and *dlx-5a/6a* genes in the development of the pituitary gland.

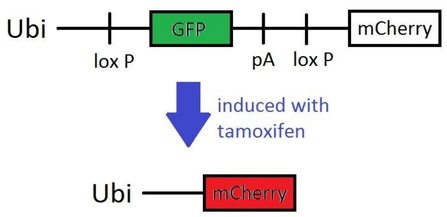

Figure 1: The effect of induction of zebrafish with tamoxifen on the Ubi switch loxP-GFP-loxP-mCherry. All cells initially express GFP. Once induced with tamoxifen, the Cre-ER^{T2} recombinase removes everything between the lox P sites. Therefore, those cells will express mCherry under the control of the Ubiquitin promoter after being exposed to tamoxifen.

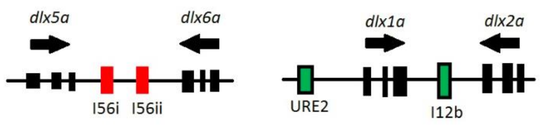

Figure 2: Genetic organization of *dlx* genes. Bigene clusters are separated by intergenic regions containing conserved enhancers (red and green boxes). The enhancers drive reporter gene expression in largely overlapping developmental domains (Solek).

Methodology

1. Double transgenic fish were obtained by crossing fish expressing the Cre-ER^{T2} recombinase under the control of *dlx* enhancers with fish containing a Ubi switch loxP-GFP-loxP-mCherry construct.
2. Embryos were screened 24 hours post fertilization (hpf) for expression of GFP under the control of the Ubi promoter.
3. Fish were induced with tamoxifen 24 hours post fertilization (Mosimann et al., 2011).
4. Embryos that expressed mCherry were selected.
5. After 14 to 45 days post fertilization, the fish were fixed in 4% paraformaldehyde and sectioned using a cryostat (Macdonald, 1999).
6. Fluorescent immunohistochemistry was carried out on sections (Macdonald, 1999) to label mCherry expressing cells and co-localize them with GABA and calretinin.
7. Samples were imaged using a Zeiss Axiophot fluorescent microscope.

Results

- Cells that expressed mCherry were observed in the anterior portion of the pituitary.
- An overlap was observed between the mCherry staining and calretinin staining in the pituitary.
- I did not observe an overlap between the mCherry staining and GABA (not shown).

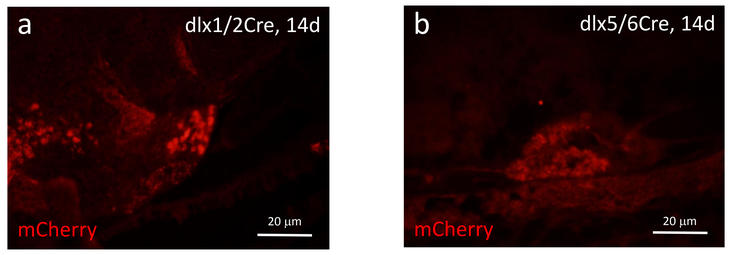

Figure 3: Detection of mCherry in anterior pituitary of double transgenic zebrafish. Sagittal sections were taken of 14dpf fish induced with tamoxifen at 24hpf. (a) mCherry staining of *dlx1a/2a*Cre double transgenics. (b) mCherry staining of *dlx5a/6a*Cre double transgenics.

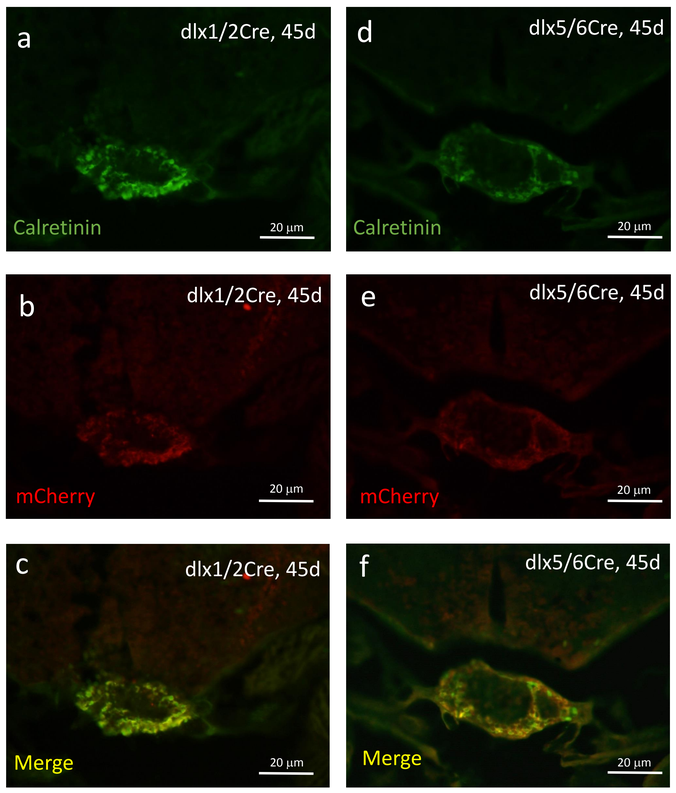

Figure 4: Detection of mCherry and calretinin in the anterior pituitary of double transgenic zebrafish. Transverse sections of 45dpf fish from *dlx1a/2aCre* fish (a-c) and *dlx5a/6aCre* fish (d-f) were used for double fluorescent immunohisto-chemistry. Calretinin staining (a, d), mCherry staining (b, e) and merge of both staining pictures (c, f) show co-expression of the calcium binding protein in the mCherry-positive cells of the pituitary.

Conclusion

- The expression of mCherry in the anterior pituitary indicates that *dlx* genes are expressed at 24 hpf in the anterior pituitary progenitor cells. This implies that *dlx* genes are involved in the development of the anterior pituitary at that time.
- I attempted fluorescent *in situ* hybridization (FISH) of 24hpf and 48hpf embryo sections of the olfactory placodes to label pituitary development markers, *Pit-1* and *lim3*. However, the experiment failed.
- The colocalization of mCherry and calretinin, a protein involved in calcium signaling, indicates that calretinin is present in those cells. The significance of this is not yet clear, although it is possible that it plays a role in hormone regulation (Jadhao & Malz, 2007).

Future Directions

- Use immunohistochemistry on the double-transgenic zebrafish to test for co-expression of mCherry with pituitary hormones such as growth hormone (GH) and luteinizing hormone (LH). This would provide further evidence that *dlx* genes are involved in the development of the pituitary.
- Repeat fluorescent *in situ* hybridization (FISH) of 24hpf or 48hpf embryo sections of the olfactory placodes to label pituitary development markers, *Pit-1* and *lim3*.
- Understand the significance of calretinin being present in pituitary cells that arose from cells that had active *dlx* genes at 24 hpf.

References

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