Expression of mRNA of the *col2a1b* gene during Zebrafish Embryogenesis

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Abstract

Cartilage is an important tissue for all vertebrate organisms; it allows for flexibility yet structure in the extracellular matrix, which translates to support across nearly all parts of the body. Most cartilage elements are composed of two major cell types: chondrocytes and perichondrial cells. The perichondrium plays an important role in signaling to chondrocytes during the conversion of cartilage to bone (ossification). Mutations in genes/proteins expressed in these cell types can lead to morbidities such as osteoarthritis, Czech dysplasia, & Langer-Saldino achondroplasia, among others.

Our lab is interested in characterizing the genetic regulation of the critical structural genes that allow for the proper formation of cartilage. We set out to characterize the expression of *col2a1b*, the zebrafish ortholog of the human *COL2A1*. In zebrafish, the *col2a1a* gene (homolog of *col2a1b*) has been shown to be expressed in both chondrocytes and perichondrial cells, our preliminary work suggests *col2a1b* is only expressed in the perichondrium of cartilage elements; thus, the protein product of *col2a1b* is a type II alpha 1 collagen chain, which promotes cartilage development and ossification of chondrocytes. Our lab is interested in understanding the differential expression of the two homologs. In this report, we document the complete spatiotemporal expression of the zebrafish *col2a1b* mRNA during the first 5 days of development via *in situ* hybridization.

Danio rerio

Figure 1: Representative example of our model organism, the zebrafish – *Danio rerio.*

In Situ Hybridization (ISH) Mechanism

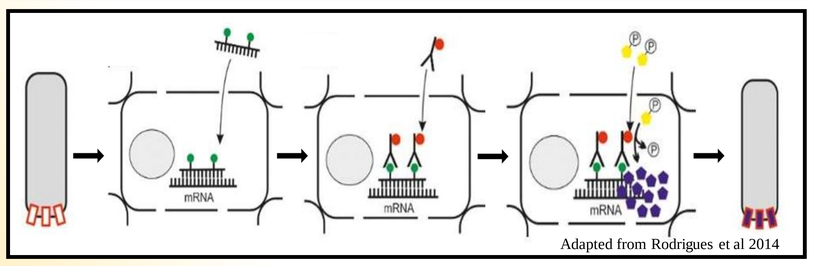
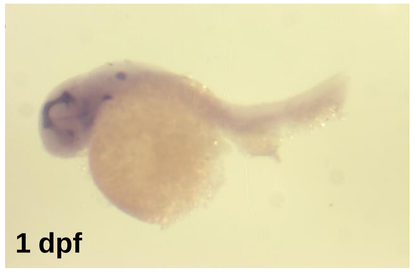


Figure 2: Schematic of the *In Situ* Hybridization technique.

In situ hybridization (ISH) is a technique which allows for the sensitive and specific localization of mRNA fragments within a model organism, ours being the zebrafish. ISH relies on the principles of Watson-Crick nucleic acid complementarity, with hybridization ideally occurring between the endogenous mRNA strand and our lab-engineered, enzyme-conjugated, complementary ribonucleotide probe. *In situ* hybridization is an excellent method to use when attempting to characterize the spatiotemporal expression of genes critical to an organism's successful embryogenesis.

In Situ Hybridization of mRNA of *col2a1b*

Sense



Antisense

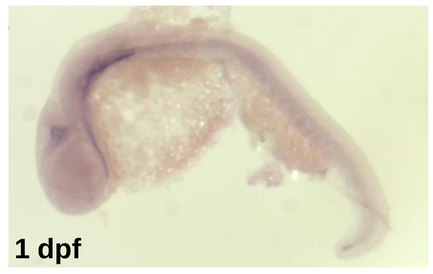


Figure 4: Expression of *col2a1b* during days 1, 2, 3, 4, 5 post-fertilization.

Post-ISH, Light & Fluorescent Microscopy

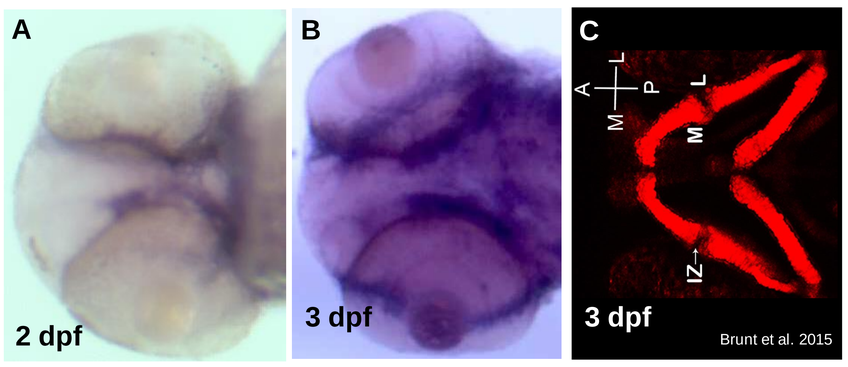


Figure 5: Ventral view of expression during day 2 & 3 post fertilization.

A. Two-day **B.** Three-day old zebrafish embryo showing craniofacial cartilage. **C.** Location of cartilage elements at 3dpf fish. Modified from Brunt, *et al*. 2016.

Gel Electrophoresis of RT-PCR Product

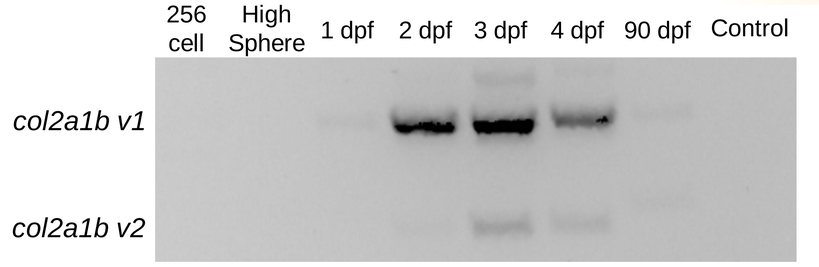


Figure 3: RT-PCR of *col2a1b mRNA* extracted during development.

Results

In situ hybridization provided a baseline to compare endogenous gene expression to regulation elements reporter plasmids. From this we can determine the region in the genome responsible for *col2a1b* perichondrial expression. We have seen variable results after performing multiple *in situ* hybridizations, so we have made adjustments to ensure more consistent results in the future.

Future Directions

1. Cryosections of whole-mount zebrafish will be performed to determine more specific tissues of staining.
2. Reverse Transcription Polymerase Chain Reaction (RT-PCR) will be performed on all stages from 4-cell to 5 dpf.
3. Establish a system to allow for the repopulation of genetic lines.

Acknowledgments

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