Cartilage is an important tissue for all vertebrate organisms because it allows for flexibility and structure to many 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. There are multiple congenital defects and diseases, such as osteoarthritis and achondroplasia, that disrupt these two cell types during development and aging.

Our lab is interested in better understanding the genetic regulation of critical structural genes that allow for the proper formation of cartilage. In this work, we set out to characterize the expression of col2a1b, one of two orthologs that are more similar to the human COL2A1 gene. While in zebrafish, the paralog col2a1a has been shown to be expressed in both chondrocytes and perichondrial cells, our preliminary work suggests col2a1b seems to only be expressed in the perichondrium of cartilage elements. This makes it a Type II Collagen that promotes cartilage development that then leads to bone development in chondrocytes. Our lab is interested in understanding this differential expression of the two paralogs. In this report, we document the complete spatiotemporal expression pattern of the zebrafish gene col2a1b during the first 5 days of development using an RNA probe and in situ hybridization.

**Figure 1:** Representative example of our Model Organism – *Danio rerio.*

**In Situ Hybridization**

In *In Situ* Hybridization (ISH) is a technique that allows for accurate localization of mRNA segments that code for a specific protein within the zebrafish. This is done by hybridizing the complementary strand of a labeled ribonucleotide probe to the mRNA of interest. *In Situ* Hybridization is an excellent method in describing temporal and spatial expression patterns in genes that are developmentally regulated.

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

In *In Situ* Hybridization (ISH) is a technique that allows for accurate localization of mRNA segments that code for a specific protein within the zebrafish. This is done by hybridizing the complementary strand of a labeled ribonucleotide probe to the mRNA of interest. *In Situ* Hybridization is an excellent method in describing temporal and spatial expression patterns in genes that are developmentally regulated.

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

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.

Special thanks to Dr. Rodney M. Dale for being our mentor throughout our research at Loyola University Chicago. And special thanks to the other members of the Dale Lab. This research project was funded by the Mulcahy Scholars Program.

**REFERENCES**