2015

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Cloning of the SOX2 gene from *Diplosoma listerianum*

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**Background**

The SOX2 gene is an important transcription factor (transcription factors help promote the expression of genes) in embryonic stem cells. SOX2 is present in many organisms (including humans) and is highly conserved, meaning the sequence is very similar in different species. It is essential for pluripotency in stem cells, which is the ability of a stem cell to differentiate into different tissue types. From my own past research, I know that the SOX2 gene is expressed in Diplosoma, but the role that it plays in this organism has not been previously studied. Our hypothesis is that this gene is important for Diplosoma's asexual development.

**Introduction**

Diplosoma listerianum is an invasive tunicate species that can reproduce asexually by pyloric budding. Our research primarily focuses on the genetic basis for the asexual development in this organism. From past research I have found that Diplosoma listerianum expresses a gene that codes for the SOX2 transcription factor, and successfully sequenced a portion of this gene. The end goal of this project is to determine where gene expression occurs in the organism. If we find that gene expression occurs in the bud tissue, we will provide evidence for the fact that this gene is involved in asexual development in Diplosoma. One way we can do this is to use a method known as in situ hybridization to localize where gene expression occurs.

**Methods**

**Overall Goal**

The goal of in situ hybridization is to find where in a fixed tissue sample a gene is being expressed. This is done by creating a labeled complementary RNA probe that will hybridize to a specific mRNA sequence within the organism. If our hypothesis is correct, our probe will hybridize to the mRNA sequence in the budding tissue of Diplosoma. The first step in this process was to insert the SOX2 gene in a plasmid vector, in order to definitively determine the sequence of the fragment.

**Results from Past Research**

My past research involved testing to see which developmental genes are expressed in Diplosoma. In order to do this, I extracted messenger RNA from tunicates, Diplosoma listerianum, and used complementary DNA (cDNA), which is composed of different primers (primers are sequences that initiate DNA synthesis) to create copies of specific genes that code for development in other species, to see if these genes are also present in *D. listerianum*. Using a method known as RT-PCR, I used these primers to make many copies of the gene, and then sequenced the entire length of the nucleotides that make up the gene. From doing this, I found that the gene encoding the SOX2 transcription factor is expressed in Diplosoma, and determined the sequence of the fragment of the gene. Then I searched for this sequence using the program BLAST, which I could compare my sequence tags (represented sequence tags) of previously sequenced genomes of other organisms. The program indicated that the sequence from our PCR product is most closely similar to genes in the SOX family. Our sequence is highly similar to the sequence that codes for SOX21 in Ciona intestinalis, another tunicate species.

**In Situ Hybridization Determines Where A Gene Is Expressed in a Fixed Tissue Sample**

In order to determine the role SOX2 plays in Diplosoma, we must look at where gene expression occurs in the organism. If the gene is important for asexual development, it will most likely be expressed in the bud tissue. In order to determine where gene expression occurs, we can use a method known as in situ hybridization.

**Results from Sequencing**

I sequenced the recombinant plasmid DNA from isolates grown on different ampicillin plates. Three of the colonies were grown on one plate, and the other three were grown on three plates. From each of the plates, I obtained two distinct sequences for the colonies grown on the same plate. The first sequence is the ampicillin resistance gene, which now contains the entire length of the gene of interest. Since the sequence is most closely similar to SOX2, we can use it as a reference sequence to find where gene expression occurs. Thus, I can use this sequence to design a primer that will allow us to clone and sequence a larger fragment of the gene.

**Data and Results**

**Table 1: Top Blast Results**

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**Figure 1:** Diagram of the Diplosoma listerianum organism showing the asexual development.

**Figure 2:** Close up of *D. listerianum* showing the pyrlosic bud.

**Figure 3:** Whole mount in situ hybridization in a fixed chick embryo. The antisense probe hybridizes with the mRNA in the embryo, causing a dark precipitate to form where gene expression occurs.

**Figure 4:** Comparison of the Diplosoma SOX2 sequences to that of Ciona intestinalis. An 85% match was found between the two sequences. (Source: ANZEESE Database)

**Figure 5:** Alignment of SOXB1 sequence in Diplosoma compared to its location in Ciona intestinalis (Source: ANZEESE Database)

**Figure 6:** Ligation of the PCR product into the plasmid vector.

**Figure 7:** Transformation of the recombinant plasmid into E. Coli cell.

**Figure 8:** Whole mount in situ hybridization in a fixed chick embryo. The antisense probe hybridizes with the mRNA in the embryo, causing a dark precipitate to form where gene expression occurs.

**Figure 9:** The graph above shows the top blast results for each of the nucleotide sequences, and compares the translated proteins to the closest results in humans and another tunicate species, Ciona intestinalis. (Sources: ANZEESE database)

**The Next Steps:**

Future research can focus on whether other genes that are essential for stem cell pluripotency are also important for asexual reproduction in Diplosoma. Examples of such genes would include other SOX2 genes, Oct genes, and VASA genes.