Cloning of the SOX2 gene from Diplosoma listerianum

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Cloning the SOX2 gene from Diplosoma listerianum

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Background

Asexual Development in the tunicate species Diplosoma listerianum

Tunicates, more commonly known as "sea squirts" or ascidians, are filter feeding marine invertebrates. D. listerianum is a colonial ascidian, meaning that thousands of individual animals live together in a colony, surrounded by a tough outer matrix, and can reproduce sexually as well as asexually through a process known as pyrlic budding. In this process, the esophagus of a single animal forms two buds that can each develop into a whole new animal, allowing for very rapid reproduction. Pyrlic budding is a unique feature of a few tunicate species including D. listerianum, where our research is to learn more about the genes that encode this organism's asexual development.

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 specific mRNA sequence within the organism. If our hypothesis is correct, our probe will hybridize to an 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 only code for specific complementary DNA (cDNA), which is extracted. Using this technique, primers are selected that invoke DNA synthesis to create copies of specific genes that code for development in other species, as well as these genes are also present in D. listerianum. Using a method known as "polymerase chain reaction", PCR, I used these primers to make many copies of the genes, and then sequenced them to determine the order of the nucleotides that make up the gene. From this, I found that the gene encoding the SOX2 transcript factor is expressed in Diplosoma, and determined the sequence of the gene by thermocycling the PCR products which I then compared to a database of previously sequenced genes of other organisms. The program indicated that the sequence from our PCR product was most closely similar to genes in the SOX family. Our sequence is highly similar to the sequence that codes for SOX2 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 animal. If the gene is important for asexual development, it will most likely be expressed in the budding tissue. In order to determine where gene expression occurs, we can use a method known as "in situ hybridization".

Introduction

Diplosoma listerianum is an invasive tunicate species that can reproduce asexually by pyrlic 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 budding 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. This project accomplished the beginning steps of this process, allowing for future research to determine where SOX2 gene expression occurs in Diplosoma.

Cloning the SOX2 gene for the in situ hybridization

Since the fragment of the SOX2 gene was too short to sequence in only 200 basepairs in length, the first step in this project is to sequence a large enough portion of the gene to create the probe for the in situ hybridization (this method typically requires the probe to be at least 2000 basepairs in length). In order to do this, I needed to get a more exact sequence for the fragment I managed to clone. Therefore, I inserted the gene into a cloning vector. This resulted in a recombinant plasmid, which can be used as a "biological" probe. Since the probe must be complementary to the mRNA, the probe can then be labeled and added to our tissue sample to hybridize with the specific mRNA sequence.

Data and Results

Results from Sequencing

I sequenced the recombinant cloned DNA fragment in order to gain a more defined amino acid sequence. The fragment was then processed to create two separate aliquots of the sequence to be sequenced the next day. We found evidence that the sequence of the SOX2 gene is more closely related but have different functions. Thus, I may be able to create two different probes, and determine a larger fragment of the sequence of two different genes within the SOX1 family.

Sequence 1 Top Blast Results:

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The Next Steps:

The next step in this project is to carry out the rest of the in situ experiment by creating the antisense RNA probe that will hybridize to the mRNA in Diplosoma adults that have been fixed in formalin.

Data and Results

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Explanation:

When DNA is translated to make a protein, it must first be transcribed into what is referred to as "messenger" RNA or mRNA. This messenger RNA is then processed and taken to a cell organelle known as a ribosome, where the ribosome reads the sequence of the mRNA and makes a protein. The mRNA for a particular gene will only be present in cells where that gene is expressed. Thus, in order to determine if a gene is being expressed in an organism we can make a probe that will hybridize to that specific mRNA sequence. This probe must be complementary to the mRNA. The probe can then be labeled and added to our tunicate sample to induce hybridization with the specific mRNA sequence. If a hybridization to mRNA in the budding tissue, this may be evidence that SOX2 may play role in the asexual development of this organism.

Future research:

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 SOX family genes, Otx genes, and VASA genes.

Acknowledgements

This project was supported by a URI Council for Research Proposal Development Grant. I would also like to thank the Coastal Fellows program at the University of Rhode Island.