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Analysis of HNF1-beta as a Possible Regulatory Protein for the Transcription of the *Col11a2* Collagen Gene

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Cartilage is a highly specialized form of connective tissue that functions as a template for most skeletal structures and aids in supporting the tension and pressure common to most animal joint function. Abnormal cartilage formation often results from defective collagen DNA and can lead to several skeletal diseases including dwarfism, cleft palate and osteoarthritis. The purpose of this research was to investigate the regulatory role of HNF proteins in the transcription of the *Col11a2* gene which codes for a collagen protein found in cartilage.

Type XI collagen is a minor, though necessary, component of the extracellular matrix found in articular cartilage. Three different protein subunits wind together into a heterotrimer triple helix to form the collagen XI molecule. The *Col11a2* codes for the production of the second of these protein subunits which is known as the alpha-2 subunit.

Dr. Laura Bridgewater studies the mechanisms that regulate how the transcription of the *Col11a2* gene is activated in cartilage. In her research she has identified three regions of DNA within the gene that are essential for transcription. These regions, termed enhancers, bind regulatory proteins that help make up the transcriptional machinery. The enhancers have been given the names B/C, D/E, and F/G.

Further studies in Dr. Bridgewater's lab have identified specific sequences within the three enhancer regions of the gene that bind proteins known as transcription factors and make transcription possible. As some of the DNA sequences that regulate *Col11a2* transcription have become identified, the next logical progression in the research was to identify the transcription factors that bind those DNA sequences. This was the project that was generously funded by an ORCA scholarship and is what I have been working on in Dr. Bridgewater's laboratory this year.

Using an internet database containing known transcription factors, it was found that a protein known as HNF3 binds an exact sequence of DNA found in the B/C enhancer element. HNF is a family of proteins that were first discovered to act as transcription factors in liver and intestinal cells. Consequently, it was determined likely that members of the HNF family could function as transcriptional activators in collagen molecular biology and that research should be performed in relation to the *Col11a2* transcription studied in our lab.

In order to further pursue the hypothesis that members of the HNF acted as transcriptional activators for the *Col11a2* gene two proteins known as HNF-1alpha and HNF-1beta were selected for testing. The DNA for these two proteins was obtained in expression plasmid form through the generosity of Dr. T.C. Simon from St. Louis, Missouri.

The technique that was selected to be used to determine whether HNF-1alpha and HNF-1beta actually activated transcription from the *Col11a2* enhancers was that of a series of transient

transfections in a chondrocytic cell line called RCS. Plasmids had been constructed that contained a luciferase reporter gene under the control of each of the three *Col11a2* enhancer elements. The HNF expression plasmids were than cotransfected in various combinations with each of the reporter plasmids to determine whether the proteins made by HNF expression plasmids were capable of binding to and activating the *Col11a2* enhancers. The reporter gene activity was assayed in a Turner Luminometer while using a cotransfected Beta galactosidase gene as an internal control.

Initial attempts to perform these experimental transient transfections were met with some difficulty as the RCS cell line died several times due to faulty ventilation in the experimental hoods used in the laboratory. Furthermore, the delicate technique involved in the transfection of microliter amounts of plasmid DNA naturally lent itself to some experimental error. Consequently, much effort was employed in attempting to correct the unknown problems that were causing the experiments to fail. The initial intent of the project to test both HNF-1alpha and HNF-1beta with each enhancer element was quickly evaluated to be too ambitious and overly time consuming. Therefore, the project was divided in half and given to another student in the lab. I was left with attempting to determine the regulatory properties of only the HNF-1beta protein.

Eventually, the inhibitory problems of the project were corrected as ventilation malfunctions were repaired and laboratory technique was refined. The result proved to be data that could be trusted and results that began to paint a picture of the role of HNF-1beta in collagen gene transcription. Contrary to what was expected, results suggest that HNF-1beta represses rather than activates transcription at both the B/C and D/E enhancers. This supports the current molecular biology theory that multiple proteins interact with each other to influence transcription. In this case, it appears that HNF-1beta downregulates transcription while another hereto unidentified protein causes the activational increases identified in the research previously accomplished by Dr. Bridgewater. Results concerning the F/G enhancer's relation with HNF1-beta are still a matter of investigation.

In the future my research will continue to attempt to elucidate the full role of the HNF transcription family in relation with to the *Col11a2* gene. In addition, research will continue to be directed at identifying the protein or group of proteins that account for the large activation of transcription found by Dr. Bridgewater's previous research. Regardless, data from such research contributes to the overall understanding of collagen gene regulation which could in turn lead eventually to new genetic treatments to correct the debilitating effects of cartilage deformation.