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BBSI Summer 2004 Research Proposal

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Identifying Splice Variants in the Treacher-Collins gene: TCOF1

Introduction

Alternative splicing of RNA transcripts is a method by which many different protein products are coded for by the same gene. It is predicted that approximately 50% of genes are alternatively spliced in humans (Brett et al, 2001). Alternative splicing often is a result of an omission or addition of an exon. Alternative splice products can be identified by expressed sequence tag (EST) analysis. One gene which undergoes alternative splicing is *TCOF1*. *TCOF1* is found on the long arm of chromosome 5 and codes for a protein called treacle, mutations in treacle (usually resulting in premature stop codons) are the cause of the craniofacial disorder called Treacher-Collins syndrome (Gladwin et al, 1996). Treacher-Collins is an autosomal dominant disorder which affects approximately 1 in 50,000 live births. The protein treacle has been shown to be a nucleolar phosphoprotein and has close structural similarities to the nucleolar phosphoprotein Nopp140 (Isaac et al, 2000 et al, 2000; Winokur and Shiang, 1998). Nucleolar phosphoproteins aid in the transport of proteins or ribosomal subunits between the cytoplasm and the nucleolus, thus they likely have a large role in ribosome construction.

The *TCOF1* gene has known orthologs in canine, bovine, mouse, and rhesus monkey genomes (unpublished). Currently little is known about the various structural

similarities and differences between the human *TCOF1* gene and its orthologs. This study will seek to analyze and compare the various splice variants that have been identified in the different organisms. This will help provide us with an idea of what the similarities and differences are in the function of the treacle protein between the species as well as information on how well the gene has been evolutionarily conserved between the species. After identifying the different splice variants and the differences between the orthologs, we can analyze the differential expression of the genes in the lab to find the role of treacle throughout the development process and hopefully help shed some light on the role that treacle plays on craniofacial development.

Materials and Methods

This summer I hope to continue the research I have been doing with characterizing the *TCOF1* orthologs in the monkey, mouse, and canine and comparing them with the *TCOF1* gene in the human. I have, to date, isolated and sequenced many splice variants of both the monkey and the mouse. This summer I hope to isolate and sequence splice variants for the canine. Then, if an exon is found in these sequences I can use it to fill in a complete gene map of each species. When completed I will study the differential expression of the splice variants of *TCOF1* during development by using whole-mount *in situ* hybridization of mouse embryos.

Results and Expectations

Through analysis of the finished sequences I have already found that some of the exons previously thought to be unexpressed are differentially expressed in the organism. Hopefully further analysis of the sequence and isolation and sequencing of more splice variants this summer will help to form a fully characterized analysis and comparison of

the TCOF1 gene across these four species. Using whole-mount *in situ* hybridization will help us learn how the *TCOF1* gene is expressed throughout development and may help to shed light on what point in development the mutated protein which causes Treacher-Collins syndrome is expressed.

Works Cited

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