Sequence Variation of the Enzyme IDH in Isolate Strains of E. coli

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Introduction

Over the school year of 2007/2008 I will be working with E. coli. My mentor at Davidson College has his microbiology class isolate their own E. coli samples via rectal swabs. Within the E. coli genome the gene *icd* encodes for the enzyme isocitrate dehydrogenase (IDH) that participates in the citric acid cycle. IDH is an important housekeeping gene and is highly conserved across and among all E. coli species. Fu-Sheng *et al* have found that sometimes a lambdoid phage 21 or a defective prophage element may be integrated into the 3' end of the *icd* gene. This integration introduces an alternative terminal segment adjacent to the segment of the *icd* gene that encodes the *icd* transcriptional terminator. I would like to investigate sequence variation between the aforementioned E. coil isolates to see if they contain the phage 21, prophage, or other insertions. This will enable me to better understand the structure and function of the *icd* gene on a genomic level.

This summer I have been working with the genetic syndrome Treacher Collins. TCOF1 has been identified as the gene behind Treacher Collins syndrome. The coding region of this gene has been mapped. However, there still exist patients who have Treacher Collins syndrome but do not have any mutations in the coding region. Therefore, we are looking to the promoter region for mutations that could cause changes in expressivity. I have been injecting Tcof1 promoter regions that are tagged with GFP into mouse embryos. We were able to find that the presence of an ATG site greatly

increased transcription. We then used the Tcof1 promoter region construct with the ATG site and preformed serial deletions to investigate the effects of different protein binding sites on expression. A similar experiment had been performed using a mouse cell line. We were able to find similar results to the mouse cell line experiments and therefore bolster our beliefs about the roles that each protein-binding site plays in expression.

Goals

Although my plans for the school year and my research over the past summer seem to have no relation, I strongly feel that one will help me in the other. Over the school year I will learn the tools necessary for performing more in depth investigations into Tcof1. Over this coming school year I will be taking both a bioinformatics course and a genomics course. Because I work in a primarily wet lab setting, I feel that my new skills and research will allow me to bring another perspective and set of possibilities into the study of Tcof1 and zebrafish.

Academic Year

In order to study the *icd* gene I will have to use a number of different techniques. To isolate the *icd* gene I will use PCR. I will then be able to clone the *icd* gene enabling me to sequence the gene. I will most likely use the dideoxynucleotide chain termination method to sequence a single stranded version of my cloned DNA. I will then use both bioinformatics and genomics tools to analyze sequence variations between the isolates.

References

Wang, F. S., T. S. Whittam, and R. K. Selander. "Evolutionary Genetics of the Isocitrate Dehydrogenase Gene (*icd*) in Escherichia Coli and Salmonella Enterica." Journal

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