Mutagenesis at Amino Acid Phosphorylation sites of the Human ERH protein

Markaylia Grant
The College at Brockport, mgran4@u.brockport.edu

Follow this and additional works at: https://digitalcommons.brockport.edu/research_posters

Part of the Biology Commons, and the Genetics and Genomics Commons

Recommended Citation
Grant, Markaylia, "Mutagenesis at Amino Acid Phosphorylation sites of the Human ERH protein" (2018). Posters@Research Events. 15.
https://digitalcommons.brockport.edu/research_posters/15

This Book is brought to you for free and open access by Digital Commons @Brockport. It has been accepted for inclusion in Posters@Research Events by an authorized administrator of Digital Commons @Brockport. For more information, please contact kmyers@brockport.edu.
INTRODUCTION

The enhancer of rudimentary, e(r), is a gene present in many organisms that encodes for the protein, enhancer of rudimentary homolog, ERH. This protein has functions in pyrimidine biosynthesis and the cell cycle. In human cancer cells, the abundance of ERH is great in comparison to that of normal cells. It is theorized that ERH may play a role in cancer progression during the cell cycle. The overall purpose of this research is to determine the effect of phosphorylation of amino acids within the human ERH protein on the activation of the protein. Drosophila melanogaster, the common fruit fly will be used as the model organism for this project due to the 76% amino-acid identity between Drosophila and human ERH, and the fact that human ERH is functionally active in Drosophila.

RESEARCH GOAL

The goal of this project is to create seven mutant clones of human ERH. The mutant genes tested are S2A, S24A, T51A, S55A, S47A S49A, Y19F Y22F, and T18A Y19F Y22F. These plasmids will then be sent to the company, BestGene Inc. (thebestgene.com) for insertion into the genome of the human ERH. The mutant genes tested are S2A, S24A, T51A, S55A, S47A S49A, Y19F Y22F, and T18A Y19F Y22F. These plasmids will then be sent to the company, BestGene Inc. (thebestgene.com) for insertion into the genome of D. melanogaster.

METHODOLOGY

The 104 amino acid sequence of the human ERH protein was studied and amino acids with the ability to be phosphorylated were selected to be tested. Amino-acid substitutions were designed for these amino acids. These mutations were synthesized by IDT DNA.com. An initial PciI-NcoI restriction enzyme digest isolated the mutant coding region. This fragment was ligated into the vector, pSMART empty e(r), Escherichia coli cells were transformed with this recombinant plasmid to increase the number of clones with the mutant plasmid. An EcoRI-Xhol digest was performed to retrieve the entire mutant e(r) gene, which was then ligated into the vector, pattB. This process was repeated for all the genes tested.

The vector, pattB, containing the 3.0-kb mutant human ERH coding region empty e(r) containing the 0.3-kb e(r) mutant ERH coding region was ligated into the vector, pattB. This plasmid has an attachment site called “attB” which allows for the insertion of the plasmid into the Drosophila genome via site-specific recombination.

EXPERIMENTAL

The remaining mutation, S2A, will be cloned into pattB. The flies that have picked up the mutant plasmids will be observed and studied for the ability of the mutant proteins to rescue mutant e(r) phenotypes.

ANALYSIS

The flies that have picked up the mutant plasmids will be observed and studied for the ability of the mutant proteins to rescue mutant e(r) phenotypes.

ACKNOWLEDGEMENTS

I would like to thank my mentor, Dr. Stuart Tsubota, for all his help with this project, the Research Foundation. I would also like to thank the Ronald E. McNair Program who funded this project and staff who guided me.

REFERENCES


