Mutagenesis at Amino Acid Phosphorylation sites of the Human *ERH* Protein

Markaylia Grant and Stuart Tsubota, Ph.D.
The Department of Biological Sciences

The College at BROCKPORT
STATE UNIVERSITY OF NEW YORK

TRIO
RONALD E. MCNAIR POST-BACCALAUREATE ACHIEVEMENT PROGRAM

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 mutant genes will be inserted into a transformation plasmid, pattB. 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 IDTDNA.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-XhoI 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.

EXPERIMENTAL

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                                1       10        20        30        40        50        60        70        80        90       100
                                .        .        .         .         .         .         .         .         .         .         .
D. melanogaster wild-type:      MSHTILLVQPGARPETRTYCDYESVNECMEGVCKIYEEHLKRRNPNTPTITYDISQLFDFIDTMVDISCMVYQKSTNTYAPYNKDWIKEKIYVLLRQAAFSSNT
          Human wild-type:      MSHTILLVQPTKRPEGRTYADYESVNECMEGVCKMYEEHLKRMNPNSPSITYDISQLFDFIDDLADLSCLVYRADTQTYQPYNKDWIKEKIYVLLRQAQQAGK

Phosphorylation Sites:
S2A:                            A
S24A:                                     A
T51A:                                                 A
S55A:                                                    A
S47A S49A:                                            A A
Y19F Y22F:                            F   F
T18A Y19A Y22F:                      AF   F
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Figure 1. The 104 amino acid sequence of the human and Drosophila ERH with amino acids with the ability to be phosphorylated highlighted in blue and the amino acid replacement underneath highlighted in red. The mutant amino acids are similar in shape and size to the wild-type amino acids, however they cannot be phosphorylated.

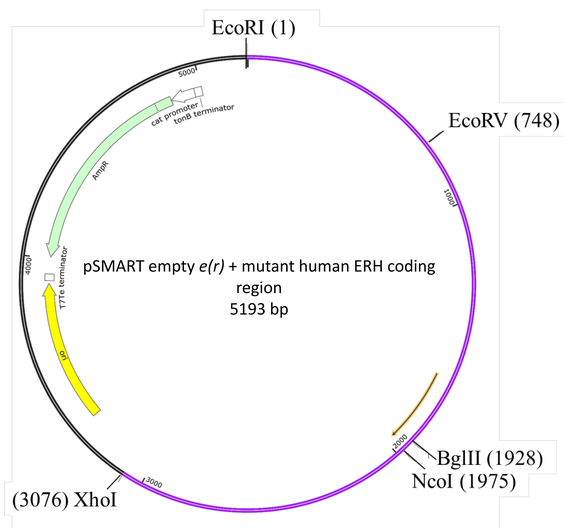

Figure 2. The vector, pSMART empty *e(r),* containing the 0.3-kb *e(r)* mutant ERH coding region empty *e(r)* containing the 0.3-kb *e(r)* mutant ERH coding region

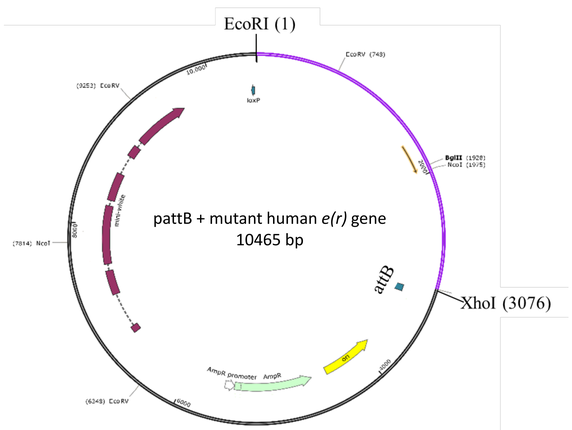

Figure 3. The vector, pattB, containing the 3.0-kb mutant human *e(r)* gene from the pSMART plasmid. This plasmid has an attachment site called "attB" which allows for the insertion of the plasmid into the Drosophila genome via site-specific recombination.

ANALYSIS

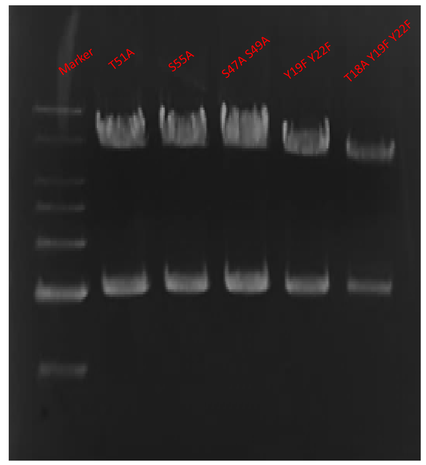

Figure 4. Currently, 6 of the 7 mutations, S24A, T51A, S55A, S47A S49A, Y19F Y22F, and T18A Y19F Y22F are successfully cloned into the pattB plasmid, evident by means of gel electrophoresis which produced a 3.0-kb band representing the desired fragment, and a 7.4-kb band representing the pattB vector.

FUTURE WORK

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.

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