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The Effects of Warming Temperatures on Rad51 Expression in Tetrahymena thermophila

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Experiment Type: Reverse transcriptase (RT) PCR
Feature: TTHERM_00142330
Gene: Rad51: DNA repair protein RAD51 containing protein; strand exchange protein; involved in homologous recombination and repair of double-stranded DNA breaks

1 Introduction

Our experiment explored whether raised temperatures affected expression of the Rad51 gene in Tetrahymena thermophila. Our hypothesis was that raised temperatures would result in a decrease in expression of the Rad51 gene. The protein product of this gene functions in double stranded DNA repair. We chose this experiment to see if the rising average temperatures around the world would affect expression of genes involved in DNA repair. Our test samples were kept in a growth medium at 37C while our control samples were kept in a growth medium at 24C. Afterwards, the transcribed mRNA was reverse transcribed into DNA and primers were used to amplify the Rad51 gene. Semi-quantitative PCR was then used to analyze expression of the Rad51 gene. Our prediction was that higher temperatures would decrease the expression of the Rad51 gene.

2 Methods

Primer synthesis: Primers for Rad51 were designed using the Tetrahymena Genome Database and Integrated DNA Technologies Oligoanalyzer. The sequences of the forward and reverse primers used to amplify Rad51 are as follows: TGGTGGTATATTATGGCTCACGC (Forward) and CGTATTTATTTGGCTCACACTTTTGTGTATT (Reverse). The sequences of the control Btu1 primers are as follows: CCCAGAGCTATCTTGATGGACTTA (Forward) and TAACACCAGACATGGCAGCA (Reverse).

Culturing Tetrahymena: T. thermophila were cultured in NEFF media and then transferred into nutrient-rich SPP media at the time of the experiment (Cassidy-Handley, 2012). For the experiment, cultures were randomly separated into a control group and an experimental group. Experimental cultures were placed in a 37C incubator and control cultures in a room temperature incubator for the course of one week.

RNA extraction: RNA was extracted from control and experimental T. thermophila cultures following the experiment using Qiagens RNeasy Mini Kit as per the manufacturer's instructions. All cultures were centrifuged for three minutes at 3,000rpm and washed with 5mL of 10mM Tris (pH 7.4) before processing.

RT-PCR: cDNA was synthesized using the RevertAid RT kit (ThermoScientific) and following the manufacturer's protocol. PCR was performed using GoTaq Green PCR Master Mix (Promega) using the manufacturer's protocol. PCR amplification of BTU1 cDNA was used as a positive control for the Tetrahymena RT-PCRs while Gapdh was used as a positive control for the entire RT-PCR experiment as the reagents for this sample were provided in the RevertAid kit. The No Template Control (NTC) reactions contained all reagents for the RT-PCR reaction, except nuclease-free water was added in place of RNA template.

Gel electrophoresis: The RT-PCR reactions were electrophoresed on a 1% agaorse gel (BioRad) in 1xTBE (VWR). The ImageJ gel analysis program was used to determine the relative intensities of the PCR products for semi-quantitative analysis.

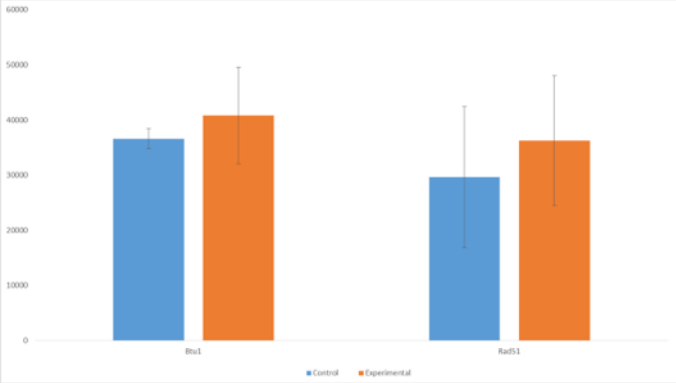
3 Results

The semi-quantitative RT-PCR results demonstrate that exposure of Tetrahymena cells 37C course of one week had no significant effect on the expression of Rad51 compared to control cells, as indicated in Figure 1 ($p = 0.40$). There was also no significant change in expression of Btu1 ($p = 0.63$).

Future studies should focus on varying the temperatures and times of the Tetrahymena cultures, as well as increasing the number of replicates. Furthermore, a more quantitative measure of gene expression should be used to better determine whether increased temperatures affect Rad51 expression.

4 Figures

4.1 Relative Expression of Rad51 in 37C-exposed Tetrahymena thermophila Cultures



The relative expression of RadD51 compared to the control gene Btu1 was measured using semi-quantitative RT-PCR across four control and four experimental (37C-exposed) Tetrahymena cultures over the course of one week. The error bars represent the standard error of the means for each condition. A two-tailed t-test assuming unequal variance was performed to determine the significance of changes in gene expression between the control and experimental cultures, with p=0.40 for Rad51 and p=0.63 for Btu1.

5 References

Cassidy-Hanley DM. Tetrahymena in the laboratory: strain resources, methods for culture,maintenance, and storage. Methods Cell Biol. 2012;109: 237-76. doi: 10.1016/B978-0-12-385967-9.00008-6.

6 Acknowledgements

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