The Effect of Exposure to UV Light 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

In this experiment, we attempted to answer the question: Does exposure to UV light affect expressionof the Rad51 gene in Tetrahymena thermophila? It was hypothesized that the expression of Rad51 would increase proportionately to the exposure of UV light. The Rad51 protein is fundamental in the repair of double-stranded DNA breaks in eukaryotes, specifically during the process of homologous recombination.

In order to test this hypothesis, Tetrahymena were randomly assigned to either a control group, which was cultured under ideal conditions, or a treatment group, which was exposed to UV light. Previous experiments have suggested that T. thermophila prosper most in environments out of direct light and at room temperature. The experimental group was exposed to incremental periods of UV light over the course of 26 hours. Immediately following the culturing and exposure, RNA extraction, reverse transcription, and gene-specific PCR was conducted on the samples from both the control and treatment groups to measure Rad51 expression. Because UV light would likely cause DNA damage and Rad51 plays a role in the repair of such damage, it was predicted that the Rad51 gene would be up-regulated in those organisms exposed to UV light.

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. The experimental group received ten minutes of UV light exposure every hour over the course of ten hours, for a total of ten exposures. The control group was placed in the same environment as the experimental groups, however they were not exposed to the UV light.

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.

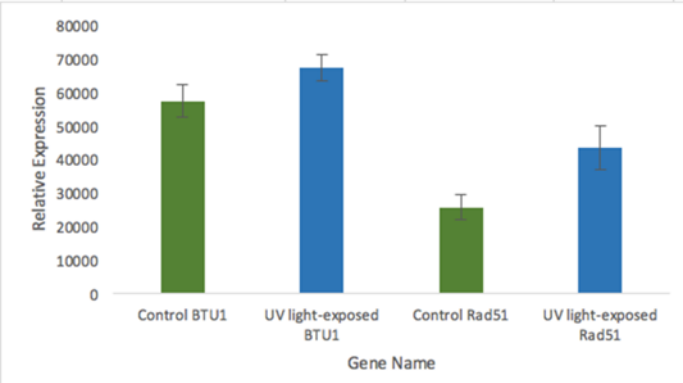
3 Results

The semi-quantitative RT-PCR results demonstrate that exposure of Tetrahymena cells to UV light over the course of 10 hours had no significant effect on the expression of Rad51 compared to control cells, as indicated in Figure 1 ($p = 0.06$).

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

4 Figures

4.1 Relative Expression of Rad51 in UV light-exposed Tetrahymena thermophila Cultures



The relative expression of Rad51 compared to the control gene BTU1 was measured using semi-quantitative RT-PCR across four control and four experimental (UV-light exposed) Tetrahymena cultures after 10 hours. 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=0.06 for Rad51 and p=0.85 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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