Analysis of FBP1 Gene Expression in UV Light-exposed Tetrahymena thermophila Cultures

Colby Henry and Stefanie Otto-Hitt, PhD
Carroll College

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Experiment Type: Reverse transcriptase (RT) PCR
Feature: UV-B Radiation
Gene: FBP1: Fructose 1,6 bisphosphatase family protein

1 Introduction

In this study, Tetrahymena thermophila cultures were exposed to an acute treatment of UV light and expression of the FBP1 gene was examined. The FBP1 gene encodes Fructose-1,6-biphosphatase which plays an important role in the process of gluconeogenesis. It was hypothesized that exposure of Tetrahymena to UV light would cause an upregulation in DNA repair enzymes, thereby causing these cells to require higher levels of glucose. The higher demand for glucose would then result in the upregulation of gluconeogenesis and FBP1.

Following acute exposure of Tetrahymena cultures to UV light, the expression of FBP1 was analyzed using reverse transcription and semi-quantitative PCR. It was predicted that the levels of FBP1 would be upregulated following acute UV light treatment.

2 Methods

Primer synthesis: Primers for FBP1 were designed using the Tetrahymena Genome Database and Integrated DNA Technologies Oligoanalyzer. The sequences of the forward and reverse primers used to amplify FBP1 are as follows: TGGTTCCATGGTTGCTGATGT (Forward) and CTAGCAAGCTGTTTTGGTAGGCTT (Reverse). The sequences of the control Btu1 primers are as follows: CCCAGAGCTATCTTGATGGACTTA (Forward) and TAACACCAGACATGGCA (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, Tetrahymena cultures were maintained in SSP media and the experimental group was exposed to two minutes of UV-B light five times over the course of 24 hours.

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.

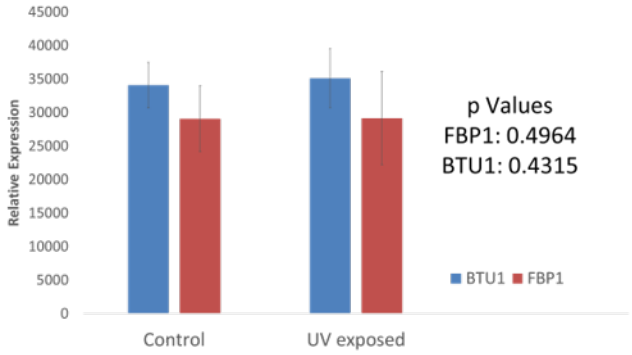
3 Results

The semi-quantitative RT-PCR results demonstrate that exposure of Tetrahymena cells to UV-B radiation over the course of 24 hours had no significant effect on the expression of FBP1 compared to control cells, as indicated in Figure 1 ($p = 0.4964$).

Future studies should focus on varying the exposure of the Tetrahymena cultures to UV-B radiation, as well as increasing the number of replicates. The expression of different genes involved in the DNA repair pathway, specifically those that are involved in repairing damage caused by UV-light exposure, should also be explored. Furthermore, a more quantitative measure of gene expression should be used to better determine whether exposure to UV-B light affects FBP1 expression.

4 Figures

4.1 Relative Expression of FBP1 in UV-B exposed Tetrahymena thermophila Cultures



The relative expression of FBP1 compared to the control gene BTU1 was measured using semi-quantitative RT-PCR across four control and four experimental (UV-B exposed) Tetrahymena cultures after one week of culturing. 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=0.4964 for FBP1 and p=0.4315 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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