

The Effect of Exposure to Ionizing Radiation on Cwf16 Expression in *Tetrahymena thermophila*

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Experiment Type: Reverse transcriptase (RT) PCR
Feature: UV-C Radiation
Gene: Cwf16: Cell cycle control protein

1 Introduction

The following research seeks to determine whether exposure to ultraviolet radiation will induce an increase in the expression of the Cwf16 gene in *Tetrahymena thermophila*. Cwf16 is a homolog of Ccgc94, whose gene product is a functioning member of the Prp19 complex, a protein complex which protects cells from committing apoptosisprogrammed cell death due to exposure to ionizing radiation. The homology between Cwf16 and Ccgc94 yields the hypothesis that Cwf16 expression in *T. thermophila* will increase in response to UV radiation exposure in a similar mechanism to that observed the Ccgc94 system.

In the present research, *T. thermophila* cultures were randomly assigned into an experimental group and a negative control group, in which the experimental group was subject to incremental exposures to ultraviolet radiation over the course of one week. RT-PCR and gel electrophoresis were used to measure the level of expression of Cwf16 and a hemocytometer was used to measure the growth rate of the control and experimental cultures.

Despite the homology between Cwf16 and Ccdc94, we predict that UV-radiation exposure will not raise *T. thermophilas* expression of Cwf16, because homology does not guarantee that the two genes products serve the same function. The Prp19 complex (the protein complex where we observe Ccdc94s expression) has not been observed in *T. thermophila*, either because researchers have not looked for it, or because *T. thermophila* do not have the Prp19 complex in its biochemical repertoire.

2 Methods

Primer synthesis: Primers for Cwf16 were designed using the Tetrahymena Genome Database and Integrated DNA Technologies Oligoanalyzer. The sequences of the forward and reverse primers used to amplify Cwf16 are as follows: AGTTCGTTGCAACACTTGTGGAAATTATC (Forward) and AGAACTTCTTCAAGCCTTTTCGTTATCACGC (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, Tetrahymena cultures were maintained in SSP media and the experimental group was exposed to UV-C light for 5 minutes each day for a total of seven days.

RNA extraction: RNA was extracted from control and experimental *T. thermophila* cultures following the weeklong incubation using Qiagen 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% agarose 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.

Growth Rate: The growth rate of control and experimental Tetrahymena cultures was determined by counting cells with a hemocytometer (Bright-line (TM), Sigma) over the course of the week-long experiment. In preparation for counting, cells were first treated with a 5% solution of glutaraldehyde (Sigma). More specifically, 190uL of cell culture was mixed with 10uL of 5% glutaraldehyde.

3 Results

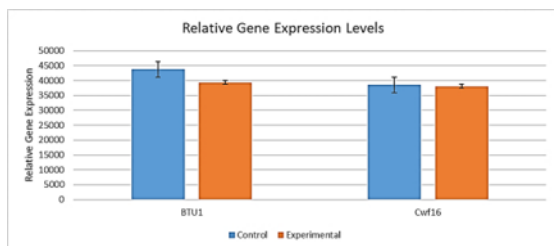
The semi-quantitative RT-PCR results demonstrate that exposure of Tetrahymena cells to UV-C radiation over the course of one week had no significant effect on the expression of Cwf16 compared to control cells, as indicated in Figure 1 ($p = 0.86$). The growth curve analysis showed that the level of UV-C exposure in this experiment did not affect

the rates of proliferation between the control and UV-C treated cultures. Collectively, these results support our hypothesis regarding UV-C exposure and its effects on Cwf16 expression; however, they do not support our hypothesis regarding UV-C exposure and its effects on cell proliferation.

Future studies should focus on varying the exposure of the Tetrahymena cultures to UV-C 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-C light affects Cwf16 expression.

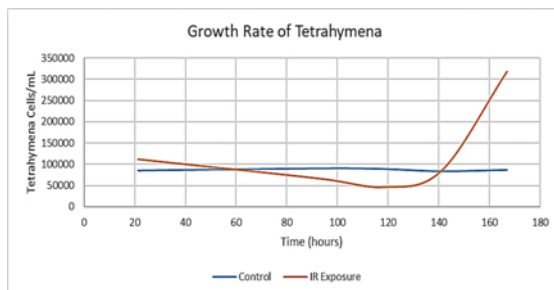
4 Figures

4.1 Relative Expression of Cwf16 in UV-C exposed Tetrahymena thermophila Cultures



The relative expression of Cwf16 compared to the control gene BTU1 was measured using semi-quantitative RT-PCR across four control and four experimental (UV-C 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 $p=0.86$ for CWF16 and $p=0.38$ for BTU1).

4.2 Growth Rate of UV-C exposed Tetrahymena Cultures



The growth rate across four control and four experimental (UV-C exposed) Tetrahymena cultures was measured over the course of the weeklong experiment.

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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