

Exploring the Effects of Environmental Temperature on CDC-7 Expression in *T. thermophila*

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Experiment Type: Reverse transcriptase (RT) PCR

Feature: TTHERM.00729170

Gene: CDC7: Protein kinase domain containing protein. Sequence similarity to the CDC7 protein kinase family (Eisen et al., 2006).”

1 Introduction

Tetrahymena thermophila are unicellular eukaryotes with a remarkably large genome: over 27,000 genes. One important aspect of studying the *Tetrahymena* genome is identifying and understanding what factors influence gene expression. This study examined the effects of temperature on the expression of CDC-7, a cell division control gene. We subjected *Tetrahymena* cultures to increased temperature and hypothesized such treatments would induce cell division and increase CDC-7 expression. Gene expression levels were measured via semi-quantitative PCR analysis after an acute heat treatment.

2 Methods

Primer synthesis: Primers for CDC7 were designed using the *Tetrahymena* Genome Database and Integrated DNA Technologies Oligoanalyzer. The sequences of the forward and reverse primers used to amplify CDC7 are as follows: TTGTTAAGCCAGCAAATATCTAACTTTTCTATTTCAA and GATCAAATTCCTTTTTCTCAGCAGTGTT (Reverse). The sequences of the control Btu1 primers are as follows: CCCAGAGCTATCTTGATGGACTTA (Forward) and TAACACCAGACATG (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 randomly placed in either a room temperature

incubator (control group) or a 37C incubator (experimental group) for 24 hours.

RNA extraction: RNA was extracted from control and experimental *T. thermophila* cultures following the experiment using Qiagen's 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.

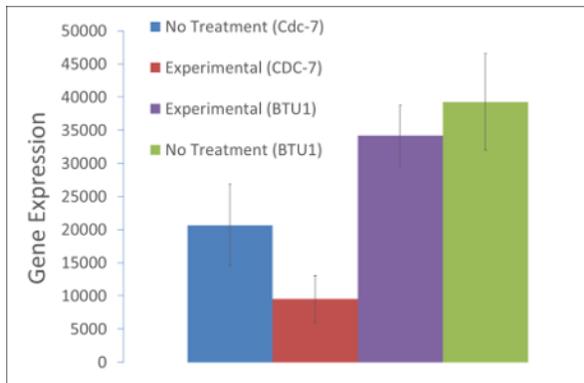
3 Results

Contrary to our hypothesis, acute exposure of *Tetrahymena* to a 37C environment resulted in the significant down-regulation of CDC7 ($p < 0.05$). This change in expression appears to be gene specific as there was no significant change in BTU-1 expression ($p > 0.05$).

Future studies should focus on varying the temperatures *Tetrahymena* cultures are exposed to, 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 heat affects CDC7 expression.

4 Figures

4.1 Relative Expression of CDC7 in Heat-exposed *Tetrahymena thermophila* Cultures



The relative expression of CDC7 compared to the control gene BTU1 was measured using semi-quantitative RT-PCR across four control and four experimental (37C-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 $p < 0.05$ for CDC7 and $p < 0.05$ 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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