Impacts of Environmental Motion on CDC2 Expression and Cell Growth in Tetrahymena thermophila

Jack Rees, Keaton Brady, and Stefanie Otto-Hitt, PhD
Carroll College

July 31, 2018

Experiment Type: Reverse transcriptase (RT) PCR
Feature: TTHERM_01207660
Gene: CDC2: Homolog of the catalytic subunit of the major cell cycle cyclin-dependent kinase (cdk); studies suggest cdc2p phosphorylates HHO1; expression positively regulated by HHO1 phosphorylation during starvation, resulting in a posi

1 Introduction

In this experiment, the eect of constant motion on expression of the cell cycle control gene CDC2 was examined in the organism Tetrahymena thermophila. We hypothesized that CDC2 expression would decrease with a corresponding increase in movement within the Tetrahymena culture environment. The CDC2 protein is primarily used in the replication of eukaryotic DNA because of its connection to HHO1, an important factor in transcriptional regulation. If the cells were subject to vegetative growth in which there are no stressors put on the cell, then HHO1 becomes phosphorylated to prevent it from interacting with chromatin. Because CDC2 is important in facilitating the phosphorylation of the HHO1, we hypothesized that, during vegetative growth, there would likely be an increase in the expression of CDC2. Moreover, when the cell is subject to stressors there would be no need to control cell growth by phosphorylating HHO1, so CDC2 expression should decrease.

To address this hypothesis, Tetrahymena thermophila were randomly assigned to a control group, which was cultured under normal lab-growth conditions, or a treatment group, which was subjected to constant movement on an orbital shaker for a period of one week. Following treatment, RNA extraction, reverse transcription, and gene-specific PCR (including gel electrophoresis) were used to analyze CDC2 expression in both the control and treatment groups. Furthermore, the growth rate of both groups was analyzed using a hemocytometer. We predicted that Tetrahymena cells subjected to constant motion would exhibit a decrease in cell growth rate and expression of CDC2.

2 Methods

Primer synthesis: Primers for CDC2 were designed using the Tetrahymena Genome Database and Integrated DNA Technologies Oligoanalyzer. The sequences of the forward and reverse primers used to amplify CDC2 are as follows: CGAGTTCATCAAGCTTGGGGCA (Forward) and GCAGATTTCGGTTTGGCTAGAGCTT (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). The experimental cultures were placed in an orbital shaker at 50rpm for one week.

RNA extraction: RNA was extracted from control and experimental T. thermophila cultures following the weeklong incubation 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 amplfication 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.

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

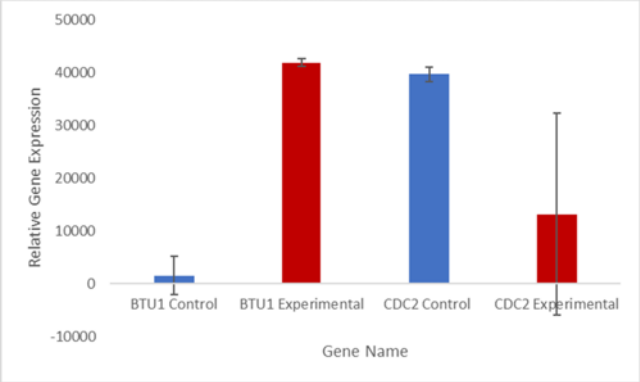
The semi-quantitative RT-PCR results demonstrate that constant motion results in the decreased expression of CDC2, as seen in Figures 1 ($p = 0.035$). Furthermore, the growth curve indicates that there was a slight decrease in the growth rate of Tetrahymena that were subjected to constant motion on the orbital shaker. Collectively, these results support our hypothesis regarding constant motion and its effects on CDC2 expression and growth rate

in Tetrahymena thermophila.

Future studies should focus on varying the constant motion parameters the Tetrahymena are subjected to, as well as increasing the number of replicates. Furthermore, a more quantitative measure of gene expression should be used to better determine whether constant motion affects CDC2 expression.

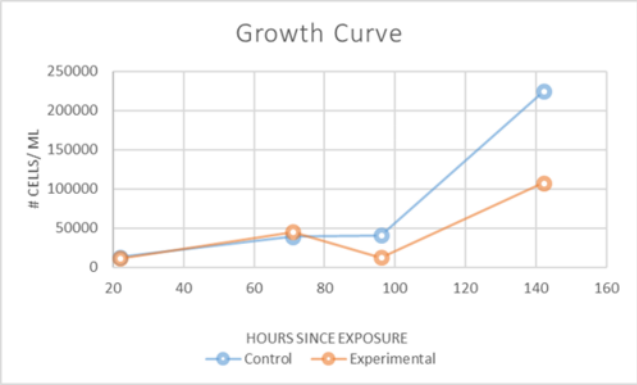
4 Figures

4.1 Relative Expression of CDC2 in Control and Constant Motion-subjected Tetrahymena thermophila Cultures



The relative expression of CDC2 compared to the control gene BTU1 was measured using semi-quantitative RT-PCR across four control and four constant motion-subjected 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=0.035 for CDC2 and p=0.20 for BTU1.

4.2 Growth Rate of Motion-subjected Tetrahymena Cultures



The growth rate across four control and four constant motion-subjected Tetrahymena cultures was 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

We would like to thank our faculty advisor, Dr. Stefanie Otto-Hitt for her guidance throughout this process. This project was funded in part by a supplies grant from the Ciliate Genomics Consortium.