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Effect of EGTA on SIT1 Scramblase Gene Expression and Cell Growth in Tetrahymena thermophila

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Experiment Type: Reverse transcriptase (RT) PCR
Feature: TTHERM_00094220
Gene: SIT1: Scramblase family protein

1 Introduction

Scramblase is an enzyme that facilitates the movement of newly synthesized phospholipids from the cytosolic side to the extracellular side of the lipid bilayer. This process is vital for cell membrane repair and growth. In Tetrahymena thermophila, the gene SIT1 encodes for the Scramblase protein, whose functionality is Ca2+-dependent. In this experiment, the concentration of accessible Ca2+ ions was decreased in order to observe whether the change had an aect on the expression of SIT1 and cell growth. It was hypothesized that expression of the SIT1 gene would increase, while cell growth would decrease.

To carry out the experiment, Tetrahymena thermophila were randomly separated into either a control or experimental group. The control groups were exposed to conditions with no Ca^{2+} inhibitor, while the experimental groups were exposed to EthyleneDiamineTetraacetic Acid (EDTA). EDTA is a commonly used chelating agent that inhibits the function of calcium ions. After exposing the experimental group to a single dose of EDTA over the course of one week, RNA extraction, reverse transcription, and semi-quantitative gene-specific PCR were performed on both the experimental and control groups to measure expression of SIT1. Cell growth was also measured throughout the week of culturing by counting cells on a hemocytometer.

Because the addition of EDTA results in a deficiency of accessible Ca^{2+} ions, it was predicted that SIT1 gene expression would increase due to decreased productivity of available Scramblase proteins, and that cell growth would decrease due to the inability of these Tetrahymena to repair their membranes.

2 Methods

Primer synthesis: Primers for SIT1 were designed using the Tetrahymena Genome Database and Integrated DNA Technologies Oligoanalyzer. The sequences of the forward and reverse primers targeting SIT1 are as follows: AATAGAGGTAATCAAGGAAAACAGCGGTTT (Forward) and TGGTAAGGCTGAATGGCCTTGCT (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). The SPP media for experimental cultures was supplemented with 5Mm EDTA.

RNA extraction: RNA was extracted from control and experimental T. thermophila cultures following one week of exposure to 5Mm EDTA 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) once a day 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.

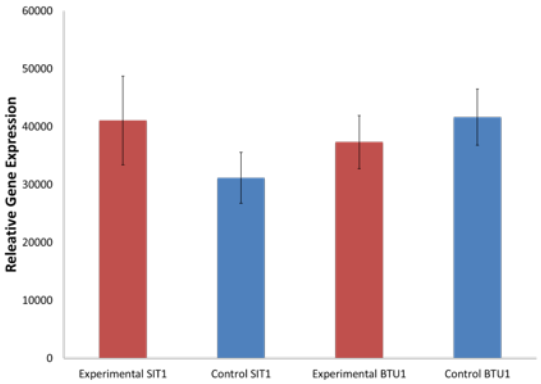
3 Results

The semi-quantitative RT-PCR results demonstrated that EDTA exposure resulted in no major change in expression of SIT1, as seen in Figure 1 ($p=0.32$). However, EDTA exposure did cause a reduction in cell growth, as indicated in Figure 2. Collectively, these results disagree with our hypothesis regarding EDTA treatment and SIT1 gene expression, but agree with our hypothesis for cell growth.

Future studies should focus on increasing the number of experimental samples and testing different EDTA concentrations; alternate calcium chelators should also be explored. Furthermore, a more quantitative measure of gene expression should be used to better determine whether decreased calcium concentrations affect SIT1 expression.

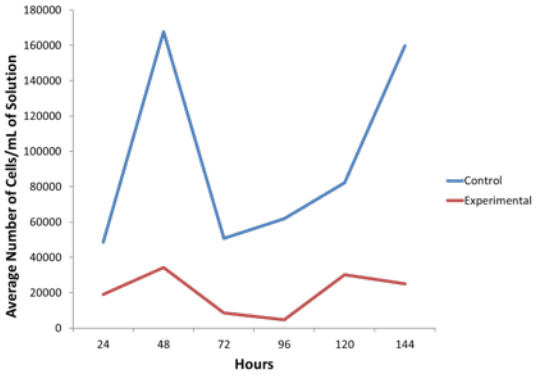
4 Figures

4.1 Relative Expression of SIT1 in Control and EDTA-treated Tetrahymena thermophila Cultures



The relative expression of SIT1 compared to the control gene BTU1 was measured using semi-quantitative RT-PCR across four control and four EDTA-treated 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=0.31 for SIT1 and p=0.54 for BTU1.

4.2 Growth Rate of EDTA-treated Tetrahymena Cultures



The growth rate across four control and four EDTA-treated Tetrahymena cultures was measured every 24 hours over the course of one week.

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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