Investigation of DNA Damage Repair by TTHERM_00439

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Abstract

Genes upregulated after DNA damage, such as Rad51, imply that they play a role in DNA damage repair. These repairs can result in mutations. By pinpointing genes that repair DNA damage and manipulating the functions of these genes, we may further research concerning cancer and various genetic diseases. We know that if the gene plays a role in DNA damage repair, the gene specific primer will bind to the DNA template which will result in greater gene expression--upregulated and brighter bands of product with DNA damaged cells compared to undamaged cells. To induce DNA damage, we add hydroxyurea to Tetrahymena thermophila—causing double strand breaks. To observe the gene expression of our gene in question, TTHERM_00439, we amplified the sample in damaged DNA and undamaged DNA using PCR and then ran gel electrophoresis. We compared the gene specific primers bands with our controls of Rad51 sample—positive control known to increase expression when there is DNA damage— and RPP0 sample—negative control that confirms that bands are specific to the primers rather than from a contamination. Our results confirmed that TTHERM_00439 gene does appear to be potentially involved in DNA damage repair. We can further research the specific function and role of this gene in the repair mechanism of the cell.

Introduction

- Tetrahymena thermophila changes gene expression, increase level of proteins after DNA damage which can be used to measure mRNA level (1). Becoming a highly attractive gene to study expression and regulation of genes during conjugation; which essentially allows us to explore primers that perform DNA damage repair.
- By observing upregulated expression during gel electrophoresis, we can see if the gene aids in DNA damage repair. DNA damage can cause mutations that lead to cell death, cancer, and more commonly links to the process of gaining and age-related diseases (i.e. diabetes) (2,3,4). By exploring what genes help repair these damages, we can understand how to prevent or treat these conditions.
- Knowing that Rad51 is a gene involved in DNA damage repair, we can compare our experimental gene's expression with Rad51's expression to measure if there was an upregulated expression after being amplified in damaged DNA
- By comparing the expected band size, we can conclude if the primer is to be further explored. The gene expression of Tetrahymena thermophila increased after DNA damage which suggests that the gene specific primer did repair the broken DNA. However, despite the upregulated expression, we still lack further research on its specific role during the repair process and how it functions.

Question or Hypothesis and/or Objectives

By manipulating and harnessing the functions of certain genes, proteins, and repair pathways, it may lead us to powerful applications in research concerning cancer and other genetic diseases.

Methods

1. 1.Validate the gene specific primer; gel electrophoresis run with PCR sample of the TTHERM_00439 primer and RPP0 as control
   2.Add hydroxyurea to DNA, creates damaged DNA. Extract RNA from both damaged and undamaged cell
2. 1.RNA Reverse transcribe to cDNA → DNA template cDNA and cDNA + Hydroxyurea
   2.Run PCR with these DNA templates with gene specific primer and with RPP0 and Rad 51 as controls. Along with samples without DNA template. Each PCR sample composed of 1X Go Taq PCR master mix, 2.5µL of forward and reverse primer, and 2µLof DNA template
3. 1.Run gel electrophoresis at 1.5% gel at 120 V for 34 minutes.
   2.Quantify the difference from damaged vs undamaged DNA template.
   3.Determine if primer was involved in DNA damage repair by observing the gene expression

Discussion

The bands in + HU cDNA (damaged DNA) were brighter and thus had greater amplification. And for lanes without a DNA template, there were no bands present. With these controls, we can confirm that the PCR was conducted correctly and can believe the results from this gel image. The multiple bands that was expressed for TTHERM_00439 gene in damaged DNA may imply alternative slicing. The amplification confirms that TTHERM_00439 gene plays a role in DNA damage repair.

Results

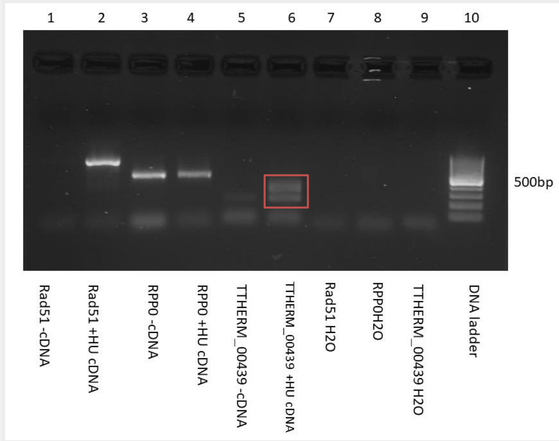


Figure 1: Polymerase chain reaction of TTHERM_00439 gene in damaged DNA shows multiple bands with amplification.

Amplified and ran on 1.5% gel at 120V for 34 minutes. The gel shows an interesting but promising results as evidenced by the multiple bands in lane 6. This suggests that there was successful amplification but may suggest alternative slicing.

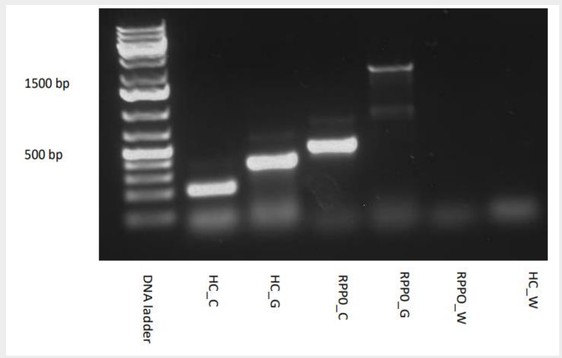
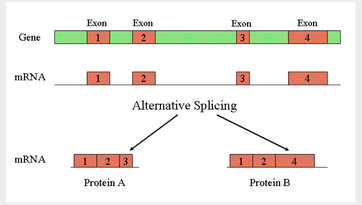


Figure 2: Gene Expression of TTHERM_00439 in cDNA and gDNA

Amplified and ran on 1.5% gel at 100V for 27 minutes. This confirms primer validation due to the PCR products being expressed at the expected sizes and there are no bands in the negative controls (samples with no DNA template).

Future Directions

. To further research about TTHERM_00439 gene's specific role in DNA damage repair and if our hypothesis about alternative splicing is true, we may run another experiment. We will rerun this set up to confirm that multiple bands will show up again. Then further explore methods to find the gene's alternative splicing function in DNA damage repair. With an alternative slicing function of TTHERM_00439 gene, we may be able to research a way to alter the gene to prevent it from ever forming a malicious sequence. With this new technology, we may be able to prevent many deaths from genetic causes.



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