

Effects of UVB light on Tfb5 gene expression in developing *Drosophila melanogaster*

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Experiment Type: Reverse transcriptase (RT) PCR

Feature: UV radiation

Gene: Tfb5: Transcription factor B5 (Tfb5) is a subunit of TFIIH, a basal transcription and DNA repair factor TFIIH.

1 Introduction

UVB light is known to cause DNA damage that results in gene mutations. Most regions of DNA damage are corrected using Nucleotide Excision Repair mechanisms (NER). In *Drosophila melanogaster*, the Tfb5 gene codes for an essential subunit of a protein involved in NER-mediated DNA repair. In this experiment, we attempted to answer the question: will overexpression of the Tfb5 gene in *Drosophila* compensate for the damage done to DNA by UVB light exposure?

In testing this hypothesis, we exposed our *Drosophila* larvae cultures to 7-minute periods of UVB light once during each stage of larval development. Our control group grew in conditions without UVB light. Larvae counts in both the experimental and control groups served as our quantitative analysis of survivability. Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed on the extracted larval RNA to determine the level of expressed Tfb5 gene in our control and experimental groups.

Because our *Drosophila* larvae were exposed to UVB light early on in development, we predicted there would be an overexpression of the Tfb5 gene in the experimental group, along with similar numbers of living larvae in both groups.

2 Methods

Primer synthesis: Primers for Tfb5 were designed using Flybase and Integrated DNA Technologies PrimerQuest. The sequences of the forward and reverse primers targeting Tfb5 are as follows: TGTTATGAAAGGAGTGCTGGTG (Forward) and TTGTCGTGCAGCGGAAAG (Reverse). The sequences of the control Gapdh primers are as follows: CGCCAAGAAGGTCATCATCTC (Forward) and CCTCGACCTTAGCCTTGATTTC (Reverse).

Culturing *Drosophila*: Wild type *Drosophila* cultures were purchased from Carolina Biological and maintained in Formula 4-24 Instant *Drosophila* Medium in culture vials as per the manufacturers protocol (Carolina Biological). Briefly, equal volumes of water and dry media were added to the culture tubes along with several grains of bakers yeast (Red Star Yeast). The cultures were incubated at room temperature and were sub-cultured every two weeks. The anesthetizing of the flies was accomplished using CO₂ FlyBeds (Azer Scientific) and The Flowbuddy CO₂ regulator (Flystuff.com). For the experiment, adult flies were applied to the culture vessels for 24hrs before being removed. Forty-eight hours after removal of the adult flies were removed the resulting larval cultures were randomly selected to be in the control or UV radiation-exposed group. For the radiation exposure, experimental cultures were subjected to six 7-minute doses of UV radiation during each larval instar stage, for a total of three exposures.

RNA extraction: RNA was extracted from control and experimental *Drosophila* larvae after 72hrs using Qiagens RNeasy Mini Kit as per the manufacturer's instructions. To prepare for RNA extraction, the larvae were transferred, using forceps, into 35mm petri dishes filled with 1x PBS (ThermoFisherScientific). The larvae were then placed in pre-chilled Eppendorf tubes and incubated in the freezer for 10 minutes. After freezing, a 1000uL micropipette tip was used to grind the larvae for 1 minute. Following the addition of Buffer RLT, the larval tissue was gently passed through an 18 gauge-needled syringe 10 times followed by a 25 gauge-needled syringe 10 times. During RNA extraction, the samples were subjected to on-column DNase treatment using an RNase-free DNase kit (Qiagen).

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 Gapdh cDNA was used as a positive control for the *Drosophila* RT-PCRs while the RevertAid 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.

Larval Counts: The number of third instar larvae in the control and UV radiation-

exposed fly cultures was determined by isolating these larvae from the culture media using a dissecting microscope and subsequent counting.

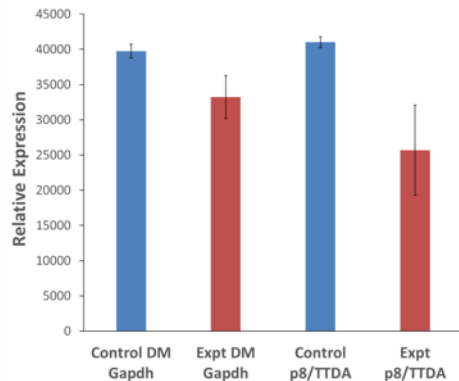
3 Results

The semi-quantitative RT-PCR results indicate that the UV radiation exposure applied to our developing *Drosophila* cultures did not result in a change in *Tfb5* expression, as seen in Figure 1 ($p = 0.14$). Furthermore, UV radiation exposure did not affect the survivability of the developing larvae, as depicted in Figure 2 ($p = 0.17$). Collectively, these results contradict our initial hypothesis that UV radiation exposure would result in increased *Tfb5* expression and decreased survivability.

Future studies should focus on optimizing the exposure time of the larvae to the UV radiation source and increasing the number of experimental replicates. Additionally, a more quantitative measure of gene expression should be used to more definitively determine the effect UV radiation has on *Tfb5* expression.

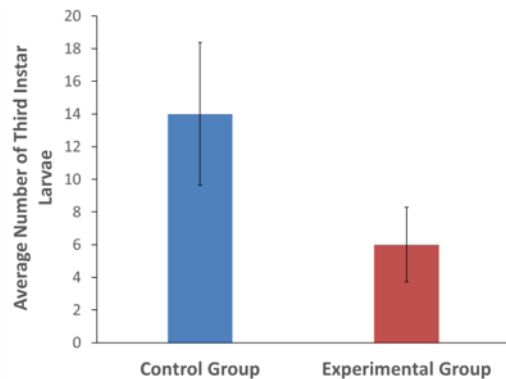
4 Figures

4.1 Relative Expression of *Tfb5* in Control and UV Radiation-exposed *Drosophila* Cultures



The relative expression of *Tfb5* compared to the control gene *Gapdh* was measured using semi-quantitative RT-PCR across four control and four UV radiation-exposed *Drosophila* cultures. 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 $p=0.14$ for *Tfb5* and $p=0.18$ for *Gapdh*.

4.2 Average Third Instar Larvae Counts from Control and UV Radiation-exposed Drosophila Cultures



The average number of third instar *Drosophila* larvae was measured across four control and four UV radiation-exposed cultures. The number of third instar larvae was not significantly altered in the UV-exposed cultures compared to control cultures ($p=0.17$). Error bars represent the standard error of the means and the measure of statistical significance (p value) was determined using a two-tailed t-test assuming unequal variance.

5 Acknowledgements

We would like to thank our faculty advisor, Dr. Stefanie Otto-Hitt for her guidance throughout this process and the energy she brought to the table