CHARACTERIZATION OF DNA REPAIR PROTEINS POLYMERASE ETA AND CG7139 IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

DNA synthesis can be stalled by many different types of DNA damage, or lesions, in the DNA. The cell must find a way to bypass these lesions in order to proceed with DNA replication so that the cell can divide. *Drosophila* cells usually prefer to tolerate DNA damage by an error-prone process known as translesion synthesis (TLS), but when this pathway is inhibited, the cell is forced to bypass the damage through template switching (TS), which is relatively error-free (Zhao, 2017). While TLS is well characterized, there is much to be learned about TS. I worked with flies that contain nonfunctional polymerase eta (PolH), CG7139 protein, and flies lacking the carboxy-terminal domain (CTD) of the REV1 protein, with the goal of determining whether these proteins may be involved in TS, learning more about what role these proteins play in DNA repair mechanisms, and determining whether PolH and CG7139 mutants are sensitive to a variety of mutagens when TLS is impaired. Double mutants were created that contained the REV1ΔCTD mutation with each of the other mutations. The REV1ΔCTD mutation strongly impairs TLS. Both the single and double mutants are being used in sensitivity assays, where they are treated with a mutagen, and the relative survival rates of the homozygotes and heterozygotes are used to draw conclusions about the sensitivity of the proteins to the mutagen. There was not enough time this summer to complete the double mutant sensitivity assays, but the single mutant sensitivity assays showed that PolH and the CG7139 protein may have mild sensitivity to the mutagen methyl methanesulfonate (MMS).

INTRODUCTION

Drosophila melanogaster was the ideal organism to use for my research project because of its excellent genetic tools, the vast array of knowledge available from decades of *Drosophila* research, and the fly's rapid generation time. One of the remarkable tools that *Drosophila* possess that makes them great for genetics research is balancer chromosomes. Balancers are specialized chromosomes whose genes have been rearranged to prevent recombination in a region of a chromosome (Tolwinski, 2017). Balancers are an important tool because they allow for fly stocks that possess multiple mutations on a single chromosome to be maintained for many generations. Without the presence of a balancer chromosome, the mutations would be able to separate from each other across generations through meiotic recombination in females. (Meiotic recombination does not occur in male fruit flies.)

D. melanogaster is used as a model organism to study DNA repair mechanisms because the DNA repair pathways used by *Drosophila* cells are remarkably similar to those found in humans. *Drosophila* cells typically prefer to bypass a stalled replication fork through an error-prone mechanism known as translesion synthesis (TLS). TLS is quick but can be mutagenic (Zhao, 2017). The alternative DNA repair pathways to TLS fall under the category of template switching (TS), which includes fork regression and homologous recombination. These pathways are slower and more error-free than the TLS pathways (Zhao, 2017). *Drosophila* cells prefer TLS to TS, so TS is generally only carried out if TLS cannot be performed.

Human cells use TLS polymerases to quickly bypass DNA lesions, which prevents cell death at the cost of increasing mutation rates and contributing to the

development and progression of cancer (Korzhnev, 2016). A lesion is the site of damage in the DNA, which can come from numerous endogenous and exogenous factors. Endogenous damage results from the chemically reactive DNA reacting with other reactive species, like water, that are present in the cell (Chatterjee, 2017). Exogenous damage is induced by external sources, such as UV light; ionizing radiation; and alkylating agents, like MMS (Chatterjee, 2017). Lesions can lead to a stalled replication fork, base mispairing, and single or double-stranded DNA breaks. TLS increases the survival of cancer cells after exposure to genotoxic chemotherapy, contributes to a greater mutation rate in tumors, and has been linked to chemotherapy resistance (Korzhnev, 2017). Cancer cells prefer to carry out TLS because it is faster than TS, and cancer cells want to grow and expand as quickly as they can. My research aimed to learn more about the proteins involved in TS and identify DNA-damaging agents that these proteins may have increased sensitivity to, which could offer insights into cancer research and chemotherapeutics.

TLS is fairly mutagenic partly because the TLS polymerases used to synthesize DNA are inexact and have low fidelity. The TLS polymerases are specialized to deal with a certain type of DNA damage. A protein known as REV1 plays a role in recruiting the TLS polymerases to the DNA lesion. The carboxyl-terminal domain (CTD) of REV1 is crucial for recruiting the TLS polymerases to the site of damage (Wojtaszek, 2012). While *Drosophila* cells prefer to tolerate DNA damage by performing TLS, the REV1ΔCTD mutants are forced to repair their DNA primarily through TS because the REV1 protein has a nonfunctional CTD that is unable to recruit TLS polymerases to the site of damage in the DNA.

Polymerase eta is one of the TLS polymerases, which is known to help repair pyrimidine dimers that result from UV light. Pyrimidines include uracil, cytosine, and thymine, whose molecule structures include one carbon-nitrogen ring. Pyrimidine dimers form when two consecutive pyrimidines bind together, which alters the normal base pairing structure of DNA. PolH is relatively error-free when repairing thymine-thymine dimers, but it is much more error-prone when used in other DNA repair methods (Venkadakrishnan et. al., 2023). The role of PolH in TLS is well characterized, but it is thought that it may also be involved in the repair of double-stranded breaks by homologous recombination (Gramates et al., 2022). The null PolH mutant flies (poleta^{12} homozygotes) have a 1.5kb deletion in the PolH gene, which results in a nonfunctional protein.

The CG7139 protein is predicted to possess DNA endonuclease activity, which means that CG7139 may cut DNA as part of a DNA repair process (Gramates, et al., 2022). The mutant stock of flies that I am working with has a 7.5 kilobase transgenic insertion into an intron in the CG7139 gene, which results in a truncated, nonfunctional protein.

METHODS

Since *Drosophila* cells typically opt for TLS over template switching to repair DNA damage, it was necessary to produce flies that cannot perform TLS to determine whether the CG7139 protein and PolH may be involved in template switching. A stock of REV1ΔCTD mutants was mated to flies with the null mutations. The REV1ΔCTD mutants have a balancer trans (on the homologous chromosome opposite) to the REV1ΔCTD mutation. This balancer chromosome, which is abbreviated as TM6B, w^+,

prevents recombination in the region of the mutation, and it includes a wildtype red eye marker, which is dominant to the allele for white eyes that is present on the X chromosome(s) of these flies. The wildtype red eye marker on the balancer allows us to infer that if the fly has white eyes, it is homozygous for the mutation, and if it has red eyes then it is heterozygous.

Double mutants with either the null CG7139 protein or the null PolH protein on the same chromosome as the REV1ΔCTD mutation were generated through three generations of crosses, as seen in Figure 1. The crosses were designed so that the genotype of the offspring could usually be determined by their phenotype, which made it easy to select the flies of interest. Although the genotype of the flies could usually be inferred from their phenotype, PCR was performed on at least two flies from all of the stocks used and from each of the initial double mutant vials to confirm that the mutations were present and the stocks had not been contaminated.

All of the males used in the third round of crosses of c) and d) (as shown in Figure 1) were genotyped by PCR to see whether the REV1ΔCTD and poleta[12] or CG7139 Minos mutations had recombined onto the same chromosome. This round of crosses was set up as single male crosses that included one male and 3 virgin females in each vial. The vials from the males that did not have both mutations were discarded. PCR was carried out again on some of the offspring from the double mutant males to once again verify that they did in fact have both mutations.

The general procedure that I followed for PCR is as follows. First, single-fly DNA preps were made to prepare the DNA of the flies to be used for PCR. These DNA preps were performed as previously described in the research paper titled *Type I Repressors*

of P Element Mobility (Gloor et al., 1993). When genotyping the males from the single male crosses, there were two PCRs that were performed consecutively on the DNA samples from the flies. The first PCR was used to see whether the males had the REV1ΔCTD mutation. Those that had the REV1ΔCTD mutation were used in another round of PCR to see if they also had either the CG7139 Minos mutation or the poleta^{12} mutation. All of these PCRs were conducted with a standard phusion protocol. The PCR products were imaged on a gel after undergoing gel electrophoresis.

The boxed crosses in Figure 1 were used in mutagen sensitivity assays, following the procedure and timeline outlined in Figure 1 of the section titled “DNA Repair in *Drosophila*: Mutagens, Models, and Missing Genes” in the *Genetics* textbook, volume 205 (Sekelsky, 2017). In the sensitivity assays the control vials were treated with 250 µL of water that did not contain any mutagen, and the experimental vials were treated with a 250µL solution that contained a specified concentration of a select DNA damaging agent diluted in water. From 10-12 days after the crosses were set the progeny were counted every other day. The heterozygotes had red eyes, and the homozygotes had white eyes. If the mutant flies were hypersensitive to the DNA damaging agent, then the relative survival rate of the mutagen treated homozygotes would be small relative to that of the control homozygotes.

Crosses a) and b) produced the single mutant flies that were used in sensitivity assays, and crosses c) and d) produced the double mutant flies to be used in sensitivity assays. Single mutant sensitivity assays for both poleta^{12} and CG7139 Minos were treated with methyl methanesulfonate (MMS) diluted to 0.03% and 0.05% MMS. MMS is an alkylating agent that damages DNA by adding methyl groups (CH_3) to DNA. MMS

preferentially methylates guanine and adenine, which can result in base mispairings and stalled replication forks (Lundin et al., 2005). The sensitivity of cells to MMS has been found to increase significantly when other DNA repair pathways are compromised (Lundin et al., 2005). It is thought that the double mutants may show increased sensitivity to MMS compared to the single mutants because the double mutants also possess the REV1ΔCTD mutation, which inhibits the flies' primary DNA repair pathway: TLS.

Since the role of the CG7139 protein is not well characterized, additional sensitivity assays were conducted with other DNA-damaging agents to see if the protein has any sensitivity to these agents. Topotecan was the DNA damaging agent used in one of the sensitivity assays. Topotecan is a topoisomerase I inhibitor that acts on topoisomerase I by intercalating, or inserting, between two DNA bases at the active site of the enzyme, which inhibits supercoil relaxation (Bali, 2018). Supercoils are topological distortions in DNA that can alter gene expression (Bali, 2018). The experimental vials in the topotecan sensitivity assay were treated with 250μL of 50μM topotecan, and the control vials received 250μL of water. Nitrogen mustard (HN_2) was also used as a mutagen in a sensitivity assay with the CG7139 Minos mutants. Nitrogen mustard is an alkylating agent that forms noncanonical nitrogen compounds with DNA (Jan, 2020). The experimental vials in the nitrogen mustard sensitivity assay were treated with 250μL of 0.005% HN_2, and the control vials received 250μL of water.

The double mutant sensitivity assays will be conducted in the fall.

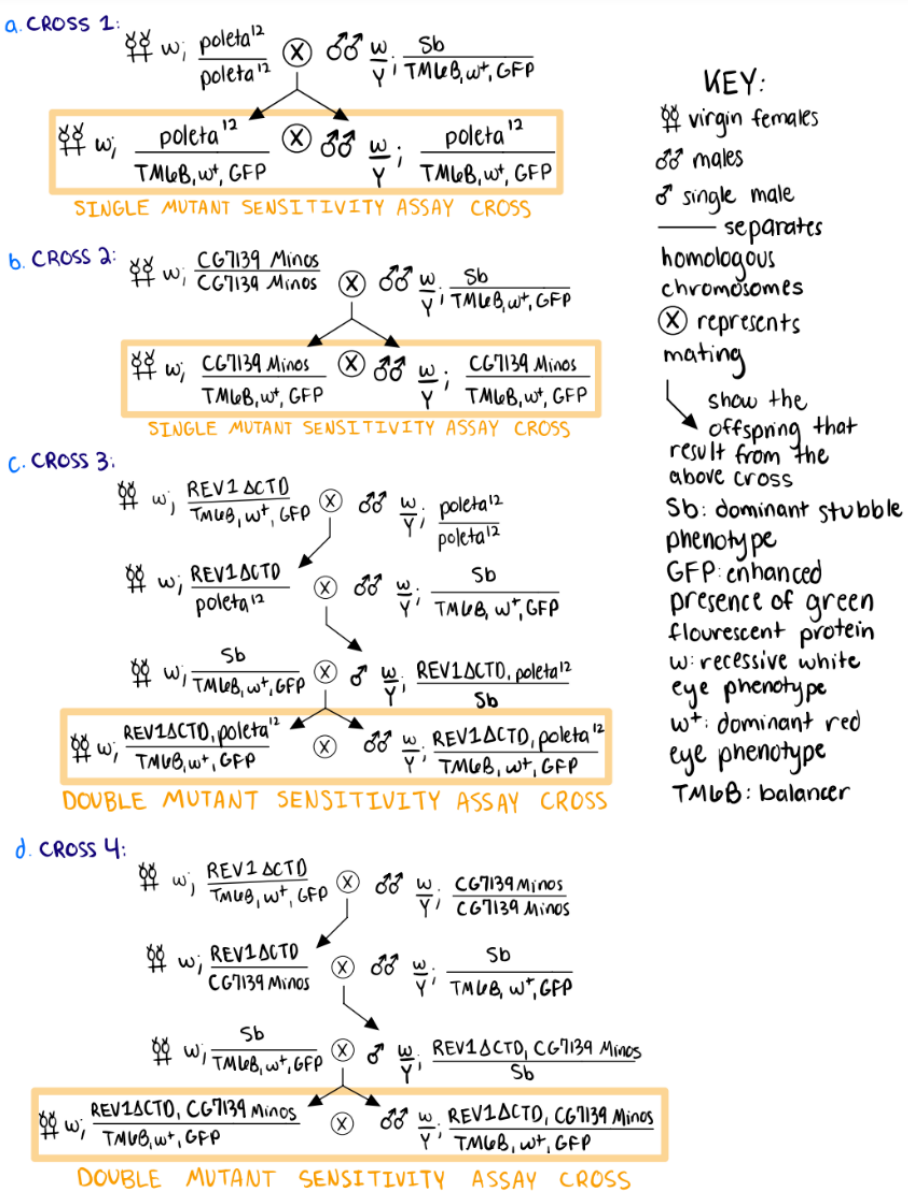

Figure 1: Single and double mutant cross scheme

(**a**, **b**) The poleta^{12}/TM6B, w^{+}, GFP and the CG7139 Minos/TM6B, w^{+}, GFP single mutants that were used in MMS sensitivity assays were each generated from one cross, and those with red eyes were selected because they had both the balancer and the poleta^{12} mutation. (**c**, **d**) The REV1ΔCTD, poleta^{12}/TM6B, w^{+}, GFP and the REV1ΔCTD, CG7139 Minos/TM6B, w^{+}, GFP double mutants that are being used in the MMS sensitivity assays were each generated from 3 crosses. The desired offspring from the

first and third generations were selected by their eye color, which corresponds to their phenotype. The genotypes of the males in the third generation were validated by PCR, and if the males did not have both mutations and stubble, then they were discarded along with their offspring.

RESULTS

The sensitivity assay results shown in Figure 2(a) indicate that PolH may have mild sensitivity to MMS. It is significant that all of the mean relative survival rates and standard deviations shown in Figure 2 for MMS are below 100%, as PolH and the CG7139 protein may have mild sensitivity to MMS. The CG7139 protein does not appear to have any sensitivity to topotecan since the mean relative survival is nearly 100%. The CG7139 protein may be very slightly sensitive to nitrogen mustard (HN_2), but more data is needed to verify the mutant's sensitivity.

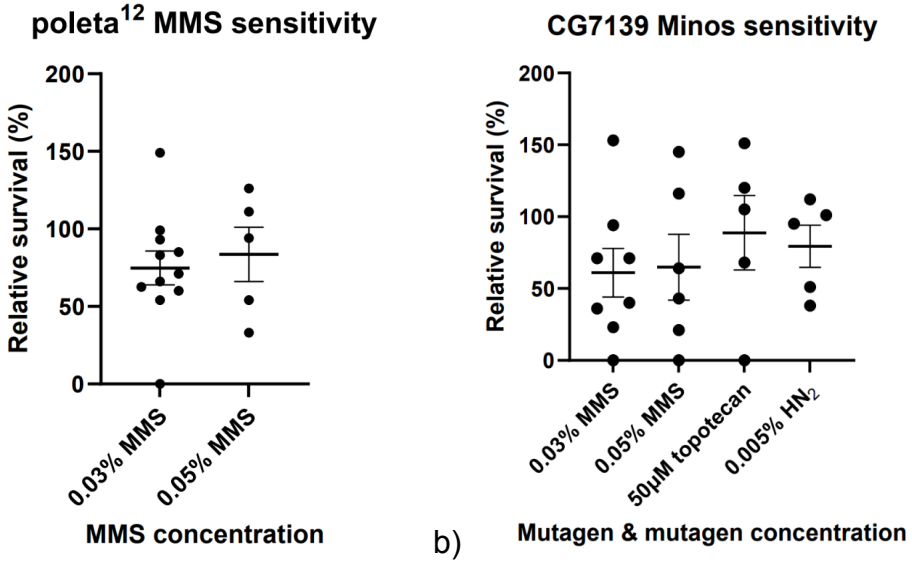

Figure 2: PolH and CG7139 Minos sensitivity assay results

Each data point represents the relative survival of homozygotes per vial, which is calculated by dividing the percent of mutagen-treated homozygotes by the percent of

control homozygotes. Mean and standard deviation are depicted by the horizontal bars in each column. Scatter plots were generated by GraphPad Prism software.

DISCUSSION

The CG7139 protein and PolH may have mild sensitivity to MMS from the results of the single mutant MMS sensitivity assays (as shown in Figure 2). It is important to note that this mild sensitivity was observed when the larvae were treated with a relatively high concentration of MMS, which means that these proteins likely have only a minor role promoting tolerance of alkylation damage.

The results of the double mutant sensitivity assays will determine whether PolH and the CG7139 protein are hypersensitive to MMS in a ΔCTD background. Comparing the respective sensitivities of the single and double mutants may also offer further insight into how the CG7139 protein and PolH are involved in DNA repair. If hypersensitivity to MMS is not observed from either of the double mutants, that would lead me to believe that the CG7139 protein and PolH likely do not play a crucial role in TS. I would arrive at this conclusion if the relative survival of the double mutant flies was about the same, or slightly more than, the single mutant flies. However, it is possible that a lack of hypersensitivity observed in the double mutant flies does not mean that the CG7139 protein or PolH are not important in one of the template switching pathways. It is possible that these proteins could be crucial to one of the TS pathways, but another TS pathway is able to adequately address the damage to compensate for the diminished functionality or accuracy of another TS pathway.

If hypersensitivity to MMS is observed in either of the double mutants, that would lead me to believe that either the CG7139 protein or PolH may play a role in TS. If this

is the case, then the goal of my future research will be to learn about how, if at all, PolH and the CG7139 protein are involved in template switching.

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