Characterization of DNA Repair Proteins Polymerase Eta and CG7139 in *Drosophila melanogaster*

Ana Dias, Mitch McVey, Ph.D.

Department of Biology, Tufts University

Introduction

We explored whether *D. melanogaster* mutants with a nonfunctional CG7139 protein or nonfunctional polymerase eta (PolH) exhibit sensitivity to a DNA damaging agent known as methyl methanesulfonate (MMS) through mutagen sensitivity assays. Sensitivity assays are currently being conducted with double mutants that have both the REV1ΔCTD mutation and either the nonfunctional CG7139 protein or the nonfunctional PolH to see if MMS sensitivity is altered when translesion synthesis (TLS) is greatly impaired.

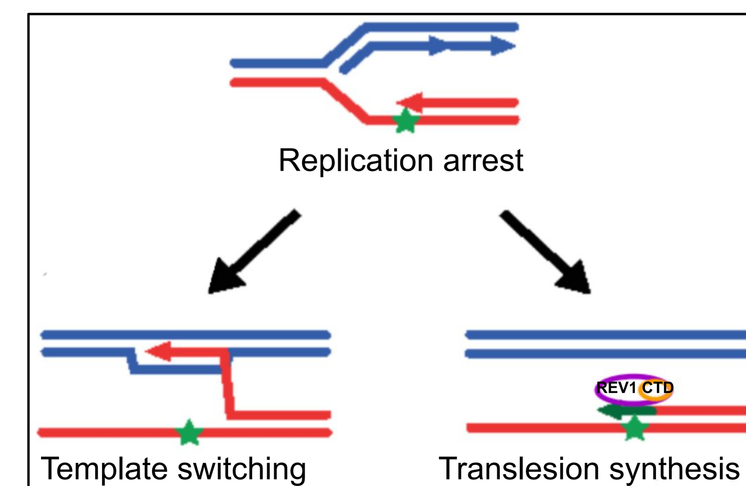


Figure 1: DNA repair pathways adapted from Sale[1]
Drosophila cells typically prefer to bypass a stalled replication fork through an error-prone mechanism known as translesion synthesis, as opposed to the alternative template Switching (TS) pathways, which are more error-free than the TLS pathways.[2]

REV1 protein
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.[3] TLS is strongly impaired without the functional CTD of REV1, so the REV1ΔCTD mutants are forced to bypass DNA damage primarily through TS.

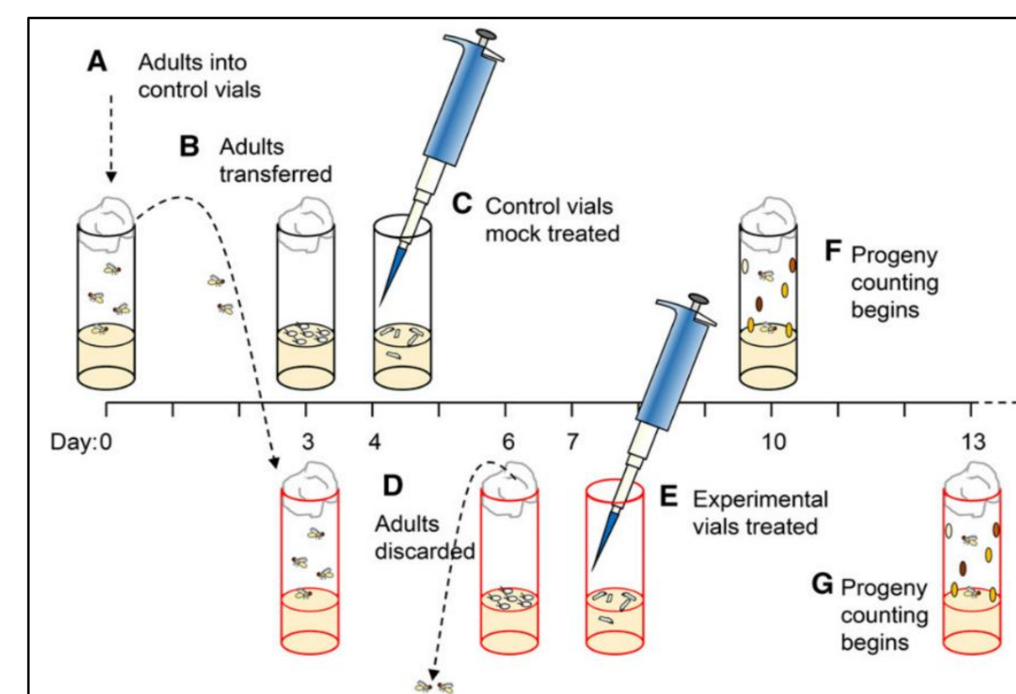
Polymerase eta
Polymerase eta is one of the TLS polymerases, which is known to help repair pyrimidine dimers that result from UV light. PolH is relatively error-free when repairing thymine-thymine dimers, but it has lower fidelity when it is used to repair other types of lesions.[4] The role of PolH 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.[5] The null PolH mutant flies (poleta[12] homozygotes) have a 1.5kb deletion in the PolH gene, which results in a nonfunctional protein.

CG7139 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.[6] The null CG7139 mutant flies (CG7139 Minos) have a 7.5 kilobase transgenic insertion into an intron in the CG7139 gene, which results in a truncated, nonfunctional CG7139 protein.

Methods

Mutagen sensitivity assays were used to evaluate the sensitivity of homozygous mutants to a mutagen. MMS was the primary mutagen used in the sensitivity assays. MMS is an alkylating agent, and the sensitivity of cells to MMS has been found to increase significantly when other DNA repair pathways are compromised.[7] Topotecan and nitrogen mustard were also used in mutagen sensitivity assays with the CG7139 Minos mutants.

Figure 1: Diagram of sensitivity assay timeline from Skelesky[8]
The control vials were treated with 250 µL of water, 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 survival rate of the mutagen treated homozygotes would be small relative to that of the homozygotes.



Results

PolH and the CG7139 protein appear to have mild sensitivity to MMS. The CG7139 protein does not appear to have any sensitivity to topotecan or nitrogen mustard (HN_2). Double mutant sensitivity assays are currently being conducted.

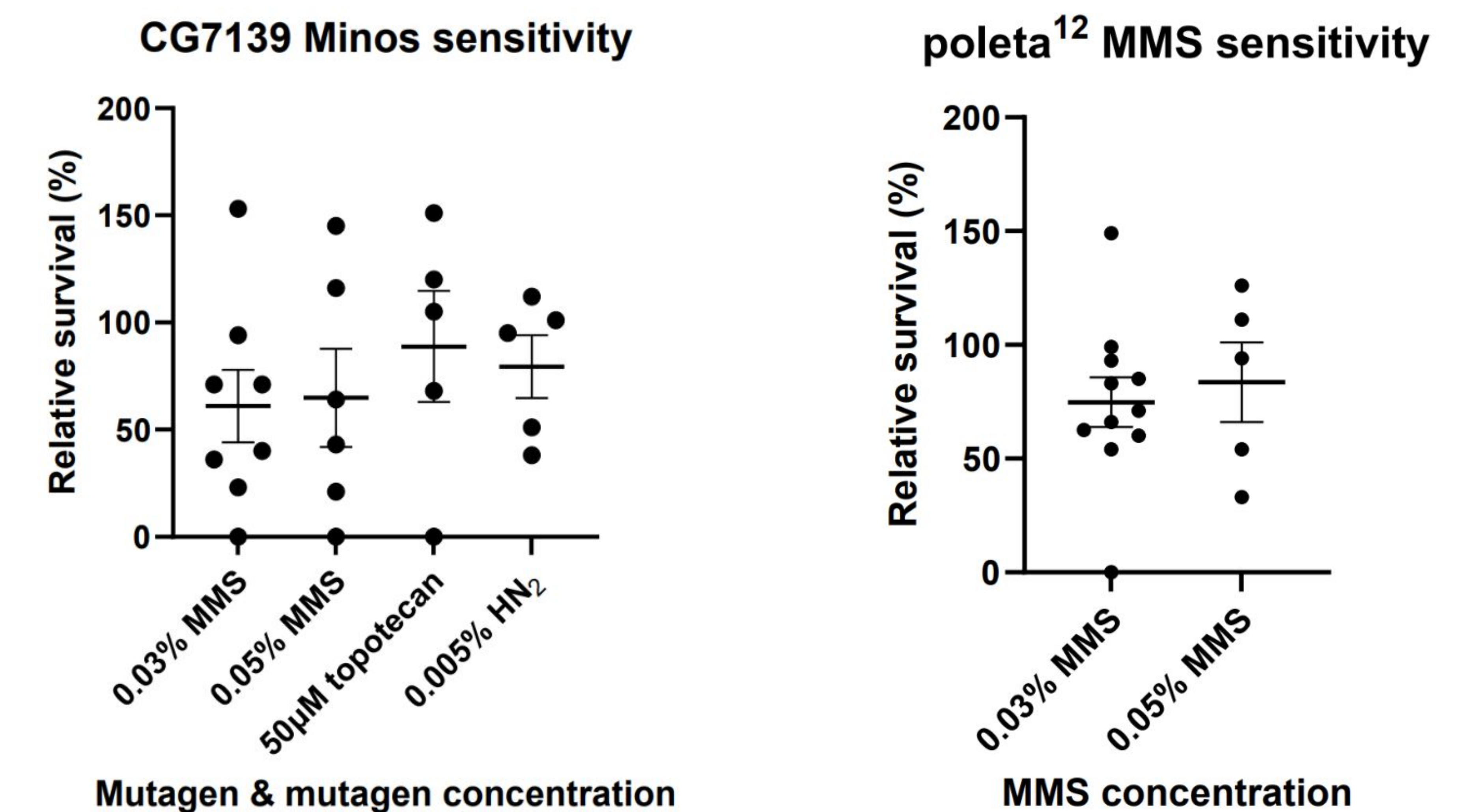


Figure 2: PolH and CG7139 Minos sensitivity assay results
Each data point represents the relative survival of homozygotes per vial, which is calculated by dividing the percent of mutagen-treated homozygotes by the percent of control homozygotes. Mean and standard deviation are shown. Scatter plots were generated by GraphPad Prism software.

Discussion

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.

This research is being conducted because of its applicability to humans. 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.[9] 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.[10] 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. **Our 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.**

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