Abstract:

It is important to study DNA damage such as double strand breaks (DSBs) because DSBs can cause cancer and genomic instability. DSB are repaired by homologous recombination which can cause a crossover or non-crossover event. Crossovers are detrimental because they can lead to genomic instability. Synthesis dependent strand annealing (SDSA) is a form of double strand break repair (DSBR) without crossovers. It is beneficial because it protects against genomic instability. This experiment seeks to determine the role of *Marcal1* in SDSA in *Drosophila melanogaster* using a P{w^a} P-element system in a fly line with a *Marcal1* deletion. *Marcal1* was studied because we have evidence that it is involved in some aspect of DSBR; however, it is unclear about which aspect of DSBR. It is hypothesized that when *Marcal1* is deleted and DSBs are induced in the fly line, there will be an increase in aberrant repair. Results show an increase in aberrant repair and a decrease in SDSA, indicating that *Marcal1* has a role in SDSA. PCR results of the aberrant repair flies found no statistical difference in the tract lengths between wild type flies and *Marcal1* mutant flies.

Introduction:

DNA damage can cause genetic instability and cancer. Therefore, it is important to understand DNA repair for disease treatment and chemotherapy development. SMARCAL1 is a replication stress response protein in the SWI/SNF1 family that has a role in repair at replication forks. It binds to stalled replication forks to prevent damage accumulation in replication. It can regress replication forks both *in vivo* and *in vitro*, so it is has a role in non-crossover DNA repair at replication forks. A mutation in *SMARCAL1* causes Schimke Immunoosseous Dysplasia

(SIOD), an autosomal recessive disorder of T-cell deficiency, nephropathy, and growth retardation (Postow *et al*. 2009).

Previous studies have found SMARCAL1 to have a role in repair during DNA replication. Silencing *SMARCAL1* makes cells hypersensitive to replication stress-inducing agents such as hydroxyurea, camptothecin, and aphidicolin (Baradaran-Heravi *et al*. 2012). During replication stress, SMARCAL1 localizes to sites of stalled replication by co-localizing with RPA2. ATM, ATR, and DNA-PK kinases phosphorylate SMARCAL1 to start a signal cascade (Bansbach *et al*. 2009). RNAi knockdown of SMARCAL1 causes an increase in DNA damage and cell cycle arrest (Bansbach *et al*. 2010).

Marcal1 is the ortholog of *SMARCAL1* in *Drosophila melanogaster*, which was used as the model system in this experiment. SMARCAL1 and Marcal1 both interact with RPA and have a conserved phosphorylation site. The main difference between SMARCAL1 and Marcal1 is that SMARCAL1 has two HARP domains while Marcal1 just has one (Figure 1). When one of the HARP domains was removed in SMARCAL1, it still functioned the same (Bétous *et al*. 2012). It is believed that the HARP domain gives SMARCAL1 polarity and handedness. The HARP domain allows for ATP-dependent annealing helicase activity which helps with stabilization of stalled replication forks and DNA repair during replication (Ghosal *et al*. 2011). There is an RPA binding motif near the N-terminus and the HARP binding domain(s) are at the N-terminal border of the ATPase SNF2 domain. The Marcal1 HARP domain is at the same location as the second HARP domain of SMARCAL1. When the first HARP domain was deleted in SMARCAL1, it still functioned, and the first HARP domain was not needed for annealing helicase activity. The area from the second HARP domain to the C-terminal end of the SNF2 ATPase domain in

SMARCAL is needed for annealing helicase activity (Bansbach *et al*. 2014). The distance between the two domains in flies is not appreciably different.

Studies with *Drosophila* Marcal1 have shown that it may be involved in transcription. Marcal1 binds to H3K4me3, which is a marker of open chromatin and an indicator of transcription rather than replication (Baradaran-heravi *et al*. 2012). However, both Marcal1 and SMARCAL1 can move Holliday junctions and dissociate D-loops *in vitro* which are template switching intermediates. Sensitivity assays performed by our lab indicate *Marcal1* mutants are not sensitive to replication stress-inducing agents (unpublished data). These data are consistent with recently published biochemical studies that show that, unlike SMARCAL1, Marcal1 cannot unwind model replication forks (Kassavetis *et al*. 2014). Altogether, these data suggest *D. melanogaster* Marcal1 may have a role in DSB repair via synthesis dependent strand annealing (SDSA) or homologous recombination (HR)—a role that has not been studied in metazoans to date.

Both HR and SDSA are forms of high fidelity double strand break repair (DSBR). SDSA is a form of HR that does not produce crossovers. When a DSB is repaired by SDSA, the 5’ ends are resected to produce 3’-ended single stranded tails (Figure 2). One of the strands invades the homologous chromosome and the strand exchange displaces a strand to make a D-loop. The invading strand synthesizes off of the homologous chromosome and dissociates. The dissociated strand anneals to the other resected end formed from the DSB. The gaps are filled or the overhangs are trimmed and the strands are ligated to complete the repair (The Sekelsky Lab Mitotic Recombination. 2014). There are several proteins that are expected to aid in *Drosophila* DSBR. Spn-A is a protein found to aid in strand invasion (Mcvey *et al.* 2004). Blm is an annealing helicase has been found to dissociate D-loops (Adams *et al*. 2003). Fancm also has

been found to dissociate D-loops, but its mutation has less of an effect on DSBR than Blm (Kuo *et al.* 2014). The results of the Marcal1 studies outlined in this project may implicate that Marcal1 plays a role in DSB repair which has not yet been explored in SMARCAL1.

Materials and Methods:

Fly Stocks

D. melanogaster were raised on standard cornmeal-based media at 25°C. The fly crosses were generated by collecting virgins stored at 18°C. Two different deletion alleles were used. The $Marcal1^{del}$ null allele was generated by the Boerkoel lab using imprecise P-element excision. The $Marcal1^{kh1}$ deletion was made via CRISPR in the Sekelsky lab by Julie Holsclaw. The CRISPR system uses chimeric RNA designed against user-specified sequences and a modified Cas9 nuclease to cause sequence specific cleavage of DNA. ChiRNA can lead Cas9 to a certain genomic sequence to cause DSBs, and when two chiRNAs are used, large deletions can occur (Overview. 2014). $Marcal1^{kh1}$ resulted from a 1088-bp deletion in the sequence beginning before the start codon and ending in the second exon causing a frame shift. The start codon was deleted; however, if an alternate start codon exists, the first 363 amino acids of the protein were deleted, so any potential protein product made would not have the RPA binding domain, HARP domain, PESH box, or part of the ATP binding domain (unpublished data). Heteroallelic mutants of $Marcal1^{del}$ and $Marcal1^{kh1}$ were used in all assays.

P{w^a} Assay

A P{w^a} P-element system previously designed by our lab was used to determine if Marcal1 plays a role in SDSA. Briefly, the system utilizes a P-element containing the *white* gene that is inserted into the X chromosome of *D. melanogaster*. *Copia* is inserted into an intron of the *w* gene and there are repeated 276-bp long terminal repeats (LTRs) at each end of the *copia* retrotransposon, resulting in the w^a allele. P transposase was used to excise the P{w^a} element, generating a DSB and large gap.

Results:

We adapted an assay published by our lab in Adams *et al.* (2003) to test SDSA efficiency in *Marcal1* mutants. Fly eye color was used to indicate the type of DSB repair utilized by *Marcal1* mutants using the P{w^a}system. The P{w^a} system consists of a P-element insertion on the X chromosome that contains the *white* (*w*) gene. In normal flies, *w+* gene causes red colored eyes; however, the P{w^a} construct has a *copia* element flanked by long terminal repeats (LTRs) inserted in an intron of the *white* gene decreasing gene expression and causing apricot-colored eyes, w^a (Figure 3). *Marcal1* mutant flies with the P{w^a} construct were crossed to a transposase source. The transposase excises the P{w^a} by a DSB at its ends, creating a large gap for repair. The fly's eye color is indicative of the way the gap is repaired. If the gap is repaired by SDSA, the synthesis from the ends of the break make areas of single stranded DNA that are complementary at the LTRs. If the new LTR sequences anneal to each other, the copia element is deleted from the repaired chromatid to form a P{w^{aLTR}} product with only one LTR in the white intron. One LTR at the *copia* insertion site causes wild type red eyes. Apricot colored eyes are

caused by the presence of the entire P{w^a} P-element. This can be caused by non-excision of the P{w^a} or by excision and complete repair to restore the P{w^a} element. Yellow eyes are an indicator of aberrant repair causing the loss of white activity (Adams *et al*. 2014). We performed this assay in male flies. Males were used because males do not have meiotic recombination, so any repair event was the result of mitotic DNA damage repair.

The percentage of red, apricot, and white-eyed *Marcal1* deletion flies were compared to the percentages of red, apricot, and white-eyed wild-type flies to determine the effect of the *Marcal1* deletion. The *Marcal1* deletion flies had an increase in aberrant repair, a decrease in complete SDSA, and an increase in overall gap repair (Figures 4 and 5).

After the flies were scored, PCR was used to determine the extent of synthesis in aberrant repair events. There was no statistical difference in the tract lengths between wild type flies and *Marcal1* mutant flies (Figure 6).

Discussion:

We hypothesized that Marcal1 is involved in SDSA. As expected, we found that *Marcal1* mutant flies had an increase in aberrant repair, a decrease in total repair, and decrease in SDSA. We hypothesized that Marcal1's role in SDSA is to facilitate the homology search by anneal the two strands of ssDNA together, but the PCR results of the aberrant repair flies found no statistical difference in the tract lengths between wild type flies and *Marcal1* mutant flies. Previous experiments found that *Blm* mutants had decreased synthesis tracts and increased flanking deletions because of its role in D-loop dissociation (Adams *et al*. 2003). There is no significant difference in the synthesis tract length between wild type flies and *Fancm* mutants,

but Fancm may be a backup repair pathway to Blm (Kuo *et al.* 2014). Because *Marcal1* mutants do not have a decrease in tract synthesis or increase in flanking deletions like Blm and because Marcal1 has known annealing activity, Marcal1 could still play a role in the annealing step of SDSA. Marcal1 could also dissociate D-loops. The role of Marcal1 remains unclear.

In the future, we plan to produce more *Marcal1* mutants to insure that our results and conclusions are accurate. More *Marcal1* mutants would also give us more PCR data to draw conclusions about the length of the synthesis track on the left side of the P-element. Also, we plan to stain *Drosophila* testis for cleaved caspase-3 which is a marker for apoptosis. This test would determine if the decrease in repair is due to an increase in cell death or an increase in precise repair. We plan to design a biochemical annealing assay using two single stranded DNA oligos with a small portion of homology to see if Marcal1 can anneal the two strands together. Finally, we would like to use the P{w^a}system for a *Marcal1* and *Blm* double mutant. Blm has been shown to have a role in D-loop dissociation. This double mutant could be used to determine if Marcal1 acts as a backup to Blm in DSBR.

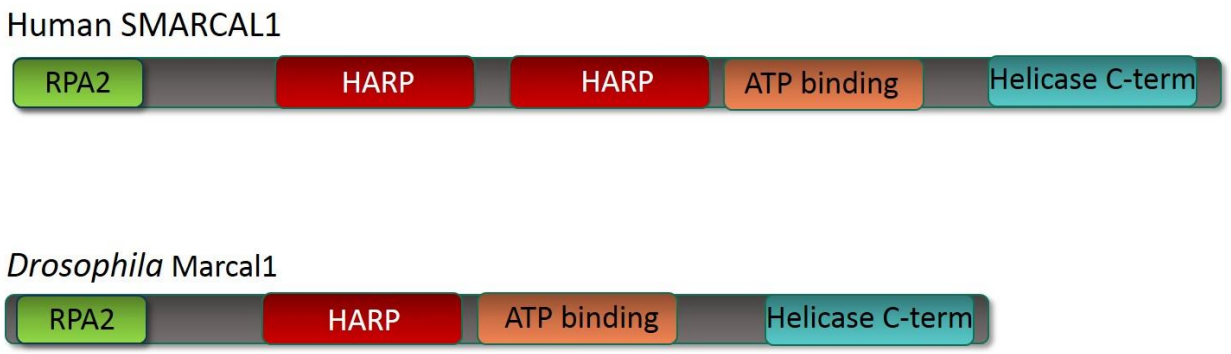

Figure 1. This figure illustrates the differences between Human SMARCAL1 and Drosophila Marcal1. SMARCAL1 has two HARP domains while Marcal1 has one HARP domain.

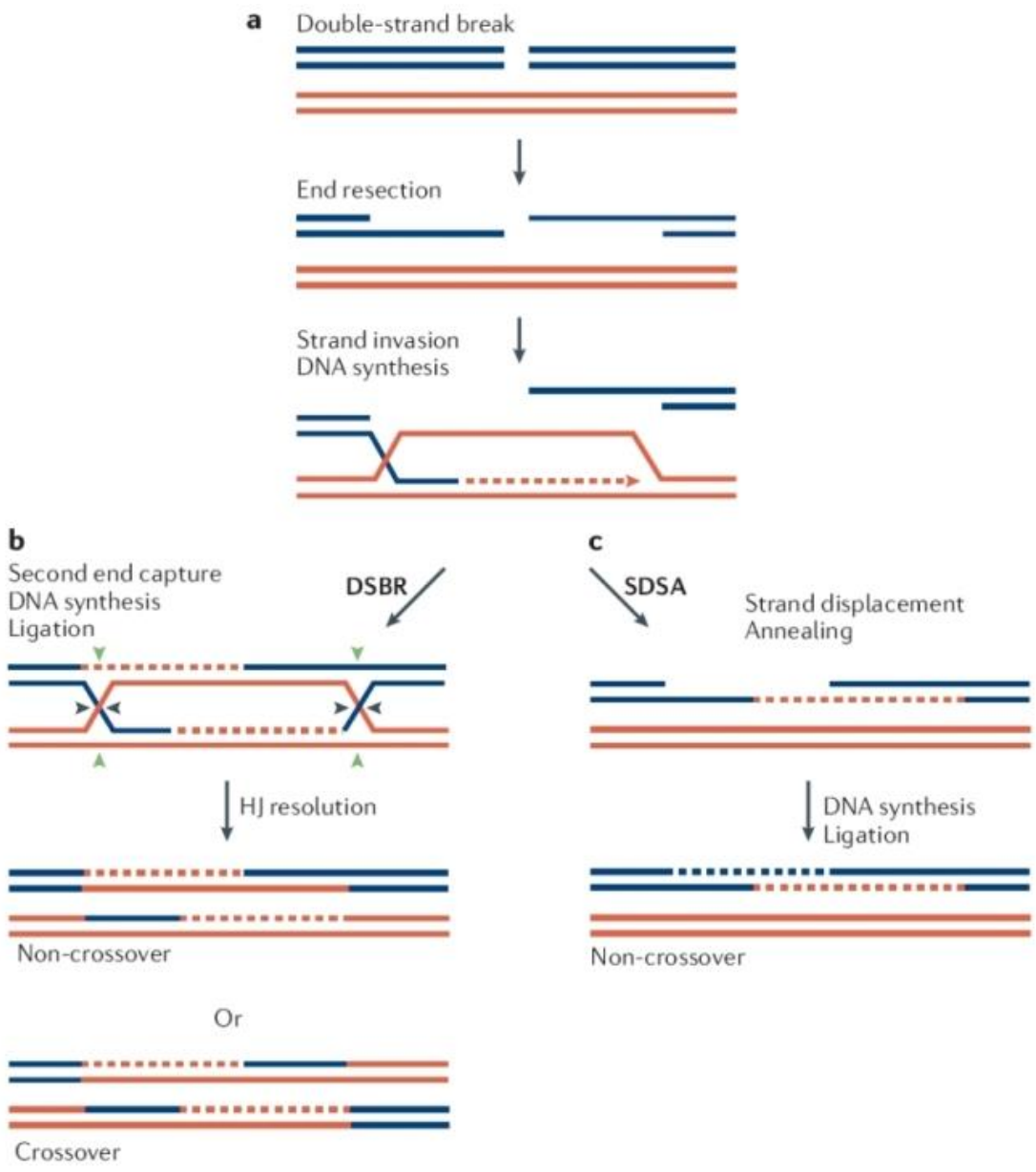

Figure 2. This figure illustrates DSB repair. The illustration on the right shows SDSA (Repair of DNA Double-Strand Breaks by DSBR and SDSA. 2014).

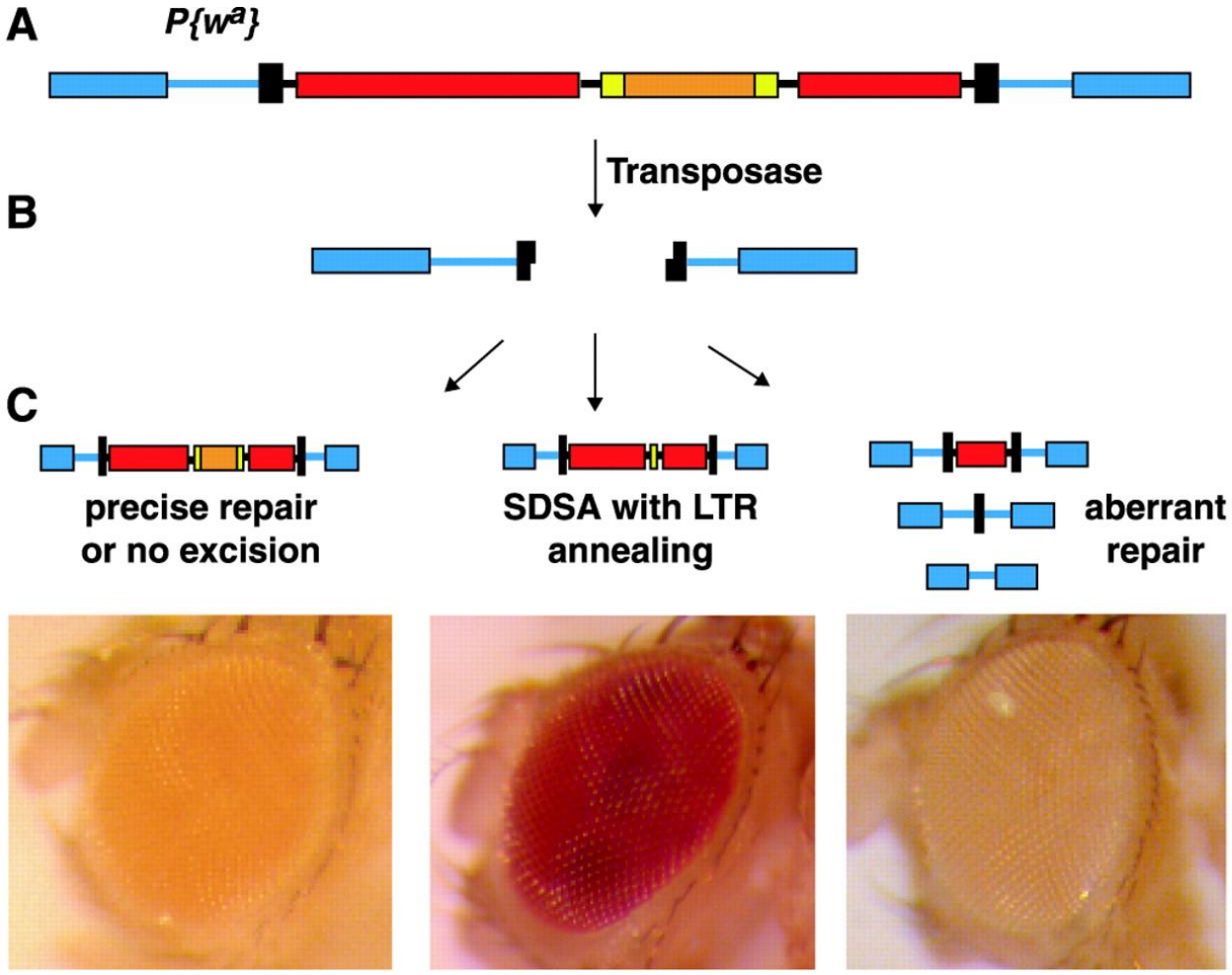

Figure 3. This figure illustrates the different eye colors that can result from the P{w^a} P-element system. Apricot eyes are caused by precise repair or no excision. Red eyes are caused by SDSA with LTR annealing. Yellow eyes are caused by aberrant repair. (A) The representation of the P{w^a} P-element system. (B) The DSB after the excision of P{w^a}. (C) The possible types of repair of P{w^a} P-element system (Adams *et al.* 2003).

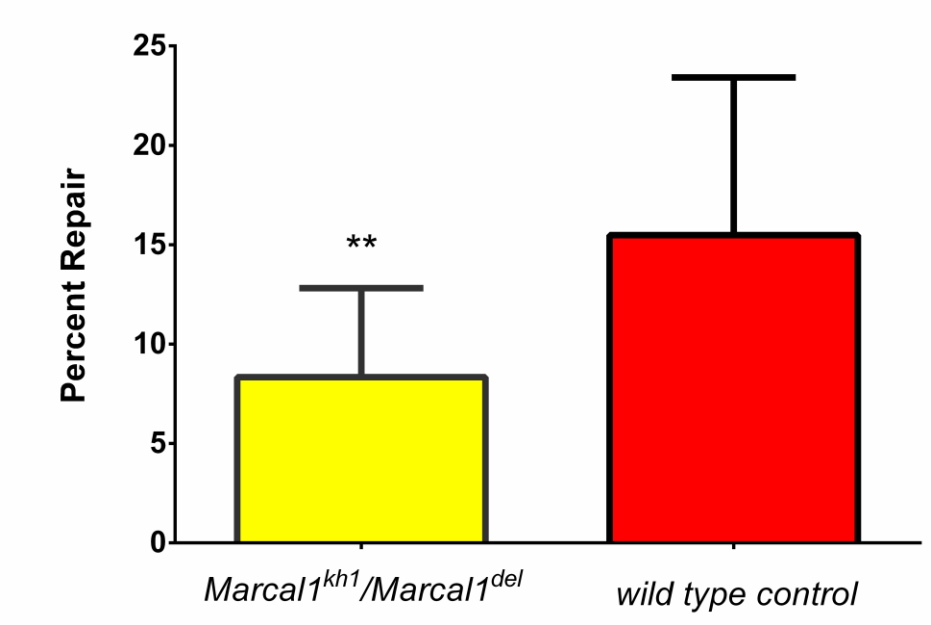

Figure 4. This figure illustrates that *Marcal1* mutants have fewer total repair events.

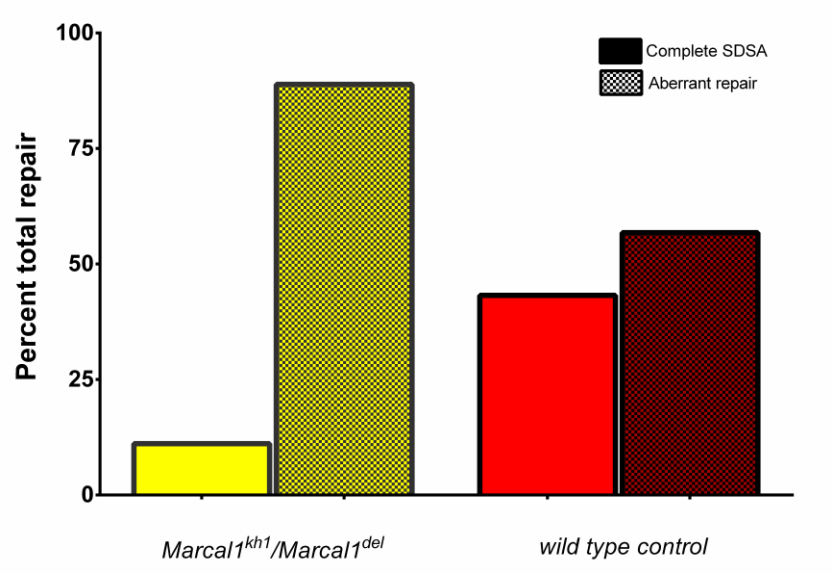

Figure 5. This figure illustrates that *Marcal1* deletion flies have an increase in aberrant repair and a decrease in SDSA.

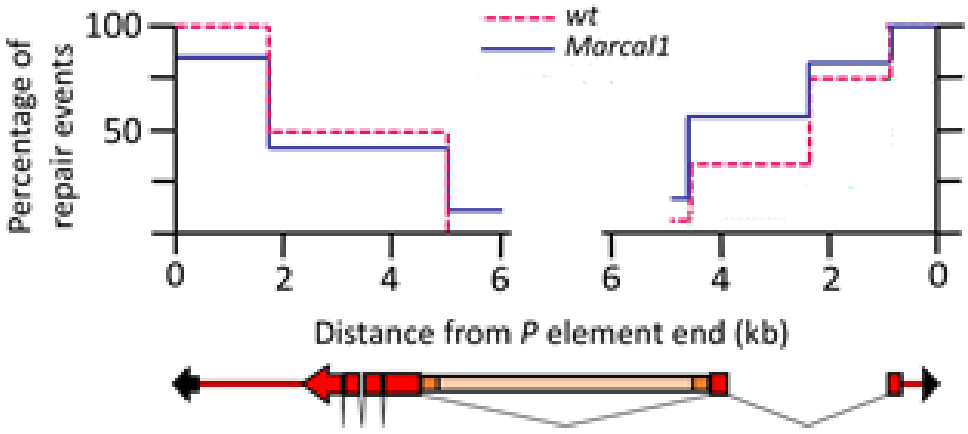

Figure 6. This figure displays the PCR results from the aberrant repair flies. *Marcal1* mutants that performed aberrant repair were had longer synthesis tracts than wild type flies.

References:

Adams, Melissa D, Mitch McVey, and Jeff J Sekelsky. "Drosophila BLM in Double-Strand Break Repair by Synthesis-Dependent Strand Annealing." *Science (New York, N.Y.)* 299.5604 (2003): 265–7. Web. 30 Nov. 2014.

Bansbach, Carol E *et al*. "The Annealing Helicase SMARCAL1 Maintains Genome Integrity at Stalled Replication Forks." *Genes & development* 23.20 (2009): 2405–14. Web. 22 Feb. 2014.

Bansbach, CE, CF Boerkoel, and David Cortez. "SMARCAL1 and Replication Stress." *Nucleus* 1.3 (2010): 245–248. Web. 22 Feb. 2014.

Baradaran-Heravi, Alireza, Kyoung Sang Cho, *et al*. "Penetrance of Biallelic SMARCAL1 Mutations Is Associated with Environmental and Genetic Disturbances of Gene Expression." *Human Molecular Genetics* 21.11 (2012): 2572–2587. Print.

Baradaran-Heravi, Alireza, Anja Raams, *et al*. "SMARCAL1 Deficiency Predisposes to Non-Hodgkin Lymphoma and Hypersensitivity to Genotoxic Agents in Vivo." *American journal of medical genetics. Part A* 158A.9 (2012): 2204–13. Web. 11 Feb. 2014.

Bétous, Rémy *et al*. "SMARCAL1 Catalyzes Fork Regression and Holliday Junction Migration to Maintain Genome Stability during DNA Replication." *Genes & development* 26.2 (2012): 151–62. Web. 22 Jan. 2014.

Holsclaw, Julie Korda. *Determining the Role of Blm and Marcal1 Helicases in Replication Fork Remodeling and Progression*. N.p. Print.

Kassavetis, George a, and James T Kadonaga. "The Annealing Helicase and Branch Migration Activities of Drosophila HARP." *PloS one* 9.5 (2014): e98173. Web. 2 Oct. 2014.

Kuo, H, Susan McMahan, Christopher Rota, Kathryn Kohl, and Jeff Sekelsky. "*Drosophila* FANCM Helicase Prevents Spontaneous Mitotic Crossovers Generated by the MUS81 and SLX1 Nucleases." *Genetics* 198 (2014): 935-945. Print.

McVey, Mitch, Jeannine LaRocque, Melissa Adams, and Jeff Sekelsky. "Formation of deletions during double-strand break repair in *Drosophila* DmBlm mutants occurs after strand invasion." *PNAS* (2004): 15694-15699. Print.

"Overview | flyCRISPR." N.p., n.d. Web. 30 Nov. 2014.

Postow, Lisa *et al*. "Identification of SMARCAL1 as a Component of the DNA Damage Response." *The Journal of biological chemistry* 284.51 (2009): 35951–61. Web. 22 Feb. 2014.

“Repair of DNA Double-Strand Breaks by DSBR and SDSA.” Web. 30 Nov. 2014.

“The Sekelsky Lab Mitotic Recombination.” Web. 30 Nov. 2014.