The Impact of Developmental Stage on DSB Repair Pathway Choice in *Drosophila melanogaster*

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Abstract

Background: Double-strand breaks (DSBs) are a common type of DNA damage that can result in large-scale genomic instability and cancer. DSBs are primarily repaired by non-homologous end joining (NHEJ) or homologous recombination (HR).

Method: To study the effect of developmental stage at time of DSB induction on repair pathway choice (NHEJ vs. HR), the DR-*white* DSB reporter assay and TIDE was used to analyze repair events in the premeiotic germline and somatic tissue.

Conclusion: Developmental stage does not affect DSB repair pathway choice in the premeiotic germline, however, the somatic tissue data suggests that 0-1 d.o. embryos perform significantly less repair by HR than 2-3 d.o. larvae.

Materials and Methods

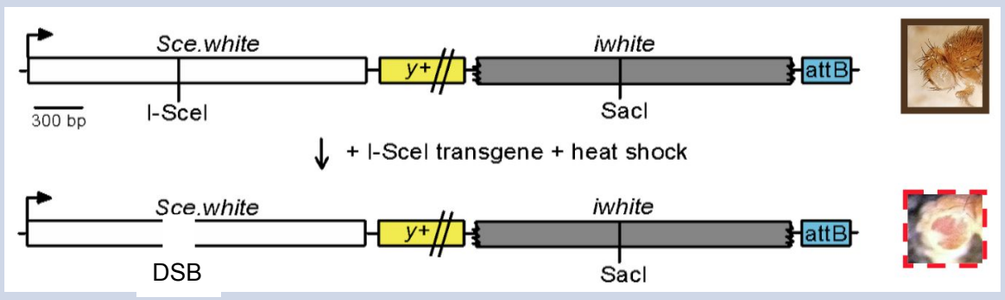

Figure 1: **The DR-*white* assay**[1]. This assay has two nonfunctional copies of the *white* gene. *Sce.white* contains an I-*SceI* recognition sequence. DSBs are induced at the I-SceI site with heat shock in flies with DR-*white* and the I-*SceI* transgene. Repair of the DSB occurs in all germline and somatic cells.

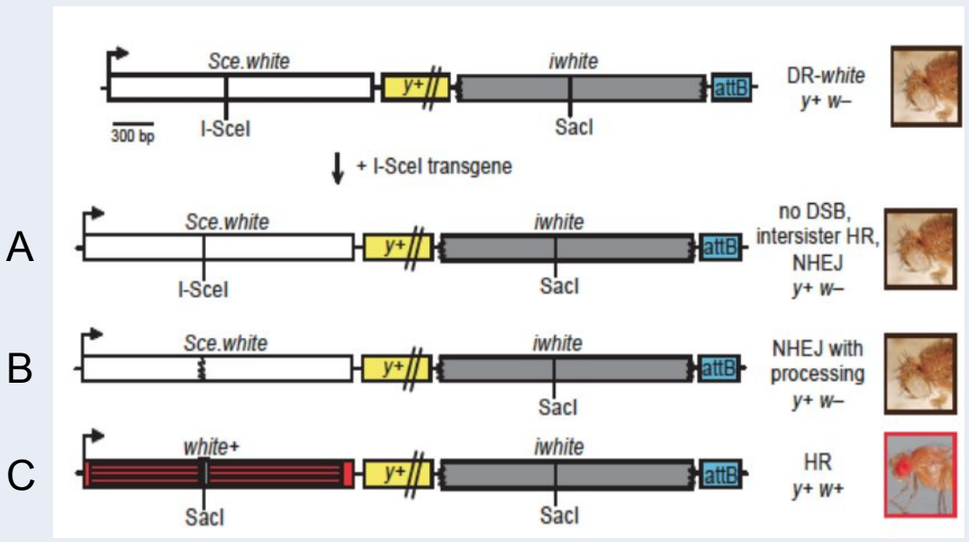

Figure 2: **Phenotypic outcomes of DSB repair events**[1]. The mosaic-eyed males (Fig. 1) are crossed to tester females and the individual germline DSB repair events can be phenotypically followed in the progeny.

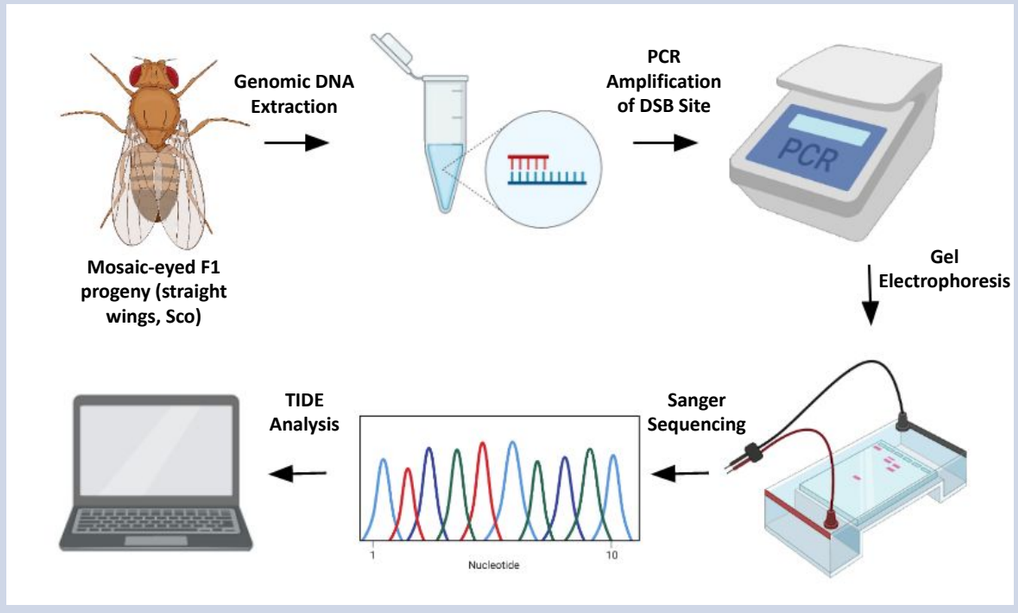

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Figure 3: **Tracking Across Indels by Decomposition (TIDE) analysis.** Genomic DNA containing DSB repair events is analyzed using TIDE. TIDE analysis looks at the nucleotides near the break site and allows for the molecular distinction between NHEJ with processing (Fig. 2B) and HR (Fig. 2C) repair in somatic tissues of whole flies[2,3].

Results

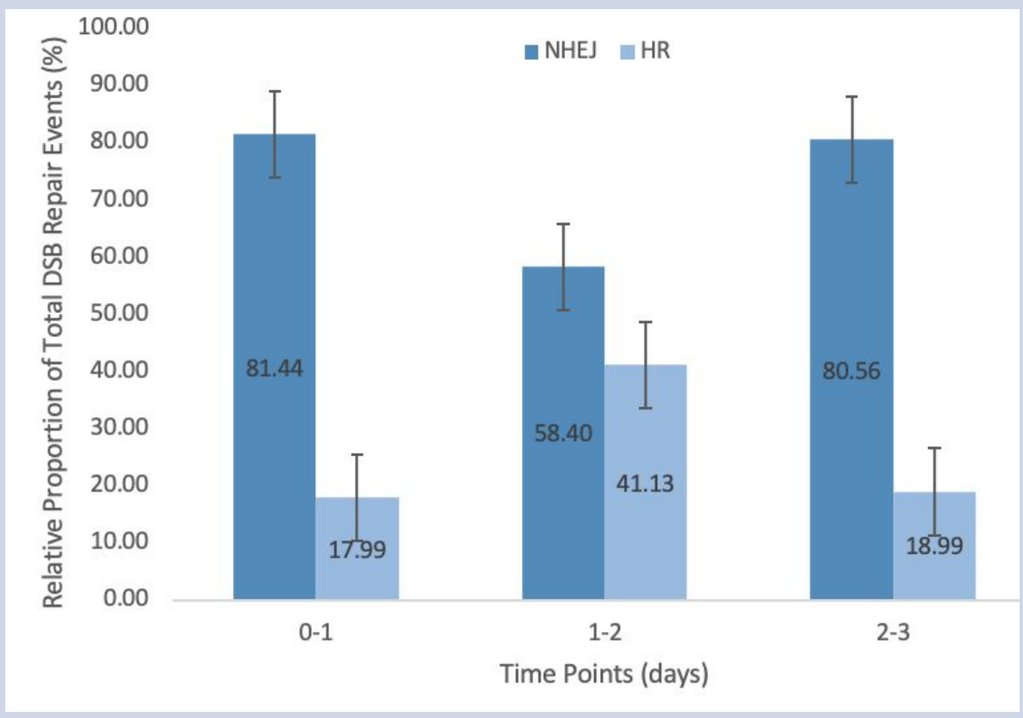

Figure 4: DSB repair in premeiotic germline. DR-*white* assay was used to examine phenotypic outcomes of DSB repair events. There is no significant impact of developmental stage on DSB repair pathway choice ($p > 0.05$, Student's T test; n = 12 germlines for each time point).

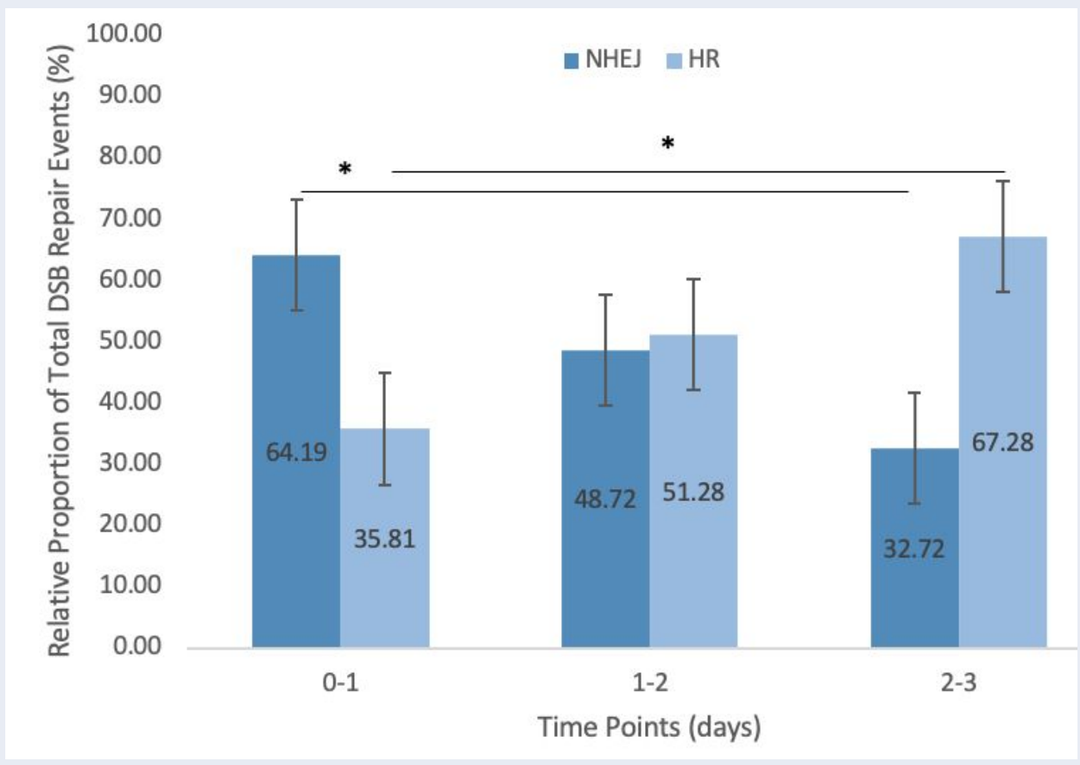

Figure 5: **Whole-fly somatic DSB repair.** DR-*white* followed with TIDE was used to examine repair pathway choice in somatic tissues. There is a significant increase in HR, and a proportional decrease in NHEJ, in 2.3 d.o. males compared to 0-1 d.o. males (*$p < 0.01$, Student's T test; n= 5-7 samples per age).

Conclusions and Future Directions

- There is no developmental stage dependent difference in DSB repair pathway choice in the premeiotic germline.
- Developmental stage plays an important role in repair pathway choice in somatic tissue, with embryos performing significantly less repair by HR than larvae.
- The use of developmental stage as a variable in DSB repair pathway choice is important for examining repair in other tissue types.
- Future Directions: More replicates of TIDE data analysis will have to be performed to confirm these findings.

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2. Brinkman, E. K., Chen, T., Amendola, M., & van Steensel, B. (2014). Easy quantitative assessment of genome editing by sequence trace decomposition. Nucleic Acids Research, 42(22), e168. doi:10.1093/nar/gku936
3. Fernandez, J., Bloomer, H., Kellam, N., & LaRocque, J. R. (2019). Chromosome preference during homologous recombination repair of DNA double-strand breaks in drosophila melanogaster. G3 (Bethesda, Md.), 9(11), 3773-3780. doi:10.1534/g3.119.400607