Functional study of *DPLG3*, a *PIF* transposable element domesticated protein, in *Drosophila melanogaster*

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Background

Transposable elements (TEs) are selfish genetic sequences that move and amplify using both their encoded proteins and the host machinery (Feschotte and Pritham, 2007). In rare cases, TE insertions are beneficial. An example is 'molecular domestication,' where a TE's protein-coding region is co-opted by the host, acquiring cellular functions (Sinzelle et al., 2008; Markova et al., 2022).

PIF/Harbinger elements (Figure 1) have been domesticated across multiple *Drosophila* lineages (Casola et al., 2007). We are investigating *DPLG3*, a *PIF*-like gene in *Drosophila melanogaster* derived from a *PIF* TE. *DPLG3* is located on the third chromosome, is intronless, and is evolving under purifying selection (dn/ds=0.09; Casola et al., 2007).

The *PIF-Harbinger-IS5* superfamily of DNA TEs: a special transposition mechanism

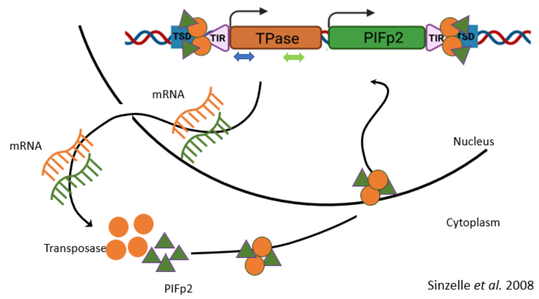

Figure 1. The *PIF/Harbinger* superfamily of DNA TEs possess two distinct open reading frames that produce two separate proteins: one being transposase, and the other protein containing a MADF domain. The complex formed by the interaction of the two proteins enters the nucleus and enables transposition (Sinzelle et al., 2008).

DPLG3 Knockout mutants show a rudimentary phenotype

Knockout (KO) flies for *DPLG3* were generated using CRISPR-Cas9 technology. The gene has been replaced by a red fluorescent protein expressed in the eyes (Ruma, unpublished; See Figures 2 and 3)

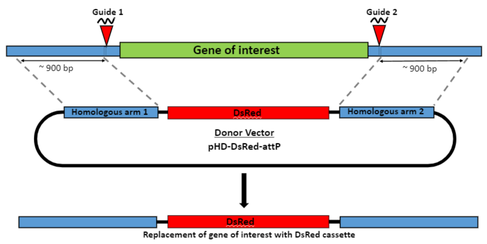

Figure 2. Approach to replace *DPLG3* gene using CRISPR-Cas9.

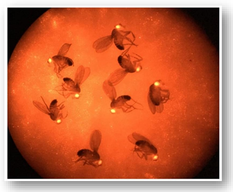

Figure 3. *DPLG3* KO flies expressing DsRed fluorescent protein.

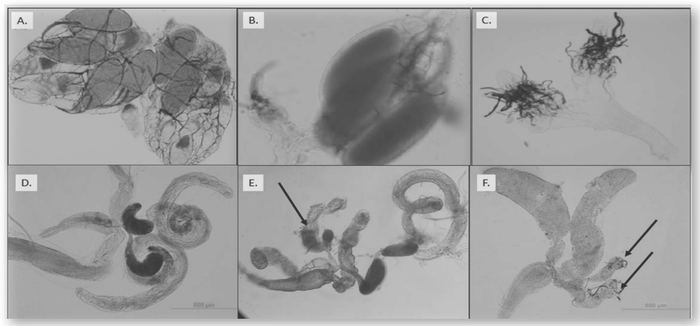

Figure 4. Categories of abnormal phenotype in *DPLG3*-KO flies

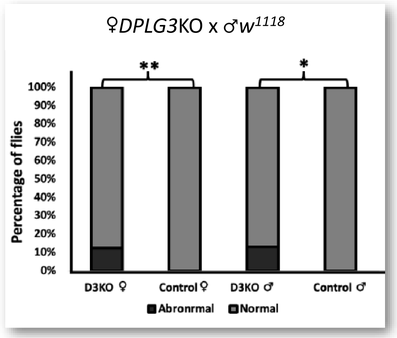

Figure 5. Frequency of abnormal phenotype in females and males. The statistical significance was tested using a Fisher's exact test and the probabilities are shown (* P<0.05; ** P<0.01; *** P<0.001, **** P<0.0001 or not significant.

DPLG3-KO phenotype is maternally inherited, always observed when the mother is *DPLG3*-KO/*DPLG3*-KO, this phenotype can be completely rudimentary or asymmetrical for both sexes (Figure 4). It is also partially penetrant, observed in 10-15% of females, and in a smaller fraction in males (Figure 5).

DPLG3 localization in oogenesis

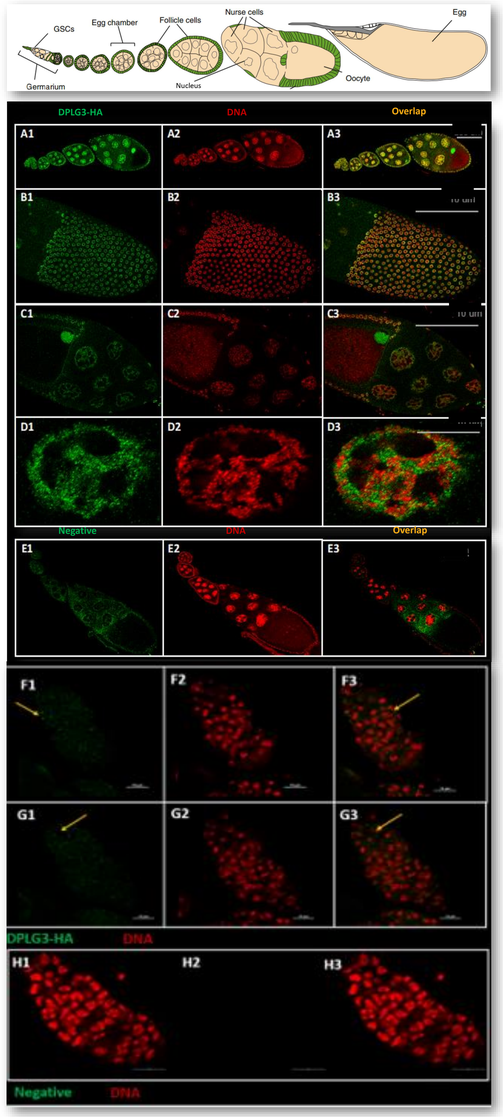

Figure 6. Oogenesis in *D. melanogaster* (Handler et al., 2013) and DPLG3-HA localization in ovaries (A-B: in germarium and somatic cell, C-D: during later stages of oogenesis, in nucleus of nurse cells and oocyte, E: negative control). (F and G) *DPLG3*-HA protein localization of the cytoplasm of the germarium. (H) Negative control. (Ruma, unpublished)

RNA-Seq analyses in normal ovaries

RNA was obtained from 40 ovaries for three independent backcrosses of *DPLG3*-KO and three controls (Ruma, unpublished). The results of the differential gene expression (DE) analyses in *DPLG3*-KO ovaries shows 86 upregulated and 37 downregulated genes (Figure 7)

- 26 digestive system+testis genes
- 14 testis-specific genes
- 20 nervous system genes
- The flamenco locus is upregulated 4 times (log2fold of 2)
- The highest downregulated gene is *CG42857,* has a role in primordial germ cells

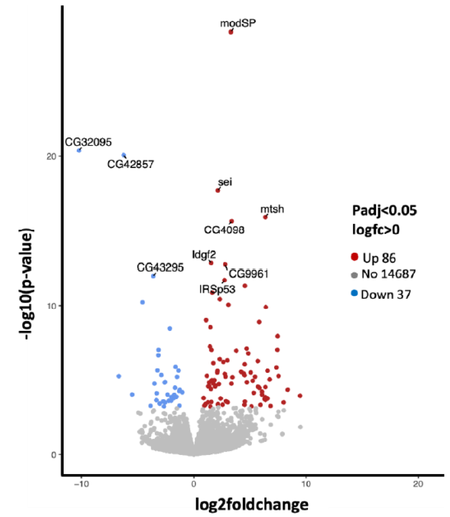

Figure 7. RNAseq differentially expressed genes in non-rudimentary *DPLG3*-KO ovaries compared to w^{1118} (Ruma unpublish).

The digestive system+testis-specific gene are part of the male-specific masculinization of the intestinal metabolism that promotes food intake and sperm development (Hudry, et al. 2019).
The rudimentary phenotype in *DPLG3*-KO ovaries could be explained by the differential expression of male-specific genes that are not typically expressed in females.

Conclusions & future experiments

- Because DPLG3 localizes in the nucleus and still conserves its DNA binding domain, it might be acting as a transcription regulator.
- The rudimentary phenotype found in *DPLG3*-KO is recessive, maternally inherited and partially penetrant affecting mainly the females flies in *D melanogaster* and smaller portion of males.
- We infer that DPLG3 is important for oogenesis. We believe that the non-rudimentary *DPLG3*-KO gonads are close to becoming rudimentary because of the expression of some male-specific genes. If that is the case, the missexpression of any of the male-specific genes should occur early in gonad development and we will test that by doing RT-PCR for the early stages of oogenesis in young *DPLG3*-KO females.
- Currently, we are trying to rescue the abnormal phenotype with a recombineering *DPLG3*-HA line. We should observe the rescue of the differentially expressed genes in the *DPLG3*-KO normal ovaries and the TEs expression changes as well.
- Using the Gal4 system, a more precise rescue experiment with a construct with only *DPLG3* will be used to confirm that *DPLG3* loss is the cause of the observed abnormal gonads and other effects.
- We also plan to produce knockdowns in germline and pole cells, i.e., germline precursors, and see if we observe the KO rudimentary phenotype.

References

1) Casola C, Lawing AM, Betran E, Feschotte C. 2007. Mol Biol Evol 24:1872-18881. **2)** Feschotte C, Pritham EJ. 2007. Annual Review of Genetics 41:331-368. **3)** Handler D, Meixner K, Pizka M, Lauss K, Schmied C, Gruber FS, Brennecke J. 2013. Molecular cell 50(5), 762-777. **4)** Hudry B, de Goeij E, Mineo A, Gaspar P, Hadjieconomou D, Studd C, Mokochinski JB, Kramer HB, Plaçais P-Y, Preat T. 2019. Sex differences in intestinal carbohydrate metabolism promote food intake and sperm maturation. Cell 178:901-918. e916. **5)** Markova DN, Ruma FB, Casola C, Mirsalehi A, Betrán E. 2022. Recurrent co-domestication of PIF/Harbinger transposable element proteins in insects. Mobile DNA 13:28. **6)** Ruma F. Unpublished. PIF transposable elements domestication and genetic novelty in insects. Ph.D. dissertation. **7)** Sinzelle L, Kapitonov VV, Grzela DP, Jursch T, Jurka J, Izsvak Z, Ivics Z. 2008. Proc Natl Acad Sci USA 105:4715-4720.

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