Exploring the functionality of *PIF* transposase-derived genes in *Drosophila*

Chathuri Devmika Wickramasinghe[1#], María del Pilar Castellanos[1], Diwash Jangam[1], Fatema B. Ruma[1], Satyam Srivastav[2], Cédric Feschotte[2] and Esther Betrán[1]

[1] Department of Biology, University of Texas at Arlington [2] Department of Molecular Biology and Genetics, Cornell University [#] Presenter

Background

Transposable Elements (TEs) are selfish genetic units with the ability to move and increase their copy numbers within a host genome, often at the expense of the host (Casola *et al*. 2007). However, TEs can benefit the host genome by providing a source of raw material for new host genes, through 'molecular domestication' (Feschotte and Pritham 2007).

We have found several *PIF*/*Harbinger* TE-derived genes in insects (Markova *et al*. 2022; Casola *et al*. 2007). In *Drosophila*, there are seven *PIF* TE-derived genes known as *Drosophila PIF-Like Genes* (*DPLG1-7)*. Only four *DPLG*s (*DPLG1-4*) are present in *D. melanogaster* (Casola *et al*. 2007). Here, we focus on the female germline functions of *DPLG1* and *DPLG4*.

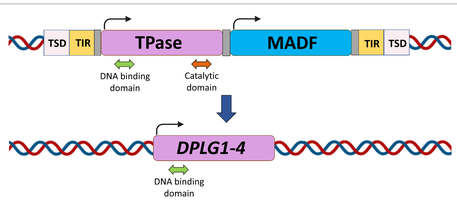

Figure 1: It is likely that *DPLG*s lost the catalytic activities of the ancestral transposases, but retained the DNA binding ability, possibly via MADF proteins.

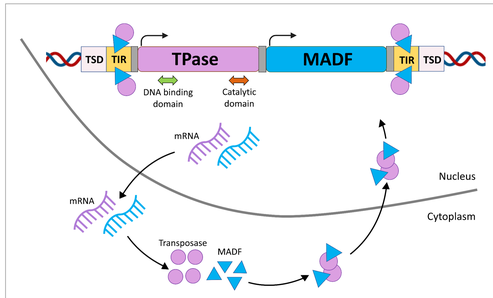

Figure 2: PIF/Harbinger superfamily of DNA TEs contain two open reading frames predicted to encode two different proteins; transposase and a protein with a MADF domain (Sinzelle *et al*. 2008; Walker *et al*. 1997; Zhang *et al*. 2004). The two proteins have been shown to interact with each other, forming a complex that enters the nucleus to facilitate transposition (Sinzelle *et al*. 2008).

DPLG1 and *DPLG4* in *Drosophila melanogaster*

DPLG1 (on Chr 2L) and *DPLG4* (on Chr 3L) are evolving under strong purifying selection (with K_A/K_S ratios of 0.07 and 0.047 respectively), and were domesticated 65 and 161 Mya, respectively (Markova *et al*. 2022; Casola *et al*. 2007). Both *DPLG1* and *DPLG4* show high expression in gonads (mainly ovaries) and nervous system (Brown *et al.*, 2014; Casola *et al.* 2007).

To study the potential functions of *DPLG1* and *DPLG4*, we have generated knock-out (KO) lines using CRISPR-Cas9 technology. In addition, we have generated DPLG1-HA and DPLG4-HA tagged proteins and have studied their localization in ovaries.

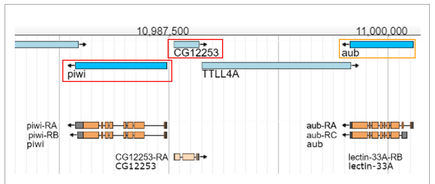

Figure 3: *DPLG1* and *piwi* are located in head-to-head orientation, on the Chr 2L, where *DPLG1* is only 402 bp upstream of *piwi*. Piwi is a major protein in piRNA pathway, involved in silencing TEs in *Drosophila* germline (Ozata *et al.*, 2019). This arrangement could have facilitated domestication of *DPLG1* and its potential involvement in the piRNA pathway.

Figure 4: KO gene replacement with DsRed.

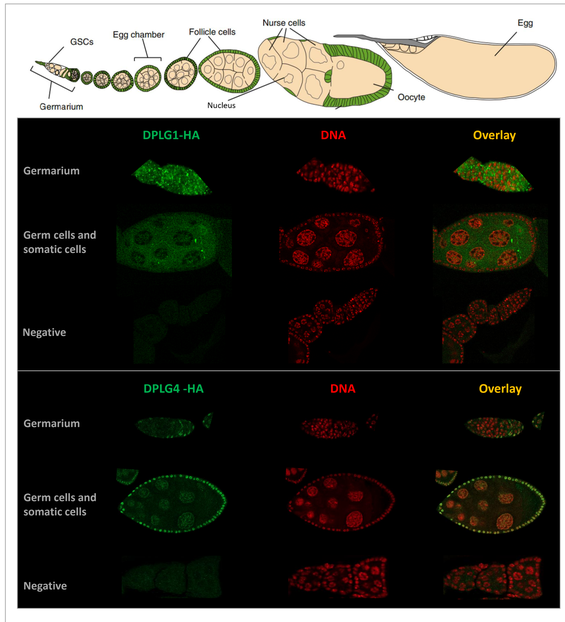

Figure 5: Oogenesis in *D. melanogaster* (Handler *et al*. 2013), DPLG1-HA and DPLG4-HA localization in ovaries. **DPLG1-HA**: germarium, nucleus of germline cells, nurse cell cytoplasm, and ring canals. **DPLG4-HA**: posterior part of the germarium, nucleus of the nurse cells, oocyte and follicular cells.

Conclusions: DPLG1 or DPLG4 tagged protein localization with DNA in the nucleus of ovarian cells suggests their role as regulators of transcription. We propose that *DPLG1* and *piwi* might share regulatory elements and be functionally associated.

Future Experiments: We aim to investigate the binding sites and molecular interactions of DPLG1 and DPLG4 with other proteins across various cell types within the gonads, using immunostaining and the CUT&RUN technology.

Potential functions of *DPLG1 and DPLG4* in gonads

DPLG1-KO flies exhibited a decrease in embryonic viability but an increase in postembryonic viability, compared to the control (w^{1118}) flies. *DPLG4* knockout flies showed significantly lower viability in both embryonic and post-embryonic stages.

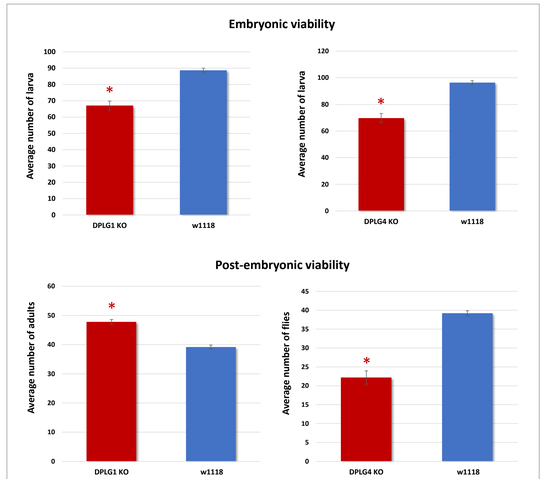

Figure 6: Embryonic and post-embryonic viability effects in *DPLG1* and *DPLG4* mutant flies. (* statistically significant)

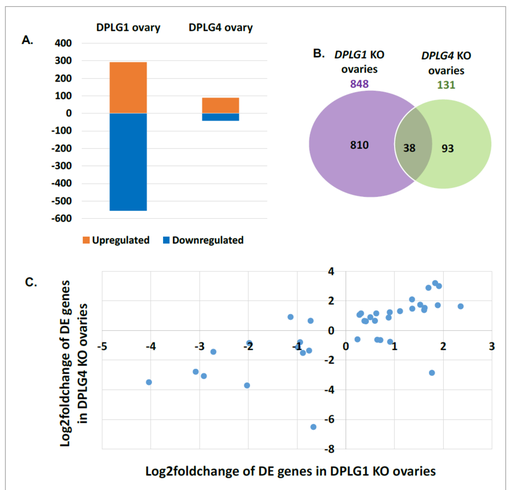

Figure 7: (A) Number of upregulated and downregulated genes in *DPLG1*-KO and *DPLG4*-KO ovaries. **(B)** Venn diagram showing overlap of DE genes in *DPLG1*-KO and *DPLG4*-KO ovaries. **(C)** Comparison of Log2foldchange of overlapping DE genes between *DPLG1*-KO and *DPLG4*-KO ovaries (upregulated DE genes were associated with general development, nervous system development, oogenesis, and gamete generation, while downregulated DE genes were related to translation, metabolic processes, and electron transport chain).

RNA-Seq analysis from ovaries of mutant flies showed a significant overlap of 38 DE genes between *DPLG1*-KO and *DPLG4*-KO ovaries, with positively correlated change in expression. Several upregulated and downregulated genes associated with piRNA pathway were also found among DE genes in *DPLG1*-KO ovaries.

In both *DPLG1*-KO or *DPLG4*-KO ovaries, various TE families showed differential expression, with *Gypsy* LTR retrotransposons being upregulated and telomeric non-LTR retrotransposons being downregulated. Interestingly, preliminary small RNA-Seq analysis revealed increased piRNAs targeting telomeric elements, possibly explaining the decrease in telomeric element expression observed.

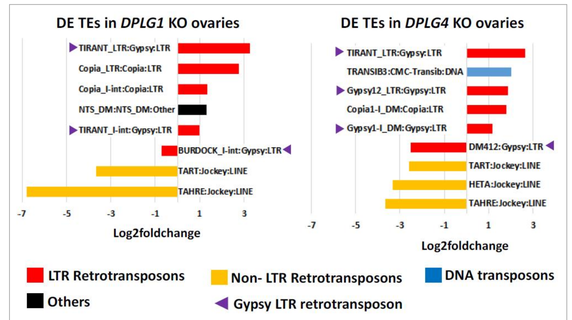

Figure 8: Differential TE expression analyses of *DPLG1*-KO and *DPLG4*-KO ovaries.

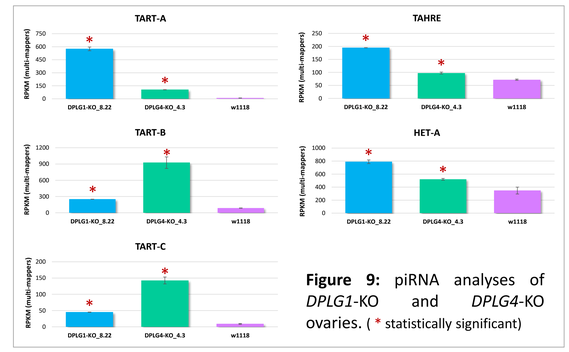

Figure 9: piRNA analyses of *DPLG1*-KO and *DPLG4*-KO ovaries. (* statistically significant)

Conclusions: Our findings imply functional interactions between *DPLG1* and *DPLG4*, and their potential regulatory functions in *Drosophila* telomere elongation via the piRNA pathway.

Future Experiments: We aim to further analyze telomeric elements in KO flies using qPCR to assess copy number changes, expression, etc., and investigate the co-localization of DPLG1 and DPLG4 with telomere proteins through immunostaining.

References

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Acknowledgements

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