Investigating the role of a somatic piRNA pathway in *Drosophila melanogaster* fat body function

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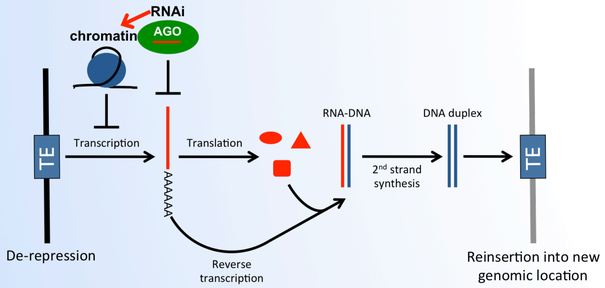
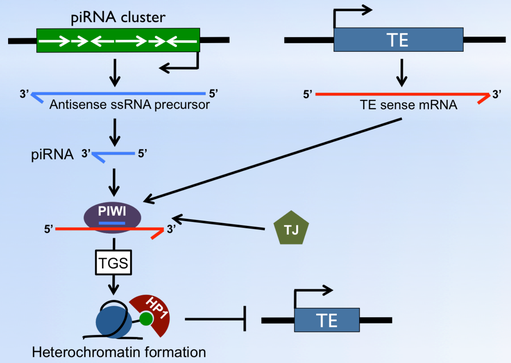
Introduction: Transposable Elements

Transposable elements (TEs) are parasitic mobile sequences of DNA that can proliferate throughout the genome by replicating and reinserting themselves into new regions of DNA. This process can disrupt normal gene function via insertional mutations, double stranded DNA breaks, apoptosis and chromosomal rearrangements. They also contribute to genetic variation and alteration to gene regulation. TEs are of medical interest as they have been associated with cancer, neurodegeneration, and natural aging. Previous studies have estimated that TEs can account for approximately 44% of the human genome. Fortunately, molecular defenses involving small RNA-mediated silencing of foreign transcripts provide genomic regulation so that less than 0.05% of all TEs are active.



The piRNA Pathway

The piRNA pathway silences TEs primarily in the gonads. piRNAs are small 23-29 nucleotide small RNAs and are transcribed from regions of the genome called piRNA clusters. piRNAs associate with the P-element induced wimpy testes (PIWI) protein. PIWI, driven by the transcription factor Traffic jam (TJ), uses its bound piRNA to target homologous nascent TE transcripts in the nucleus and recruits the chromatin-silencing factors H3K9me3 and HP1. TE suppression by the piRNA pathway has long been considered to be specific to the gonadal tissues, serving as vital protection of genomic integrity during gametogenesis and development. Here we present evidence that this pathway is also present and functional in the *Drosophila* abdominal fat body and that it is necessary for normal fat body function.



1. TJ drives PIWI expression
2. piRNAs are loaded onto PIWI and this complex then finds its homologous TE target
3. The PIWI complex recruits HP1 and the trimethylation of H3k9 (an epigenetic mark), resulting in transcriptional gene silencing
4. TEs are repressed

Results

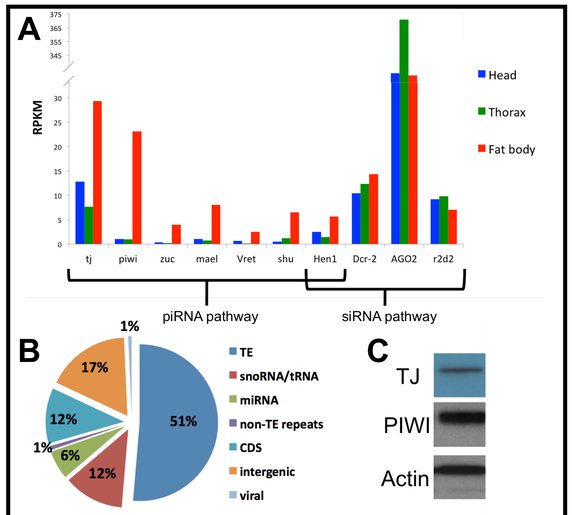


Figure 1. A somatic piRNA pathway is present in the fat body. **A)** Total RNA-seq data from three somatic tissues indicates that many gene transcripts of the piRNA pathway are highly expressed in the fat body relative to thorax and head tissues. **B)** 51% of 23-29nt smRNAs in the fat body were found to map to TEs. **C)** Western blot indicates that both PIWI protein and its transcription factor TJ are present in the fat body tissue.

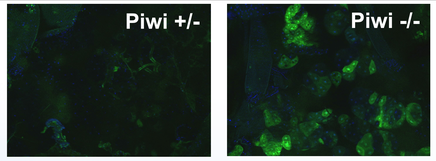


Figure 2. Loss of Piwi results in increased transposition in the fat body as shown by a GFP reporter. GFP-positive cells indicate transposition of the *gypsy* TE.

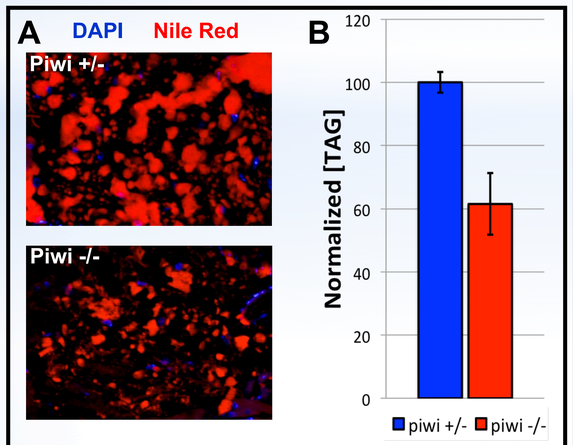


Figure 3. piRNA pathway mutants are deficient in lipid stores. **A)** Visualization of the lipid droplets from dissected fat body using Nile Red staining indicates that Piwi mutants have smaller lipid droplets. **B)** Metabolic assays indicate a significant decrease in triacylglyceride (TAG) concentrations in homozygous mutants relative to heterozygotes.

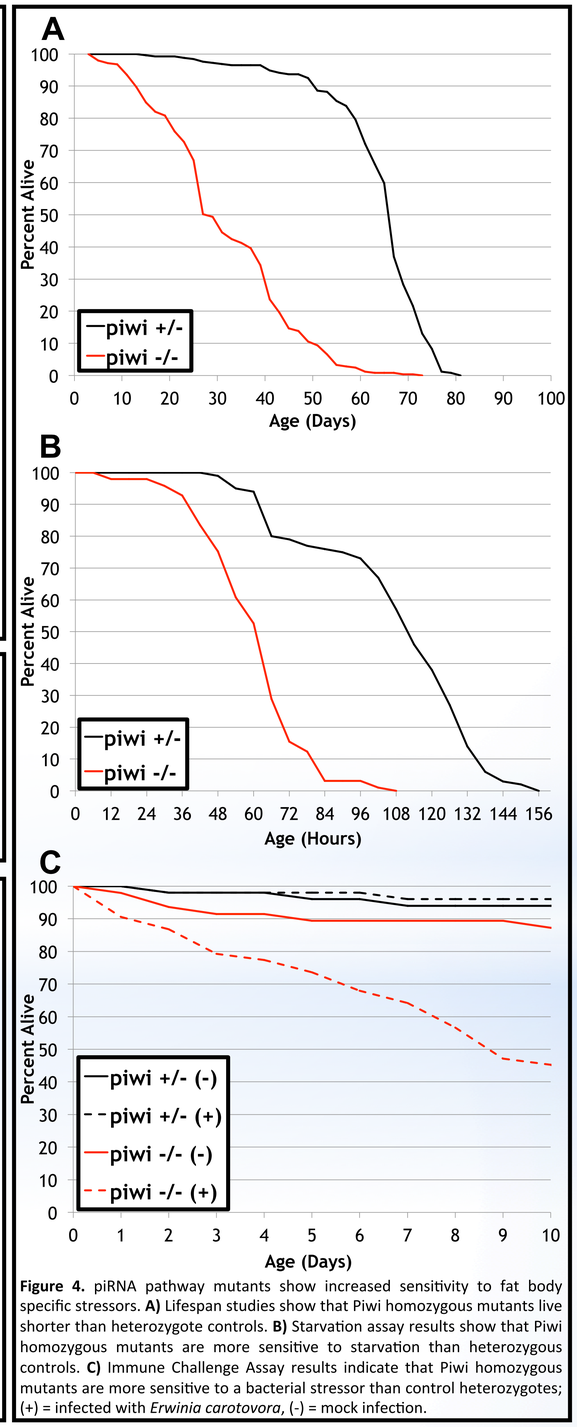


Figure 4. piRNA pathway mutants show increased sensitivity to fat body specific stressors. **A)** Lifespan studies show that Piwi homozygous mutants live shorter than heterozygote controls. **B)** Starvation assay results show that Piwi homozygous mutants are more sensitive to starvation than heterozygous controls. **C)** Immune Challenge Assay results indicate that Piwi homozygous mutants are more sensitive to a bacterial stressor than control heterozygotes; (+) = infected with *Erwinia carotovora*, (-) = mock infection.

Hypothesis

If the piRNA pathway has an impact on fat body function in *Drosophila melanogaster*, then I hypothesize that piRNA pathway mutants will have compromised fat body health. I will test this by performing experiments that measure physiological health as a proxy for fat body function: lifespan, stress resistance, and lipid droplet size.

Conclusions

As an analogue to the adipose tissue and liver of vertebrates, the fat body of *Drosophila melanogaster* is an important organ for energy storage, metabolism, and enantiostasis. We have shown the presence of elements of the piRNA pathway in the somatic fat body (piRNA, RNA transcripts, and protein). Comparable transcript levels of the siRNA pathway, which also silences TEs, suggest that the increased enrichment of the piRNA pathway observed in the fat body is specific to this pathway and tissue. The lipid droplets of Piwi mutants were visibly smaller than the heterozygote controls and a metabolic assay confirmed a significant decrease in relative % concentration of TAG in Piwi -/-. In starvation assays, Piwi -/- starve faster when compared to their heterozygous counterparts. Similarly, when exposed to a bacterial stressor, homozygous mutants exhibited increased sensitivity.

For future directions of this research, the potential for Piwi to target coding genes will be investigated. It is thought that TEs are the primary targets of Piwi. However, recent research has shown that genic piRNAs derived from 3'UTRs are able to target and deadenylate protein-coding mRNAs with incomplete complementarity. This may allow for Piwi to target protein-coding transcripts, despite mismatches between the transcripts and piRNA. We will determine whether Piwi can target coding genes in the fat body and subsequently whether mutation of the piRNA pathway would result in dysregulation of the coding genes.

In order to better understand the observed relationship between increased transposition and decreased fat body function, TEs will be overexpressed and knocked down in wild type flies using the GAL4/UAS system and resulting fat body function and lifespan will be evaluated.

References

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