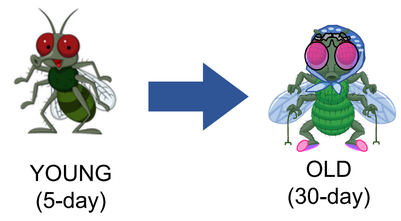
The 'moving target' of transposon landscape changes in aging Drosophila

Nachen Yang[1], Satyam Srivistav[1], Qicheng Ma[1], Reazur Rahman[2], Madoka Chien[3], Elissa P Lei[3], and Nelson C. Lau[1]

1: Boston University School of Medicine, Department of Biochemistry

2: Brandeis University, Department of Biology, 3: NIH / NIDDK

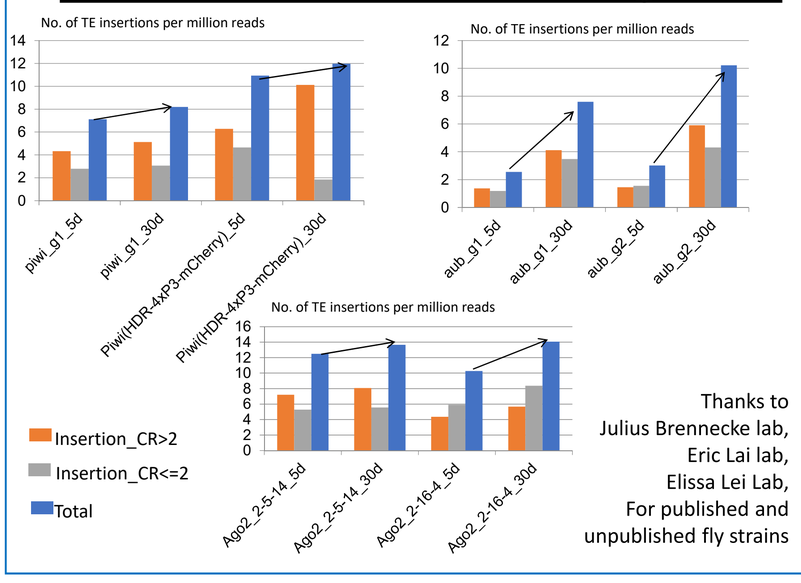


Abstract

Genetic mechanisms that strongly repress transposable elements (TEs) in young animals decline during aging because TE transcripts become reactivated. Does TE transcriptional reactivation during aging then alter and damage the genome? To test this hypothesis, we quantified Transposon Landscape (TLs) via deeply sequencing genomes of young and aged *Drosophila* strains of wild-type and mutant backgrounds. We quantified TLs in aging whole flies as well as dissected brains, and we validated the feasibility of our approach in detecting increases in new TE insertions in aging *Drosophila* genomes when RNAi and Piwi pathways are compromised.

By also incorporating droplet digital PCR as an important validation methodology for measuring genomic TE loads, we now show that genetic mutations that strongly reactivate TE RNA expression only exhibit modest genomic TL changes. Additionally, we examine a new frontier of extra-chromosomal DNA circles (eccDNAs) as a source of accumulating TE copies and describe new sequencing methods to quantify eccDNAs in *Drosophila*. Our analysis suggests that small RNA surveillance mechanisms still prevent genomic TL expansion despite the increase in transposon transcripts during aging. However, to combat the natural progression of increased TE expression during animal aging we show that knocking down the PAF1 complex that regulates RNA Pol II elongation and transcription termination, can reduce aging related TE expression increases.

Increased TE Insertions can be detected by WGS in some RNAi mutant backgrounds



Thanks to
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Eric Lai lab,
Elissa Lei Lab,
For published and unpublished fly strains

Can we intervene in TE transcript activation during fly aging with Gal4-UAS transgenic system?

- Gal4 (yeast transcriptional co-activator)
  Using Tublin-Gal4 ubiquitous driver
- Upstream Activation Sequence: UAS

enhancer to which Gal4 specifically bind to and activate gene expression

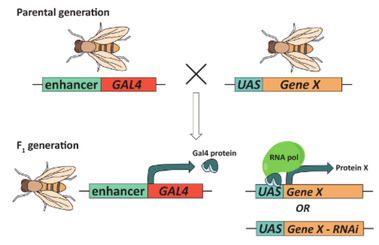
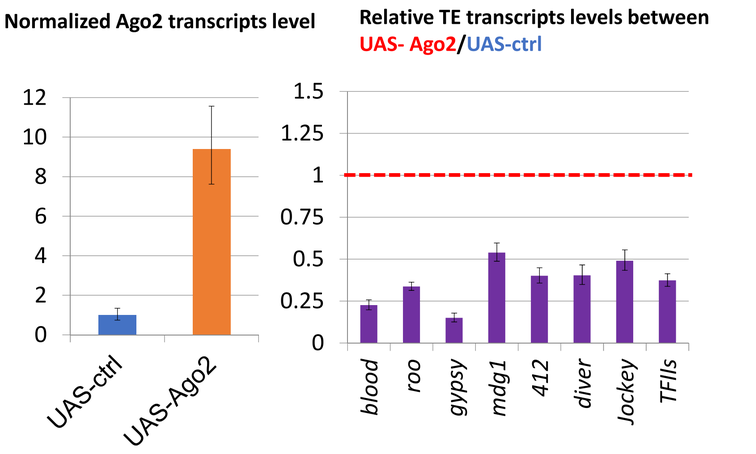


Image Citation: Kelly, S. M., Elchert, A., Kahl, M. *J. Vis. Exp.* (129), e56174, doi:10.3791/56174 (2017).

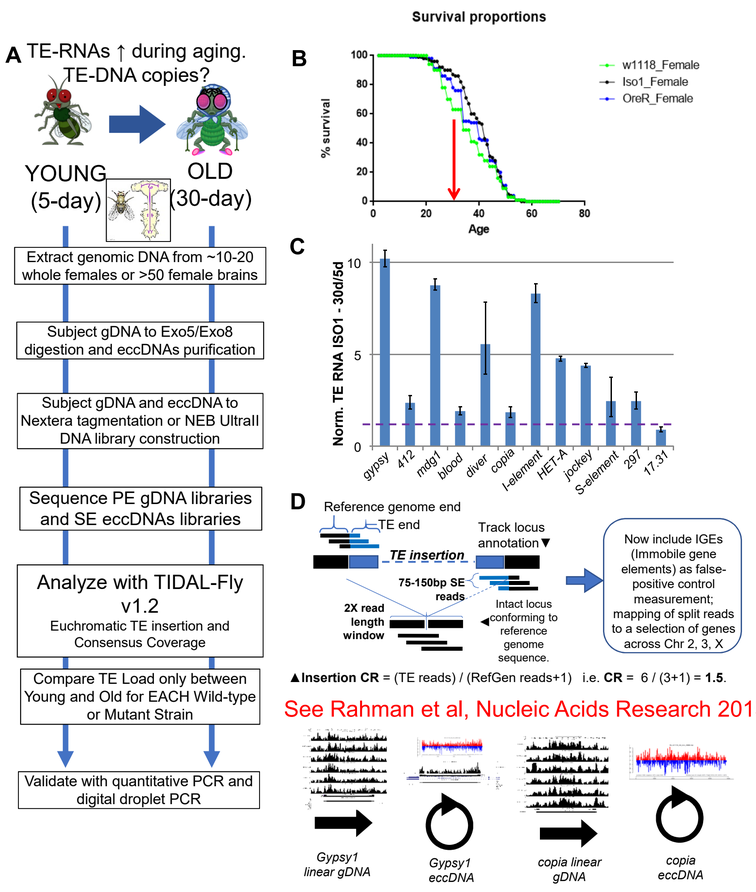
Turn up a target gene

Turn off a target gene

Overexpressing *dAgo2* can increase TE silencing in young adult flies

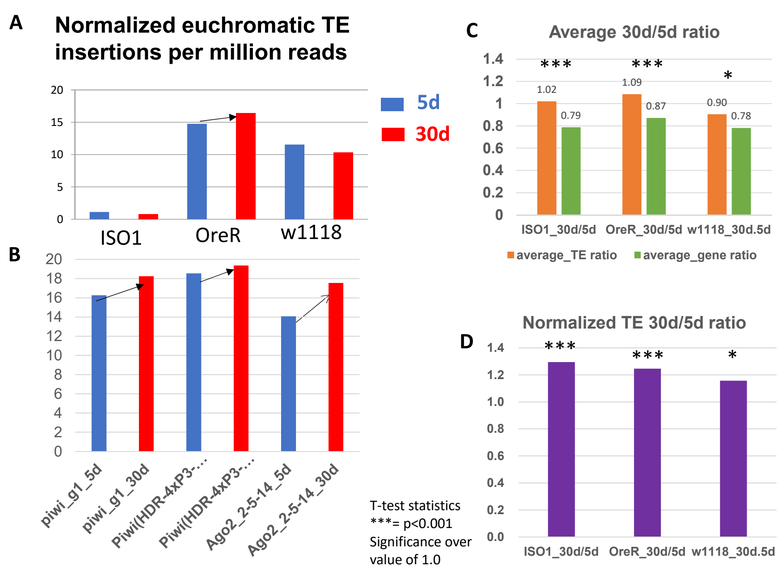


Testing the Transposon Hypothesis of Aging in *Drosophila*



TE RNAs clearly increase during aging, but how extensively are genomes affected?

WGS of fly brains can also detect somatic TE insertions during aging



T-test statistics
***= p<0.001
Significance over value of 1.0

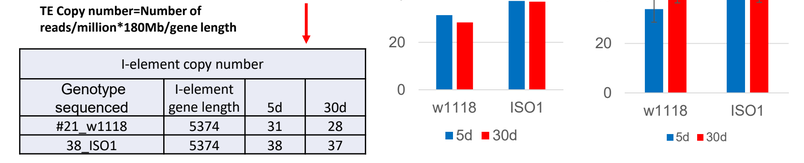
Quantification of TE copies by droplet digital PCR (ddPCR) as validation of WGS

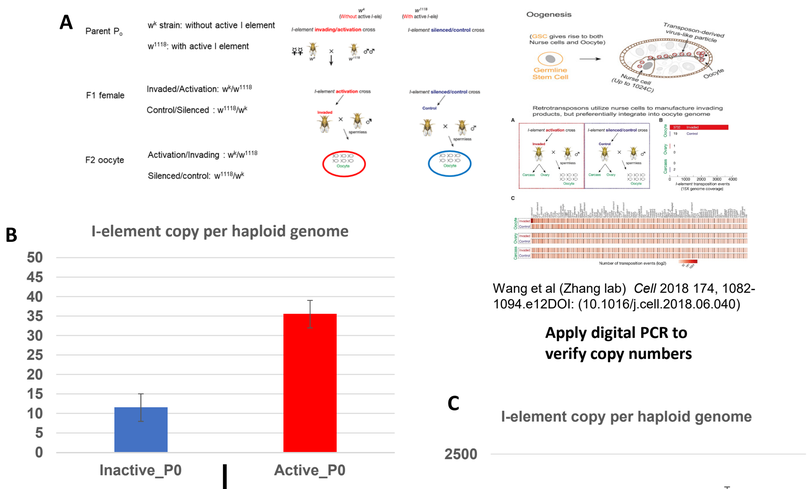
A

TIDAL consensus table RPM (reads per million)			
Genotype sequenced	I-element gene length	5d	30d
#21_w1118	5374	940	850
#38_ISO1	5374	1121	1111

TE Copy number=Number of reads/million*180Mb/gene length

I-element copy number			
Genotype sequenced	I-element gene length	5d	30d
#21_w1118	5374	31	28
38_ISO1	5374	38	37

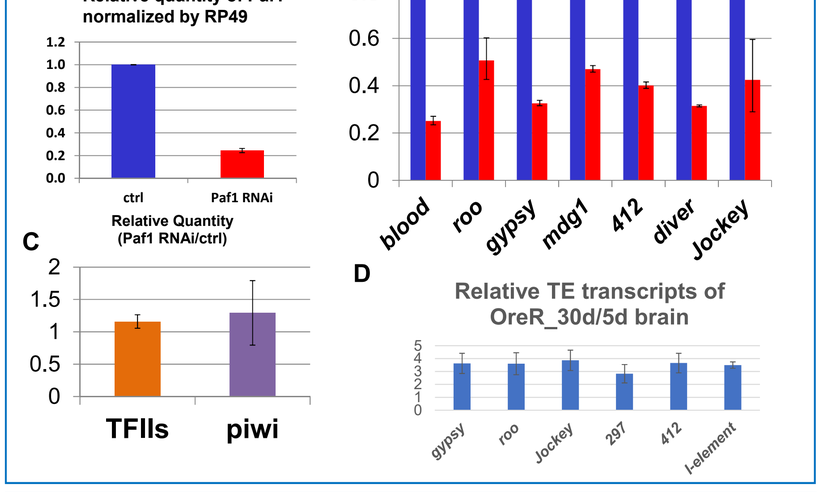




Wang et al (Zhang lab) *Cell* 2018 174, 1082-1094.e12DOI: (10.1016/j.cell.2018.06.040)

Apply digital PCR to verify copy numbers

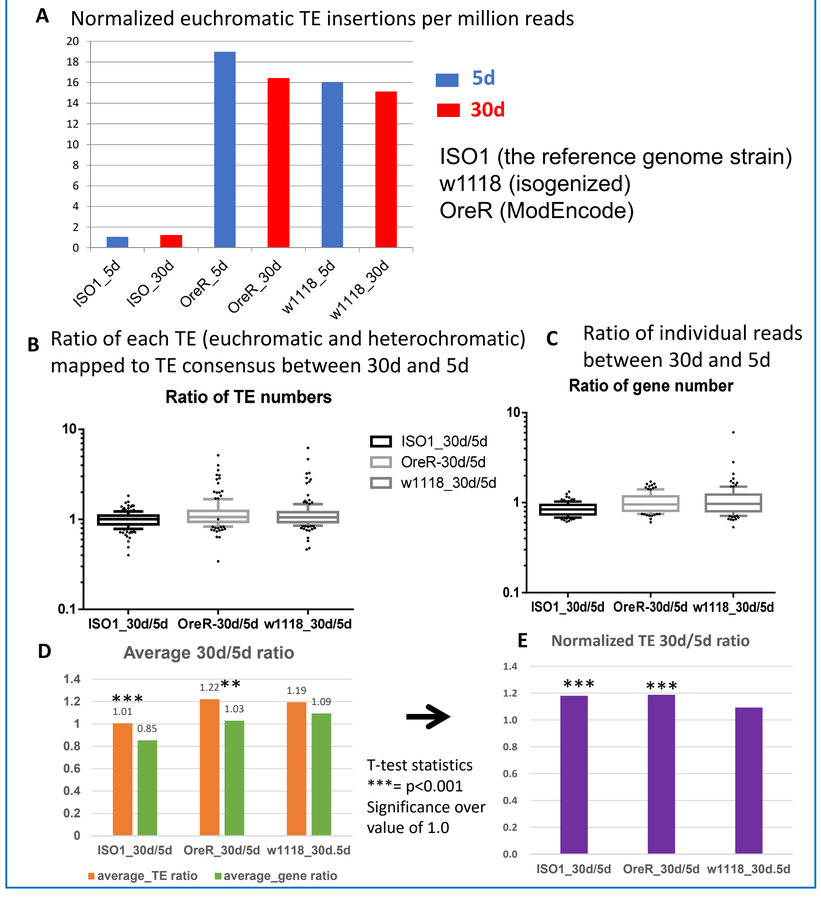
PAF1 complex antagonizes PIWI/piRNA silencing of transposons



Polymerase Associated Factor 1 (Paf1) complex regulates RNA Pol II transcription elongation rate and termination

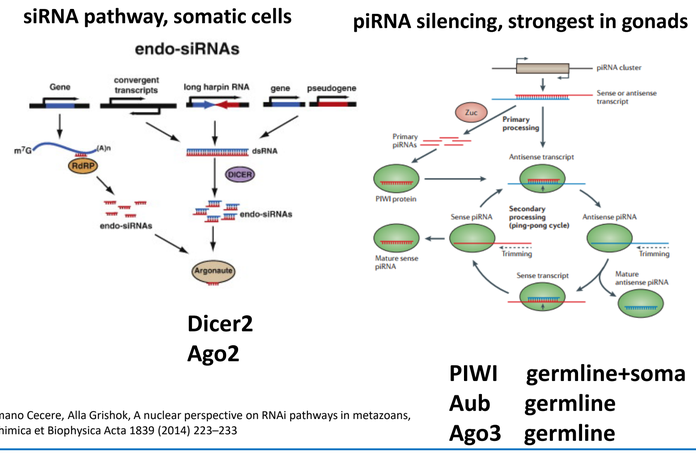
Clark et al, Current Biology, 2017

A WGS approach to measure genomic TE load in aging flies



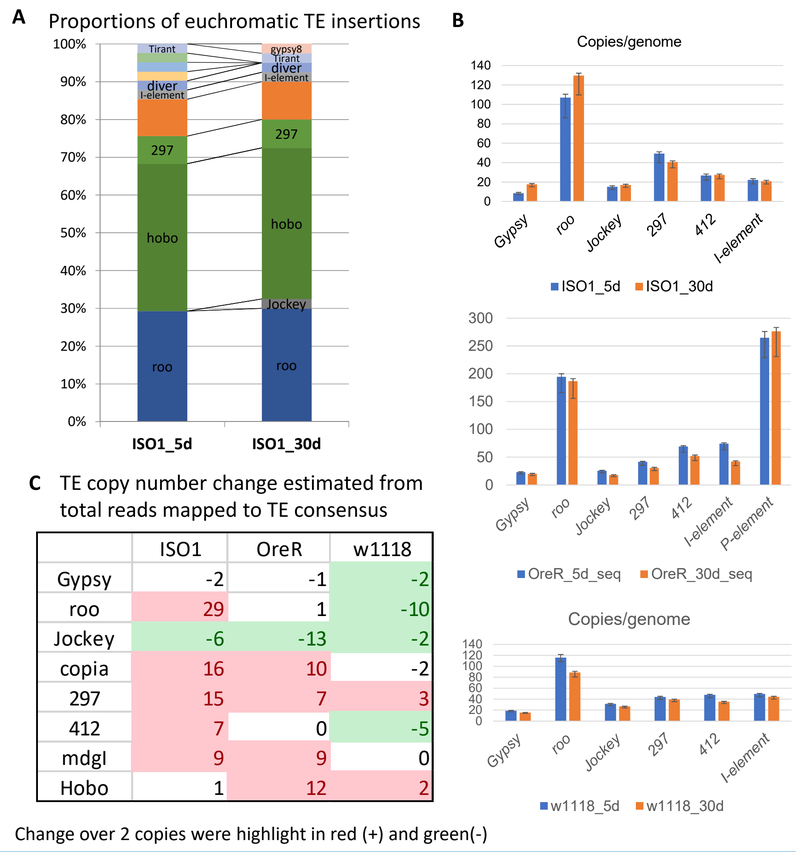
Knocking down PAF1 in flies also enhances silencing of transposons

Two major RNAi pathways for TE silencing in the fly soma and germlines



Germano Cecere, Alla Grishok, A nuclear perspective on RNAi pathways in metazoans, Biochimica et Biophysica Acta 1839 (2014) 223–233

Applying ddPCR and focused analysis on specific TE families during fly aging

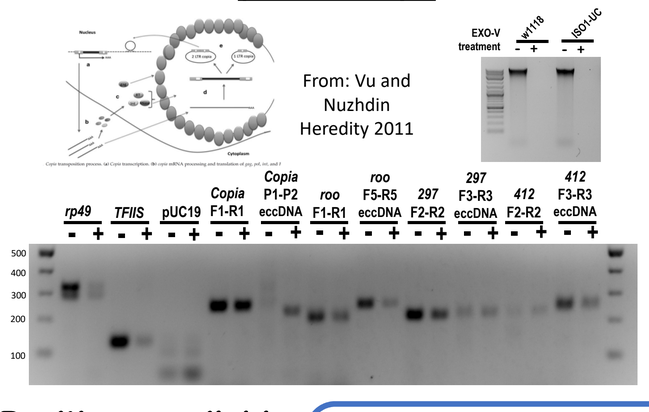


C TE copy number change estimated from total reads mapped to TE consensus

	ISO1	OreR	w1118
Gypsy	-2	-1	-2
roo	29	1	-10
Jockey	-6	-13	-2
copia	16	10	-2
297	15	7	3
412	7	0	-5
mdgl	9	9	0
Hobo	1	12	2

Change over 2 copies were highlight in red (+) and green(-)

Future direction: examine expanding TE copies via extra-chromosomal circular DNAs (eccDNAs)?



Positions available in Lau lab email: nclau@bu.edu

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