A Dual-activity Topoisomerase Complex Interacts with piRNA Machinery to Promote Transposon Silencing and Germ Cell Function

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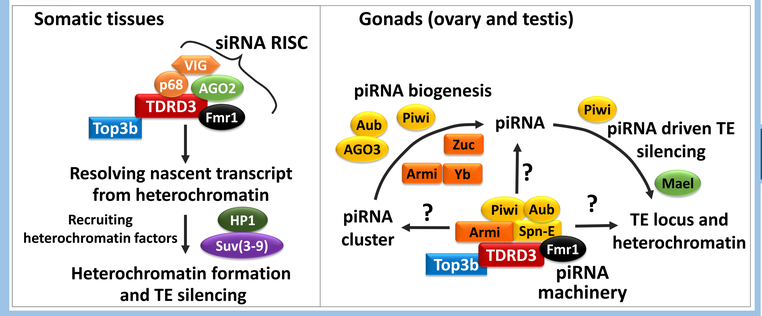
Background

Topoisomerase 3 beta (Top3β) is the only eukaryotic dual-activity topoisomerase that can change topology for both DNA and RNA. Current evidence suggests that Top3β can facilitate transcription on DNA and translation of mRNAs. Top3β forms a complex with Tudor domain containing 3 (TDRD3), which interacts with the Fragile X Mental Retardation Protein (FMRP) to regulate mRNA translation. *Top3β* mutation in human has been linked to schizophrenia, autism, and cognitive impairment, whereas Top3β inactivation in mice results reduced lifespan and abnormal neurodevelopment. However, the mechanism of how Top3β maintains normal life-span and mental health remains largely unclear.

We have recently shown that the Top3β-TDRD3 complex interacts with the siRNA machinery to facilitate heterochromatin formation and transcriptional silencing of genes and transposable elements (TEs). PIWI-interacting RNAs (piRNAs) are the other major class of small RNAs that are germ-cell specific and suppresses TEs. Here we demonstrate the functions of Top3β biochemically and genetically interact with piRNA mediated machinery in germ cells. First, Top3β and Tdrd3 form stable complexes with the piRNA machinery, including PIWI, in germline tissues of both mouse and *Drosophila*. Second, mutation of *Top3β* results in de-silencing of multiple transposons in gonads. Third, *Top3β* and piRNA machinery genetically interact to suppress expression of TEs. Together, our data reveal a novel role of Top3β-TDRD3 complex—interacting with the piRNA machinery to promote silencing of TEs and germ cell function

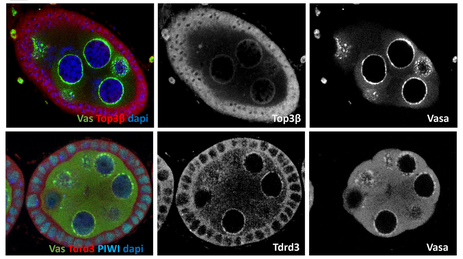


I. Top3β and TDRD3 interact with Piwi in *Drosophila* ovary

A Top3β and Tdrd3 are highly expressed in ovary and testis

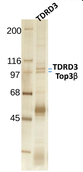
Loaded 3ug of total lysate/lane

B Localization of Top3β and Tdrd3 in fly ovary



C Top3β-TDRD3 biochemically interact with piRNA machinery including PIWI

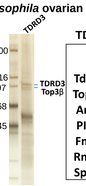
Drosophila ovary



TDRD3 IP-MS

	Unique peptides
Tdrd3	71
Top3β	54
Fmr1	32
AGO2	26
Rm62	22
Aub	19
PIWI	15

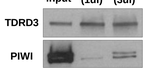
Drosophila ovarian somatic sheath cells



TDRD3 IP-MS

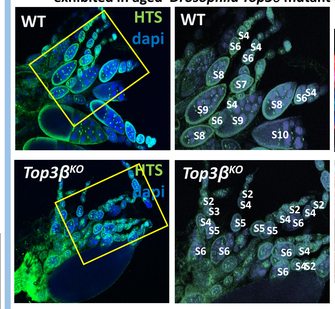
	Unique peptides
Tdrd3	65
Top3β	52
Armi	45
PIWI	32
Fmr1	31
Rm62	28
Spn-E	17

TDRD3 IP-WB



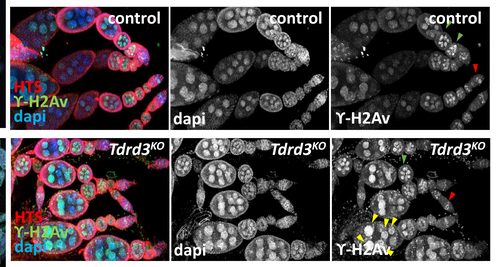
II. *Top3β* and *TDRD3* mutants show reduced fertility and delayed oogenesis in age-dependent manner

A Developmental delay and defective oogenesis are exhibited in aged *Drosophila Top3β* mutant ovary



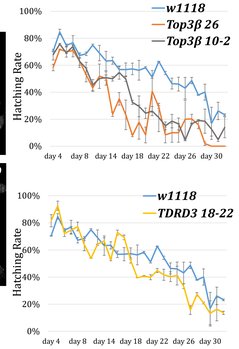
Top: 2 weeks old WT ovary show progress of development
Bottom: the same age Top3bKO exhibits stalling of development at stage 6-7

B *Tdrd3* mutant displays increased DNA damage in ovary



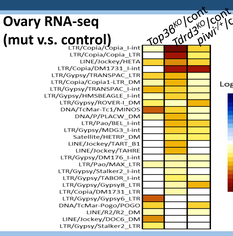
Top: 2 weeks old WT ovary show DNA DSB marker g-H2Ax only parts of germalium (red arrow) and early eggchamber (green arrow)
Bottom: the same age Tdrd3KO occationally exhibits a strong signals of r-H2Ax throughout all eggchambers

C Egg hatching rate is reduced in *Drosophila Top3β* and *Tdrd3* mutants in age-dependent manner

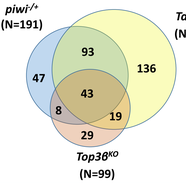


III. *Top3β* and *TDRD3* mutants exhibit de-silencing of TE

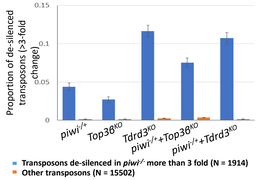
Ovary RNA-seq (mut v.s. control)



Overlap of de-silenced TEs in mutants

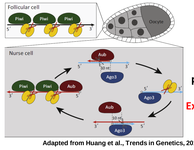


De-silencing of PIWI targeted v.s. PIWI non-targeted TEs



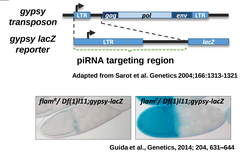
IV. *Top3β* and *Tdrd3* mutants exhibit de-silencing of TE and genetically interact with *piwi*

A piRNA biogenesis in germline and somatic origin



Adapted from Huang et al., Trends in Genetics, 2017, 33, 11

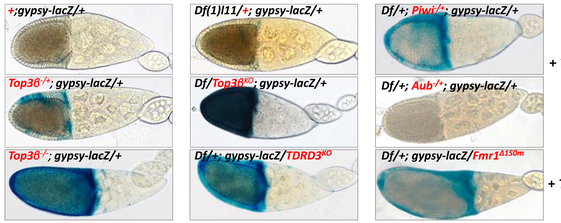
B *gypsy-lacZ* sensor



Adapted from Sarot et al. Genetics 2004;166:1313-1321

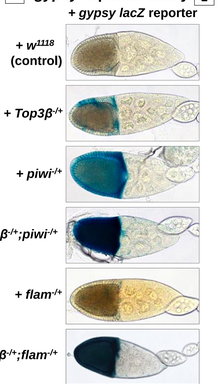
Guida et al., Genetics, 2014; 204, 631–644

C *Top3β* and *Tdrd3* mutant induces a strong *gypsy-lacZ* reporter activity

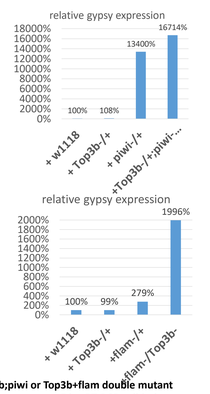


A-C: Using gypsy sensor reporter, *Top3b, Tdrd3, piwi* mutant dominantly de-silence gypsy lacZ sensor shown by b-gal staining (blue).

D *gypsy* reporter assay



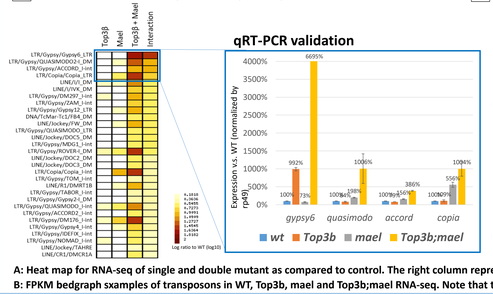
E qRT-PCR (*gypsy* transposon)



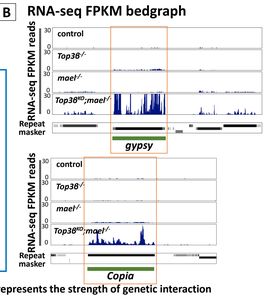
D-E: Using the same reporter, Top3b;piwi or Top3b+flam double mutant exhibit enhanced de-silencing of gypsy reporter. E is qRT-PCR validation

V. *Top3β* and *mael* mutant show synergistic effect on transposon silencing and oogenesis

A Genetic interaction by RNA-seq (ovary)



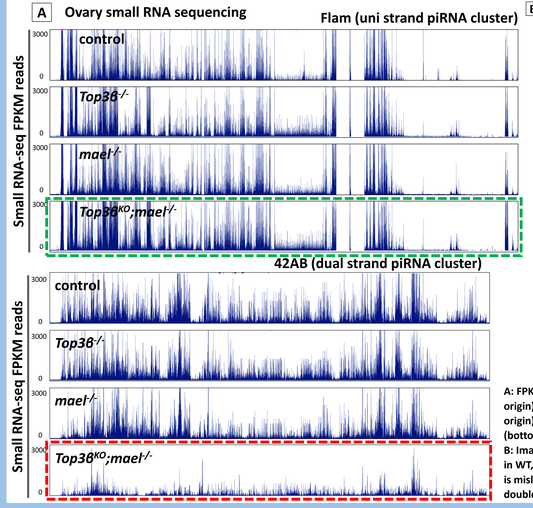
B RNA-seq FPKM bedgraph



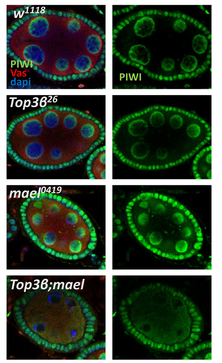
A: Heat map for RNA-seq of single and double mutant as compared to control. The right column represents the strength of genetic interaction
B: FPKM bedgraph sxamples of transposons in WT, Top3b, mael and Top3b;mael RNA-seq. Note that the double mutant has a very strong de-silencing

VI. *Top3β;mael* double mutant exhibits defective piRNA biogenesis and Piwi localization

A Ovary small RNA sequencing



B Oogenesis defect in *Top3β;mael*



A: FPKM bedgraph of flam (top, uni-strand, somatic origin) and 42AB (bottom, dual-strand, germline origin). Note that piRNA levels are low only in 42AB (bottom) but not flam locus (top)
B: Images of eggchamber stained with Piwi (green) in WT, *Top3b, mael* and *Top3b;mael*. Note that Piwi is mislocalized from nucleus to cytoplasm in the double mutant

Conclusions

- Top3β and TDRD3 interact with Piwi in mouse and *Drosophila* gonads
- Loss of Top3β and TDRD3 results in defective oogenesis and embryogenesis
- Top3β and Tdrd3 promote silencing of transposons in association with piRNA machinery
- *Top3β* genetically interacts with *mael* for transposon silencing and piRNA biogenesis

References

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Sarot, E., G. Payen-Groschene, A. Bucheton, and A. Pe´lisson. Evidence for a piwi-dependent RNA silencing of the gypsy endogenous retrovirus by the *Drosophila melanogaster flamenco* gene. Genetics, 166 (2004),1313–1321

V. Guida, F. Cernilogar, A. Filograna, R. De Gregorio, H. Ishizu, M. Siomi, G. Schotta, G. Bellenchi and D. Andrenacci. Production of small noncoding RNAs from the *flamenco* locus is regulated by the *gypsy* retrotransposon of *Drosophila melanogaster. Genetics*, 204 (2017), 631-644