Tudor5-like promotes germline differentiation through post-transcriptional gene regulation and maternal RNA regulation

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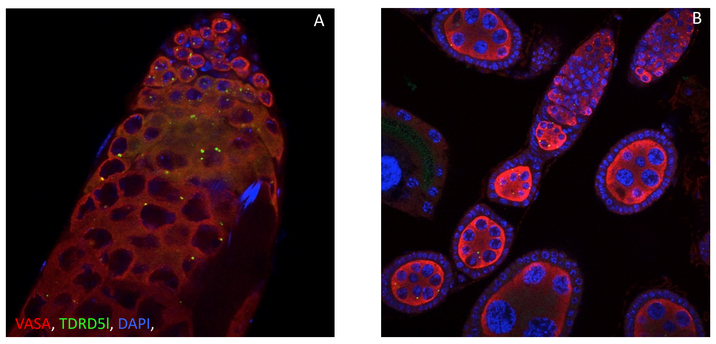
Abstract

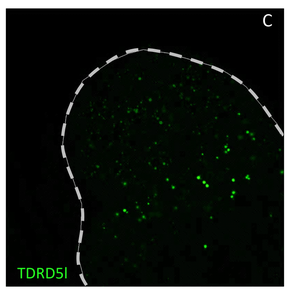
Tudor-domain containing proteins are conserved across the animal kingdom for their necessary functions in germline development including post-transcriptional gene regulation. Recent work in our lab identified Tudor5-like (Tdrd5l), which promotes male germline identity in germline stem cells (GSCs) in the testis, but is repressed by the RNA binding protein Sex lethal (SXL) in female GSCs. Interesting, Tdrd5l is also expressed in the differentiating germline in both sexes, indicating that it may also act to control germline differentiation in both sexes.

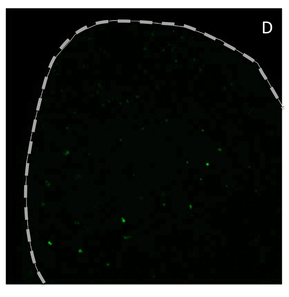
Previously we reported that Tdrd5l localizes to the periphery of a previously uncharacterized germline RNA granule in both sexes. In the males, numerous smaller Decapping protein 1 (Dcp1) granules co-localize with the periphery of the Tdrd5l granule. This suggests that Tdrd5l granules are docking with processing bodies, and could play a role in post-transcriptional gene regulation. To understand what RNA regulatory pathway Tdrd5l functions in, we conducted RNAi against the deadenylase *twin* in mutant gonads, which revealed a genetic interaction between *tdrd5l* and the CCR4-NOT deadenylation complex. In both males and females, knockdown of *twin* in *tdrd5l* mutants results in sterility. Additionally, we found similar genetic interactions between *tdrd5l* and both *dcp1* further suggesting a role for *tdrd5l* in post-transcriptional gene regulation. Recent investigation into the role Tdrd5l plays in the female germline suggests that Tdrd5l could function in maternal RNA deposition. Interestingly, Tdrd5l localizes to a single large granule at the anterior of the oocyte directly adjacent to the ring canals. To further investigate this possibility we stained for Gurken(Grk) in *tdrd5l* mutant ovaries and wild type ovaries. In wild type flies we see normal Grk translation in the anterior dorsal corner of the oocyte, while in *tdrd5l* mutant ovaries we see translation of Grk in the nurse cells. Additionally eggs laid by *tdrd5l* mutants have a decreased hatch rate and dorsal appendage defects consistent with *grk* misregulation. This suggests the loss of maternal RNA regulation by Tdrd5l could result in patterning defects. Lastly, we immunostained for Oskar(osk) in *tdrd5l* mutants and saw mislocalized osk, suggesting that Tdrd5l could be responsible for regulating multiple maternal RNAs. Currently we are conducting FISH to investigate at what point maternal RNAs such as grk and osk are mis-regulated in *tdrd5l* mutants.

Tdrd5l localizes to RNA dependent granules



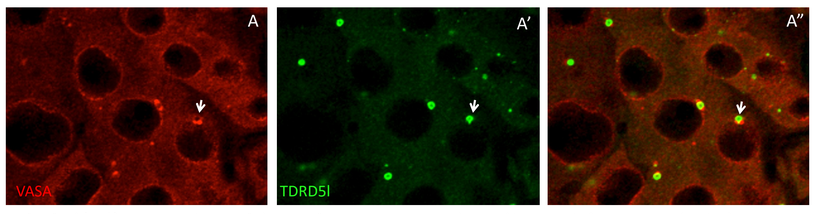
Tdrd5l localizes to cytoplasmic granules in both the male(A) and female(B) germline. Tdrd5l granules were seen starting in the germline stem cells(GSCs) in males, however they were absent from the female GSCs.



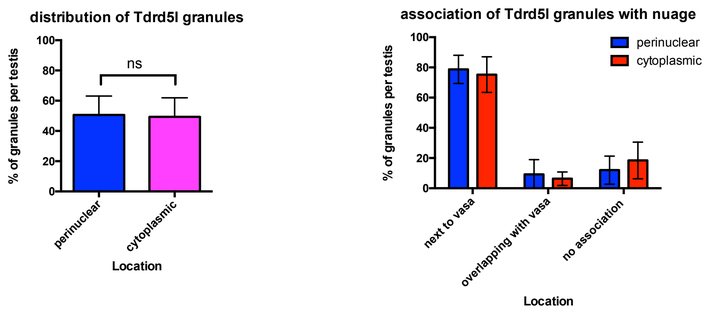


RNase treatment resulted in a loss of Tdrd5l granules(D) compared to controls suggesting that Tdrd5l localizes to an RNA granule

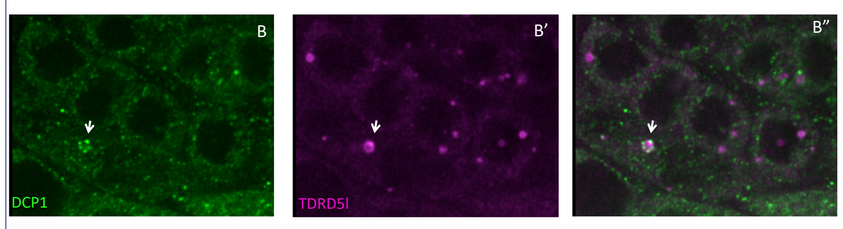
Tdrd5l localizes to the periphery of granules associated with nuage in the male germline



Tdrd5l(A') associated but does not co-localize with VASA bright punctae(A, A''). This suggests Tdrd5l could occupy a unique granule.

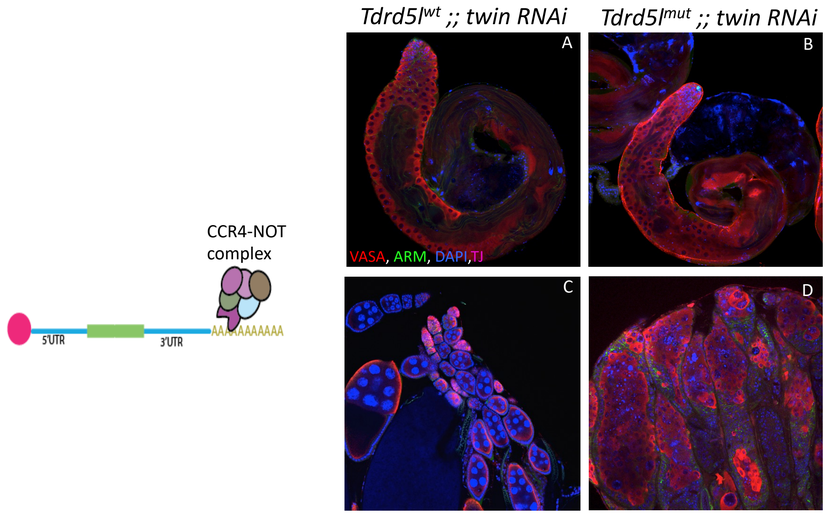


Tdrd5l granules also localize with Dcp1

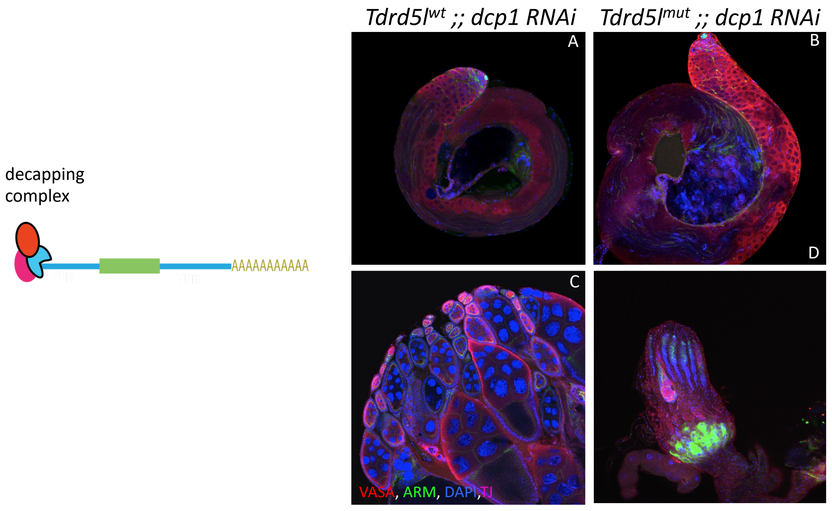


Confocal imaging with an airyscan detector showed that numerous Dcp1 granules(B) localize to the periphery of Tdrd5l granules(B',B'') in the male germline.

tdrd5l genetically interacts with decapping and deadenylation proteins

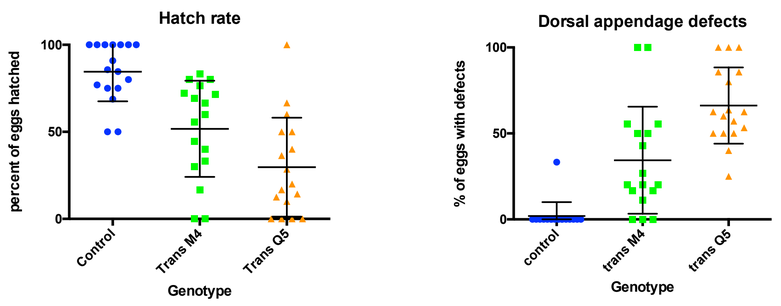


Knockdown of the CCR4 homolog *twin* in *Tdrd5l* mutant ovaries(D) and testes (B) resulted in sterility and an enhanced phenotype compared to *twin* knockdown in wild type gonads(A,C).

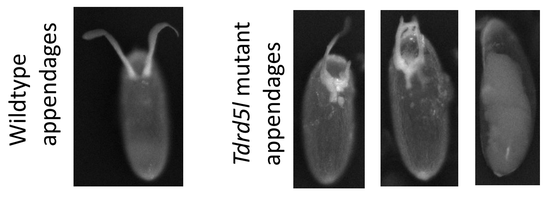


Knockdown of *dcp1* in *Tdrd5l* mutant ovaries(D) resulted in sterility and an enhanced phenotype compared to *dcp1* knockdown in wild type ovaries(C). There was no difference in phenotype of *dcp1* RNAi between *Tdrd5l* mutant testes(B) and wild type testes (A).

tdrd5l mutant eggs have Dorsal/Ventral defects

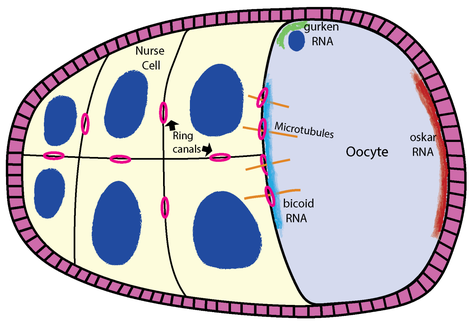


Tdrd5l trans heterozygous mutant females laid eggs with a decreased hatch rate compared to control females. These mutant eggs also had a higher percentage of dorsal appendage defects

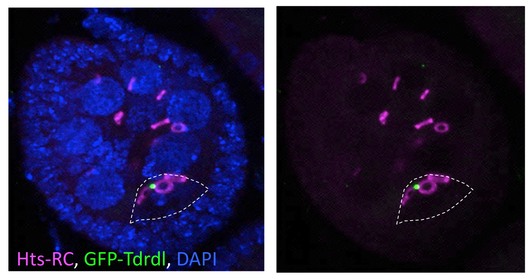


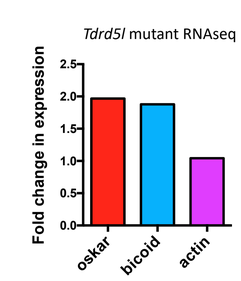
Dorsal appendage defects seen in *tdrd5l* mutants vary from only one appendage present, stubby appendages, to no appendages at all

tdrd5l regulates maternally deposited RNAs in the female germline

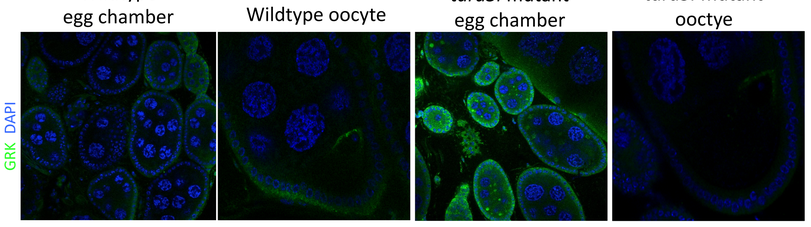


Model for maternal RNA deposition in the *Drosophila* egg chamber



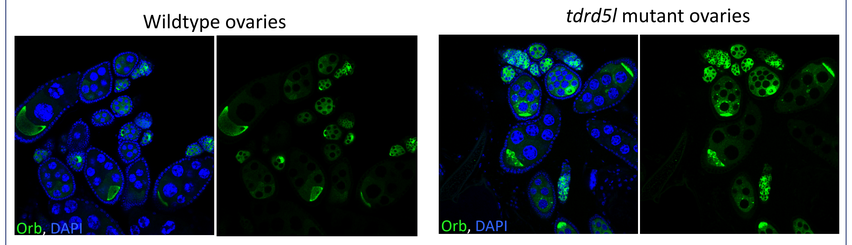


A single large Tdrd5l granule was found adjacent to the ring canals used for maternal RNA deposition at the anterior of the oocyte. Additionally, RNAseq of *tdrd5l* mutant ovaries showed an increase in maternal RNA levels.

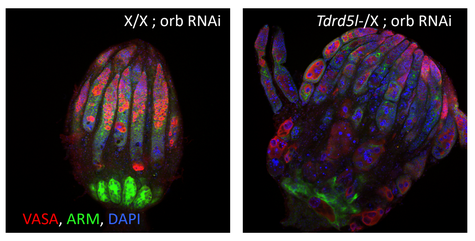


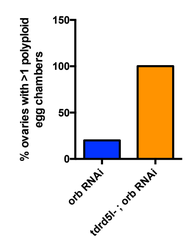
gurken which was repressed post-transcriptionally in wild type nurse cells was translated in *tdrd5l* mutant nurse cells

Tdrd5l represses Orb protein levels



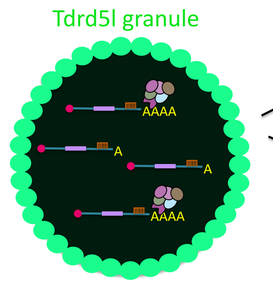
Orb protein expression was only seen in the oocyte and faintly in the most posterior nurse cell in wildtype ovaries. In *tdrd5l* mutants, Orb was seen throughout the nurse cells which could cause the increase in Grk protein levels





Knockdown of *orb* in wildtype ovaries resulted in loss of the developing germline. When this knockdown was conducted in *tdrd5l* mutant ovaries some development is rescued

Model and future directions



Post transcriptional regulation of genes required for gametogenesis

Maternal RNA trafficking

We are currently generating reagents to conduct RIP-seq which will identify RNAs that localize to Tdrd5l granules

Lastly we plan to conduct FISH to maternal RNAs such as *gurken* to see if these RNAs reside in Tdrd5l granules and if their localization is altered in Tdrd5l mutants

Acknowledgements

We would like to thank the members of the Van Doren lab for their helpful discussion, as well as the Johnston, Chen, Gall, and Kim labs advice and reagents. We would also like to thank the Chou lab for providing us with the Dcp1:YFP flies, and Zhao Zhang and Fred Tan for help with RNA sequencing. C.P. is supported by an NSF GRFP fellowship.