Reduced insulin signaling promotes germline stem cell maintenance under P-element hybrid dysgenesis

Efren Silva, Terion Griffiths, and Erin S. Kelleher

Department of Biology and Biochemistry, University of Houston, Houston, TX

BACKGROUND

Transposable elements (TEs) are selfish mobile DNA segments that proliferate throughout the genome by transposition in gamete nuclei, thus allowing for vertical transmission to offspring. While often suppressed by the host, the consequences of transposition are severe, inducing deleterious mutations and disrupting the production of gametes. In the case of the P-element, unregulated transposition leads to hybrid dysgenesis, characterized by atrophied ovaries containing little to no germline stem cells (GSCs). We hypothesize that DNA damage from P-element transposition impairs GSCs. We test this by manipulating the differentiation signals that a GSC receives of 16 differentiation genes. Decreasing differentiation signals is predicted to suppress atrophy by sustaining GSCs in the stem cell niche.

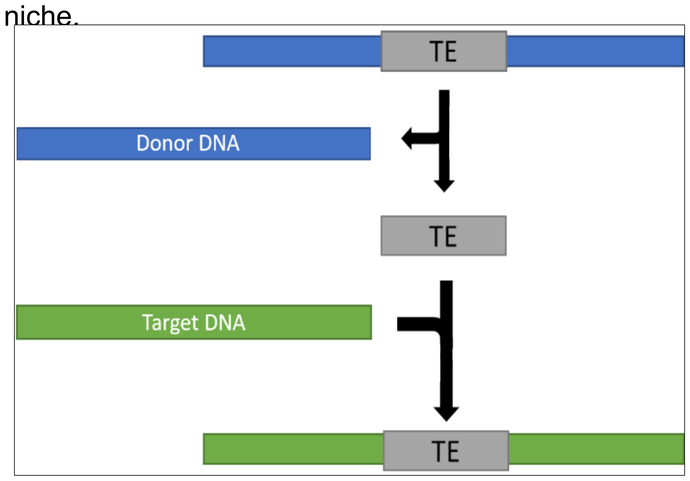

Figure 1. "Cut and Paste" Transposition. TEs "cut" themselves out of the donor DNA and "paste" themselves into the target DNA. Transposition into coding genes can induce deleterious mutations and exert lethal, genotoxic stress on cells that result in double-stranded breaks.

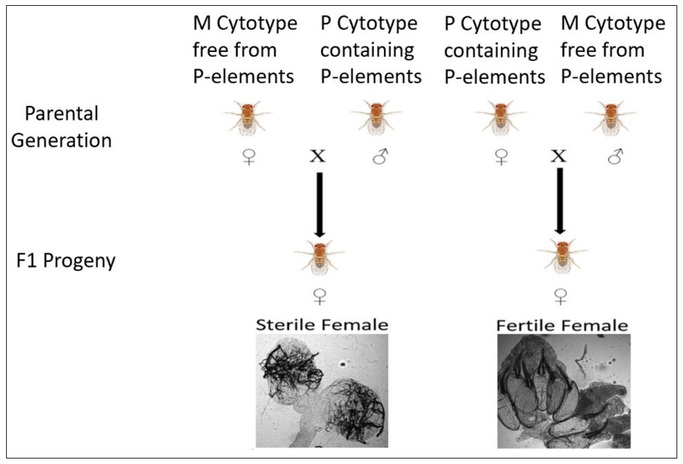

Figure 2. Hybrid Dysgenesis. Sterility occurs amongst progeny in a cross between lab-bred female flies and WT male flies. Progeny contain dysgenic ovaries. The reciprocal cross yields normal and fertile progeny. Progeny contain normal ovaries.

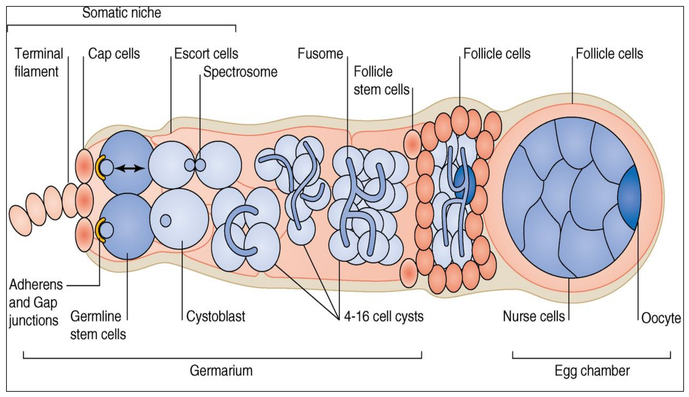

Figure 3. Stem Cell Niche. A GSC divides asymmetrically to produce a self-renewing GSC and a cystoblast, committed to differentiate. The cystoblast further divides 4 times to form a 16-cell cyst. Of these, one cell will differentiate into an oocyte whereas the rest adopt a nurse cell fate. Slaidina et al. 2014.

METHODS

The *Gal4*-UAS system was utilized to knock-down the gene of interest. The driver line stock contains the *Gal4* transcription factor gene under the regulation of the native ovary specific enhancer, nanos, and a GFP marker. The responder line stock contains an UAS controlling a dsRNAi- transgene that targets the gene of interest for knock-down. The responder lines were crossed with stocks containing balancer chromosomes (*CyO* or *TM3*). The resulting progeny were then crossed to the driver line. Fruit flies containing both *Gal4* and UAS-RNAi transgenes exhibit gene silencing, specifically in the germline. Fertility assay was carried out to assess ovarian atr[illegible]

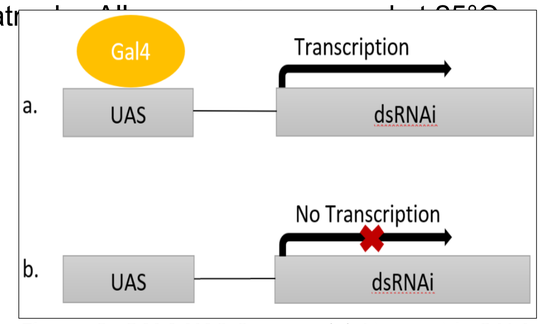

Figure 6. GAL4-UAS System. (a) Lines with GAL4 & UAS-RNAi transgenes will observe gene KD. (b) Lines without GAL4 will not observe gene KD.

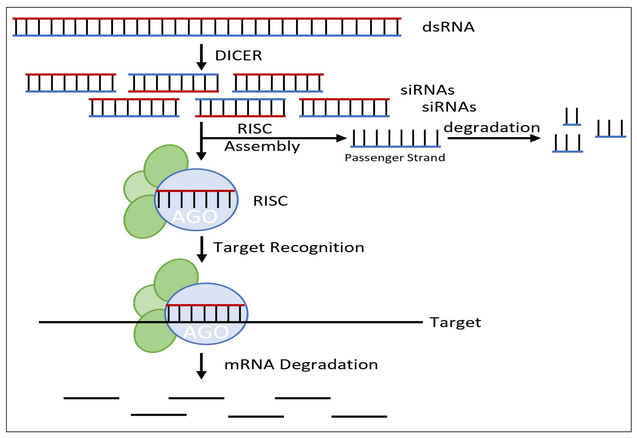

Figure 7. RNA Interference Mechanism. DICER cleaves the double stranded-RNA into siRNAs, interwoven with other structures to produce the RISC complex. It then targets an mRNA matching the guide strand and degrades it.

RESULTS

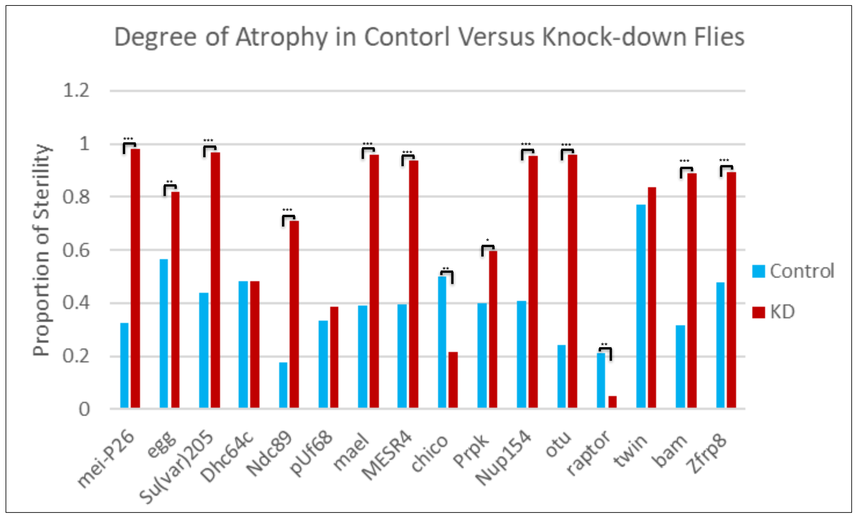

Figure 8. Degree of Sterility. Graph depicts the proportion of sterility of 16 genes that were knocked-down. The blue represents control flies and red represents the knock-down flies.

CONCLUSION

All genes except for *raptor* and *chico*, showed greater ovarian atrophy in the knockdown line versus the control lines. Knock-down of *raptor* and *chico* resulted in germline rescue in dysgenic ovaries against the P-elements, evidence to suggest that atrophy was suppressed and a pool of GSCs sustained to differentiate into eggs. Chico and Raptor are components of the insulin signaling pathway, which promotes the G2/M transition in GSCs. Our observations therefore suggest that G2 arrest may be critical for the recovery of GSCs from P-element induced DNA-damage. Further work should be conducted to solidify the relationship between insulin signaling and egg development within a genome where TEs are transposing at a high rate, specifically in gametes. Future work to be conducted should look at other components of the insulin signaling pathway and factors not directly required to elucidate their role as potential regulators in GSC maintenance.

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