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BRUNO AND P-ELEMENT TRANSPOSITION: POSITIVE REGULATOR OR CELLULAR RESPONDER?

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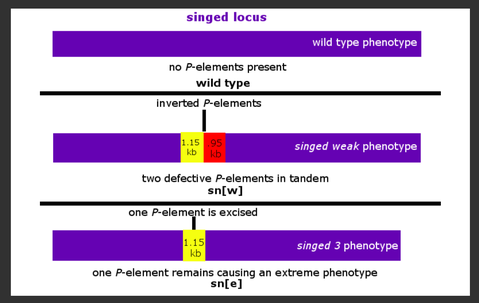
Introduction

Transposable elements (TEs) are obligate genetic parasites that spread throughout genomes and guarantee their transmission to offspring by replicating in germline cells. TE's are abundant in the genomes of every organism, so understanding ways organisms tolerate or repress them is imperative.

The P-element causes ovarian atrophy in female flies. It has been proven that maternally inherited piRNAs have an important role in transposition repression since they are antisense to TEs, and the absence of these piRNAs in dysgenic crosses allow us to study mechanisms
of tolerance.

We isolated a gene, bruno, as a possible source of natural variation in P-element tolerance. bruno is a protein coding gene and has no known function in TE regulation.

Materials and Methods



Fertility assays led to the hypothesis that bruno is a source of natural variation in P-element tolerance or resistance. Based on preliminary data from this experiment, we expect that bruno is a tolerance factor.
(Figures 1, 2 and 3)

By performing RNA extraction on Drosophila ovaries, cDNA synthesis and qPCR amplification we can observe P-element expression relative to rpl32. (Figure 3)

By crossing flies with specific genotypes, we can determine rates of P-element excision and whether bruno confers tolerance or resistance to P-element activity. If excision rates are higher or similar, it is likely that bruno is conferring tolerance. If they are lower it is likely bruno is conferring resistance. (Figure 4)

Results

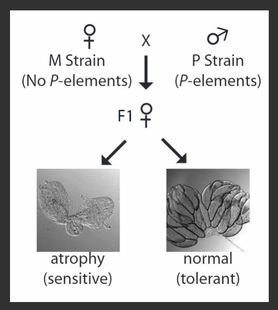


Figure 1: A dysgenic cross between a naive maternal strain and a P-element containing paternal strain results in offspring with atrophied ovaries in sensitive strains and normal ovaries in tolerant strains.

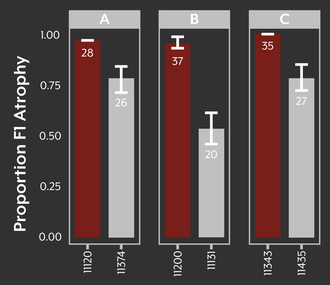


Figure 2: Proportion of ovarian atrophy in F1 offspring is much lower in tolerant strains than in sensitive strains.

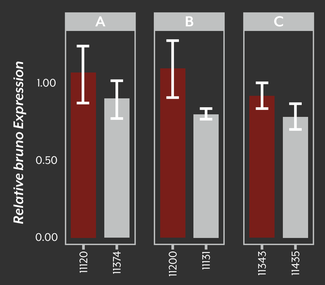


Figure 3: Sensitive and tolerant bruno alleles differ significantly in bruno expression.

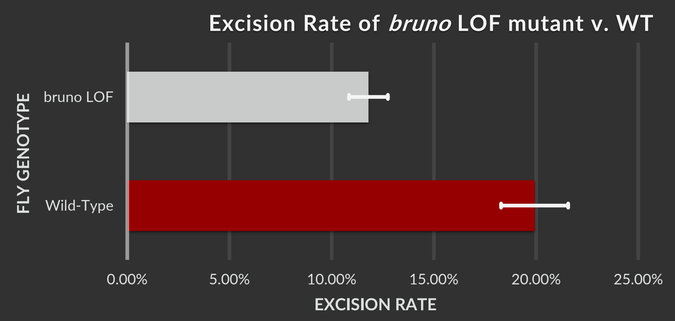


Figure 4: Excision rate in bruno LOF mutants [aretQB] is much lower than excision rate in WT

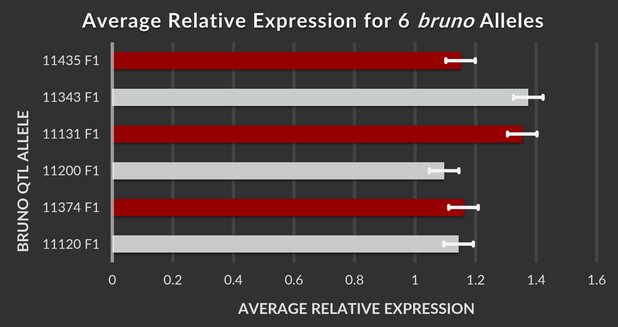


Figure 5: P-element expression relative to rpl32 is not significantly different.

Conclusion

Through qPCR, we found that in the 2 naturally occurring alleles from 3 different genetic backgrounds(Kelleher et al 2018), bruno function does NOT play a role in P-element expression. P-element expression between bruno sensitive and tolerant pairs was not significantly different. These results strongly suggest that bruno is acting as a P-element tolerance factor.

In lab generated bruno mutants (aretQB), we found that the P-element excision rates were surprisingly much lower than wild-type excision rates. This suggests that bruno might be an upstream regulator of P-element activity.

Next…

Run qPCR expression assay on dysgenic bruno (aretRM and aretQB) offspring to determine the difference in P-element expression between the mutants and the wild type.

Run excision assays on the 2 naturally occurring alleles from 3 different genetic backgrounds alleles to determine if P-element excision rates differ between sensitive and tolerant allele pairs.

References

Kelleher, E. 2016, "Reexamining the P-Element Invasion of Drosophila melanogaster Through the Lens of piRNA Silencing", Genetics, p.1513-1531.

Kelleher, E., Jaweria, j. Akoma, U., Ortega, L., Tang, W.,2018, "QTL mapping of natural variation reveals that the developmental regulator bruno reduces tolerance to P-element transposition in the Drosophila female Germline.", PLOS Biology

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