**A natural transposon affects gene regulation and fitness-related traits depending on the developmental stage and environmental conditions in D. melanogaster**

Gonzalez-Lab
Evolutionary and Functional Genomics

Míriam Merenciano and Josefa González
Institute of Evolutionary Biology (CSIC-UPF), Barcelona, Spain
miriam.merenciano@ibe.upf-csic.es
@mirimerenciano

**Introduction**

FBti0019985 is a transposable element belonging to the soo family that overlaps with the SUTRI region of Lime gene. This gene encodes a zinc finger transcription factor that links immunity and metabolism, and it has also been involved in cold-stress resistance.

Moreover, FBti0019985 adds an alternative transcription start site to Lime producing a longer transcript in both nonstress and after an infection with the bacteria P. entomaphila. FBti0019985 has been associated with Lime upregulation in embryos and with increased egg-to-adult viability in different natural populations under nonstress and cold-stress conditions.

**Objectives**

To fully characterize the molecular and phenotypic effects of FBti0019985 in different stress conditions that are relevant for D. melanogaster in nature: immune- and cold-stress.

To do that, we performed in vivo reporter assays and expression analysis to investigate the role of FBti0019985 in its near vicinity gene: Furthermore, to check whether FBti0019985 has an associated phenotypic effect in the different stress responses, we also performed phenotypic experiments using laboratory-outbred populations and CRISPR/Cas9 mutant strains.

**FBti0019985 acts as an enhancer in adults under immune-stress conditions, and in embryos in nonstress conditions**

**Immune stress (adult gut)**

FBti0019985 acts as an enhancer in guts after an infection with the bacteria P. entomaphila. The deletion of three predicted immune-related binding sites in the FBti0019985 sequence reduces the expression of the reporter gene, suggesting that the deleted binding sites are the responsible for the enhancer activity of FBti0019985 in infected conditions. These results confirm that FBti0019985 harbors functional binding sites responsible for its enhancer activity in infected conditions.

**Cold stress (embryos)**

FBti0019985 also acts as an enhancer in embryos in nonstress conditions, while it does not under cold-stress conditions. The deletion of the predicted binding sites related with developmental processes in the FBti0019985 sequence does not reduce the expression of this reporter gene. These results suggest that the binding sites related with developmental processes are not responsible for the enhancer activity of FBti0019985 in embryos in nonstress conditions.

**FBti0019985 upregulates its nearby gene Lime in adults under immune-stress conditions, and in embryos in nonstress conditions**

**Immune stress (adult gut)**

FBti0019985 is associated with Lime upregulation under immune-stress conditions in both female and male guts from outbred flies. These results are consistent with FBti0019985 acting as an enhancer in immune-stress conditions, but not in nonstress. Moreover, as expected, CRISPR/Cas9 deletion of the insertion results in reduced Lime expression compared with the maternal strain with FBti0019985 only in infected conditions. These results confirm that FBti0019985 is the causative mutation affecting the expression of Lime in infected conditions.

**Cold stress (embryos)**

FBti0019985 is associated with Lime upregulation in nonstress but not in cold-stress conditions in embryos from outbred flies. These results are consistent with FBti0019985 acting as an enhancer only in nonstress conditions. Moreover, in nonstress, CRISPR/Cas9 mutants show reduced Lime expression. These results confirm that FBti0019985 is the responsible mutation for Lime upregulation in embryos in nonstress conditions.

**FBti0019985 is associated with tolerance to infection and with increased viability in cold**

**Conclusions**

- FBti0019985 affects the expression of its nearby gene Lime depending on the developmental stage and the environmental conditions.
- In guts after an infection with P. entomaphila, FBti0019985 is associated with an upregulation of Lime due to the presence of immune-related binding sites in its sequence, causing an increase tolerance to infection.
- In embryos, FBti0019985 is associated with an upregulation of Lime in nonstress. Furthermore, the presence of FBti0019985 results in increased viability in both nonstress (previous works) and cold-stress conditions.
- Importance to consider the effect of a candidate adaptive mutation under different context to fully characterize its molecular and functional effects.

**References**

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