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

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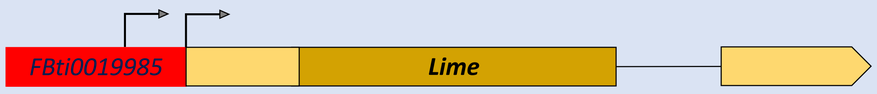
Introduction

FBti0019985 is a transposable element belonging to the *roo* family that overlaps with the 5'UTR 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. entomophila*. *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.

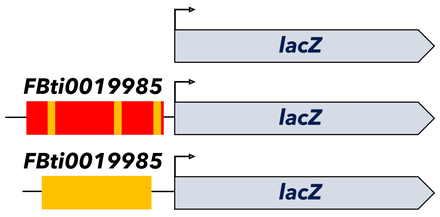
Objectives

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 nearby gene expression. 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

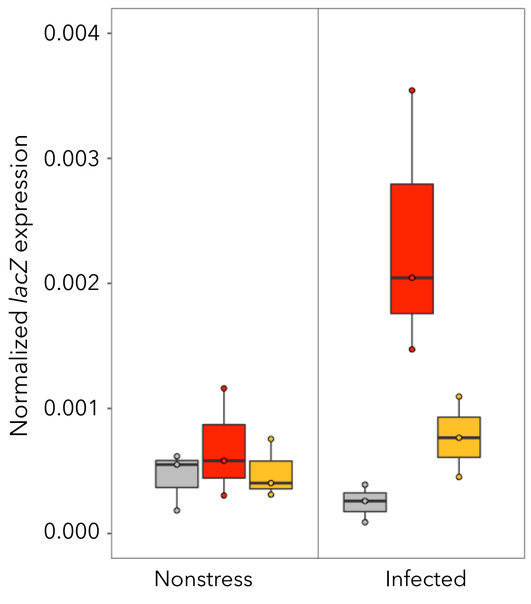
Immune stress (adult gut)



DEAF-1, tin, and Dorsal binding sites

***In vivo* enhancer assay**

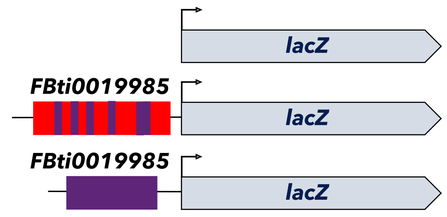
FBti0019985 acts as an enhancer in guts after an infection with the bacteria *P. entomophila*.
The deletion of three predicted immune-related binding sites in the *FBti0019985* sequence reduces the expression of the reporter gene, suggesting that the deleted binding sites are the responsible for the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



Empty vector
FBti0019985
***FBti0019985* ΔTFBSs**

Two-Way ANOVA
Genotype *p-values* < 0.05

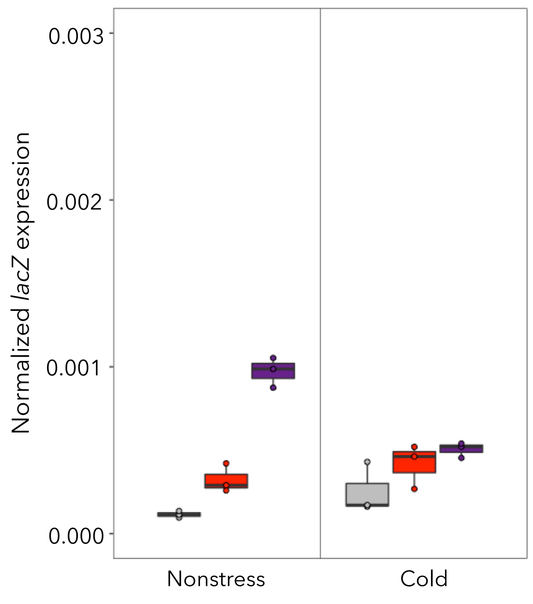
Cold stress (embryos)



ara/mirr, Caup, Bap, Vnd, Btd, and Nub binding sites

***In vivo* enhancer assay**

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 the 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.

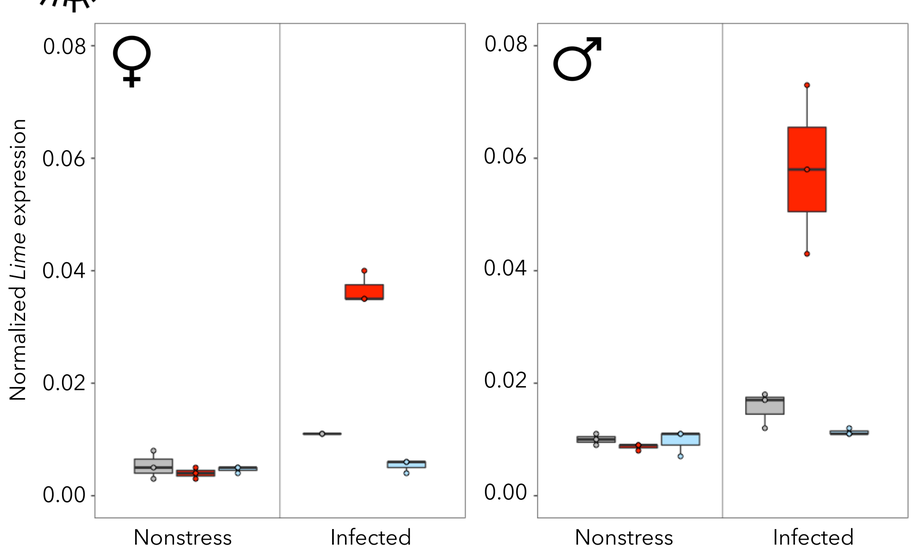


Empty vector
FBti0019985
***FBti0019985* ΔTFBSs**

Two-Way ANOVA
Genotype *p-values* < 0.05

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

Immune stress (adult gut)

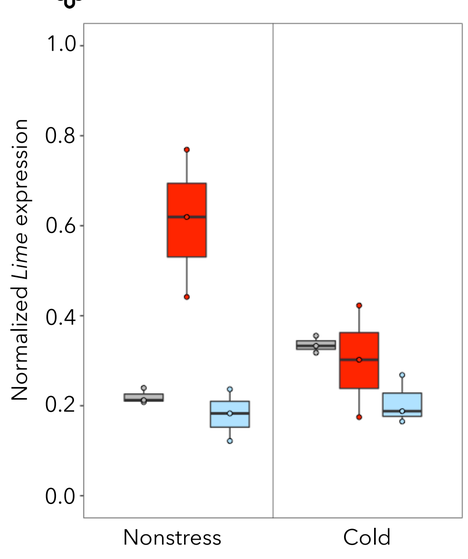


FBti0019985 is associated with *Lime* upregulation under immune-stress conditions in both female and male guts from outbred flies. These results are consistent with *FBti0019985* acting as an enhancer in immune stress conditions but not in nonstress. Moreover, as expected, CRISPR/Cas9 deletion of the 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.

Outbred *FBti0019985* (-)
Outbred *FBti0019985* (+)
CRISPR/Cas9 mutant Δ*FBti0019985*

Two-Way ANOVA
Genotype*Treatment *p-values* < 0.001

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 under nonstress.

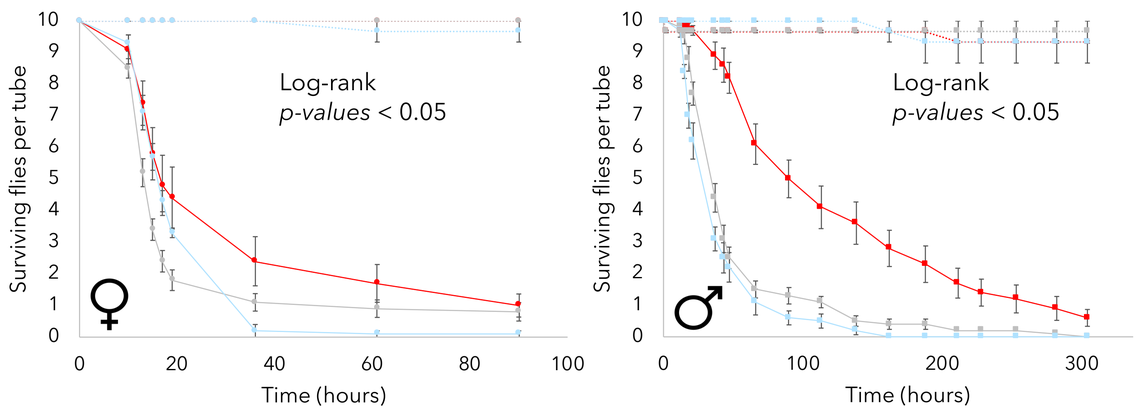
Outbred *FBti0019985* (-)
Outbred *FBti0019985* (+)
CRISPR/Cas9 mutant Δ*FBti0019985*

Two-Way ANOVA
Genotype*Treatment *p-values* < 0.05

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

Immune stress

Infection tolerance assay



Outbred flies with *FBti0019985* show increased tolerance to *P. entomophila* infection compared with flies without the insertion (females and males). Moreover, CRISPR/Cas9 mutants also show reduced tolerance compared to the maternal strain with the *FBti0019985*, suggesting that the presence of the insertion confers tolerance to *P. entomophila* infection.

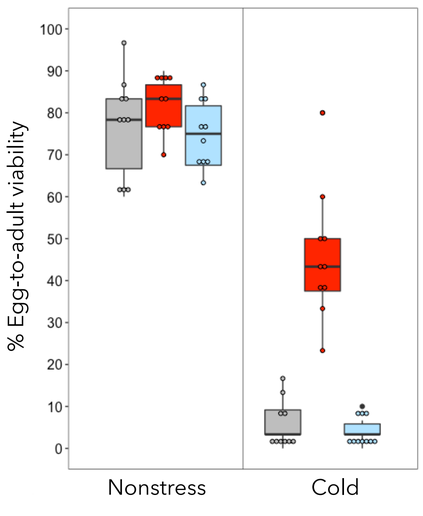
Outbred *FBti0019985* (-)
Outbred *FBti0019985* (+)
CRISPR/Cas9 mutant Δ*FBti0019985*

Cold stress

Outbred flies with *FBti0019985* have increased egg-to-adult viability under cold-stress. Moreover, CRISPR/Cas9-mutants show reduced viability compared with the outbred strain with the element in cold-stress conditions, suggesting that *FBti0019985* is the responsible mutation conferring this phenotype.

Outbred *FBti0019985* (-)
Outbred *FBti0019985* (+)
CRISPR/Cas9 mutant Δ*FBti0019985*

Two-Way ANOVA
Genotype*Treatment *p-values* < 0.05



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. entomophila, 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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