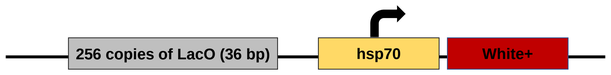
Regulation of repeat-induced silencing and position-effect variegation by genomic position and the TOR pathway in *Drosophila melanogaster*

Emily A. Holmquist[1], Adam T. Smiley[1], Nhi T. Vuong[1], Spencer A. Tye[2] and Andrew M Arsham[1]

Bemidji State University- Biology Baccalaureate Partnership at North Hennepin Community College[1], Brooklyn Park, MN and University of Kansas Department of Molecular Biosciences[2]

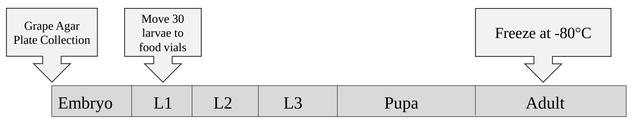
Introduction

Large percentages of eukaryotic genomes are composed of repetitive DNA sequences, including tandem repeats and interspersed repeats. The transcriptional silencing of repetitive sequence elements by heterochromatin is integral for the maintenance of gene expression and genomic integrity. It is unknown if the silencing triggered by different types of repetitive elements occurs through distinct or similar mechanisms. A 256-copy tandem array of the E. coli lac operator sequence (LacO) can trigger variegation of a downstream *white+* reporter gene in *Drosophila melanogaster* when inserted into certain euchromatic genomic locations proximal to large blocks of heterochromatin. Using different lines of LacO-induced variegating flies [Poster 846C], we investigated how silencing is influenced by environmental cues. We reared flies at 18°C and 25°C, and in the presence of the metabolism inhibitory drug rapamycin and found that both rapamycin and low temperatures suppress variegation and increase eye pigmentation. Concentrations of rapamycin that are insufficient to delay development are nonetheless sufficient to suppress variegation suggesting that the mechanism is independent from drug-dependent developmental delay and that both temperature and rapamycin induced suppression of variegation may operate through distinct molecular mechanisms converging on the establishment and/or maintenance of heterochromatin.

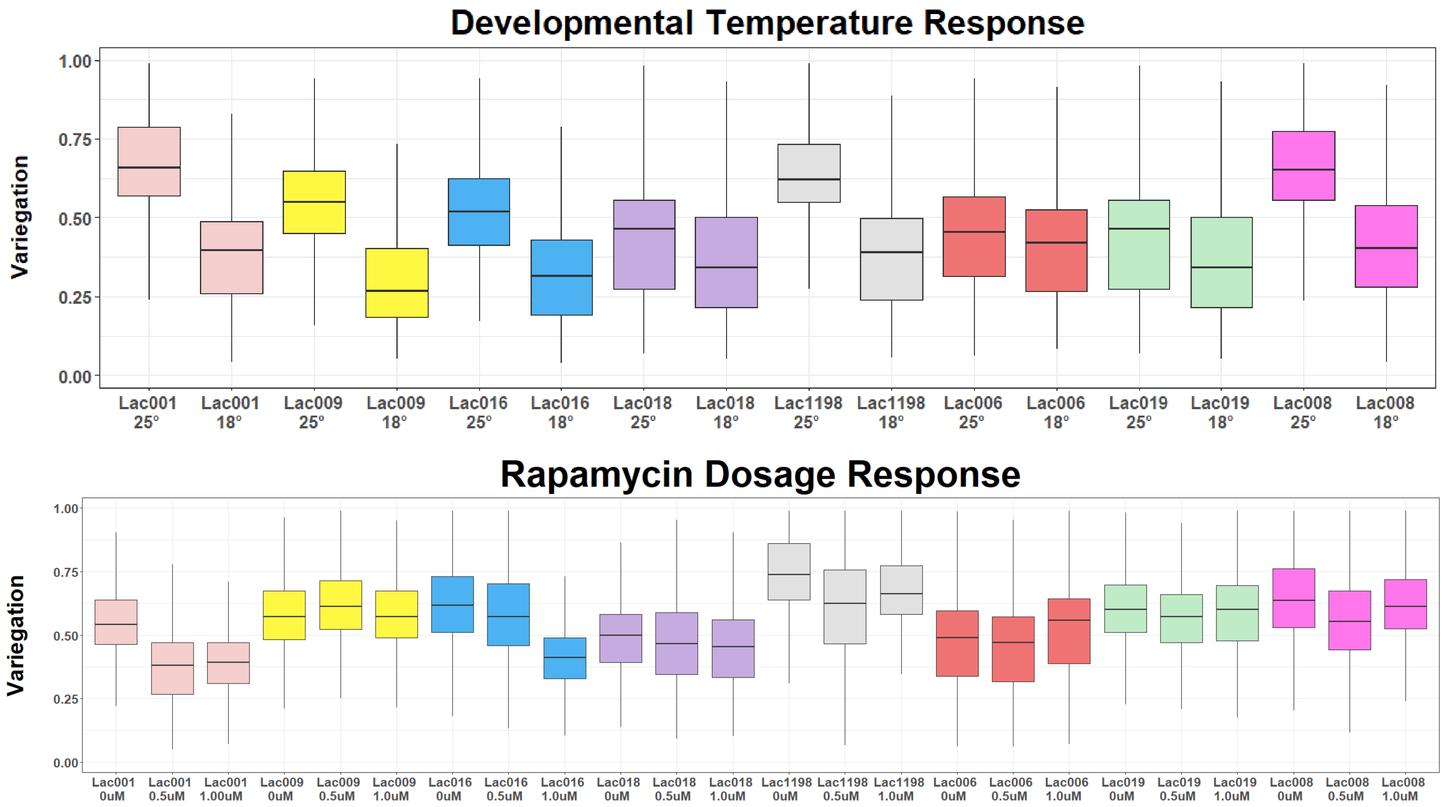


Materials and Methods

Expression of a *white+* transgene is driven by a constitutively active hsp70 promoter downstream of 256 repeats of LacO. This sequence triggers partial stochastic silencing of the transgene resulting in variegation in the fly eye. Several highly variegating lines were produced from P Element transposition and their phenotypic plasticity was tested under different concentrations of rapamycin and developmental temperatures. Images were collected from 2-4 day old female flies heterozygous for the transgenic construct using a dissecting stereomicroscope with a C-mount digital camera at 45x zoom with a dual LED spotlight and Infinity Capture software (Gain=1.39, Resolution=1392x1040, image files saved as .tiff format). Rapamycin was dissolved in DMSO and concentrations of 0.0, 0.5, 1.0 and 2.0μM were prepared in Bloomington food. Fly embryos to be raised on rapamycin were collected in population cages on grape agar plates and transferred to vials of experimental media at 25° C during the 1st instar. Flies for temperature comparisons were raised on standard Bloomington food in either 18°C or 25°C. Flies were frozen 2-4 days post eclosure at -80° C to facilitate standardized batch collection of image data. Eye images were analyzed as text images by the TIF package in R and plotted excluding the values of the black background and white glare.



Results



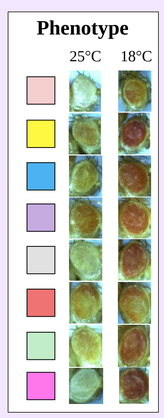


Figure One: The varying levels of response to temperature and rapamycin concentrations for each of the LacO insert's locations are representative of the phenotypic plasticity. Higher values correspond to higher levels of variegation in pigmentation and therefore lighter eyes. Fly eye pictures to the right show the different phenotypic means for each insert at both temperatures.

Discussion

Previous experiments in the *Lac1198* line revealed a substantial suppression of variegation by rearing at 18°C compared to 25°C. We further explored this phenotypic plasticity by testing if other conditions which slow development have a similar effect. We found the growth-inhibitor rapamycin to suppress variegation independent of developmental delay as concentrations insufficient to delay development were sufficient to suppress variegation and thus increase expression of our transgene – suggesting a change in the establishment or maintenance of heterochromatin. This effect's independence of developmental timing suggests either that rapamycin and temperature suppress variegation through different mechanisms or that they share a mechanism unrelated to developmental timing.

We have generated additional highly variegating lines at unique genomic positions across all three *Drosophila melanogaster* autosomes through P element transposition. We have tested the effect of developmental temperature and rapamycin on the expression of the LacO-linked eye color transgene in seven of our novel lines alongside the original *Lac1198*.

Our single transgenic insertion on the largely heterochromatic fourth chromosome was found to have suppressed variegation when reared at 18°C compared to 25°C. Additionally, the variegation in this novel insertion was slightly suppressed under both 0.5, and 0.1 μM concentrations of rapamycin.

Both insertions on the third chromosome have very little suppression of variegation from both temperature and rapamycin. The low phenotypic plasticity could be due to the locations of the insert on the chromosome.

All second chromosome insertions are temperature-sensitive but the magnitude varies widely. However, not all temperature-sensitive insertions are also sensitive to rapamycin, again suggesting distinct mechanisms of heterochromatin regulation. Among rapamycin-sensitive lines the dose-response varied, for instance Lac001 shows suppression starting at the 0.5 μM concentration, where Lac016 does not show significant change until 1.0 μM.

Across all conditions tested we observe consistently greater variegation in females vs males, confirming observations of other investigators which suggest that the largely heterochromatic Y chromosome serves as a sink for trans-acting components of heterochromatin, reducing variegation elsewhere in the genome by drawing away key structural proteins, reading or writing enzymes, or metabolites required for heterochromatin formation or maintenance.

Our results show that both reduced temperature and metabolic inhibition by rapamycin suppresses variegation, but our data shows non-dose response results for different concentrations of rapamycin.

We intend to generate additional highly variegating lines through P element transposition experiments to further investigate which genomic positions allow for suppression of our LacO transgene by temperature and rapamycin. Additionally, we plan to further analyze our existing and newly generated insertion sites for specific proteins, chromatin marks, proximity to constitutive heterochromatin, and characteristic DNA sequences that are allowing for this observed suppression of variegation.

References

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Tye, S.A, et al., (2019). Regulation of Repeat Induced Silencing and Position-Effect Variegation by Nutrition and the TOR Pathway. Poster presentation at the 60th Annual Drosophila Research Conference, Dallas, TX.

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