Investigating the Regulation of Position-Effect Variegation by Cis-Acting Repetitive Elements and Transgene Expression in *Drosophila melanogaster*

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Introduction

Euchromatin and heterochromatin are two epigenetic states arising from complex DNA-protein interactions. When normally euchromatic genes are placed in proximity to heterochromatin by transposition or chromosomal rearrangement, they can be partially silenced. This phenomenon is known as position-effect variegation (PEV) and was first observed in *Drosophila melanogaster* by Hermann Muller in the 1930s. Previous results showed that transposon remnant 1360 can trigger formation of ectopic heterochromatin leading to stochastic silencing and PEV. Replacing a variegating 1360 insertion with a 256-copy tandem array of the *E. coli* Lac operator (*LacO*) through recombination mediated cassette exchange effectively triggers heterochromatin formation, but it remained unclear whether this effect could be observed in other genomic locations. By mobilizing the RCME-generated LacO P element and isolating variegating insertion mutants we assessed the potency of the LacO array to trigger silencing.

Methods

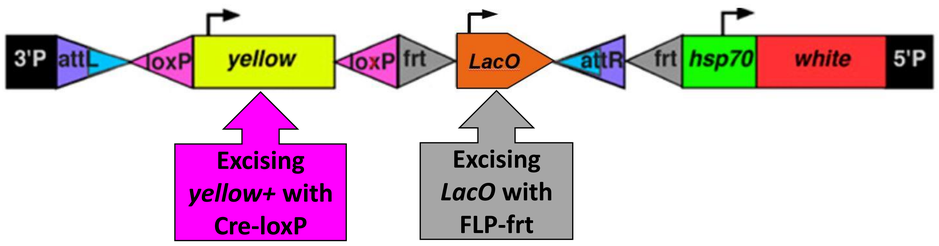


Figure 1. Excision of *LacO* and *yellow+*
After isolating new insertional mutants with variegating reporter expression, elements of the reporter that are flanked by cis-acting recombinase target sequences can be excised by crossing to FLP or Cre recombinases to assess their contributions to silencing. We will test whether the inclusion of the strongly active *yellow* gene or the LacO repeat array are the triggers for silencing.

Figure 2. Investigation of trans-silence effect between PEV lines.
Counterintuitively, in some PEV lines homozygous expression of the reporter construct results in less pigmentation than heterozygous. Analysis of this observation is complicated by the presence of potent enhancers and suppressors of variegation on common balancer chromosomes. We outcrossed mutants to an unbalanced *yw* stock to assess reporter expression as a function of gene dosage and to look for allele-specific trans-effects.

A. $\frac{XLacOA2}{Y} \times \frac{XLacOA2}{XLacOA2}$

B. $\frac{y-w-}{Y}\frac{1198\text{LacO}}{CyO} \times \frac{XLacOA2}{XLacOA2}$

C. $\frac{y-w-}{Y}\frac{1198\text{LacO}}{CyO} \times \frac{y-w-}{y-w-}\frac{1198\text{LacO}}{CyO}$

D. $\frac{y-w-}{Y}\frac{1198\text{LacO}}{CyO} \times \frac{y-w-}{y-w-}\frac{\text{Lac01}-001}{CyO}$

E. $\frac{y-w-}{Y}\frac{\text{Lac01}-001}{CyO} \times \frac{y-w-}{y-w-}\frac{\text{Lac01}-001}{CyO}$

F. $\frac{y-w-}{Y}\frac{\text{Lac01}-001}{CyO} \times \frac{y-w-}{y-w-}$

G. $\frac{y-w-}{Y}\frac{\text{Lac01}-001}{CyO} \times \frac{XLacOA2}{XLacOA2}$

Results

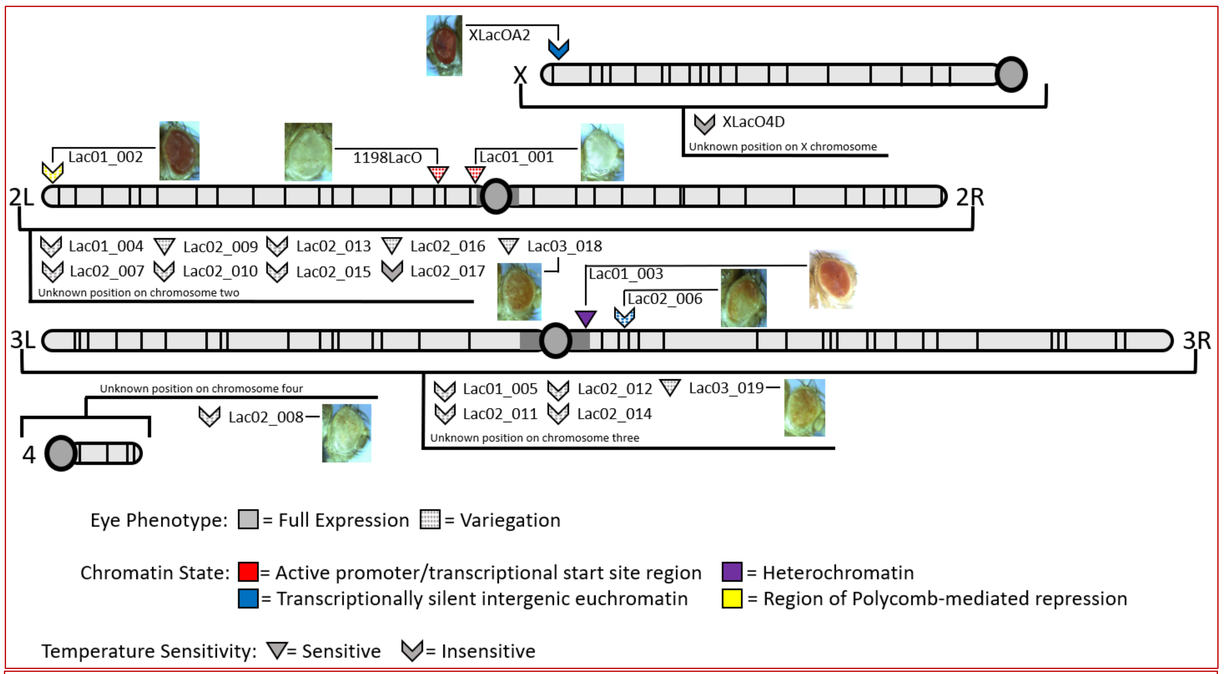


Figure 3. Mapping novel LacO P-element insertions.
Variegating insertions are marked according to their location and phenotype. Some insertions have been molecularly mapped by inverse PCR and sequencing while others have not. Marker color indicates modEncode 9-state chromatin model (Kharchenko *et al.*, 2011). Marker shape corresponds to the temperature sensitivity of reporter variegation. While low temperatures (18^0C) suppresses variegation in some insertions, others are non-responsive to changes in temperature. Images are representatives of phenotype at 25^0C.

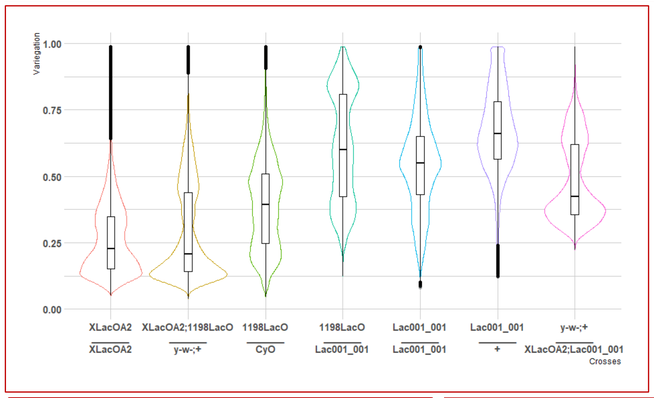
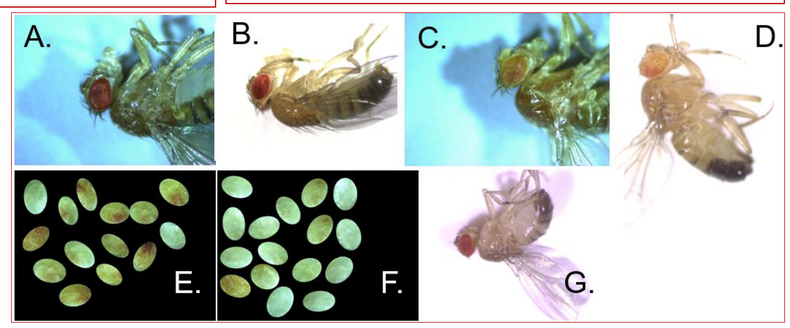


Figure 4. Investigating trans-silence effect
F2 progeny from **(A)** to **(G)** were collected and images were saved in RGB TIF format and the green channel was used as a proxy for overall pigmentation. Variegation in each image was measured by plotting grayscale pixel brightness in a composite of eyes from a single genotype using the ggplot2 package in R. 0 is black and 1 is white, so more variegated eyes are characterized by more light/bright pixels and a higher distribution along the Y axis. Violin plots show the distribution of pixel intensity (excluding background) for the composite images as shown in 5E and 5F, with box-plotted median and interquartile ranges, whiskers show 1.5* IQR, and outliers outside 1.5*IQR.

Figure 5. Representatives of trans-silence experiment.
(A) XLacOA2/ XLacOA2
(B) XLacOA2;1198LacO/y-w-;+
(C) /CyO
(D) 1198LacO/ Lac001_001
(E) Composite eye images of Lac001_001/ Lac001_001
(F) Composite eye images of Lac001_001/ +
(G) XLacOA2; Lac001_001/ y-w-;+



Discussion

- We isolated 20 novel variegating insertions of the *LacO* reporter construct on all 3 autosomes.
- Reporter expression of the original RCME-generated *LacO* insertion is inversely correlated with rearing temperature, *i.e.,* lower temperatures suppress variegation; this temperature sensitivity is replicated in some but not all novel insertions.
- While initial phenotypic analysis suggested that allelic trans-silencing similar to transduction might be happening in line Lac001_001, subsequent analysis revealed that homozygous expression of the *LacO-white+* transgene in these flies produces more red pigments than the unbalanced heterozygotes. Since homozygotes have two copies of the reported gene, *white+* gene, this is not too exciting. Initial results indicating the possibility of trans-silencing appear to be caused by one or more unidentified suppressors of variegation on the second chromosome *CurlyO* balancer.
- We noticed a persistent spatial expression pattern in the eye where red pigment clusters in the dorsal posterior quadrant of the adult eye. Such clusters can be observed in figure 5C, D, and E.
- Future experiments will focus on completing the molecular mapping of all novel insertions and looking for cis-acting sequence attributes or chromatin states that contribute to induction of ectopic heterochromatin by exogenous repeats, to variegation in general, and to temperature sensitivity.
- Future experiments will also include excision of *yellow+* and *LacO* segments of the reporter transgene to assess their contributions to silencing.

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Wang, S.H., Elgin, S.C.R. The impact of genetic background and cell lineage on the level and pattern of gene expression in position effect variegation. Epigenetics & Chromatin 12, 70 (2019).
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