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The effect of *JIL-1* on position-effect variegation is proportional to the total amount of heterochromatin in the genome

Jack Girton,* Chao Wang, Jørgen Johansen and Kristen M. Johansen

Department of Biochemistry; Biophysics and Molecular Biology; Iowa State University; Ames, IA USA

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In this study, we have taken advantage of recent whole genome sequencing studies that have determined the DNA content in the heterochromatic regions of each *Drosophila* chromosome to directly correlate the effect on position-effect variegation of a pericentric insertion reporter line, *118E-10* with the total amount of heterochromatic DNA. Heterochromatic DNA levels were manipulated by adding or subtracting a Y chromosome as well as by the difference in the amount of pericentric heterochromatin between the X and Y chromosome. The results showed a direct, linear relationship between the amount of heterochromatic DNA in the genome and the expression of the *w* marker gene in the *118E-10* pericentric reporter line and that increasing amounts of heterochromatic DNA resulted in increasing amounts of pigment/gene activity. In *Drosophila,* heterochromatic spreading and gene silencing is counteracted by H3S10 phosphorylation by the JIL-1 kinase, and we further demonstrate that the haplo-enhancer effect of *JIL-1* is proportional to the amount of total heterochomatin, suggesting that JIL-1's activity is dynamically modulated to achieve a more or less constant balance depending on the levels of heterochromatic factors present.

Introduction

Position-effect variegation (PEV) was discovered by Muller,[1] who observed a mosaic pattern of repression of euchromatic genes juxtaposed with blocks of heterochromatin in chromosomal rearrangements. The early observation that gene inactivation is inversely proportional to the distance of the variegating gene from the breakpoint suggested that heterochromatic factors spread from the breakpoint into the adjacent euchromatin, and that variegation in gene inactivation results from variation in the distance of this spreading.[2-4] The level of expression of the variegating genes in PEV genotypes can be modified by genetic mutations that either increase [Su(var)s] or decrease [E(var)s] gene expression.[5,6] Su(var) and E(var) genes have been shown to code for components of chromatin or enzymes that epigenetically modify components of chromatin. In addition, the level of expression of the variegating genes can be modified by chromosomal mutations that add or subtract blocks of heterochromatic DNA. Increasing heterochromatic DNA gives a Su(var) phenotype and decreasing it gives an E(var) phenotype suggesting that heterochromatic DNA modifies PEV by titrating heterochromatic factors.[4,6,7] That these modifying effects extend to the modulation of gene expression of transgenic P-element insertion lines was demonstrated by Wallrath and Elgin[8] who compared *hsp70-white* expression in X^X/Y, X/O and X/Y flies. In this study we have taken advantage of recent whole genome sequencing studies that have determined the DNA content in the heterochromatic regions of each *Drosophila* chromosome[9] to directly correlate the effect on PEV of a pericentric insertion reporter line, *118E-10*[8,10-12] with the total amount of heterochromatic DNA. Heterochromatic DNA levels were manipulated by adding or subtracting a Y chromosome as in Wallrath and Elgin[8] as well as by the difference in the amount of pericentric heterochromatin between the X and Y chromosome. We show that there is a linear correlation between the total amount of heterochromatic DNA and gene expression of the *118E-10* reporter line in X/O, X/Y, X/X and X^Y/X flies. Furthermore, in *Drosophila*, histone H3S10 phosphorylation by the JIL-1 kinase functions to maintain euchromatic domains by counteracting heterochromatization and gene silencing,[12-18] and we provide evidence that this effect is proportional to the amount of total heterochromatin.

Results and Discussion

In the absence of H3S10 phosphorylation by the JIL-1 kinase the major heterochromatin markers H3K9me2, HP1a, and Su(var)3-7 spread to ectopic locations on the chromosome arms of *Drosophila* polytene chromosomes.[14-16] These observations suggested a model for a dynamic balance between euchromatin and heterochromatin,[11,13,14,16] where the level of gene expression is determined by antagonistic functions of the euchromatic H3S10ph mark and the heterochromatic H3K9me2 mark.[10-12,16,19]

*Correspondence to: Jack Girton; Email: bpatch@iastate.edu
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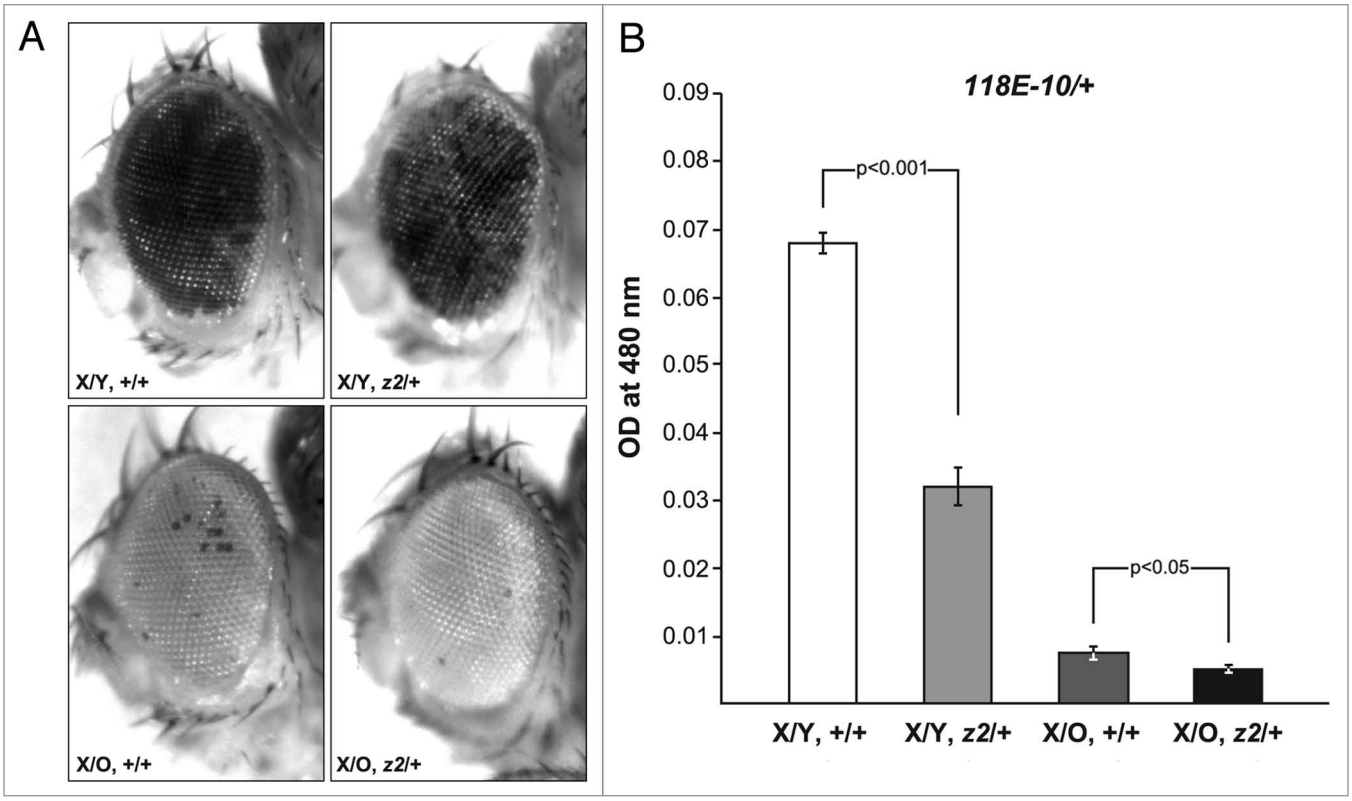

Figure 1. *JIL-1* is a haplo-enhancer of PEV in *118E-10*/+ male flies with or without a Y chromosome. (**A**) Examples of the degree of PEV in the eyes of X/Y; +/+, X/Y; *JIL-1*^{z2}/+ (X/Y; *z2*/+), X/O; +/+, and X/O; *JIL-1*^{z2}/+ (X/O; *z2*/+) flies. (**B**) Histograms of the levels of eye pigment in X/Y; +/+, X/Y; *JIL-1*^{z2}/+ (X/Y; *z2*/+), X/O; +/+, and X/O; *JIL-1*^{z2}/+ (X/O; *z2*/+) flies. The average eye pigment levels with SD are shown and comparisons between levels in +/+ and *JIL-1*^{z2}/+ backgrounds were done using a two-tailed Student's t-test.

This model has recently been tested in the variegating pericentric insertion line *118E-10* where loss-of-function alleles of *JIL-1* act as enhancers of PEV and the haplo-enhancer effect of *JIL-1* can counterbalance the haplo-suppressor effect of both *Su(var)3-9* and *Su(var)2-5* on position-effect variegation, providing strong evidence that a finely tuned balance between the levels of JIL-1 and the major heterochromatin components contribute to regulation of gene expression.[10,12] However, how this effect of *JIL-1* interacts with other types of modifiers of PEV is not understood, nor is it known whether the counterbalancing effect of JIL-1 is at a set level or whether it is proportional to the total amount of heterochromatic DNA.

To address these issues, we explored the effect on PEV caused by the P-element insertion line *118E-10* by the different heterochromatic levels in males and females as well as by adding or subtracting a Y chromosome[8,20] in wild-type and in *JIL-1*^{z2}/+ mutant backgrounds. The *JIL-1*^{z2} allele is a true null allele[21,22] and insertion of the *118E-10* P-element (*P[hsp26-pt, hsp70-w]*) into a known heterochromatin region of the fourth chromosome results in a variegating eye phenotype.[10,12,23] Thus, in the experiments we examined *w* expression in +/+; *118E-10*/+, and *JIL-1*^{z2}/+; *118E-10*/+ male and female flies as well as in X/O and X^Y/Y mutant backgrounds. Eye pigment levels of the various genotypes were determined essentially as in Kavi and Birchler[24] and Wang et al.[11,12] using three sets of 10 pooled fly heads from each genotype. As illustrated in **Figures 1 and 2**, the heterozygous *JIL-1*^{z2}/+ genotype enhances PEV as indicated by the increased proportion of white ommatidia and a decrease in the optical density of the eye pigment levels as compared with +/+ flies in both X/O, X/X, X/Y and X^Y/X flies. The reduction in all cases was statistically significant (**Figs. 1 and 2**) and averaged 40 ± 12%. Additionally, the difference in eye pigment levels between X/O, X/X, X/Y and X^Y/X flies in both +/+ and *JIL-1*^{z2}/+ backgrounds was statistically significant (**Tables 1 and 2**). These results indicate that the haplo-enhancer effect of *JIL-1* is consistent in all genotypes, decreasing the amount of pigment in genotypes where changes in heterochromatic DNA give a Su(var) phenotype and also decreasing the amount of pigment in genotypes in which changes in heterochromatic DNA give an E(var) phenotype. To further explore the correlation between the haplo-enhancer effect of *JIL-1* with the amount of heterochromatin present we plotted the *118E-10*/+ eye pigment levels determined above as a function of total heterochromatin levels in X/O, X/X, X/Y and X^Y/X flies in both +/+ and *JIL-1*^{z2}/+ backgrounds (**Fig. 3**). Based on the results of Hoskins et al.,[9] the Y chromosome contains approximately 40.9 Mb, the X 19.9 Mb, the second 18.3 Mb, the third 17.5 Mb and the fourth 3.1 Mb of pericentric heterochromatic DNA. Consequently, the total amount of heterochromatic DNA in the different genotypes used in this study is 97.7 Mb for X/O males, 117.6 Mb for X/X females, 138.6 Mb for X/Y males and

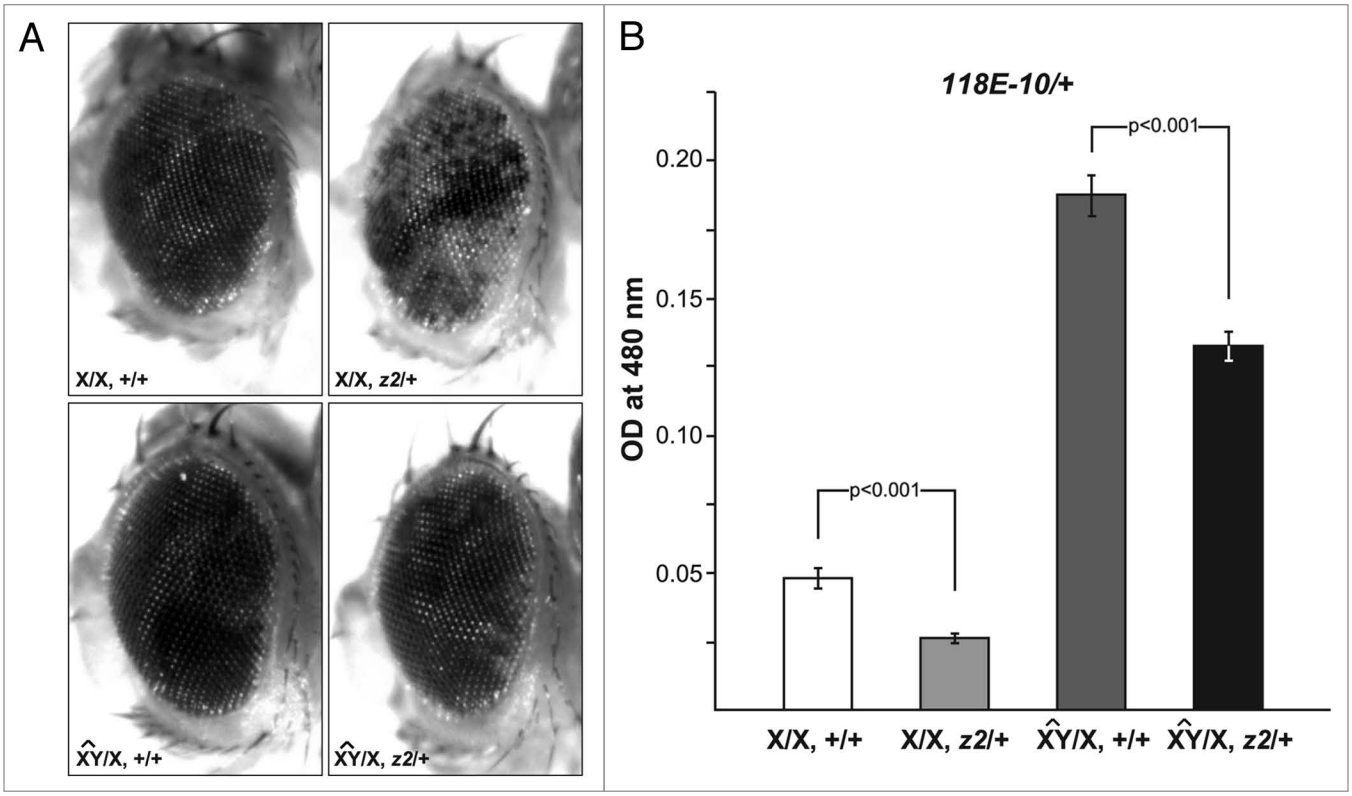

Figure 2. *JIL-1* is a haplo-enhancer of PEV in *118E-10*/+ female flies with or without a Y chromosome. (**A**) Examples of the degree of PEV in the eyes of X/X; +/+, X/X; *JIL-1*^{z2}/+ (X/X; *z2*/+), X^Y/X; +/+, and X^Y/X; *JIL-1*^{z2}/+ (X^Y/X; *z2*/+) flies. (**B**) Histograms of the levels of eye pigment in X/X; +/+, X/X; *JIL-1*^{z2}/+ (X/X; *z2*/+), X^Y/X; +/+, and X^Y/X; *JIL-1*^{z2}/+ (X^Y/X; *z2*/+) flies. The average eye pigment levels with SD are shown and comparisons between levels in +/+ and *JIL-1*^{z2}/+ backgrounds were done using a two-tailed Student's t-test.

158.5 Mb for X^Y/X females. As illustrated in **Figure 3A** there was a linear relationship (r = 0.929) between eye pigment levels and the total amount of heterochromatin in these genotypes in the wild-type background confirming previous findings that the degree of PEV depends on overall levels of heterochomatin. Interestingly, this relationship was also linear (r = 0.874) in the *JIL-1*^{z2}/+ background strongly suggesting that the haplo-enhancer effect of *JIL-1* is proportional to the total amount of heterochromatin.

Thus we found a direct, linear relationship between increasing and decreasing the amount of heterochromatic DNA in the genome and the expression of the *w* marker gene in the *118E-10* pericentric reporter line. These results are consistent with current models of heterochromatic spreading.[5,6] These models predict that in X/O males, which have the least amount of pericentric heterochromatic DNA to titrate a constant amount of heterochromatic factors, there will be an increased amount of heterochromatic silencing factors near the *118E-10* insertion. This increases the extent of heterochromatic spreading and decreases gene expression. In contrast, in X/X, X/Y, and X^Y/X flies there are increasing amounts of heterochromatic DNA to titrate the heterochromatic factors reducing their concentration in the region of the *118E-10* insertion. This decreases the extent of heterochromatic spreading and increases gene expression. These findings also may explain the difference in PEV between males and females as attributable to differences in their respective amount of total heterochromatin. Furthermore, we found that the decreased concentration of JIL-1 and the euchromatic H3S10ph mark in *JIL-1*^{z2}/+ flies[17,18] caused increased heterochromatic spreading and increased gene

Table 1. Statistical comparison of eye pigment levels in *118E-10*/+ flies with different amounts of heterochromatin wild-type for *JIL-1*

Genotype	X/X	X/Y	X^Y/X
X/O	p < 0.001	p < 0.001	p < 0.001
X/X		p < 0.001	p < 0.001
X/Y			p < 0.001

For each genotype the average pigment levels from three sets of measurements were compared using a two-tailed Student's t-test.

Table 2. Statistical comparison of eye pigment levels in *118E-10*/+, *JIL-1*^{z2}/+ flies with different amounts of heterochromatin

Genotype	X/X	X/Y	X^Y/X
X/O	p < 0.001	p < 0.001	p < 0.001
X/X			p < 0.001
X/Y			p < 0.001

For each genotype the average pigment levels from three sets of measurements were compared using a two-tailed Student's t-test.

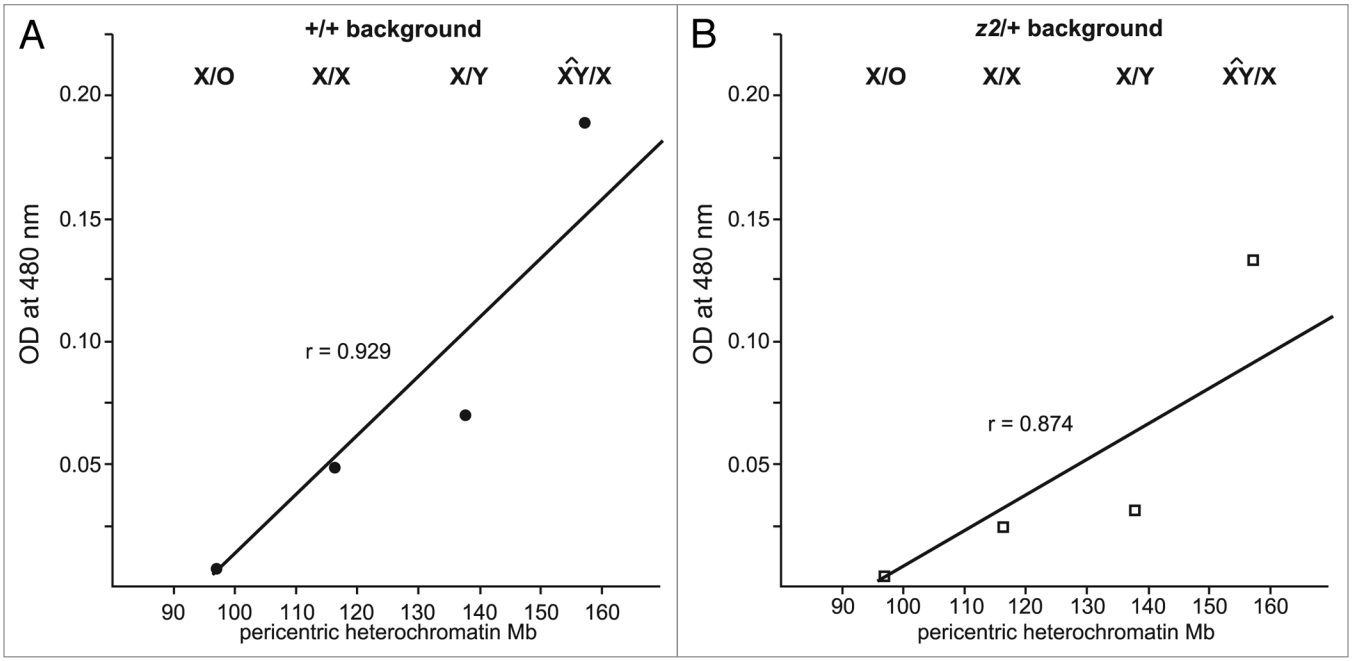

Figure 3. The relationship between eye pigment levels in *118E-10*/+ flies and the total amount of heterochromatin present is linear. (**A**) Eye pigment levels plotted as a function of total heterochromatin levels in X/O, X/X, X/Y and X^Y/X flies from a wild-type *JIL-1* (+/+) background. (**B**) Eye pigment levels plotted as a function of total heterochromatin levels in X/O, X/X, X/Y and X^Y/X flies from a *JIL-1*^{z2}/+ (*z2*/+) background. r indicates the correlation coefficient for each plot.

silencing in both X/O, X/X, X/Y and X^Y/X backgrounds. The lack of any indication of an epistatic interaction between the effect of the loss of function *JIL-1* allele and the changes in heterochromatic DNA, and the observation that the haplo-enhancer effect of *JIL-1* is proportional to the amount of total heterochromatin suggest that JIL-1's activity is dynamically modulated to achieve a more or less constant balance depending on the levels of heterochromatic factors present. It will be of interest in future studies to determine the molecular mechanisms for how this balance is regulated.

Materials and Methods

***Drosophila melanogaster* stocks.** Fly stocks were maintained and crosses made at 22°C according to standard protocols.[25] Canton S. was used for wild-type preparations. The *JIL-1*^{z2} null allele is described in Wang et al.[21] as well as in Zhang et al.[22] The insertion line *118E-10*[8] used in these experiments contains the P-element (*P[hsp26-pt, hsp70-w]*) and was the generous gift of Dr. L. Wallrath. Balancer chromosomes and markers are described in Lindsley and Zimm.[26] The compound chromosome C(1;Y)1 was obtained from the Bloomington stock center. The C(1;Y)1 chromosome contains the short (Ys) and long (Yl) arms of the Y chromosome attached to a complete X chromosome. C(1;Y)1/0 flies are viable and fertile males with no free Y chromosome. This chromosome was modified by a double crossover in an X^Y/X female to replace the w^+ allele with the w^{1118} allele.

PEV assays. PEV assays were performed as previously described.[10,12,16,19] To quantify the variegated phenotype adult flies were collected from the respective crosses at eclosion, aged 6 d at 22°C, frozen in liquid nitrogen, and stored at -80°C until assayed. The pigment assays were performed essentially as in Kavi and Birchler[24] using three sets of 10 fly heads of each genotype collected from males and females, respectively. For each sample the heads from the 10 flies were homogenized in 200 µl of methanol with 0.1% hydrochloric acid, centrifuged, and the optical density of the supernatant spectrophotometrically measured at a wavelength of 480 nm. Statistical comparisons were performed using a two-tailed Student's t-test. Eyes from representative individuals from these crosses were photographed using an Olympus Stereo Microscope and a Spot digital camera (Diagnostic Instruments).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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