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Note

Loss-of-Function Alleles of the JIL-1 Histone H3S10 Kinase Enhance Position-Effect Variegation at Pericentric Sites in Drosophila Heterochromatin

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ABSTRACT

In this study we show that loss-of-function alleles of the JIL-1 histone H3S10 kinase act as enhancers of position-effect variegation at pericentric sites whereas the gain-of-function *JIL-1*$^{Su(var)3\text{-}1[3]}$ allele acts as a suppressor strongly supporting a functional role for JIL-1 in maintaining euchromatic chromatin and counteracting heterochromatic spreading and gene silencing.

HIGHER-order chromatin structure is important for epigenetic regulation and control of gene activation and silencing. Transgenes inserted into centromeric regions of chromosomes in Drosophila can exhibit position-effect variegation (PEV), a mosaic silencing of transcription of euchromatic genes as a result of their placement in or near heterochromatin (reviewed by Wallrath 1998; Henikoff 2000; Schotta *et al.* 2003; Girton and Johansen 2007). It has recently been demonstrated that alleles of the *JIL-1* locus are important regulators of chromatin structure and gene expression (Wang *et al.* 2001; Ebert *et al.* 2004; Deng *et al.* 2005; Lerach *et al.* 2005, 2006). The JIL-1 histone H3S10 tandem kinase localizes specifically to euchromatic interband regions of polytene chromosomes in Drosophila (Jin *et al.* 1999) and analysis of a *JIL-1* null allele, *JIL-1*^{z2}, has shown that *JIL-1* is essential for viability (Wang *et al.* 2001; Zhang *et al.* 2003). Furthermore, loss of JIL-1 results in the spreading of the major heterochromatin markers histone H3K9 dimethylation (H3K9me2) and HP1 to ectopic locations on the chromosome arms, and genetic interaction assays have shown that JIL-1 functions antagonistically to Su(var)3-9, which is the major catalyst for dimethylation of the histone H3K9 residue (Schotta *et al.* 2002; Zhang *et al.* 2006). On the basis of these findings, Zhang *et al.* (2006) suggested a model in which JIL-1 histone H3S10 kinase activity functions to maintain euchromatic domains and counteracts heterochromatization and gene silencing. A prediction of this model is that loss-of-function *JIL-1* alleles will act as enhancers of PEV resulting in increased silencing of gene expression reporter constructs inserted into normally transcriptionally repressive regions such as pericentric heterochromatin (Johansen and Johansen 2006).

To test this model we examined the effect of decreased levels of JIL-1 protein on expression of a *white* reporter gene in four *P*-element transgenic insertion lines (Wallrath and Elgin 1995; Wallrath *et al.* 1996; Cryderman *et al.* 1998) (Table 1). Insertion of the *P* element (*P[hsp26-pt, hsp70-w]*) into euchromatic sites results in a uniform red eye phenotype whereas insertion into known centromeric heterochromatin regions of the fourth chromosome (line *118E-10*), the X chromosome (lines *118E-25* and *118E-32*), and the second chromosome (line *39C-3*) results in a variegating eye phenotype (Wallrath and Elgin 1995; Wallrath *et al.* 1996; Cryderman *et al.* 1998). This silencing within the heterochromatic domains has been correlated with local alterations in chromatin structure and/or shifts in chromatin packaging and suggests a dynamic balance between factors promoting repression and activation of gene expression (Wallrath and Elgin 1995; Cryderman *et al.* 1998; Sun *et al.* 2000). Both unique and repetitive DNA sequences were found adjacent to these variegating transgenes, suggesting that PEV does not require that the transgenes be surrounded by repetitive sequences (Cryderman *et al.* 1998). Furthermore, all of the transgenes show suppression of PEV in response to a mutation in the gene encoding HP1 (Cryderman *et al.* 1998).

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TABLE 1

Centromeric heterochromatin *P*-element insertion lines

Line[a]	Insertion site
118E-10	Fourth chromosome
118E-25	X chromosome
118E-32	X chromosome
39C-3	Second chromosome

[a] The *P*-element insertion lines contained the *hsp26* gene tagged with a fragment of barley cDNA and with a *hsp70-white* gene as a reporter. These lines are further characterized in Wallrath and Elgin (1995), Wallrath *et al.* (1996), and Cryderman *et al.* (1998).

In the experiments the four transgenic reporter lines were crossed into different *JIL-1* mutant backgrounds that combined hypomorphic and null *JIL-1* alleles (*JIL-1^{z60}* and *JIL-1^{z2}*) to generate progeny expressing decreased amounts of wild-type JIL-1 protein. The *JIL-1^{z60}* allele is a strong hypomorph producing only 0.3% of wild-type JIL-1 protein levels, whereas the *JIL-1^{z2}* allele is a true null and homozygous animals do not survive to adulthood (Wang *et al.* 2001; Zhang *et al.* 2003). The *JIL-1^{z2}/JIL-1^{z60}* heteroallelic combination is semilethal and only a limited number of eclosed animals from large-scale crosses could be analyzed. In addition, we compared the effect of the loss-of-function *JIL-1* alleles to that of the dominant gain-of-function *JIL-1$^{Su(var)3\text{-}1[3]}$* allele that is one of the strongest suppressors of PEV so far described (Ebert *et al.* 2004). The *JIL-1$^{Su(var)3\text{-}1[3]}$* allele generates truncated proteins with COOH-terminal deletions that mislocalize to ectopic chromosome sites (Ebert *et al.* 2004; Zhang *et al.* 2006). Flies from each of the four transgenic lines with the different JIL-1 genotypes were scored for the percentage of the eye that had red ommatidia and compared to flies containing wild-type levels of JIL-1 protein (Figures 1 and 2 and Table 2). Although both male and female flies were scored, due to sex differences results from only female flies are shown. However, the trend observed in male flies was identical to that in female flies. As illustrated in Figure 1, hypomorphic allelic combinations of the *JIL-1* alleles *JIL-1^{z60}* and *JIL-1^{z2}* lead to a strong enhancement of PEV as

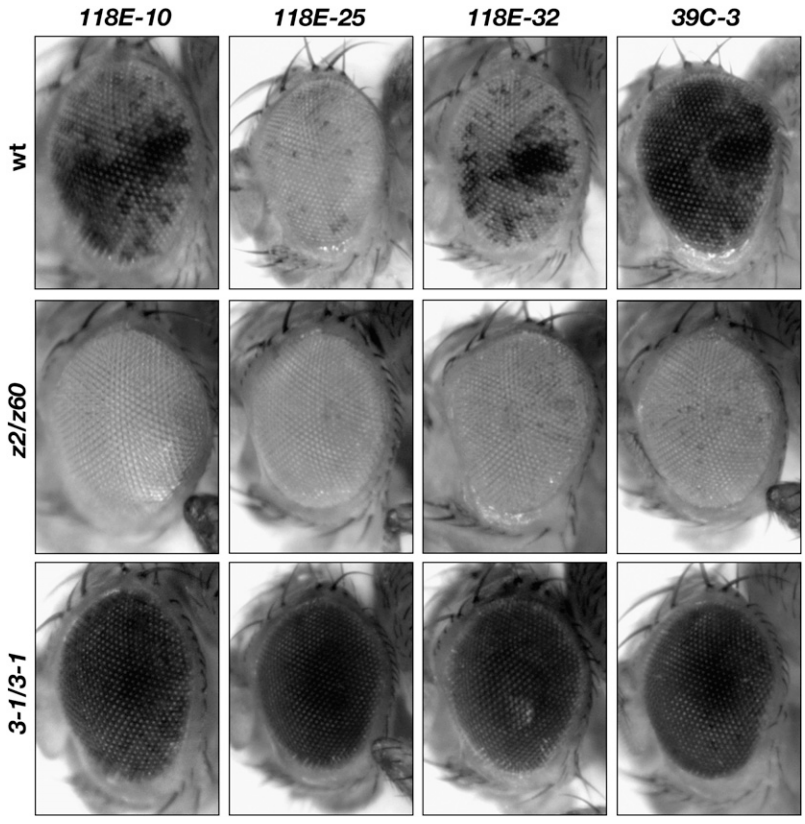

Figure 1.—The effect of *JIL-1* loss-of-function alleles and the gain-of-function *JIL-1$^{Su(var)3\text{-}1[3]}$* allele on PEV of the *P*-element lines *118E-10*, *118E-25*, *118E-32*, and *39C-3*, which were inserted into pericentric heterochromatin. (Top) PEV in the eyes of control flies with a wild-type *JIL-1* allele (+/+). (Middle) A hypomorphic allelic combination of the *JIL-1* alleles *JIL-1^{z60}* (*z60*) and *JIL-1^{z2}* (*z2*) leads to a strong enhancement of PEV as indicated by the nearly completely white eye phenotype. (Bottom) In contrast, the homozygous gain-of-function *JIL-1$^{Su(var)3\text{-}1[3]}$* allele (*3-1/3-1*) leads to strong suppression of PEV as indicated by the nearly completely red eye phenotype. All images are from female flies. *Drosophila melanogaster* fly stocks were maintained according to standard protocols (Roberts 1998). Canton-S was used for wild-type preparations. The *JIL-1^{z2}* and *JIL-1^{z60}* alleles are described in Wang *et al.* (2001) and in Zhang *et al.* (2003). The *JIL-1$^{Su(var)3\text{-}1[3]}$/TM3 Sb Ser* stock was obtained from the Bloomington Stock Center. Balancer chromosomes and markers are described in Lindsley and Zimm (1992). Strains containing X chromosomes with the *w^{1118}* allele and a loss-of-function *JIL-1* allele (either *JIL-1^{z2}* or *JIL-1^{z60}*) or the gain-of-function *JIL-1$^{Su(var)3\text{-}1[3]}$* allele heterozygous with the *TM6 Sb Tb e* third chromosome balancer were produced by standard crossing. Subsequent crosses between these strains generated flies with different *JIL-1* allelic combinations in a background homozygous for *P[hsp26-pt, hsp70-w]* insertions that were introduced from the *P*-element lines *118E-10*, *118E-25*, *118E-32*, or *39C-3* (Wallrath and Elgin 1995; Wallrath *et al.* 1996; Cryderman *et al.* 1998) by standard crosses. As a control, PEV of each of these transgenes was analyzed in flies homozygous for a Canton-S wild-type third chromosome. The *hsp70* promoter is leaky and promotes sufficient expression to generate a variegated eye phenotype under non-heat-shock conditions (Wallrath and Elgin 1995). To quantify the variegated phenotype, newly eclosed adults were collected, aged for 4–5 days at 25°, and then sorted into different classes on the basis of the percentage of the eye that was red. Eyes from representative individuals from these crosses were photographed using an Olympus stereo microscope and a Spot digital camera (Diagnostic Instruments).

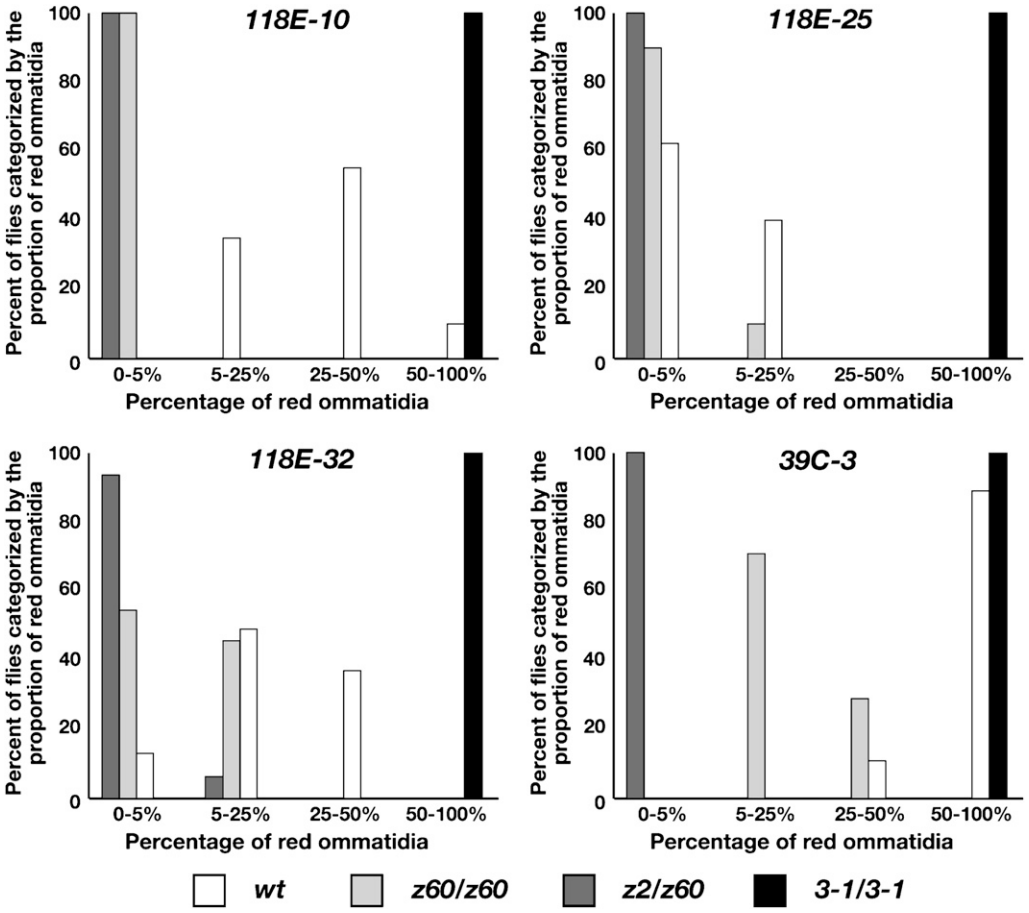

FIGURE 2.—*JIL-1* loss-of-function alleles and the gain-of-function *JIL-1*$^{Su(var)3\text{-}1[3]}$ allele affect the distribution of the percentage of red ommatidia in female flies homozygous for the *P*-element lines *118E-10*, *118E-25*, *118E-32*, and *39C-3*, which were inserted into pericentric heterochromatin. In the histograms the eyes from wild-type *JIL-1* flies (wt), homozygous *JIL-1*^{z60} flies (*z60/z60*), *JIL-1*^{z2}/*JIL-1*^{z60} flies (*z2/z60*), and *JIL-1*$^{Su(var)3\text{-}1[3]}$/*JIL-1*$^{Su(var)3\text{-}1[3]}$ flies (*3-1/3-1*) were sorted into different classes on the basis of the percentage of the eye that was red. The data suggest that for each *P*-element insertion line PEV is enhanced by *JIL-1* loss-of-function alleles as indicated by the increased proportion of white ommatidia whereas PEV is suppressed by the gain-of-function *JIL-1*$^{Su(var)3\text{-}1[3]}$ allele as indicated by the increased proportion of red ommatidia.

indicated by the nearly completely white eye phenotype, whereas in contrast, the homozygous gain-of-function *JIL-1*$^{Su(var)3\text{-}1[3]}$ allele leads to strong suppression of PEV as indicated by the nearly completely red eye phenotype. The effect of the JIL-1 alleles on the distribution of the proportion of red ommatidia in the four centromeric *P*-element insertion lines is shown in Figure 2 and Table 2. As indicated in Figure 2, the strongly hypomorphic allelic combination of *JIL-1*^{z2}/*JIL-1*^{z60} leads to a greater enhancement of PEV than the less severe *JIL-1*^{z60}/*JIL-1*^{z60} allelic combination. In addition, we compared the mean proportion of the eyes that had red ommatidia between *JIL-1*^{z2}/*JIL-1*^{z60}, +/+, and *JIL-1*$^{Su(var)3\text{-}1[3]}$/*JIL-1*$^{Su(var)3\text{-}1[3]}$ flies, respectively, for each of the four transgenic lines using a Student's *t*-test. In each case the mean proportions were significantly different ($P < 0.01$). Thus, these results show that while loss-of-function *JIL-1* alleles act as enhancers of PEV at pericentric sites the gain-of-function *JIL-1*$^{Su(var)3\text{-}1[3]}$ allele acts as a suppressor of PEV strongly supporting the model for JIL-1's function in counteracting heterochromatic spreading and gene silencing.

Interestingly, it has previously been demonstrated that both *JIL-1* hypomorphic loss-of-function mutations and gain-of-function *JIL-1*$^{Su(var)3\text{-}1}$ alleles act as strong suppressors of PEV of the w^{m4} allele (EBERT *et al.* 2004; LERACH *et al.* 2006). The *In(1)w*^{m4} X chromosome contains an inversion that juxtaposes the euchromatic *white* gene and heterochromatic sequences adjacent to the centromere (MULLER 1930; SCHULTZ 1936). Studies of PEV of this allele suggest that the degree of silencing may depend on the amount of heterochromatic factors at the breakpoint (reviewed in WEILER and WAKIMOTO 1995; GIRTON and JOHANSEN 2007). In this model a reduction in the amount of these factors in centromeric regions would limit heterochromatic spreading over long distances but would be predicted to not affect short range silencing. Thus, the finding that both *JIL-1* hypomorphic loss-of-function mutations and gain-of-function *JIL-1*$^{Su(var)3\text{-}1}$ alleles act as suppressors of PEV of the w^{m4} allele, whereas they have opposite effects on PEV of transgenes inserted directly into centromeric heterochromatin, supports this model. In the case of w^{m4} the redistribution of the major heterochromatin markers H3K9me2 and HP1 to ectopic locations on the chromosome arms and the resulting decrease in the concentration of these factors at centromeric regions occurring in *JIL-1* loss-of-function mutants (ZHANG *et al.* 2006) diminishes the ability of pericentric heterochromatin to spread long distances, leading to a reduction of w^{m4} silencing (LERACH *et al.* 2006). In contrast, the results of this study indicate that in the absence of JIL-1 histone H3S10 kinase activity transcription of euchromatic transgenes in direct proximity to pericentric heterochromatin is almost completely suppressed.

TABLE 2

The effect of *JIL-1* alleles on PEV of centromeric *P*-element insertion lines

Genotype[a]	*n*	% of flies categorized by the proportion of red ommatidia			
		0–5% red	5–25% red	25–50% red	50–100% red
118E-10					
+/+	207	0.0	35.0	55.3	9.7
z60/z60	57	100.0	0.0	0.0	0.0
z2/z60	47	100.0	0.0	0.0	0.0
3-1/3-1	304	0.0	0.0	0.0	100.0
118E-25					
+/+	97	59.8	40.2	0.0	0.0
z60/z60	151	90.1	9.9	0.0	0.0
z2/z60	56	100.0	0.0	0.0	0.0
3-1/3-1	101	0.0	0.0	0.0	100.0
118E-32					
+/+	53	13.2	49.1	37.7	0.0
z60/z60	90	54.4	45.6	0.0	0.0
z2/z60	77	93.5	6.5	0.0	0.0
3-1/3-1	54	0.0	0.0	0.0	100.0
39C-3					
+/+	83	0.0	0.0	10.8	89.2
z60/z60	62	0.0	71.0	29.0	0.0
z2/z60	30	100.0	0.0	0.0	0.0
3-1/3-1	113	0.0	0.0	0.0	100.0

[a] Only results from female flies homozygous for the *P*-element inserts as well as for w^{1118} in the different *JIL-1* allelic backgrounds are tabulated.

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