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Note

Loss-Of-Function Alleles of the JIL-1 Kinase Are Strong Suppressors of Position Effect Variegation of the w^{m4} Allele in Drosophila

Stephanie Lerach, Weiguo Zhang, Xiaomin Bao, Huai Deng, Jack Girton, Jørgen Johansen and Kristen M. Johansen[1]

Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, Iowa 50011

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ABSTRACT

In this article we show that hypomorphic loss-of-function alleles of the JIL-1 histone H3S10 kinase are strong suppressors of position effect variegation (PEV) of the w^{m4} allele and that lack of JIL-1 activity can counteract the effect of the dominant enhancer *E(var)2-1* on PEV.

HIGHER-ORDER chromatin structure is important for epigenetic regulation and control of gene activation and silencing. In Drosophila euchromatic genes can be transcriptionally silenced as a result of their placement in or near heterochromatin, a phenomenon known as position effect variegation (PEV) (reviewed by Wallrath 1998; Henikoff 2000; Schotta *et al.* 2003). Repression typically occurs in only a subset of cells and can be heritable, leading to mosaic patterns of gene expression (Schotta *et al.* 2003; Delattre *et al.* 2004). PEV in Drosophila has served as a major paradigm for the identification and genetic analysis of evolutionarily conserved determinants of epigenetic regulation of chromatin structure through the isolation of mutations that act as suppressors [*Su(var)*] or enhancers [*E(var)*] of variegation (Schotta *et al.* 2003). Some of the strongest suppressors of PEV described, *Su(var)3-1* mutations, were recently identified to be alleles of the *JIL-1* locus that generate proteins with COOH-terminal deletions (Ebert *et al.* 2004). JIL-1 is a tandem kinase that localizes specifically to euchromatic interband regions of polytene chromosomes (Jin *et al.* 1999). Analysis of *JIL-1* null and hypomorphic alleles has shown that *JIL-1* is essential for viability and that reduced levels of JIL-1 protein lead to a global disruption of chromosome structure (Jin *et al.* 2000; Wang *et al.* 2001; Zhang *et al.* 2003; Deng *et al.* 2005). These defects are correlated with severely decreased levels of histone H3S10 phosphorylation demonstrating that JIL-1 is the predominant kinase regulating the phosphorylation state of this residue at interphase (Wang *et al.* 2001). Ebert *et al.* (2004) provided evidence that the *Su(var)3-1* alleles of *JIL-1* consist of dominant gain-of-function mutations that may antagonize the expansion of heterochromatin formation; however, these experiments did not directly address JIL-1's normal function.

Thus, to examine the role that JIL-1 plays in higher-order chromatin structure and gene expression, we examined the effect of an allelic series of hypomorphic *JIL-1* alleles on PEV of the w^{m4} allele. 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). The resulting somatic variegation of w^{m4} expression occurs in clonal patches in the eye reflecting heterochromatic spreading from the inversion breakpoint that silences w^{m4} expression in the white patches and euchromatic packaging of the *w* gene in those patches that appear red (reviewed in Grewal and Elgin 2002). Studies of this effect suggest that the degree of spreading may depend on the amount of heterochromatic factors at the breakpoint (reviewed in Weiler and Wakimoto 1995). In these experiments the $In(1)w^{m4}$ chromosome was crossed into different *JIL-1* mutant backgrounds that combined hypomorphic and null *JIL-1* alleles ($JIL\text{-}1^{z28}$, $JIL\text{-}1^{z60}$, and $JIL\text{-}1^{z2}$) to generate progeny expressing different amounts of wild-type JIL-1 protein. The $JIL\text{-}1^{z28}$ allele is a weak hypomorph producing 45% of the normal level of wild-type JIL-1 protein; the $JIL\text{-}1^{z60}$ allele is a strong hypomorph producing only 0.3% of wild-type JIL-1 protein levels, whereas the $JIL\text{-}1^{z2}$ allele is a true null and homozygous animals do not survive to adulthood (Wang *et al.* 2001;

[1]*Corresponding author:* Department of Biochemistry, Biophysics, and Molecular Biology, 3154 Molecular Biology Building, Iowa State University, Ames, IA 50011. E-mail: kristen@iastate.edu

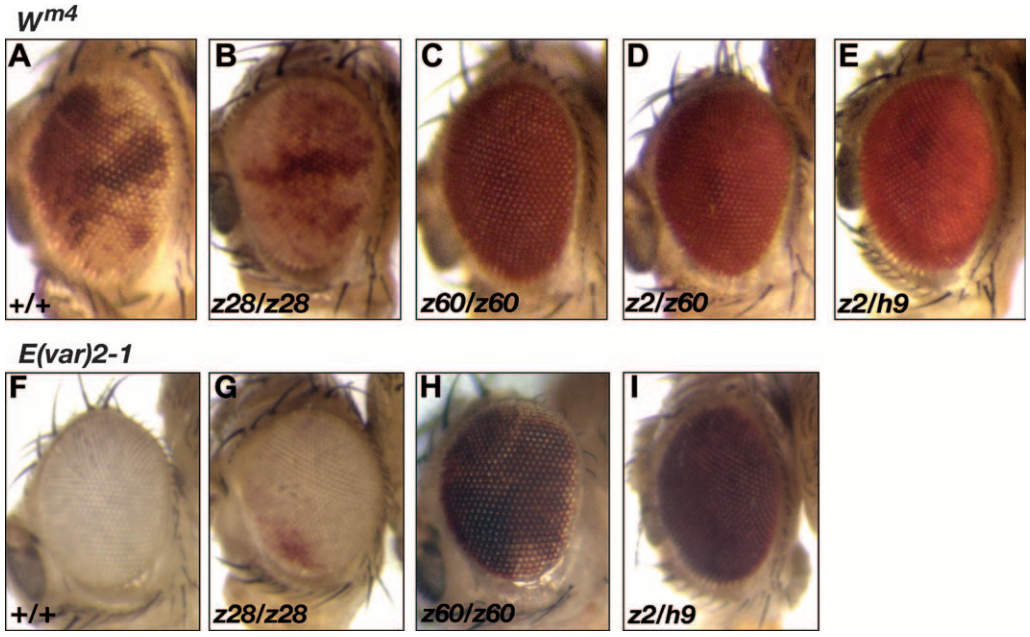

FIGURE 1.—The effect of *JIL-1* hypomorphic alleles on PEV. (A–E) Suppression of PEV in the eyes of $ln(1)w^{m4}$ (w^{m4}) flies with hypomorphic allelic combinations of the *JIL-1* alleles $JIL\text{-}1^{z28}$ (*z28*), $JIL\text{-}1^{z60}$ (*z60*), $JIL\text{-}1^{h9}$ (*h9*), and $JIL\text{-}1^{z2}$ (*z2*). Strong suppression of PEV is indicated by a completely red eye phenotype. (F–I) Hypomorphic allelic combinations of the *JIL-1* alleles $JIL\text{-}1^{z28}$ (*z28*), $JIL\text{-}1^{z60}$ (*z60*), $JIL\text{-}1^{h9}$ (*h9*), and $JIL\text{-}1^{z2}$ (*z2*) overcome the effects of *E(var)2-1* on PEV in the eyes of $ln(1)w^{m4}$ flies. Fly stocks were maintained according to standard protocols (ROBERTS 1998). Canton-S was used for wild-type preparations. The $JIL\text{-}1^{z2}$, $JIL\text{-}1^{z60}$, and $JIL\text{-}1^{h9}$ alleles are described in WANG *et al.* (2001) and in ZHANG *et al.* (2003). $In(1)w^{m4}$*; Pr Dr/TM3 Sb Ser* and $In(1)w^{m4}$*; CyO E(var)2-1/Sco* stocks were generously provided by G. Reuter. $In(1)w^{m4}$ and $Su(var)3\text{-}1^{3}$*/TM3 Sb Ser* stocks were obtained from the Bloomington Stock Center. Balancer chromosomes and markers are described in LINDSLEY and ZIMM (1992). Strains containing the $In(1)w^{m4}$ X chromosome and a loss-of-function *JIL-1* allele ($JIL\text{-}1^{z2}$, $JIL\text{-}1^{z60}$, $JIL\text{-}1^{28}$, or $JIL\text{-}1^{h9}$) 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 w^{m4} background. As a control, w^{m4} PEV was analyzed in flies homozygous for a Canton-S wild-type third chromosome. A *CyO* second chromosome containing the *E(var)2-1* allele was introduced into the $In(1)w^{m4}$*; JIL-1/ TM6* stock by standard crosses. As a control for PEV in these stocks, individuals that carried the *E(var)2-1 CyO* chromosome were compared with siblings that did not. To quantify the variegated phenotype newly eclosed adults were collected, aged for 4–5 days at 25°, and were 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).

ZHANG *et al.* 2003). The $JIL\text{-}1^{h9}$ allele expresses a truncated JIL-1 protein that lacks part of the second kinase domain and the entire COOH-terminal domain (ZHANG *et al.* 2003). The $JIL\text{-}1^{z2}/JIL\text{-}1^{z60}$ heteroallelic combination is semilethal and only a few eclosed animals from large-scale crosses could be analyzed. Flies with the different genotypes were scored for the percentage of the eye that was red and variegated w^{m4}; +/+ flies containing wild-type levels of JIL-1 protein were used as controls (Figure 1, A–E and Table 1). As JIL-1 protein levels were reduced, an increasing percentage of flies showed fully pigmented eyes, with 100% of the $JIL\text{-}1^{z2}/JIL\text{-}1^{z60}$ and $JIL\text{-}1^{z2}/JIL\text{-}1^{h9}$ animals showing completely red eyes (Figure 1, D and E and Table 1). This is in contrast to the control crosses in which none of the flies exhibited completely red eyes (Figure 1A and Table 1). These results strongly indicate that loss of JIL-1 protein results in suppression of PEV of the w^{m4} allele.

To confirm that loss of the JIL-1 protein produces a *bona fide Su(var)* phenotype, we examined the effect of decreased levels of JIL-1 protein in a w^{m4} genetic background that also carries the dominant enhancer

TABLE 1

***JIL-1* alleles suppress PEV of w^{m4}**

		% of flies categorized by the proportion of red ommatidia				
Genotype[a]	*n*	0% red	0–25% red	25–75% red	75–99% red	100% red
+/+	542	0.0	36.3	41.5	22.1	0.0
z28/z28	160	0.0	48.1	24.4	25.6	1.9
z60/z60	397	0.0	0.0	0.0	8.3	91.7
z2/z60	48	0.0	0.0	0.0	0.0	100.0
z2/h9	57	0.0	0.0	0.0	0.0	100.0

[a] Genotype of the third chromosome. In addition, all flies were homozygous (females) or hemizygous (males) for w^{m4} on the X chromosome.

TABLE 2

***JIL-1* alleles overcome the effects of *E(var)2-1* on PEV of w^{m4}**

Genotype[a]	*n*	% of flies categorized by the proportion of red ommatidia				
		0% red	0–25% red	25–75% red	75–99% red	100% red
+/+	1209	89.7	10.3	0.1	0.0	0.0
z28/z28	283	58.3	40.3	1.5	0.0	0.0
z60/z60	41	0.0	0.0	9.8	39.0	51.2
z2/h9	10	0.0	0.0	0.0	10.0	90.0

[a] Genotype of the third chromosome. In addition, all flies were homozygous (females) or hemizygous (males) for w^{m4} and were *E(var)*$^{2\text{-}1}$*CyO/+*.

E(var)2-1. This enhancer results in nearly completely white-eyed flies (Figure 1F) and has proven useful in identifying and characterizing strong *Su(var)* mutations in genetic screens (Schotta *et al.* 2003). As levels of JIL-1 protein decreased in this background, a corresponding increase in pigmentation was observed (Figure 1, G–I and Table 2) with 51.2% of *JIL-1*^{z60}*/JIL-1*^{z60} and 90.0% of *JIL-1*^{z2}*/JIL-1*^{h9} flies showing completely red eyes compared to 0% in control flies. Thus, lack of JIL-1 activity strongly counteracts the effect of the dominant enhancer *E(var)2-1* on PEV of the w^{m4} allele.

Zhang *et al.* (2006) recently demonstrated that a reduction in the levels of the JIL-1 histone H3S10 kinase results in a redistribution of the major heterochromatin markers H3K9me2 and HP1 to ectopic locations on the chromosome arms with the most pronounced increase on the X chromosomes. Interestingly, overall levels of heterochromatic factors remained unchanged, implying a concomitant reduction in the levels of pericentric heterochromatic factors (Zhang *et al.* 2006). On the basis of these findings a model was proposed wherein JIL-1 kinase activity functions to maintain euchromatic regions by antagonizing Su(var)3-9 mediated heterochromatization (Zhang *et al.* 2006). Thus, in the absence of JIL-1 function the dispersion of the H3K9me2 mark and HP1 to ectopic locations on the chromosomes would be expected to lead to heterochromatization and repression of gene expression at these sites, suggesting that loss of JIL-1 would result in an *E(var)* phenotype if the reporter locus were located at such a site. However, the results of Zhang *et al.* (2006) also showed that ectopic heterochromatization was not uniform and that not all active gene loci were repressed. This implies that certain chromatin sites are molecularly distinct and may be preferentially modified by Su(var)3-9 to recruit HP1 in the absence of JIL-1. Paradoxically, as a consequence

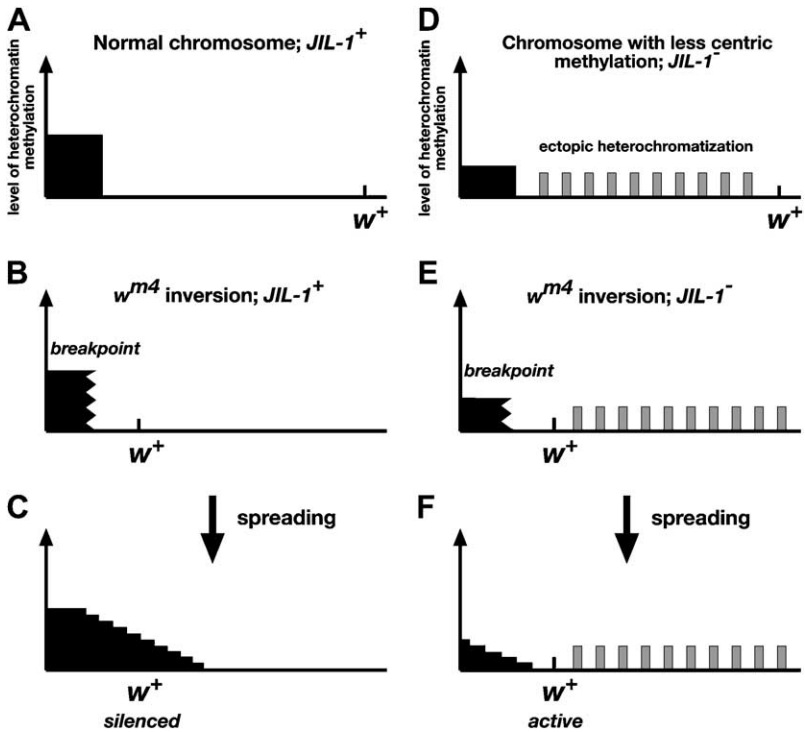

Figure 2.—Model for suppression of PEV of the w^{m4} allele by *JIL-1* null and hypomorphic alleles. (A–C) Spreading of heterochromatic factors (solid area) in a wild-type *JIL-1* background. With normal levels of pericentric heterochromatic factors present the spreading across the inversion breakpoint can reach the *w* gene and silence gene expression. (D–F) Spreading of heterochromatic factors (solid area) in a *JIL-1* null and hypomorphic allelic background. Shaded boxes indicate the redistribution to ectopic chromosome sites of heterochromatic markers occurring in *JIL-1* hypomorphic mutants. Because of the reduced levels of pericentric heterochromatic factors in the JIL-1 mutant background the spreading is attenuated and does not extend far enough from the breakpoint to silence *w* expression.

of this combined with the redistribution of a fixed level of heterochromatic factors, a reduction in JIL-1 activity would be predicted to lead to suppression—not enhancement—of PEV at loci not affected by ectopic Su(var) 3-9 activity but sensitive to the levels of heterochromatic factors at the pericentromeric chromatin, such as has previously been demonstrated for the w^{m4} allele (reviewed in Weiler and Wakimoto 1995). The results of this study support this hypothesis by demonstrating that *JIL-1* hypomorphic loss-of-function alleles are strong suppressors of PEV of the w^{m4} allele and that lack of JIL-1 activity can counteract the effect of the dominant enhancer *E(var)2-1* on PEV. We propose that the suppression of PEV of the w^{m4} allele in *JIL-1* hypomorphic backgrounds is due to a reduction in the level of heterochromatic factors at the pericentromeric heterochromatin near the inversion breakpoint site that reduces its potential for heterochromatic spreading and silencing (Figure 2).

It has recently been demonstrated that the *Su(var)3-1* alleles of *JIL-1* consist of dominant gain-of-function alleles that also strongly suppress PEV (Ebert *et al.* 2004). However, $JIL\text{-}1^{Su(var)3\text{-}1}$ alleles are characterized by deletions of the COOH-terminal domain that do not affect JIL-1 kinase activity or the spreading of heterochromatin markers (Ebert *et al.* 2004; Zhang *et al.* 2006). Furthermore, the results of Zhang *et al.* (2006) indicated that the COOH-terminal domain of JIL-1 is required for proper chromosomal localization and that $JIL\text{-}1^{Su(var)3\text{-}1}$ proteins are mislocalized to ectopic chromosome sites. Thus, the dominant gain-of-function effect of the $JIL\text{-}1^{Su(var)3\text{-}1}$ alleles may be attributable to JIL-1 kinase activity at ectopic locations possibly through phosphorylation of novel target proteins or by misregulated localization of the phosphorylated histone H3S10 mark (Ebert *et al.* 2004; Zhang *et al.* 2006). Consequently, the molecular mechanism of suppression of PEV of the w^{m4} allele is likely to be different for the dominant gain-of-function *Su(var)3-1* alleles and for the hypomorphic loss-of-function *JIL-1* alleles.

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