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Reduced Levels of Su(var)3-9 But Not Su(var)2-5 (HP1) Counteract the Effects on Chromatin Structure and Viability in Loss-of-Function Mutants of the JIL-1 Histone H3S10 Kinase

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ABSTRACT

It has recently been demonstrated that activity of the essential JIL-1 histone H3S10 kinase is a major regulator of chromatin structure and that it functions to maintain euchromatic domains while counteracting heterochromatization and gene silencing. In the absence of JIL-1 kinase activity, the major heterochromatin markers histone H3K9me2 and HP1 spread in tandem to ectopic locations on the chromosome arms. In this study, we show that the lethality as well as some of the chromosome morphology defects associated with the null JIL-1 phenotype to a large degree can be rescued by reducing the dose of the *Su(var)3-9* gene. This effect was observed with three different alleles of *Su(var)3-9*, strongly suggesting it is specific to *Su(var)3-9* and not to second site modifiers. This is in contrast to similar experiments performed with alleles of the *Su(var)2-5* gene that codes for HP1 in Drosophila where no genetic interactions were detectable between *JIL-1* and *Su(var)2-5*. Taken together, these findings indicate that while Su(var)3-9 histone methyltransferase activity is a major factor in the lethality and chromatin structure perturbations associated with loss of the JIL-1 histone H3S10 kinase, these effects are likely to be uncoupled from HP1.

IN Drosophila initiation of heterochromatin formation and repression of transcription has been linked to the RNAi machinery (Pal-Bhadra *et al.* 2004) and involves covalent modifications of histone tails and/or the exchange of histone variants (Swaminathan *et al.* 2005). In addition, heterochromatin formation requires several nonhistone chromatin proteins (Schotta *et al.* 2002; Greil *et al.* 2003; Delattre *et al.* 2004). Two of these, Su(var)2-5 (HP1) and Su(var)3-9 (a histone methyltransferase), are predominantly found at pericentric heterochromatin (James *et al.* 1989; Schotta *et al.* 2002) and are important components for silencing of reporter genes by heterochromatic spreading (for review, see Weiler and Wakimoto 1995; Girton and Johansen 2007). Su(var)3-9 has been shown to catalyze most of the dimethylation of the histone H3K9 residue, which in turn can promote HP1 binding (Schotta *et al.* 2002). In addition, Su(var)3-9 and HP1 can directly interact, suggesting a model where interdependent interactions between Su(var)3-9, HP1, and histone H3K9 dimethylation lead to heterochromatin assembly and gene silencing (Lachner *et al.* 2001; Schotta *et al.* 2002; Elgin and Grewal 2003). However, this model does not address the mechanism for how heterochromatin formation is restricted to certain parts of the genome and for how heterochromatic spreading is regulated (Greil *et al.* 2003).

It has recently been demonstrated that activity of the JIL-1 histone H3S10 kinase (Jin *et al.* 1999; Wang *et al.* 2001) is a major regulator of chromatin structure (Deng *et al.* 2005) and that it functions to maintain euchromatic domains while counteracting heterochromatization and gene silencing (Ebert *et al.* 2004; Lerach *et al.* 2006; Zhang *et al.* 2006; Bao *et al.* 2007). In the absence of JIL-1 kinase activity, the major heterochromatin markers H3K9me2 and HP1 spread in tandem to ectopic locations on the chromosome arms with the most pronounced increase on the X chromosomes (Zhang *et al.* 2006). However, overall levels of the H3K9me2 mark and HP1 were unchanged, suggesting that the spreading was accompanied by a redistribution that reduces the levels in pericentromeric heterochromatin. Genetic interaction assays demonstrated that *JIL-1* functions *in vivo* in a pathway with *Su(var)3-9* and that JIL-1 activity and localization are not affected by the absence of Su(var)3-9 activity, indicating that *JIL-1* is upstream to *Su(var)3-9* in this pathway (Zhang *et al.* 2006). Furthermore, the results of Zhang *et al.* (2006) suggested the possibility that the lethality of *JIL-1* null mutants may be due to repression of essential genes at these ectopic sites as a consequence of the spreading of Su(var)3-9 activity and HP1 recruitment. In this study, we have tested this hypothesis and examined the relative contributions of

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Su(var)3-9 and HP1. We show that while Su(var)3-9 histone methyltransferase activity is a major factor in the lethality and chromatin structure perturbations associated with loss of the JIL-1 histone H3S10 kinase, these effects are likely to be uncoupled from HP1.

MATERIALS AND METHODS

***Drosophila melanogaster* stocks:** Fly stocks were maintained according to standard protocols (Roberts 1998). Canton-S was used for wild-type preparations. The *JIL-1^{z2}* allele is described in Wang *et al.* (2001) and in Zhang *et al.* (2003). The *Su(var)3-9^{06}*, *Su(var)2-5^{04}*, and *Su(var)2-5^{05}* alleles are described in Schotta *et al.* (2002) and in Eissenberg *et al.* (1992) and were generously provided by. L. Wallrath. The *Su(var)3-9^{1}* and *Su(var)3-9^{2}* stocks were obtained from the Umeå Stock Center. Recombinant *JIL-1^{z2} Su(var)3-9^{1}*, *JIL-1^{z2} Su(var)3-9^{2}*, and *JIL-1^{z2} Su(var)3-9^{06}* chromosomes were identified by generating recombinants as described in Ji *et al.* (2005) except that the dominant *Su(var)3-9* phenotype was selected for in a *w^{m4}* background and the presence of *JIL-1^{z2}* was confirmed by PCR as in Zhang *et al.* (2003). Balancer chromosomes and markers are described in Lindsley and Zimm (1992).

Immunohistochemistry: Polytene chromosome squash preparations were performed as in Kelley *et al.* (1999) using the 5-min fixation protocol and labeled with antibody as described in Jin *et al.* (1999). Primary antibodies used include affinity purified Hope rabbit antiserum raised against JIL-1 residues 886–1013 (Jin *et al.* 1999), H3K9me2 rabbit antiserum (Upstate Biotechnology), and anti-HP1 mAb C1A9 (Developmental Studies Hybridoma Bank, University of Iowa). DNA was visualized by staining with Hoechst 33258 or with propidium iodide (Molecular Probes, Eugene, OR) in PBS. The appropriate species- and isotype-specific Texas Red-, TRITC-, and FITC-conjugated secondary antibodies (Cappel/ICN, Southern Biotech) were used (1:200 dilution) to visualize primary antibody labeling. The final preparations were mounted in 90% glycerol containing 0.5% *n*-propyl gallate. The preparations were examined using epifluorescence optics on a Zeiss Axioskop microscope and images were captured and digitized using a high resolution Spot charge-coupled device camera. Images were imported into Photoshop where they were pseudocolored, image processed, and merged. In some images, nonlinear adjustments were made for optimal visualization of Hoechst labeling of chromosomes.

RESULTS

Viability and chromosome morphology in *JIL-1* and *Su(var)3-9* double mutants: In a previous study, Zhang *et al.* (2006) used genetic assays to explore interactions between mutant alleles of *Su(var)3-9* and *JIL-1* by generating double mutant individuals. Since both *Su(var)3-9* and *JIL-1* are located on the third chromosome, a *Su(var)3-9^{1}*, *JIL-1^{z60}* chromosome was generated by recombination. However, in these experiments, only the +/*Su(var)3-9^{1}* allelic combination was examined (Zhang *et al.* 2006) and the *JIL-1^{z60}* allele, although a strong hypomorph, still had low levels of histone H3S10 kinase activity (Zhang *et al.* 2003). We have therefore extended these studies by recombining the true null *JIL-1^{z2}* allele (Wang *et al.* 2001; Zhang *et al.* 2003) with three different loss-of-function *Su(var)3-9* alleles. The *Su(var)3-9^{1}* allele consists of a frameshift at the N terminus of the protein upstream of the chromo- and SET domains while the *Su(var)3-9^{2}* allele has two missense mutations, and both alleles result in a null phenotype (Reuter *et al.* 1986; Tschiersch *et al.* 1994; Ebert *et al.* 2004). The null *Su(var)3-9^{06}* allele is due to a DNA insertion and immunoblot analysis has shown that histone H3K9 dimethylation is greatly reduced in homozygous animals (Schotta *et al.* 2002). Homozygous null *Su(var)3-9* mutants are viable and fertile (Tschiersch *et al.* 1994).

To determine whether reduction of Su(var)3-9 levels will rescue the lethality normally associated with a null *JIL-1^{z2}/JIL-1^{z2}* mutant background, we crossed *JIL-1^{z2} Su(var)3-9*/TM6 Sb Tb* males [where *Su(var)3-9** denotes the *Su(var)3-9^{1}*, *Su(var)3-9^{2}*, or *Su(var)3-9^{06}* allele] with *JIL-1^{z2}/TM6 Sb Tb* virgin females generating *JIL-1^{z2} Su(var)3-9*/JIL-1^{z2}* progeny identified as non-*Sb* (Table 1). In control experiments in which Su(var)3-9 activity was not altered, we crossed *JIL-1^{z2}/TM6 Sb Tb* males with *JIL-1^{z2}/TM6 Sb Tb* virgin females generating *JIL-1^{z2}/JIL-1^{z2}* progeny. In the control crosses, of a total of 685 eclosed flies, we observed no flies of the *JIL-1^{z2}/JIL-1^{z2}* genotype, indicating complete lethality (Table 1). However, in crosses that generate the double mutant combination [*JIL-1^{z2}/JIL-1^{z2} Su(var)3-9**] with one copy of either of the *Su(var)3-9* mutant alleles, the number of surviving flies with the *JIL-1^{z2}/JIL-1^{z2}* genotype increased dramatically. In these crosses, one-third of the eclosed flies would be expected to be of the *JIL-1^{z2}/JIL-1^{z2} Su(var)3-9** genotype, assuming full rescue, indicating that the reduction of Su(var)3-9 activity in these animals resulted in a 50.7–87.4% viability rate compared to a rate of 0% for *JIL-1^{z2}/JIL-1^{z2}* flies without the reduction in Su(var)3-9 activity (Table 1). However, it should be noted that both rescued male and female flies are sterile. We also performed crosses to generate *JIL-1^{z2} Su(var)3-9^{1}/JIL-1^{z2} Su(var)3-9^{2}* progeny. As indicated in Table 1, the further reduction in the dose of *Su(var)3-9* did not lead to an additional increase in viability of homozygous *JIL-1* null flies. Taken together, these results suggest that the lethality in null *JIL-1* mutant backgrounds to a substantial degree is mediated by *Su(var)3-9* activity. Furthermore, since this effect was observed with three different alleles of *Su(var)3-9* it is likely to be specific to *Su(var)3-9* and not to second site modifiers.

We further investigated whether a reduction in the dose of *Su(var)3-9* would also affect the severely perturbed polytene chromosome morphology observed in null *JIL-1^{z2}* homozygous larvae (Wang *et al.* 2001; Deng *et al.* 2005). For this analysis, we prepared squashes of polytene chromosomes labeled with Hoechst or with propidium iodide from *JIL-1^{z2}* homozygous null and wild-type third instar larvae and compared them with squashes from double mutant homozygous *JIL-1^{z2}* larvae with various combinations of the *Su(var)3-9* alleles described above. As illustrated in Figure 1A, loss of JIL-1

TABLE 1

Genetic interaction between *JIL-1* and *Su(var)3-9* alleles

Cross	Genotypes (no. of adult flies)		% expected ratio[a]
$JIL\text{-}1^{z2}/TM6 \times JIL\text{-}1^{z2}/TM6$	$JIL\text{-}1^{z2}/TM6$ (685)	$JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ (0)	0.0
$JIL\text{-}1^{z2}/TM6 \times JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{1}/TM6$	$JIL\text{-}1^{z2}/TM6$ or $JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{1}/TM6$ (350)	$JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{1}$ (144)	87.4
$JIL\text{-}1^{z2}/TM6 \times JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{2}/TM6$	$JIL\text{-}1^{z2}/TM6$ or $JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{2}/TM6$ (280)	$JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{2}$ (57)	50.7
$JIL\text{-}1^{z2}/TM6 \times JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{06}/TM6$	$JIL\text{-}1^{z2}/TM6$ or $JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{06}/TM6$ (209)	$JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{06}$ (53)	60.7
$JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{1}/TM6 \times JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{2}/TM6$	$JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{1}/TM6$ or $JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{2}/TM6$ (892)	$JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{1}/JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{2}$ (204)	55.8

[a] In these crosses, the *TM6* chromosome was identified by the *Stubble* marker. Consequently, the experimental genotypes could be distinguished from balanced heterozygotic flies by absence of the *Stubble* marker. The expected Mendelian ratio of non-*Stubble* to *Stubble* flies was 1:2 since *TM6/TM6* is embryonic lethal. The percentage of expected genotypic ratios was calculated as observed non-*Stubble* flies × 300/total observed flies.

histone H3S10 kinase activity leads to misalignment of the interband chromatin fibrils, which is further associated with coiling of the chromosomes and an increase of ectopic contacts between nonhomologous regions. This results in a shortening and folding of the chromosomes with a nonorderly intermixing of euchromatin and the compacted chromatin characteristic of banded regions (Deng *et al.* 2005). The extreme of this phenotype is exhibited by the male X polytene chromosome where no remnants of coherent banded regions can be observed (Figure 1A). However, in homozygous $JIL\text{-}1^{z2}$ double mutant combinations with a reduced dosage of Su(var)3-9, there was a marked improvement of polytene chromosome morphology of both male and female autosomes (Figure 1, B and C). The chromosome arms were to a large extent unfolded with reduced ectopic contacts and a clearly discernible banding pattern that in some cases attained near wild-type morphology (Figure 1D). In contrast, the morphology of the male X chromosome was largely unaffected by the reduction in Su(var)3-9, suggesting that ectopic Su(var)3-9 activity does not contribute to the "puffed" X chromosome phenotype in *JIL-1* null mutant backgrounds. It is well documented that the male X chromosome is unique because of the activity of the MSL complex and the MOF histone acetyltransferase that leads to hyperacetylation of histone H4 (Bone *et al.* 1994; Hilfiker *et al.* 1997). Therefore, the effects on chromosome morphology by *Su(var)3-9* may only be observable on autosomes and the female X chromosome. It has previously been shown that both morphology and JIL-1 localization in *Su(var)3-9* null polytene chromosomes are indistinguishable from that observed in wild-type chromosomes (Zhang *et al.* 2006). Furthermore, HP1 binding is severely reduced at the chromocenter and does not spread to the chromosome arms in either a homozygous null *Su(var)3-9* background (Schotta *et al.* 2002) or a homozygous null *Su(var)3-9, JIL-1* background (Figure 2). Taken together, these data indicate that the lethality as well as some of the chromosome morphology defects observed in *JIL-1* null mutant backgrounds may be mediated by ectopic Su(var)3-9 activity.

Genetic interactions between *JIL-1* and *Su(var)2-5 (HP1)*: The second component of the ectopic heterochromatic spreading observed in loss-of-function *JIL-1* mutant animals is the dimethylated histone H3K9-binding protein, HP1 (Zhang *et al.* 2006), which is intrinsic to pericentric heterochromatin formation (reviewed in Elgin and Grewal 2003). In contrast to homozygous null *Su(var)3-9* animals that are viable, transheterozygous null *Su(var)2-5* animals die as third instar larvae (Eissenberg *et al.* 1992; Eissenberg and Hartnett 1993). Furthermore, tethering of HP1 to euchromatic sites has shown that HP1 is sufficient to nucleate the formation of silent chromatin and that it can cause the formation of ectopic chromatin associations (Seum *et al.* 2001; Li *et al.* 2003; Danzer and Wallrath 2004) similar to those observed in *JIL-1* mutants. These findings suggest that the lethality as well as the disorganization of chromosomes in *JIL-1* null mutant backgrounds may be affected by ectopic HP1 binding and that the partial rescue of animals with reduced Su(var)3-9 activity is a consequence of impaired HP1 recruitment resulting from the reduction in histone H3K9 dimethylation. To explore this possibility, we performed genetic interaction assays between *JIL-1* and *Su(var)2-5*.

We first tested whether JIL-1 localization was affected in a functionally null mutant *Su(var)2-5* background by generating $Su(var)2\text{-}5^{04}/Su(var)2\text{-}5^{05}$ heteroallelic third instar larvae (Eissenberg *et al.* 1992). The $Su(var)2\text{-}5^{04}$ allele is due to a nonsense mutation leading to a truncated HP1 protein that degrades, whereas the $Su(var)2\text{-}5^{05}$ allele is associated with a frameshift resulting in a nonsense peptide containing only the first 10 amino

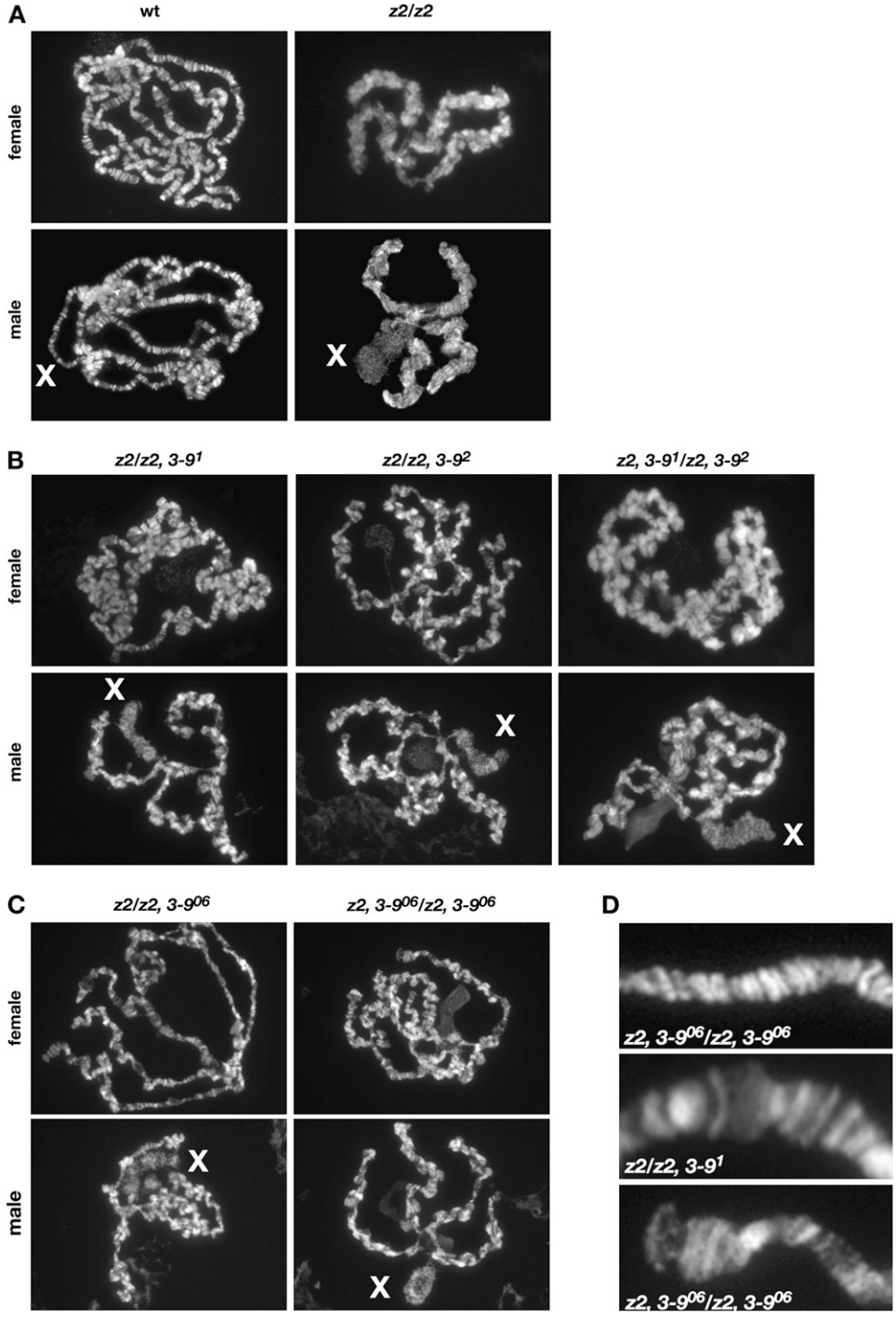

FIGURE 1.—Morphology of polytene chromosomes in *JIL-1* and *Su(var)3-9* double mutant backgrounds. Polytene chromosome preparations from third instar larvae were labeled with Hoechst or with propidium iodide to visualize the chromatin. (A) Polytene chromosome preparations from wild-type (wt) male and female larvae and from male and female homozygous $JIL\text{-}1^{z2}$ larvae (z2/z2). Note the misalignment and intermixing of interband and banded regions and the extensive coiling and folding of the chromosome arms in $JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ mutant chromosomes. The male X chromosome (X) is particularly affected and no remnants of banded regions are discernable. (B) Polytene chromosomes from male and female $JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{1}/JIL\text{-}1^{z2}$ (*z2/z2, 3-9*^{1}), $JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{2}/JIL\text{-}1^{z2}$ (*z2/z2, 3-9*^{2}), and $JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{1}/JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{2}$ (*z2, 3-9*^{1}*/z2, 3-9*^{2}) larvae. (C) Polytene chromosomes from male and female $JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{06}/JIL\text{-}1^{z2}$ (*z2/z2, 3-9*^{06}) and $JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{06}/JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{06}$ (*z2, 3-9*^{06}*/z2, 3-9*^{06}) larvae. Note the marked improvement of polytene chromosome morphology of both male and female autosomes in B and C. The chromosome arms were to a large extent unfolded with reduced ectopic contacts and a clearly discernible banding pattern that in some cases attained near wild-type morphology. In contrast, there was no discernible improvement in the morphology of the male X chromosome. (D) High magnification examples of regions of polytene chromosomes from $JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{1}/JIL\text{-}1^{z2}$ (*z2/z2, 3-9*^{1}) and $JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{06}/JIL\text{-}1^{z2}$ $Su(var)3\text{-}9^{06}$ (*z2, 3-9*^{06}*/z2, 3-9*^{06}) larvae with near wild-type morphology of band and interband regions.

acids of HP1 (EISSENBERG *et al.* 1992). Figure 3 shows polytene squashes from wild-type and $Su(var)2\text{-}5^{04}/Su(var)2\text{-}5^{05}$ heteroallelic larvae double labeled with Hoechst and JIL-1 antibody. Polytene chromosomes from loss-of-function HP1 mutant larvae partly lose the distinct pattern of band–interband regions of wild-type chromosomes, a phenotype that is especially pronounced for the male X chromosome (SPIERER *et al.* 2005) (Figure 3). However, JIL-1 localizes to both male and female chromosomes and is upregulated on the male X chromosome, as in wild-type preparations (Figure 3), suggesting that JIL-1 distribution is not altered by loss of HP1 function.

We further examined whether a reduction in HP1 levels could improve chromosome morphology of JIL-1 null animals and in reciprocal experiments whether a reduction in JIL-1 levels could improve chromosome morphology of HP1 null animals. Since the HP1 gene is located on the second chromosome while *JIL-1* is on the third chromosome, such double mutant animals were

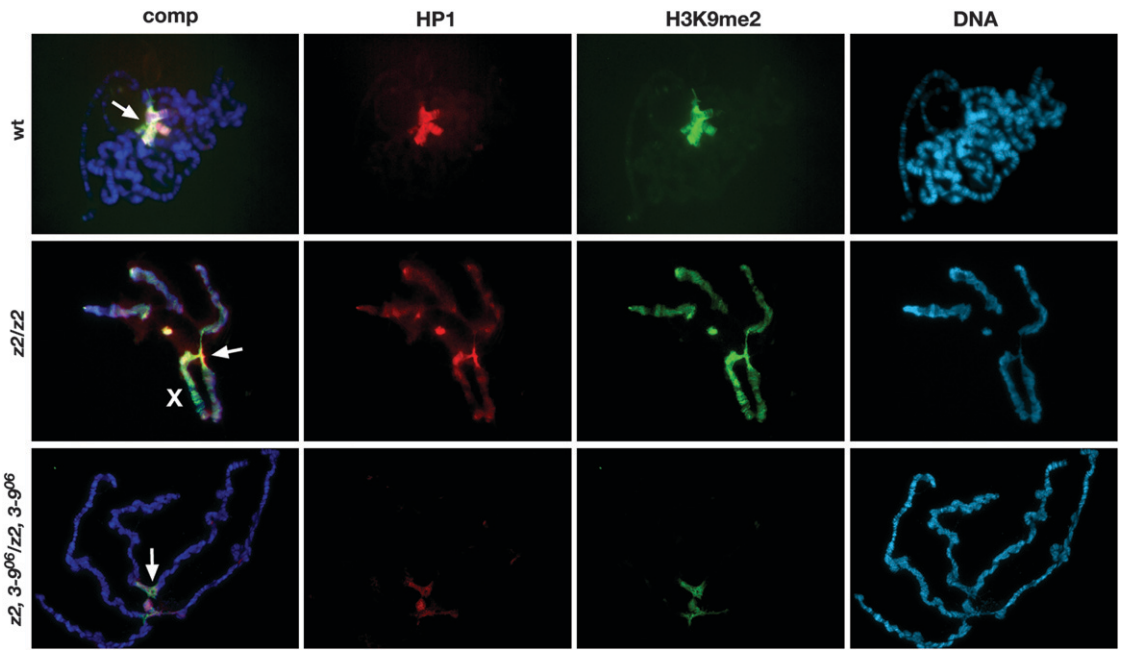

Figure 2.—Localization of HP1 and histone H3K9me2 in polytene chromosomes from *JIL-1* and *Su(var)3-9* mutant third instar larvae. The polytene squashes were triple labeled with antibodies to HP1 (red) and H3K9me2 (green) and with Hoechst (DNA, blue). The X chromosome is indicated by an X and the chromocenter by an arrow. Preparations from wild-type (wt), $JIL\text{-}1^{z2}$ homozygous (*z2/z2*), and $JIL\text{-}1^{z2}$ and $Su(var)3\text{-}9^{06}$ double homozygous (*z2, 3-9*^{06}*/z2, 3-9*^{06}) larvae are shown. In wild-type preparations, HP1 and H3K9me2 labeling was mainly localized to and abundant at the chromocenter; however, in the absence of the JIL-1 kinase, the HP1 and H3K9me2 labeling spread to the autosomes and particularly to the X chromosome (see also Zhang *et al.* 2006). In *z2, 3-9*^{06}*/z2, 3-9*^{06} double mutant larvae, the HP1 and H3K9me2 labeling were greatly reduced and confined to the chromocenter.

generated by standard genetic crosses. Polytene squashes from third instar larval salivary glands from these double mutant combinations were double labeled with Hoechst and an antibody to histone H3K9me2 and compared to wild-type preparations (Figure 4). In HP1 null animals, histone H3K9 dimethylation is dramatically upregulated on all the chromosome arms (Schotta *et al.* 2002) (Figure 4B), whereas in JIL-1 null mutants, the upregulation is most pronounced on the X chromosome (Zhang *et al.* 2006) (Figure 4C). As illustrated in Figure 4D, a reduction in the dose of JIL-1 affected neither chromosome morphology nor the spreading of H3K9 dimethylation in $JIL\text{-}1^{z2}/+$; $Su(var)2\text{-}5^{04}/Su(var)2\text{-}5^{05}$ mutant larvae. Likewise, chromosome morphology and H3K9me2 distribution in $Su(var)2\text{-}5^{04}/+$; $JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ (Figure 4F) and $Su(var)2\text{-}5^{05}/+$; $JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ (Figure 4E) double mutant larvae were indistinguishable from those of homozygous $JIL\text{-}1^{z2}$ mutant larvae. In the double mutant null *JIL-1* and *Su(var)2-5* combination, the chromosome morphology resembled that of JIL-1 null polytene chromosomes, whereas the H3K9me2 distribution resembled that of *Su(var)2-5* null chromosomes (Figure 4G). The similarity of the spreading of the H3K9me2 marker in HP1 loss-of-function mutants in both wild-type *JIL-1* and *JIL-1* null mutant backgrounds indicates that this ectopic redistribution is independent of JIL-1 kinase activity. Furthermore, in viability assays, there was neither rescue of $JIL\text{-}1^{z2}$ homozygous lethality by reducing the dose of wild-type HP1 by either the $Su(var)2\text{-}5^{04}$ or the $Su(var)2\text{-}5^{05}$ allele nor rescue of *Su(var)2-5* mutant lethality by reducing the dose of wild-type *JIL-1* (Table 2). Unlike the situation for *Su(var)3-9* in which loss of one wild-type allele results in significant rescue of the null $JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ lethality, loss of a *Su(var)2-5* wild-type allele has no effect. Taken together, these results suggest that there are no genetic interactions detectable in these assays between *JIL-1* and *Su(var)2-5* and that HP1 in contrast to Su(var)3-9 does not contribute to the lethality or disruption of chromosome morphology observed in *JIL-1* loss-of-function mutants.

DISCUSSION

Interdependent interactions between the HP1 and Su(var)3-9 proteins as well as histone H3K9 dimethylation are thought to be major factors in heterochromatin formation and gene silencing in Drosophila (Lachner *et al.* 2001; Schotta *et al.* 2002; Elgin and Grewal 2003). Recently, it has been demonstrated that the essential JIL-1 histone H3S10 kinase antagonizes heterochromatization and functions to maintain the chromatin structure of euchromatic regions (Wang *et al.* 2001; Ebert *et al.* 2004; Zhang *et al.* 2006). In the absence of JIL-1 kinase activity, the heterochromatin

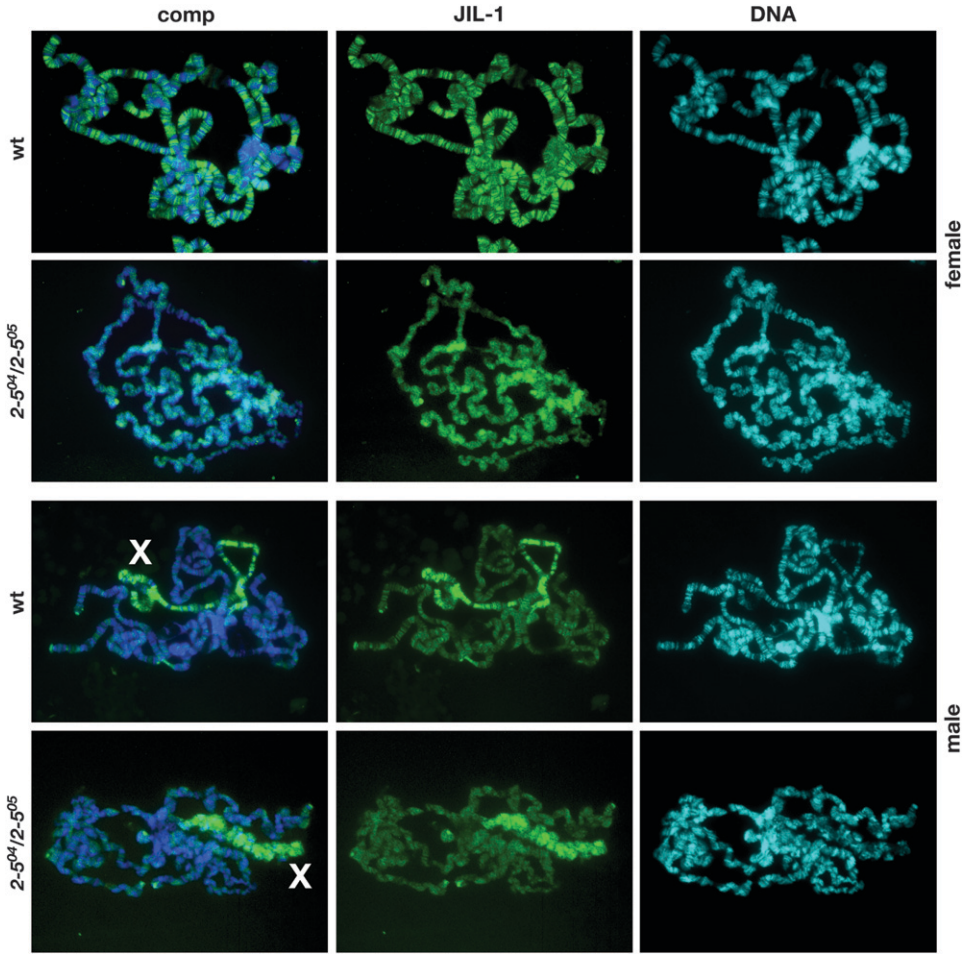

FIGURE 3.—JIL-1 localization in HP1 mutant larvae. Polytene chromosome preparations from male and female wild-type (wt) and *Su(var)2-5*^{04}/*Su(var)2-5*^{05} (*2-5*^{04}/*2-5*^{05}) third instar larvae were double labeled with JIL-1 antibody and Hoechst to visualize the chromatin. The male X chromosome is indicated with an X. Although polytene chromosomes from loss-of-function HP1 mutant larvae partly lose the distinct pattern of band–interband regions of wild-type chromosomes, a phenotype that is especially pronounced for the male X chromosome, JIL-1 localizes to both male and female chromosomes and is upregulated on the male X chromosome as in wild-type preparations.

markers H3K9me2 and HP1 spread in tandem to ectopic locations on the chromosomes (ZHANG *et al.* 2006). Furthermore, loss of JIL-1 histone H3S10 kinase activity results in an increase of ectopic contacts between nonhomologous regions leading to a shortening and folding of the chromosomes with a nonorderly intermixing of euchromatic and compacted chromatin regions (DENG *et al.* 2005). In this study, we show that the lethality as well as some of the chromosome morphology defects associated with the null *JIL-1* phenotype to a large degree can be rescued by reducing the dose of the *Su(var)3-9* gene. This effect was observed with three different alleles of *Su(var)3-9*, strongly suggesting it is likely to be specific to *Su(var)3-9* and not to second site modifiers. This is in contrast to similar experiments performed with alleles of the *Su(var)2-5* gene that codes for HP1 in Drosophila. In these assays, no genetic interactions were detectable between *JIL-1* and *Su(var)2-5*, suggesting that the lethality and disruption of chromosome morphology observed when JIL-1 levels are decreased are not due to increased HP1 on the chromosomal arms but rather are associated with ectopic Su(var)3-9 activity. How Su(var)3-9 may mediate these effects is unknown and will require additional studies.

While Su(var)3-9 and HP1 reciprocal interactions are well documented at pericentric regions, they are not universal (SCHOTTA *et al.* 2002; GREIL *et al.* 2003; DANZER and WALLRATH 2004). For example, HP1 binding on the fourth chromosome has been shown to be independent of Su(var)3-9 (SCHOTTA *et al.* 2002). Furthermore, a large scale survey of Su(var)3-9 and HP1 binding at coding regions demonstrated exclusive enrichment of Su(var)3-9 at the majority of such nonpericentric regions that map to the chromosome arms (GREIL *et al.* 2003). Strikingly, while both Su(var)3-9 and HP1 preferentially associate with genes of low expression levels, this preference is more prominent for Su(var)3-9 than for HP1, suggesting that where Su(var)3-9 is actively involved in silencing of its target genes, these Su(var)3-9 complexes may be more potent silencers if they lack HP1 (GREIL *et al.* 2003). Conversely, DANZER and WALLRATH (2004), using a tethering system to recruit HP1 to euchromatic sites, have shown that HP1-mediated silencing can operate in a Su(var)3-9-independent manner. These findings indicate that although Su(var)3-9 and HP1 cooperate in heterochromatin formation and gene silencing at pericentric chromosome sites, they may function independently at other regions such as the

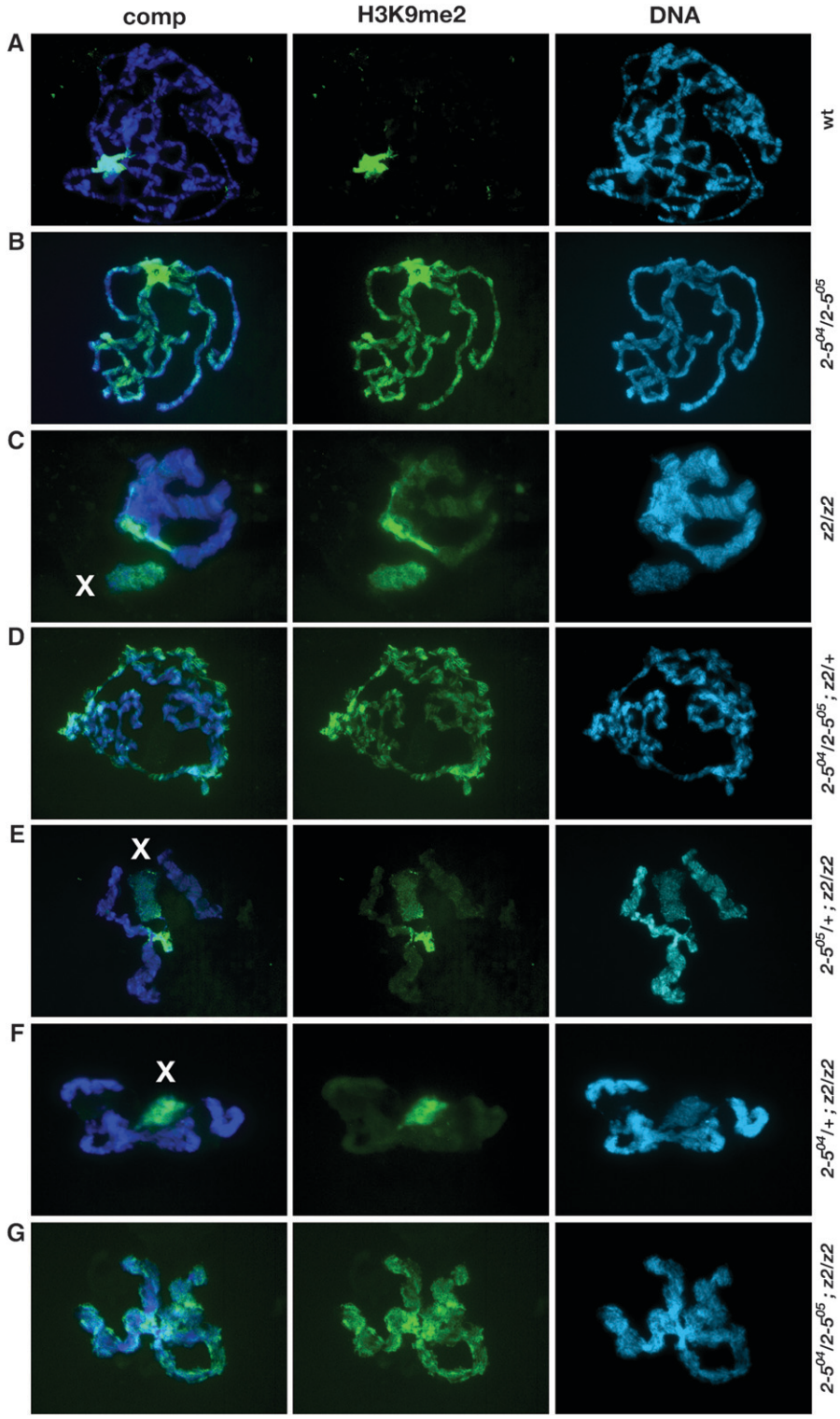

FIGURE 4.—Polytene chromosome morphology and distribution of the H3K9me2 marker in *JIL-1* and *Su(var)2-5* mutants. Polytene chromosome preparations from third instar larvae were double labeled with histone H3K9me2 antibody (green) and Hoechst (DNA in blue) to visualize the chromatin. The male X chromosome is indicated with an X. Preparations from wild-type (wt, A), *Su(var)2-5*^{04}/*Su(var)2-5*^{05} (*2-5*^{04}/*2-5*^{05}; B), *JIL-1*^{z2}/*JIL-1*^{z2} (*z2/z2*; C), *Su(var)2-5*^{04}/*Su(var)2-5*^{05}; *JIL-1*^{z2}/+ (*2-5*^{04}/*2-5*^{05}; *z2*/+; D), *Su(var)2-5*^{05}/+; *JIL-1*^{z2}/*JIL-1*^{z2} (*2-5*^{05}/+; *z2/z2*; E), *Su(var)2-5*^{04}/+; *JIL-1*^{z2}/*JIL-1*^{z2} (*2-5*^{04}/+; *z2/z2*; F), and *Su(var)2-5*^{04}/*Su(var)2-5*^{05}; *JIL-1*^{z2}/*JIL-1*^{z2} (*2-5*^{04}/*2-5*^{05}; *z2/z2*; G) larvae are shown.

chromosome arms. This is underscored by the finding that whereas Su(var)3-9 is necessary for HP1 recruitment to pericentric chromatin, Su(var)3-9 spreads and is dramatically upregulated on the chromosome arms in the absence of HP1 (SCHOTTA *et al.* 2002). Interestingly, the spreading in HP1 loss-of-function mutants is independent of JIL-1 kinase activity, indicating that at least two different molecular mechanisms regulate Su(var)3-9 localization, one dependent on HP1 and one dependent on the JIL-1 kinase.

The results of this study suggest that the lethality of *JIL-1* null mutants may be due to the repression of

TABLE 2

Genetic interaction between *JIL-1* and *Su(var)2-5* alleles

Cross	Genotypes (no. of adult flies)		% expected ratio[a]
JIL-1^{z2}/TM6 × *JIL-1^{z2}/TM6*	*JIL-1^{z2}/TM6* (685)	*JIL-1^{z2}/JIL-1^{z2}* (0)	0.0
Su(var)2-5^{04}/CyO ; *JIL-1^{z2}/TM6* × *Su(var)2-5^{04}/CyO* ; *JIL-1^{z2}/TM6*	*Su(var)2-5^{04}/CyO* ; *JIL-1^{z2}/TM6* (231)	*Su(var)2-5^{04}/CyO* ; *JIL-1^{z2}/JIL-1^{z2}* (0)	0.0
Su(var)2-5^{05}/CyO ; *JIL-1^{z2}/TM6* × *Su(var)2-5^{05}/CyO* ; *JIL-1^{z2}/TM6*	*Su(var)2-5^{05}/CyO* ; *JIL-1^{z2}/TM6* (328)	*Su(var)2-5^{05}/CyO* ; *JIL-1^{z2}/JIL-1^{z2}* (0)	0.0
Su(var)2-5^{04}/CyO ; *JIL-1^{z2}/TM6* × *Su(var)2-5^{05}/CyO* ; *JIL-1^{z2}/TM6*	*Su(var)2-5^{04}/CyO* ; *JIL-1^{z2}/TM6* or *Su(var)2-5^{05}/CyO* ; *JIL-1^{z2}/TM6* (636)	*Su(var)2-5^{04}/Su(var)2-5^{05}* ; *JIL-1^{z2}/JIL-1^{z2}* or *Su(var)2-5^{04}/CyO* ; *JIL-1^{z2}/JIL-1^{z2}* or *Su(var)2-5^{05}/CyO* ; *JIL-1^{z2}/JIL-1^{z2}* (0)	

[a] In these crosses, the *TM6* chromosome was identified by the *Stubble* marker. Consequently, the experimental genotypes could be distinguished from balanced heterozygotic flies by absence of the *Stubble* marker. The expected Mendelian ratio of non-*Stubble* to *Stubble* flies was 1:2 since *TM6/TM6* and *CyO/CyO* are embryonic lethal and *Su(var)2-5^{04}/Su(var)2-5^{04}*, *Su(var)2-5^{05}/Su(var)2-5^{05}*, and *Su(var)2-5^{04}/Su(var)2-5^{05}* die before or at the third instar larval stage. The percentage of expected genotypic ratios was calculated as observed non-*Stubble* flies × 300/total observed flies.

essential genes as a result of ectopic Su(var)3-9 activity unrelated to HP1 recruitment. At interphase, JIL-1 phosphorylates the histone H3S10 residue in euchromatic regions of polytene chromosomes (Jin *et al.* 1999; Wang *et al.* 2001), suggesting as a plausible model that this phosphorylation during interphase prevents recruitment of Su(var)3-9 to these sites. At present, we do not know whether the observed spreading of Su(var)3-9 in JIL-1 hypomorphic backgrounds occurred preferentially to specific euchromatic sites (Zhang *et al.* 2006). In *JIL-1* null animals, the morphology of polytene chromosomes is greatly perturbed and there is not only an intermixing of euchromatin and the compacted chromatin characteristic of banded regions but also a looping of nonhomologous chromatid regions that become fused and confluent (Deng *et al.* 2005). However, in support for this model for JIL-1's function in counteracting heterochromatic spreading and gene silencing, it was recently demonstrated that loss-of-function *JIL-1* alleles act as enhancers of position-effect-variegation (PEV) at centric sites, whereas the gain-of-function *JIL-1$^{Su(var)3-1}$* allele acts as a suppressor of PEV (Bao *et al.* 2007). The *JIL-1$^{Su(var)3-1}$* allele is one of the strongest suppressors of PEV so far described (Ebert *et al.* 2004) and it generates truncated proteins with COOH-terminal deletions that mislocalize to ectopic chromosome sites (Ebert *et al.* 2004; Zhang *et al.* 2006). Thus, the dominant gain-of-function effect of the *JIL-1$^{Su(var)3-1}$* alleles may be attributable to JIL-1 kinase activity at ectopic locations, leading to misregulated localization of the phosphorylated histone H3S10 mark counteracting the spreading of Su(var)3-9. In future experiments, it will be of interest to determine the molecular mechanisms for how Su(var)3-9 activity can effect the lethality and changes in chromatin structure observed in the absence of JIL-1.

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