A *Drosophila* insulator protein facilitates dosage compensation of the X chromosome *mini-white* gene located at autosomal insertion sites

Robin R. Roseman, Jeffrey M. Swan and Pamela K. Geyer
Department of Biochemistry, University of Iowa, College of Medicine, Iowa City, IA 52242, USA

SUMMARY

The *suppressor of Hairy-wing* [*su(Hw)*] gene encodes a zinc finger protein that binds to a repeated motif in the gypsy retrotransposon. These DNA sequences, called the *su(Hw)*-binding region, have properties of an insulator region because they (1) disrupt enhancer/silencer function in a position-dependent manner and (2) protect the *mini-white* gene from both euchromatic and heterochromatic position effects. To gain further insights into the types of position effects that can be insulated, we determined the effects of the *su(Hw)*-binding region on dosage compensation of the X-linked *mini-white* gene. Dosage compensation is the process that equalizes the unequal content of X-linked genes in males and females by increasing the X-linked transcription level twofold in males. Transposition of X-linked genes to the autosomes commonly results in incomplete dosage compensation, indicating that the distinct male X chromatin environment is important for this process. We found that dosage compensation of autosomally integrated *mini-white* genes flanked by *su(Hw)*-binding regions was greatly improved, such that complete or nearly complete compensation was observed at the majority of insertion sites. The *su(Hw)* protein was essential for this enhanced dosage compensation because in a *su(Hw)* mutant background compensation was incomplete. These experiments provide evidence that the *su(Hw)*-binding region facilitates dosage compensation of the *mini-white* gene on the autosomes. This may result from protection of the *mini-white* gene from a negative autosomal chromatin environment.

Key words: *Drosophila*, *su(Hw)* protein, domain boundaries, dosage compensation, gene expression, position-effects, X chromosome, *mini-white*

INTRODUCTION

Mutations in the *suppressor of Hairy-wing* [*su(Hw)*] gene reverse the phenotype of tissue-specific mutations caused by the insertion of the gypsy retrotransposon (Modolell et al., 1983). This modifier locus is specific for gypsy-induced mutations and does not affect mutations caused by other transposons (Rutledge et al., 1988). The *su(Hw)* locus encodes a protein that contains a zinc-finger DNA-binding domain, a leucine zipper region and two acidic domains and is expressed in most tissues throughout development (Parkhurst et al., 1988; Harrison et al., 1993). The *su(Hw)* protein binds to gypsy sequences located 3' of the 5' LTR, called the *su(Hw)*-binding region (Spana et al., 1988; Mazo et al., 1989). This binding region contains twelve copies of a 27 bp binding site, which are embedded in A-rich sequences (Marlor et al., 1986).

Insertions of gypsy disrupt gene expression by inactivating control elements in the target gene (Geyer et al., 1986; Peifer and Bender, 1986; Simon et al., 1990; Jack et al., 1991; Dorsett, 1993). The portion of gypsy responsible for these effects is the *su(Hw)*-binding region. Mutant phenotypes associated with gypsy insertions are reproduced by the binding region alone (Holdridge and Dorsett, 1991; Geyer and Corces, 1992). Inactivation of enhancers depends upon the position of the binding region within a gene. The *su(Hw)*-binding region represses enhancer function only when inserted between the affected enhancer and its promoter (Holdridge and Dorsett, 1991; Geyer and Corces, 1992). All twelve *su(Hw)*-binding sites are required for complete enhancer inactivation, as insertion into or deletion of these sequences partially restores enhancer activity (Geyer et al., 1988; Peifer and Bender, 1988; Smith and Corces, 1992).

The effects of the *su(Hw)* protein are general. This is supported by the fact that a number of unrelated genes are mutated either by association of the *su(Hw)* protein with gypsy (Modolell et al., 1983) or an isolated *su(Hw)*-binding region (Holdridge and Dorsett, 1991; Geyer and Corces, 1992; Roseman et al., 1993). In addition, *su(Hw)*-binding regions flanking the *mini-white* gene protect *white* expression from inhibitory and stimulatory position-effects when inserted throughout the genome (Roseman et al., 1993).

Taken together, these experiments indicate that the *su(Hw)*-binding region has properties similar to those associated with domain boundaries (Kellum and Schedl, 1992). Boundary elements are proposed to organize chromosomes into separate domains such that each domain represents an independent unit of gene activity (reviewed in Eissenberg and Elgin, 1991; Wolfe, 1994). In this way, genes within a domain are subject to its regulatory environment but are protected from control elements present in surrounding domains.
To gain insights into the capacity of the su(Hw) protein to establish independent chromosomal domains, we were interested in determining the spectrum of position-effects that are insulated by this protein. In these studies, we examined the effects of the su(Hw) protein on dosage compensation of an X-linked gene integrated at autosomal sites. Dosage compensation is the process by which the unequal content of X chromosomes in males and females is equalized. In Drosophila, dosage compensation is accomplished by transcribing the male X chromosome at twice the level of each of the two female X chromosomes (Mukherjee and Beermann, 1965; reviewed in Lucchesi and Manning, 1987). These effects are associated with changes in chromatin structure (Dobzansky, 1957) and require at least four genes, maleless (mle) and the male-specific lethal-1, -2, -3 genes (msl-1, msl-2, msl-3), collectively known as the msls (Fukunaga et al., 1975; Belote and Lucchesi, 1980; Uchida et al., 1981; Lucchesi et al., 1982). Proteins encoded by these genes appear to play a direct role in dosage compensation, as evidenced by their preferential localization along the length of the male X chromosome (Kuroda et al., 1991; Palmer et al., 1993; Gorman et al., 1995). Association of MLE, MSL-1 and MSL-3 with the male X chromosome requires the presence of the other MSLs, suggesting that these proteins assemble into a multimeric complex (Gorman et al., 1993, 1995; Hilftiker et al., 1994; Palmer et al., 1994). In addition to the MSL complex, the male X chromosome preferentially contains a specific histone isoform, histone H4 acetylated at lysine 16 (Turner et al., 1992). The enrichment of the acetylated histone H4 requires all of the msl genes (Bone et al., 1994). This has led to the model that these proteins form a male-specific complex which associates with the X chromosome and alters its chromatin structure, thereby causing hypertranscription (reviewed in Kuroda et al., 1993; Baker et al., 1994).

Germline transformation provided insights into cis-linked sequences required for dosage compensation of several X-linked genes (reviewed in Lucchesi and Manning, 1987; Qian and Pirrotta, 1995). These studies showed that X-linked genes inserted at an autosomal location retain some ability to dosage compensate because heterozygous males had a higher level of gene expression than heterozygous females. This indicates that cis-acting sites important for dosage compensation are relatively small and lie near the promoter or within the gene. However, an autosomal location inhibited complete compensation. In most cases, expression of the X-linked gene in heterozygous males was not as high as that in homozygous females.

Two models were proposed to explain the repressive autosomal position-effects (Qian and Pirrotta, 1995). The first model suggests that transposons carrying X-chromosome genes lack sufficient dosage compensation determinants. An alternative model proposes that the autosomal chromatin environment over-rides or compromises the ability of the X-linked gene to dosage compensate. To examine this issue, we compared the level of pigmentation of heterozygous males and homozygous females carrying a mini-white gene flanked by the su(Hw)-binding regions integrated on an autosome. We found that, in the majority of lines studied, expression of the male transgene was close to that found in homozygous females. These experiments indicate that the su(Hw) protein enhances dosage compensation of an X-linked gene inserted on an autosome, perhaps by protecting this transgene from a negative chromatin environment. These findings suggest that the su(Hw)-binding regions may facilitate the identification of dosage compensation determinants by insulation of genes to be assayed from repressive position-effects on the autosomes.

**MATERIALS AND METHODS**

**Drosophila stocks**

Flies were raised at 25°C, 70% humidity on standard corn meal/agar medium. The mutations and chromosomes used in this study are described in Lindsley and Zimm (1992). The original transgenic line carrying the transposon shown in Fig. 1A was generated in Roseman et al. (1993) and was called B.R.> white >B.R. This transposon was renamed SUPor-P-it for suppressor-P element, light eyes. SUPor-P-it carries the mini-white gene, which contains approximately 300-bp of 5’ flanking DNA, 630 bp of 3’ flanking DNA and lacks regulatory sequences required for high level expression in the eye (Pirrotta 1988). The mini-white gene is flanked by two 430 bp su(Hw)-binding regions (gypsy sequences between nucleotides 647 and 1077, as numbered in Marlor et al., 1986). In addition, this transposon carries the X-linked yellow gene, which was used as a marker for germline transformation. This gene is 5.2 kb in length, contains 2.8 kb of 5’ and 0.13 kb of 3’ flanking DNA and lacks the intron that is the location of the tissue-specific bristle and tarsal claw enhancers (Geyer and Corces, 1987).

Additional independent insertion lines were obtained using a chromosomal source of transposase (P[ry+ Δ2-3]/(99B); Robertson et al., 1988). The chromosomal location of each P element was determined by a set of crosses to appropriate balancer stocks. The effects of a mutant su(Hw) background on the degree of dosage compensation of autosomes are illustrated in Fig. 1B. The transposon shown in Fig. 1A was integrated into the genome of the yellow mini-white stock. In this stock, the mini-white gene is flanked by two 430 bp su(Hw)-binding regions. The mini-white gene is flanked by two 430 bp su(Hw)-binding regions.
transformants carrying SUPor-P-lt were determined by crossing male transformants to females of the stock $y^{ac-}u^{67} c^{6} v^{1} f^{1}$/2/CyO; bx$^{34e}$ su(Hw)'/TM6, su(Hw)'/Ubx. This combination of su(Hw) alleles reverses the phenotypes associated with gypsy insertions and is female fertile. su(Hw)' is a deletion of the su(Hw) gene (Harrison et al., 1992), whereas su(Hw)'/ is a point mutation in one of the Zn fingers which retains some ability to bind DNA (Harrison et al., 1993). The X chromosome in this stock carries two gypsy-induced alleles, $c^{6} f^{1}$ that are suppressed by su(Hw) mutations which allowed the identification of homozygous su(Hw) mutant flies. The su(Hw) mutant stocks were established in the following manner. For second chromosome insertion lines, transformed males were mated to $y^{ac-}u^{67} c^{6} v^{1} f^{1}$/2/CyO; bx$^{34e}$ su(Hw)'/TM6, su(Hw)'/Ubx females and the resulting male progeny that were $y^{+}$, $w^{+}$, curly-winged (CyO) and heterozygous for a su(Hw) mutation were backcrossed to females of the su(Hw) mutant stock. Progeny of this cross that were su(Hw)'/ su(Hw)'/ were selected based on the suppressed phenotypes of the X-linked gypsy-induced mutations. These flies were then used to establish a stock. For X-linked SUPor-P-lt lines and the hypomorphic white alleles, the $c^{6}$ and $f^{1}$ mutations were crossed onto the X-chromosome that contained the transposon or white mutation, so that a su(Hw) mutant background could be identified. Males carrying the recombinant chromosome were used to establish su(Hw) mutant lines, as described above. The degree of dosage compensation was determined for the recombinant chromosome in both the su(Hw)'/su(Hw)'/ background and in a su(Hw) wild-type background.

Flies carrying the drifter, mini-white transposon were generously provided by Dr Wayne Johnson. This transposon contains a drifter-lac Z fusion gene in which 2.4 kb of drifter 5' regulatory sequences were placed upstream of the hsp 70-lac Z gene. This fusion gene was inserted at the 5' end of the mini-white gene in the CaSpeR vector (Anderson et al., 1995). The drifter gene encodes a POU-domain DNA-binding protein localized to 65D on the third chromosome. Flies carrying the yp 1,2 mini-white transposon were kindly provided by Kristin Scott. This transposon contains a 380 bp region of the yolk protein (yp) 1,2 intergenic region cloned upstream of the Adh promoter in the vector pCaSpeR-AUG-β-gal (Thummel et al., 1988). The yp 1,2 genes encode vitellogenins and are located on the X chro-
mosome. Flies carrying the otu-β-gal mini-white transposon were kindly provided by Scott Patton. This transposon contains an approximately 1.1 kb fragment of 5’ otu regulatory sequences inserted upstream of the Adh promoter in the vector pCaSpeR-AUG-β-gal (Thummel et al., 1988) as described in Rodesch et al. (1995). The otu gene encodes two proteins expressed exclusively in the germline that are required for oogenesis. This gene is located on the X chromosome. The mini-white gene, which is present in all of these vectors, is the same as that in SUPor-P-lt.

### Dosage compensation

The degree of dosage compensation of each transformed line was assessed by comparing the eye phenotype of males that carried one copy of the transgene with females that carried two copies. In most cases, females were homozygous for the P element transposon. A major source of variation in the level of eye pigmentation is the age and body size of individual flies. For this reason, extreme care was taken during the culturing of flies. To avoid overcrowded culture conditions that can lead to large differences in body size, six pairs of flies were allowed to lay eggs for 4 days, the parents were removed and the progeny were grown at 25°C. The eye phenotype of flies that were less than 4 hours old were compared. These flies were frozen at −80°C and photographed at a later time. Heterozygous males and homozygous females were cultured in parallel. Visual inspection was used to compare phenotypes, as in our hands pigment assays gave variable results and did not always agree with the observed phenotype. Eye phenotypes were determined from several different fly cultures in blind studies.

### In situ localization

For most lines, the cytological location of the mini-white transposon was determined. These experiments were done using white DNA as a probe, as described by Lim (1993). This probe recognizes both the endogenous white gene at 3C and the transposon. P-element positions were determined with resolution to the lettered interval.

## RESULTS

### The addition of su(Hw)-binding regions to a mini-white transposon facilitates dosage compensation on the autosomes

We studied the effects of the su(Hw)-binding region on dosage compensation of the mini-white gene using the P element vector shown in Fig. 1A. This vector, called SUPor-P-lt, carries an intronless yellow gene and a mini-white gene that is flanked by su(Hw)-binding regions. The modified yellow gene confers dark pigmentation to larval mouthparts, denticle hairs, the adult wing and body cuticle (Geyer and Corces, 1987) and was used as a transformation marker. The white gene is necessary for eye pigmentation and encodes a transport protein required for the import of pigment precursors (Dreesen et al., 1988). Flies transformed with SUPor-P-lt have a yellow eye color, independent of the chromosomal insertion site (Roseman et al., 1993).

The white gene is an excellent reporter for studies of dosage compensation. Although the level of white gene expression roughly correlates with eye color, this relationship is non-linear. Small changes in gene expression produce clearly distinct eye phenotypes. The non-linearity of this relationship amplifies differences in white expression, thereby facilitating studies on dosage compensation. Using this system, partial degrees of dosage compensation can be detected which would be difficult to observe using northern analysis. We chose to
study dosage compensation of flies carrying SUPor-P-lt, since pigmentation is low and not near saturation.

Previous studies indicated that dosage compensation of genes transposed to an autosomal location is usually incomplete (reviewed in Lucchesi and Manning, 1987; Qian and Pirrotta, 1995). To verify this result, we obtained a number of transgenic lines that carried different mini-white transposons inserted on the autosomes and examined the level of dosage compensation. These transposons carry between 6.5 and 4.3 kb of DNA inserted 5’ to the white gene (Fig. 1B-D). In two cases, these transgenes contain DNA from an X-linked gene, either the yolk protein 1,2 genes (yp) or the ovarian tumor (otu) gene. The presence of these X-linked sequences is unlikely to increase the degree of dosage compensation from the mini-white gene, as both genes show female-associated expression. In fact, the yp genes are not dosage compensated (Lucchesi and Manning, 1987). The degree of dosage compensation of the mini-white gene in the drifter, yp 1,2 or otu-β gal transposon was determined by comparing the eye phenotype of heterozygous males carrying one copy of the mini-white gene with that of comparably aged homozygous females with two copies. In all cases (10/10), the mini-white gene in these transposons showed incomplete dosage compensation when inserted on the second or third chromosome (Table 1; Fig. 2). In contrast, flies carrying insertions of two of these transposons onto the X chromosome (yp 1,2 mini-white and otu-β gal mini-white) had complete or hyperdosage compensation of the mini-white gene; such that hemizygous males had either an equal or greater level of pigmentation than homozygous females (Table 1). Hypercompensation was observed previously, and is believed to arise from enhancement of dosage compensation of the mini-white gene by the X chromosome flanking sequences (Qian and Pirrotta, 1995). These results support the conclusion that dosage compensation of the mini-white transposon is chromosome dependent and provide a framework for comparison of phenotypes observed in flies transformed with SUPor-P-lt.

To test whether insulation of the mini-white gene by the su(Hw)-binding region produced complete dosage compensation on autosomes, we mobilized SUPor-P-lt and obtained seven X chromosome lines, twenty-one second chromosome lines and six third chromosome lines. The cytological location of the transposon in most lines was determined to ensure that the distribution of insertion sites was random (Table 2). These studies showed that there was some clustering of integration events. We obtained three insertions into 28C, three into 50F, two into 60E and two into 82E. Thus, we estimate that all of the seven X chromosome, at least thirteen of the second chromosome and at least four of the third chromosome insertion sites are unique.

Next, we examined the degree of dosage compensation for lines carrying the SUPor-P-lt transposon on the X chromosome by comparing the eye phenotype of hemizygous males with comparably aged homozygous females. We found that all of the X-chromosome insertion lines showed complete dosage compensation (Table 2; Fig. 3). These results indicate that the mini-white gene can dosage compensate when su(Hw)-binding regions flank it.

We then compared the eye phenotypes of males and females carrying SUPor-P-lt inserted on the second or third chromosome. We found that all of the autosomal lines showed some degree of dosage compensation; such that heterozygous males had a darker eye color than heterozygous females (data not shown). However, the critical test was whether heterozygous males and homozygous females had the same eye color. When these individuals were compared, we found that our lines could be separated into three phenotypic classes (Table 2; Fig. 3). Lines in the first class showed complete dosage compensation; males with one copy of the transgene had the same eye color as females with two copies. We classified 48% (13/27) of our lines in this class. The second class showed nearly complete compensation. In this class, males with one copy of the transgene had a slightly lighter eye color than homozygous females. We classified 37% (10/27) of our lines in this class. Members in the third class of phenotypes did not dosage compensate completely and heterozygous males had a distinctly lighter eye color than homozygous females. We classified 15% (4/27) of our lines in this class. These results suggest that the mini-white gene surrounded by su(Hw)-binding regions had an enhanced ability to dosage compensate at autosomal locations. The majority of our lines (85%) show better dosage compensation than was observed for any of the other mini-white containing transposons inserted on an autosome (Tables 1, 2). It is interesting to note that lines inserted at the same cytological location did not necessarily fall into the same phenotypic class. This indicates that, although the SUPor-P-lt transposons are in the same region of the chromosome, they are in unique positions and are likely to be influenced by different chromatin contexts.

**Effects of a su(Hw) mutant background on dosage compensation of the SUPor-P-lt transposon**

If the su(Hw) protein was responsible for the improved dosage compensation, then crossing the transgenes into a su(Hw) mutant background should lower the degree of dosage compensation. We crossed several second chromosome lines into a su(Hw) mutant background and assessed the eye phenotype of heterozygous males and homozygous females. We found that the level of dosage compensation was sensitive to the allelic

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**Table 3. Effects of a su(Hw) mutant background on dosage compensation of hypomorphic white alleles**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Lesion</th>
<th>Eye phenotype of M1 vs F2 in a su(Hw)+ bkgd</th>
<th>Eye phenotype of M1 vs F2 in a su(Hw)+ bkgd</th>
</tr>
</thead>
<tbody>
<tr>
<td>w[a]</td>
<td>Copia retrotransposon inserted in intron 2^a</td>
<td>Equal</td>
<td>Equal</td>
</tr>
<tr>
<td>w[a3]</td>
<td>Point mutation^b</td>
<td>Equal</td>
<td>Equal</td>
</tr>
<tr>
<td>w[a1l]</td>
<td>P-M hybrid dysgenic revertant of w[1]</td>
<td>Almost equal</td>
<td>Almost equal</td>
</tr>
<tr>
<td>w[b1f]</td>
<td>B104 inserted in intron 4^d</td>
<td>Equal</td>
<td>Equal</td>
</tr>
<tr>
<td>w[b1l]</td>
<td>Blood retrotransposon insertion in intron 2^e</td>
<td>Equal</td>
<td>Equal</td>
</tr>
<tr>
<td>w[e1]</td>
<td>pogo insertion into w[1]f</td>
<td>female greater</td>
<td>female greater</td>
</tr>
</tbody>
</table>

Abbreviations are the same as used for Table 2.

^aBingham and Judd (1981).

^bZachar and Bingham (1982).

^1in Birchler et al. (1994).

^2Zachar and Bingham (1982).


^4Zachar and Bingham (1982).
state of the su(Hw) gene (Table 2; Fig. 4). Lines in which the mini-white gene was completely or almost completely compensated in a su(Hw)+ background became incompletely compensated in a su(Hw)− background, such that homozygous females had a darker eye color than heterozygous males. Dosage compensation of the transposon was not eliminated in a su(Hw) mutant background because heterozygous males still had darker eyes than heterozygous females (data not shown). Thus, in a su(Hw) mutant background, the degree of dosage compensation of the mini-white gene in SUPor-P-lt is similar to that of a mini-white transposon that lacks the su(Hw)+-binding region (Table 1, 2). From these experiments, we conclude that the su(Hw) protein facilitates dosage compensation of X-linked genes removed from the X chromatin environment.

We also crossed several of the X-linked SUPor-P-lt transposons into a su(Hw) mutant background. Previous studies demonstrated that transposons carrying mini-white displayed two classes of phenotypes when inserted on the X chromosome, hypercompensation in which hemizygous males have more eye pigmentation than homozygous females or complete compensation (Qian and Pirrotta, 1995). Lines corresponding to these classes were isolated at nearly equal frequency. Thus, we expected that similar phenotypes would be observed for the X-linked SUPor-P-lt lines in a su(Hw) mutant background, as the mini-white gene would no longer be insulated. The degree of dosage compensation of all seven X-linked SUPor-P-lt lines was determined by comparing the eye phenotypes of mutant, hemizygous males and homozygous females (Table 2; Fig. 4). We found that dosage compensation was not changed in three of the X-linked SUPor-P-lt lines. In two cases, we found that hemizygous males had an eye color very close to, but less than, that of the homozygous female and these lines were classified as nearly completely compensated. Finally, in two lines, the homozygous females had more pigmentation than the hemizygous male, indicating incomplete dosage compensation. Thus, the majority of X chromosome insertion lines (5/7) showed nearly complete dosage compensation in a su(Hw) mutant background. However, none of the lines showed hypercompensation. These results indicate that in a su(Hw) mutant background, dosage compensation of SUPor-P-lt is largely unaffected on the X chromosome, in contrast to what was observed for the autosomes.

**su(Hw) mutations do not affect dosage compensation of the endogenous white gene**

It was somewhat surprising that, in a su(Hw) mutant background, none of the X-linked SUPor-P-lt transgenes became hypercompensated and that two lines became incompletely compensated. We considered two alternatives to explain these results; either a su(Hw) mutant background lowers the overall level of white dosage compensation or the nature of DNA flanking the SUPor-P-lt insertion site influences the expression of the enhancerless mini-white gene. To address this question, the effects of a su(Hw) mutant background on dosage compensation of the endogenous white gene was determined. In these experiments, we studied dosage compensation of several hypomorphic white mutations whose eye color is similar to that of flies carrying SUPor-P-lt, since pigment levels are low and not near saturation. In this way, small differences in white expression can be more easily observed than for the wild-type gene, where lowering the wild-type level of white expression by half does not cause a discernible change in eye phenotype (Lindsley and Zimm, 1992). We examined dosage compensation of the hypomorphic white mutations in both a su(Hw) wild-type and mutant background. As expected, all of the white
alleles tested, except \( w^e \), showed complete dosage compensation in \( su(Hw)^+ \) background (Table 3; Fig. 5; Lucchesi and Manning, 1987; Baker et al., 1994). Similarly, in the \( su(Hw) \) mutant background, all of the hypomorphic alleles remained completely dosage compensated with the exception of \( w^e \) (Table 3; Fig. 5). It is unclear why there is a dramatic decrease in pigmentation of \( \text{wbl} \) and \( w^e \) in the \( su(Hw) \) mutant background. However, this affect does not interfere with the dosage compensation system because compensation of these alleles does not depend on the allelic state of \( su(Hw) \). These results indicate that the \( su(Hw) \) mutant background does not affect dosage compensation of the endogenous \( \text{white} \) gene and support the hypothesis that lack of detection of hypercompensation is a consequence of the sequences into which SUPor-P_lt was integrated.

**The \( su(Hw) \)-binding region flanking the mini-white gene does not promote negative pairing effects on \( \text{white} \) expression.**

One possible explanation for the increased compensation of the mini-white gene in SUPor-P_lt was that the \( su(Hw) \) protein promotes interactions between paired genes. If a pairing caused a slight repression of mini-white gene expression, then this effect could equalize the amount of eye pigment produced in homozygous females and heterozygous males. Pairing-dependent repression of \( \text{white} \) expression has been observed for other insulator regions (Chung et al., 1993; Vazquez et al., 1993). To test this idea, we compared the eye phenotype of females that were homozygous for the SUPor-P_lt transposon at a given chromosomal location with the eye phenotype of females that were trans-heterozygous for two SUPor-P_lt transposons inserted at different chromosomal sites. If repression occurred in the paired state, the pigmentation level of the homozygous females should be lower than that of the doubly, trans-heterozygous female. In contrast to this prediction, we found that the eye phenotype of trans-heterozygous females was identical to that seen in females homozygous for either transposon (Fig. 6). Thus, we conclude that there is no pairing-dependent repression of mini-white gene expression in the homozygous female.

**DISCUSSION**

We determined whether insulator sequences could enhance the degree of dosage compensation of an X-linked gene located at
autosomal positions. In our investigations, dosage compensation of the mini-white gene flanked by su(Hw)-binding regions was examined. Previous studies on white dosage compensation indicated that white transgenes do not completely dosage compensate at autosomal locations (Hazelrigg et al., 1984; Levis et al., 1985; Pirrotta et al., 1985; Qian and Pirrotta, 1995). For example, although heterozygous male flies, which carry a variety of white transposons with different amounts of 5′ flanking DNA, had a two- to three-fold higher level of eye pigment than heterozygous females, these males had two to three times less pigment than homozygous females.

In our studies, we found that the majority (86%) of autosomal SUPor-P-lt lines showed nearly complete dosage compensation (Table 2), as compared to 11% reported for transgenic lines carrying the mini-white gene (Qian and Pirrotta, 1995). Although the mini-white gene was identical in these two studies, the SUPor-P-lt transposon carried additional X-linked sequences. Thus, the improved compensation of SUPor-P-lt at autosomal sites may be explained by several mechanisms, including: (1) protection of repressive autosomal position-effects by the su(Hw) insulators bordering the mini-white gene, (2) inclusion of additional dosage compensation determinants in SUPor-P-lt which might be present on the X-linked yellow gene or (3) a combination of both factors. We favor a model in which insulator sequences have a protective influence on dosage compensation of the autosomally located transgene for the following reasons. First, the yellow gene was inserted outside the su(Hw)-binding regions. Thus, the additional dosage compensation determinants present on this gene may be in a different domain than the mini-white gene and would not be expected to directly influence white expression. Second, autosomal SUPor-P-lt transposons in a su(Hw) mutant background lose the ability to completely dosage compensate (Table 2; Fig. 4). In this background, the transgene remained partially dosage compensated, as males with one copy showed a darker eye phenotype than females with one copy, but this color was not equivalent to that of a homozygous female. These results are similar to what is observed for mini-white transgenes that lack su(Hw)-binding regions (Table 1; Fig. 2). We conclude that enhanced dosage compensation did not result solely from the inclusion of the yellow gene in our test transposon. However, we cannot rule out the possibility that the additional X-linked sequences played a role. For example, it is conceivable that dosage compensation determinants on the yellow gene partially establish an X-chromatin environment outside the domain containing the white gene. In this case, the chromatin context surrounding the mini-white gene would be less repressive than the general autosomal environment, making it easier to insulate.

We examined dosage compensation of several X-linked SUPor-P-lt lines in a su(Hw) mutant background. Interestingly, two X-linked SUPor-P-lt lines were no longer completely compensated in a su(Hw) mutant background; homozygous females had a darker eye phenotype than hemizygous males (Table 2; Fig. 4). In the absence of the su(Hw) protein, expression of the enhancerless mini-white gene becomes influenced by enhancers/silencers present in the surrounding environment (Roseman et al., 1993). Thus, in these two lines, the mini-white transposon may be subjected to position-effects. For example, these transgenes may be integrated near a female-specific enhancer that preferentially increases expression of the white gene in females. An alternative explanation may be that dosage compensation is not uniform along the length of the X chromosome, as suggested by the fact that the MSL proteins are in a banded pattern along the X chromosome (Kuroda et al., 1991; Palmer et al., 1993; Gorman et al., 1995). In this case, the SUPor-P-lt transposons may be in regions of the X chromosome that have a chromatin environment similar to that on the autosomes. In a wild-type su(Hw) background, the negative influences of this neighboring chromatin on the SUPor-P-lt transposon are insulated but this protection is lost in the su(Hw) mutant background.

Hyperdosage compensation of any X-linked SUPor P-lt line in a su(Hw)− background was not observed. The absence of this phenotypic class was surprising considering the frequency in which hyperdosage compensating lines carrying the mini-white gene were isolated (Qian and Pirrotta, 1995). One explanation for these results is that only a small number of SUPor-P-lt lines were studied which by chance were insertions in regions of the X chromosome that enhance dosage compensation. Alternatively, the SUPor-P-lt transposon may integrate into a different spectrum of X chromosome sites than the mini-white transposon. Insertional specificity of P elements can be altered by sequences within the P element vector (Kassiss et al., 1992). Perhaps sequences within SUPor-P-lt, including the su(Hw)-binding region, change the insertional specificity of this transposon from that of the mini-white gene. Preferential insertion of SUPor-P-lt at non-repressive autosomal sites is unlikely to cause the enhanced dosage compensation on these chromosomes because all sixteen lines became incompletely compensated in a su(Hw) mutant background.

Insulators are not uniform in their ability to affect dosage compensation. A transposon carrying the mini-white gene flanked by the 87A7 heat shock locus special chromatin sequences (scs/scs′) was not completely compensated on the autosomes (Kellum and Schedl, 1991; Vazquez and Schedl, 1994). Dosage compensation of the scs/scs′-containing transposon was the same as that observed for transposons carrying the mini-white gene without insulators. The reason for the difference in insulator properties is unclear. The scs/scs′ sequences lack su(Hw)-binding regions (Kellum and Schedl, 1992). Thus, characteristics of the scs/scs′ insulator are most likely conferred by a distinct set of proteins that may not be able to protect against position-effects exerted on the dosage compensation system.

Our results provide evidence that autosomal chromatin is inhibitory for dosage compensation. One model for dosage compensation proposes that male-specific association of a multimeric complex of MSL-1,-2,-3 and MLE with the X chromosome remodels nucleosome structure by catalyzing the specific acetylation of histone H4 on Lys 16. This histone modification facilitates decondensation of chromatin, leading to hypertranscription (Kuroda et al., 1993; Baker et al., 1994). In this context, the su(Hw) protein may prevent negative autosomal effects by stabilizing the interaction of the MSL complex with the transposed mini-white gene, allowing the histone H4 modification to take place. A second manner in which the su(Hw) protein may increase compensation is by preventing access of deacetylases to histones positioned on the mini-white gene, thereby increasing the degree of nucleosome modification surrounding the transposed gene. Finally, the su(Hw) protein may separate the mini-white gene into a topo-
logically distinct domain which facilitates decondensation of the modified chromatin.

Although a great deal of progress has been made towards understanding the role of the trans-acting factors required for dosage compensation, less is known about the cis-linked DNA sequence requirements. Our studies suggest that the insulation of autosomal position-effects by the su(Hw) protein may facilitate the elucidation of these dosage compensation determinants. These insulator sequences may provide sufficient buffering capacity against negative autosomal influences, such that dosage compensation can be conferred by these determinants to a previously non-compensated gene at an autosomal location.

The su(Hw) protein protects transgenes from several types of position-effects in addition to those described here. These include position-effects caused by enhancers and silencers present in euchromatin, centric heterochromatin and telomeric chromatin (Roseman et al., 1955). The mechanism by which the su(Hw) protein insulates against these diverse types of position-effects is unclear. It will be interesting to determine whether insulation from these effects shares the same underlying molecular mechanisms.

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