Polycomb Group Repression Is Blocked by the Drosophila *suppressor of Hairy-wing* [*su(Hw)*] Insulator

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ABSTRACT

The *suppressor of Hairy-wing* [SU(HW)] binding region disrupts communication between a large number of enhancers and promoters and protects transgenes from chromosomal position effects. These properties classify the SU(HW) binding region as an insulator. While enhancers are blocked in a general manner, protection from repressors appears to be more variable. In these studies, we address whether repression resulting from the Polycomb group genes can be blocked by the SU(HW) binding region. The effects of this binding region on repression established by an *Ultrabithorax* Polycomb group Response Element were examined. A transposon carrying two reporter genes, the *yellow* and *white* genes, was used so that repression and insulation could be assayed simultaneously. We demonstrate that the SU(HW) binding region is effective at preventing Polycomb group repression. These studies suggest that one role of the *su(Hw)* protein may be to restrict the range of action of repressors, such as the Polycomb group proteins, throughout the euchromatic regions of the genome.

IN Drosophila, several tissue-specific mutations are caused by insertion of a *gypsy* retrotransposon (Modolell *et al.* 1983). *gypsy* is a potent mutagen, exerting effects on resident gene expression even when positioned at distances of 10–80 kb from a promoter (Peifer and Bender 1986; Jack *et al.* 1991). The mutant phenotypes associated with several *gypsy*-induced alleles require a wild-type copy of the second-site modifier gene, *suppressor of Hairy-wing* [*su(Hw)*] (Modolell *et al.* 1983). This gene encodes a ubiquitously expressed Zn-finger protein, which binds to *gypsy* sequences called the SU(HW) binding region (Parkhurst *et al.* 1988; Spana *et al.* 1988; Mazo *et al.* 1989; Harrison *et al.* 1993).

The mutagenic effects of *gypsy* result when the *su(Hw)* protein associates with its binding region and blocks enhancer activity in the resident gene (Geyer *et al.* 1986; Peifer and Bender 1986; Jack *et al.* 1991; Dorsett 1993). The SU(HW) binding region contains 12 copies of a sequence similar to the octameric motif, a mammalian enhancer element (5′PyPuTTGCATAC3′). DNA-footprinting experiments suggest more than one *su(Hw)* molecule associates with this region; as many as 12 molecules may associate if it binds as a monomer (Spana *et al.* 1988; Mazo *et al.* 1989). Altering the SU(HW) binding region by insertion or deletion causes partial suppression of *gypsy* mutations (Geyer *et al.* 1988; Peifer and Bender 1988; Flavell *et al.* 1990; Smith and Corces 1992). Furthermore, *gypsy* effects can be reproduced by the SU(HW) binding region alone (Holdridge and Dorsett 1991; Geyer and Corces 1992). These studies demonstrate that the SU(HW) binding region is both necessary and sufficient for *gypsy* mutagenesis.

A wide variety of enhancers can be affected by interposition of the SU(HW) binding region between an enhancer and promoter, indicating that these effects are general (Holdridge and Dorsett 1991; Geyer and Corces 1992; Roseman *et al.* 1993; Cai and Levine 1995; Scott and Geyer 1995). The block imposed on an enhancer does not result in enhancer inactivation, suggesting that the *su(Hw)* protein interferes with communication between enhancers and promoters (Cai and Levine 1995; Scott and Geyer 1995).

An insulation assay, in which SU(HW) binding regions flanked the *white* reporter gene, demonstrated that the *su(Hw)* protein can block position effect variegation (PEV) associated with the insertion of transposons into repressive chromatin (Roseman *et al.* 1995). The *su(Hw)* protein may not confer general protection from silencer or repressor elements, as this region failed to block silencing from the VR600 element, which is dependent on *dorsal* protein function (Cai and Levine 1995). The basis for this differential ability to block repressors is unknown, but might reflect the mechanism by which repressors affect gene expression.

We tested whether the SU(HW) binding region could protect from repression conferred by the Polycomb group (PcG) genes to examine how generally the *su(Hw)* protein blocks repressors. The PcG genes en-

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code repressors required for maintenance of initial expression boundaries of homeotic gene expression (reviewed in Paro 1990; Pirrotta and Rastelli 1994; Kennison 1995; Simon 1995; Pirrotta 1997). Mutations in PcG genes cause ectopic expression of homeotic genes because of the loss of maintenance of the early pattern of homeotic gene expression (Struhl and Akam 1985; McKeon and Brock 1991; Simon *et al.* 1992). Although a number of similarities exist between repression conferred by PcG genes and heterochromatic PEV, these repression systems appear to be independent (Pirrotta 1997). The PcG proteins may interfere with transcriptional activity in several ways, including preventing transcription factor access by the formation of repressive complexes (McCall and Bender 1996) or by inhibiting activities such a nucleosome remodeling that may be necessary for active transcription (Pirrotta 1997).

The PcG proteins exert their effects by interacting with specific *cis*-elements called Polycomb group response elements (PREs; Simon *et al.* 1993; Chan *et al.* 1994; Chiang *et al.* 1995; Gindhart and Kaufman 1995). Identification of these sequences has been based on three criteria: (1) they promote continued repression of homeotic reporter genes, (2) they create new binding sites for PcG proteins in polytene chromosomes, and (3) they cause PEV of a linked *white* gene. In our experiments, we modified the *SUPor P* element (Roseman *et al.* 1995), which contains both the *white* and *yellow* reporter genes, by inserting a PRE from the *Ultrabithorax* (*Ubx*) gene downstream of *yellow.* Using this transposon, we examined whether the SU(HW) binding region could block repression conferred by PcG proteins. These experiments provide additional information concerning the spectrum of position effects insulated by the SU(HW) binding region and expand the possible functions for the *su(Hw)* protein in the organization of chromatin domains within the genome.

MATERIALS AND METHODS

Drosophila stocks: Flies were raised at 25°, 70% humidity on standard corn meal and agar medium. The mutations and chromosomes used in this study are described in Lindsley and Zimm (1992).

DNA constructions: The PRE tested in these studies corresponds to the principal *Ubx* PRE (Chan *et al.* 1994; Chiang *et al.* 1995; Poux *et al.* 1996). This PRE is encompassed in a 1.6-kb *Sty*I-*Eco*RI fragment (a gift from V. Pirrotta). The *Ubx* PRE was used to modify the *SUPor P* element (Roseman *et al.* 1995; Figure 1). This *P* element contains two marker genes. The first is a variation of the mini-*white* gene. Mini-*white* contains 305 bp of 5′ and 500 bp of 3′ flanking DNA and a deletion of most of the first intron (Pirrotta 1988). The mini-*white* gene was modified by insertion of *white* regulatory sequences (−1084 and −1465 relative to the transcription start site) upstream of −305 bp. This regulatory region contains both the eye and testes enhancers (Qian *et al.* 1992) and directs a high level of *white* expression in the eye. Two 430-bp SU(HW) binding regions (*gypsy* sequences between nucleotides 647 and 1077, as numbered in Marlor *et al.* 1986) flank the *white* gene and provide insulation of this gene from chromosomal position effects (Roseman *et al.* 1993). The second marker gene is the intronless *yellow* gene. This *yellow* gene is 5.2 kb in length, contains 2.8 kb of 5′ and 0.13 kb of 3′ flanking DNA, and lacks the tissue-specific bristle and tarsal claw enhancers (Geyer and Corces 1987). The PRE was inserted into *SUPor P* at the 3′ end of the *yellow* gene to create PRE 6. A second construct (PRE 2) was created in which the orientation of the *yellow* gene containing the PRE was reversed, such that the PRE was juxtaposed with a SU(HW) binding region (Figure 1).

Germ-line transformation: Germ-line transformation was carried out as described by Rubin and Spradling (1982). The host strain used in these experiments was *y ac* w^{1118}, which carries a deletion of a portion of the *X* chromosome containing the *yellow* and *achaete* loci and the 5′ region of the *white* gene. DNA concentrations used in these experiments were 400 μg/ml of the PRE construct and 200 μg/ml of the "wings clipped" helper plasmid pπ25.7 (Karess and Rubin 1984). Transformants were recognized by changes in body and/or eye color, and these flies were used to establish stocks. Initially, a single PRE 2- and two PRE 6-transformed lines were obtained. Additional independent insertion lines were generated by mobilizing the transposon from one line with the *Sb* ry^{506} P($ry^{+}\Delta$2-3) (99B) chromosome (Robertson *et al.* 1988). For each independent line, the number of insertions and the integrity of the transposon were determined by DNA Southern analysis. Only lines with single insertions were analyzed.

Pigmentation scale: The effects of the PRE insertion on *yellow* and *white* gene expression were assessed by examining the level of cuticle and eye pigmentation of 2- to 4-day-old flies. Wild type *yellow* expression produces a black pigmentation, intermediate levels produce brown pigmentation, and no expression produces yellow pigmentation. Wild-type *white* expression produces red eyes, whereas no *white* expression produces white eyes. Intermediate levels of pigmentation produce yellow, orange, and brown eyes, which reflect low, intermediate, and high levels of gene expression, respectively. PRE repression was associated with variegation, most prominently observed in the abdominal cuticle of males. The degree of variegation in the abdomen was scored using a 5-point scale, where 1 referred to a loss of pigmentation, 2 extreme variegation, 3 intermediate variegation, 4 mild variegation and 5 no variegation. This scale was also used to score *white* variegation in adult eyes.

Effects of mutations in PcG genes on marker gene expression: Several PcG mutations were tested to determine whether they altered the variegated pattern of *yellow* expression. In these studies, we tested the dominant effects of the following mutations (provided by J. Simon): *Additional sex combs*, Asx^{XT129}; *Polycomb*, Pc^{3}; *Polycomblike*, Pcl^{5}; *Posterior sex combs*, Psc^{IIN48}; *Sex combs extra*, Sce^{D1}; and *Sex combs on mid leg*, Scm^{D1}. The effect of each PcG mutation on *yellow* gene expression was determined by mating male flies containing the PcG mutation carried over a balancer chromosome (*CyO*, *TM1*, or *TM3*) to virgin y^{-} ac^{-} w^{1118}, *P[PRE]* females. Two- to 4-day-old males were scored for pigmentation in the abdominal cuticle, as described above. Males containing the PcG mutation and the PRE transposon were compared to siblings containing the PRE transposon and balancer chromosome to verify that suppression of variegation was associated with the chromosome carrying the PcG mutation. These crosses were conducted three times, and at least 50 progeny scored each time.

Effects of the *su(Hw)* protein on marker gene expression: To test the effects of *su(Hw)* protein on *white* gene expression, lines containing the PRE transposons were crossed into a $su(Hw)^{v}/su(Hw)^{f}$ mutant background. This combination of

su(Hw) alleles reverses the phenotypes associated with *gypsy* insertions and is female fertile. $su(Hw)^v$ is a deletion of the *su(Hw)* gene (Harrison *et al.* 1992), whereas $su(Hw)^f$ is a point mutation in one of the Zn fingers that retains some ability to bind DNA (Harrison *et al.* 1993). Males carrying the PRE transposons were crossed to females of the stock $y^- ac^- w^{67}$ ct^6 v^1 f^1; *2/CyO;*bx^{34e} $su(Hw)^v$/*TM6,* $su(Hw)^f$ Ubx. The resulting male progeny that were $y^- ac^- w^{67}$ ct^6 v^1 f^1; *PRE 2* or *PRE 6* and either $su(Hw)^v$ or $su(Hw)^f$/*3* were backcrossed to females of the *su(Hw)* mutant stock. Flies that were $su(Hw)^v/su(Hw)^f$ were selected based on the phenotypes of the *X*-linked ct^6 f^1 *gypsy*-induced mutations. The eye phenotype of these flies was scored.

***In situ* hybridizations:** Determination of the cytological location of *SUPor P* was done according to the procedure of Lim (1993) using *white* DNA as a probe. This probe recognized both the endogenous *white* gene at 3C and the transposon. *P*-element positions were determined with resolution to the lettered interval.

RESULTS

Repression of *yellow* expression in transposons carrying the *Ubx* PRE: The *Ubx* gene contains a principal PRE positioned approximately 24 kb upstream from the transcription start site (Chan *et al.* 1994; Chiang *et al.* 1995; Poux *et al.* 1996). This PRE was initially identified within larger fragments, centered around positions -20 to -30 kb upstream of the *Ubx* transcription start site (Simon *et al.* 1993; Chan *et al.* 1994; Chiang *et al.* 1995) and was subsequently localized to a smaller 1.6-kb fragment (Chan *et al.* 1994; Poux *et al.* 1996). Many PREs, including this 1.6-kb *Ubx* PRE, repress *white* gene expression. Flies transformed with *white* reporter genes cloned next to a PRE generally show a variegated eye phenotype (Fauvarque and Dura 1993; Kassis 1994; Chan *et al.* 1994; Gindhart and Kaufman 1995; Zink and Paro 1995; Poux *et al.* 1996). Repression of *white* expression is useful to monitor the activity of particular PRE sequences. We used this assay to determine whether the SU(HW) binding region blocked PcG dependent repression.

The *SUPor P* transposon was chosen for these studies because it carries two reporter genes, *yellow* and *white.* The *white* gene is placed between two SU(HW) binding regions, which protect its expression from chromosomal position effects (Roseman *et al.* 1995), whereas the *yellow* gene is located outside the binding regions (Figure 1). *SUPor P* was modified by the addition of the 1.6-kb *Ubx* PRE into the 3′ end of the *yellow* gene. We reasoned that if the SU(HW) binding regions insulated the *white* gene from PcG-induced repression, then this gene would be expressed at a high level and transformants would have a uniform red eye color. It was unclear whether inclusion of the PRE downstream of *yellow* would cause repression of *yellow* gene expression, as PRE effects on *yellow* have not been previously tested. Recent studies suggest that the expression state of a gene carrying a PRE can influence whether PcG-depen-

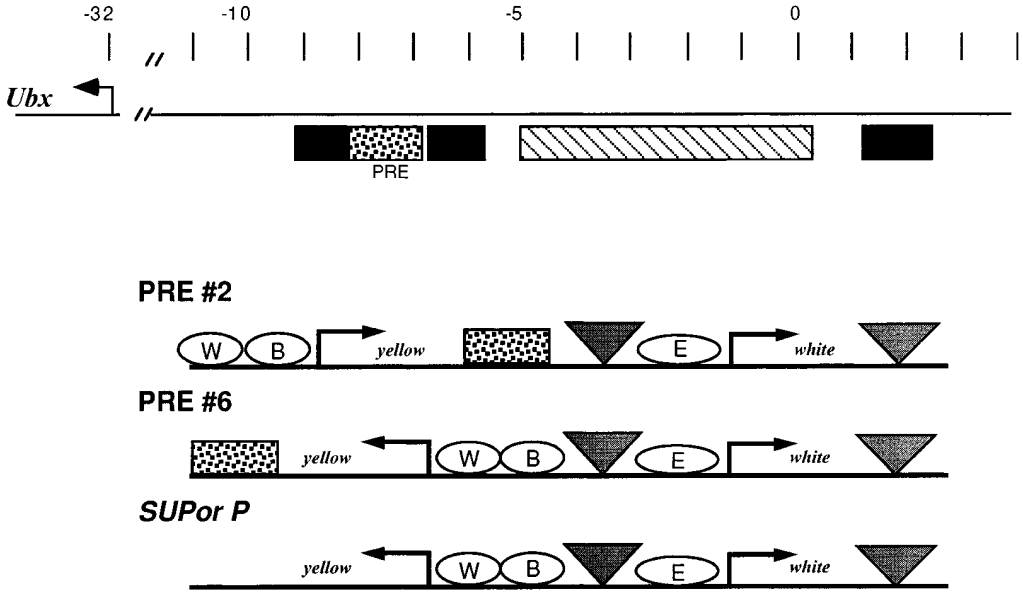

Figure 1.—Structure of the PRE and *SUPor P* transposons. The top panel diagrams the location of the 1.6-kb PRE (dotted square) in the *Ubx* gene (redrawn from Chan *et al.* 1994). The PRE is located approximately 24 kb upstream from the transcription start site (arrow). The numbering scale indicates kb and is based on that of Bender *et al.* (1983). The PRE is located nearby embryonic enhancers (black boxes) and imaginal disc enhancers (striped boxes). The *Ubx* PRE was inserted into the 3′ end of the *yellow* gene in the *SUPor P* transposon. Enhancers within the *yellow* gene are indicated by ovals; the W refers to the wing, and B refers to the body enhancer. In addition, the transposon carries the *white* gene with the eye enhancer (E oval). Two SU(HW) binding regions (shaded triangles) flank the *white* gene. The *yellow* gene carrying the PRE was placed in the same transcriptional orientation to *white* (PRE 2) or the opposite transcriptional orientation (PRE 6). In this way, the distance between the SU(HW) binding region and PRE was altered.

dent silencing is established. Repression occurred when the reporter gene was inactive during the establishment of PcG complex formation but not if the gene was active (Poux *et al.* 1996). Transcription of the *yellow* gene begins approximately 12 hr after egg laying (P. K. Geyer, unpublished result), which corresponds to a time after PcG repression is established. Thus, we predicted that *yellow* expression would be sensitive to PcG repression.

The effects of the PRE were tested using two constructs (Figure 1). These transposons differ in the position of the PRE relative to the SU(HW) binding regions. In this way, we obtained information concerning the effects of distance on protection by the SU(HW) binding region. Transformation with both transposons PRE 2 and PRE 6 produced transgenic lines with two phenotypes (Table 1; Figure 2). Class I (5 out of 14 lines) displayed wild-type eye and body pigmentation. Class II (9 out of 14) had a wild-type eye color but a variegated, low level of body pigmentation (Figure 2). The level of *yellow* variegation in repressed lines did not change when the *P* transposon was made homozygous. The high frequency of transformed lines showing *yellow* variegation suggests the PRE can repress *yellow* expression. Furthermore, the absence of *white* repression in these lines indicates that the SU(HW) binding regions block PcG repression. Finally, we saw no enhanced sensitivity to repression in lines carrying PRE 2 relative to PRE 6, even though in the former case the PRE abutted the SU(HW) binding region. These results suggest that SU(HW) insulation is not sensitive to the distance of the PRE relative to the SU(HW) binding region.

Previous transformation of the *SUPor P* transposon lacking a PRE generated lines with phenotypes similar to the class II PRE lines (Roseman *et al.* 1995). In these cases, *yellow* repression was associated with insertion of *SUPor P* into the second or third chromosome telomeres. To determine whether the PRE lines with non-wild-type body pigmentation reflected a similar position effect, *in situ* localization was carried out. We found that the majority of variegating lines (six of nine) had insertions at euchromatic sites (Table 1). The remaining three lines were inserted into telomeric regions; one localized to 1A (line PRE 6-69) and two localized to 60F (PRE 6-69M2, PRE 6-69M10). Previously several transgenic lines were obtained in which *SUPor P* was integrated into the X telomere of flies isogenic with those used in this study (Roseman *et al.* 1995). In these lines, *yellow* gene expression was not repressed, although repression of *white* was detected in a *su(Hw)*

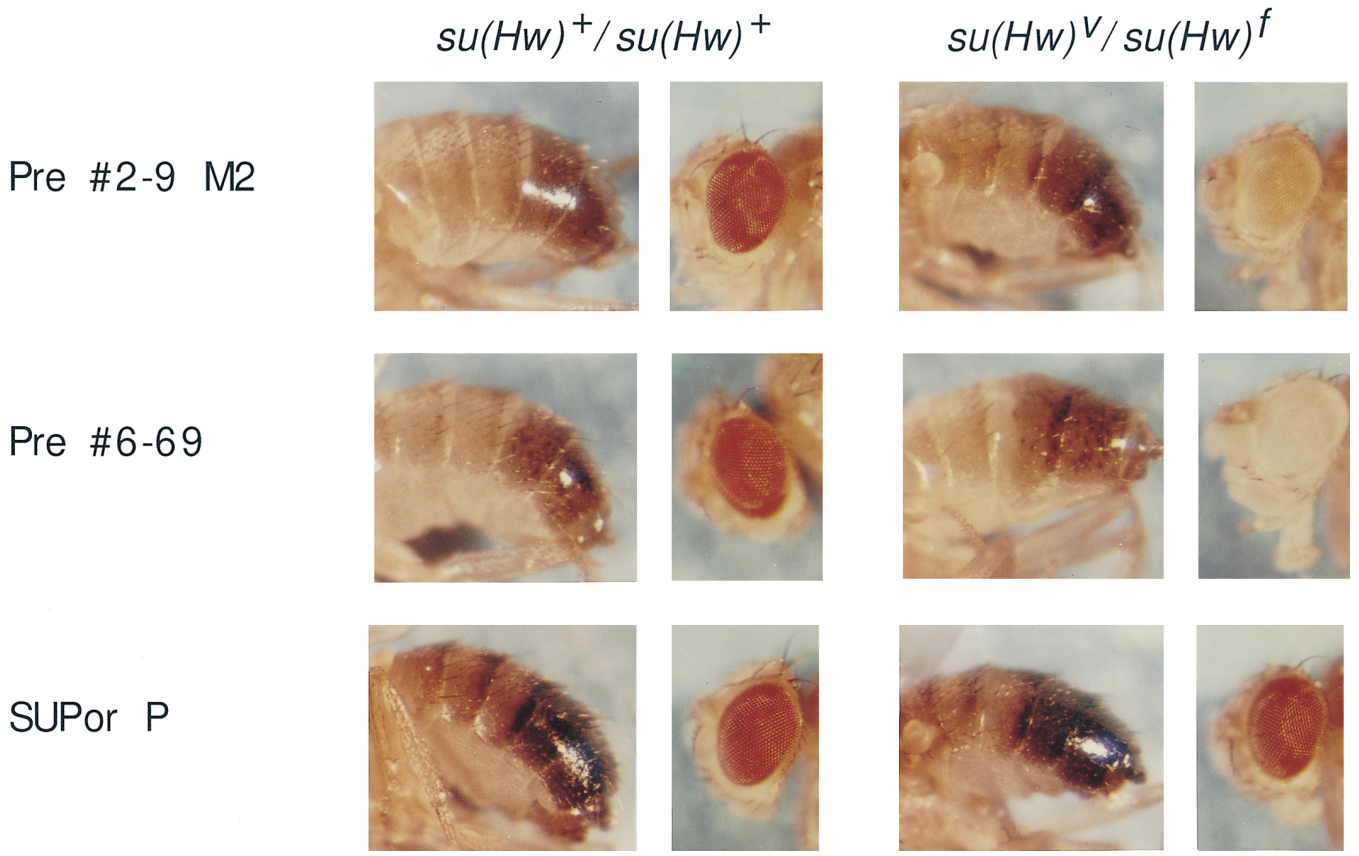

Figure 2.—Phenotype of class II PRE lines. The cuticle and eye phenotypes of two representative class II lines and one *SUPor P* line are shown. Flies in the top panels carry PRE 2; those in the middle panels carry PRE 6, and those in the bottom panels carry *SUPor P*. The phenotype of flies either in a *su(Hw)*$^{+}$ background (left panels) or *su(Hw)v*/*su(Hw)*^{f} background (right panels) is shown.

mutant background. This suggests that the *yellow* repression in line PRE 6-69 results, at least in part, from the PRE present on the transposon. PcG proteins are associated with several telomeres (Zink and Paro 1989; DeCamillis *et al.* 1992; Rastelli *et al.* 1993), and mutations in the *Psc-Su(z)2* gene complex suppress telomeric position effects (cited in Pirrotta 1997). Thus, phenotypes associated with the 2R and 3R telomeric insertions may also reflect the effects of PcG repression.

Repression of *yellow* expression is dependent on PcG genes: To verify that *yellow* repression was dependent on PcG genes, we tested for dominant suppression of the variegated expression of *yellow* in the male abdominal cuticle. We chose eight class II lines for analysis. The phenotypes associated with flies carrying the PcG mutation and PRE transposon were compared to siblings that carried the balancer chromosome and the PRE transposon. As a control, a *SUPor P* line was similarly crossed to the collection of PcG alleles. None of the PcG mutations affected *yellow* expression in *SUPor P*, demonstrating that mutations in these genes do not decrease expression of the *yellow* gene lacking a PRE in a dominant fashion (Figure 3; Table 2). We found that for each PRE transposon line tested, at least one PcG mutant background suppressed the variegated *yellow* phenotype, producing flies with darker body pigmentation (Figure 3; Table 2). In most cases, suppression was incomplete, causing a shift to less variegation but not enough of a shift to restore pigmentation to the wild-type level. This partial suppression may reflect the fact that the PcG mutant background tested results from a 50% reduction in gene activity and not a complete loss of PcG function. We were not surprised by the lack of uniform effect of a particular PcG mutation on *yellow* gene expression because in previous studies suppression of variegated *white* expression was similarly variable, showing differential sensitivity depending on the location of the *white* gene (Chan *et al.* 1994; Zink and Paro 1995). From these studies, we conclude that repression of *yellow* gene expression results from effects of the PcG complex.

The *su(Hw)* protein insulates the *white* gene from PRE repression: Several lines carrying the PRE transposon were crossed into a *su(Hw)* mutant background to address whether the wild-type eye phenotype resulted from protection from the PRE by the *su(Hw)* protein. Lines from both classes of transformants were analyzed. The eye phenotype of flies in class I lines remained red in a *su(Hw)* mutant background (Table 1). This suggests that the lack of repression of *yellow* reflects a general inability of the PRE to establish repression at these genomic locations. In contrast, the class II lines became variegated for *white* expression in the mutant *su(Hw)* background. These results support the conclusion that the *su(Hw)* protein blocks PRE-induced repression. In the *su(Hw)* mutant background, the lines displayed the same degree of *yellow* variegation (Figure 2; Table 1), suggesting that the PRE effects on *yellow* expression are not insulated by the SU(HW) binding region.

Interestingly, repression of the *yellow* and *white* genes always correlated (Table 1). At a given insertion site, either both genes were repressed, or both genes were expressed at a wild-type level. However, the degree of re-

TABLE 1

Summary of phenotypes associated with transgenic PRE lines

PRE construct-line no.	Location	Class	*yellow* phenotype		*white* phenotype	
			$su(Hw)^+/su(Hw)^+$	$su(Hw)^v/su(Hw)^f$	$su(Hw)^+/su(Hw)^+$	$su(Hw)^v/su(Hw)^f$
PRE 2-9A	ND	I	black	ND	red	ND
PRE 2-9M2	94F	II	brown, 1 var	brown, var	red	yellow, 1.5 var
PRE 2-9M68	57F	I	black	ND	red	ND
PRE 6-4	41F	II	brown, 3 var	brown, var	red	yellow, 2 var
PRE 6-69	1A	II	brown, 1.5 var	brown, var	red	white, 1 var
PRE 6-69M1	22A	II	brown, 1 var	brown, var	red	orange, 3.5 var
PRE 6-69M2	60F	II	brown, 2 var	brown, var	red	white, 1 var
PRE 6-69M3	82D	II	brown, 1 var	brown, var	red	orange, 2 var
PRE 6-69M4	64B	I	black	black	red	red
PRE 6-69M5	82E	I	black	black	red	red
PRE 6-69M6	93D	II	brown, 1.5 var	ND	red	ND
PRE 6-69M7	22A	II	brown, 1 var	brown, var	red	orange, 3 var
PRE 6-69M10	60F	II	brown, 1 var	brown, var	red	orange, 3 var
PRE 6-69M11	78A	I	black	black	red	red

The wild-type level of *yellow* gene expression produces flies with black pigmentation. Brown coloration indicates repression of *yellow* gene expression. The wild-type level of *white* gene expression produces flies with a red eye color. Orange coloration indicates repression of *white* gene expression. Pigmentation was uniform in the cuticle or eye, unless noted otherwise. var, variegation; ND, not determined.

pression between the two genes was not always the same. In some genomic positions, *white* expression was more severely affected than *yellow*, while in other positions *yellow* expression showed more repression. For example, in lines PRE 6-69 and PRE 6-69M2, *white* expression was completely repressed when the transposon was crossed into a *su(Hw)* mutant background, whereas the *yellow* gene was still expressed. Similarly, lines PRE 2-9M2, PRE 6-69M1, PRE 6-69M3, PRE 6-69M7, and PRE 6-69M10 displayed severe *yellow* repression but the *su(Hw)* mutant background only showed intermediate silencing of *white* expression. These differences in degree of repression illustrate that the flanking genomic DNA contributes to the effect of the *Ubx* PRE on gene expression.

DISCUSSION

In these studies, we tested whether the SU(HW) binding region provided protection from the repressive effects caused by a PRE. We used a dual gene transposon (*SUPor P*) to facilitate the identification of transformants. This transposon carries a *white* gene flanked by SU(HW) binding regions. The *white* gene is an excellent reporter of PRE effects, as there is a strong correlation between degree of *white* repression and the ability of a particular PRE to maintain appropriate homeotic gene expression (Chan *et al.* 1994; Poux *et al.* 1996). In addition, *SUPor P* carries the *yellow* gene into which the principal *Ubx* PRE was inserted. Transformation using *SUPor P* derivatives carrying the PRE showed a variegated cuticle pigmentation in the majority of lines (Class II), even though most transposons were inserted into euchromatic sites. The variegated phenotype was suppressed by mutations in the PcG genes, indicating that the observed repression was PcG dependent. In all cases, lines with a variegated cuticle phenotype had a red eye color, suggesting that the SU(HW) binding region was able to protect against PcG repression. Consistent with this conclusion, placing the trans-

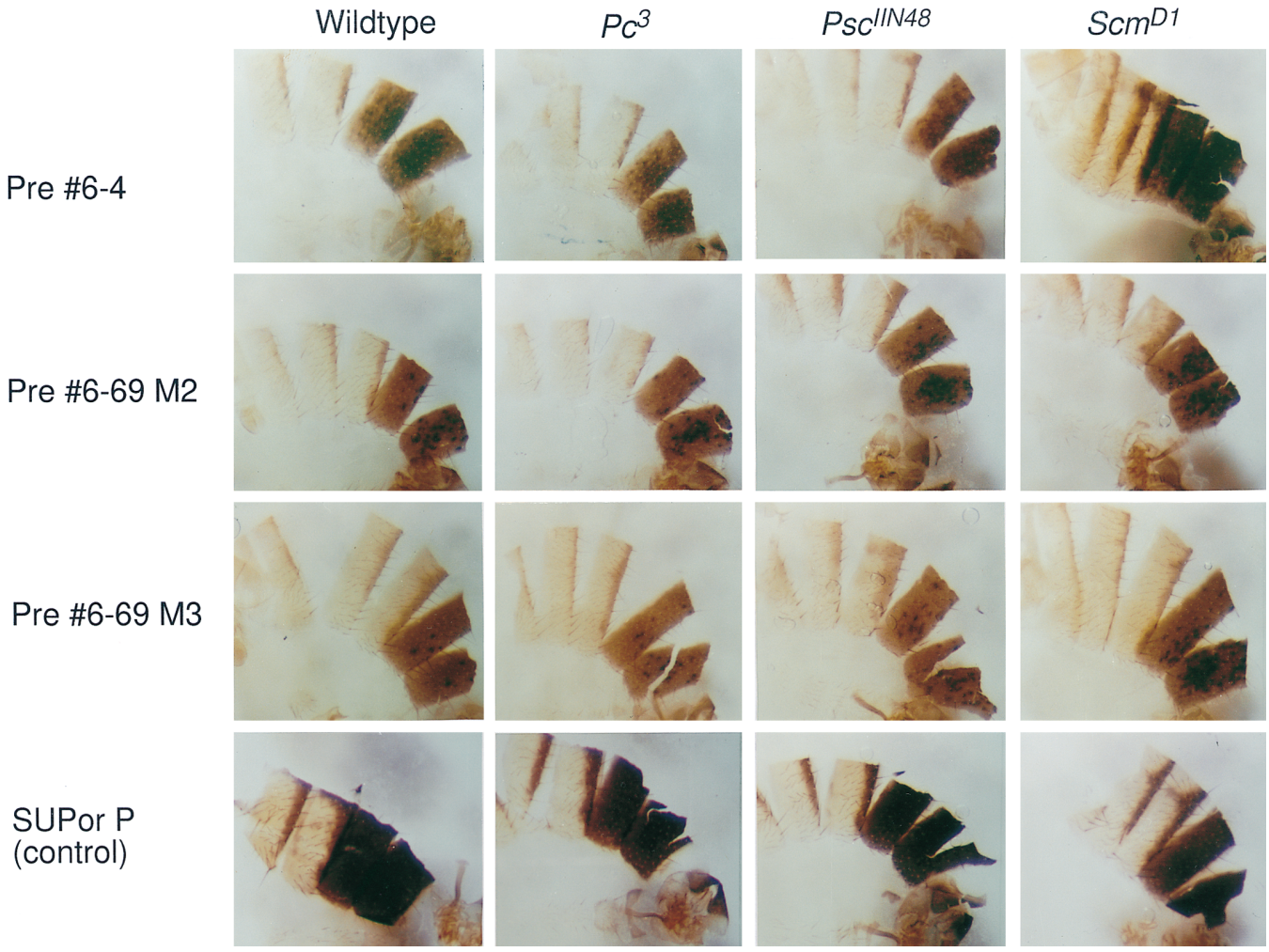

Figure 3.—Phenotypes of PRE 6 and *SUPor P* (control) lines in a wild-type or *Pc-G* mutant background. The abdominal cuticle phenotype of males isolated from three independent class II PRE 6 lines and *SUPor P* are shown in different genetic backgrounds, including wild type or mutant for *Polycomb* (Pc^3), *Posterior sex combs* (Psc^{IIN48}), or *Sex combs on midleg* (Scm^{D1}).

poson into a *su(Hw)* mutant background caused a variegated- or white-eye phenotype. The *white* gene was insulated from repression even when the PRE was placed directly next to the SU(HW) binding region (PRE 2), indicating that the SU(HW) binding region is extremely effective at blocking PcG effects.

The observation that the SU(HW) binding region protects gene expression from the repressive effects of a PRE suggests that this chromatin insulator may provide a useful tool in further analysis of PREs and other DNA elements that negatively affect gene expression. Our analysis was further assisted by the use of a transposon carrying two reporter genes. One gene was protected from repression so that transformants could be identified, and the second gene was used to study repression.

The repressive effects of the PRE are dependent on genomic location: The 1.6-kb fragment containing the *Ubx* PRE site did not always confer repression upon the two reporter genes. Previous experiments using this fragment similarly found that repression occurred only in a subset of the insertion sites (Chan *et al.* 1994; Poux *et al.* 1996). There are several possible explanations for variable effects of the PRE at different genomic locations. It is possible that certain insertion sites are neutral for repression because these transposons are integrated nearby or into genes that are actively transcribed, which may interfere with formation or extension of PRE silencing. This prediction is consistent with observations that the GAL4 activator can overcome Pc protein association with chromatin (Zink and Paro 1995) and that silencing may be dependent upon the state of gene activity during the time of establishment of the PcG complex (Poux *et al.* 1996). Second, the genomic DNA nearby these neutral sites may be devoid of weak binding sites for PcG protein. These weak binding sites have been proposed to participate in the formation and stabilization of repressive chromatin that is initiated at a PRE (Pirrotta and Rastelli 1994; Pirrotta 1997). Third, it is possible that these neutral sites are regions of the genome that are located in a nuclear subcompartment that is incompatible with the formation of repressive chromatin. Recent studies indicate that nuclear compartmentalization impacts the degree of repression associated with yeast silencers (Maillet *et al.* 1996) and in heterochromatic silencing associated with Drosophila PEV (Wakimoto and Hearn 1990; Csink and Henikoff 1996; Dernburg *et al.* 1996).

Interestingly, in genomic locations where the PRE caused repression of gene expression, both genes were always affected but not to equal degrees. We observed that lines displaying the greatest degree of *yellow* silencing were not necessarily the lines showing the lowest amount of *white* expression. In addition, the effects of PcG mutations depended upon genomic location. These results support the model that flanking genomic DNA contributes to repression and suggest the possibility that at a given genomic location a distinct PcG complex exists that assembles a unique type or extent of repressive chromatin. Additional evidence supports the suggestion that PcG complexes assembled at PREs may vary in composition. Effective repression of two segmentation genes is dependent upon only some of the PcG genes (Pelegri and Lehmann 1994). Additionally, PRE containing transposons displaying silencing effects show a variable association of PcG proteins, as determined by *in situ* localization to polytene chromosomes (Chan *et al.* 1994).

Mutations caused by *gypsy* may involve disruption of PRE function, as well as enhancers: There are a large number of *gypsy*-induced mutations of *Ubx* (Bender *et al.* 1983; Peifer and Bender 1986; Peifer and Bender 1988). These mutations are associated with a loss of *Ubx* function, and are proposed to result from the blocking of enhancers present in the large regulatory regions of this gene (Peifer and Bender 1986; Peifer and

TABLE 2

Summary of effects of *Pc-G* mutations on *yellow* expression in Class II lines

		Pc-G mutation					
PRE-construct line	None	*Asx*^{XT129}	*Pc*^{3}	*Pcl*^{5}	*Psc*^{IIN48}	*Sce*^{D1}	*Scm*^{D1}
PRE 2-9 M2	1	1	1	1	1	**2**	1
PRE 6-4	3	3	3	**4**	3	**4**	**4**
PRE 6-69	1.5	1.5	1.5	1.5	**2.5**	1.5	**2.5**
PRE 6-69 M1	1	1	1	1	**1.5**	1	**2**
PRE 6-69 M2	2	2	2	2	**3**	2	**3**
PRE 6-69 M3	1	1	**2**	1	**2**	**2**	**3**
PRE 6-69 M7	1	**1.5**	1	1	1	1	1
PRE 6-69 M10	1	1	1	1	**3**	1	1
SUP or P	5	5	5	5	5	5	5

The level of variegation is based on a 5-point scale, with 1 corresponding to a lack of pigmentation and 5 to no variegation. Numbers in bold represent a suppressed phenotype.

Bender 1988). Our results demonstrate a second possible effect of the *gypsy* insertions; that of interference with the establishment of PcG repression. In this case, *gypsy* insertion would be predicted to produce gain-of-function phenotypes, similar to that seen by mutations in PcG genes. Gain-of-function phenotypes may not be observed because of redundancy in the sequences involved in PRE repression. There are at least two PREs within *Ubx* (Chan *et al.* 1994; Chiang *et al.* 1995) and other weak binding sites may exist (Strutt *et al.* 1997). Additionally, the loss of PRE repression resulting from *gypsy* insertion would be coupled to a loss of enhancer activation, which are offsetting effects.

Insulators may be involved in establishing domains of repression within the genome: The effects of the *su(Hw)* protein bound to its *gypsy* binding region on gene expression are well characterized. However, it is unclear whether this protein acts similarly at the several-hundred euchromatic positions to which it localizes (Harrison *et al.* 1993). The simplest model suggests that the *su(Hw)* protein functions as an insulator protein at these endogenous locations, possibly in concert with other insulator proteins to limit the effectiveness of enhancer-promoter communications. Our present studies suggest that the *su(Hw)* protein may work to limit repression. It is widely appreciated that repression is a commonly used mechanism to regulate gene expression. The PcG proteins are involved in the regulation of several genes, in addition to the homeotic loci (Moased and O'Farrell 1992; Pelegri and Lehmann 1994; McKeon *et al.* 1994). PcG repression appears to involve the cooperation of multiple PREs to promote the formation of repressive chromatin (Pirrotta 1997). This suggests that maintenance of repressed domains may require the activity of insulators distributed throughout the genome. Thus, a primary function of the *su(Hw)* protein and other insulator proteins may be to limit domains of repression. The presence of insulator sequences within the bithorax complex, such as in the *Fab-7* insulator located in the *Abdominal B* gene, supports this contention (Hagstrom *et al.* 1996; Zhou *et al.* 1996).

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