Enhancer of Polycomb Is a Suppressor of Position-Effect Variegation in Drosophila melanogaster

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ABSTRACT

Polycomb group (PcG) genes of Drosophila are negative regulators of homeotic gene expression required for maintenance of determination. Sequence similarity between Polycomb and Su(var)205 led to the suggestion that PcG genes and modifiers of position-effect variegation (PEV) might function analogously in the establishment of chromatin structure. If PcG proteins participate directly in the same process that leads to PEV, PcG mutations should suppress PEV. We show that mutations in E(Pc), an unusual member of the PcG, suppress PEV of four variegating rearrangements: In(l)mw³, B⁵V, T (2;3)Sb⁵, and In(2R)bwm⁴. Using reversion of a P element insertion, deficiency mapping, and recombination mapping as criteria, homeotic effects and suppression of PEV associated with E(Pc) co-map. Asx is an enhancer of PEV, whereas nine other PcG loci do not affect PEV. These results support the conclusion that there are fewer similarities between PcG genes and modifiers of PEV than previously supposed. However, E(Pc) appears to be an important link between the two groups. We discuss why Asx might act as an enhancer of PEV.

The Polycomb group (PcG) genes in Drosophila include over a dozen described members (Simon 1995), although it has been estimated that there may be up to 40 members (Jurgens 1985; Landecker et al. 1994). Locke et al. (1988) proposed that PcG products form multimeric complexes. This view has been supported by observations that polyhomeotic (Ph), Polycomb (Pc), Polycomblike (Pcl), and Posterior sex combs (Psc) have overlapping binding sites on polytene chromosomes (Zink and Paro 1989; DeCamillis et al. 1992; Lonie et al. 1994; Martin and Adler 1993; Rastelli et al. 1993), and also by the finding that PH and PC immunoprecipitate as members of a multimeric complex (Franke et al. 1992). PcG genes are negative regulators of homeotic genes (McKeon and Brock 1991; Simon et al. 1992). Many PcG genes have pleiotropic phenotypes, so their function is not limited to regulation of homeotic genes (Ingham 1984; Breen and Duncan 1986; Smouse et al. 1988; Wu et al. 1989; Jones and Gelbart 1990). This idea is supported by the observations that PH, PC, PCL, and PSC bind to about 100 sites on polytene chromosomes (Zink and Paro 1989; DeCamillis et al. 1992; Lonie et al. 1994; Martin and Adler 1993; Rastelli et al. 1993).

Position-effect variegation (PEV) occurs when a euchromatic gene is transposed adjacent to a broken segment of heterochromatin. Expression of the transposed gene is repressed in some cells but not in others, producing a mosaic phenotype. Repression is probably caused by spreading of heterochromatin into the euchromatic gene, causing inactivation (Henikoff 1990; Hayashi et al. 1990; Reuter and Spierer 1992), although models invoking nuclear localization have been gaining support (Wakimoto and Hearn 1990; Kenpén 1994; Dernburg et al. 1996; Csík and Henikoff 1996). Several groups have screened for mutations that enhance or suppress PEV, referred to as E(var)s or Su(var)s, respectively (Reuter et al. 1982; Sinclair et al. 1983; Locke et al. 1988). Most modifiers of PEV are thought to be nonhistone chromatin proteins (Hayashi et al. 1990; Eissenberg et al. 1990; Reuter et al. 1990). Mutations in these genes may interfere with or increase the ability of heterochromatin to spread, or alternatively, to target regions to transcriptionally inactive compartments.

Paro and Hogness (1991) reported that Pc shares a region of sequence similarity, termed the chromobox, with Su(var)205, which encodes the heterochromatin protein HP1. Another domain, the SET domain, is shared by Su(var)309 and another PcG gene, Enhancer of zeste (E(z)), as well as by trithorax, a gene required for gene activation (Tsien et al. 1994). Both PcG genes and modifiers of PEV are required for the maintenance of determination (Struhl and Akam 1985; Wu et al. 1989; Reuter et al. 1990). This has led to speculation

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that both the PcG genes and modifiers of PEV might contribute to the establishment of repressive chromatin domains (Reuter et al. 1990; Paro and Hogness 1991; Gaunt and Singh 1990; Alberts and Stern 1990; Paro 1990). In addition, Dorn et al. (1993) report that an E(var) is an apparent member of the trithorax group (Kennison 1993), suggesting that it is possible for regulators of homeotic genes to be modifiers of PEV. Very recently, Larson et al. (1996) reported that mutations in the Su(z)5 locus, which encodes the gene for S-adenosylmethionine synthetase, act both as Su(var)s and as enhancers of Pc.

If PcG proteins participate directly in the same process mediated by modifiers of PEV, then PcG mutations should cause suppression of PEV. So far, the possibility that previously described PcG genes are modifiers of PEV has not been tested directly, although Kennison (1995) reports unpublished data suggesting that PcG genes do not suppress PEV. Fauvarque and Dura (1993) show that variegation of a miniwhite gene in a P transposon containing regulatory sequences from ph is sensitive to PcG mutations. Interestingly, most modifiers of PEV, or removal of the Y chromosome, do not affect this variegation, suggesting that these two kinds of variegation are different.

All PcG genes show posteriorly directed homeotic transformations in embryos and adults, resulting from ectopic expression of homeotic genes (McKeon and Brock 1991; Simon et al. 1992). Enhancer of Polycomb (E(Pc)) occupies an unusual position in the PcG because it does not have a zygotic homeotic phenotype, even though it is a homoyzogous embryonic lethal (Sat o et al. 1983). Even when embryos lacking the maternal and zygotic product are examined, their cuticles appear normal, and only a very minor ectopic expression of Abdominal-B is detected (Sat o et al. 1995). However, mutations in E(Pc) enhance homeotic phenotypes of several PcG genes (Sat o et al. 1984), so its status as a PcG gene is unclear. We report here that mutations in E(Pc) are Su(var)s.

We go on to test the hypothesis that some PcG genes might be suppressors of PEV by crossing chromosomes containing mutations in Additional sex combs (Asx; Sinclair et al. 1992), extra sex combs (esc; Struhl 1981), E(z) (Wu et al. 1989; Jones and Gilbert 1990; Phillips and Shearn 1990), l(4)102EFc (Geering 1970), renamed pleiohomeotic (Girton and Jean 1994), Pc (Lewis 1978), Pcl (Duncan 1982), ph (Dura et al. 1985), Psc (Jurgens 1985), Sex combs extra (Sce; Breun and Duncan 1986), Sex combs on midleg (Scm; Jurgens 1985), and super sex combs (ssc; Ingham 1984) to variegating rearrangements. A number of chromosomes containing PcG mutations enhance PEV. With the exception of Asx, however, we were unable to co-map modification of PEV and homeotic phenotypes. We discuss the degree of overlap between PEV and repression exhibited by PcG proteins.

MATERIALS AND METHODS

Drosophila strains and culture: The PcG strains used in this study are described in Lindsley and Zimm (1992), except where indicated: Asx1, Asx2 (Sinclair et al. 1992), Df(2)lrix; esc; E(Pc)1; E(z)1; l(4)102EFc; Pcl, Pcl1; Pcl2; Pc1, Df(2R)Pcl-W5; ph; Psc1; Psc2; Psc3; Su(z)11; (Brunke et al. 1991), Df(2R)v glb; Scm; Scm1, Su(z)302; ssc1, ssc2. We also used deletions in the region of 48A that are described in Lindsley and Zimm (1992): Df(2R)en-A, Df(2R)en-B, Df(2R)en-SFX31, Df(2R)en28, Df(2R)en30, or in Chase and Baker (1993): Df(2R)x8713, Df(2R)xwba9x1, Df(2R)xwba9x3, and Df(2R)xwba9x4.

We used Su(var)210 and Dpl(2;2)Mh as examples of Su(var)s and E(var)s, respectively. Flies were raised and crossed on standard cornmeal-sucrose medium at 22° or 25°.

Phenotypic characterization of PEV: Variegation assays were performed as outlined previously (Sinclair et al. 1983). Male PcG mutants were crossed to females bearing In(1)wm4, T(2;3)Sb, and In(2)bw10E, hereafter referred to as wm4, Sb, and bw1, respectively. A minimum of 25 flies of each genotype were scored for each cross described below. For tests with Psc/Psc, PcG/Balancer males were crossed to wm4/Y females, and wm4/Y; PcG/+ flies were scored visually compared to their wm4/Y, Balancer/ +. Because Sb1 is dominant and mutant, Su(var)s increase the severity of the variegating phenotype, whereas E(var)s decrease its severity. The opposite is true of modifiers of bw1. Both Sb1 and bw1 show sex-specific differences, so males and females were scored separately. For Sb1, 14 defined bristles on each fly were scored as being wild type or Sb, and the mean percentage of Sb bristles ± SEM was determined for 25 control and 25 experimental flies. Note that bristle length is a continuously varying phenotype, so that each scorer had to make an arbitrary decision for each bristle scored as to whether it was wild-type or Sb. For bw1, the amount of pigment in five samples of five flies each was determined for control and experimental genotypes, and expressed as an mean percentage of wild-type pigment ± SEM. The general form of the crosses to test PcG mutations on variegation of Sb1 or bw1 if both mutations are on the same chromosome as follows: PcG/Balancer × Variegator/Balancer, and PcG/Variegator progeny were compared to their Balancer/ Variegator siblings. If the PcG and variegating rearrangement are on different chromosomes, the general form of the crosses was PcG/Balancer1; +/- × +/-; +/+; Variegator/ Balancer2, and PcG/+; Variegator/+; flies were compared to their Balancer1/ +; +/+; Variegator/ +; siblings (“Balancer” refers to any balancer, and “Balancer1” and “Balancer2” refer to different balancers). B54 is a variegating allele of B on the Y chromosome. Like Sb1, Su(var)s enhance and E(var)s decrease the severity of the variegating phenotype. PcG females were crossed to B54 males. No internal controls were generated, so the results were compared to the mean of results obtained for control crosses carried out in two different backgrounds. For each cross, 25 eyes were traced using a camera lucida, and the tractings were cut out and weighed individually to obtain an estimate of their area. The results were expressed as a percentage of wild-type area ± SEM.

Screen for P-induced mutations and revertants: Generation of line 4-12E has been described by Kassir (1994). To recover E(Pc)1 revertants, 4-12E/Cyo females were crossed to Sp/Cyo; Δ2-3 Sb/TM 6 males, and 4-12E/Cyo; Δ2-3 Sb/+ males were recovered and crossed to Bc Gla/Cyo Roi females. Single 4-12E/Cyo Roi males were crossed to E(Pc)1/SM 5 females, and their progeny were scored for the presence of straight-winged flies. The reversion frequency was <10%, and 15 lines were
established by backcrossing to E(Pc)^1. Five of these lines were retained for further analysis.

Mapping: After testing the mapping chromosome for absence of modifiers, progeny derived from mating w^m4; E(Pc)^1/Sp Tft n w^D Pin^T females to w^m7/Y males were scored for suppression of PEV and segregation of the other dominant mutations. Map position was calculated to ±95% confidence limits. A similar strategy was used to attempt to map enhancement of PEV exhibited by Asx^1, Pd^112, and Psc^1 chromosomes. This approach failed, however. We reasoned that if PcG mutations exhibited strong maternal effects so that all progeny derived from Pcg/GS Sp Tft n w^D Pin^T exhibit enhancement of PEV, then it might be necessary to cross the Pcg recombinants into a w^m4 background to be able to map the enhancement of PEV. Therefore, recombinant males derived from crossing w^m4; Asx^1/S Sp Tft n w^D Pin^T females were crossed individually to w^m7 females, and their recombinant progeny were compared to w^m7 sibling controls for enhancement of PEV. This strategy allowed us to map the enhancement of PEV associated with the Asx chromosome, but it did not allow us to map enhancement of PEV associated with Pd^112 or Psc^1, suggesting that multiple loci contribute to this phenotype.

To show that the E(Pc)^2 chromosome did not contain any additional Su(var) loci, the E(Pc)^2 mutation (and the P[w^+] transposon) was crossed off. This allowed us to score the effect of recombinant chromosomes on w^m4 in the absence of the E(Pc)^2 mutation. E(Pc)^1/Sp^1 B^1 L^m B^1 Pu^2 Pin^R females were crossed to Dif(2R)en-A/CyO males. Straight-winged flies carrying recombinants between B1 and L are heterozygous for Dif(2R)en-A, and must therefore have lost E(Pc)^2 by recombination, since E(Pc)^2 is lethal over this deficiency. These straight-winged recombinant males were crossed to w^m7 females, and their progeny were scored for suppression of PEV. Dif(2R)en-A is a Su(var), providing an internal control for suppression of the PEV associated with w^m7, and these flies were compared to their recombinant siblings. No suppression of PEV was seen in 255 recombinants between B1 and L (+ L Bc Pu Pin or Sp B1 +++++). The 95% confidence limits are ±1.0 cm.

Molecular analysis: Preparation of phage DNA, subcloning, mapping, blotting, hybridization, labeling probes, and preparation of poly(A)^+ RNA were carried out using standard techniques. Genomic DNA was prepared from adult females as described by Sinclair et al. (1992), and 10 μg was used for each lane in Southern analysis. Embryonic and adult mRNA were prepared from ~1 g of material, using Trizol under conditions recommended by the manufacturer (Bethesda Research Laboratories, Gaithersburg, MD). About 2 μg of poly(A)^+ per lane was used in the gels for Northern analysis.

RESULTS

Co-mapping of E(Pc) and suppression of PEV: Wustmann et al. (1989) showed that a Su(var) mapped to the overlap of Dif(2R)en-A and Dif(2R)en-B, but the region was not further characterized. As noted in the Introduction, proteins with a common function in repression established by PcG proteins and PEV should suppress PEV, so it seemed possible that E(Pc) might be the Su(var) identified by deficiency mapping. Satô et al. (1983) had shown that E(Pc) lay between the breakpoints of Dif(2R)en-SFX31 and Dif(2R)en30, in the region between bands 48A1-2 and 48A3-4, and proximal to engrailed (en). As a preliminary step, we confirmed these results, and we performed an analysis of deficiencies in the region for their ability to suppress PEV and for failure to complement E(Pc)^1. The results of this analysis are shown in Figure 1. There is a perfect correspondence between deficiencies that uncover E(Pc) and those that suppress PEV. These data confine the Su(var) and E(Pc) to the same two- to four-band interval.

During the course of a genomic walk through the region proximal to en, Kuner et al. (1985) mapped the breakpoint of Dif(2R)en-SFX31 and located en on the walk. Subsequently, Drs. et al. (1987) mapped three transcription units in the region between the Dif(2R)en-SFX31 breakpoint and invected (inv), the gene immediately proximal to en, suggesting that E(Pc) must correspond to one of these three transcription units. We obtained the phages that cover this interval as a gift from T. Kornberg. First, we checked the restriction map of this region, and found some difference in order and fragment size compared to those published previously (Kuner et al. 1985), and our revised map is shown in Figure 2.

Next, we performed Northern analysis on embryo and adult RNA using restriction fragments obtained from the walk, and we confirmed the presence of at least three different transcription units in this region, although the molecular weights of the transcripts differ from those published (Dr. et al. 1987). For convenience, we refer to them as the proximal, middle, and distal transcription units. Examples of the Northern analysis and a transcription map of the region are shown in Figure 2. Two transcripts of 1.4 and 1.5 kb hybridize to a single 4.1 kb EcoRI fragment delimiting the proximal transcription unit. During the course of these studies, we isolated a cDNA that hybridizes to both of these transcripts. Sequence analysis (D. A. R. Sinclair, N. J. Clegg and H. W. Brock, unpublished data) of the partial cDNA revealed that this transcription unit en-

![Figure 1](image_url)
codes a Drosophila homologue of a water channel protein, alternatively termed aquaporin or channel-forming integral protein (CHIP; Sabolic and Brown 1995). This gene was not further studied, on the assumption that it did not encode E(Pc). For the middle and distal transcription units, several contiguous EcoRI fragments hybridize to multiple transcripts, as indicated in Figure 1. For the middle transcription unit, we do not know if the multiple transcripts represent alternative processing of one transcription unit, or less likely, transcripts from nested transcription units. As shown below, the distal transcription unit is alternatively spliced.

Until recently, only E(Pc)1 had been isolated. No rearrangements visible upon Southern analysis are detected with this allele (unpublished data), so it was not possible to easily determine which of the middle and distal transcription units corresponds to E(Pc). One of us (J.A.K.) recovered a lethal P element mutation uncovered by Df(2R)en-SFX31 as a byproduct of another screen. This mutation, termed 4-12E, was crossed to E(Pc)3 and failed to complement. No Cy+ and 171 Cy flies were recovered from a cross of 4-12E/CyO and E(Pc)3/SM5. The 4-12E mutation was crossed to Pc6, and the progeny were scored for enhancement of the extra sex combs phenotype. As shown in Table 1, en-
Table 1: Analysis of E(Pc) and revertants on enhancement of Pc1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of flies scored</th>
<th>Average no. of legs with sex combs</th>
</tr>
</thead>
<tbody>
<tr>
<td>E(Pc1)/ +; Pc1/ +</td>
<td>117</td>
<td>3.4</td>
</tr>
<tr>
<td>SM5/ +; Pc1/ +</td>
<td>102</td>
<td>2.0</td>
</tr>
<tr>
<td>E(Pc1)/ +; TM3/ +</td>
<td>86</td>
<td>2.0</td>
</tr>
<tr>
<td>E(Pc1)/ +; Pc1/ +</td>
<td>75</td>
<td>4.8</td>
</tr>
<tr>
<td>Cyo/ +; Pc1/ +</td>
<td>58</td>
<td>2.0</td>
</tr>
<tr>
<td>E(Pc1)/ +; TM3/ +</td>
<td>94</td>
<td>2.0</td>
</tr>
<tr>
<td>E(Pc1)/ +; Pc1/ +</td>
<td>75</td>
<td>2.2</td>
</tr>
<tr>
<td>E(Pc1)/ +; Pc1/ +</td>
<td>99</td>
<td>2.04</td>
</tr>
<tr>
<td>E(Pc1)/ +; Pc1/ +</td>
<td>102</td>
<td>2.1</td>
</tr>
</tbody>
</table>

For each cross, E(Pc)/Bal females were crossed to Pc1/TM3 males to yield the indicated genotypes. Males were scored for the number of legs with sex combs, and they are reported as the average number of legs with sex combs. A fly with complete transformation to T1 would thus have a score of 6.0 and a wild-type would score of 2.0.

Figure 3.—Mapping of P element insertion in the 4.5-kb EcoRI fragment from phage E2 and of revertant lines showing loss of the P element. Genomic DNA was obtained from adult females, digested with EcoRI, electrophoresed, and blotted to a nylon membrane (Hybond N; Amersham, Arlington Heights, IL), probed with the 4.5-kb EcoRI fragment, and autoradiographed. (Lane 1) Wild-type, showing hybridization to the 4.5-kb EcoRI fragment. (Lane 2) 4-12E/+. (Lane 3) E(PcRev)/ +. (Lane 4) E(PcRev)/ + E(PcRev)/ +. The insertion of the P element gives two new bands of 7.0 and 2.9 kb in 4-12E/+, owing to the presence of a single EcoRI site in the P element itself. These bands are absent in the wild type and revertants. Partial digestion products are faintly visible in lanes 3 and 4, but are clearly different from the new bands seen in lane 2.

Figure 4.—Northern analysis of E(Pc) mutants. Poly(A)+ was prepared from adult females, electrophoresed on agarose gels containing formaldehyde, blotted to a Hybond-N membrane, and hybridized with the 4.5-kb EcoRI fragment of phage E2 (lanes 1 and 2, 5 and 6), or with the 3.6-kb EcoRI fragment of phage E2 (lanes 3 and 4). The bottom panels of lanes 5 and 6 were hybridized with RP-49 as a loading control. The sizes of transcripts are indicated in kilobases for E(Pc). The sizes of the transcripts from the middle transcription unit are given in Figure 2. Note that lanes 1 and 2 come from a different blot than lanes 3 and 4. Odd-numbered lanes are wild-type. Lanes 2 and 4 are E(Pc)/+, and lane 6 is E(Pc)/+. Note the presence of an additional band of 5.0-kb in lane 2 compared to lane 1, but the absence of any change in lane 4 compared to lane 3. This identifies the distal transcription unit as E(Pc). To confirm this inference, lane 6 shows that the amount of mRNA is greatly reduced in E(Pc) flies relative to the wild type, especially when compared to the RP-49 loading control. Quantitation of this and similar autoradiographs shows that E(Pc)/+ adults have between 10 and 21% of the 8.5-kb mRNA relative to the wild type.

The location of the P element in the 4-12E mutant strain was mapped by Southern hybridization, and was found to lie in a 4.5-kb EcoRI fragment of phage E2 that encodes the distal transcription unit (Figure 3). As a confirmation, poly(A)+ RNA from heterozygous 4-12E mutants was subjected to Northern analysis. As shown in Figure 4, in 4-12E/+, mRNA transcripts with altered molecular weight are detected in the distal but not in the middle transcription unit. Furthermore, the amount of transcript is severely reduced in E(Pc)/+ mRNA relative to the wild type. The difference was quantified using a scanning densitometer, and E(Pc)/+ has only 10–21% of the mRNA found in wild-type flies. If E(Pc) was a null mutation, it would be expected that heterozygotes would have 50% of the wild-type level. We have no explanation for the latter data, but this could result from a direct or indirect upset of a positive feedback loop that regulates E(Pc). Together, these data indicate that the distal transcription unit corresponds to E(Pc).

To confirm this suggestion, the 4-12E mutant was reverted by mobilizing the P element and scoring for survival over E(Pc) chromosomes. As shown in Figure 3, the revertant lines tested lost the P element associated with the 4.5-kb EcoRI fragment, indicating that the revertant lines are Pc1/TM3.
with the 4.5-kb EcoRI fragment in this region. The revertants show zero to slight enhancement of the extra sex combs phenotype of Pc compared to the strong enhancement seen in the parent chromosome (Table 1). The revertants may be weak hypomorphs, perhaps because of imprecise excision of the P element. We suppose that there is enough E(Pc) function in the revertants to be viable over E(Pc); but enough function is impaired to be detectable in the enhancement assay. Because the Southern data show that all revertants have lost the P element (Figure 3), these data show that loss of the P element is correlated with loss of the homeotic phenotype. Accordingly, 4-12E has been renamed E(Pc)2.

Knowing that the distal transcription unit corresponded to E(Pc), we tested E(Pc)1 and E(Pc)2 for their ability to modify PEV. As shown in Table 2, both of these alleles, and Df(2R)en-A suppressed PEV at all four loci tested, consistent with E(Pc) being a Su(var). Df(2R)en-SFX31 generally had a weaker effect than Df(2R)en-A, presumably because of the presence of background modifiers, but nevertheless acted as a Su(var). We used recombination to map the suppression of w⁶PEV associated with the E(Pc)1 chromosome to 2–63 ± 1.6, consistent with the published map position of E(Pc) of 2–61.9. In this case, recombinants were tested to ensure that the expected chromosomes failed to complement E(Pc)1. As outlined in the materials and methods, we also checked for the presence of modifiers on the E(Pc)2 chromosome. In this case, we recovered recombinants that had lost E(Pc)2, and tested for presence of modifiers of w⁶ PEV. No modifiers were found in 255 recombinants between Bl and L, so if there is a second site modifier on this chromosome, it is within 1.0 cM of E(Pc)².

Finally, we tested E(Pc)² revertants for loss of suppression of PEV of w⁶ and B⁵⁷. All revertants of E(Pc) tested showed either partial or complete reversion of the Su(var) phenotypes. The reversion of B⁵⁷ was quantified, and it correlated well with the amount of loss of enhancement of the extra sex combs phenotype of Pc seen in Table 1. As discussed above, we assume that complete revertants are examples of precise excision of the P element, whereas the partial revertants represent imprecise excisions, although this possibility has not been tested directly. Together with the molecular and genetic tests described above, the reversion data establish that E(Pc) is a Su(var).

Tests of chromosomes containing PcG mutations for their ability to modify PEV: To determine if E(Pc) is a special case, or if other PcG mutations also suppress PEV, we crossed chromosomes carrying PcG mutations from 10 loci to w⁶ and B⁵⁷. For comparison, we crossed Su(var)210 and the strong enhancer Dp(2;2)M dh to these variegators, and selected results are shown in Table 3. Where possible, we crossed a null mutation, a deficiency, and a gain of function mutation for each PcG locus, reasoning that because the PcG mutations were selected for various phenotypes, not all alleles would necessarily modify PEV. In a screen of modifiers of the zeste-white interaction, only gain-of-function PcG muta-

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**TABLE 2**

Effects of E(Pc) mutations on PEV

<table>
<thead>
<tr>
<th>Genotype</th>
<th>w⁶</th>
<th>B⁵⁷</th>
<th>Sb⁶/Mutation</th>
<th>Sb⁶/+</th>
<th>bw⁶/Mutation</th>
<th>bw⁶/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>Mottled</td>
<td>61 ± 3</td>
<td>—</td>
<td>65 ± 4</td>
<td>—</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>Su(var)210/+</td>
<td>Red</td>
<td>29 ± 1</td>
<td>71 ± 3</td>
<td>67 ± 3</td>
<td>65 ± 2</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Dp(2;2)M dh/+</td>
<td>White</td>
<td>88 ± 1</td>
<td>26 ± 3</td>
<td>72 ± 4</td>
<td>3 ± 0</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>E(Pc)¹+/+</td>
<td>Red</td>
<td>23 ± 1</td>
<td>88 ± 2</td>
<td>75 ± 3</td>
<td>107 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>E(Pc)²+/+</td>
<td>Red</td>
<td>27 ± 2</td>
<td>89 ± 3</td>
<td>73 ± 4</td>
<td>97 ± 2</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>Df(2R)en-A</td>
<td>Red</td>
<td>27 ± 2</td>
<td>88 ± 2</td>
<td>68 ± 3</td>
<td>57 ± 3</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>Df(2R)en-FX31</td>
<td>Red mottled</td>
<td>43 ± 3</td>
<td>84 ± 2</td>
<td>67 ± 3</td>
<td>22 ± 1</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>E(Pc)rv⁴/+/+</td>
<td>Mottled</td>
<td>45 ± 4</td>
<td>—</td>
<td>—</td>
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<td></td>
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<tr>
<td>E(Pc)rv⁶/+/+</td>
<td>Mottled</td>
<td>62 ± 4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
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<tr>
<td>E(Pc)rv¹⁰/+/</td>
<td>Mottled</td>
<td>57 ± 2</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>

Crosses and scoring are described in materials and methods. Wild-type is Oregon R. For w⁶, eyes were scored visually and compared to internal controls. E(Pc) contains a P transposon with the mini white gene. E(Pc) normally has dark orange eyes. If E(Pc) did not suppress w⁶, we would expect to see red mottled eyes. Instead, we see red eyes, suggesting that E(Pc) suppresses w⁶. Note that the revertants have lost the mini white gene, and thus it is possible to score modification of w⁶. For B⁵⁷, the data are reported as the mean eye size as a percentage of wild-type ± SEM, and the experimental data were compared to the mean of control values. For Sb⁶ and bw⁶, only male data are reported here because there are sex differences. Female data were also collected, and they exhibit the same trends as shown above for males. For both variegators, PcG mutants were compared to their balanced siblings. The Sb⁶ data are expressed as the mean percentage of full Sb expression ± SEM. The bw⁶ data are expressed as mean percentage of wild type pigment ± SEM (measured spectrophotometrically). Dashes represent crosses that were not done.
tions were recovered (Wu et al. 1989), suggesting that mutants carrying gain-of-function PcG mutations might be more likely to interact with chromatin proteins.

As shown in Table 3, chromosomes containing all alleles of Asx, E(z), Pcl, Psc, and Scm enhanced variegation of w^m_, and most also enhanced variegation of B^S_. It is striking that different alleles can modify variegation in different directions. This could either represent allele-specific differences or indicate the presence of modifiers in the background. The esc, l(4)102Efx, Pc, ph, and sxc mutations had no effect on the variegation of w^m_ and B^S_ (data not shown) and were not tested further. PcG loci that modified variegation of w^m_ and B^S_ were crossed to Sb^v_ and bw^v_, and these results are shown in Table 3. Because most strong modifiers of PEV modify all variegating rearrangements (Sinclair et al. 1989), we expected that strong modifiers would affect all four variegating loci tested. Of the alleles tested, only Pd^I_, Pd^Z, and Psc^I, met this criterion.

The data above are consistent with the possibility that some PcG mutations modify PEV. However, the data are also consistent with the possibility that the observed modification of PEV results from dominant modifiers in the background, or from recessive modifiers uncovered by deletions, rather than being attributable to PcG mutations themselves. We attempted to recombinationally map the enhancement of PEV for the three loci that showed the strongest effects: Asx, Pcl, and Psc, but we did not examine E(z) or Scm. Provided that we introduced the Asx mutation via males into the w^m_ background in females, we were able to map the enhancement of PEV associated with the Asx^I chromosome to 2–71 ± 1.1, in reasonable agreement with the published map position of 2–72 (Jurgen's 1985). However, we were unable to map the enhancement of PEV associated with the Pd^I_ and Psc^I_ chromosomes to any defined interval, showing that there are multiple modifiers on the mutant chromosomes. For the remaining PcG alleles examined in Table 3, there are no mapping data, so we have not excluded the possibility that the untested mutations do modify PEV.

**DISCUSSION**

Our most important finding is that E(Pc) provides a link between the PcG and the Su(var)s. Mutations in or deletions of E(Pc) are Su(var)s. Our data show that the Su(var) associated with Df(2R)en-A described by Wustmann et al. (1989) co-maps by cytogenetics, recombinational mapping, and co-reversion of a transposable element-induced mutation to E(Pc). The most straightforward interpretation of these results is that E(Pc) has a joint role in the repression established by PcG and Su(var) proteins. One model is that E(PC) is a structural component of heterochromatin and PcG complexes, and therefore mutations perturb the establishment of both structures. Alternatively, E(Pc) might be necessary to modify precursors or proteins required for repression by PcG proteins and modifiers of PEV. Kennison (1995) has made the interesting suggestion that E(Pc) might interact with or modify the chromodomain, thus accounting for its dual role in the PcG and in modification of PEV. E(PC) might mediate assembly of repressive chromatin by acting as a template or a chaperone protein. Larsson et al. (1996) have shown that Su(z)5, which encodes S-adenosylmethionine syn-

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**TABLE 3**

Effects of chromosomes containing PcG mutations on PEV

<table>
<thead>
<tr>
<th>Genotype</th>
<th>w^m_</th>
<th>B^S_</th>
<th>Sb^v_</th>
<th>Sb^v_ Mutation</th>
<th>Sb^v_ +</th>
<th>bw^v_</th>
<th>bw^v_ Mutation</th>
<th>bw^v_ +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asx^I_/+</td>
<td>White</td>
<td>83 ± 1</td>
<td>38 ± 3</td>
<td>73 ± 4</td>
<td>8 ± 3</td>
<td>4 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asx^I^12_/+</td>
<td>White</td>
<td>86 ± 1</td>
<td>52 ± 4</td>
<td>67 ± 3</td>
<td>4 ± 1</td>
<td>8 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Df(2R)trix/+</td>
<td>White</td>
<td>78 ± 2</td>
<td>81 ± 4</td>
<td>76 ± 3</td>
<td>24 ± 1</td>
<td>19 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E(z)^I_/+</td>
<td>Mottled</td>
<td>79 ± 2</td>
<td>42 ± 3</td>
<td>Lethal</td>
<td>7 ± 1</td>
<td>7 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E(z)^I^0_/+</td>
<td>White</td>
<td>44 ± 2</td>
<td>49 ± 4</td>
<td>Lethal</td>
<td>14 ± 1</td>
<td>6 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pd^I_/+</td>
<td>White</td>
<td>84 ± 2</td>
<td>48 ± 4</td>
<td>53 ± 5</td>
<td>9 ± 1</td>
<td>24 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pd^Z_/+</td>
<td>White</td>
<td>47 ± 4</td>
<td>59 ± 3</td>
<td>69 ± 4</td>
<td>12 ± 1</td>
<td>20 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pd-W6/+</td>
<td>White</td>
<td>30 ± 2</td>
<td>54 ± 3</td>
<td>69 ± 4</td>
<td>7 ± 1</td>
<td>13 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Df(2R)Pd-W5/+</td>
<td>White</td>
<td>57 ± 3</td>
<td>—</td>
<td>—</td>
<td>10 ± 1</td>
<td>19 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psc^I_/+</td>
<td>White</td>
<td>81 ± 2</td>
<td>53 ± 4</td>
<td>67 ± 4</td>
<td>5 ± 1</td>
<td>4 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psc^I^I^2_/+</td>
<td>White</td>
<td>92 ± 2</td>
<td>50 ± 3</td>
<td>65 ± 3</td>
<td>2 ± 0</td>
<td>4 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psc^I^I^2_/+</td>
<td>White</td>
<td>45 ± 2</td>
<td>77 ± 3</td>
<td>69 ± 5</td>
<td>40 ± 1</td>
<td>28 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Df(2R)vg-B/+</td>
<td>Mottled</td>
<td>45 ± 3</td>
<td>48 ± 4</td>
<td>79 ± 3</td>
<td>19 ± 1</td>
<td>33 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scm^I^1_/+</td>
<td>Mottled</td>
<td>73 ± 2</td>
<td>48 ± 4</td>
<td>67 ± 3</td>
<td>18 ± 1</td>
<td>28 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scm^I^2_/+</td>
<td>Mottled</td>
<td>86 ± 2</td>
<td>69 ± 3</td>
<td>Lethal</td>
<td>11 ± 1</td>
<td>7 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scm^I^3_/+</td>
<td>White</td>
<td>44 ± 3</td>
<td>63 ± 4</td>
<td>Lethal</td>
<td>11 ± 1</td>
<td>31 ± 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Details and controls are the same as those given in the legend to Table 2.
thetase, can also act as a Su(var) and enhance the extra sex combs phenotype of \( P \). They suggest that mutations of this enzyme may lead to a decrease in spermine concentration, which in turn leads to problems in establishing DNA or protein structures needed for repression.

Our data do not allow us to conclude that \( E(Pc) \) acts directly or indirectly on chromatin or chromatin proteins. \( E(Pc) \) could have a role in nuclear compartmentalization or the establishment of nuclear architecture that is required for the establishment of repressive chromatin. Alternatively, \( E(Pc) \) may regulate genes encoding Su(var)s or E(var)s so that its role in the suppression of PEV is indirect. If wild-type products of PEV and PcG gene modifiers interact with common intermediary proteins, alterations in the amount or quality of PcG products may have an indirect effect on PEV by changing properties of the intermediary proteins. It is also formally possible that \( E(Pc) \) mutations might influence PEV through subtle changes in the developmental rate. Molecular characterization of \( E(Pc) \) function will be required to distinguish among these possibilities.

\( E(Pc) \) occupies an unusual position in the PcG because it does not itself have a homeotic phenotype, even though it strongly enhances homeotic transformations of mutations at other PcG loci. Even in embryos lacking maternal and zygotic proteins, only a very minor, localized ectopic expression of Abd-B is detectable. The simplest interpretation of these data is that \( E(Pc) \) is largely redundant, and that another protein can substitute for its function when required. This would be analogous to Su(z)2, which is a homologue of Psc. Mutations in Su(z)2 can enhance some phenotypes of mutations in PcG genes, but do not themselves have strong homeotic phenotypes (Wu and Howe, 1995). Alternatively, \( E(Pc) \) either may have an indirect effect on PcG function, or else play a limited role in the regulation of homeotic genes, even though it may interact with PcG proteins in the regulation of other targets (Soto et al. 1995).

PEV can be modified by many loci in the background, making simple conclusions about the correlation of modification of PEV seen with a chromosome carrying a mutation in a given locus difficult. Mapping the phenotype by meiotic recombination is necessary, but is not sufficient, since one cannot eliminate the possibility of closely linked modifiers next to the locus of interest. The presence of maternal effects or genuine allele-specific differences complicate interpretation. Despite our initial enthusiasm for the idea that some PcG mutations are modifiers of PEV (Grigliatti 1991; DeCamillo et al. 1992), our attempts to co-map homeotic effects and modification of PEV have had very mixed results. We have no evidence for co-mapping of homeotic effects of Psc and Pd mutations and modification of PEV. Interestingly, a transformed line containing a cosmid that includes Pd\(^+\) (\( Pcos5.1 \), obtained from D. St. Johnston) acts as a Su(var), as expected if Pdd is a dosage-dependent modifier of PEV (Locke et al. 1988; unpublished results). Notwithstanding the observation that many chromosomes containing PcG mutations are E(var)s our current evidence does not permit the conclusion that mutations in PcG genes (except E(Pc) and Asx) modify PEV. Recently, Laibl et al. (1997) reported that E(z)\(^2\) is a weak Su(var), and that duplications of E(z) are E(var)s, based on modification of PEV, associated with \( w^\text{min} \). We cannot account for the differences between their results and ours, although different modifiers may have accumulated in our strain relative to theirs.

It was possible to co-map enhancement of PEV and Asx\(^1\), but as noted above, the data do not rule out the possibility that there is an E(var) closely linked to Asx\(^1\). If PEV arises from the spreading of heterochromatin into euchromatin, then one would expect PcG mutations to be Su(var)s if they work analogously to establish repressive target domains. This argument depends on the assumptions that PcG genes function only as repressors, and that mutations in PcG genes directly affect PEV. If these assumptions are incorrect, then Asx could be an E(var). The following observation provides support for the idea that Asx might be an E(var). The trithorax group (trx-G), which are required for activation of homeotic targets, are thought to act in opposition to PcG genes. Mutations in trx-G genes cause anterior transformations (Kennison 1993), and mutations in trithorax-like, which encodes the GAGA factor, are E(var)s (Farkas et al. 1994). Some Asx alleles show anterior as well as posterior transformations (Sinclair et al. 1992), suggesting that this locus may have a role in gene activation as well as repression. If so, Asx mutations could act as E(var)s, not Su(var)s.

Kennison (1995) has argued that PcG mutations do not suppress PEV. The finding that most PcG mutations do not suppress PEV suggests that the mechanisms underlying PEV and the function of the PcG might be different. Fauvarque and Duran (1993) showed that most modifiers of PEV do not affect variegation of the miniwhite gene in a P transposon containing ph regulatory sequences, whereas this variegation is affected by PcG mutations. Nevertheless, some modifiers of PEV do modify variegation of the miniwhite gene, although not always in the same direction as for modification of PEV, reminiscent of the results observed above with Asx. They suggested that variegation of the miniwhite construct differs from classical PEV, but that the two processes might share some common proteins. Consistent with the idea that different proteins can be used for different silencing processes, Wallrath and Elgin (1995) have shown that telomeric position effects are different from those associated with insertion into heterochromatin. Finally, Chan et al. (1994) have shown that Su(var)3-7 modifies expression from a reporter regulated by a PcG response element, but they suggest...
that this effect might be indirect. This problem of
deciding whether the genetic results are direct or indirect
will only be answered when the molecular mechanism of
PcG and PEV repression are understood.

Our results showing that most PcG mutations are
not suppressors of PEV suggest that there are not many
proteins required for PcG-mediated homeotic
gene repression and PEV, will help us understand how
these proteins function in these important processes.

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