Identification of *Polycomb* and *trithorax* Group Responsive Elements in the Regulatory Region of the Drosophila Homeotic Gene *Sex combs reduced*

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ABSTRACT

The Drosophila homeotic gene *Sex combs reduced* (*Scr*) is necessary for the establishment and maintenance of the morphological identity of the labial and prothoracic segments. In the early embryo, its expression pattern is established through the activity of several gap and segmentation gene products, as well as other transcription factors. Once established, the *Polycomb* group (Pc-G) and *trithorax* group (trx-G) gene products maintain the spatial pattern of *Scr* expression for the remainder of development. We report the identification of DNA fragments in the *Scr* regulatory region that may be important for its regulation by *Polycomb* and *trithorax* group gene products. When DNA fragments containing these regulatory sequences are subcloned into *P*-element vectors containing a *white* minigene, transformants containing these constructs exhibit mosaic patterns of pigmentation in the adult eye, indicating that *white* minigene expression is repressed in a clonally heritable manner. The size of pigmented and nonpigmented clones in the adult eye suggests that the event determining whether a cell in the eye anlagen will express *white* occurs at least as early as the first larval instar. The amount of *white* minigene repression is reduced in some *Polycomb* group mutants, whereas repression is enhanced in flies mutant for a subset of *trithorax* group loci. The repressor activity of one fragment, normally located in *Scr* Intron 2, is increased when it is able to homologously pair, a property consistent with genetic data suggesting that *Scr* exhibits transvection. Another *Scr* regulatory fragment, normally located 40 kb upstream of the *Scr* promoter, silences ectopic expression of an *Scr-lacZ* fusion gene in the embryo and does so in a *Polycomb*-dependent manner. We propose that the regulatory sequences located within these DNA fragments may normally mediate the regulation of *Scr* by proteins encoded by members of the *Polycomb* and *trithorax* group loci.

WILD-TYPE function and expression of the *Sex combs reduced* (*Scr*) locus is necessary for the specification of the segmental identity of the labial and prothoracic segments. Scr protein is first detected in portions of parasegment 2 at ~3:45 hr after egg deposition (AED) (LEMOTTE *et al.* 1989; MAHAFFEY *et al.* 1989). As development proceeds, Scr protein accumulates in the ventral and lateral ectoderm of the labial and prothoracic segments, with additional expression in the visceral mesoderm of the anterior and posterior midgut and the subesophageal ganglion of the ventral nerve cord (MAHAFFEY and KAUFMAN 1987; RILEY *et al.* 1987; CARROLL *et al.* 1988; LEMOTTE *et al.* 1989; TREMML and BIENZ 1989; REUTER and SCOTT 1990). During imaginal development, high levels of Scr protein are detected in the labial discs and prothoracic leg discs, with additional expression in other imaginal tissues (MAHAFFEY and KAUFMAN 1987; GLICKSMAN and BROWER 1988; PATTATUCCI and KAUFMAN 1991).

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The establishment of individual homeotic gene expression patterns during embryogenesis is primarily controlled by the gap and segmentation class genes (reviewed in INGHAM and MARTINEZ ARIAS 1992). In the absence of individual gap and segmentation loci, patterns of homeotic gene expression are altered such that expression in normal domains is reduced or, alternatively, transcription occurs outside of normal expression domains. Because the gap and segmentation gene products are expressed transiently during embryogenesis, they are unable to maintain the established patterns of homeotic gene expression necessary for proper segmental identity of the larva and adult. Genetic analysis has demonstrated that two broad groups of *trans*-acting factors encoded by the *Polycomb* group (Pc-G) and *trithorax* group (trx-G) genes are necessary for the maintenance of transcriptionally repressed and active states of homeotic gene expression (reviewed in PARO 1990; KENNISON 1993). The pattern of homeotic gene expression in individuals containing mutations in Pc-G or trx-G genes is initially normal, but later in development the pattern degenerates (INGHAM 1985; STRUHL and AKAM 1985). This degeneration appears to temporally coincide with a decrease in the levels of gap and seg-

mentation gene products (GAUL *et al.* 1987; TAUTZ 1988). *Polycomb* group mutations cause varying degrees of ectopic homeotic gene expression later in embryogenesis and during larval development, whereas *trithorax* group mutations cause a reduction of homeotic gene expression in their normal spatiotemporal domains.

A current model for the mechanism by which active and inactive states of homeotic gene expression are developmentally maintained is that the Pc-G and trx-G genes recognize "closed" and "open" chromatin conformations corresponding to transcriptional repression and activation, respectively (LOCKE *et al.* 1988; PARO 1990; KENNISON 1993). These chromatin conformations reflect the active and inactive transcriptional states of each homeotic gene, in an individual cell, at the time of gap and segmentation gene activity. Differences in the chromatin structure of transcriptionally silent closed domains or transcriptionally active open domains are somehow recognized by subsets of Pc-G and trx-G gene products. This event "imprints" the transcriptional state of each homeotic gene in every cell of the embryo in a clonally heritable manner. Recent molecular data suggest that Pc-G gene products form multimeric chromatin complexes by interacting with DNA in the *Antennapedia* complex (ANT-C) and *bithorax* complex (BX-C) (ZINK and PARO 1989; ZINK *et al.* 1991; FRANKE *et al.* 1992; RASTELLI *et al.* 1993).

The goal of this analysis was to gain a better understanding of how *Scr* is regulated by *trans*-acting factors during Drosophila development. By identifying *cis*-regulatory sequences necessary for normal *Scr* expression, the mechanism by which *trans*-acting factors, such as the Pc-G and trx-G gene products, interact with the *Scr* locus to control its global regulation can be further elucidated.

Previous analyses have suggested that the developmental regulation of *Scr* expression is complex, because *Scr* mutations map to an 80-kb interval that includes over 40 kb of upstream regulatory DNA (reviewed in KAUFMAN *et al.* 1990). An additional level of *Scr* regulatory complexity is its sensitivity to the degree of homologue pairing at the *Scr* locus. The chromosomes of Drosophila and other Dipterans are homologously paired in somatic tissues (METZ 1916). Disruption of chromosome pairing is usually of little consequence to the fly, but pairing-sensitive phenomena such as transvection (reviewed in WU 1993) demonstrate the significance of homologue pairing in certain genetic contexts. The penetrance and expressivity of *Scr* gain-of-function alleles are enhanced when homologue pairing at the *Scr* locus is disrupted by chromosomal rearrangements (PATTATUCCI and KAUFMAN 1991). Additionally, the level of ectopic *Scr* expression during imaginal development in $Pc^3/+$ larvae is enhanced when chromosome pairing at *Scr* is disrupted (PATTATUCCI 1991). Under normal conditions it appears that pairing-sensitive negative regulation of *Scr* is redundant, but its role in *Scr* regulation is revealed in situations that compromise the ability of Pc-G loci such as *Polycomb* to repress ectopic *Scr* expression.

To gain a better understanding of the molecular mechanisms controlling *Scr* expression, an attempt has been made to identify *cis*-regulatory sequences necessary for proper *Scr* regulation during embryonic and larval development. The genetically defined *Scr* regulatory region was subdivided into several overlapping DNA fragments ranging from 2 to 10 kb in length. These fragments were then subcloned into *P*-element vectors containing either an *hsp70-lacZ* or an *Scr-lacZ* fusion reporter gene, as well as a *white* minigene (PIRROTTA 1988) for transformant screening. A subset of DNA fragments from the *Scr* regulatory region were found to contain sequences that induce mosaic repression of *white* mingene expression in the adult eye, a tissue in which the *white* minigene is normally expressed in a uniform pattern. In some cases repression is enhanced when transformants are made homozygous, suggesting that these regulatory sequences are pairing-sensitive negative regulatory elements. The size and shape of pigmented and nonpigmented sectors in these adult eyes indicate that the time at which an individual cell makes a developmental decision regarding the transcriptional state of the *white* minigene is at least as early as first larval instar and is epigenetically inherited for the remainder of development. Genetic interactions suggest that *white* minigene repression observed in transformants is due to the interaction of a subset of Pc-G and trx-G gene products with *Scr* sequences present in the transformant construct. These results may help us understand the molecular mechanisms responsible for phenomena such as transvection, pairing-sensitive negative regulation and how patterns of homeotic gene expression are maintained by *Polycomb* and *trithorax* group gene products.

MATERIALS AND METHODS

Plasmid construction: The *P*-element vectors used in this analysis include P{w^{+m}, *hsp70:lacZ*=HZR} (HZR Reporter gene), P{w^{+m}, *Scr:lacZ*=SSRN} (Small *Scr* Reporter gene with Nuclear localization sequence), P{w^{+m}, *Scr:lacZ*=BSRN} (Big *Scr* Reporter gene with Nuclear localization sequence), as well as SSR and BSR, which are identical to SSRN and BSRN, respectively, but lack a nuclear localization sequence. Their construction is described in GINDHART *et al.* (1995). Genomic DNA fragments from the *Scr* regulatory region were subcloned into the unique *Not*I site 5′ of the *lacZ* fusion genes in HZR, SSRN and BSRN.

***P*-element mediated transformation:** Germline transformation of P{w^+} constructs was performed essentially as described in ROBERTSON *et al.* (1988). A solution of 0.5–1.0 mg ml^{-1} of each construct was injected into 0- to 45-min AED embryos resulting from the cross *w*P{ry^+,Δ2,3} females × *w*; *TM3/ TM6B* males. Embryos that survived microinjection

were allowed to develop at 24°, and the resulting G_0 adults were crossed to w^1. Transformants were isolated on the basis of the rescue of the *white* phenotype. Multiple transformant lines were isolated for each construct. The linkage of individual transformant lines was determined by the segregation of P{w^+} from dominant markers present on *SM5* and *TM3*. A subset of transformants was localized to salivary gland polytene chromosomes using standard techniques.

Immunohistochemistry: Embryo and imaginal disc staining were performed essentially as described in MAHAFFEY and KAUFMAN (1987) and PATTATUCCI and KAUFMAN (1991), respectively. Polyclonal rabbit antisera recognizing Scr protein were provided by MARIE MAZZULLA and used at a 1:150 dilution, whereas a monoclonal mouse antibody recognizing β-galactosidase was purchased from Boehringer-Mannheim, rehydrated in 500 μl H_2O and used at a 1:2000 dilution. Goat-anti-mouse IgG and goat-anti-rabbit IgG secondary antibodies coupled to horseradish peroxidase (Bio-Rad) were used at a 1:200 (mouse) or 1:150 dilution (rabbit). Diaminobenzidene was the chromogenic substrate used in all reactions and was used in the presence of 0.003% hydrogen peroxide.

Mutant strains: A complete list of *Polycomb* group and *trithorax* group mutants tested, along with references, is contained in Table 1.

Examination of eye pigmentation: Transformants containing *Scr* regulatory fragments linked to a *white* minigene were tested for interactions with *Pc*-G and *trx*-G loci by crossing 6–8 w^1P{w^+} virgin females to 10–12 males from balanced stocks containing Pc-G and trx-G mutant loci in half-pint milk bottles containing standard Drosophila medium supplemented with dry yeast. Flies were allowed to mate at 24° for 1 week and then the parents were discarded. The level and pattern of eye pigmentation in the progeny classes w^1P{w^+} mutant and w^1P{w^+}Balancer were compared. A minimum of 50 flies of each progeny class were collected at random in the 3 days after the commencement of eclosion. In some cases, mutant females were crossed to P{w^+} males, instances of which are noted in the text. Representative flies of the same sex and same age ±24 hr from the same culture were aged 3–5 days and then photographed at ×63 magnification. In most cases, at least two insertion lines of each construct were tested for genetic interactions.

Pigment assays were performed according to EPHRUSSI and HEROLD (1944) and HAZELRIGG *et al.* (1984), with some modifications. Male progeny of all four genotypic classes of the cross w^1, P{w^{+m}, *Scr:lacZ*=SSRN+8.2Xb}16D; +/+; Scm^{D1} *st in ri/TM3* × +/Y; *In(2R)Pcl*^{W4}*/SM5* and +/+ were aged 10 days and then frozen at −80°. Heads from 240 of each genotypic class were subdivided into 12 1.5-ml polypropylene tubes, with each tube containing 20 heads. The heads were homogenized with a Teflon pestle in 100 μl of AEA buffer (30% ethanol, 0.1% concentrated HCl). After homogenization, 900 μl of AEA buffer was added to each sample and the tubes were vortexed for 30 min. After vortexing, each sample was spun 10 min in a tabletop centrifuge at top speed. Twenty microliters of 0.5% hydrogen peroxide was added to the supernatant to oxidize extracted pigments. Each sample was then vortexed briefly (5 sec) and spun again. The mean OD_{480} of each genotype was estimated by computing the average optical density of the 12 samples tested. The absolute average for each genotype was computed by subtracting the optical density of a w^1 control from the computed averages. The 95% confidence interval for each estimated mean is the standard error multiplied by a factor of two.

Reporter gene expression: To determine the expression pattern of the *Scr-lacZ* reporter gene in the transformant line P{w^{+m}, *Scr:lacZ*=BSRN+10.0Xb}II in a Pc^3/Pc^3 mutant background, P{w^{+m}, *Scr:lacZ*=BSRN+10.0Xb}II flies were crossed to *w*; $Pc^3/TM3$ and then the resulting P{w^{+m}, *Scr:lacZ*=BSRN+10.0Xb}II; $Pc^3/+$ flies were backcrossed to *w*; $Pc^3/TM3$. Embryos 0–24 hr of age from this cross were collected and stained with anti-β-galactosidase. Similarly, the expression pattern of P{w^{+m}, *Scr:lacZ*=BSRN+10.0Xb}II in a ph^{503} mutant background was determined by crossing ph^{503}/*Binsinscy* females to P{w^{+m}, *Scr:lacZ*=BSRN+10.0Xb}II males, collecting 0- to 24-hr embryos and then staining the embryos with anti-β-galactosidase.

Recombination of *P*-element insert onto $In(1)m^{38c}$ and $In(1)y^4$ chromosomes: The SSRN+8.2Xb insert P{w^{+m}, *Scr:lacZ*=SSRN+8.2Xb}16A, an insert at 16A on the X chromosome, was recombined onto $In(1)m^{38c}$, *w m* (*In(1)10E1–2;13B*) and $In(1)y^4$, *y cv v f* (*In(1)1A8-B1;18A3–4*). $In(1)m^{38c}$, *w m* males were crossed to w^1 P{w^{+m}, *Scr:lacZ*=SSRN+8.2Xb}16A females. Heterozygous female progeny were backcrossed to $In(1)m^{38c}$, *w m* males, and *m* P{w^+} recombinants were selected. In the second experiment, $In(1)y^4$, *y cv v f* males were crossed to w^1 P{w^{+m}, *Scr:lacZ*=SSRN+8.2Xb}16A females, and heterozygous female progeny were crossed to *y w f* tester males. One *y w v f* P{w^+} chromosome was recovered out of 7500 chromosomes screened; upon further analysis it was shown that this chromosome had simultaneously recombined both w^1 and the *P*-element insert onto the $In(1)y^4$ chromosome.

RESULTS

***Scr* genomic DNA sequences repress *white* minigene expression:** The *white* minigene is used in *P*-element transformation vectors as a marker for germline transformants in a *white* background (PIRROTTA 1988). Ordinarily, mutations that remove function of the endogenous *white* gene are recessive, so the level of eye pigmentation in w^+/w females is similar to wild type. However, positive regulatory elements important for high levels of *white* expression in the adult eye are absent from the *white* minigene (LEVIS *et al.* 1985b). Therefore, the amount of eye pigmentation seen in *white* minigene transformants is proportional to the number of transgenic copies in the genome (PIRROTTA 1988). The level of eye pigmentation in P{w^+} transformants is also affected by sequences flanking the site of chromosome insertion (LEVIS *et al.* 1985a; HAZELRIGG and PETERSEN 1992), so that the eye color of transformants can vary from pale yellow to deep red. The function of the *white* gene product in pigment deposition is cell autonomous, making single cell differences in *white* gene expression readily detectable. The combination of gene dosage dependence, cell autonomy and an easily observable adult phenotype makes the *white* minigene an excellent reporter gene for assaying the function of both positive and negative regulatory sequences that act during larval and pupal development in the eye.

The *white* minigene present in the *P*-element constructs SSRN, BSRN and HZR (GINDHART *et al.* 1995) is uniformly expressed throughout the eye, and the quantity of eye pigmentation seen in transformants is

TABLE 1

Pc-G and trx-G alleles used in this study

	Alleles tested	Phenotype	References
Polycomb group genes			
Additional sex combs (Asx)	Asx^{D1}	Hypomorph	1
Enhancer of Polycomb (E(Pc))	*E(Pc)*	Amorph	2–4
Enhancer of zeste (E(z))	$E(z)^{S1}$	Antimorph	5–7
	$E(z)^{S6}$	Amorph	
extra sex combs (esc)	esc^{2}	Amorph	8–10
	esc^{21}	Amorph	
pleiohomeotic (ph)	pho^{1}	Amorph	3, 11–13
Polycomb (Pc)	Pc^{1}	Amorph	14, 15
	Pc^{3}	Antimorph	
Polycomblike (Pcl)	Pcl^{10}	Amorph	3, 13, 16
	Pcl^{W4}	Amorph	
polyhomeotic (ph)	ph^{503}	Amorph	17, 18
Posterior sex combs (Psc)	Psc^{IIN48}	Hypomorph	19, 20
Sex combs extra (Sce)	Sce^{D1}	Unknown	1
Sex combs on midleg (Scm)	Scm^{D1}	Amorph	1
super sex combs (sxc)	sxc^{1}	Amorph	21
trithorax group genes			
ash-1	$ash\text{-}1^{RF605}$	Amorph	22, 23
	$ash\text{-}1^{B1}$	Hypomorph	
ash-2	$ash\text{-}2^{1803}$	Hypomorph	22, 23
brahma (brm)	brm^{2}	Amorph	24
	brm^{5}	Hypomorph	
	brm^{20}	Hypomorph	
Brista (Ba)	$Df(2R)^{Ba}$	Amorph	25, 26
devenir (dev)	dev^{1}	Hypomorph	24
	dev^{2}	Hypomorph	
kismet (kis)	kis^{2}	Hypomorph	24
kohtalo (kto)	kto^{1}	Hypomorph	24
l(3)87Ca	$l(3)87Ca^{12}$	Hypomorph	24
moira (mor)	mor^{1}	Hypomorph	24
	mor^{2}	Hypomorph	
osa (osa)	osa^{1}	Hypomorph	24
	osa^{2}	Hypomorph	
sallimus (sam)	sam^{1}	Hypomorph	24
skuld (skd)	skd^{1}	Hypomorph	24
	skd^{2}	Hypomorph	
Su(Pc)37D	*Df(2L)JK12*	Amorph	24
trithorax (trx)	trx^{E2}	Amorph	27–29
	trx^{1}	Hypomorph	24, 30
	trx^{3}	Hypomorph	
urdur (urd)	urd^{2}	Hypomorph	24
verthandi (vtd)	vtd^{5}	Hypomorph	24

The Pc-G and trx-G mutant alleles used in this analysis, their phenotype relative to wild type and references are shown. The references listed are as follows: (1) BREEN and DUNCAN (1986); (2) RUSSELL and EBERLEIN (1979); (3) SATO *et al.* (1983); (4) SATO *et al.* (1984); (5) WU *et al.* (1989); (6) PHILLIPS and SHEARN (1990); (7) JONES and GELBART (1990); (8) STRUHL (1981); (9) STRUHL and BROWER (1982); (10) STRUHL (1983); (11) HOCHMAN *et al.* (1964); (12) GEHRING (1970); (13) DUNCAN (1982); (14) LEWIS (1978); (15) DUNCAN and LEWIS (1982); (16) KENNISON and RUSSELL (1987); (17) DURA *et al.* (1985); (18) DURA *et al.* (1987); (19) JURGENS (1985); (20) ADLER *et al.* (1989); (21) INGHAM (1984); (22) SHEARN *et al.* (1987); (23) SHEARN (1989); (24) KENNISON and TAMKUN (1988); (25) SUNKEL and WHITTLE (1987); (26) COHEN *et al.* (1989); (27) INGHAM and WHITTLE (1980); (28) INGHAM (1980); (29) INGHAM (1981); (30) INGHAM (1985).

dependent upon its number of copies in the genome. Thus, heterozygous transformants have more lightly pigmented eyes than homozygotes (compare the SSRN heterozygote (A) and homozygote (B) in Figure 2). DNA fragments from throughout the genetically defined *Scr* regulatory region were subcloned into SSRN, BSRN and HZR to identify and characterize sequences responsible for the developmental regulation of *Scr* ex-

TABLE 2

Eye phenotype of transformants containing *Scr* genomic DNA fragments

DNA fragment	No. of variegating transformant lines/total no. of lines	
	SSRN	HZR
SSR[a]	1/8	
BSR[b]	0/5	
6.8 kb *Xba*I	0/6	0/3
6.7 kb *Bam*HI	0/9	0/5
8.2 kb *Xba*I	7/10	1/8
5.4 kb *Bam*HI	3/5	0/5
3.0 kb *Eco*RI	0/8[c]	NT[d]
2.4 kb *Hin*dIII	0/4	NT[d]
3.0 kb *Xba*I	0/3	NT[d]
5.5 kb *Hin*dIII	4/8	0/4
6.5 kb *Kpn*I/*Sal*I	3/7	0/5
3.5 kb *Kpn*I/*Sal*I	0/8	0/5
5.6 kb *Hin*dIII	0/2	0/1
3.7 kb *Hin*dIII	0/4	0/3
7.0 kb *Eco*RI	1/7	0/3
7.6 kb *Hin*dIII	1/5	0/5
10.0 kb *Xba*I	3/3	3/5

Column 1 identifies each fragment tested by subcloning into SSRN and then generating transformants containing each construct [see GINDHART *et al.* (1995) for fragment position in the *Scr* regulatory region]. Column 2 shows the number of independent transformant lines exhibiting variegation of *white* minigene expression in the adult eye as heterozygote or homozygote divided by the total number of independent lines tested. Column 3 displays data in the same format as column 2 but the fragments are subcloned into HZR. Whenever possible, homozygotes were used, with the exception of homozygous lethal inserts and inserts on *TM3* or *TM6B*.

[a] Includes SSR and SSRN.

[b] Includes BSR and BSRN.

[c] Tested in BSR.

[d] Subcloned into HZ50PL, a ry^+ vector, instead of HZR, thus preventing the examination of eye phenotype.

pression. This analysis identified several *Scr cis*-regulatory elements distributed throughout the *Scr* locus (GINDHART *et al.* 1995). A serendipitous byproduct of this analysis was the discovery that some DNA fragments from the *Scr* regulatory region, when subcloned into SSRN or HZR, cause the *white* minigene present in these vectors to be expressed in a mosaic pattern of pigmented and nonpigmented eye tissue in adults (Table 2). For example, when an 8.2-kb *Xba*I fragment normally located in *Scr* intron 2 (Figure 1) is placed 7.5 kb upstream of the *white* promoter in SSRN, the *white* minigene is expressed in a mosaic pattern (Figure 2D). This suggests that the 8.2-kb *Xba*I fragment may contain sequences that repress *white* minigene expression. The size and shape of pigmented and nonpigmented eye sectors are similar in size and shape to mitotic clones induced by X-irradiation during the first larval instar (BAKER 1967; BECKER 1976). Although repression of the *white* minigene by 8.2 *Xba*I is enhanced when transformants are made homozygous (3 of 10 insertion lines; compare the heterozygote in Figure 2C with the homozygote in D), repression does not require homozygosity, as some insertion lines (4 of 10) exhibit mosaicism as heterozygotes (Figure 3D). The pattern of mosaicism seen is somewhat variable from fly to fly, as well as between eyes of the same fly. Pigmented sectors in homozygotes are darker than pigmented sectors in heterozygotes (compare Figure 2, C and D). In addition, the extent of mosaic *white* minigene repression in transformants appears to be independent of the orientation of the 8.2 *Xba*I fragment, suggesting that this effect is not due to the novel juxtaposition of vector and insert sequences (data not shown). A 5.4-kb *Bam*HI fragment that partially overlaps the 8.2 *Xba*I fragment also exhibits pairing-sensitive (2 of 5 inserts) repression of *white*, but, like the 8.2 *Xba*I fragment, pairing is not required, as one of five insertion lines shows variegation as a heterozygote. These results suggest that sequences in the 8.2 *Xba*I fragment repress *white* minigene expression early in development in a clonally heritable fashion but do not affect the dosage dependence of the *white* minigene in pigmented (nonrepressed) sectors. The eyes of transformants containing the 8.2 *Xba*I fragment subcloned into HZR have a patterned eye color (4 of 8 inserts), whereas transformants containing HZR alone do not. In HZR+8.2Xb transformants, the posterior 10–20% of the eye is darkly pigmented, whereas the anterior part of the eye is lighter in color (data not shown). This patterning does not appear to be enhanced when transformants are made homozygous. The 5.4 *Bam*HI fragment does not share eye patterning with the 8.2 *Xba*I fragment. Although one HZR+8.2Xb transformant line exhibits variegation, it is not enhanced when made homozygous and may be caused by the influence of nearby sequences, as this is the only insertion line of eight analyzed that variegates. Differences in the pattern of *white* minigene repression seen when the 8.2 *Xba*I fragment is subcloned into SSRN compared with HZR may reflect the fact that this fragment is 7.5 kb closer to the *white* promoter in HZR than in SSRN. Alternatively, the regulatory activity of sequences in the 8.2 *Xba*I fragment may require other sequences in SSRN, such as the *Scr* promoter region, that are absent from HZR.

Similar regulatory interactions were also observed when a 10.0-kb *Xba*I fragment from the *ftz-Antp* interval was subcloned just 5′ of the *white* promoter in HZR (Figures 1; 2, E and F, and also see Figure 3A). Transformants containing this construct exhibit eye pigment variegation in three of five insertion lines tested, demonstrating that sequences in the 10.0 *Xba*I fragment can repress expression of the *white* minigene in HZR. Repression of *white* minigene expression by the 10.0

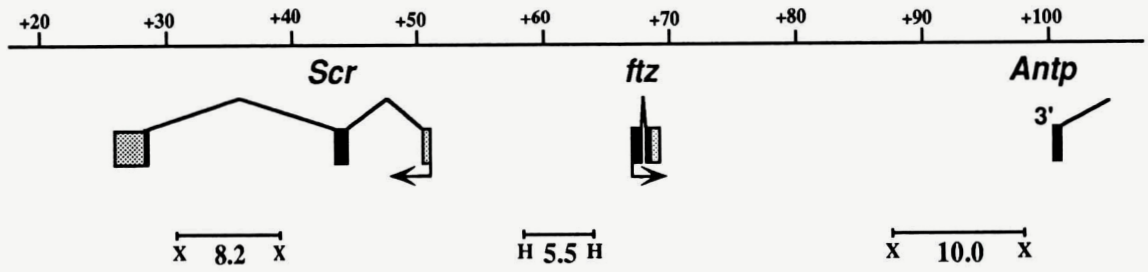

FIGURE 1.—Molecular organization of the *Scr* locus and location of genomic DNA fragments that heritably repress *white*. The coordinates at the top of the figure are in kilobases and are numbered according to SCOTT *et al.* (1983). *Scr* is transcribed from right to left; its exons are represented by three boxes. Filled boxes indicate coding regions and hatched boxes indicate noncoding transcripts. The *ftz* transcription unit is located 15 kb 5′ of the *Scr* transcript and is transcribed from left to right. The 3′ end of the *Antp* transcript is shown at right. It is located 50 kb 5′ of the *Scr* transcript. Below the molecular map is the location of three genomic DNA fragments that heritably repress *white* minigene expression. The number above each line indicates the size of that fragment in kilobases. The letters below each line indicate the restriction sites that define each fragment. X, *Xba*I; H, *Hin*dIII. The positions of other *Scr* genomic DNA fragments, as well as maps of constructs used in this analysis, are shown in GINDHART *et al.* (1995).

*Xba*I fragment occurs when transformants are heterozygous or homozygous. Unlike the 8.2 *Xba*I fragment, repression by the 10.0 *Xba*I fragment is not enhanced when transformants are made homozygous. Differences observed between heterozygous and homozygous HZR+10.0Xb transformants are due to a dosage-dependent increase in the pigmentation of *white*$^+$ sectors, whereas the pigment level of *white* sectors remains low (compare Figure 2, E and F; data not shown). In addition, sequences in the 10.0 *Xba*I fragment also repress the *white* minigene in SSRN in a pairing-independent manner in three of three insertion lines tested (data not shown).

Figure 1 shows the position of a third DNA fragment that appears to repress the *white* minigene in SSRN. This 5.5-kb *Hin*dIII fragment is located ~9 kb 5′ of *Scr* in the region between the *Scr* and *ftz* transcription units. Mosaic patterns of repression of *white* transcription by sequences in the 5.5 *Hin*dIII fragment is pairing dependent, because all eight transformant lines containing SSRN+5.5H have uniform eye pigmentation as heterozygotes (Figure 2, G and H). Like the 8.2 and 10.0 *Xba*I fragments, the level of *white* minigene repression by 5.5 *Hin*dIII varies among independent transformant lines. For example, some SSRN+5.5H transformant lines show almost complete repression of the *white* minigene

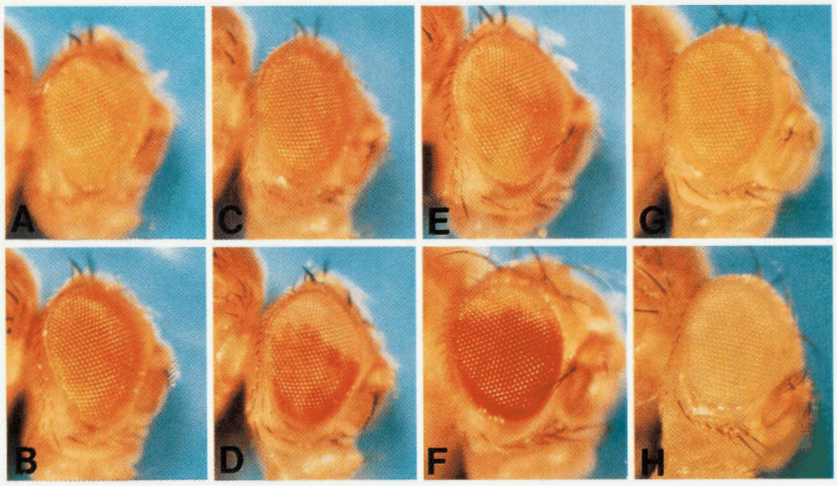

FIGURE 2.—Genomic DNA fragments from the genetically defined *Scr* regulatory region clonally represses *white* minigene expression. (A and B) Eye pigmentation of SSRN transformants is uniform throughout the eye and is sensitive to *white* minigene dosage, as heterozygotes (A) have lighter eyes than homozygotes (B). (C and D) When an 8.2-kb *Xba*I fragment from the *Scr* regulatory region is subcloned 7.5 kb 5′ of the *white* promoter in SSRN, the eyes of heterozygotes are uniformly pigmented (C), but homozygotes have mosaic eyes composed of pigmented and nonpigmented sectors (D). Note that in this autosomal insertion line, pigmented sectors of homozygotes are darker than heterozygotes. (E and F) When a 10.0-kb *Xba*I fragment from the *Scr* regulatory region is placed next to the *white* minigene in HZ-white, mosaic repression of *white* is seen in heterozygotes (E) and homozygotes (F). (G and H) When a 5.5-kb *Hin*dIII fragment from the region between the *ftz* and *Scr* promoters is subcloned into SSRN, it causes *white* to be expressed in a mosaic pattern in homozygotes (H) but not in heterozygotes (G).

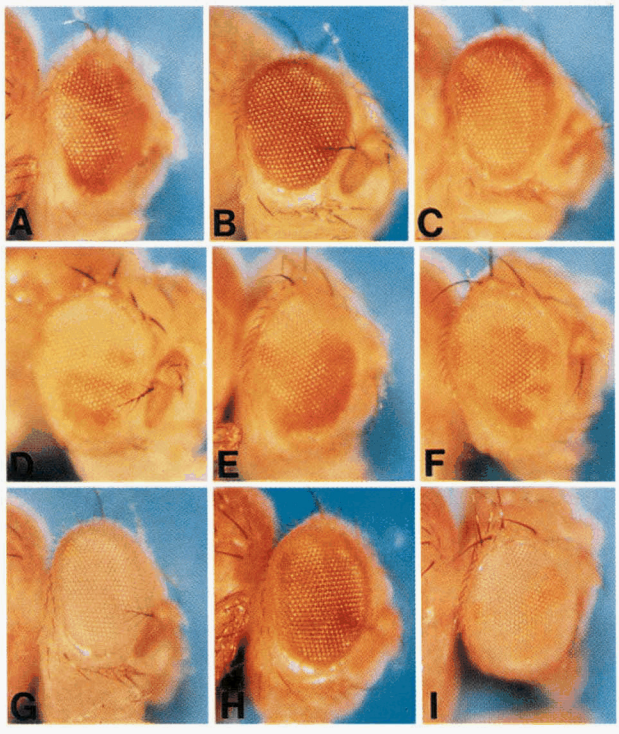

FIGURE 3.— *Polycomb* and *trithorax* group mutations modify the repression of *white* by sequences in the 10.0 and 8.2 *Xba*I fragments. Males from balanced stocks containing mutations in individual Pc-G or trx-G loci were crossed to female HZR+10.0Xb or SSRN+8.2Xb transformants, and the eye phenotypes of wild type and mutant male progeny were compared. Although there is some overlap in the phenotype of wild-type and mutant transformants, the distribution of pigment levels is shifted significantly when the dosage of Pc-G or trx-G loci is altered. (A) A representative HZR+10.0Xb heterozygote in a wild-type background. (B) The phenotype of the same transformant line in a heterozygous $E(z)^{S1}$ background. (C) The level of pigmentation observed when this HZR+10.0Xb transformant line is in a dev^{1}/+ background. (d) A typical expression pattern of *white* in SSRN+8.2Xb transformants. In a Pc-G^{+} background, ~25–50% of the ommatidia are pigmented in heterozygotes, and this pigmentation is usually restricted to the ventral part of the eye. (E and F) The effect on SSRN+8.2Xb transformants in Pcl^{W4}/+ and Scm^{D1}/+ backgrounds, respectively. (G) The level of pigmentation observed when this transformant line is in a brm^{2}/+ background. The amount of pigmentation in this mutant is greatly reduced relative to that observed in a trx-G^{+} background. (H) Lowering the dosage of both *Pcl* and *Scm* results in almost complete suppression of 8.2 *Xba*I-mediated repression of *white* as *Pcl* and *Scm* interact with the 8.2 *Xba*I fragment in an additive manner. Whereas brm^{2}/+ flies (G) have fewer pigmented ommatidia than controls (D) and Pcl^{W4}/+ (E) flies have more eye pigmentation than control flies, the Pcl^{W4}/+;brm^{2}/+ flies (I) have an intermediate phenotype that resembles the control (D).

as a homozygote, whereas other transformant lines exhibit weak mosaicism when homozygous (data not shown). Similar results are obtained when a 6.5-kb *Kpn*I / *Sal*I fragment that overlaps the 5.5 *Hin*dIII fragment was subcloned into SSRN, as three of seven insertion lines exhibit *white* repression in a pairing-sensitive manner. Repression of the *white* promoter by sequences in the 5.5 *Hin*dIII fragment does not occur when this fragment, or the 6.5 *Kpn*I / *Sal*I fragment, is subcloned into HZR. The lack of *white* repression by the 5.5 *Hin*dIII fragment in this context may be caused by moving this fragment to just upstream of the *white* promoter in HZR, whereas this fragment is 7.5 kb upstream of the *white* promoter in BSRN. Alternatively, this result may reflect a need for additional *Scr* sequences absent from HZR but present in SSRN. These results suggest that sequences in the 5.5 *Hin*dIII fragment can heritably repress *white* minigene expression, but only when transgenes are made homozygous, and that the amount of repression observed is influenced by chromosomal sequences surrounding the site of *P*-element insertion.

Mutations in *Polycomb* and *trithorax* group genes modify *white* minigene repression by the 10.0 and 8.2 *Xba*I *Scr* regulatory fragments: The phenotype of transformants containing a *white* minigene linked to DNA fragments from the *Scr* locus suggests that regulatory sequences in the 8.2 *Xba*I, 10.0 *Xba*I and 5.5 *Hin*dIII fragments repress *white* transcription in a clonally heritable manner and that the decision determining whether a progenitor cell will express the *white* minigene occurs early in development. Is there a link between the maintenance of *white* transcriptional repression by these regulatory sequences and the maintenance of regulated *Scr* expression by *Polycomb* and *trithorax* group gene products? To test this possibility, transformants containing the appropriate constructs were crossed to flies mutant for individual Pc-G or trx-G loci (Table 3). Reducing the zygotic dosage of certain Pc-G loci appears to suppress *white* transcriptional repression by sequences in the 10.0 and the 8.2 *Xba*I fragments, that is, certain Pc-G mutants cause the eyes of transformant flies to be more fully pigmented. For example, lowering the dosage of the Pc-G gene *E(z)* in HZR+10.0Xb transformants (Figure 3B) results in an increase in the amount of eye pigmentation relative to wild type (Figure 3A). The increase in pigmentation in mutants is caused by an increase in the number of pigmented ommatidia and not an increase in pigment levels of individual ommatidia. The 8.2 *Xba*I fragment interacts with Pc-G loci in a similar manner. Figure 3, E and F shows that the Pc-G mutations *Pcl* and *Scm* both interact genetically with the 8.2 *Xba*I fragment, as these mutants suppress *white* transcriptional repression by 8.2 *Xba*I normally observed in a wild-type background (Figure 3D). Table 3 shows that the 10.0 *Xba*I fragment interacts genetically with *E(z)*, *Pc*, *Pcl*, *Psc*, *Scm* and *ph*, whereas sequences in the 8.2 *Xba*I fragment interact with *Pc*, *Pcl*, *ph*, *esc* and *Scm*. The effect of these mutations on *white* minigene expression parallels ectopic *Scr* expression observed in Pc-G mutants.

TABLE 3

Genetic interactions of Pc-G and trx-G loci with sequences in 8.2 and 10.0 *Xba*I

Mutant genotype	Regulatory element[a] 8.2 *Xba*I	10.0 *Xba*I
Polycomb group		
Additional sex combs (*Asx*)	0	0
Enhancer of Polycomb (*E(Pc)*)	0	0
Enhancer of zeste (*E(z)*)	0	+[b]
extra sex combs (*esc*)	+[c]	0
pleiohomeotic (*pho*)	0	0
Polycomb (*Pc*)	+[d]	+
Polycomblike (*Pcl*)	+	+
polyhomeotic (*ph*)	+	+
Posterior sex combs (*Psc*)	0	+
Sex combs extra (*Sce*)	0	NT
Sex combs on midleg (*Scm*)	+	+
super sex combs (*sxc*)	0	0
trithorax group		
ash-1	–	–
ash-2	0	0
brahma (*brm*)	–	–[b]
Brista (*Ba*)	0	0
devenir (*dev*)	0	–
kismet (*kis*)	0	0
kohtalo (*kto*)	0	0
l(3)87Ca	0	0
moira (*mor*)	–	0
osa (*osa*)	–	0
sallimus (*sam*)	0	0
skuld (*skd*)	+	0
su(Pc)37D	+	0
trithorax (*trx*)	–	–
urdur (*urd*)	0	0
verthandi (*vtd*)	0	0

+, mutations at this locus increase the amount of eye pigmentation (suppression of *white* minigene repression); –, less eye pigmentation relative to wild type (enhancement of *white* minigene repression); 0, no effect; NT, not tested.

[a] the *P*-element constructs tested were SSRN+8.2Xb and HZR+10.0Xb.

[b] (allele-specific) denotes that some alleles genetically interact with this fragment whereas others do not.

[c] (maternal effect) denotes that *esc* only suppresses *white* minigene repression when inherited from the mother. The maternal effect of *esc* on the 10.0 *Xba*I fragment has not been tested.

[d] (insert-specific) denotes that *Pc* mutations interact with one 8.2 *Xba*I insert line but not another.

However, not all Pc-G mutants modify the mosaic repression of *white* transcription by the 10.0 or the 8.2 *Xba*I fragment (Table 3). These results suggest that sequences in the 10.0 and 8.2 *Xba*I fragments interact directly or indirectly with a subset of Pc-G loci to clonally repress *white* minigene expression and that these sequences may normally mediate the interaction of Pc-G *trans*-acting factors with the *Scr* locus. Initial experiments with the 5.5 *Hin*dIII fragment have shown that its mosaic repression of *white* transcription in homozygotes is unaffected by *Pc* or *E(z)* mutations (data not shown). Based on these results, the 5.5 *Hin*dIII fragment will not be the subject of further discussion.

To test the effect of altering the dosage of trx-G loci on mosaicism of *white* minigene expression in 10.0 and 8.2 *Xba*I constructs, transformants were crossed to flies heterozygous for individual trx-G loci and then the patterns of *white* mosaicism in trx-G$^+$ and trx-G mutant progeny were compared. Figure 3 and Table 3 show that a subset of trx-G mutations enhance the repression of *white* minigene expression in HZR+10.0Xb and SSRN+8.2Xb transformants. Lowering the dosage of some trx-G loci causes fewer ommatidia in the eye to be pigmented, suggesting that the formation or maintenance of pigmented sectors in these transformants is dependent upon trx-G gene function. For example, the trx-G mutation *dev* causes the eyes of HZR+10.0Xb transformants to be less pigmented (Figure 3C) than wild type (Figure 3A). Similarly, a reduction in the dosage of the trx-G gene *brm* enhances 8.2 *Xba*I-mediated repression of *white* (Figure 3G) relative to wild type (Figure 3D). Table 3 shows that the trx-G loci *ash-1*, *brm*, *dev* and *trx* interact genetically with the 10.0 *Xba*I fragment, whereas the repressor activity of the 8.2 *Xba*I fragment is enhanced by mutations in the trx-G loci *ash-1*, *brm*, *mor*, *osa* and *trx*. The modification of *white* mosaicism in a subset of trx-G mutants parallels changes in *Scr* expression observed in these mutants (TAMKUN *et al.* 1992; BREEN and HARTE 1993). Interestingly, mutations in the trx-G loci *Su(Pc)37D* and *skuld* (*skd*) suppress rather than enhance repression of the *white* minigene by the 8.2 *Xba*I fragment (Table 3). This effect is not allele specific, because both *skd*^{1}, an EMS-induced mutation, and *skd*^{2}, a gamma-ray-induced *skd* allele (KENNISON and TAMKUN 1988), exhibit similar effects on 8.2 *Xba*I-mediated repression of *white* expression. Allelism tests could not be performed with *Su(Pc)37D*, because only one allele exists (KENNISON and TAMKUN 1988). The genetic interaction of a subset of trx-G loci with the 10.0 and 8.2 *Xba*I fragments suggests that the gene products encoded by these loci interact directly or indirectly with sequences in these fragments to maintain transcriptionally active states of *white* minigene expression and perhaps act through these sequences to maintain *Scr* expression in its normal domain.

Pc-G mutations modify *white* transcriptional repression by the 8.2 *Xba*I fragment in an additive manner, but Pc-G and trx-G mutations mutually suppress one another: One of the criteria used to classify a gene as a member of the *Polycomb* group or *trithorax* group is that a mutation in one locus enhances the phenotype of other loci in the same group, whereas mutations in one group suppress the phenotype of mutations in the

TABLE 4

Quantitation of eye pigment in SSRN+8.2Xb transformants in wild-type and Pc-G mutant backgrounds

Genotype	OD_{480}	Mutant/wild-type pigment ratio
$w^1/+$	1.22 ± 0.05	
w^1/Y	0.023 ± 0.001	
$P\{w^{+m}\};+/+;+/+$	0.087 ± 0.009	1
$P\{w^{+m}\};Pcl^{W4}/+;+/+$	0.118 ± 0.009	1.76
$P\{w^{+m}\};+/+;Scm^{D1}/+$	0.115 ± 0.006	1.70
$P\{w^{+m}\};Pcl^{W4}/+;Scm^{D1}/+$	0.180 ± 0.010	2.85

Each data point in the OD_{480} column represents the average amount of red eye pigment in 12 samples of 20 fly heads. The mutant/wild-type pigment ratio was computed by subtracting the OD_{480} of the w^1/Y control from the OD_{480} of transformants in Pc-G^+ and mutant backgrounds and then dividing this number by the OD_{480} of Pc-G^+ transformants.

other group (reviewed in PARO 1990; KENNISON 1993). To determine whether similar genetic interactions could be observed in the eyes of transformants containing SSRN+8.2Xb, transformants containing single or pairwise combinations of Pc-G and trx-G mutations were generated, and the level of 8.2 *Xba*I-mediated *white* repression in wild type, single mutants and double mutants were compared. Whereas *Pcl*/+ (Figure 3E) or *Scm*/+ (Figure 3F) mutants have more fully pigmented eyes than Pc-G^+ controls, the eyes of *Pcl*/+; *Scm*/+ double heterozygotes (Figure 3H) are almost fully pigmented. The combination of *ph* and *Scm* mutations exhibited similar effects, that is, the double mutations suppressed the regulatory activity of 8.2 *Xba*I more than either single mutation (data not shown). The extraction of red eye pigments from SSRN+8.2Xb transformants shows that *Pcl* and *Scm* mutations individually cause a statistically significant increase in pigment levels relative to Pc-G+ controls and that *Pcl*, *Scm* double heterozygotes have significantly more red eye pigments than either individual heterozygote (Table 4). To determine whether the effects of Pc-G mutations on 8.2 *Xba*I-mediated *white* minigene repression could be suppressed by trx-G mutations, the level of *white* minigene repression in *Pcl*/+ and *brm*/+ heterozygotes was compared with the level observed in *Pcl*/+; *brm*/+ double heterozygotes. The *brm* mutation enhances *white* minigene repression by 8.2 *Xba*I sequences (Figure 3G), whereas *Pcl* suppresses it (Figure 3E). *Pcl*/+; *brm*/+ double heterozygotes (Figure 3I) have intermediate levels of eye pigmentation that are similar to $P\{w^+\}$ controls (Figure 3D); therefore, the enhancement of *white* minigene repression in *brm* mutants appears to be offset by the suppression observed in *Pcl* mutants. These results demonstrate that dosage-dependent genetic interactions observed among Pc-G and trx-G loci, which cause predictable changes in *Scr* expression, modulate the expression of a *white* minigene linked to an 8.2 *Xba*I fragment from the *Scr* regulatory region in a similar manner.

Pc-G and trx-G mutations interact with pairing-sensitive SSRN+8.2Xb transformant lines: Experiments described thus far demonstrating the interaction of Pc-G and trx-G loci with *Scr* regulatory sequences in the 8.2 *Xba*I fragment have used transformant lines that exhibit mosaic patterns of *white* minigene repression when the *P*-element insert is heterozygous. However, a subset of SSRN+8.2Xb transformant lines displays mosaic *white* minigene repression only when the insert is homozygous. Do these lines interact with Pc-G and trx-G mutations in the same way as lines that have non-pairing-sensitive inserts? To answer this question, the eye pigmentation phenotype of two pairing-sensitive SSRN+8.2Xb transformant lines were compared as heterozygotes and homozygotes in both wild-type and Pc-G or trx-G mutant backgrounds. When a pairing-sensitive SSRN+8.2Xb insertion line was crossed to flies mutant for the Pc-G gene *Pcl*, both *Pcl*$^+$ and *Pcl* heterozygous transformants had fully pigmented eyes (Figure 4, A and C); however, in homozygous transformants *Pcl*/+ suppresses *white* minigene repression by the 8.2 *Xba*I fragment (compare Figure 4, B and D). Similar results were obtained using mutant alleles of *Pc* and *Scm* (not shown), suggesting that pairing-sensitive SSRN+8.2Xb insert lines interact with Pc-G mutations in the same way as non-pairing-sensitive lines, but suppression by Pc-G mutations is observable only when the pairing-sensitive insert is homozygous. An analogous result was obtained when a pairing-sensitive SSRN+8.2Xb insertion line was made doubly heterozygous for the trx-G mutations *brm* and *trx*, which greatly enhanced 8.2 *Xba*I-mediated repression of *white*, as expected. However, this effect is only observed when the insert is homozygous (compare Figure 4, E and F, with G and H). Similar genetic interactions were also observed in *brm* and *trx* single mutants, as well as in *ash-1*, *mor* and *osa* mutants (data not shown). Collectively, these results suggest that the pairing-sensitive repression of *white* by the 8.2 *Xba*I fragment is Pc-G dependent and that the relative dosage of Pc-G and trx-G gene products influence the amount of repression observed.

Pairing-sensitive SSRN+8.2Xb transformant lines obey the rules of transvection: Transvection is manifested as partial or complete interallelic complementation observed only when the alleles are able to pair (recently reviewed in WU 1993). Transvection phenomena are probably caused by the ability of enhancer sequences on one homologue to interact with promoter sequences located on the other homologue (GEYER *et al.* 1990). Previous genetic results suggest that, in certain contexts, homologue pairing influences both positive and negative regulation at the *Scr* locus (HAZELRIGG and KAUFMAN 1983; PATTATUCCI and KAUFMAN 1991).

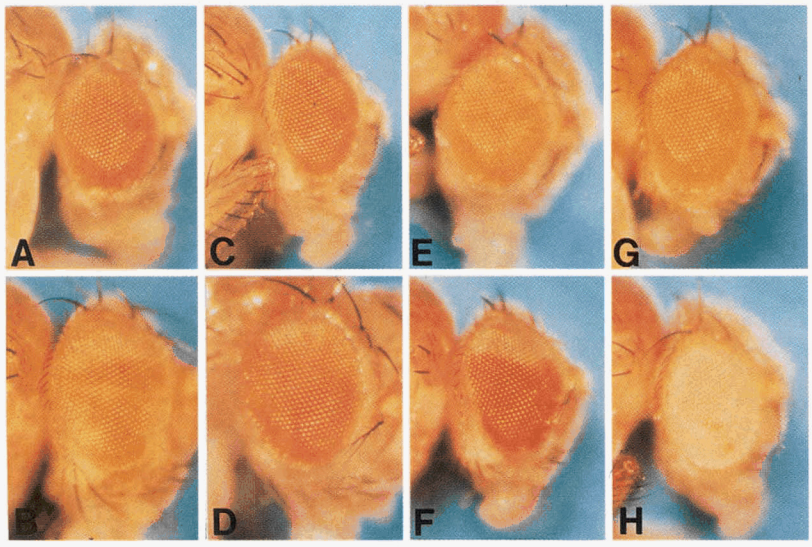

FIGURE 4.—Pc-G and trx-G loci also interact with a pairing-sensitive 8.2 *Xba*I transformant line. (A and B) The eye pigmentation phenotype of an X-linked pairing-sensitive SSRN+8.2Xb transformant line in heterozygous and homozygous females, respectively. Occasionally weak mosaicism is observed in heterozygotes, but it is greatly enhanced in homozygotes. (C and D) The eye phenotype as a heterozygote (C) or a homozygote (D) in a $Pcl^{W4}/+$ background. Note that there is no obvious difference between Pcl^{+} (A) and $Pcl^{W4}/+$ (C) in heterozygotes. In homozygous transformants, $Pcl^{W4}/+$ (D) suppresses the *white* mosaicism observed in a Pcl^{+} background (B). This suppression causes the homozygote to more fully resemble the heterozygote, in which little or no repression is seen. (E–H) The pattern of *white* mosaicism observed in heterozygous (E) and homozygous (F) transformants for an autosomal SSRN+8.2Xb inset line is shown. When this transformant line is crossed into a brm^{2} $trx^{E2}/++$ background, the eye pigmentation of heterozygotes (G) is unaffected relative to brm^{+} trx^{+}, whereas 8.2 *Xba*I-mediated repression of the *white* minigene (F) is greatly enhanced in homozygotes (H) mutant for *brm* and *trx*.

To test the hypothesis that pairing-sensitive regulatory sequences in the 8.2 *Xba*I fragment may influence observed transvection phenomena at *Scr*, a pairing-sensitive SSRN+8.2Xb insert line at chromosomal location 16A was recombined onto two inversion chromosomes: $In(1)m^{38c}$ (*In(1)10E1–2; 13B*) and $In(1)y^{4}$ (*In(1)1A8-B1; 18A3–4*). Both inversion breakpoints of $In(1)m^{38c}$ are distal to 16A with respect to the centromere and therefore should not disrupt pairing between an insert on the inversion chromosome and an insert on a cytologically normal chromosome. In contrast, $In(1)y^{4}$ has an inversion breakpoint between the insert at 16A and the centromere and therefore should disrupt homologous pairing at the site of *P*-element insertion. A comparison of the eye phenotype of homozygous transformants in which the insert is paired or unpaired is shown in Figure 5. The eye phenotype of $In(1)m^{38c}$, P{w^{+}}/$In(1)m^{38c}$, P{w^{+}} (Figure 5A) and $In(1)m^{38c}$, P{w^{+}}/P{w^{+}} (Figure 5B) are similar, which is consistent with the assumption that this inversion does not disrupt chromosome pairing at 16A, the site of *P*-element insertion. However, the level of eye pigmentation of $In(1)y^{4}$, P{w^{+}}/P{w^{+}} heterozygotes (Figure 5D) is much greater than $In(1)y^{4}$, P{w^{+}}/$In(1)y^{4}$, P{w^{+}} homozygotes (Figure 5C). Therefore, pairing-sensitive 8.2 *Xba*I-mediated repression of *white* minigene expression is suppressed when chromosome pairing is disrupted.

Sequences in the 10.0 *Xba*I fragment repress ectopic *Scr* reporter gene expression in a Pc-G gene-dependent manner during embryogenesis: The gene products encoded by Pc-G and trx-G loci are necessary for maintaining the patterns of homeotic gene expression established by gap and segmentation gene products (reviewed in PARO 1990; KENNISON 1993). For example, the accumulation pattern of *Scr* protein in Pc^{-} embryos is initially normal, but ectopic *Scr* protein accumulation can be detected both anterior and posterior to its normal domain before the end of germ-band extension (RILEY *et al.* 1987; MCKEON and BROCK 1991) (Figure 6, A and B). *Scr* regulatory sequences in the 10.0 and 8.2 *Xba*I fragments repress *white* minigene expression in the eyes of transformants containing the appropriate constructs, and a subset of the Pc-G and trx-G gene products are able to modify this phenomenon. However, these results do not demonstrate that sequences in the 10.0 and 8.2 *Xba*I are important for the spatiotemporal regulation of *Scr*. To test the hypothesis that these fragments contain *Scr* regulatory sequences, they were subcloned into BSRN, transformants were generated and the expression patterns of these constructs were compared with BSRN expression. The *Scr-lacZ* reporter gene in BSRN is expressed in a broad domain from the procephalon to A8, with highest levels of expression in the thorax (Figure 6, C and D). This broad domain of reporter gene expression is similar to Scr accumulation in Pc^{3} mutant embryos

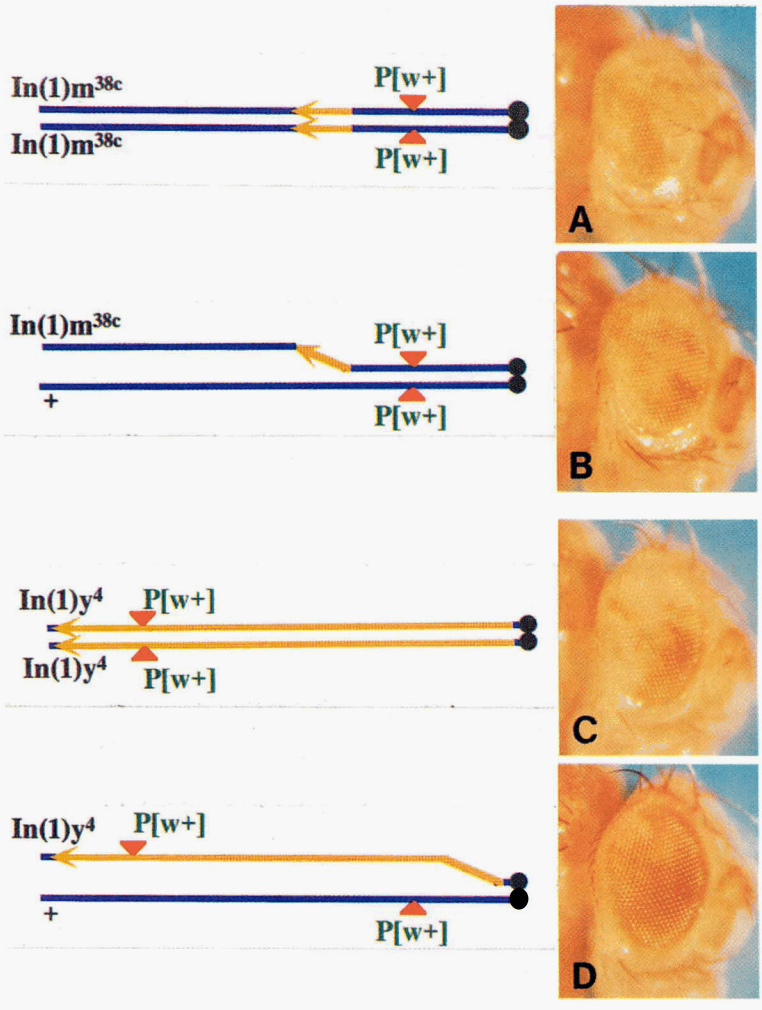

FIGURE 5.—Disruption of homologue pairing suppresses 8.2 *Xba*I-mediated repression of *white*. The lines on the left represent the genotype of each fly shown to the right. Blue lines indicate normal sequences, whereas yellow arrows indicate inverted sequences. P{w^+} shows the location of the pairing-sensitive SSRN+8.2Xb insert at 16A. (A and B) Structural heterogeneity distal of the insert at 16A has no effect on *white* minigene repression, as the eye pigmentation of $In(1)m^{38c}$ homozygotes (A) is similar to $In(1)m^{38c}$ heterozygotes (B). When $In(1)y^4$ is homozygous (C), the 8.2 *Xba*I fragment-containing insert is paired and a moderate level of *white* minigene repression is observed. If chromosome pairing is disrupted by structural heterogeneity proximal of 16A (D), 8.2 *Xba*I-mediated repression of *white* transcription is suppressed, resulting in more fully pigmented eyes. This effect is similar to the suppression seen in Pc-G mutants (Figure 4).

(Figure 6, A and B). This suggests that the *Scr-lacZ* fusion gene in BSRN contains enhancers that direct reporter gene expression in a broad domain but is missing negative regulatory elements that normally repress ectopic *Scr* activation. However, when the 10.0 *Xba*I fragment is inserted into BSRN 2.3 kb 5′ of the Scr promoter, ectopic reporter gene expression is turned off posterior of T1 and anterior of the labial segment (Figure 6, E and F). This result suggests that regulatory sequences in the 10.0 *Xba*I fragment repress transcriptional activation of the *Scr* promoter outside of its normal expression domain. To determine what role, if any, Pc-G gene products have in transcriptional repression of the *Scr* promoter by the 10.0 *Xba*I fragment, the pattern of reporter gene expression was assayed in embryos homozygous for *Polycomb* or *polyhomeotic* mutations. The *Scr-lacZ* fusion gene is ectopically expressed in these embryos (Figure 6, G–J). Reporter gene expression is initially normal, but by stage 12 ectopic expression is seen along the A-P axis from the procephalon to A8. The derepressed pattern of reporter gene expression is similar to the pattern seen in transformants containing an *Scr-lacZ* fusion that lacks the 10.0 *Xba*I fragment (Figure 6, C and D). These results suggest that the 10.0 *Xba*I fragment contains regulatory sequences that mediate the initial establishment of a posterior boundary of *Scr-lacZ* expression in T1, as well as sequences that maintain this boundary in a *Pc*- and *ph*- dependent manner. However, sequences in the 10.0 *Xba*I fragment are not sufficient for truncation of the broad pattern of BSRN reporter gene expression during larval development (data not shown). To determine whether the 8.2 *Xba*I fragment also contains embryonic regulatory sequences, this fragment was subcloned 2.3 kb 5′ of the *Scr-lacZ* fusion gene in BSRN, and reporter gene expression was assayed in transformant embryos. The 8.2 *Xba*I fragment had no effect on the expression of the *Scr-lacZ* fusion gene (data not shown). Therefore, the 8.2 *Xba*I fragment does not appear to contain sequences sufficient for repression of the *Scr* promoter outside of its normal expression domain.

DISCUSSION

The homeotic gene *Scr* is developmentally regulated in two distinct phases: the establishment of its expression pattern during embryogenesis by the gap and seg-

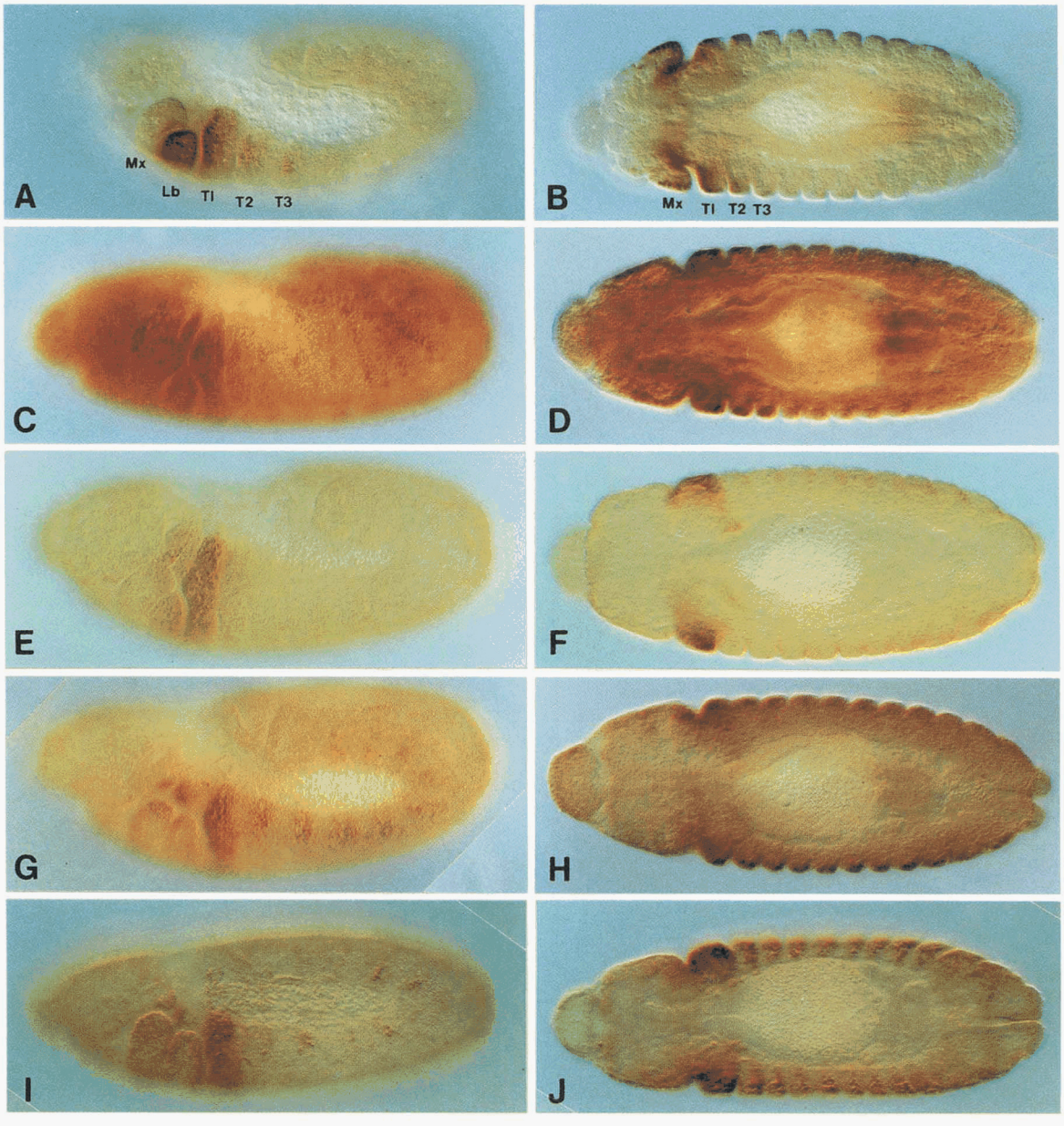

FIGURE 6.—Sequences in the 10.0 *Xba*I fragment repress ectopic *Scr* reporter gene expression during embryogenesis in a Pc-G-dependent manner. All embryos in this figure were stained with antibodies recognizing β-galactosidase, with the exception of A and B, which were stained with polyclonal Scr antisera. The left column of embryos are sagittal views of stage 12 embryos (7:20–9:20 hr AED), whereas the right column of embryos are horizontal views of stage 14 embryos (10:20–11:20 hr AED) (CAMPOS-ORTEGA and HARTENSTEIN 1985). The anterior end of all embryos is to the left. (A and B) *Polycomb*⁻ embryos exhibit ectopic *Scr* expression in the mesothoracic (T2) and metathoracic (T3) segments at stage 12 of embryogenesis, with additional ectopic expression in the maxillary (Mx) segment at stage 14. *Pc*- embryos also express *Scr* in its normal domain, the labial (Lb) and prothoracic (T1) segments. (C and D) The expression pattern of the *Scr-lacZ* fusion gene in the *P*-element vector BSRN. At both stage 12 and 14, β-galactosidase accumulation is detected from anterior of the maxillary segment to the posterior abdominal segments. High background levels are due to low-level ubiquitous expression of the insertion line pictured here. (E and F) When the 10.0 *Xba*I fragment is subcloned into BSRN, the expression pattern of this new construct, BSRN+10.0Xb, is dramatically different than the original construct pictured in C and D. Ectopic reporter gene expression in the head and posterior of T1 appears to be blocked by sequences in the 10.0 *Xba*I fragment. The restricted pattern of reporter gene expression observed in BSRN+10.0Xb transformants becomes derepressed when this construct is crossed into a ph^- (G and H) or Pc^- (I and J) background, in which β-galactosidase is detected throughout the ectoderm in a pattern similar to the reporter construct lacking the 10.0 *Xba*I fragment (C and D).

mentation gene products and the maintenance of its expression pattern for the rest of development by the *Polycomb* and *trithorax* groups of gene products. The establishment of *Scr* expression is dependent on a developmental cascade that transforms broad gradients of maternally supplied morphogens into precise patterns of periodic expression that define parasegmental boundaries (reviewed in INGHAM and MARTINEZ ARIAS 1992). The interaction of the gap and segmentation gene products results in the expression of *Scr* in a discrete spatiotemporal pattern. This phase of *Scr* regulation is essentially complete by germ-band retraction, as the gene products necessary for the establishment of the *Scr* expression pattern have dissipated by this point of development. The Pc-G and trx-G gene products act at this point in development to "imprint" the transcriptional state of *Scr* in every cell, thus maintaining the established pattern of *Scr* expression in embryonic tissues and the small number of cells set aside in each segment to form the imaginal disc anlagen.

This paper describes the identification and genetic characterization of putative regulatory sequences involved in the maintenance of *Scr* expression by *Polycomb* and *trithorax* group gene products. These sequences, located 15 kb downstream and 40 kb upstream of the *Scr* transcription start site, heritably repress the expression of a *white* minigene in the anlagen of the adult eye. The mosaic pattern of *white* transcription established early in development by these sequences is maintained for the rest of development in a Pc-G and trx-G dependent manner. Repression by the 3′ fragment, 8.2 *Xba*I, is enhanced when it is able to homologously pair with itself, a quality consistent with genetic evidence indicating that *Scr* regulation is sensitive to homologue pairing in certain genetic contexts. In addition to repressing *white*, the 5′ 10.0 *Xba*I fragment can block ectopic *Scr* reporter gene expression during embryogenesis in a *Pc* and *ph* dependent manner. We conclude that these DNA fragments contain *Scr cis*-regulatory sequences that interact with Pc-G and trx-G gene products and may be important for the developmental regulation of *Scr* expression.

***white* transcriptional repression by *Scr* regulatory sequences is phenotypically similar to position-effect variegation:** Three DNA fragments from the *Scr* regulatory region, when placed in *P*-element vectors containing a *white* minigene, cause this minigene to be expressed in mosaic patterns. The size and shape of pigmented and nonpigmented sectors in the eye suggest that regulatory sequences in these fragments inactivate *white* in a subset of the ~20 cells comprising the first larval instar eye disc and that this inactivation event is clonally inherited for the remainder of development. It does not appear that these regulatory sequences function by interpreting a gradient of gene activity in the eye, because the position of pigmented sectors is relatively random. This inactivation phenomenon, at the phenotypic level, is similar to the variegating phenotype of chromosomal rearrangements that place the *white* locus next to heterochromatin (reviewed in SPOFFORD 1976; REUTER and SPIERER 1992), most notably *In(1)w*^{m4} (TARTOF *et al.* 1984, 1989). Dozens of loci have been identified as enhancers and suppressors of position-effect variegation of *white* (reviewed in GRIGLIATTI 1991; REUTER and SPIERER 1992), and some of these have additional phenotypes similar to Pc-G mutations, suggesting there may be a functional link between homeotic gene regulation and heterochromatin formation (REUTER *et al.* 1990). A collection of over 30 *Su(var)* and *E(var)* mutations were tested for modifying effects on 8.2 *Xba*I-mediated *white* repression, but these mutations had no effect on eye pigmentation in transformants (data not shown). Although position-effect variegation and *white* minigene repression by *Scr* regulatory sequences are phenotypically similar, this result suggests that the same gene products or regulatory sequences are not responsible for both phenomena.

Pc-G and trx-G mutations interact with the *Scr* locus: Our results demonstrate that a subset of Pc-G and trx-G loci directly or indirectly interact with *Scr* regulatory sequences in the 10.0 *Xba*I fragment located near *Antp* and the 8.2 *Xba*I fragment in *Scr* intron 2. Altering the dosage of a subset of Pc-G or trx-G loci in transformants containing these regulatory fragments linked to a *white* minigene either suppresses (Pc-G) or enhances (trx-G) the repression of *white* minigene expression. These effects parallel ectopic homeotic gene expression observed in Pc-G mutants, as well as reductions in homeotic gene expression seen in trx-G mutants. The genetic interaction of Pc-G and trx-G loci with the same *Scr* regulatory fragments suggests that sequences necessary for *Scr* regulation by Pc-G and trx-G gene products are clustered in the 80-kb *Scr* regulatory interval rather than distributed throughout. One possible model for positive regulation of *Scr* by trx-G gene products is that the DNA binding of a subset of trx-G gene products to *Scr* regulatory sequences in the 10.0 and 8.2 *Xba*I fragments is somehow favored over Pc-G gene product binding in cells that transcribe *Scr* during early embryogenesis. The trx-G gene products then form a clonally heritable "open" chromatin domain by preventing the binding of Pc-G gene products to nearby or overlapping DNA binding sites through either steric (short-range) effects or by the linear propagation of chromatin structures that favor the formation of open chromatin instead of "closed" chromatin domains (long-range effects). Outside of the normal *Scr* expression domain, Pc-G gene product binding to sequences in the 10.0 and 8.2 *Xba*I fragments may, in a similar manner, preclude trx-G gene product binding to the *Scr* regulatory interval. An example of short-range repression in Drosophila is the formation of *even-skipped*

(*eve*) stripe 2, where binding sites of the transcriptional repressors *Kruppel* (*Kr*) and *giant* (*gt*) block the binding of the transcriptional activators *bicoid* (*bcd*) and *hunchback* (*hb*) to nearby and overlapping binding sites when the concentration of BCD and HB proteins fall below critical levels (STANOJEVIC *et al.* 1991). Similar "long-range" models have been proposed to describe the formation of centric heterochromatin (LOCKE *et al.* 1988), as well as the regulation of homeotic gene expression (PARO 1990; KENNISON 1993). Molecular evidence supporting this model has been recently provided by ORLANDO and PARO (1994), who demonstrated that PC protein is bound to nearly the entire length of *Ubx* in cells that repress *Ubx* transcription. If, as suggested by genetic data, Pc-G proteins bind to the 8.2 and 10.0 *Xba*I fragments, then these fragments may represent nucleation sites at which the spreading of the Pc-G complex over *Scr* begins, resulting in Pc-G protein binding of the *Scr* locus in a manner similar to that observed at *Ubx*.

Why do only a subset of Pc-G and trx-G loci genetically interact with regulatory sequences in the 8.2 and 10.0 *Xba*I fragments? One possibility is that the Pc-G and trx-G loci that regulate *Scr* but do not interact with these two fragments may interact with regulatory sequences located elsewhere in the *Scr* locus. Another possibility is some loci have stronger phenotypes than others, and weak interactions may not be detected by this assay. Although some of the loci that do not interact with these fragments may not function in the eye anlagen, all of the Pc-G and trx-G loci tested thus far appear to be expressed in an unrestricted pattern (PARO 1990; KENNISON 1993). An interesting possibility is that some Pc-G and trx-G loci categorized on the basis of mutant phenotype may regulate homeotic genes posttranscriptionally, either in splicing, translation or as transcriptional cofactors (KENNISON 1993). Some of the genes that do not interact with the 8.2 and 10.0 *Xba*I fragments might regulate homeotic genes posttranscriptionally and therefore may not affect *white* expression.

Other genes contain regulatory sequences that heritably repress *white*: Regulatory DNA fragments from other loci cause *white* minigene mosaicism in a manner similar to that seen in our analysis of *Scr* fragments. A 1.9-kb DNA fragment from the regulatory region of the *engrailed* (*en*) locus represses *white* minigene expression in homozygous transformants but not in heterozygotes (KASSIS *et al.* 1991; KASSIS 1994). This effect is not pairing dependent *per se* but is proximity dependent; two closely linked inserts can interact in *cis* when on the same chromosome, and independent insert lines that are located at similar cytological locations can repress *white* transcription in double heterozygotes (KASSIS 1994). The maintenance of normal patterns of *en* expression during embryogenesis and larval development, like *Scr*, is dependent upon Pc-G gene function (BUSTURIA and MORATA 1988; DURA and INGHAM 1988; MOAZED and O'FARRELL 1992). However, it does not appear that the *en* pairing-sensitive sites are responsible for its regulation by Pc-G gene products, as Pc-G mutations do not modify their regulatory effects on *white* (KASSIS 1994). Additional loci containing regulatory sequences that heritably repress *white* minigene expression include *polyhomeotic* (FAUVARQUE and DURA 1993), *proboscipedia* (*pb*) (A. KAPOUN and T. KAUFMAN, personal communication), *decapentaplegic* (*dpp*) (W. GELBART, personal communication), *abdominal-B* (*abd-B*) (M. MÜLLER, personal communication) and *Ubx* (CHAN *et al.* 1994). The *pb* regulatory fragment does not appear to genetically interact with Pc-G or trx-G loci (A. KAPOUN, personal communication), whereas the *abd-B* fragment interacts with Pc-G and trx-G mutations (M. MÜLLER, personal communication) and the *Ubx* fragment interacts with Pc-G mutations, although interactions with trx-G mutations have not been tested (CHAN *et al.* 1994). The level of sequence similarity between these regulatory sequences and *Scr* regulatory sequences is unknown; however, these results suggest that these *Scr*, *abd-B* and *Ubx* regulatory fragments may be functionally related.

Pairing sensitivity and *Scr* regulation: The discovery of pairing-sensitive regulatory elements at the *Scr* locus may shed some light on complex allelic interactions at *Scr* (HAZELRIGG and KAUFMAN 1983; PATTATUCCI 1991; PATTATUCCI and KAUFMAN 1991; PATTATUCCI *et al.* 1991). In an otherwise wild-type background, the disruption of chromosome pairing at *Scr* has no phenotypic consequences. However, in a sensitized genetic background that compromises *Scr* regulation through the removal of *Scr* regulatory sequences or by mutations in *trans*-regulators of *Scr* such as *Pc*, chromosome pairing at *Scr* negatively regulates expression outside of its normal domain (PATTATUCCI and KAUFMAN 1991). For example, the gain-of-function allele Scr^{ScxW} has an enhanced phenotype when it is in a genetic background that disrupts chromosome pairing at *Scr* but is otherwise wild type for *Scr* (PATTATUCCI and KAUFMAN 1991). This enhancement is similar to that observed when Pc-G mutations are introduced into Scr^{ScxW} heterozygotes. We propose that pairing-sensitive *white* repression by the 8.2 *Xba*I fragment is due to the enhanced ability of Pc-G proteins to interact with regulatory sequences in this fragment when those sequences are paired and that this pairing sensitivity may reflect the pairing-sensitive regulation of *Scr* observed in certain genetic contexts. Repression by regulatory sequences in the 8.2 *Xba*I fragment is enhanced when they are able to homologously pair. This enhancement of repressor activity is suppressed when transformants are introduced into Pc-G mutant backgrounds, as well as when homologous pairing is inhibited by chromosomal rearrangements that inhibit synapsis. The result of the disruption of chromo-

some pairing and lowering the dosage of Pc-G gene products is phenotypically similar; *white* gene expression is increased, resulting in more fully pigmented eyes. Although these results do not eliminate the possibility that independent pathways are responsible for pairing sensitivity and Pc-G-dependent repression, we prefer a model in which pairing of regulatory sequences in the 8.2 *Xba*I fragment increases the efficiency of formation of a Pc-G protein complex and that either disruption of pairing or lowering the dosage of one of the components of the Pc-G complex result in less complex formation and therefore less repression. According to the mass action model proposed by LOCKE *et al.* (1988) to explain the formation of heterochromatin, the rate at which a multimeric complex is formed is related to the concentration of its constituent parts and lowering the concentration of any one component results in a decrease in the amount of complex formed. This model has also been used to describe how Pc-G gene products form higher order complexes that stably repress homeotic gene expression (FRANKE *et al.* 1992). Although none have been described thus far, it is not unreasonable to expect that a subset of Pc-G genes encode sequence-specific DNA binding proteins that direct the formation of Pc-G complexes to specific regions of the genome, including the ANT-C and BX-C. Chromosome pairing would, in effect, double the local concentration of binding sites for these Pc-G proteins and therefore double the local concentration of proteins bound by them. The doubling of the local concentration of Pc-G proteins bound to DNA would, according to the LOCKE *et al.* (1988) model, drive Pc-G complex formation at this site at an exponentially greater rate than when the binding sites are unpaired, resulting in more Pc-G multimeric complex and more repression.

If there is a link between chromosome pairing and Pc-G complex formation, the following questions need to be addressed: Why are some 8.2 *Xba*I inserts pairing sensitive, whereas other inserts repress *white* when unpaired? Why does the 10.0 *Xba*I fragment repress *white* when unpaired? One explanation for why the 8.2 *Xba*I fragment represses *white* in a pairing-sensitive manner in some cases but not others is that some transgenes are able to interact with Pc-G proteins bound to DNA near the chromosomal site of insertion, resulting in the ability to repress *white* when unpaired, whereas other transgenes are inserted in regions which Pc-G proteins are absent, resulting in a pairing-sensitive phenotype. It has been shown that a pairing-sensitive 8.2 *Xba*I insertion can become pairing independent when, by a conservative transposition event, another copy of the transgene is placed near the original insertion site (J. POWERS and T. KAUFMAN, unpublished results). This demonstrates that sequences in the 8.2 *Xba*I fragment can "pair" in *cis* as well as in *trans* and suggests that 8.2 *Xba*I fragment-containing transgenes may interact with heterologous nearby Pc-G binding sites.

The 10.0 *Xba*I fragment may repress *white* in a pairing-independent manner because multiple Pc-G protein binding sites are present in this fragment, thereby eliminating the necessity of chromosome pairing. This appears to be the case, as multiple independent *white* repressor elements have been identified in the 10.0 *Xba*I fragment (J. POWERS and T. KAUFMAN, unpublished results). The presence of multiple Pc-G responsive elements in the *Scr* regulatory region may also provide an explanation for why disruption of chromosome pairing in an otherwise wild-type fly does not alter *Scr* expression. If, as suggested by our results, Pc-G responsive elements can interact in *cis*, then perhaps the 8.2 and 10.0 *Xba*I fragments can interact with each other or with unidentified Pc-G responsive elements at *Scr* when chromosomes are unpaired, thereby permitting Pc-G protein complexes to form at a rate similar to that observed when chromosome pairing can occur. In this model of *Scr* regulation, the role of chromosome pairing is normally redundant, but when the regulatory mechanism controlling *Scr* expression is sufficiently compromised, such as with the mutation Scr^{ScxW}, the role of chromosome pairing in *Scr* regulation becomes apparent.

Regulation of *Antennapedia* and *Ultrabithorax* by *Polycomb* group genes: Molecular analyses have identified the presence of sequences in the *Antennapedia* (*Antp*) and *Ultrabithorax* (*Ubx*) regulatory regions that act during embryogenesis to maintain expression boundaries established by gap and segmentation gene products. Immunolocalization of ANTP protein in embryos lacking individual Pc-G loci shows that, in the absence of Pc-G gene function, *Antp* is ectopically expressed anterior of its normal parasegment 4 boundary (CARROLL *et al.* 1986; WEEDEN *et al.* 1986; MCKEON and BROCK 1991). ZINK *et al.* (1991) identified regulatory sequences surrounding the *Antp* P1 and *Antp* P2 promoters that direct *Antp-lacZ* reporter gene expression in an *Antp*-like pattern during embryogenesis. In *Pc* mutants, the P1 and P2 promoter-driven patterns degenerate after ~10 hr of embryogenesis, whereas the anterior boundary is maintained in wild-type embryos. Polycomb protein appears to bind to sequences in these constructs, as assayed by the presence of new PC binding sites on salivary gland chromosomes at the cytological location of construct insertion (ZINK *et al.* 1991). Polycomb protein binding to these sequences is likely to be indirect, as PC does not appear to bind DNA directly (PARO and HOGNESS 1991). The function of PC-binding sequences surrounding the *Antp* P1 and P2 promoters is quite similar to the function of *Scr* regulatory sequences in the 10.0 *Xba*I fragment. The 10.0 *Xba*I fragment maintains the posterior boundary of *Scr* reporter gene expression in a *Pc*-dependent manner, whereas *Antp* regulatory sequences maintain the anterior boundary of *Antp* reporter gene expression in a *Pc*-

dependent manner. These results, in conjunction with the observed modification of *white* minigene repression by the 10.0 *Xba*I fragment in Pc^3 mutants, suggest that the 10.0 *Xba*I fragment contains PC binding sites.

Molecular dissection of the large *Ubx* regulatory region has identified regulatory sequences both 5′ and 3′ of the *Ubx* promoter that direct reporter gene expression in a *Ubx*-like pattern during embryogenesis and larval development (SIMON *et al.* 1990; IRVINE *et al.* 1991; MÜLLER and BIENZ 1991; QIAN *et al.* 1991; ZHANG and BIENZ 1992; CASTELLI-GAIR *et al.* 1992). A combinatorial model has emerged in which Pc-G response elements (PREs) maintain spatial patterning of *Ubx* established by the gap and segmentation genes through regulatory elements distinct from the PREs (ZHANG and BIENZ 1992; SIMON *et al.* 1993; CHAN *et al.* 1994). However, the PREs do not contain positional information, as they confer maintenance of expression boundaries to heterologous enhancer elements. It has also been demonstrated that the maintenance function of the *Ubx* PREs is Pc-G dependent and that one PRE heritably represses *white* (CHAN *et al.* 1994). By analogy, the *Scr* regulatory fragment 10.0 *Xba*I contains two activities: the establishment of an expression boundary in the prothoracic segment and a PRE-like function that maintains the T1 boundary in a Pc-G dependent manner. The 8.2 *Xba*I fragment may contain PRE-like regulatory elements important for the maintenance of established patterns of *Scr* expression but does not contain enhancers that help establish a restricted pattern of *Scr* expression. A comparative analysis of PREs in *Scr*, *Ubx* and other genes regulated in a similar fashion may result in the identification of a common mechanism governing the maintenance of developmentally regulated patterns of gene expression by the Pc-G and trx-G gene products.

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LITERATURE CITED

ADLER, P. N., J. CHARLTON and B. BRUNK, 1989 Genetic interactions of the *suppressor 2 of zeste* region genes. Dev. Genet. **10:** 249–260.

BAKER, W. K., 1967 A clonal system of differential gene activity in *Drosophila*. Dev. Biol. **16:** 1–17.

BECKER, H. J., 1976 Mitotic recombination, pp. 1019–1087 in *Genetics and Biology of Drosophila*, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

BREEN, T. R., and I. DUNCAN, 1986 Maternal expression of genes that regulate the *bithorax* complex of *Drosophila melanogaster*. Dev. Biol. **118:** 442–456.

BREEN, T. R., and P. J. HARTE, 1993 *trithorax* regulates multiple homeotic genes in the *bithorax* and *Antennapedia* complexes and exerts different tissue-specific, parasegment-specific and promoter-specific effects on each. Development **117:** 119–134.

BUSTURIA, A., and G. MORATA, 1988 Ectopic expression of homeotic genes caused by the elimination of the *Polycomb* gene in *Drosophila* imaginal epidermis. Development **104:** 713–720.

CAMPOS-ORTEGA, J. A., and V. HARTENSTEIN, 1985 *Embryonic Development of Drosophila melanogaster*. Springer-Verlag, Berlin.

CARROLL, S. B., R. A. LAYMON, M. A. MCCUTCHEON, P. D. RILEY and M. P. SCOTT, 1986 The localization and regulation of *Antennapedia* protein expression in *Drosophila* embryos. Cell **47:** 113–122.

CARROLL, S. B., S. DINARDO, P. H. O'FARRELL, R. A. H. WHITE and M. P. SCOTT, 1988 Temporal and spatial relationships between segmentation and homeotic gene expression in *Drosophila* embryos: distributions of the *fushi tarazu*, *engrailed*, *Sex combs reduced*, *Antennapedia*, and *Ultrabithorax* proteins. Genes Dev. **2:** 350–360.

CASTELLI-GAIR, J., J. M;AUULLER and M. BIENZ, 1992 Function of an *Ultrabithorax* minigene in imaginal cells. Development **114:** 877–886.

CHAN, C.-S., R. RASTELLI and V. PIRROTTA, 1994 A *Polycomb* response element in the *Ubx* gene that determines an epigenetically inherited state of repression. EMBO J. **13:** 2553–2564.

COHEN, S. M., G. BRONNER, F. KUTTNER, G. JURGENS and H. JACKLE, 1989 *Distal-less* encodes a homeodomain protein required for limb development in *Drosophila*. Nature **338:** 432–434.

DUNCAN, I., 1982 *Polycomblike*: a gene that appears to be required for the normal expression of the *bithorax* and *Antennapedia* gene complexes of *Drosophila melanogaster*. Genetics **102:** 49–70.

DUNCAN, I., and E. B. LEWIS, 1982 Genetic control of body segment differentiation in *Drosophila*, pp. 533–554 in *Developmental Order: Its Origin and Regulation*, edited by S. SUBTELNY and P. B. GREEN. Alan R. Liss, Inc., New York.

DURA, J.-M., and P. INGHAM, 1988 Tissue- and stage-specific control of homeotic and segmentation gene expression in *Drosophila* embryos by the *polyhomeotic* gene. Development **103:** 733–741.

DURA, J.-M., H. W. BROCK and P. SANTAMARIA, 1985 *Polyhomeotic*: a gene of *Drosophila melanogaster* required for correct expression of segmental identity. Mol. Gen. Genet. **198:** 213–220.

DURA, J.-M., N. B. RANDSHOLT, J. DEATRICK, I. ERK, P. SANTAMARIA *et al.*, 1987 A complex genetic locus, *polyhomeotic*, is required for segmental specification and epidermal development in *D. melanogaster*. Cell **51:** 829–839.

EPHRUSSI, B., and J. L. HEROLD, 1944 Studies of eye pigments of Drosophila. I. Methods of extraction and quantitative estimation of the pigment components. Genetics **29:** 148–175.

FAUVARQUE, M.-O., and J.-M. DURA, 1993 *polyhomeotic* regulatory sequences induce developmental regulator-dependent variegation and targeted P-element insertions in *Drosophila*. Genes Dev. **7:** 1508–1520.

FRANKE, A., M. DECAMILLIS, B. ZINK, N. CHENG, H. W. BROCK *et al.*, 1992 *Polycomb* and *polyhomeotic* are constituents of a multimeric protein complex in chromatin of *Drosophila melanogaster*. EMBO J. **11:** 2941–2950.

GAUL, U., E. SEIFERT, R. SCHUH and H. JACKLE, 1987 Analysis of *Krüppel* protein distribution during early *Drosophila* development reveals posttranscriptional regulation. Cell **50:** 639–647.

GEHRING, W. J., 1970 A recessive lethal *l(4)29* with a homeotic effect in *D. melanogaster*. Dros. Inf. Serv. **45:** 103.

GEYER, P. K., M. M. GREEN and V. G. CORCES, 1990 Tissue-specific transcriptional enhancers may act in *trans* on the gene located in the homologueous chromosome: the molecular basis of transvection in *Drosophila*. EMBO J. **9:** 2247–2256.

GINDHART, J. G., A. N. KING and T. C. KAUFMAN, 1995 Characteriza-

tion of the *cis* regulatory region of the Drosophila homeotic gene *Sex combs reduced*. Genetics **139:** 781–795.

GLICKSMAN, M. A., and D. L. BROWER, 1988 Expression of the *Sex combs reduced* protein in *Drosophila* larvae. Dev. Biol. **127:** 113–118.

GRIGLIATTI, T., 1991 Position-effect variegation—an assay for nonhistone chromosomal proteins and chromatin assembly and modifying factors. Methods Cell Biol. **35:** 587–627.

HAZELRIGG, T., and T. C. KAUFMAN, 1983 Revertants of dominant mutations associated with the *Antennapedia* gene complex of *Drosophila melanogaster*: cytology and genetics. Genetics **105:** 581–600.

HAZELRIGG, T., and S. PETERSEN, 1992 An unusual genomic position effect on *Drosophila white* gene expression: pairing dependence, interactions with *zeste*, and molecular analysis of revertants. Genetics **130:** 125–138.

HAZELRIGG, T., R. LEVIS and G. M. RUBIN, 1984 Transformation of *white* locus DNA in *Drosophila*: dosage compensation, *zeste* interaction, and position effects. Cell **36:** 469–481.

HOCHMAN, B., H. GLOOR and M. M. GREEN, 1964 Analysis of chromosome 4 in *Drosophila melanogaster*. I. Spontaneous and X-ray-induced lethals. Genetica **35:** 109–126.

INGHAM, P. W., 1980 Genetic analysis of *trithorax*, *trx*, a new homoeotic mutant of *Drosophila melanogaster*. Dros. Inf. Serv. **55:** 63–64.

INGHAM, P. W., 1981 *Trithorax*: a new homoeotic mutation of *Drosophila melanogaster* II. The role of *trx*+ after embryogenesis. Wilhelm Roux's Arch. Dev. Biol. **190:** 365–369.

INGHAM, P. W., 1984 A gene that regulates the *bithorax* complex differentially in larval and adult cells of *Drosophila*. Cell **37:** 815–823.

INGHAM, P. W., 1985 A clonal analysis of the requirement for the *trithorax* gene in the diversification of segments in *Drosophila*. J. Embryol. Exp. Morphol. **89:** 349–365.

INGHAM, P., 1988 The molecular genetics of embryonic pattern formation in *Drosophila*. Nature **335:** 25–34.

INGHAM, P. W., and A. MARTINEZ ARIAS, 1992 Boundaries and fields in early embryos. Cell **68:** 221–235.

INGHAM, P. W., and R. WHITTLE, 1980 *Trithorax*: a new homoeotic mutation of *Drosophila melanogaster* causing transformations of abdominal and thoracic imaginal segments I. Putative role during embryogenesis. Mol. Gen. Genet. **179:** 607–614.

IRVINE, K. D., S. L. HELFAND and D. S. HOGNESS, 1991 The large upstream control region of the *Drosophila* homeotic gene *Ultrabithorax* (*Ubx*). Development **111:** 407–424.

JONES, R. S., and W. M. GELBART, 1990 Genetic analysis of the *Enhancer of zeste* locus and its role in gene regulation in *Drosophila melanogaster*. Genetics **126:** 185–199.

JURGENS, G., 1985 A group of genes controlling the spatial expression of the *bithorax* complex in *Drosophila*. Nature **316:** 153–155.

KASSIS, J. A., 1994 Unusual properties of regulatory DNA from the *Drosophila engrailed* gene: three "pairing-sensitive" sites within a 1.6 kb region. Genetics **136:** 1025–1038.

KASSIS, J. A., E. P. VANSICKLE and S. M. SENSABAUGH, 1991 A fragment of *engrailed* regulatory DNA can mediate transvection of the *white* gene in *Drosophila*. Genetics **128:** 751–761.

KAUFMAN, T. C., M. A. SEEGER and G. OLSON, 1990 Molecular and genetic organization of the *Antennapedia* gene complex of *Drosophila melanogaster*. Adv. Genet. **27:** 309–362.

KENNISON, J. A., 1993 Transcriptional activation of *Drosophila* homeotic genes from distant regulatory elements. Trends Genet. **9:** 75–78.

KENNISON, J. A., and M. A. RUSSELL, 1987 Dosage-dependent modifiers of homoeotic mutations in *Drosophila melanogaster*. Genetics **116:** 75–86.

KENNISON, J. A., and J. W. TAMKUN, 1988 Dosage-dependent modifiers of *Polycomb* and *Antennapedia* mutations in *Drosophila*. Proc. Natl. Acad. Sci. USA **85:** 8136–8140.

LEMOTTE, P. K., A. KUROIWA, L. I. FESSLER and W. J. GEHRING, 1989 The homeotic gene *Sex combs reduced* of *Drosophila*: gene structure and embryonic expression. EMBO J. **8:** 219–227.

LEVIS, R., T. HAZELRIGG and G. M. RUBIN, 1985a Effects of genomic position on the expression of transduced copies of the *white* gene of *Drosophila*. Science **229:** 558–561.

LEVIS, R., T. HAZELRIGG and G. M. RUBIN, 1985b Separable cis-acting control elements for expression of the *white* gene of *Drosophila*. EMBO J. **4:** 3489–3499.

LEWIS, E. B., 1978 A gene complex controlling segmentation in *Drosophila*. Nature **276:** 565–570.

LOCKE, J., M. A. KOTARSKI and K. D. TARTOF, 1988 Dosage-dependent modifiers of position effect variegation in *Drosophila* and a mass action model that explains their effect. Genetics **120:** 181–198.

MAHAFFEY, J. W., and T. C. KAUFMAN, 1987 The distribution of the *Sex combs reduced* gene product during development of *Drosophila melanogaster*. Genetics **117:** 51–60.

MAHAFFEY, J. W., R. J. DIEDERICH and T. C. KAUFMAN, 1989 Novel patterns of homeotic protein accumulation in the head of the *Drosophila* embryo. Development **105:** 167–174.

MCKEON, J., and H. W. BROCK, 1991 Interactions of the *Polycomb* group of genes with homeotic loci of *Drosophila*. Roux's Arch. Dev. Biol. **199:** 387–396.

METZ, C. W., 1916 Chromosome studies on the Diptera II. The paired association of chromosomes in the Diptera, and its significance. J. Exp. Zool. **21:** 213–279.

MOAZED, D., and P. H. O'FARRELL, 1992 Maintenance of the *engrailed* expression pattern by *Polycomb* group genes in *Drosophila*. Development **116:** 805–810.

MÜLLER, J., and M. BIENZ, 1991 Long range repression conferring boundaries of *Ultrabithorax* expression in the *Drosophila* embryo. EMBO J. **10:** 3147–3155.

ORLANDO, V., and PARO, R. 1994 Mapping Polycomb-repressed domains in the bithorax complex using in vivo formaldehyde cross-linked chromatin. Cell **75:** 1187–1198.

PARO, R., 1990 Imprinting a determined state into the chromatin of *Drosophila*. Trends Genet. **6:** 416–421.

PARO, R., and D. S. HOGNESS, 1991 The *Polycomb* protein shares a homologueous domain with a heterochromatin-associated protein of *Drosophila*. Proc. Natl. Acad. Sci. USA **88:** 263–267.

PATTATUCCI, A. M., 1991 A genetic analysis of the homeotic gene *Sex combs reduced* of the *Antennapedia* complex in *Drosophila melanogaster*: towards an understanding of the nature in which developmental processes are regulated. Ph.D. thesis, Indiana University, Bloomington.

PATTATUCCI, A. M., and T. C. KAUFMAN, 1991 The homeotic gene *Sex combs reduced* of *Drosophila melanogaster* is differentially regulated in the embryonic and imaginal stages of development. Genetics **129:** 443–461.

PATTATUCCI, A. M., D. C. OTTESON and T. C. KAUFMAN, 1991 A functional and structural analysis of the *Sex combs reduced* locus of *Drosophila melanogaster*. Genetics **129:** 423–441.

PHILLIPS, M. D., and A. SHEARN, 1990 Mutations in *Polycombeotic*, a *Drosophila Polycomb*-group gene, cause a wide range of maternal and zygotic phenotypes. Genetics **125:** 91–101.

PIRROTTA, V., 1988 Vectors for P-mediated transformation in *Drosophila*. Biotechnology **10:** 437–456.

QIAN, S., M. CAPOVILLA and V. PIRROTTA, 1991 The *bx* region enhancer, a distal *cis*-control element of the *Drosophila Ubx* gene and its regulation by *hunchback* and other segmentation genes. EMBO J. **10:** 1415–1425.

RASTELLI, L., C. S. CHAN and V. PIRROTTA, 1993 Related chromosome binding sites for zeste, suppressors of zeste and Polycomb group proteins in *Drosophila* and their dependence on *Enhancer of zeste* function. EMBO J. **12:** 1513–1522.

REUTER, G., and P. SPIERER, 1992 Position effect variegation and chromatin proteins. BioEssays **14:** 605–612.

REUTER, G., M. GIARRE, J. FARAH, J. GAUSZ, A. SPIERER *et al.*, 1990 Dependence of position-effect variegation in *Drosophila* on dose of a gene encoding an unusual zinc-finger protein. Nature **344:** 219–223.

REUTER, R., and M. P. SCOTT, 1990 Expression and function of the homeotic genes *Antennapedia* and *Sex combs reduced* in the embryonic midgut of *Drosophila*. Development **109:** 289–303.

RILEY, P. D., S. B. CARROLL and M. P. SCOTT, 1987 The expression and regulation of *Sex combs reduced* protein in *Drosophila* embryos. Genes Dev. **1:** 716–730.

ROBERTSON, H. M., C. R. PRESTON, R. W. PHILLIS, D. M. JOHNSON-SCHLITZ, W. K. BENZ *et al.*, 1988 A stable genomic source of *P*-element transposase in *Drosophila melanogaster*. Genetics **118:** 461–470.

RUSSELL, M., and S. EBERLEIN, 1979 New mutants of *engrailed* in *Drosophila melanogaster*. Genetics **91:** s109.

SATO, T., P. H. HAYES and R. E. DENELL, 1984 Homeosis in *Drosophila*: maternal effect of the *Enhancer of Polycomb* locus and its interaction with *Polycomb* and related loci. Dev. Genet. **4:** 185–198.

SATO, T., M. A. RUSSELL and R. E. DENELL, 1983 Homoeosis in Drosophila: a new enhancer of *Polycomb* and related homoeotic mutations. Genetics **105:** 357–370.

SCOTT, M. P., A. J. WEINER, T. I. HAZELRIGG, B. A. POLISKY, V. PIRROTTA *et al.*, 1983 The molecular organization of the *Antennapedia* locus of *Drosophila*. Cell **35:** 763–776.

SHEARN, A., 1989 The *ash-1*, *ash-2* and *trithorax* genes of *Drosophila melanogaster* are functionally related. Genetics **121:** 517–525.

SHEARN, A., E. HERSPERGER and G. HERSPERGER, 1987 Genetic studies of mutations at two loci of *Drosophila melanogaster* which cause a wide variety of homeotic transformations. Wilhelm Roux's Arch. Dev. Biol. **196:** 231–242.

SIMON, J., M. PEIFER, W. BENDER and M. O'CONNOR, 1990 Regulatory elements of the *bithorax* complex that control expression along the anterior-posterior axis. EMBO J. **9:** 3945–3956.

SIMON, J., A. CHIANG, W. BENDER, M. J. SHIMELL and M. O'CONNOR, 1993 Elements of the *Drosophila* bithorax complex that mediate repression by *Polycomb* group products. Dev. Biol. **158:** 131–144.

SPOFFORD, J. B., 1976 Position-effect variegation in *Drosophila*, pp. 955–1018 in *Genetics and Biology of Drosophila*, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

STANOJEVIC, D., S. SMALL and M. LEVINE, 1991 Regulation of a segmentation stripe by overlapping activators and repressors in the *Drosophila* embryo. Science **254:** 1385–1387.

STRUHL, G., 1981 A gene product required for correct initiation of segmental determination in *Drosophila*. Nature **293:** 36–41.

STRUHL, G., 1983 Role of the *esc*$^+$ gene product in ensuring the selective expression of segment-specific homeotic genes in *Drosophila*. J. Embryol. Exp. Morphol. **76:** 297–331.

STRUHL, G., and M. E. AKAM, 1985 Altered distributions of *Ultrabithorax* transcripts in *extra sex combs* mutant embryos of *Drosophila*. EMBO J. **4:** 3259–3264.

STRUHL, G., and D. L. BROWER, 1982 Early role of the *esc*$^+$ gene product in the determination of segments in *Drosophila*. Cell **31:** 285–292.

SUNKEL, C. E., and J. R. S. WHITTLE, 1987 *Brista*: a gene involved in the specification and differentiation of distal cephalic and thoracic structures in *Drosophila melanogaster*. Roux's Arch. Dev. Biol. **196:** 124–132.

TAMKUN, J. W., R. DEURING, M. P. SCOTT, M. KISSINGER, A. M. PATTATUCCI *et al.*, 1992 *brahma*: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcription factor SNF2/SWI2. Cell **68:** 561–572.

TARTOF, K. D., C. HOBBS and M. JONES, 1984 A structural basis for variegating position effects. Cell **37:** 869–878.

TARTOF, K. D., C. BISHOP, M. JONES, C. A. HOBBS and J. LOCKE, 1989 Towards an understanding of position effect variegation. Dev. Genet. **10:** 162–176.

TAUTZ, D., 1988 Regulation of the *Drosophila* segmentation gene *hunchback* by two maternal morphogenetic centres. Nature **332:** 281–284.

TREMML, G., and M. BIENZ, 1989 Homeotic gene expression in the visceral mesoderm of *Drosophila* embryos. EMBO J. **8:** 2677–2685.

WEEDEN, C., K. HARDING and M. LEVINE, 1986 Spatial regulation of *Antennapedia* and *Ultrabithorax* gene expression by the *Polycomb* locus in *Drosophila melanogaster*. Cell **44:** 739–748.

WU, C.-T., 1993 Transvection, nuclear structure, and chromatin proteins. J. Cell Biol. **120:** 587–590.

WU, C.-T., R. S. JONES, P. F. LASKO and W. M. GELBART, 1989 Homeosis and the interaction of *zeste* and *white* in *Drosophila*. Mol. Gen. Genet. **218:** 559–564.

ZHANG, C.-C., and M. BIENZ, 1992 Segmental determination in *Drosophila* conferred by *hunchback* (*hb*), a repressor of the homeotic gene *Ultrabithorax* (*Ubx*). Proc. Natl. Acad. Sci. USA **89:** 7511–7515.

ZINK, B., and R. PARO, 1989 *In vivo* binding pattern of a trans-regulator of homoeotic genes in *Drosophila melanogaster*. Nature **337:** 468–471.

ZINK, B., Y. ENGSTROM, W. J. GEHRING and R. PARO, 1991 Direct interaction of the *Polycomb* protein with *Antennapedia* regulatory sequences in polytene chromosomes of *Drosophila melanogaster*. EMBO J. **10:** 153–162.

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