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Genetic Analysis of the *Enhancer of zeste* Locus and Its Role in Gene Regulation in *Drosophila melanogaster*

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ABSTRACT

The *Enhancer of zeste* [*E*(*z*)] locus of *Drosophila melanogaster* is implicated in multiple examples of gene regulation during development. First identified as dominant gain-of-function modifiers of the *zeste*[1]-*white* (*z-w*) interaction, mutant *E*(*z*) alleles also produce homeotic transformations. Reduction of $E(z)^+$ activity leads to both suppression of the *z-w* interaction and ectopic expression of segment identity genes of the Antennapedia and bithorax gene complexes. This latter effect defines *E*(*z*) as a member of the Polycomb-group of genes. Analysis of $E(z)^{S2}$, a temperature-sensitive *E*(*z*) allele, reveals that both maternally and zygotically produced $E(z)^+$ activity is required to correctly regulate the segment identity genes during embryonic and imaginal development. As has been shown for other Polycomb-group genes, $E(z)^+$ is required not to initiate the pattern of these genes, but rather to maintain their repressed state. We propose that the *E*(*z*) loss-of-function eye color and homeotic phenotypes may both be due to gene derepression, and that the $E(z)^+$ product may be a general repressing factor required for both examples of negative gene regulation.

ONE approach toward understanding the proper control of differential gene expression is to identify and characterize *trans*-acting regulatory factors. Two general methods are often employed for this purpose. Many *trans*-acting factors have been identified by isolating proteins that bind *cis*-regulatory regions in a sequence specific manner. Alternatively, a genetic approach may be used to identify regulatory factors on the basis of their functional rather than physical qualities. By isolating mutations that disrupt or modify phenotypes which are the result of specific examples of gene regulation, one can identify genes whose wild-type products are components of the regulatory mechanisms involved. In addition to those factors which specifically regulate a small number of genes, factors affecting expression of multiple and very different genetic functions also merit attention. Such factors may play more general roles in regulating gene expression. We are focusing on factors affecting two distinct and well defined genetic systems in Drosophila, both of which are very sensitive to modulation: the *zeste-white* interaction and anterior-posterior segment identity gene expression.

The z^1 allele of the *zeste* (*z*) locus represses expression of the *white* (*w*) gene in the eye (GANS 1953; JACK and JUDD 1979; GELBART and WU 1982; ZACHAR, CHAPMAN and BINGHAM 1985). The *X*-linked *white* locus is required for pigmentation of the *Drosophila* eye. Because in *Drosophila*, homologous chromosomes are paired in somatic cells (METZ 1916), females normally have two copies of the *w* gene in close apposition, while males have a single unpaired *w* gene. Homozygous $z^1\ w^+$ females have yellow eyes, whereas the eyes of hemizygous $z^1\ w^+$ males are wild type. This difference reflects the ability of z^1 protein to repress expression of the w^+ gene more strongly when two or more copies of the *white* gene's 5′-flanking regulatory region are in close proximity (JACK and JUDD 1979; ZACHAR, CHAPMAN and BINGHAM 1985; DAVISON *et al.* 1985; LEVIS, HAZELRIGG and RUBIN 1985b; RIRROTTA, STELLAR and BOZZETTI 1985).

Mutations in a number of loci, including several Minutes, have been isolated which act in *trans* to modify the z^1 eye color (KALISCH and RASMUSON 1974; PERSSON 1976a,b; WU *et al.* 1989). Several other modifiers of the z^1 eye color display no Minute phenotypes. These include *Suppressor of zeste 2* [*Su*(*z*)*2*], *Enhancer of zeste* [*E*(*z*)], *Suppressor of zeste 4* [*Su*(*z*)*4*] (KALISCH and RASMUSON 1974; PERSSON 1976b; WU *et al.* 1989) and *Sex comb on midleg* (*Scm*) (WU *et al.* 1989). At least some of these are likely to encode products that are involved in the repression of *white* gene expression by the z^1 protein.

Both Drosophila larvae and adults are segmented along the anterior-posterior body axis. The developmental fates of the cells within each segment are largely determined by the active states of the segment identity genes of the *Antennapedia* (*ANT-C*) and *bithorax* (*BX-C*) gene complexes [see AKAM (1987) for review]. The pattern in which the segment identity genes are expressed is initiated by the segmentation

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genes early in embryogenesis (WHITE and LEHMAN 1986; INGHAM and MARTINEZ-ARIAS 1986; INGHAM, ISH-HOROWICZ and HOWARD 1986; MARTINEZ-ARIAS and WHITE 1988; HARDING and LEVINE 1988). Once the pattern is established, it is maintained in at least two ways. First, the spatial domains of segment identity genes are restricted by negative regulatory interactions with other segment identity genes. For example, *BX-C* genes repress expression of *Antennapedia* in more posterior segments (HAFEN, LEVINE and GEHRING 1984; HARDING *et al.* 1985). Second, a group of loci collectively known as Polycomb-group genes, after the prototype *Polycomb* (*Pc*) locus, are required to maintain the segment-specific repression of the segment identity genes (STRUHL and AKAM 1985; WEDEEN, HARDING and LEVINE 1986). Mutations inactivating functions of any of the Pc-group loci allow ectopic expression of segment identity genes, resulting in a general syndrome of homeotic transformations (STRUHL 1981, 1983).

So far three loci have been identified in which mutations can both produce homeotic transformations typical of Pc-group mutations and modify the z^1 eye color [*Su(z)2*, *Scm* and *E(z)*: WU *et al.* (1989)]. In this paper, we describe a more thorough characterization of the role that one of these, *E(z)*, plays in modulating the z^1-*w* interaction and in producing homeotic phenotypes. We suggest that in both cases, the phenotypes produced in the absence of sufficient $E(z)^+$ activity are due to derepression of gene activity.

MATERIALS AND METHODS

Mutations and strains: Both *Df(3L)lxd*^{6} and *Df(3L)lxd*^{15} (Table 1), which were kindly provided by V. FINNERTY, fail to complement the recessive phenotypes elicited by *E(z)* mutations. In the text, they are referred to generically as *Df(3L)E(z)*$^-$. *Dp(3;3)S2a3* is a tandem duplication which contains two copies of $E(z)^+$ and is referred to as $E(z)^+$, *Dp(3;3)E(z)*$^+$. *In(3LR)TM3, Sb Ser* and *In(3LR)TM6B, Tb* are third chromosome balancers which will be referred to as *TM3* and *TM6B*, respectively. The *l(3)67Fa* alleles were kindly provided by MARK PHILLIPS and ALLEN SHEARN and are described by SHEARN, HERSPERGER and HERSPERGER (1978) and LINDSLEY and ZIMM (1986). The *l(3)67Fa*^{1}, *l(3)67Fa*^{3} and *l(3)67Fa*^{7} alleles were previously named *l(3)1902*, *l(3)MK436* and *l(3)MR127*, respectively (SHEARN, HERSPERGER and HERSPERGER 1978). All other mutations and chromosomes are described by LINDSLEY and GRELL (1968) or LINDSLEY and ZIMM (1985, 1986, 1987).

Culture conditions: Flies were cultured as previously described (SMOLIK-UTLAUT and GELBART 1987). Unless otherwise specified, crosses were maintained at 25°.

Mutagenesis procedures: In experiments designed to revert the dominant gain-of-function eye color phenotypes of $E(z)^1$ or $E(z)^{S1}$, males were irradiated with approximately 4000 rad from a ^{137}Cs γ-ray source. Males bearing one of these alleles were aged the equivalent of 3–5 days at 25° before being mutagenized. After mutagenesis, they were allowed to mate for 1 or 2 days for ethylmethane sulfonate (EMS) and γ-ray mutageneses, respectively, and were then discarded. Inseminated females were brooded every 2–3 days. In F_2 lethal screens designed to isolate mutations which fail to complement the recessive lethality of $E(z)^{S1}$, aged males isogenic for an *ebony*^{11} (*e*^{11}) third chromosome were exposed to 0.3% (v/v) EMS in 0.3 M sucrose for 24 hr (LEWIS and BACHAR 1968), and mated to females bearing a *TM3, Sb Ser* balancer chromosome. F_1 **e*^{11}*/TM3, Sb Ser* males were individually mated to two *sc z*^{1} *w*^{is}; $E(z)^{S1}$*/TM3, Sb Ser* females. Each cross was scored for presence or absence of Sb^+ progeny.

Tests for allelic recessive lethality: Females which carried either $E(z)^1$ or $E(z)^{S1}$ in *trans* with the *TM3* balancer chromosome were crossed to males carrying one of the *l(3)67Fa* alleles balanced over *TM3*. In each lethal test in which no *E(z)/l(3)67Fa* progeny were recovered, at least 350 *TM3*-bearing sibs were scored.

Determination of lethal phases and homeotic leg phenotypes: The developmental stages at which individuals hemizygous for *E(z)* mutant alleles die were determined by crossing males carrying an *E(z)* allele in trans to either the *TM3* or *TM6B* balancer chromosome to *Df(3L)lxd*^{15}*/TM3* females at 25°. The numbers of dead third instar larvae, pupae, or viable nonbalancer adults were then compared to the numbers of viable adults carrying either the *E(z)* mutant allele or *Df(3L)lxd*^{15} in trans to a third chromosome balancer. Homeotic leg transformations produced by mutant *E(z)* alleles in trans with $E(z)^{S2}$ were determined by crossing males carrying an *E(z)* mutant allele in *trans* with *TM3* or *TM6B* to *sc z*^{1} *w*^{is}; $E(z)^{S2}$*/TM3* females, rearing the progeny at 25° or 29°, and scoring the legs of $E(z)^x/E(z)^{S2}$ pharate or eclosed adult males.

Cuticle preparations: Embryonic cuticles were mounted essentially as previously described (IRISH and GELBART 1987). Eclosed or pharate adults, which were dissected out of their pupal cases, were stored and dissected in 70% ethanol, then mounted in GMM, a 2:1 Canada balsam:methyl salicylate mixture (LAWRENCE, JOHNSON and MORATA 1986).

Antibody staining: Immunohistochemical staining of imaginal discs and larval central nervous system (CNS) was performed according to the protocol of BROWER (1987) except that antibody localization was visualized by horseradish peroxidase staining (MACDONALD and STRUHL 1986) and photographed using Nomarski-DIC optics. For embryo staining, females were allowed to lay eggs for 0–14 hr at 25° or 29°, as specified. The embryos were then washed with water, dechorionated in 50% hypochlorite bleach, rinsed extensively with water, and fixed for 20 min in 4% formaldehyde in PEM [0.1 M Pipes (pH 6.9), 2 mM $MgSO_4$, 1 mM EGTA): heptane (1:3). The fix-heptane mixture was removed, followed by heptane washes and devitellinization in methanol:heptane (1:1). Devitellinized embryos were then washed several times with 100% methanol and stored in methanol at −20°. The *Scr* (GLICKSMAN and BROWER 1988b) and *Ubx* (WHITE and WILCOX 1985) monoclonal antibodies were kindly provided by M. GLICKSMAN and D. BROWER, and by G. STRUHL, respectively. Biotinylated goat anti-mouse immunoglobulin G was purchased from Vector Labs.

RESULTS

Isolation and initial characterization of *E(z)* alleles

In this report we will deal with four classes of *E(z)* alleles: a gain-of-function enhancer of z^1 [$E(z)^1$], gain-of-function suppressors of z^1 [*e.g.*, $E(z)^{S1}$], revertants of the dominant eye color phenotypes produced by

TABLE 1

List of mutations

	Cytology in 67E region	Origin
$E(z)^1$	Normal	a
$E(z)^{S1}$	Normal	b
$E(z)^{1R1}$	T(2;3)21C1-2; 67E3-4	c
$E(z)^{1R2}$	Df(3L)67E3-4; 67F1-3	c
$E(z)^{1R3}$	In(3L)64B; 67E + Df(3L)67E1-2; 67E5-7	c
$E(z)^{1R4}$	Normal	c
$E(z)^{1R5}$	Normal	c
$E(z)^{1R6}$	Normal + In(3L)64E-F; 75C-76B	c
$E(z)^{1R7}$	Normal	c
$E(z)^{1R8}$	Normal	c
$E(z)^{1R9}$	Df(3L)67E3-4; 67E6-7	c
$E(z)^{1R10}$	Normal	c
$E(z)^{1R11}$	Normal	c
$E(z)^{1R12}$	Df(3L)67E1-4; 68A1-2	c
$E(z)^{1R13}$	Df(3L)67D9-13; 68F	c
$E(z)^{1R14}$	Normal	c
$E(z)^{S1R1}$	Df(3L)67E1-2; 67E3-5	d
$E(z)^{S1R2}$	Normal	d
$E(z)^{S1R3}$	Normal	d
$E(z)^{S2}$	Normal	e
$E(z)^{S3}$	Normal	e
$E(z)^{S4}$	Normal	e
$E(z)^{S5}$	Normal	e
$E(z)^{S6}$	Df(3L)67E1-4; 67F1-3	e
Df(3L)1xd^6	Df(3L)67E1-2; 68Cl-2	f
Df(3L)1xd^{15}	Df(3L)67E; 68C10-15	f
Dp(3;3)S2a3	Dp(3;3)67D9-11; 68A1-2	g

a, EMS-induced dominant Enhancer of z^1 w^+/Y eye color (KALISCH and RASMUSON 1974); b, EMS-induced dominant Suppressor of z^1 w^{is}/Y eye color (WU *et al.* 1989); c, γ-ray-induced revertants of the gain-of-function $E(z)^1$ eye color; d, γ-ray-induced revertants of the gain-of-function $E(z)^{S1}$ eye color; e, EMS-induced allelic recessive lethals; f, X-ray-induced recessive alleles of *1xd* (SCHOTT, BALDWIN and FINNERTY 1986); g, X-ray-induced derivative of *Dp(3;3)S2* (CRAYMER 1984).

TABLE 2

Effects of *E(z)* alleles on the z^1 w^{is}/Y eye color

	$E(z)^y$		
	$E(z)^+$	$E(z)^{S2}$	
$E(z)^x$	25°	25°	29°C
$E(z)^+$	Orange	Orange	Orange
$E(z)^1$	Yellow	Red/yellow	Wild type
$E(z)^{S1}$	Reddish	Wild type	†
$E(z)^{1R1}$	Dark orange	Reddish	Wild type
$E(z)^{1R2}$	Dark orange	Reddish	Wild type
$E(z)^{1R3}$	Dark orange	Reddish	Wild type
$E(z)^{1R4}$	Dark orange	Wild type	Wild type
$E(z)^{1R5}$	Dark orange	Reddish	Wild type
$E(z)^{1R6}$	Dark orange	Reddish	Wild type
$E(z)^{1R7}$	Dark orange	Reddish	Wild type
$E(z)^{1R8}$	Light orange	Red/yellow	Wild type
$E(z)^{1R9}$	Dark orange	Reddish	Wild type
$E(z)^{1R10}$	Dark orange	Reddish	Wild type
$E(z)^{1R11}$	Dark orange	Reddish	Wild type
$E(z)^{1R14}$	Dark orange	Reddish	Wild type
$E(z)^{1R15}$	Dark orange	Reddish	Wild type
$E(z)^{S1R1}$	Dark orange	Reddish	Wild type
$E(z)^{S1R2}$	Dark orange	Reddish	Wild type
$E(z)^{S2}$	Orange	Reddish	Wild type
$E(z)^{S3}$	Reddish orange	Wild type	†
$E(z)^{S4}$	Dark orange	Reddish	Wild type
$E(z)^{S5}$	Dark orange	Reddish	†
$E(z)^{S6}$	Dark orange	Reddish	Wild type
1(3)67Fa^1	Dark orange	Reddish	Wild type
1(3)67Fa^3	Dark orange	Reddish	Wild type
1(3)67Fa^7	Dark orange	Reddish	Wild type
Df(3L)1xd^6	Dark orange	Reddish	Wild type

sc z^1 w^{is}; $E(z)^{S2}$ e^{11}/*TM3, Sb Ser* females were crossed to $E(z)^x$/*TM3, Sb Ser* males and the progeny reared at 25° or 29°. Eye colors of *sc* z^1 w^{is}/Y; $E(z)^x/E(z)^+$ and *sc* z^1 w^{is}/Y; $E(z)^x/E(z)^{S2}$ males were determined by scoring *Sb Ser* and Sb^+ Ser^+ males, respectively. †, die prior to eye pigmentation.

the gain-of-function $E(z)^1$ and $E(z)^{S1}$ alleles, and mutations isolated on the basis of their allelic recessive lethality. Cytogenetic properties of these alleles are summarized in Table 1.

Dominant gain-of-function mutations: The $E(z)$ gene can be mutationally altered to either enhance or suppress the z eye color phenotype. These states are represented by the cytologically normal alleles $E(z)^1$ and $E(z)^{S1}$, respectively. Normally, z^1 w^+/Y males are wild type in eye color, but when heterozygous for $E(z)^1$, z^1 w^+/Y males display brownish eyes (KALISCH and RASMUSON 1974). This allele can thus be termed a dominant enhancer of *zeste* (from which the name of the locus derives). Females homozygous for z^1 and w^+ are yellow-eyed. In the presence of the $E(z)^{S1}$ allele, such females are orange-eyed (WU *et al.* 1989). This second type of allele, $E(z)^{S1}$, can thus be termed a dominant suppressor of *zeste*. $E(z)^1$ and $E(z)^{S1}$ map within the same chromosomal region (WU *et al.* 1989 and see below), and the *trans*-heterozygote of the two mutations are inviable, indicating that they share a recessive lethal defect. Thus we conclude that $E(z)^1$ and $E(z)^{S1}$ are lesions in the same genetic unit.

The eye color of z^1 w^{is}/Y males is a particularly sensitive indicator of alterations in $E(z)$ function. w^{is} confers a wild-type eye color in a z^+ background. However, it is hypersensitive to z^1 such that even a single unpaired copy of w^{is} in a z^1 background (*e.g.*, in z^1 w^{is}/Y males: RASMUSON 1962) produces orange eyes. In the presence of $E(z)^1/+$, z^1 w^{is}/Y males display yellow eyes [PERSSON (1976b) and Table2]. Likewise, the eye color of z^1 w^{is}/Y males is suppressed to a reddish, but not wild-type color, by heterozygosity for $E(z)^{S1}$ (Table 2).

Reduction of $E(z)^+$ activity suppresses the z^1 eye color. Deletions of the $E(z)$ gene act as weak dominant suppressors of *zeste*, as indicated by the observation that z^1 w^{is}/Y; *Df(3L)*$E(z)^-/E(z)^+$ males have an eye color that is a darker shade of orange than that produced by z^1 w^{is}/Y; $E(z)^+/E(z)^+$ males (Table 2). In contrast to this weak loss-of-function dominance, the dominant effects of $E(z)^1$ and $E(z)^{S1}$ are both due to gain-of-function activities. $E(z)^{S1}$ is antimorphic, since z^1 $w^{is}/$

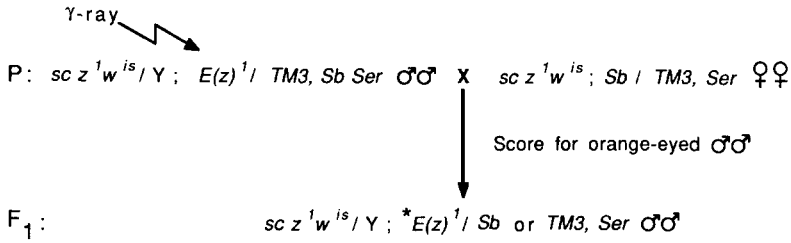

FIGURE 1.—Reversion of $E(z)^1$ gain-of-function eye color phenotype. *TM3, Sb Ser* is lethal in *trans* with both *Sb* and *TM3, Ser*. Therefore, all progeny carry the mutagenized $E(z)^1$ chromosome and, with the exception of $E(z)^1$ revertant males, have yellow eyes. Similar mutageneses were employed to isolate revertants of the $E(z)^{S1}$ antimorphic eye color phenotype. However, females bearing different third chromosomes were used.

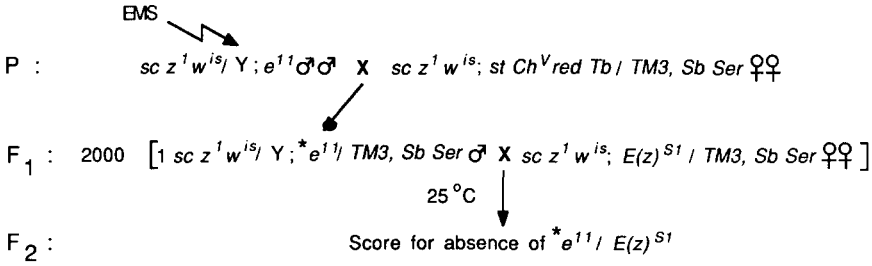

FIGURE 2.—F_2 lethal mutagenesis used to isolate $E(z)$ alleles. Of the 2000 crosses of individual males to two tester females, ~1800 were fertile. From those crosses in which no $*e^{11}/E(z)^{S1}$ progeny were recovered, $*e^{11}/TM3$ males were crossed to $E(z)^{S1}/TM3$ and $E(z)^1/TM3$ females to confirm the failure of the respective $*e^{11}$ chromosomes to complement the recessive lethality of these $E(z)$ alleles.

Y; $E(z)^{S1}/E(z)^+$, $Dp(3;3)E(z)^+$ males have dark orange-colored eyes, that are lighter than $z^1\ w^{is}/Y$; $E(z)^{S1}/E(z)^+$ but darker than $z^1\ w^{is}/Y$; $E(z)^+/E(z)^+$ males. $E(z)^1$ may be neomorphic in its enhancement of the z^1 eye color as $z^1\ w^{is}/Y$; $E(z)^1/E(z)^+$ $Dp(3;3)E(z)^+$ males have eyes which are very similar in color to $z^1\ w^{is}/Y$; $E(z)^1/+$. However, since this effect of an extra dose of $E(z)^+$ on $E(z)^1$ is somewhat variable, we will simply refer to $E(z)^1$ as a gain-of-function allele.

Reversions of dominant *E(z)* alleles: The dominant eye color phenotypes of both $E(z)^1$ and $E(z)^{S1}$ can be reverted by mutationally inactivating the locus, again arguing that these dominant phenotypes are due to gain-of-function. By γ-irradiating males carrying the $E(z)^1$ allele and scoring their $z^1\ w^{is}/Y$ sons for loss of the gain-of-function yellow eye color phenotype, we have isolated 14 $E(z)^1$ revertants among ~86,000 flies scored (Figure 1 and Table 1). Comparable screens have produced 3 revertants of the $E(z)^{S1}$ eye color among ~34,000 F_1 flies scored (Table 1). With two exceptions, as will be described below, these revertants all behave as null alleles of $E(z)$. In addition, 3 second site mutations that at least partially suppress the $E(z)^1$ eye color phenotype, but which are easily separable from the original $E(z)^1$ allele by recombination, were recovered (data not shown). When separated from $E(z)^1$, these act as weak dominant suppressors of z^1.

Mutations isolated as allelic recessive lethal alleles: Additional mutant alleles of the $E(z)$ locus were induced with EMS and identified by their failure to complement the recessive lethality of $E(z)^{S1}$ (Figure 2). Out of ~1800 lines tested, 5 possessed new $E(z)$ alleles designated $E(z)^{S2}$ through $E(z)^{S6}$ (Table 1). At 29°, each of these is lethal in *trans* with $E(z)^1$, $Df(3L)lxd^6$ and $Df(3L)lxd^{15}$, and *inter se* (Table 3 and data not shown). $E(z)^{S2}$ through $E(z)^{S5}$ are cytologically normal, while $E(z)^{S6}$ is a deficiency of polytene bands 67E1–4 to 67F1–3. $E(z)^{S4}$ through $E(z)^{S6}$ behave essentially as amorphic alleles. $E(z)^{S2}$ is a temperature-sensitive allele, which will be described more fully below. $E(z)^{S3}$ is a weakly antimorphic allele, as the eye color of $z^1\ w^{is}/Y$; $E(z)^{S3}/E(z)^+$ males is intermediate between the phenotypes of $z^1\ w^{is}/Y$; $E(z)^{S1}/E(z)^+$ and $z^1\ w^{is}/Y$; $Df(3L)E(z)^-/E(z)^+$ (Table 2).

Chromosomal localization of *E(z)*: $E(z)^1$ has been recombinationally mapped to position 34.0 on the left arm of the third chromosome (KALISCH and RASMUSON 1974). Cytologically aberrant revertants of the gain-of-function $E(z)^1$ and $E(z)^{S1}$ alleles have allowed us to refine the location of $E(z)$, previously localized to polytene bands 67E1–2 through 68A2–3 (WU *et al.* 1989). $E(z)^{1R2}$, $E(z)^{1R3}$, $E(z)^{1R9}$, $E(z)^{1R12}$, $E(z)^{1R13}$ and $E(z)^{S1R1}$ are deficiencies which remove some or all of the 67E-F region (Table 1). Two of these, $E(z)^{S1R1}$ and $E(z)^{1R9}$, are deficiencies which extend from 67E1–2 to 67E3–5 and 67E3–4 to 67E6–7, respectively. $E(z)^{1R1}$ is a translocation between chromosomes *2* and *3*, with its third chromosome breakpoint in 67E3–4. Thus, all available data are consistent with the localization of the $E(z)$ gene in 67E3–4.

***E(z)* is allelic to *l(3)67Fa*:** The *l(3)67Fa* locus (LINDSLEY and ZIMM 1986), formerly named *l(3)1902*, was identified on the basis of its early pupal lethal phenotype (SHEARN 1977; SHEARN, HERSPERGER and HERSPERGER1978; SHEARN *et al.* 1978). Individuals which are homozygous for *l(3)67Fa* null alleles die shortly after pupation with undersized imaginal discs. Because of similar lethal phases, homeotic phenotypes (see below) and chromosomal locations, we tested $E(z)^1$ and $E(z)^{S1}$ for complementation of three cytologically normal, EMS-induced recessive lethal alleles of *l(3)67Fa* [$l(3)67Fa^1$, $l(3)67Fa^3$ and $l(3)67Fa^7$] (LINDSLEY and ZIMM 1986). Both $E(z)^1$ and $E(z)^{S1}$ die in *trans* with each of these *l(3)67Fa* alleles and therefore appear to be allelic. In addition, these three alleles of

TABLE 3

Lethal phases and homeotic phenotypes produced by *E(z)* alleles

		$E(z)^{S2}$			
		25°		29°	
	$Df(3L)1xd^{15}$	MESO/META basitarsi	PRO 2nd tarsal segment	MESO/META basitarsi	PRO 2nd tarsal segment
$E(z)^{1}$	P	+	−	−	++
$E(z)^{S1}$	<L3	+++	−	†	†
$E(z)^{1R1}$	<L3	+	−	+++	−
$E(z)^{1R2}$	<L3	+	−	+++	−
$E(z)^{1R3}$	<L3	+	−	+++	−
$E(z)^{1R4}$	<L3	+	−	+++	−
$E(z)^{1R5}$	L/P	+	−	+++	−
$E(z)^{1R6}$	L/P	+	−	+++	+
$E(z)^{1R7}$	L/P	+	−	+++	−
$E(z)^{1R8}$	P	+	−	+++	+
$E(z)^{1R9}$	<L3	+	−	+++	−
$E(z)^{1R10}$	L/P	+	−	+++	−
$E(z)^{1R11}$	L/P	+	−	+++	−
$E(z)^{1R14}$	L/P	+	−	+++	−
$E(z)^{1R15}$	L/P	+	−	+++	−
$E(z)^{S1R1}$	<L3	+	−	+++	−
$E(z)^{S1R2}$	L/P	+	−	+++	−
$E(z)^{S2}$	AV	+	−	+++	−
$E(z)^{S3}$	L	−	++*	†	†
$E(z)^{S4}$	P	+	−	+++	−
$E(z)^{S5}$	P	+	−	†	†
$E(z)^{S6}$	<L3	+	−	+++	−
$l(3)67Fa^{1}$	P	+	−	+++	+
$l(3)67Fa^{3}$	P	+	−	+++	+
$l(3)67Fa^{7}$	P	+	−	+++	+
$Df(3L)1xd^{6}$	<L3	+	−	+++	−

<L3, lethal prior to late third instar larvae; L, primarily late third instar lethality; L/P, mixture of late third instar and early pupal lethality; P, early pupal lethality; AV, adult viable. MESO/META basitarsi, ectopic sex comb teeth on basitarsi of male mesothoracic and metathoracic legs: +, a fraction of males have one or a few ectopic sex comb teeth (see Figure 4a); ++, all males have at least several ectopic sex comb teeth (see Figure 4b); +++, virtually complete sex combs on mesothoracic and metathoracic legs (see Figure 3e, f). PRO 2nd tarsal segment, ectopic sex comb teeth on the second tarsal segment of male prothoracic legs: −, essentially wild type; +, a small percentage of males with one or two ectopic sex comb teeth; ++, virtually 100% penetrance with one to several ectopic sex comb teeth per prothoracic leg. *, reduced number of sex comb teeth on basitarsi and partial loss of transverse rows on the tibia and basitarsi of the prothoracic legs. †, early pupal lethality.

l(3)67Fa display mutant eye color and adult homeotic phenotypes in *trans* to the temperature-sensitive $E(z)^{S2}$ allele (Tables 2 and 3). Because of its chronological priority, we will refer to the locus by its original published name, *E(z)* (KALISCH and RASMUSON 1974).

Modification of the *zeste-white* interaction by *E(z)* alleles

Modification of eye pigmentation by all tested *E(z)* alleles occurs only in z^{1}-containing genotypes (with the exception of the *P[(w,ry)A^R]4–24 white* transposon, which will be described in the DISCUSSION). Although only w^{+}, w^{is} and w^{a} have been tested, it seems that this modification of eye color is independent of a specific *white* allele. For example, $E(z)^{1}$ enhances and $E(z)^{S1}$ suppresses the z^{1} w^{a} phenotype, but they do not modify z^{+} w^{a} and have very little, if any, effect on z^{a} w^{a} (KALISCH and RASMUSON 1974 and our observations). z^{a} alleles are hypomorphic or amorphic *zeste* alleles (KAUFMAN, TASAKA and SUZUKI 1974; GOLDBERG, COLVIN and MELLIN 1989).

Temperature-sensitive suppression of the *zeste*^{1} eye color: The $E(z)^{S2}$ mutation has proven to be an extremely valuable allele for examining the phenotypic effects of the loss of $E(z)^{+}$ activity. As will be documented below, $E(z)^{S2}$ is a temperature-sensitive allele that behaves as if it has reduced $E(z)^{+}$ activity at restrictive temperatures (25°–29°). Because of this residual $E(z)^{+}$ activity, *trans*-heterozygotes of many other *E(z)* alleles with $E(z)^{S2}$ survive to pharate pupal or adult stages. In contrast, those same alleles would engender earlier death when heterozygous with an *E(z)* null allele, precluding the observation of adult cuticular phenotypes. Thus, more severe adult eye color and homeotic abnormalities can be examined in an $E(z)^{S2}$ background than in any other genotypes viable to adulthood. In addition, the phenotypes produced by $E(z)^{S2}$ in trans to other *E(z)* alleles has aided in our classification of these alleles with respect to their effects on specific functions. For example, antimorphic alleles produce more severe mutant pheno-

types in *trans* to $E(z)^{S2}$ than do amorphic alleles or deficiencies which uncover the $E(z)$ locus.

$E(z)^{S2}$ is a temperature-sensitive recessive suppressor of the z^1 eye color. $z^1\ w^{is}/Y$; $E(z)^{S2}/E(z)^{S1}$ males reared at 25°, and $z^1\ w^{is}/Y$; $E(z)^{S2}/Df(3L)E(z)^-$ males reared at 29° die during pupation, generally developing to pharate adults with wild-type eye color (Table 2). Under similar conditions, $z^1\ w^+$ or $z^1\ w^{is}$ females with these $E(z)$ genotypes, in which even greater repression of the *white* gene by z^1 presumably occurs, also show complete suppression of the mutant eye color in pharate adults. At 25°, hemizygosity for $E(z)^{S2}$ produces an intermediate level of suppression of the z^1 eye color; specifically, $z^1\ w^{is}/Y$; $E(z)^{S2}/Df(3L)E(z)^-$ males have reddish, but not wild-type, eyes (Table 2). At 22°, such males have eyes indistinguishable from those that are hemizygous for $E(z)^+$. $E(z)^{S4}$ through $E(z)^{S6}$ show interactions with $E(z)^{S2}$ similar to those engendered by known deletions of the locus (Table 2), and are therefore considered to be amorphic with respect to eye color modification. However, at 29°, $E(z)^{S5}$ in *trans* to $E(z)^{S2}$ produces early pupal lethality (Tables 2 and 3) prior to pigmentation of the eyes. As exemplified by the phenotype of $z^1\ w^{is}/Y$; $E(z)^{S3}/E(z)^{S2}$ males reared at 25°, the antimorphic $E(z)^{S3}$ allele produces pharate adult lethality and suppression of the $z^1\ w^{is}$ eye color to wild type.

An intriguing aspect of the $E(z)^1$ allele is revealed by its interaction with $E(z)^{S2}$. At 29°, $z^1\ w^{is}/Y$ males (as well as $z^1\ w^{is}$ females) which are $E(z)^1/E(z)^{S2}$ have wild-type eye color (Table 2). The strong suppression of z^1 in these *trans*-heterozygotes suggests that the gain-of-function enhancement of z^1 by $E(z)^1$ requires $E(z)^+$ activity. When insufficient amounts of $E(z)^+$ are produced by the homolog, $E(z)^1$ behaves as a loss or reduction of function allele resulting in suppression of z^1. At 25°, males of this same genotype have eyes which are wild type in the anterior 2/3 to 3/4 and yellow in the remaining posterior (Table 2). We presume that at this lower temperature, $E(z)^{S2}$ produces more $E(z)^+$ activity than it does at 29°, leading to expression of the gain-of-function $E(z)^1$ eye color in the posterior portion of the eye. While the ontogeny of such anterior-posterior mosaic eye colors are not understood, several examples of such patterns resulting from misregulation of the *white* gene have been identified (HAZELRIGG, LEVIS and RUBIN 1984; LEVIS, HAZELRIGG and RUBIN 1985a; and our observations). It is possible that this reflects the posterior to anterior direction of differentiation across the eye disc and effects on the timing of *white* gene expression.

With the exception of $E(z)^{1R4}$ and $E(z)^{1R8}$, revertants of $E(z)^1$ and $E(z)^{S1}$ generally behave as null alleles in their $z^1\ w^{is}$ eye color interactions with $E(z)^{S2}$ (Table 2). $E(z)^{1R4}$ is weakly antimorphic in that at 25° $z^1\ w^{is}/Y$; $E(z)^{1R4}/E(z)^{S2}$ *trans*-heterozygous males show a stronger suppression than do $z^1\ w^{is}/Y$; $Df(3L)E(z)^-/E(z)^{S2}$ males (Table 2). Thus, in the case of $E(z)^{1R4}$, $E(z)^1$ has been mutated from a gain-of-function Enhancer of z^1 to a weakly antimorphic state which suppresses the z^1 eye color. $E(z)^{1R8}$ behaves as a partial revertant of $E(z)^1$ in that (1) $z^1\ w^{is}/Y$; $E(z)^{1R8}/E(z)^+$ males have eyes of a much lighter shade of orange than do such males that are hemizygous for $E(z)^+$, and (2) $z^1\ w^{is}$; $E(z)^{1R8}/E(z)^{S2}$ flies produce temperature-sensitive eye colors very similar to those of $z^1\ w^{is}$; $E(z)^1/E(z)^{S2}$ *trans*-heterozygotes (Table 2).

Effects of *E(z)* mutations on homeotic gene expression

Pupal/adult homeotic phenotypes due to altered zygotic expression of *E(z)*: The antimorphic $E(z)^{S1}$ allele causes a weak dominant homeotic phenotype. Approximately 35% of $E(z)^{S1}/+$ males exhibit ectopic sex comb teeth on their mesothoracic legs (WU *et al.* 1989). $E(z)^{S2}/E(z)^{S1}$ animals reared at 25° die as pharate adults with severe homeotic transformations (Table 3 and Figure 3, d–f) similar to those described for animals homozygous for temperature-sensitive alleles of *l(3)67Fa* (SHEARN, HERSPERGER and HERSPERGER 1978). The mesothoracic and metathoracic legs are transformed toward the prothoracic state as shown by the nearly complete sex combs on both mesothoracic and metathoracic legs of males. In addition, their external abdomens are poorly developed. The hemitergites and hemisternites often fail to fuse, leaving large gaps in the cuticle (data not shown). It is not clear if this latter phenotype is homeotic. $E(z)^{S2}$ homozygotes and hemizygotes are viable at 25°; a small percentage of hemizygous $E(z)^{S2}$ males display at least one sex comb tooth on their mesothoracic legs (Table 3 and Figure 4a) and a variable transformation of abdominal segments toward a more posterior state. The fourth abdominal segment (A4) is partially transformed toward the fifth (A5), as evidenced by the ectopic black pigmentation on A4 in males (Figure 5b). A6 is partially transformed toward A7. In females this is shown by the A7-like appearance of sternite hairs on A6 (Figure 5e). Males show a reduction in the size of A6 (Figure 5b). Since adult males do not normally have an A7 tergite, this may also be a transformation of A6 to A7. At 29°, $E(z)^{S2}$ hemizygotes die as early to late pupae (Table 3). Those surviving to pharate stages display essentially the same phenotypes as do $E(z)^{S2}/E(z)^{S1}$ individuals at 25°. Approximately 1/5 to 1/3 of the individuals homozygous for $E(z)^{S2}$ at 29° survive to adulthood with the remainder dying as pharate adults, suggesting that $E(z)^{S2}$ is somewhat leaky even at 29°. At 18° or 22°, $E(z)^{S2}$ homozygotes and hemizygotes display wild-type morphology. In contrast, even at 18°, $E(z)^{S2}/E(z)^{S1}$ adult males display an elevated frequency of ectopic sex comb teeth on their mesothoracic legs compared to $E(z)^{S1}/$

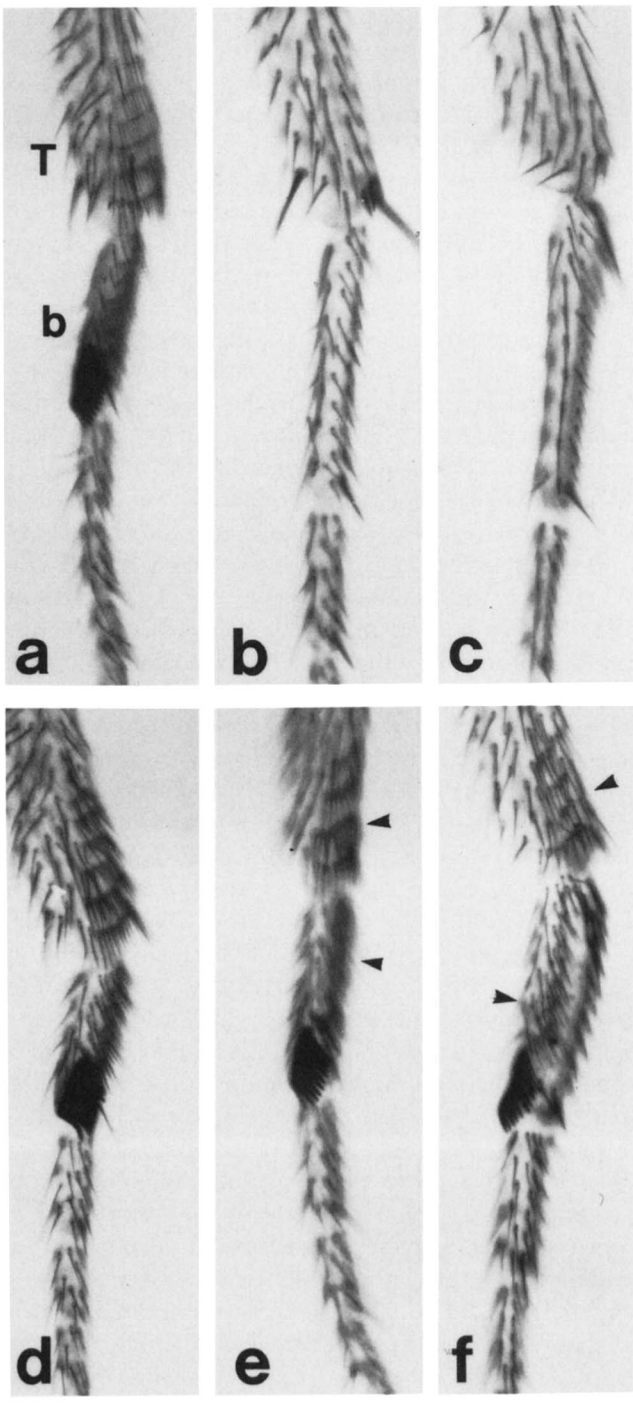

FIGURE 3.—Anteriorly directed homeotic transformations of the mesothoracic and metathoracic legs towards the prothoracic state by $E(z)$ mutations. Legs of (a–c) *Canton-S* and (d–f) $E(z)^{S1}$ /$E(z)^{S2}$ males reared at 25°. The tibia and basitarsus are designated T and b, respectively. (a and d) Prothoracic; (b and e) mesothoracic; (c and f) metathoracic; (e and f) in addition to virtually complete sex combs on their basitarsi, these mesothoracic and metathoracic legs have transverse rows on the tibia and basitarsus, characteristic of prothoracic legs. Anterior is oriented to the left.

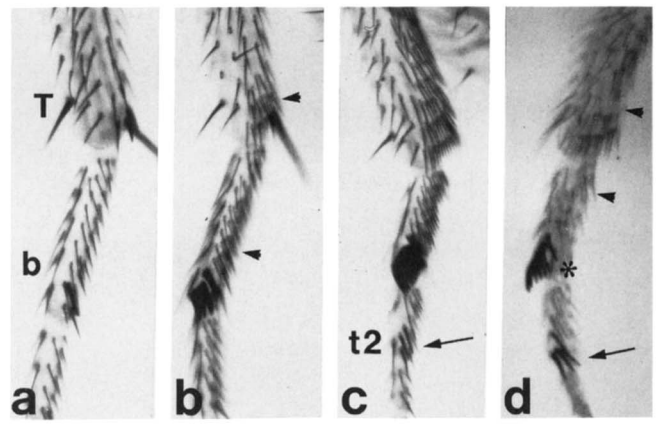

FIGURE 4.—Additional leg phenotypes produced by $E(z)$ mutations. All are the legs of males reared at either (a, b, and d) 25° or (c) 29°. The tibia, basitarsus and second tarsal segment are designated T, b and t2, respectively. (a) $E(z)^{S2}/Df(3L)lxd^{15}$ mesothoracic leg with a single sex comb tooth on the basitarsus. (b) $E(z)^{S2}/E(z)^{1R4}$ mesothoracic leg with ~7 sex comb teeth on the basitarsus in addition to a few transverse rows on the tibia and basitarsus (arrowheads). (c) Prothoracic leg of $E(z)^{S2}$ /$E(z)^{1}$ with ectopic sex comb teeth on the second tarsal segment (arrow). (d) Prothoracic leg of $E(z)^{S2}$ /$E(z)^{S3}$ with loss of transverse rows on the tibia and basitarsus (arrowheads) and sex comb teeth on the basitarsus (*), in addition to ectopic sex comb teeth on the second tarsal segment (arrow).

$E(z)^{+}$ (recall that $E(z)^{S1}$ is antimorphic). Therefore, it seems that $E(z)^{S2}$ is not fully functional at 18° nor amorphic at 29°.

$E(z)^{1}/E(z)^{S2}$ *trans*-heterozygotes at 29° are pharate adult lethal. Unlike individuals which are hemizygous for $E(z)^{S2}$, these pharates display little or no transformation of the mesothoracic or metathoracic legs toward the prothoracic state. However, $E(z)^{1}/E(z)^{S2}$ male pharates do display an ectopic sex comb, comprising 1–3 teeth, on the second tarsal segment of their prothoracic legs in addition to a normal sex comb on their basitarsi (Table 3 and Figure 4c). This phenotype is very similar to that produced by mutant alleles of *cramped* (LINDSLEY and ZIMM 1985), also known as *sparse arista* (RAYLE and GREEN 1968).

The antimorphic $E(z)^{S3}$ allele also produces unusual phenotypes in combination with $E(z)^{S2}$. At 25°, individuals which are $E(z)^{S3}/E(z)^{S2}$ die as pharate adults, show a very weak, if any, anteriorly directed transformation of their mesothoracic or metathoracic legs, and display sex comb teeth on the second tarsal segment of their prothoracic legs (Table 3 and Figure 4d). Unlike $E(z)^{1}/E(z)^{S2}$ reared at 29°, $E(z)$ $^{S3}/E(z)^{S2}$ pharates reared at 25° have reduced numbers of sex comb teeth on the basitarsi and transverse rows on the tibia and basitarsi (Figure 4d). This phenotype is suggestive of a partial transformation of prothoracic legs towards the mesothoracic state similar to that produced by hemizygosity for the Scr^{+} gene (STRUHL 1982).

Revertants of the dominant modifiers of the *z-w* interaction generally act as loss-of-function mutations with regard to homeotic phenotypes: With two exceptions, mutant $E(z)$ alleles, which were selected on the basis of reverting the gain-of-function eye color phenotypes of $E(z)^{1}$ behave as loss-of-function alleles

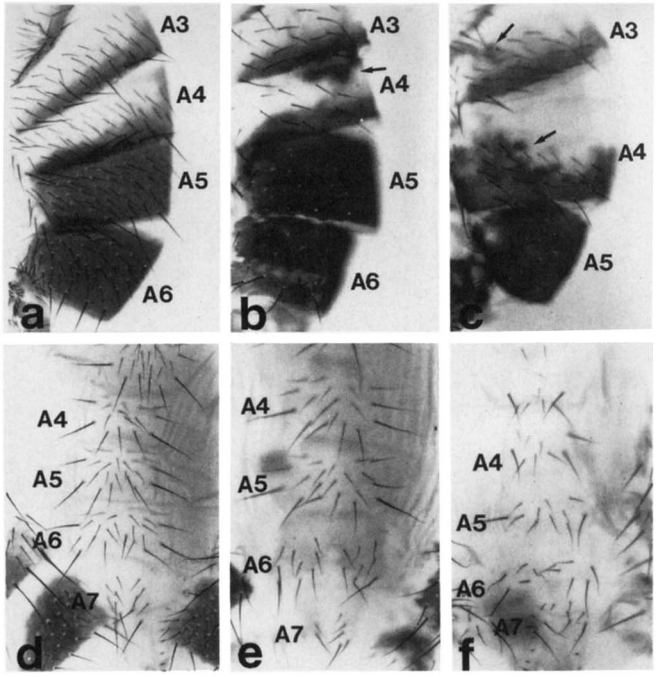

FIGURE 5.—Abdominal homeotic phenotypes produced by $E(z)$ mutations. (a–c) Male tergites and (d–f) female sternites. (a and d) *Canton-S*; (b and e) $E(z)^{S2}/E(z)$1R3 reared at 25°; (c and f) $E(z)^{S2}/E(z)^{1R4}$ reared at 25°. (a) Wild-type size and pigmentation of the third to sixth (A3–A6) male abdominal tergites. (b) Mild posteriorly directed transformation of A4 and A6 is shown by patches of dark pigmentation in A4 (arrow) and slight reduction in size of A6. (c) More extensive dark pigmentation in A4 and patches of pigmentation in A3 (arrows), and virtual absence of A6 are characteristic of stronger posteriorly directed abdominal transformation. (d) Wild-type laterally directed sternite hairs of A4–A6 and posteriorly oriented hairs of A7. (e) Posteriorly oriented A6 sternite hairs suggests a partial homeotic transformation of A6 toward A7. (f) More anterior sternites show posteriorly oriented hairs, suggesting a stronger transformation toward A7.

with respect to the homeotic phenotypes they produce in trans with $E(z)^{S2}$ (Table 3). That is, at 29° these *trans*-heterozygotes die as pharate adults and display strong transformations of mesothoracic and metathoracic legs toward the prothoracic state, and a reduced penetrance (in most cases a virtual elimination) of the second tarsal segment sex comb phenotype. One exception is $E(z)^{1R4}$, which as described above behaves as a weakly antimorphic suppressor of the z^1 w^{is} eye color. $E(z)^{1R4}$ is also antimorphic with respect to the homeotic phenotypes it produces in *trans* with $E(z)^{S2}$. At 25°, approximately two-thirds of $E(z)^{1R4}/E(z)^{S2}$ *trans*-heterozygotes die as pharate adults while one-third survive as viable adults. These *trans*-heterozygotes show more severe leg and abdominal homeotic phenotypes than do flies which are hemizygous for $E(z)^{S2}$ (Table 3 and Figures 4b and 5, c and f). At 29°, $E(z)^{1R4}/E(z)^{S2}$ individuals die as early pupae (Table 3). The other exception is $E(z)^{1R8}$, which acts as a loss-of-function allele in that at 29° $E(z)^{1R4}/E(z)^{S2}$ males show an extreme mesothoracic and metathoracic extra sex comb phenotype (Table 3). However, $E(z)^{1R8}/E(z)^{S2}$ males are not reverted for the ectopic second tarsal sex comb teeth of $E(z)^1/E(z)^{S2}$. This ectopic sex comb is absent in males heterozygous for $E(z)^{S2}$ and for revertant alleles associated with deletion of the gene (Table 3).

Revertants of the antimorphic $E(z)^{S1}$ eye color phenotype also revert its antimorphic homeotic phenotypes to the null state (Table 3). Both $E(z)^{S1R1}/E(z)^{S2}$ and $E(z)^{S1R2}/E(z)^{S2}$ animals reared at 25° survive to adulthood and produce homeotic phenotypes essentially identical to those of $E(z)^{S2}$ hemizygotes. Indeed, $E(z)^{S1R1}$ is a cytologically visible deficiency (Table 1).

Embryonic phenotypes in the absence of $E(z)^+$ maternal product: The requirement for maternally produced $E(z)^+$ was examined using the $E(z)^{S2}$ allele. At permissive temperatures, male and female $E(z)^{S2}$ homozygotes, hemizygotes, and *trans*-heterozygotes with other mutant $E(z)$ alleles are viable and fertile. When such adult females are elevated to 29°, resulting embryos reared at this restrictive temperature exhibit embryonic lethality. This maternal-effect phenotype is modulated by the zygotic $E(z)$ genotype. This is apparent from a comparison of the results of three crosses, in which females hemizygous or homozygous for $E(z)^{S2}$ were crossed to males homozygous for $E(z)^{S2}$ (cross 1), homozygous for $E(z)^+$ (cross 2), or heterozygous for $E(z)^+$ *Dp(3;3)*$E(z)^+$ and an $E(z)^+$ balancer chromosome (cross 3). As exemplified in Figure 6, b and c, all of the embryos from crosses 1 and 2 died as late embryos with cuticle patterns exhibiting homeotic transformations characteristic of Polycomb-group mutations (STRUHL 1981). Lack of both maternal and paternal $E(z)^+$ (cross 1) produces embryos in which T1 through A8 all develop with the cuticular structures characteristic of A8 (Figure 6b). In addition, an A8-like ventral setal belt appears on a head segment and another patch of abdominal-like denticles appears on the dorsal side of the head. A9 also appears to be partially transformed toward A8 as evidenced by the patch of denticle teeth anterior to the anal pads. This phenotype is essentially identical to that produced by complete lack of maternal and zygotic *extra sex comb*$^+$ (*esc*) (STRUHL 1981). Embryos carrying one copy of a paternally contributed $E(z)^+$ exhibit partial rescue of the transformations at the anterior end (inferred from the phenotype of all the progeny of cross 2 and half of the progeny of cross 3) (Figure 6c). In these embryos, the head still fails to involute, but some rudimentary gnathal structures develop and the thoracic segments are often slightly less abdominal-like. The phenotypes of embryos containing two paternally derived copies of $E(z)^+$ show even greater rescue. Half of the progeny of cross 3 are expected to be of this genotype. Of this progeny class, ~10% hatch but die as first instar larvae. Some of these larvae have a virtually wild-type cuticular

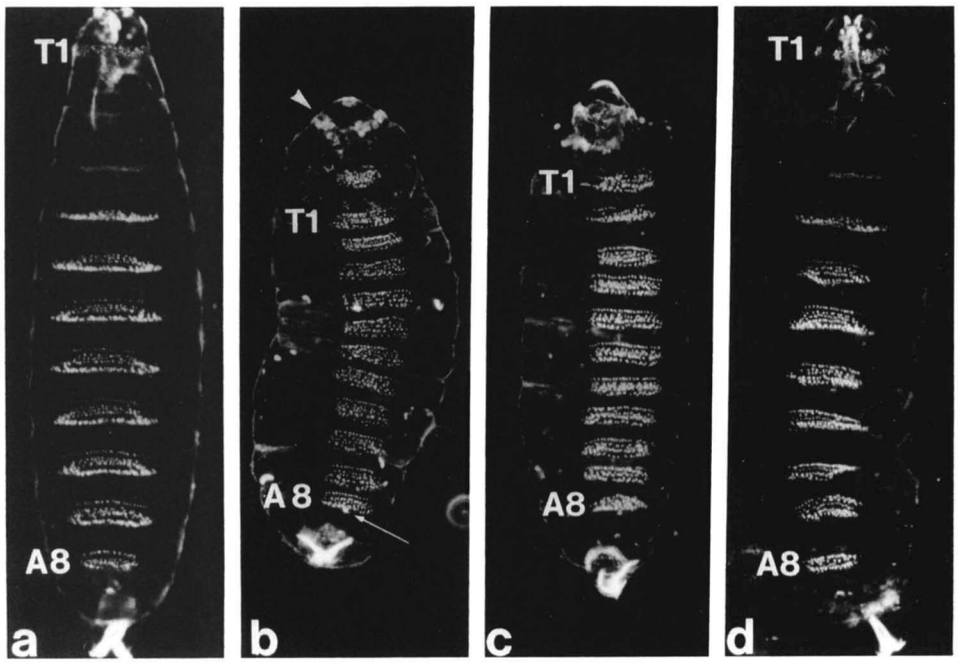

FIGURE 6.—Embryonic homeotic phenotypes. (a) Ventral view of a *cn*; ry^{42} embryo with wild-type cuticular morphology. The first thoracic and eighth abdominal segments are designated T1 and A8, respectively. (b–d) Embryos produced at 29° by homozygous $E(z)^{S2}$ mothers mated to (b) homozygous $E(z)^{S2}$, (c) *Canton-S*, or (d) $E(z)^{+}$, $Dp(3;3)E(z)^{+}/TM3$ males. (b) Abdominal-like denticle teeth on the dorsal side of the head and anterior to the anal pads are pointed out by an arrowhead and arrow, respectively.

phenotype, while others show mild posteriorly directed transformations (Figure 6d).

E(z) adult homeotic phenotypes are due to misregulation of segment identity genes

Homeotic transformations produced by other Pc-group mutations have been shown to result from the ectopic expression of segment identity genes of the *ANT-C* and *BX-C* (BEACHY, HELFAND and HOGNESS 1985; STRUHL and AKAM 1985; WEDEEN, HARDING and LEVINE 1986; GLICKSMAN and BROWER 1988a; BUSTURIA and MORATA 1988). The *Sex combs reduced* (*Scr*) gene is required to specify the developmental fate of the cells in the prothoracic leg discs (STRUHL 1982). Normally, the Scr protein is expressed in the prothoracic leg disc epithelial cells, but not in the mesothoracic or metathoracic leg discs (GLICKSMAN and BROWER 1988b; and Figure 7, a–c). Ectopic expression of the Scr protein in mesothoracic and metathoracic leg discs correlates with the homeotic transformation of those legs toward the prothoracic state (GLICKSMAN and BROWER 1988a). In order to ascertain whether the temperature-sensitive homeotic adult leg transformations associated with the $E(z)^{S2}$ allele are due to misregulation of the *Scr* gene, imaginal discs and CNS from $E(z)^{S2}/Df(3L)E(z)^{-}$ larvae reared at 25° and at 29° were stained with an antibody specific for the Scr product (GLICKSMAN and BROWER 1988b). At 25°, essentially no expression of the Scr protein is observed in the mesothoracic or metathoracic leg discs (data not shown). In contrast, at 29°, the Scr protein is expressed at high levels in both mesothoracic and metathoracic leg discs (Figure 7, e and f). Thus, the transformation of mesothoracic and metathoracic legs toward the prothoracic state at 29° *vs.* 25° in $E(z)$ mutants correlates with the ectopic expression of the Scr protein. In addition, some, but not all, wing discs from these larvae show ectopic expression of Scr in a region destined to produce wing hinge structures (Figure 7h). (It is uncertain if $E(z)$ mutant genotypes cause any homeotic transformation in this portion of the wing.) No additional ectopic expression of Scr is observed in the major imaginal discs or CNS of these larvae.

Normally, the Ubx protein is expressed in the metathoracic discs (*i.e.*, the metathoracic leg and halter discs), and in the peripodial membrane, but not epithelial cells, of the wing discs (BROWER 1987 and Figure 8a). Partial transformations of the wings into halteres by other Pc-group mutations is due to ectopic expression of the Ubx protein in the epithelial cells of the wing discs (CABRERA, BOTAS and GARCÍA-BELLIDO 1985; GLICKSMAN and BROWER 1988a). Adult viable $E(z)$ mutants show no mutant wing phenotype. However, since it is difficult to examine the unfolded wings of pharate adults, it is possible that ectopic expression of Ubx in such pharates might not produce an observable phenotype. In order to ascertain if reduction of $E(z)^{+}$ activity also produces ectopic expression of Ubx, we stained the CNS and major imaginal discs of $E(z)^{S2}/Df(3L)E(z)^{-}$ larvae reared at 25° and 29° with an antibody specific for the Ubx protein (WHITE and WILCOX 1985). At 25°, these larvae display a wild-type pattern of Ubx expression. At 29°, in addition to normal expression in the metathoracic leg and halter discs, the Ubx protein is ectopically expressed in the wing disc epithelial cells (Figure 8b). This expression is limited to the posterior compartment of the wing pouch, which is essentially identical to the

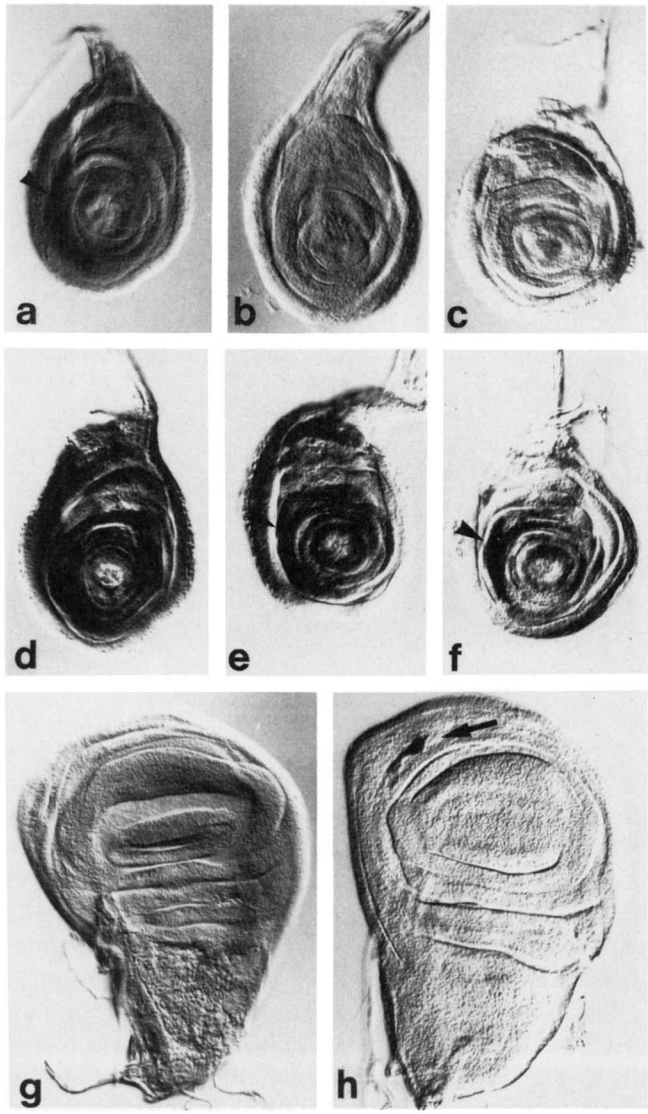

FIGURE 7.—Ectopic imaginal disc expression of the Scr protein caused by reduction of $E(z)^+$ activity. (a and d) Prothoracic leg discs; (b and e) mesothoracic leg discs; (c and f) metathoracic leg discs. (a–c and g) Wild type pattern of Scr expression in discs of a *Canton-S* larva. As described by GLICKSMAN and BROWER (1988b), the Scr protein is most abundant in a crescent shaped pattern in the anterior compartment of the prothoracic leg disc (arrowhead) and is undetectable in the (b) mesothoracic and (c) metathoracic leg discs. (d–f and h) discs from $E(z)^{S2}/Df(3L)E(z)^-$ larvae reared at 29°. In addition to normal expression in the (d) prothoracic leg disc, the Scr protein is also detected in a similar crescent shaped pattern in the anterior compartments of the (e) mesothoracic and (f) metathoracic leg discs (arrowheads). (g) *Canton-S* wing disc showing no detectable expression of the Scr protein. (h) Ectopic expression of Scr in the portion of the wing disc which will give rise to wing hinge structures (arrowhead) (BRYANT 1978).

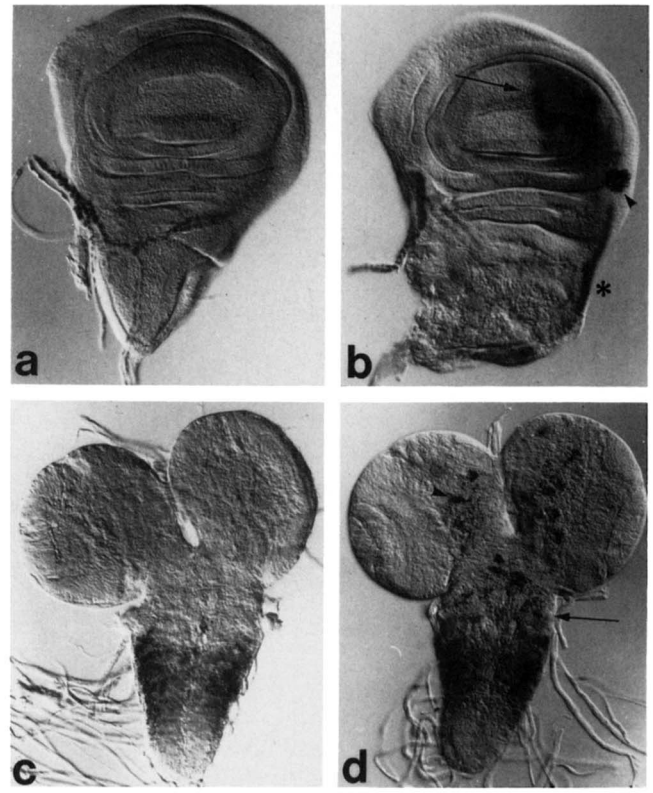

FIGURE 8.—Ectopic expression of the Ubx protein in $E(z)$ mutant larvae. (a) Ubx protein is not detected in the epithelial cells of a *Canton-S* wing disc. (b) Wing disc of an $E(z)^{S2}/Df(3L)E(z)^-$ larva reared at 29° showing ectopic expression in the posterior compartment of the wing pouch (arrow) and the alar lobe (arrowhead) and postnotal (*) regions (BRYANT 1978). (c) Wild-type pattern of Ubx expression in the CNS of a *Canton-S* larva. (d) CNS from an $E(z)^{S2}/Df(3L)E(z)^-$ larva showing, in addition to the normal pattern of expression in the ventral ganglion, ectopic expression in more anterior cells of the ventral ganglion (arrow) and the brain (arrowhead).

pattern observed in esc^- larvae (GLICKSMAN and BROWER 1988a), and the regions from which the alar lobe and postnotum will be derived. As has been shown for *esc* mutant larvae (GLICKSMAN and BROWER 1988a), $E(z)^{S2}/Df(3L)E(z)^-$ larvae reared at 29° produce ectopic expression of the Ubx protein in the CNS (Figure 8d). In addition to its normal pattern of expression, Ubx is expressed more anteriorly in the ventral ganglion as well as in the brain lobes. We have not observed mutant phenotypes associated with any of these patterns of ectopic Ubx expression.

Embryonic homeotic phenotypes are due to misregulation of *BX-C* and *ANT-C* segment identity genes: To determine if the embryonic homeotic phenotypes produced by $E(z)$ mutations are also the result of misregulation of *ANT-C* and *BX-C* genes, we probed embryos produced at restrictive temperature by $E(z)^{S2}$ females with antibodies specific for the Ubx or Scr products. As with the esc^- maternal effect, the pattern of Ubx expression is normal until stage 11 (~6–7 hr postfertilization) (STRUHL and AKAM 1985) (Figure 9e). At this time, in addition to its normal pattern of accumulation, Ubx protein is detected ectopically in more anterior segments of the germ band (Figure 9f). Initially this ectopic signal is strongest in the second parasegment, then becomes uniformly intense throughout the germ band. Upon germ band shortening, Ubx levels in the epidermis decrease while they

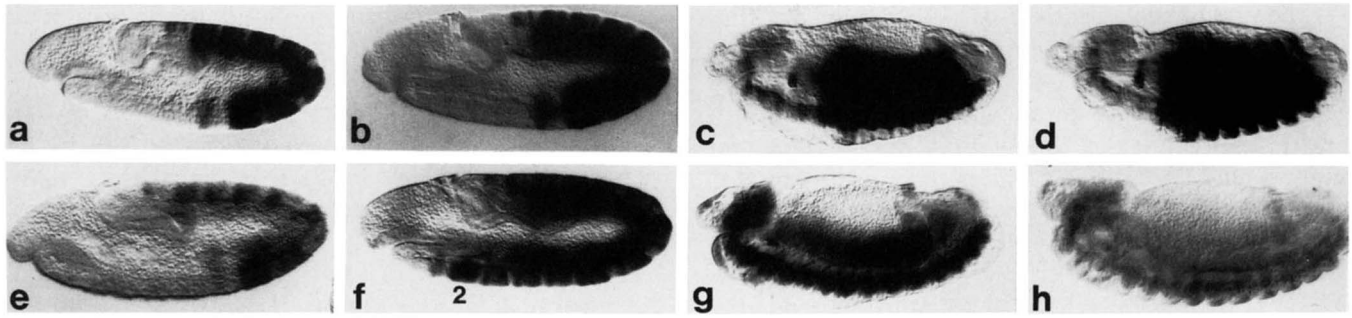

FIGURE 9.—Misregulation of Ubx embryonic expression caused by reduction of maternally provided $E(z)^+$ activity. (a–d) Wild-type pattern of Ubx expression in *cn*; ry^{42} embryos. (e–f) Embryos produced at 29° by homozygous $E(z)^{S2}$ mothers mated to homozygous $E(z)^{S2}$ males. (a and e) Stage 10 embryos; (b and f) stage 11 embryos. Note the strong signal in ps2 (2). (c and g) Stage 13 embryos; (d and h) late stage 13 or early stage 14 embryos. Embryonic stages are as described by CAMPOS-ORTEGA and HARTENSTEIN (1985).

FIGURE 10.—Misregulation of Scr embryonic expression. (a) *cn*; ry^{42} embryo showing wild type pattern of Scr expression in the labial lobe (L) and anterior T1 (arrowhead), and no signal in the maxillary lobe (M). (b) Homozygous $E(z)^{S2}$ embryo produced by a homozygous $E(z)^{S2}$ mother at 29°. In addition to normal expression in the labial lobe (L), Scr is ectopically expressed in the maxillary lobe (M). (c) $E(z)^{S2}$/+ embryo produced by a homozygous $E(z)^{S2}$ mother at 29°. Scr protein is only detected in the labial lobe (L). The embryos in b and c correspond to the embryo cuticles shown in Figure 6, b and c, respectively.

remain high in the CNS (Figure 9g). Late in stage 13, or early stage 14 (~10–11 hr postfertilization) the level of Ubx protein in the CNS decreases to that characteristic of the normal parasegment 13 (ps13) (Figure 9h). This reduction of stable Ubx expression is generally consistent with the transformation of more anterior segments to an A8-like cuticular phenotype.

The partial rescue of embryos derived from $E(z)^{S2}$ mothers by one copy of paternally contributed $E(z)^+$ is reflected in the pattern of accumulation of the Scr product. Normally, Scr is found primarily in the labial segment and anterior T1 (or ps3) (RILEY, CARROLL and SCOTT 1987; MAHAFFEY and KAUFMAN 1987) (Figure 10a). At 29°, $E(z)^{S2}$ homozygous embryos derived from homozygous $E(z)^{S2}$ mothers accumulate Scr normally in the labial lobe but not in T1. In addition, it is found ectopically in the maxillary lobe (Figure 10b). In contrast, $E(z)^+/E(z)^{S2}$ embryos derived from homozygous $E(z)^{S2}$ mothers show only the labial site of expression, with no detectable Scr protein in either the maxillary lobe or T1 segments (Figure 10c). These results differ somewhat from the distribution of Scr protein in Pc^- embryos (RILEY, CARROLL and SCOTT 1987), in which, in addition to expression in the maxillary and labial lobes, Scr is also detected in more posterior segments.

DISCUSSION

Loss or reduction of $E(z)^+$ activity results in both suppression of the z^1 eye color and ectopic expression of at least some of the segment identity genes of the *ANT-C* and *BX-C* gene complexes. We have shown that the latter is due to derepression of these genes, similar to that produced by mutations in other Polycomb-group loci.

It is likely that the suppression of the *zeste*^{1}-*white* interaction by $E(z)$ mutant alleles is also due to gene derepression. $E(z)$ mutant alleles only modify expression of *white* under conditions in which *white* is being repressed. Normally, the *white* gene is expressed in the developing eyes of late larvae and pupae (ZACHAR, CHAPMAN and BINGHAM 1985). The neomorphic z^1 allele partially represses this *white* expression. The *zeste* protein is a weak transcription factor (BIGGIN *et al.* 1988) which binds *cis*-regulatory sequences of the *w* gene (BENSON and PIRROTTA 1987; MANSUKHANI *et al.* 1988). It has been suggested that the z^+ protein facilitates transcription of the *white* gene, but that the altered z^1 protein acts to repress *white* (PIRROTTA *et al.* 1987). In general, $E(z)$ mutant alleles detectably only modify eye color in a z^1-containing genotype. For example, $E(z)^1$ and $E(z)^{S1}$ have no effects on the eye colors of z^+ w^+, z^+ w^a or z^a w^a flies. The one exception involves the effects of $E(z)$ on expression of the $P[(w,ry)A^R]4$–*24* transposon, as observed by T. HAZELRIGG (personal communication). $P[(w,ry)A^R]4$–*24* contains a fully functional copy of the *white* gene which has been reintroduced into the genome by *P* element transformation (LEVIS *et al.* 1985a). At the $P[(w,ry)A^R]$

4–24 site (polytene bands 24D1–2), the *white* gene within the transposon is repressed in the dorsal portion of the eye by flanking chromosomal sequences. As with the *zeste*^{1}*-white* interaction, $E(z)^{1}$ enhances and $E(z)^{S1}$ suppresses this $P[(w,ry)A^{R}]4\text{–}24$ phenotype. Thus, while $E(z)^{+}$ activity seems to be required for repression of *white*, it can also be mutated to a gain-of-function state (*i.e.*, $E(z)^{1}$) which increases this repression.

The basis for the ectopic sex comb teeth on the second tarsal segment in $E(z)^{1}/E(z)^{S2}$ males reared at 29° is less clear. This may represent a homeotic transformation of cells within the second tarsal segment toward a more proximal state, in this case that of first tarsal segment cells. By analogy to the other effects of *E(z)* mutations, this distal into proximal transformation may be due to misregulation of genes involved in determining or elaborating positional information along the proximal-distal axis within the leg disc. Candidates for such genes include the *Distal-less* (COHEN and JURGENS 1989) and *rotund* (KERRIDGE and THOMAS-CAVALLIN 1988; AGNEL *et al.* 1989) loci, which have been implicated in proximal-distal pattern formation.

Absence of zygotic $E(z)^{+}$ product results in early pupal lethality (SHEARN 1977). The mutant larvae, which eventually die as pupae, are normal in external appearance. However, their imaginal discs are reduced in size (SHEARN 1977; SHEARN, HERSPERGER and HERSPERGER 1978; SHEARN *et al.* 1978). Metaphase spreads of mutant larval neuroblasts, which normally are undergoing rapid cell division, reveal a very low mitotic index (GATTI and BAKER 1989). The few metaphase chromosomes observed show chromosome breakage and irregularities in condensation. Our observations and those of SHEARN, HERSPERGER and HERSPERGER (1978) demonstrate the requirement of maternal $E(z)^{+}$ activity for embryonic development. Thus, maternally contributed $E(z)^{+}$ product from $E(z)^{+}/E(z)^{-}$ females is sufficient for normal embryogenesis, but zygotically produced $E(z)^{+}$ is required for continued cell proliferation during larval development. The prepupal lethality produced by some cytologically normal alleles in *trans* to $Df(3L)E(z)^{-}$ may be due to a poisoning effect on the maternal *E(z)* product. In addition, some alleles disrupt adjacent lethal complementation groups (data not shown).

E(z), and possibly other Pc-group and/or Su(z) genes, may encode *trans*-acting factors which are functionally analogous to the products of the *SIR1, 2, 3* and *4* loci and the *RAP1* (also known as *GRF1*) and *ABF1* proteins in yeast (RINE and HERSKOWITZ 1987; SHORE and NASMYTH 1987; BUCHMAN *et al.* 1988). These yeast gene products are required for the activity of specific silencer elements at the silent mating type loci. Silencers act in *cis* to counteract the activation of promoters by enhancer elements (BRAND *et al.* 1985). Mutations either in the silencers themselves, or in the *SIR1–4* loci in yeast, result in derepression of the genes which they regulate (KIMMERLY *et al.* 1988; RINE and HERSKOWITZ 1987). In addition, these yeast genes are required for other cellular functions. For example, the *SIR2* product is also required to prevent recombination between rDNA repeats (GOTTLIEB and ESPOSITO 1989); and the products of the SIR genes and the *RAP1* and *ABF1* proteins are required for mitotic spindle independent segregation of yeast plasmids (KIMMERLY and RINEO 1987; KIMMERLY *et al.* 1988). The RAP1 protein is a component of the nuclear matrix (HOFMANN *et al.* 1989). HOFMANN *et al.* (1989) have proposed that RAP1 acts to anchor silencers to the nuclear matrix, which may facilitate the formation or stabilization of chromatin domains in a repressed state. Blockage of cell proliferation and disruption of chromosome organization by *E(z)* null mutations (GATTI and BAKER 1989) suggests that the $E(z)^{+}$ product, in addition to being required for certain examples of gene repression, may also have more general functions in the genome. As with all other phenotypes associated with *E(z)* mutations, we cannot as yet determine if the $E(z)^{+}$ product acts directly to maintain chromosome organization, or whether disruption of chromosome organization and blockage of cell proliferation is a secondary effect, possibly due to misregulation of other genes.

As previously described (BEACHY *et al.* 1985; CABRERA, BOTAS and GARCÍA-BELLIDO 1985; STRUHL and AKAM 1985; WEDEEN, HARDING and LEVINE 1986; RILEY, CARROLL and SCOTT 1987; GLICKSMAN and BROWER 1988a), Polycomb-group mutations allow limited ectopic expression of *ANT-C* and *BX-C* genes. STRUHL and AKAM (1985) showed that the *esc*$^{+}$ product is not involved in determining the initial embryonic pattern of Ubx expression, but rather is required to maintain the negative regulation of *Ubx*. Both the maternal-effect embryonic phenotypes produced by *esc* and *E(z)* mutations and their effects on the expression of *Ubx* are extremely similar. Embryos which lack maternally contributed $E(z)^{+}$ show the wild-type pattern of Ubx expression in ps5–13 until about 6–7 hr into development. Roughly coincident with the appearance of the parasegmental grooves (stage 11, CAMPOS-ORTEGA and HARTENSTEIN 1985), Ubx becomes expressed in the remaining anterior parasegments of the germ band. Initially this expression is greatest in ps2, before attaining equal levels of expression throughout the CNS. This unequal expression may reflect the distribution and strengths of *trans*-acting factors capable of inducing *Ubx* expression, which normally are prevented by the $E(z)^{+}$ product from activating *Ubx* in these cells. This progression

of ectopic *Ubx* expression is different from that produced by *esc* mutations, in which Ubx protein gradually spreads anteriorly from its normal domain [STRUHL and AKAM (1985) and our observations]. This difference may reflect different molecular mechanisms by which $E(z)^+$ and esc^+ products repress *Ubx*. Although *Ubx* is normally expressed in a similar pattern in both the CNS and the epidermis in germ band shortened stages, embryos lacking maternally contributed $E(z)^+$ show greatly reduced epidermal expression while retaining strong *Ubx* expression in the CNS. Eventually, *Ubx* is expressed throughout the germ band at levels normally seen in ps13, which explains the cuticular transformations toward A8. In the case of *esc*, STRUHL and WHITE (1985) have shown that this final level of *Ubx* expression is due to repression of *Ubx* by the *Abd-B* gene, which is also derepressed in *Pc* mutant embryos (WEDEEN, HARDING and LEVINE 1986).

Why maternally provided $E(z)^+$ activity is not required for regulation of *Ubx* until stage 11 is not clear. The initial pattern of segment identity gene expression is determined by the segmentation genes, which are precisely localized in the early embryo (AKAM 1987). Later embryonic expression may be controlled by more generally expressed transcription factors, which are distributed throughout the germ band. These later-acting factors may not be expressed, or may in some way not have access to their targets, until later in embryogenesis. The products of $E(z)$, and possibly other Pc-group genes, may negatively regulate the segment identity genes by maintaining them in a repressed configuration initially determined by the segmentation genes, and thus blocking their activation by these later factors. In this scenario, the products of Pc-group genes do not initiate repression, but only maintain a gene in the repressed state once it is turned off by a specific transcription factor. It is also possible that different *Ubx* enhancers are involved in early *vs.* later embryonic expression and that the $E(z)$ product does not control the early enhancers. It should again be noted that $E(z)^{S2}$ does not behave zygotically as a completely amorphic allele even at 29°. Therefore, it is possible that $E(z)^{S2}$ females may produce residual $E(z)^+$ activity at 29° and that complete lack of maternally contributed $E(z)^+$ might result in a more severe embryonic phenotype.

$E(z)$ mutations cause neither *Ubx* nor *Scr* to become constitutively expressed. In both embryos and larvae, their ectopic expression is limited. In embryos, it is restricted to the germ band. In larvae, *Scr* and *Ubx* are also ectopically expressed only within very restricted domains. In each case these domains are analogous to those in which the genes are normally expressed. For example, *Scr* is normally expressed in the prothoracic leg disc. In addition to its normal pattern of expression, reduced $E(z)^+$ activity allows *Scr* to be ectopically expressed in the anterior compartments of the mesothoracic and metathoracic leg discs, occasionally in the hinge region of the wing disc, but nowhere else. *Ubx* is ectopically expressed in the CNS and the posterior compartment of the wing disc. The pattern in the wing pouch is very similar to that produced by the gain-of-function Cbx^1 allele (WHITE and AKAM 1985), in which the *pbx cis*-regulatory region of *Ubx* appears to be ectopically activated (LEWIS 1982). One possible explanation is that insufficient levels of $E(z)^+$ makes *Scr* and *Ubx cis*-regulatory elements available to *trans*-activating factors in cells in which these *cis*-regulatory elements are normally not accessible. If these factors themselves are expressed in a restricted pattern, the absence of $E(z)^+$ will result only in ectopic expression of *Scr* and *Ubx* within this pattern, which agrees with our observations. Negative regulation by other segment identity genes may also restrict the expression of *Scr* and *Ubx*. It is also possible that the $E(z)$ gene is expressed in a limited pattern and only controls expression of the *Scr* and *Ubx* genes within this domain. We have cloned the $E(z)$ gene and are pursuing its molecular characterization (manuscript in preparation).

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Note: After submission of this manuscript, PHILLIPS and SHEARN (1990) reported similar phenotypes produced by $E(z)$ mutations, although they refer to the locus as *polycombeotic*.

LITERATURE CITED

AGNEL, M., S. KERRIDGE, C. VOLA and R. GRIFFIN-SHEA, 1989 Two transcripts from the *rotund* region of *Drosophila* show similar positional specificities in imaginal disc tissues. Genes Dev. **3:** 85–95.

AKAM, M., 1987 The molecular basis for metameric pattern in the *Drosophila* embryo. Development **101:** 1–22.

BEACHY, P. A., S. L. HELFAND and D. S. HOGNESS, 1985 Segmental distribution of *bithorax* complex proteins during *Drosophila* development. Nature **313:** 545–551.

BENSON, M., and V. PIRROTTA, 1987 The product of the *Drosophila zeste* gene binds to specific DNA sequences in *white* and *Ubx*. EMBO J. **6:** 1387–1392.

BIGGIN, M. D., S. BICKEL, M. BENSON, V. PIRROTTA and R. TJIAN, 1988 *Zeste* encodes a sequence-specific transcription factor that activates the *Ultrabithorax* promoter in vitro. Cell **53:** 713–722.

BRAND, A. H., L. BREEDEN, J. ABRAHAM, R. STERNGLANZ and K. NASMYTH, 1985 Characterization of a "Silencer" in yeast: a DNA sequence with properties opposite to those of a transcriptional enhancer. Cell **41:** 41–48.

BROWER, D. L., 1987 *Ultrabithorax* gene expression in *Drosophila* imaginal discs and larval nervous system. Development **101:** 83–92.

BRYANT, P. J., 1978 Pattern formation in imaginal discs, pp. 230–

335 in *Genetics & Biology of Drosophila* Vol. 2c, edited by M. ASHBURNER and T. R. F. WRIGHT. Academic Press, London.

BUCHMAN, A. R., W. J. KIMMERLY, J. RINE and R. D. KORNBERG, 1988 Two DNA-binding factors recognize specific sequences at silencers, upstream activating sequences, autonomously replicating sequences, and telomeres in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:** 210–225.

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

CABRERA, C. V., J. BOTAS and A. GARCÍA-BELLIDO, 1985 Distribution of *Ultrabithorax* proteins in mutants of *Drosophila bithorax* complex and its transregulatory genes. Nature **318:** 569–571.

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

COHEN, S., and G. JURGENS, 1989 Proximal-distal pattern formation in *Drosophila*: graded requirement for *Distal-less* gene activity during limb development. Roux's Arch. Dev. Biol. **198:** 157–169.

CRAYMER, L., 1984 Drosophila Inform. Serv. **60:** 234–236.

DAVISON, D., C. H. CHAPMAN, C. WEDEEN and P. M. BINGHAM, 1985 Genetic and physical studies of a portion of the *white* locus participating in transcriptional regulation and in synapsis-dependent interactions in *Drosophila* adult tissues. Genetics **110:** 479–494.

GANS, M., 1953 Étude genétique et physiologique du mutant z de *Drosophila melanogaster*. Bull. Biol. Fr. Suppl. **38:** 1–90.

GATTI, M., and B. S. BAKER, 1989 Genes controlling essential cell-cycle functions in *Drosophila melanogaster*. Genes Dev. **3:** 438–453.

GELBART, W. M., and C-T. WU, 1982 Interactions of *zeste* mutations with loci exhibiting transvection effects in *Drosophila melanogaster*. Genetics **102:** 179–189.

GLICKSMAN, M. A., and D. L. BROWER, 1988a Misregulation of homeotic gene expression in *Drosophila* larvae resulting from mutations at the *extra sex combs* locus. Dev. Biol. **126:** 219–227.

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

GOLDBERG, M. L., R. A. COLVIN and A. F. MELLIN, 1989 The Drosophila *zeste* locus is nonessential. Genetics **123:** 145–155.

GOTTLIEB, S., and R. E. ESPOSITO, 1989 A new role for a yeast transcriptional silencer gene, *SlR2*, in regulation of recombination in ribosomal DNA. Cell **56:** 771–776.

HAFEN, E., M. LEVINE and W. J. GEHRING, 1984 Regulation of the *Antennapedia* transcript distribution by the *bithorax* complex in *Drosophila*. Nature **307:** 287–289.

HARDING, K., and M. LEVINE, 1988 Gap genes define the limits of *Antennapedia* and *Bithorax* gene expression during early development in *Drosophila*. EMBO J. **7:** 205–214.

HARDING, K., C. WEDEEN, W. MCGINNIS and M. LEVINE, 1985 Spatially regulated expression of homeotic genes in *Drosophila*. Science **229:** 1236–1242.

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.

HOFMANN, J. F.-X., T. LAROCHE, A. H. BRAND and S. M. GASSER, 1989 RAP-1 factor is necessary for DNA loop formation in vitro at the silent mating type locus *HML*. Cell **57:** 725–737.

INGHAM, P. W., D. ISH-HOROWICZ and K. R. HOWARD, 1986 Correlative changes in homoeotic and segmentation gene expression in *Krüppel* mutant embryos of *Drosophila*. EMBO J. **5:** 1659–1665.

INGHAM, P. W., and A. MARTINEZ-ARIAS, 1986 The correct activation of *Antennapedia* and *bithorax* complex genes requires the *fushi tarazu* gene. Nature **324:** 592–597.

IRISH, V. F., and W. M. GELBART, 1987 The *decapentaplegic* gene is required for dorsal-ventral patterning of the *Drosophila* embryo. Genes Dev. **1:** 868–879.

JACK, J. W., and B. H. JUDD, 1979 Allelic pairing and gene regulation: a model for the *zeste-white* interaction in *Drosophila* melanogaster. Proc. Natl. Acad. Sci. USA **76:** 1368–1372.

KALISCH, W.-E., and B. RASMUSON, 1974 Changes of *zeste* phenotype induced by autosomal mutations in *Drosophila melanogaster*. Hereditas **78:** 97–104.

KAUFMAN, T. C., S. E. TASAKA and O. T. SUZUKI, 1973 The interaction of two complex loci, *zeste* and *bithorax* in *Drosophila melanogaster*. Genetics **75:** 299–321.

KERRIDGE, S., and M. THOMAS-CAVALLIN, 1988 Appendage morphogenesis in *Drosophila*: a developmental study of the *rotund* (*rn*) gene. Wilhelm Roux's Arch. Dev. Biol. **197:** 19–26.

KIMMERLY, W. J., and J. RINE, 1987 Replication and segregation of plasmids containing *cis*-acting regulatory sites of silent mating-type genes in *Saccharomyces cerevisiae* are controlled by the *SIR* genes. Mol. Cell. Biol. **7:** 4225–4237.

KIMMERLY, W., A. BUCHMAN, R. KORNBERG and J. RINE, 1988 Roles of two DNA-binding factors in replication, segregation and transcriptional repression mediated by a yeast silencer. EMBO J. **7:** 2241–2253.

LAWRENCE, P. A., P. JOHNSTON and G. MORATA, 1986 Methods of marking cells, pp. 229–242 in *Drosophila: A Practical Approach*, edited by D. B. ROBERTS. IRL Press Limited, Oxford.

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., and F. BACHAR, 1968 Method for feeding ethylmethane sulfonate (EMS) to *Drosophila* males. Drosophila Inform. Serv. **43:** 193.

LEWIS, E. B., 1982 Control of body segment differentiation in *Drosophila* by the *bithorax* gene complex, pp. 269–288 in *Proceedings of the 9th Congress of the Internatonal Society of Developmental Biology*, edited by M. B. BURGER. Alan R. Liss, New York.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

LINDSLEY, D. L., and G. ZIMM, 1985 The genome of *Drosophila melanogaster*. Part 1: Genes A-K. Drosophila Inform. Serv. **64.**

LINDSLEY, D. L., and G. ZIMM, 1986 The genome of *Drosophila melanogaster*. Part 2: lethals; maps. Drosophila Inform. Serv. **65.**

LINDSLEY, D. L., and G. ZIMM, 1987 The genome of *Drosophila melanogaster*. Part 3: rearrangements. Drosophila Inform. Serv. **66.**

MACDONALD, P. M., and G. STRUHL, 1986 A molecular gradient in early *Drosophila* embryos and its role in specifying the body pattern. Nature **324:** 537–545.

MAHAFFEY, J. W., and T. C. KAUFMAN, 1987 Distribution of the *Sex combs reduced* gene products in *Drosophila melanogaster*. Genetics **117:** 51–60.

MANSUKHANI, A., A. CRICKMORE, P. W. SHERWOOD and M. L. GOLDBERG, 1988 DNA-binding properties of the *Drosophila melanogaster zeste* gene product. Mol. Cell. Biol. **8:** 615–623.

MARTINEZ-ARIAS, A., and R. A. H. WHITE, 1988 *Ultrabithorax* and *engrailed* expression in *Drosophila* embryos mutant for segmentation genes of the pair-rule class. Development **102:** 325–338.

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.

PERSSON, K., 1976a A Minute mutant with suppressor effect on the eye-colour gene *zeste* in *Drosophila melanogaster*. Hereditas **82:** 57–62.

PERSSON, K., 1976b Modification of the eye colour mutant *zeste* by Suppressor, Enhancer and Minute genes in *Drosophila melanogaster*. Hereditas **82:** 111–120.

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., H. STELLER and M. P. BOZZETTI, 1985 Multiple upstream regulatory elements control the expression of the *Drosophila white* gene. EMBO J. **4:** 3501–3508.

PIRROTTA, V., E. MANET, E. HARDON, S. E. BICKEL and M. BENSON, 1987 Structure and sequence of the *Drosophila zeste* gene. EMBO J. **6:** 791–799.

RASMUSON, B., 1962 An intragenic duplication in *Drosophila melanogaster* and its significance for gene function. Hereditas **48:** 587–611.

RAYLE, R. E., and M. M. GREEN, 1968 A contribution to the genetic fine structure of the region adjacent to *white* in *Drosophila melanogaster*. Genetica **39:** 497–507.

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.

RINE, J., and I. HERSKOWITZ, 1987 Four genes responsible for a position effect on expression from *HML* and *HMR* in *Saccharomyces cerevisiae*. Genetics **116:** 922.

SCHOTT, D. R., M. C. BALDWIN and V. FINNERTY, 1986 Molybdenum hydroxylases in *Drosophila*. III. Further characterization of the *low xanthine dehydrogenase* gene. Biochem. Genet. **24:** 509–527.

SHEARN, A., 1977 Mutational dissection of imaginal disc development in *Drosophila melanogaster*. Am. Zool. **17:** 585–594.

SHEARN, A., G. HERSPERGER and E. HERSPERGER, 1978 Genetic analysis of two allelic temperature-sensitive mutants of *Drosophila melanogaster* both of which are zygotic and maternal-effect lethals. Genetics **89:** 341–353.

SHEARN, A., G. HERSPERGER, E. HERSPERGER, E. S. PENTZ and P. DENKER, 1978 Multiple allele approach to the study of genes in *Drosophila melanogaster* that are involved in imaginal disc development. Genetics **89:** 355–370.

SHORE, D., and K. NASMYTH, 1987 Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. Cell **51:** 721–732.

SMOLIK-UTLAUT, S. M., and W. M. GELBART, 1987 The effects of chromosomal rearrangements on the *zeste-white* interaction in *Drosophila melanogaster*. Genetics **116:** 285–298.

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

STRUHL, G., 1982 Genes controlling segmental specification in the *Drosophila* thorax. Proc. Natl. Acad. Sci. USA **79:** 7380–7384.

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 R. A. H. WHITE, 1985 Regulation of the *Ultrabithorax* gene of *Drosophila* by other bithorax complex genes. Cell **43:** 507–519.

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

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

WHITE, R. A. H., and M. E. AKAM, 1985 *Contrabithorax* mutations cause inappropriate expression of *Ultrabithorax* products in *Drosophila*. Nature **318:** 567–569.

WHITE, R. A. H., and R. LEHMAN, 1986 A gap gene, *hunchback*, regulates the spatial expression of *Ultrabithorax*. Cell **47:** 311–321.

WHITE, R. A. H., and M.WILCOX, 1984 Protein products of the *bithorax* complex in *Drosophila*. Cell **39:** 163–171.

WHITE, R. A. H., and M. WILCOX, 1985 Distribution of *Ultrabithorax* proteins in *Drosophila*. EMBO J. **4:** 2035–2043.

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.

ZACHAR, Z., C. H. CHAPMAN and P. M. BINGHAM, 1985 On the molecular basis of transvection effects and the regulation of transcription. Cold Spring Harbor Symp. Quant. Biol. **50:** 337–346.

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