A Role of Polycomb Group Genes in the Regulation of Gap Gene Expression in Drosophila

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ABSTRACT

Anteroposterior polarity of the Drosophila embryo is initiated by the localized activities of the maternal genes, *bicoid* and *nanos*, which establish a gradient of the *hunchback* (*hb*) morphogen. *nanos* determines the distribution of the maternal Hb protein by regulating its translation. To identify further components of this pathway we isolated suppressors of *nanos*. In the absence of *nanos* high levels of Hb protein repress the abdomen-specific genes *knirps* and *giant*. In *suppressor-of-nanos* mutants, *knirps* and *giant* are expressed in spite of high Hb levels. The suppressors are alleles of *Enhancer of zeste* (*E(z)*) a member of the Polycomb group (Pc-G) of genes. We show that *E(z)*, and likely other Pc-G genes, are required for maintaining the expression domains of *knirps* and *giant* initiated by the maternal Hb protein gradient. We have identified a small region of the *knirps* promoter that mediates the regulation by *E(z)* and *hb*. Because Pc-G genes are thought to control gene expression by regulating chromatin, we propose that imprinting at the chromatin level underlies the determination of anteroposterior polarity in the early embryo.

ESTABLISHMENT of pattern along the anteroposterior axis in Drosophila is initiated by maternal gene products which are synthesized during oogenesis. These maternal gene products direct the spatial expression of gap genes which are transcribed from the embryonic genome and whose products are expressed in large, overlapping domains [reviewed in HÜLSKAMP and TAUTZ (1991)]. Precise transition from maternal to zygotic control of gene expression is critical for the initiation and maintenance of a stable pattern of gap gene expression.

Transition between maternal and zygotic information along the anteroposterior axis is in part achieved by the transition from a concentration gradient of maternally derived Hunchback protein (Hb^{mat}) to a gradient of zygotic Hb protein (Hb^{zyg}) expressed by the embryo. The maternal gene *nanos* (*nos*) establishes the maternal Hb protein gradient (see Figure 1A). *nanos* RNA is synthesized during oogenesis and becomes localized to the posterior pole of the mature oocyte (WANG and LEHMANN 1991). After fertilization a posterior to anterior concentration gradient of Nanos protein emanates from the local RNA source (BARKER *et al.* 1992). *Hb* RNA is also synthesized during oogenesis and is distributed uniformly throughout the freshly laid egg. Nanos protein is a repressor of *hb* translation and thereby establishes a concentration gradient of Hb^{mat} complementary to that of Nos (TAUTZ 1988; HÜLSKAMP *et al.* 1989; IRISH *et al.* 1989a; STRUHL 1989; TAUTZ and PFEIFLE 1989; WANG and LEHMANN 1991; WHARTON and STRUHL 1991). Zygotic expression of *hb*, on the other hand, is controlled by the transcription factor *bicoid* (*bcd*). Like *nos*, *bcd* RNA is synthesized during oogenesis and is localized within the oocyte, but to the anterior pole (FROHNHÖFER and NÜSSLEIN-VOLHARD 1986; BERLETH *et al.* 1988). *Bcd* RNA translation results in an anterior to posterior concentration gradient of Bcd protein (DRIEVER and NÜSSLEIN-VOLHARD 1988). This protein, in turn, activates *hb* and other genes in the anterior half of the embryo in a concentration-dependent manner (SCHRÖDER *et al.* 1988; TAUTZ 1988; DRIEVER and NÜSSLEIN-VOLHARD 1989; DRIEVER *et al.* 1989; STRUHL *et al.* 1989).

Thus, both the anterior morphogen *bcd* and the posterior determinant *nos* achieve, by different mechanisms, a similar end result: the formation of an anterior to posterior gradient of Hb protein. Although there are a large number of additional regulatory interactions between maternal signals and gap genes, and between gap genes themselves [reviewed in HÜLSKAMP and TAUTZ (1991); see also ELDON and PIRROTTA (1991), KRAUT and LEVINE (1991a,b), CAPOVILLA *et al.* (1992), and STRUHL *et al.* (1992)], the Hb protein gradient stands out as a major organizer of the embryonic gap gene expression pattern. Hb protein can act both as a transcriptional activator and repressor, and the Hb protein gradient determines the expression domains of gap genes thereby dividing the embryo into anterior (*hb*-expressing), middle (*Krüppel* (*Kr*)-expressing) and posterior (*knirps* (*kni*)- and giant (*gt*)-expressing) regions (see Figure 1A) (HÜLSKAMP *et al.* 1990; KRAUT and LEVINE

1991a,b; STRUHL *et al.* 1992). A gradient of either Hb^{mat} or Hb^{zyg} protein, which are identical in primary sequence (TAUTZ *et al.* 1987), is sufficient on its own to organize the embryo into this basic (*Kr-kni-gt*) gap gene pattern (although only Hb^{zyg} protein attains the high levels necessary for *Kr* repression) (HÜLSKAMP *et al.* 1990; STRUHL *et al.* 1992).

Changes in the distribution of Hb disrupt normal embryonic patterning. This is demonstrated most directly in embryos from *nos* mutant females in which Hb^{mat} is translated throughout the embryo. Uniformly high levels of Hb^{mat} repress transcription of the abdomen-specific gap gene *kni* and *gt* and therefore these embryos lack abdomen. Since Hb is the major repressor of gap gene expression in *nos* mutants (HÜLSKAMP *et al.* 1989; IRISH *et al.* 1989a; STRUHL 1989), we reasoned that additional genes required for the production or the activity of the Hb^{mat} protein could be identified as suppressors of *nos* (Figure 1).

We report here the identification and characterization of three such *suppressor-of-nos* (abbreviated *son*) mutations. We show that these mutations are alleles of the previously characterized gene *Enhancer of zeste* (*E(z)*) (JONES and GELBART 1990; PHILLIPS and SHEARN 1990). We investigated the role of *E(z)* in the determination of the anteroposterior pattern and conclude that *E(z)* is required to maintain transcriptional repression of the gap genes *kni* and *gt* once repression has been initiated by the Hb^{mat} protein.

MATERIALS AND METHODS

Nomenclature: Throughout the text we refer to embryos from mutant females as "mutant embryos" which describes their maternal and not their zygotic genotype. Marker mutations and balancer chromosomes are described in LINDSLEY and ZIMM (1992). Staging of embryos is as in FOE and ALBERTS (1983).

Screen for suppressors of *nos*: hb^{7M} is a protein null (LEHMANN and NÜSSLEIN-VOLHARD 1987; TAUTZ 1988), nos^{L7} behaves as a loss-of-function mutation with respect to abdomen formation although it still retains *nos* function required for oogenesis (LEHMANN and NÜSSLEIN-VOLHARD 1991). $hb^{7M}nos^{L7}/TM3$ females were crossed to nos^{L7} homozygous males mutagenized with either 35 mM or 45 mM ethyl methylsulfonate (EMS). F_1 $hb^{7M}nos^{L7}/nos^{L7}$ females were tested in groups for producing hatching embryos. Single females were retested and lines were established from the F_2 males. We tested 12,854 half genomes, *i.e.*, *hb nos/nos* females (9,372 from the 35 mM batch and 3,482 from the 45 mM batch). To determine the efficiency of mutagenesis we also screened for newly induced *p* alleles and *cu* alleles. The estimated yield of our mutagenesis is about 1.7 hits per locus per 1000 chromosomes at 35 mM (not determined for 45 mM).

Genetic analysis and strains: Suppression of the *nos* phenotype by all suppressors including the *E(z)* mutations is stronger at higher temperatures and is observed more frequently in the layings of younger females. Therefore, to assure consistency in different experiments, all genetic tests were carried out at 25° (except where noted), and only the progeny from the first four days of laying were analyzed. Embryos were allowed to develop cuticle structures (24 hr at 25°) and the number of abdominal segments was scored either directly under a dissecting scope (the embryos being cleared by a film of mineral oil) or as cuticle preparations embedded in Hoyer's medium (WIESCHAUS and NÜSSLEIN-VOLHARD 1986).

$E(z)^{son}$ mutations are alleles of *Enhancer of zeste* (synonym: *polycombeotic*).

Map position: son^1 and son^2 map within 5 map units of *E(z)* (3–34.0), son^3 to the 3–26.5 to 3–43.2 interval.

Associated phenotypes: the $E(z)^{son}$ alleles, like other *E(z)* alleles are larval lethal when *trans*-heterozygous. The alleles $E(z)^{son1}$ and $E(z)^{son3}$ are lethal in *trans* to *E(z)* null alleles or deficiencies, $E(z)^{son2}$ is viable and fertile in trans to those alleles but males exhibit ectopic sex combs on the second and third legs, a phenotype associated with *E(z)* mutations (WU *et al.* 1989; JONES and GELBART 1990; PHILLIPS and SHEARN 1990). In addition, these three alleles, like antimorphic *E(z)* alleles, act as strong suppressors of the *zeste-white* interaction (data not shown) (JONES and GELBART 1990).

Complementation: a *P* element carrying only the *E(z)* transcription unit (JONES and GELBART 1993) suppresses the lethality associated with the $E(z)^{son}$ alleles (zygotic genotypes tested: $E(z)^{son3}/E(z)^{65}$, $E(z)^{son1}/E(z)^{son3}$, and $E(z)^{son2}/E(z)^{son3}$), and reduces their *suppressor-of-nos* effect (maternal genotypes tested: in a hb^{mat} heterozygous background, $E(z)^{son2}/+$ and $E(z)^{son3}/+$; in a wild-type hb^{mat} background, $E(z)^{son1}/E(z)^{son3}$ and $E(z)^{son2}/E(z)^{son3}$). Thus these gain-of-function (g-o-f) alleles are antimorphic ("poison").

E(z) alleles are referred to according to LINDSLEY and ZIMM (1992): *Df(3L)Ez6*, $E(z)^{65}$, $E(z)^{66}$ (deficiencies, $E(z)^{66}$ partial deletion, weakly antimorphic, JONES and GELBART 1993), $E(z)^{63}$, $E(z)^{64}$(nulls, $E(z)^{64}$ slightly antimorphic); $E(z)^{61}$ (temperature sensitive, see legend of Table 1), $E(z)^{60}$, $E(z)^{1}$ (g-o-f, suppressor and enhancer of the *zeste-white* interaction, respectively).

Suppressor of zeste-2 (Su(z)-2) complex mutations [except where otherwise stated (see BRUNK *et al.* 1991)]: $Su(z)2^{1.b8}$, *Df(2R)vg-B* [deletions of *Psc*, *Su(z)2* and *Su(z)2-Distal* (*Su(z)2-D*)]; *Su(z)-2*: $Su(z)2^{1.b7}$ (null), $Su(z)2^{1}$ (g-o-f, suppressor of the *zeste-white* interaction); *Posterior sex combs*: Psc^{1445} (hypomorph, possibly maternal-effect g-o-f [see ADLER *et al.* (1989)], Psc^{24}, Psc^{1433} [null and g-o-f, respectively (C.-T. WU and M. HOWE, personal communication)] Psc^{1} (g-o-f, suppressor of the *zeste-white* interaction); *Su(z)-2-D*: $Su(z)3^{1}$(g-o-f, suppressor of the *zeste-white* interaction). Other Pc-G mutations are *Additional sex combs*, Asx^{D1} [g-o-f (SINCLAIR *et al.* 1992)]; *extra sex combs*, esc^{2} (null), esc^{10} (deletion) (STRUHL 1981) [recovery of *esc* null embryos was essentially as described in STRUHL and AKAM (1985)]; *pleiohomeotic*, pho^{c} (hypomorph) (DUNCAN 1982); *Df(4)G* (deletion) (BREEN and DUNCAN 1986); *Polycomb*, *Df(3L)Pc* (deletion) (HAYNIE 1983); *Polycomblike*, Pcl^{D5} (null) (BREEN and DUNCAN 1986); *polyhomeotic*, ph^{503} (null) (DURA *et al.* 1987); *Sex combs extra*, Sce^{D1} (nature unknown) (BREEN and DUNCAN 1986); *Sex combs on midleg*, Scm^{D1} (null) (BREEN and DUNCAN 1986); *super sex combs*, sxc^{1} (null) (INGHAM 1984). The partial loss-of-function allelic combination ph^{410}/ph^{br} (DURA *et al.* 1987) did not show significant suppression of the *nos* phenotype.

Pole cell transplants: Ovo^{D1} females were used as hosts for pole cell transplants as described in LEHMANN and NÜSSLEIN-VOLHARD (1987). Ovo^{D1} causes degeneration of the female germ line (OLIVER *et al.* 1987). Donor embryos were derived from the following crosses: (1) $E(z)^{son1}nos$/TM3 × $E(z)^{son3}nos$/TM3 to obtain $E(z)^{son1}nos/E(z)^{son3}nos$ clones; (2) $E(z)^{son2}nos$/TM3 × $E(z)^{son3}nos$/TM3 to obtain $E(z)^{son2}$ $nos/E(z)^{son3}nos$ clones ($nos = nos^{L7}$). The genotype of the transplanted pole cells was determined by the phenotype of the progeny.

$E(z)^{son1}nos/E(z)^{son3}nos$ germ cells did not lead to adult progeny but their genotypes were inferred by the rescue of the *nos*

phenotype and/or other associated defects. At 25°, 8/16 fertile females (presumably $E(z)^{son1}nos/E(z)^{son3}nos$) produced very few, abnormal eggs, most of which did not form cuticles. At 18° 3/4 fertile adult females (presumably $E(z)^{son1}nos/E(z)^{son3}nos$) laid eggs which developed into larvae (71 larvae/147 eggs). All embryos formed six to eight abdominal segments. Most of the developed embryos (69/71) failed to hatch and none reached adulthood. Many (84%, $n = 32$) showed head involution defects, similar to those observed in embryos with mild homeotic transformations [see, for example, JÜRGENS (1985)].

$E(z)^{son2}nos/E(z)^{son3}nos$ germ line clones: 8/25 fertile females exhibited phenotypes similar to those observed in females carrying $E(z)^{son1}nos/E(z)^{son3}nos$ germ line clones. Two females produced embryos which reached adulthood. In these cases the genotype of the transplanted pole cells was unambiguously identified.

The additional phenotypes associated with these *E(z)son* germ line clones, such as egg laying defects, were also observed when similar germ line clones were obtained in the presence of functional *nos* product ($E(z)^{son3}nos^{+}$). A more detailed description of these germ line clone results will appear elsewhere.

Analysis of expression patterns: $E(z)^{son2}/E(z)^{61}$ females were grown at 25° and eggs were collected from young females. Mutant backgrounds: nos^{L7} homozygotes, bcd^{E1} homozygotes, gt^{YA} hemizygotes, tsl^{035}/tsl^{138} *trans*-heterozygotes.

Whole-mount *in situ* hybridization with digoxigenin-labeled RNA probes for *kni*, *gt*, *Kr* and *lac Z* was performed as described in GAVIS and LEHMANN 1992.

The transgenes which contain the *kni* promoter-*lac Z* fusions were kindly provided by M. PANKRATZ and H. JÄCKLE (PANKRATZ *et al.* 1992). Males carrying the transgene were crossed to females of the appropriate genotype. Two insertion lines led to identical results.

Whole-mount antibody staining was performed as in GAVIS and LEHMANN (1992). The rabbit anti-Hb antibody, a gift from G. STRUHL, was diluted 1:50 and preadsorbed against 4–18-hr embryos. Biotinylated secondary antibody (from Vector Laboratories) was diluted 1:15 and preadsorbed against 8–14-hr embryos.

RESULTS

Identification of *Enhancer of zeste* alleles as suppressors of *nanos*: Embryos that lack functional Nos product lack all eight abdominal segments. In the absence of both Nos and Hb^{mat} embryonic patterning can proceed normally if *hb* is expressed zygotically (HÜLSKAMP *et al.* 1989; IRISH *et al.* 1989; STRUHL 1989). Thus, the major role of Nos during early embryogenesis is to establish the Hb protein gradient. We therefore reasoned that further components of the *nos-hb* regulatory pathway could be identified as *suppressor-of-nos* mutants (Figure 1). To sensitize the selection system we searched for suppressor mutants in a background heterozygous for *hb*. This reduction of the maternal *hb* gene dosage weakens the *nos* mutant phenotype, such that one to three abdominal segments are formed in the progeny of *hb nos/+ nos* females (Table 1) (HÜLSKAMP *et al.* 1989; IRISH *et al.* 1989; STRUHL 1989). We screened for EMS-induced mutations that allowed embryos from *hb nos/+ nos* females to develop into adults (Figure 1). After screening approximately 13,000 F_1 females, five suppressor mutations were isolated. These mutants cause a strong dominant suppression of the *nos* phenotype in a maternal background heterozygous for *hb*, and to a lesser extent, they also suppress the *nos* phenotype in a maternal background of normal *hb* dosage (Table 1).

Two of the suppressor mutations represent single hits in as yet unidentified genes and will be described elsewhere. Three other mutations are alleles of the gene *Enhancer of zeste* (*E(z)*) (JONES and GELBART 1990; PHILLIPS and SHEARN 1990) (see MATERIALS AND METHODS for details). We refer to these three mutations collectively as the $E(z)^{son}$ alleles and individually as $E(z)^{son1}$, $E(z)^{son2}$ and $E(z)^{son3}$. Mutations in *E(z)* have previously been analyzed in detail and *E(z)* has been shown to play a role in regulation of expression of homeotic genes in the Antennapedia and Bithorax gene complexes. Moreover, *E(z)* is required for proliferation of imaginal disc cells and the development of egg chambers past early stages of oogenesis (JONES and GELBART 1990; PHILLIPS and SHEARN 1990). Our results suggest that *E(z)* function is also involved in establishing the abdominal anlagen in the early embryo.

$E(z)^{son}$ alleles are specific for abdomen formation: Suppression of the *nos* phenotype by *E(z)* alleles depends on the maternal genotype, thus *nos* embryos form a normal abdomen only when the *E(z)* mutations are present in the mother. Newly synthesized zygotic *E(z)* gene product provided by the paternal genome has no effect on the *nos* mutant phenotype (data not shown). Thus, consistent with a role in the regulation of expression of the first tier of segmentation genes, functions affected by the $E(z)^{son}$ mutations are provided only maternally. In contrast, homeotic transformations which affect a later stage in the segmentation process are caused by lack of maternal *E(z)* function, but they also depend on the embryonic genotype (JONES and GELBART 1990; PHILLIPS and SHEARN 1990).

$E(z)^{son}$ mutations are semidominant, and suppression of the *nos* phenotype is much stronger in embryos from *nos* females homozygous for $E(z)^{son}$ (see Table 1). Table 1 shows the dominant maternal effect of $E(z)^{son}$ alleles compared with other *E(z)* alleles that had been isolated previously on the basis of other phenotypes. Although many of the previously known alleles, including *E(z)* deficiencies, have a significant dominant effect, none of them can suppress the *nos* phenotype to the same extent as the $E(z)^{son}$ alleles. Thus the $E(z)^{son}$ alleles are gain-of-function mutations that appear to code for aberrant proteins (see also MATERIALS AND METHODS).

To determine whether the phenotype of the gain-of-function $E(z)^{son}$ alleles reflects a requirement of wild-type *E(z)* function for early pattern formation, we tested the effect of *E(z)* loss-of-function allelic combinations in a *nos* mutant background. Since *E(z)* null mutations are homozygous lethal, we used the temperature sensitive allele $E(z)^{61}$ which has significantly reduced *E(z)* activity at the restrictive temperature (JONES and GELBART 1990).

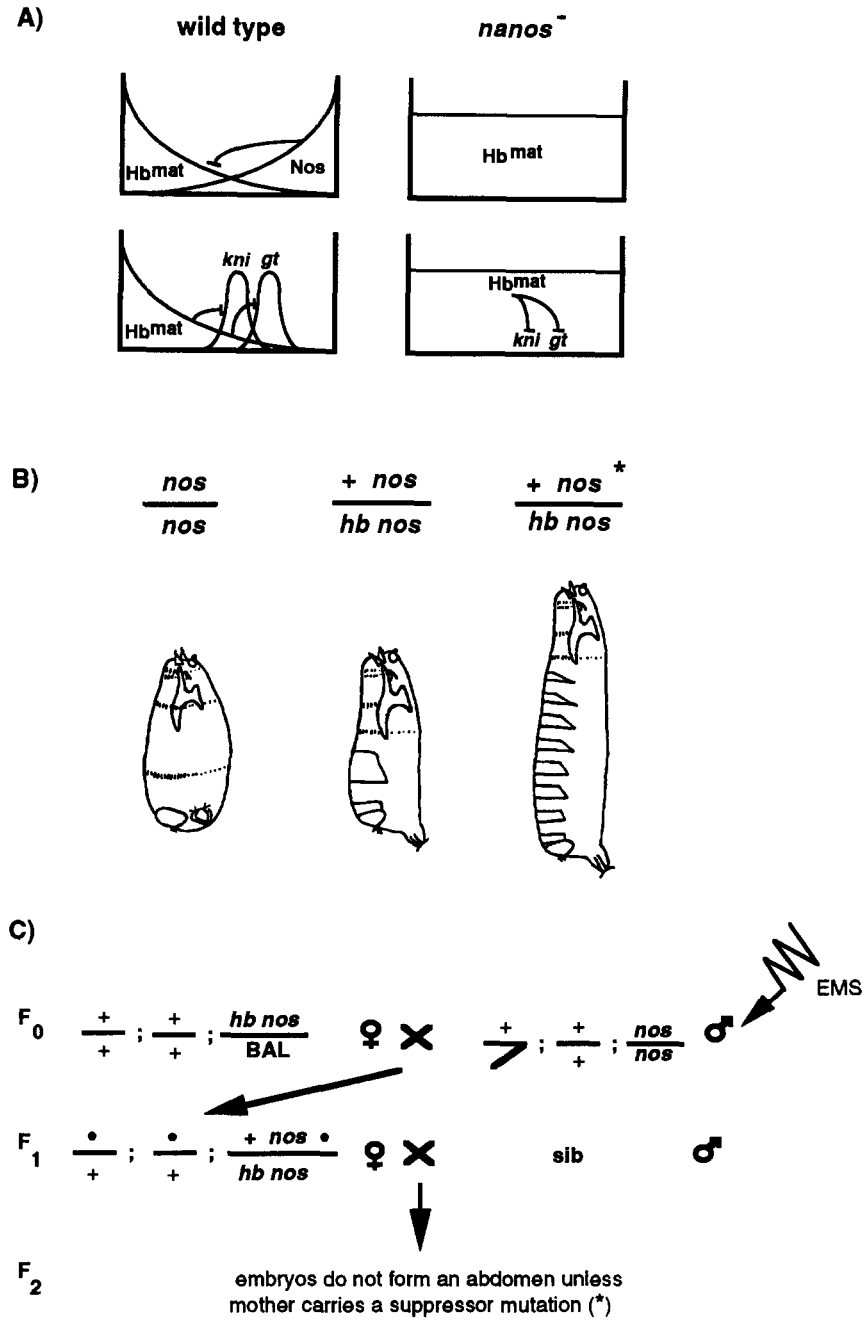

FIGURE 1.—Screen for suppressors of *nanos*. (**A**) Regulatory interactions at the early cleavage stage (top diagrams) and at the syncytial/cellular blastoderm (bottom diagrams) in embryos from wild-type and *nos* mutant females. In wild-type embryos the Nos protein represses translation of hb^{mat} RNA (top, left). The concentration of Hb determines the anterior boundaries of *kni* and *gt* expression (bottom, left). In embryos that lack functional Nos protein, the hb^{mat} RNA is translated throughout the egg (top, right). High levels of Hb^{mat} repress *kni* and *gt* transcription in the prospective abdominal region and thus no abdominal development occurs (bottom, right). (**B**) Phenotypes of embryos from *nos* mutant females. *Nos* mutant embryos lack abdominal segments, a reduction of the hb^{mat} gene dosage (+ *nos/hb nos*) weakens the *nos* phenotype. We identified suppressors of *nos* in this "sensitized" background (+ *nos */hb nos*). In principle, we should also recover new *hb* alleles, however, this is difficult since *hb* is also required zygotically (LEHMANN and NÜSSLEIN-VOLHARD 1987). (**C**) Screen for dominant suppressors of *nos*.

We found that homozygosity or hemizygosity for $E(z)^{61}$ at semipermissive (25°) and restrictive (29°) temperatures causes a significant suppression of the *nos* phenotype (Table 1, Figure 2C). We conclude that the wild-type maternal *E(z)* product is required for repression of abdominal development in a *nos* mutant background.

The phenotypes of *E(z)* null mutations suggest multiple requirements for *E(z)* wild-type product(s) at different stages of development (JONES and GELBART 1990; PHILLIPS and SHEARN 1990). In contrast, the $E(z)^{son}$ mutations are specific for abdomen formation. This specificity of the $E(z)^{son}$ alleles is demonstrated most clearly by the phenotype of embryos derived from germ line cells mutant for the two strongest $E(z)^{son}$ alleles ($E(z)^{son1}$ and $E(z)^{son3}$). Since these alleles are lethal in trans, we generated females whose germ line is homozygous for *nos* and *trans*-heterozygous for these alleles by pole cell transplants (see MATERIALS AND METHODS). Embryos from such females frequently develop a complete set of abdominal segments, but do not display the strong homeotic transformations characteristic of mutations in *E(z)* and other Pc-G genes (Table 1, Figure 2B). On the other hand, embryos from $E(z)^{61}/Df(E(z))$ females develop only some abdominal segments but show very strong homeotic transformations (Table 1, Figure 2C). This specificity of the $E(z)^{son}$ alleles is inconsistent with a model in which different levels of wild-type *E(z)* activity are required for different functions of *E(z)*. Rather, we

TABLE 1

***E(z)* mutations suppress the *nos* abdominal phenotype**

E(z) maternal genotype[a]	Maternal genotype: *nos/nos* Percent rescue[b]	*nos/nos* *n*	*hb nos/+ nos* Percent rescue[b]	*hb nos/+ nos* *n*
+/+	0	338	2	160
$E(z)^{son1}$/+	11	156	76	186
$E(z)^{son2}$/+	6	160	42	145
$E(z)^{son3}$/+	13	895	88	179
Df(3L)Ez6/+	0	525	9	768
$E(z)^{65}$/+	0	356	2	473
$E(z)^{66}$/+	0	253	10	229
$E(z)^{63}$/+	0	361	9	293
$E(z)^{64}$/+	0	575	12	353
$E(z)^{1}$/+	0	297	1	422
$E(z)^{60}$/+	0	562	4	318
$E(z)^{61}$/+	0	619	8	251
$E(z)^{61}/E(z)^{61}$ (25°)	2	216	ND	
(29°)	6	84	ND	
$E(z)^{61}/E(z)^{65}$ (25°)	24	270	ND	
(29°)	38	39	ND	
$E(z)^{son2}/E(z)^{61}$	95	366	ND	
$E(z)^{son1}/E(z)^{son3c}$	100	29	ND	
$E(z)^{son2}/E(z)^{son3d}$	100	14	ND	

Tests were performed in a genetic background with a full or half dosage of hb^{mat} (*nos/nos* and *hb nos/+ nos* columns, respectively). All tests are at 25° except where otherwise stated. The temperature-sensitive allele $E(z)^{61}$ retains some wild-type activity even at the "restrictive" temperature (29°) since suppression of the *nos* phenotype is stronger in hemizygotes ($E(z)^{61}/E(z)^{65}$) than in homozygotes [see also JONES and GELBART (1990)]. For a description of alleles see Materials and Methods.

[a] A "+" refers to the wild-type *E(z)* copy (*i.e.*, $E(z)^{+}$).

[b] Percent of embryos with ≥3 abdominal segments.

[c] From germ line clones. Test performed at 18°; 86% of the embryos scored had 8 abdominal segments.

[d] From germ line clones; 50% of the embryos scored had 8 abdominal segments.

propose that the E(z) protein is a complex molecule and that the $E(z)^{son}$ mutations affect a specific function of this protein.

***E(z)* is required for maintenance of gap gene repression:** To understand how $E(z)^{son}$ mutations alter the *nos* phenotype, we studied the expression patterns of gap genes in embryos derived from *E(z) nos* double mutant females. As a source of mutant *E(z)* embryos we used females of the genotype $E(z)^{son2}/E(z)^{61}$, which is a viable allelic combination that strongly suppresses the *nos* phenotype even in the presence of the normal maternal *hb* gene dosage (Table 1). As observed previously, *nos* mutant embryos fail to express the gap genes *kni* and *gt* in the prospective abdominal region (in Figure 3, compare E, F with B, C, respectively) (ROTHE *et al.* 1989; ELDON and PIRROTTA 1991; KRAUT and LEVINE 1991a). In contrast, *E(z)* mutant embryos express these gap genes in spite of the absence of *nos* function (Figure 3, H and I). Thus, *E(z)* wild-type function is required for the repression of abdomen-specific gap genes.

E(z) mutations suppress the abdominal phenotype of embryos derived from females mutant for *oskar* and

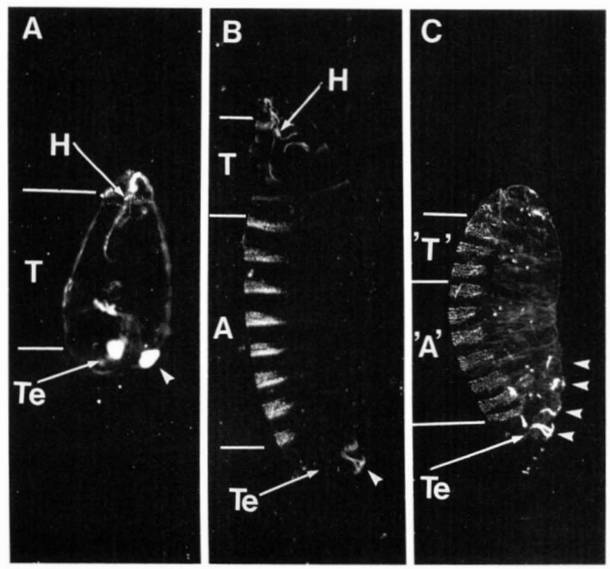

FIGURE 2.—Suppression of the *nanos* phenotype by *E(z)* mutations. (**A**) *Nos* embryos form a normal head and thorax but lack abdominal segments. (**B**) *E(z)* mutations suppress the *nos* phenotype. Embryo from $E(z)^{son1}nos/E(z)^{son3}nos$ germ line with a complete abdomen. (**C**) Embryo with suppressed *nos* phenotype from $E(z)^{61}nos/E(z)^{65}nos$ female at 29°. All segments show strong homeotic transformations toward an A8 identity. Note that the *E(z)* mutant combination in (B) shows no obvious homeotic transformations. Filzkörper (arrowheads) unstretched in (A), stretched in (B), repeated in more anterior segments in (C). Darkfield optics. Anterior up, ventral left. H, head skeleton; T, thorax; A, abdomen; Te, telson; 'T' and 'A' are thoracic and abdominal regions; all segments transformed into an A8 identity.

vasa alleles, which cause a failure to localize *nos* RNA (data not shown; WANG *et al.* 1994). Likewise, *E(z)* mutations suppress the abdominal defect of embryos from females which lack *nos* RNA and protein (data not shown) (WANG *et al.* 1994). This indicates that *E(z)* mutations act downstream of *nos* function and thus must affect a function involved in the production, the stability, or the activity of the Hb^{mat} protein. Using anti-Hb antibody staining of embryos, we have not detected any significant effect of *E(z)* mutations on either the distribution or the levels of the uniformly distributed Hb^{mat} protein present in *nos* mutants (data not shown, see also Figure 4, D and I). Thus, *E(z)* most likely acts downstream of, or in conjunction with Hb^{mat}.

It has been proposed that *E(z)* and other Pc-G genes are required for the maintenance but not for the initiation of the repressed state of homeotic genes (STRUHL and AKAM 1985; GLICKSMAN and BROWER 1990; JONES and GELBART 1990; MCKEON and BROCK 1991; SIMON *et al.* 1992). To determine whether *E(z)* is also required for the maintenance of transcriptional repression of *kni* and *gt*, we compared the time when these two genes are first expressed in wild-type embryos to the timing of their expression in embryos from *E(z) nos* double mutant females. The posterior expression of both *kni* and *gt* is first detectable in *E(z) nos* mutant embryos at the mid-

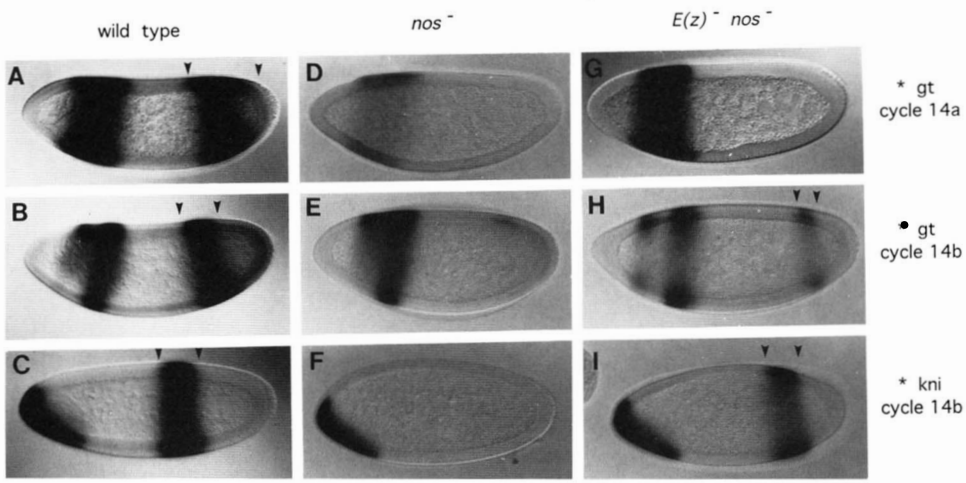

FIGURE 3.—*E(z)* mutations allow abdominal gap gene expression in the absence of *nos* function. Expression patterns of *kni* and *gt* RNA in embryos from wild-type, *nos* and *E(z) nos* females. Only the posterior-most domain of expression of these two genes (flanked by arrowheads) is required for abdomen formation and depends on *nos* function. In wild-type embryos *kni* and *gt* posterior expression is initiated at nuclear cycles 11–12 (MOHLER *et al.* 1989; ROTHE *et al.* 1989; ELDON and PIRROTTA 1991; KRAUT and LEVINE 1991a) [shown in (A) at cycle 14a]). Posterior expression is undetectable at early stages in *E(z) nos* embryos [shown in (G) at cycle 14a], but becomes detectable at a later stage [shown in (H), cycle 14b]. (Data shown only for *gt*, although a similar delay in expression is observed for *kni* in *E(z) nos* embryos.) Weak expression of *gt* in *nos* embryos (E) (KRAUT and LEVINE 1991a) is variable and low compared with wild-type and *E(z) nos* embryos. The posterior domains of both *kni* and *gt* are shifted slightly posteriorly in *E(z) nos* embryos. This may be due to incomplete penetrance of the *E(z)* mutations, and/or partial repression of *kni* and *gt* by *Kr* gene activity. In *nos* and *E(z) nos* embryos *Kr* is activated more posteriorly than normal due to the high concentration of Hb^{mat} protein in the abdominal region (data not shown). This suggests that activation of *Kr* by Hb is not affected by *E(z)* mutations. Nomarski optics of whole-mount *in situ* hybridizations. Anterior left, dorsal up.

cellular blastoderm stage, at least one nuclear cycle delayed with respect to the onset of wild-type expression (in Figure 3, compare B, H with A, G, respectively). The time at which *kni* and *gt* are expressed in *E(z) nos* embryos roughly coincides with the disappearance of the Hb^{mat} protein (TAUTZ 1988) (our own observations). Thus, our results are consistent with a role for *E(z)* in the maintenance of the repressed state initiated by the Hb^{mat} protein.

Role of *E(z)* in anteroposterior patterning: In wild-type embryos Nos protein emanates from the posterior pole and generates a complementary distribution of Hb^{mat} protein. At the syncytial blastoderm stage, when the maternal Hb^{mat} protein is no longer detectable, hb^{zyg} is transcribed in the anterior half of the embryo under the control of *bcd*. The concentration of either Hb^{mat} or Hb^{zyg} along the anteroposterior axis establishes the anterior boundaries of *kni* and *gt* expression (HÜLSKAMP *et al.* 1990; ELDON and PIRROTTA 1991; KRAUT and LEVINE 1991a,b; STRUHL *et al.* 1992). Since *E(z)* is required for the continued repression of these gap genes in *nos* mutant embryos, we asked whether *E(z)* might also be required in wild-type embryos for the proper positioning of the anterior boundaries of *kni* and *gt* expression. In embryos that are mutant for *E(z)*, but are otherwise wild-type, the anterior boundaries of *kni* and *gt* are normal (data not shown). This result could imply that the E(z) product is required for the maintenance of gap gene boundaries established by Hb^{mat} but is dispensable if anteriorly expressed genes, such as Hb^{zyg}, are activated by *bcd*.

Therefore, to determine whether *E(z)* is required for the determination of the anterior boundaries of *kni* and *gt* initiated by the Hb^{mat} gradient, we tested the effect of *E(z)* in *bcd* mutant embryos. In these embryos, the only source of anteroposterior polarity is the Hb^{mat} gradient. We find that *E(z) bcd* double mutant females produce embryos in which the anterior boundaries of *kni* and *gt* expression are shifted anteriorly (compare Figure 4, G, H with B, C, respectively). Thus, *E(z)* is required for the proper formation of the anterior boundaries of *kni* and *gt* expression by the Hb^{mat} protein gradient.

The new boundaries of *kni* and *gt* expression in *E(z) bcd* double mutant embryos are determined solely by cross-regulatory gap gene interactions. The anterior and posterior boundaries of *gt* in *E(z) bcd* mutant embryos depend upon repression by *tailless* (*tll*) and other terminal gap genes (compare Figure 4G with 5A). Negative regulation by terminal gap genes also determines the anterior border of *kni* (compare Figure 4H with 5B). The posterior boundary of *kni* in *E(z) bcd* embryos is negatively controlled by *gt* (compare Figure 4H with 5C).

The effect of *E(z)* mutations on *kni* and *gt* transcription is not observed when these genes are first expressed: anterior boundaries of expression in *E(z) bcd* embryos are established at positions similar to those found in *bcd* embryos. Later, at the cellular blastoderm stage, *kni* and

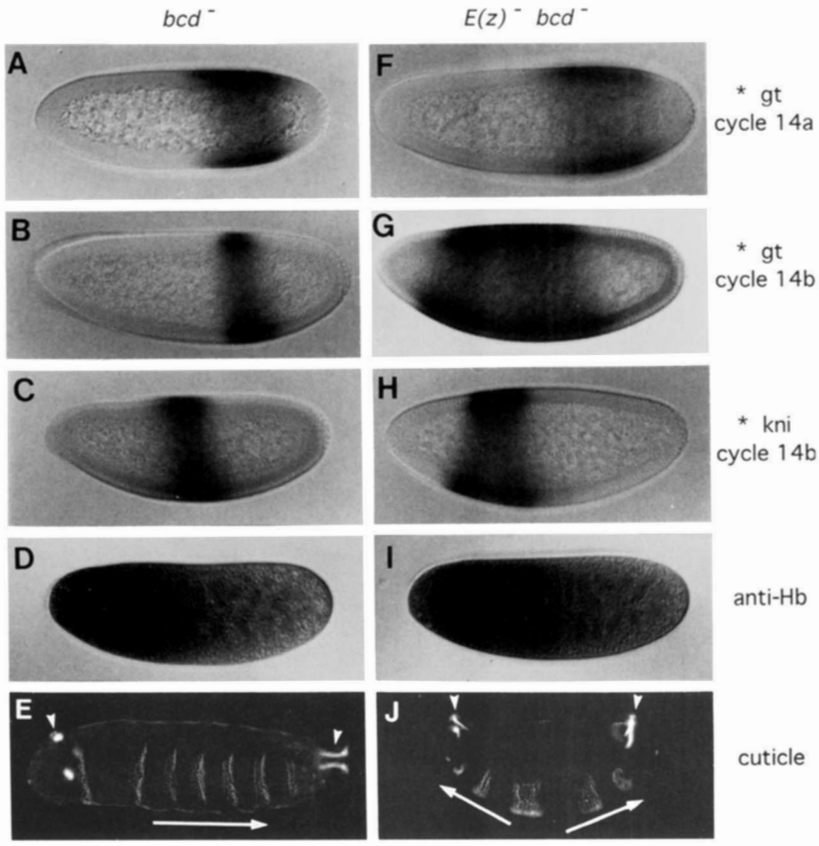

FIGURE 4.—*E(z)* maintains the anterior boundaries of *kni* and *gt* expression in *bcd* embryos. (**A–C, F–H**) *gt* and *kni* RNA expression in whole-mount embryos from *bcd* and *E(z) bcd* mutant females. Until nuclear cycle 14a the anterior boundaries of *gt* and *kni* are similar in *bcd* and *E(z) bcd* mutant embryos (A, F data shown for *gt*). At cycle 14b, however, these boundaries have shifted anteriorly in *E(z) bcd* embryos (B, G and C, H). The embryo in (G) shows the most extreme effect of *E(z)* mutations on *gt* expression. In less extreme cases *E(z) bcd* embryos show a gap of variable extent within the *gt* expression domain (not shown). This gap is likely caused by hb^{mat}-dependent activation of *Kr*, a mutual repressor of *gt* (HÜLSKAMP *et al.* 1990; ELDON and PIRROTTA 1991; KRAUT and LEVINE 1991b; STRUHL *et al.* 1992). The variability in the expression of *gt* is likely due to the incompletely penetrant *E(z)* background. The expression patterns of *Kr* and *kni* are similar and within a single embryo the patterns of *kni* and *Kr* are roughly complementary to that of *gt* (data not shown). It is therefore probable that expression of *Kr* affects *gt* expression in embryos that display the less extreme phenotype and thereby indirectly leads to activation of *kni* (H) (CAPOVILLA *et al.* 1992). (**D, I**) Antibody staining at a pre-pole-bud stage shows no detectable difference in Hb^{mat} protein levels in *bcd* and *E(z) bcd* embryos. *E(z)* does not affect the levels but rather the activity of Hb^{mat} protein: in embryos from *bcd* mutant females heterozygous for a protein null, hb^{7M}, the levels of Hb^{mat} are lower than in *E(z) bcd* embryos even though abdominal pattern duplications are not observed (see below, data not shown). Hb is detected by anti-Hb antibody. Nomarski optics. Anterior left, dorsal up. (**E, J**) Cuticular phenotypes of larvae from *bcd* and *E(z)bcd* females. (E) *Bcd* mutant larvae lack head and thoracic structures and form a second telson with unstretched Filzkörper (left arrowhead). (J) *E(z) bcd* larvae develop two short abdomens in mirror image and stretched Filzkörper at both ends (arrowheads). The anterior abdomen of *E(z) bcd* embryos is usually shorter than the posterior one; this asymmetry may be caused by the off-centered expression of *kni* which overlaps with a domain of residual *Kr* activity (H, not shown). Arrows indicate anterior to posterior polarity of abdomens. Darkfield optics. Anterior left, ventrolateral view.

gt expression is expanded anteriorly in *E(z) bcd* embryos (in Figure 4, compare B, G with A, F, respectively). This suggests that the relative concentration of Hb^{mat} protein initiates the restrictions of *kni* and *gt* expression. At the cellular blastoderm stage, when Hb^{mat} protein is no longer detectable, *E(z)* function is required to maintain a stably repressed state.

***Hb* and *E(z)* act on the same *cis*-acting sequences in the *kni* promoter:** Our experiments suggest that *E(z)* and *hb* act in conjunction to restrict expression of *kni* and *gt* within the prospective abdominal region. To analyze a possible molecular interaction between the two gene products we asked whether sequences within the *kni* promoter, known to be required for abdomen-specific expression, contain sequences required for Hb^{mat} and *E(z)* mediated regulation. A 1.8-kbp fragment of the *kni* promoter fused to the *lac Z* reporter gene confers abdomen specific expression that is identical to that of the endogenous *kni* gene (PANKRATZ *et al.* 1992) (see Figure 6A). This region contains at least five potential binding sites for the Hb protein (PANKRATZ *et al.* 1992).

To determine whether this reporter construct responds to repression by Hb^{mat} we first examined the expression of the transgene in the progeny of *nos* and *bcd* mutant females. Like the endogenous *kni* expression,

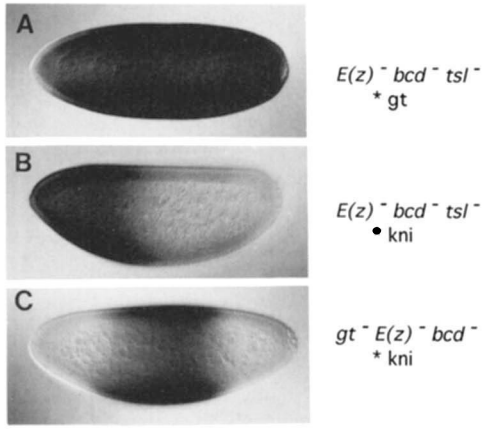

FIGURE 5.—Regulation of *gt* and *kni* expression by gap gene interactions in *E(z) bcd* embryos. Expression patterns of *kni* and *gt* RNA in *E(z) bcd tsl* and *gt, E(z) bcd* embryos. (**A**) *gt* is ubiquitously expressed in *E(z) bcd tsl* embryos (cf. Figure 4G). This embryo shows the most extreme effect of *E(z)* mutations on *gt* expression in this background. The anterior expansion of the *gt* domain is less extreme in other *E(z) bcd tsl* embryos. (**B**) The anterior boundary of *kni* expression expands anteriorly in *E(z) bcd tsl* embryos (*cf.* Figure 4H). The expression pattern in (A) represents a more extreme situation than that in (B) (see legend of Figure 4). (**C**) *kni* expands posteriorly in a *gt, bcd* embryo (*cf.* Figure 4H).

lac Z RNA is absent from the abdominal region in embryos from *nos* mutant females (Figure 6B) and is expressed as a single band in embryos from *bcd* mutant females (Figure 6D). We then asked whether these sequences are also sufficient to confer *E(z)* dependent regulation. We find that *kni-lac Z* is expressed in the prospective abdominal region in embryos from *E(z) nos* females (Figure 6C) and that the domain of expression is expanded anteriorly in embryos from *E(z) bcd* females (Figure 6E). Thus, the regulation of the transgene is identical to that of the endogenous posterior *kni* domain. We conclude that this region of the *kni* promoter contains all sequences required for *E(z)*-dependent transcriptional repression.

Other Polycomb group genes are also involved in the maternal to zygotic transition of gene expression: Based on the similar homeotic phenotypes of Pc-G genes, it has been proposed that their gene products act in conjunction. To determine whether these genes also interact during the maternal-zygotic transition of gene expression we tested mutations in additional Pc-G genes for suppression of the *nos* phenotype.

We first determined whether mutations in any of the known Pc-G genes show a dominant maternal effect similar to that of *E(z)*. Mutations in the genes *Additional sex combs*, *Polycomb*, *Polycomblike*, *polyhomeotic*, *Sex combs on midleg* and *Sex combs extra* do not show any significant dominant suppression of the *nos* phenotype (data not shown; for these and other Pc-G genes, see MATERIALS AND METHODS for specific alleles used and references). We did, however, detect significant dominant suppression of the *nos* phenotype by mutations in the *Su(z)2* complex (*Su(z)2-C*) (Figure 7A, Table 2). Interestingly, deletions of the entire complex, which includes the genes *Posterior sex combs (Psc)*, *Suppressor of zeste 2* (*Su(z)2*) and *Suppressor of zeste 2-D* (*Su(z)2-D*) show significantly stronger suppression than single mutations in any of the genes. Thus, reducing the dosage of more than one *Su(z)2-C* genes may imbalance a multicomponent "repression complex," or alternatively, the genes within the *Su(z)2-C* may be partially redundant in function. In addition, we detected dominant suppression of *nos* by mutant alleles of the gene *pleiohomeotic* (*pho*) (Table 2, see below).

Since mutations in most Pc-G genes lead to homozygous lethality, it is not simple to determine the recessive maternal effect of these genes. Nevertheless, we were able to test two genes for which homozygous mutant allele combinations are viable: null alleles of the gene *extra sex combs* (*esc*) and a hypomorphic allele of *pho*. Embryos from *nos* mutant females, which also lack maternal and zygotic *esc* product, do not form segments in the abdominal region (Figure 7C). Since a loss-of-function *E(z)* background results in suppression of the *nos* phenotype, whereas a null *esc* background has no effect, we conclude that *esc* is not involved in the repression of gap genes by Hb^{mat}. On the other hand, embryos from *nos;pho* females can form a complete set of segments in the abdominal region (Figure 7B). Thus, in addition to *E(z)* several Pc-G genes are required for the repression of gap genes by Hb^{mat}. We conclude that maintenance of gap gene boundaries, like the maintenance of homeotic gene boundaries, may involve several Pc-G group gene products.

DISCUSSION

Screens for dominant suppressors or enhancers of specific mutations have proven to be very successful for identifying interacting products. This is especially applicable to unicellular organisms where a large number of individuals can be tested to detect rare events (see, for example, STEARNS and BOTSTEIN 1988). In higher eukaryotes such as Drosophila, where the number of individuals that can be screened is limiting, highly selective suppressor and enhancer screens have been successfully used for the identification of microtubule-associated proteins (reviewed in FULLER *et al.* 1989), activators and repressors of homeotic genes (KENNISON and TAMKUN 1988), and products involved in signal transduction during photoreceptor cell determination (SIMON *et al.* 1991) or during embryonic patterning (DOYLE and BISHOP 1993).

We carried out a screen for suppressors of the *nos* phenotype and discovered that *Enhancer of zeste* (*E(z)*), a member of the Polycomb group of genes (Pc-G), is a negative transcriptional regulator of the abdomen-specific gap genes *knirps* (*kni*) and *giant* (*gt*). Our genetic analysis indicates that *E(z)* maintains the proper

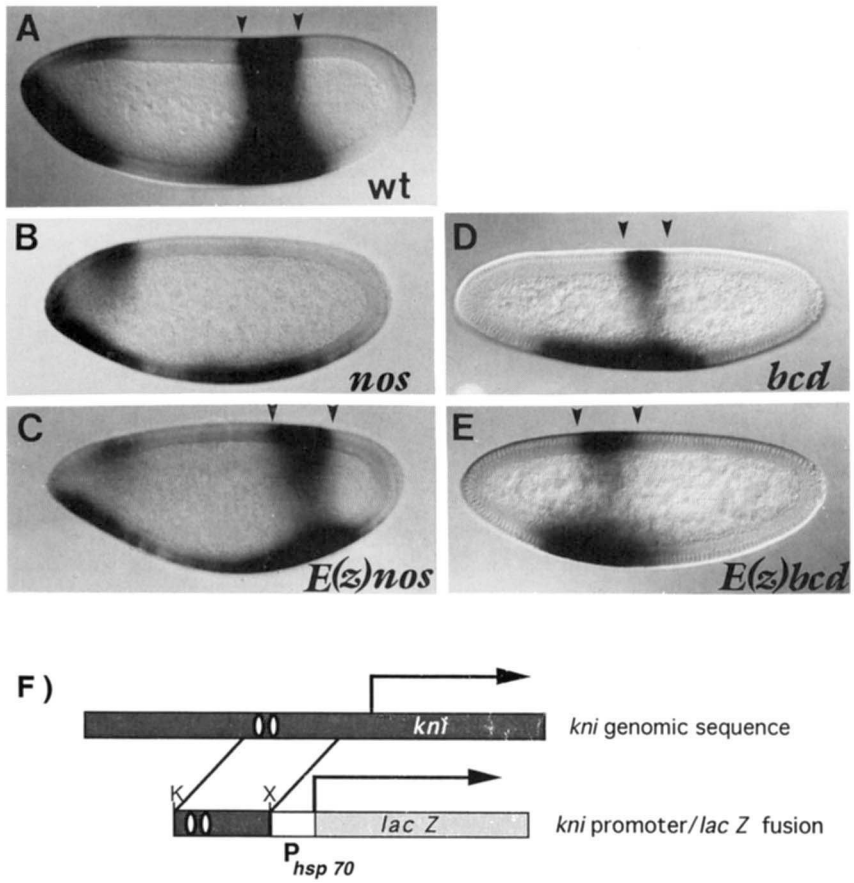

FIGURE 6.—A 1.8-kbp fragment of the *kni* regulatory region fused to *lac Z* faithfully mediates regulation by hb^{mat} and *E(z)*. (**A–E**) Whole-mount *in situ* hybridization showing expression from the *kni-lac Z* fusion construct in (F). Posterior domain of expression is marked by arrowheads. (A) Expression of the transgene in a wild-type embryo (*cf.* Figure 3C). (B) *Nos* embryo lacks the posterior domain of expression (*cf.* Figure 3F). (C) *E(z) nos* embryo expresses the transgene in the posterior domain (*cf.* Figure 3I). (D) In *bcd* embryos the anterior domain is missing and the posterior domain is shifted slightly anteriorly (*cf.* Figure 4C). (E) In *E(z) bcd* embryos the posterior domain is shifted further anteriorly than in *bcd* embryos (*cf.* Figure 4H). Ectopic expression in the ventral region is likely due to a mesoderm enhancer element present in the *ry* gene, which is part of the *P* element transformation vector (DOYLE *et al.* 1989). (**F**) Schematic diagram showing the *kni/lac Z* fusion construct. A 1.8-kbp region of the *kni* regulatory region fused to a basal heat shock promoter directs *lac Z* expression [KX construct from PANKRATZ *et al.* (1992)]. The double ovals mark a cluster of at least five potential binding sites for Hb protein (PANKRATZ *et al.* 1992). All embryos at cycle 14b, Nomarski optics, anterior left, dorsal up.

anterior boundaries of *kni* and *gt* expression once the initial domains of expression have been set according to the concentration gradient of the repressor Hunchback (Hb). Thus, the suppression-of-*nos* screen uncovered an involvement of Pc-G genes in the early patterning of the embryo.

***E(z)* is required for repression of gap genes:** We have studied the effect of *E(z)* mutations in embryos from *nos* and *bcd* mutant females. In *nos* embryos translation of hb^{mat} RNA is deregulated resulting in high levels of Hb^{mat} protein throughout the prospective abdominal region (TAUTZ 1988; WANG and LEHMANN 1991). Hb^{mat} in turn represses transcription of the gap genes *kni* and *gt*, inhibiting abdominal development (HÜLSKAMP *et al.* 1989; IRISH *et al.* 1989a; STRUHL 1989; ELDON and PIRROTTA 1991; KRAUT and LEVINE 1991a,b). We show that *E(z)* mutations lead to expression of *kni* and *gt* in the prospective abdominal region of *nos* embryos. It is this effect of *E(z)* mutations that constitutes the basis for suppression of the *nos* phenotype in the presence of Hb^{mat}. In *bcd* embryos, hb^{zyg} is not expressed, and the Hb^{mat} protein gradient is the major organizer that determines the anterior boundaries of *kni* and *gt* (HÜLSKAMP *et al.* 1990; STRUHL *et al.* 1992). We show that the E(z) product is required for the proper maintenance of these boundaries.

What is the molecular basis for the effect of *E(z)* mutations on transcriptional repression of *kni* and *gt*? *E(z)* mutations, as any other *suppressor-of-nos* mutation, may in principle affect either the production or the activity of the Hb^{mat} protein. We were unable to detect any significant reduction in the levels of Hb^{mat} in embryos from *E(z)* females. Thus, *E(z)* does not seem to affect the production of Hb^{mat} protein. In addition, the effects of *E(z)* mutations in embryos are only apparent when the Hb^{mat} protein is no longer detectable. We therefore conclude that *E(z)* affects a subsequent step of gene regulation that involves the stable maintenance of a transcriptionally repressed state.

In theory, *E(z)* mutations could affect the levels of Kr, which would then alter the boundaries of *kni* and *gt*. This idea seems unlikely, however, since the effects of *E(z)* mutations are more extreme than those caused by a lack of Kr: first, *Kr* mutations do not restore abdominal pattern in *nos* embryos (KRAUT and LEVINE 1991a), and second, the anterior border of *gt* is shifted further anteriorly in *E(z)bcd* embryos than in *Kr;bcd* double mutant embryos [this report and STRUHL *et al.* (1992)]. We favor the idea that *E(z)*, like hb^{mat}, directly affects *kni* and *gt* expression. This is consistent with the finding that the phenotype of *E(z)* mutations most closely resembles that of deleting hb^{mat} (HÜLSKAMP *et al.* 1989, 1990; IRISH *et al.* 1989a; STRUHL 1989).

We have mapped *cis*-acting sequences required for *E(z)* mediated repression to a small fragment in the *kni* regulatory region that contains binding sites for Hb pro-

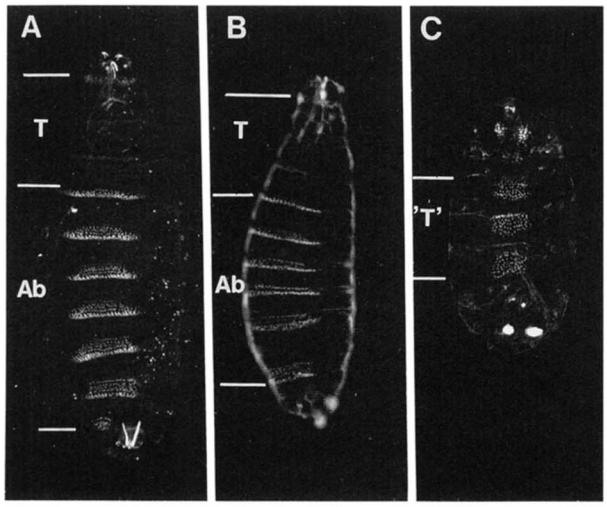

FIGURE 7.—Suppression of the *nos* phenotype by other Polycomb group genes. Cuticle preparations of larvae derived from females mutant for *nos* and various Pc-G group genes. (**A**) Embryo from a *nos* mutant female that is also heterozygous for a deficiency of the entire *Su(z)2* complex. (**B**) Embryo from a *nos;pho* double mutant female. *Nos;pho* embryos show variable degrees of homeotic transformations (not shown). (**C**) Strong *nos* phenotype in *esc*; *nos* embryo; note complete transformation of the three thoracic segments ('T') into an A8 identity. Darkfield optics. Anterior up, ventral view. T, thorax; Ab, abdomen.

TABLE 2

Suppression of *nos* abdominal phenotype by Pc-G genes

	Maternal genotype			
	nos/nos		*hb nos/+ nos*	
Pc-G maternal genotype[a]	Percent rescue[b]	*n*	Percent rescue[b]	*n*
Wild type	0	594	0	614
Df(Su(z)2-C)/+[c]	2	266	27	445
Psc^{1}/+	0	352	0	382
Psc^{1445}/+	2	350	7	256
Psc^{1433}/+	0	651	12	532
Psc^{e24}/+	0	132	0	632
$Su(z)2^{1}$/+	0	52	3	316
$Su(z)2^{1.b7}$/+	0	866	0	689
$Su(z)3^{1}$/+	0	212	0	266
Df(4)G/+	0	378	5	587
pho^{c}/+	0	455	10	659
pho^{c}/pho^{c}[d]	89	18	ND	
esc^{2}/esc^{10}	0	181	ND	

Tests were performed in a genetic background with a full or half dosage of hb^{mat} (*nos/nos* and *hb nos/+ nos* columns, respectively). All tests are at 25° except where otherwise stated. For a description of alleles see MATERIALS AND METHODS.

[a] A "+" refers to the wild-type copy of the particular Pc-G gene tested.

[b] Percent of embryos with ≥3 abdominal segments.

[c] Data shown is for the deficiency $Su(z)2^{1.b8}$. A similar result was obtained using the deficiency *Df(2R)vg-B*.

[d] Test performed at 18°.

tein (PANKRATZ *et al.* 1992). It is possible that *E(z)* and other Pc-G genes interact with the DNA region present in this transgene. Alternatively, binding sites for E(z) may be distributed throughout the genome and interactions between these sites and specific sequences bound by Hb may be required for stable repression.

Our conclusion that the E(z) product, and other Pc-G products (see below), are required for the transcriptional repression of gap genes agrees well with the previously postulated role of *E(z)* as a transcriptional repressor of the *white* and *engrailed* genes (JONES and GELBART 1990; MOAZED and O'FARRELL 1992) and of homeotic genes (JONES and GELBART 1990; PHILLIPS and SHEARN 1990; SIMON *et al.* 1992). Similar to its role in gap gene regulation, a function of Pc-G genes has been implied in the maintenance of *engrailed* repression in anterior cells within each segment (MOAZED and O'FARRELL 1992) and in the maintenance of ordered expression of homeotic genes along the anteroposterior axis (STRUHL and AKAM 1985; GLICKSMAN and BROWER 1990; JONES and GELBART 1990; MCKEON and BROCK 1991; SIMON *et al.* 1992; ZHANG and BIENZ 1992).

The determination of anterior boundaries of gap gene expression by Hb^{mat} appears analogous to the later determination of anterior boundaries of homeotic gene expression by gap genes (WHITE and LEHMANN 1986; HARDING and LEVINE 1988; IRISH *et al.* 1989b; REINITZ and LEVINE 1990; QIAN *et al.* 1991; ZHANG *et al.* 1991; ZHANG and BIENZ 1992; BUSTURIA and BIENZ 1993). In both cases transient, spatially restricted repressors initiate boundaries of expression and those boundaries persist in a Pc-G-dependent process after the original repressors are no longer present (Figure 8). The difference between the two processes is that in the blastoderm embryo additional independent regulators (*e.g.*, Hb^{zyg}) obviate the absolute requirement for the Pc-G function in the determination of gap gene boundaries. Regulators like Hb^{zyg} may not require a Pc-G-dependent maintenance function because they are present throughout the time that *kni* and *gt* are expressed. In the later embryo at the extended germ band stage, no similar redundant mechanisms exist for the proper regulation of homeotic gene boundaries in the absence of Pc-G genes.

Polycomb group genes and the chromatin link: The Pc-G genes are estimated to comprise about 40 genes (JÜRGENS 1985), of which only about a dozen are known. Here, we show that mutations in several other Pc-G genes, such as *pho*, and genes in the Su(z)2-complex, can also act as suppressors of *nos*. We speculate that these and perhaps other Pc-G genes are involved in the negative regulation of gap genes by Hb^{mat}. At least one Pc-G gene, *esc* is clearly not required for this process. *Esc* may be specific for homeotic regulation, as it is also not required for regulation of *engrailed* (MOAZED and O'FARRELL 1992).

It is likely that other Pc-G genes are involved in the repression of gap genes but were not identified in our screen which selected for rare dominant gain-of-function mutations. A rigorous test for a role of maternal Pc-G genes in gap gene regulation will require testing the effect of homozygosity for null mutations in these genes on the *nos* phenotype. These tests will require the

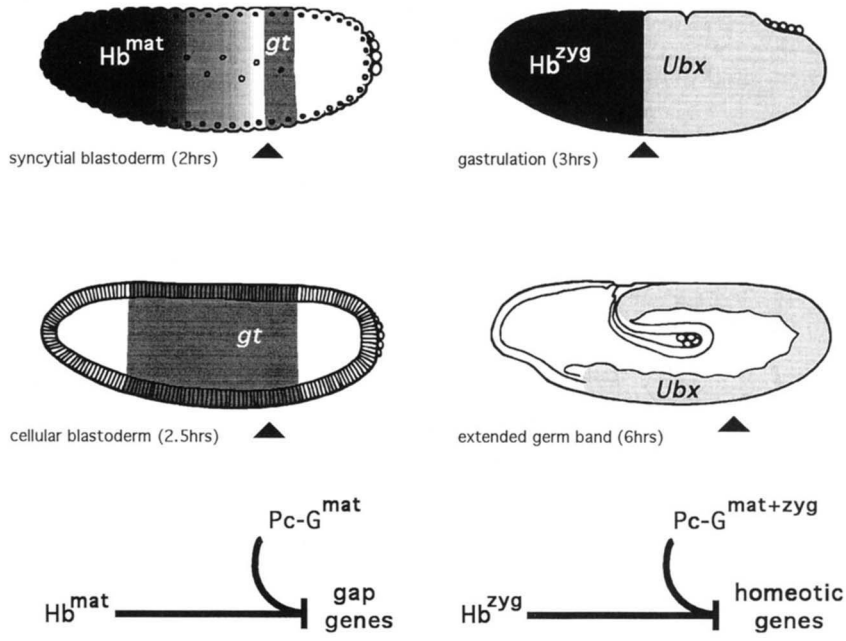

FIGURE 8.—Polycomb group genes are required for two anteroposterior patterning pathways during embryogenesis. (Left side) Model for gap gene regulation by Hb^{mat} and Pc-G genes as proposed in this article (top, syncytial stages; bottom, cellular blastoderm stage). The situation depicted is that in *bcd* mutant embryos, where the only source of anteroposterior polarity is the hb^{mat} gradient. During the syncytial stages Hb^{mat} establishes the boundaries of gap genes such as *gt* (shown) and *kni*. By the end of the blastoderm stage, when Hb^{mat} is no longer detectable, the original boundaries remain at their original positions in wild-type embryos (arrowhead), but are not maintained in Pc-G mutant embryos (as drawn). (Right side) Model for homeotic regulation by gap genes such as hb^{zyg} and Pc-G genes as previously proposed (top, beginning of gastrulation stage; bottom, germ band extended stage). Gap gene products (*e.g.*, Hb^{zyg}) are present until the early gastrulation stages, and establish the boundaries of homeotic genes such as *Ubx*. In wild-type embryos these boundaries remain at their original locations (arrowhead in the extended germ band embryo shown) when gap gene products are no longer present. In Pc-G mutant embryos, though, these boundaries are not properly maintained at these stages. In both models, we propose that at an early stage a specific repressor is present in a spatially restricted manner where it initiates boundaries of gene expression. At a later stage, when the initial repressors are no longer present, Pc-G gene products are required to maintain those boundaries. In embryos that lack Pc-G gene activity gene expression occurs indiscriminately throughout the embryo (the remaining gene boundaries are determined by cross-regulatory interactions among zygotic genes–see text).

generation of germ line chimeras since the function of most Pc-G genes is required at different times during development and thus mutations in these genes are homozygous lethal.

It has been proposed that Pc-G genes may be involved in the formation of a condensed, or "closed" chromatin structure that is less accessible to transcription factors [reviewed in PARO (1990), KENNISON and TAMKUN (1992), WINSTON and CARLSON (1992) and KENNISON (1993)]. Promotion of a stably repressed chromatin state may involve the formation of large multi-subunit complexes composed of several Pc-G gene products. This was first suggested by the similar homeotic phenotypes caused by mutations in these genes, the sensitivity of these phenotypes to dosage imbalances and the apparently synergistic action of these genes (JÜRGENS 1985; KENNISON and RUSSELL 1987; KENNISON and TAMKUN 1988). Recently, biochemical studies have corroborated this idea (ZINK and PARO 1989; DECAMILLIS *et al.* 1992; FRANKE *et al.* 1992; RASTELLI *et al.* 1993; MARTIN and ADLER 1993). Our results suggest that the Pc-G repression machinery may also regulate gap gene expression.

Redundant gradients in AP axis determination: The discovery that *nos* function is dispensable for abdomen formation in the absence of functional Hb^{mat} product raises the question about the importance of the *nos*-dependent patterning system. In particular, Hb^{zyg}, which is activated by the anterior morphogen *bicoid*, can determine the anterior boundaries of *kni* and *gt* and thus fully compensate for the lack of Hb^{mat} (HÜLSKAMP *et al.* 1990; STRUHL *et al.* 1992). At present we can only speculate about the evolution of these two maternal systems that can independently specify the proper positioning of gap gene expression domains along the anteroposterior axis.

The finding that the maintenance of repression of both gap genes and homeotic genes utilizes Pc-G products suggests an ancestral scenario for the determination of the anteroposterior axis in insects. In this model, a local source of *nos* would establish a complementary concentration gradient of Hb. Hb would then regulate both the subdivision (*e.g.*, gap gene pattern) and identity (*e.g.*, homeotic gene expression) of the first embryonic regions. A Pc-G gene dependent process would maintain this prepattern throughout embryogenesis. Indeed, homeotic genes are known to respond to regulation by the Hb^{mat} protein (IRISH *et al.* 1989b; ZHANG *et al.* 1991). Further comparative molecular studies will help

clarify the evolutionary history of the "*nos-hb*-Pc-G" system of anteroposterior patterning.

It has been proposed that redundant systems of positional information may be favored through evolutionary time, since independent overlapping functions would make the patterning process more resilient to external or internal fluctuations (TAUTZ 1992). In this context, a role of Pc-G genes in the determination of transcriptional state of gap genes suggests that the Hb^{mat} gradient may organize the embryo along the anteroposterior axis by "imprinting" the promoters of gap genes with a particular chromatin conformation. This implies that cell fate determination along the anteroposterior axis is regulated at the chromatin level, by reducing access to the promoters of posterior-promoting gap genes in anterior regions. A precedent for spatial imprinting of genes has been reported recently in mouse muscle cells (DONOGHUE *et al.* 1992). In the Drosophila embryo, such a chromatin scaffold would provide a robust base for further refinement by the overlying network of zygotic gene interactions.

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