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Ten different *Polycomb* group genes are required for spatial control of the *abdA* and *AbdB* homeotic products

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Summary

Mutations in genes of the *Polycomb (Pc)* group cause abnormal segmental development due to ectopic expression of the homeotic products of the *Antennapedia* and bithorax complexes. Here the requirements for *Pc* group genes in controlling the *abdA* and *AbdB* products of the bithorax complex are described. Embryos containing mutations in the genes *Polycomb (Pc), extra sex combs (esc), Enhancer of zeste [E(z)], polyhomeotic (ph), Sex comb on midleg (Scm), Polycomb-like (Pcl), Sex comb extra (Sce), Additional sex combs (Asx), Posterior sex combs (Psc) and pleiohomeotic (pho)* were examined. In every case, both *abdA* and *AbdB* are expressed outside of their normal domains along the anterior-posterior (A-P) axis, consistent with these *Pc* group products acting in a single pathway or molecular complex. The earliest detectable ectopic expression is highest in the parasegments immediately adjacent to the normal expression boundary. Surprisingly, in the most severe *Pc* group mutants, the earliest ectopic *AbdB* is distributed in a pair-rule pattern. At all stages, ectopic *abdA* in the epidermis is highest along the anterior edges of the parasegments, in a pattern that mimics the normal *abdA* cell-specific pattern. These examples of highly patterned mis-expression show that *Pc* group mutations do not cause indiscriminate activation of homeotic products. We suggest that the ectopic expression patterns result from factors that normally activate *abdA* and *AbdB* only in certain parasegments, but that in *Pc* group mutants these factors gain access to regulatory DNA in all parasegments.

Key words: *Polycomb* group, bithorax complex, homeotic, *Drosophila*, anterior-posterior axis.

Introduction

Differentiation of segments along the anterior-posterior (A-P) axis in *Drosophila* is controlled by genes of the *Antennapedia* and bithorax complexes (Lewis, 1978; Kaufman et al., 1980). These gene complexes encode homeotic proteins that are expressed in precise domains along the A-P axis. The bithorax complex (BX-C) controls segment identity in the posterior half of the fly (Lewis, 1978; Bender et al., 1983; Karch et al., 1985). The BX-C executes this function with three homeotic proteins, *Ubx*, *abdA* and *AbdB* (Regulski et al., 1985; Sanchez-Herrero et al., 1985; Tiong et al., 1985), which are deployed differentially along the A-P axis. *Ubx* primarily controls the identities of the third thoracic and first abdominal segments and is expressed in parasegments 5 through 13 (White and Wilcox, 1985; Beachy et al., 1985), *abdA* controls the middle abdomen and is expressed in parasegments 7 through 13 (Karch et al., 1990; Macias et al., 1990) and *AbdB* controls the posterior abdomen and is expressed in parasegments 10 through 15 (Celniker et al., 1989; DeLorenzi and Bienz, 1990; Boulet et al., 1991). The restriction of *Ubx*, *abdA* and *AbdB* to their respective anterior boundaries is critical since development is perturbed in BX-C mutants that mis-express these products in more anterior locations (White and Akam, 1985; Karch et al., 1990; Celniker and Lewis, 1987; Celniker et al., 1990).

The genes of the BX-C are first transcribed in 2-hour blastoderm stage embryos (Akam and Martinez-Arias, 1985; Harding and Levine, 1988; Kuziora and McGinnis, 1988; Sanchez-Herrero and Akam, 1989). It is likely that the initial anterior expression boundaries are set by segmentation gene products such as *hunchback* and *Kruppel* (White and Lehmann, 1986; Harding and Levine, 1988; Irish et al., 1989; Reinitz and Levine, 1990; Qian et al., 1991). However, the early expression patterns of segmentation products decay by about 4 hours of embryogenesis. In contrast, the BX-C products are expressed continuously, within their proper boundaries, throughout subsequent larval and pupal development over a period of about 10 days (White and Wilcox, 1985; Brower, 1987). This expression within A-P boundaries is required continually for proper segmental development (Lewis, 1964; Morata et al., 1983). Thus, a mechanism must exist to maintain the expression boundaries after they are set in early embryos.

The product of the *Polycomb (Pc)* gene (P.H. Lewis, 1947; E.B. Lewis, 1978; Duncan and Lewis, 1982) is a

likely component of this maintenance machinery. *Pc* product is required in embryos and during postembryonic stages for proper control of the BX-C products (Struhl, 1981; Duncan and Lewis, 1982; Busturia and Morata, 1988). Embryos that lack zygotic *Pc* product die with all segments transformed towards the eighth abdominal segment (A8). The gene is named for the dominant adult phenotype, segmental transformation of second and third thoracic legs into first thoracic leg, causing duplications of the male sex comb (Duncan and Lewis, 1982). These phenotypes result from ectopic expression of homeotic products outside of their normal A-P boundaries. In particular, *Pc* mutant embryos mis-express the *Antennapedia (Antp)* and *Sex combs reduced (Scr)* genes of the *Antennapedia* complex (ANT-C) as well as *Ubx*, *abdA* and *AbdB* (Beachy et al., 1985; Wedeen et al., 1986; Carroll et al., 1986; Riley et al., 1987; Kuziora and McGinnis, 1988; Celniker et al., 1989). The recessive embryonic phenotype results primarily from the mis-expression of *AbdB* in anterior parasegments (Celniker et al., 1989). The dominant adult leg transformation is thought to result from posterior mis-expression of *Scr* (Glicksman and Brower, 1988). Molecular studies have shown that *Pc* protein binds to the ANT-C and BX-C loci in polytene chromosomes (Zink and Paro, 1989) although it fails to bind directly to DNA in vitro (Zink et al., 1991).

Another likely component of the maintenance machinery is the *extra sex combs (esc)* gene (Struhl, 1981, 1983). Embryos lacking both maternal and zygotic *esc* product show segmental transformations similar to those in *Pc* mutants. Animals lacking only zygotic *esc* product develop into adults bearing leg transformations like in *Pc*/+ adults (Struhl, 1981). *esc* mutants ectopically express *Ubx* and *Antp* in embryos as well as *Ubx*, *Antp* and *Scr* in imaginal discs (Struhl and White, 1985; Carroll et al., 1986; Glicksman and Brower, 1988, 1990). Careful analysis of the timing of *Ubx* mis-expression in *esc* null embryos has shown that *esc* is required to maintain, but not to set up, the anterior *Ubx* expression boundary (Struhl and Akam, 1985).

A number of other mutations have been described that cause phenotypes similar to those in *Pc* and *esc* mutants (Gehring, 1970; Duncan, 1982; Ingham, 1984; Jürgens, 1985; Breen and Duncan, 1986; Dura et al., 1987; Adler et al., 1989; Jones and Gelbart, 1990; Phillips and Shearn, 1990). The genes defined by these mutations have become known collectively as the *Pc* group (reviewed in Paro, 1990). It is generally assumed that these *Pc* group genes are trans-regulators of the ANT-C and BX-C genes and that, like *Pc* and *esc*, they maintain homeotic boundaries by repressing expression in inappropriate A-P axis positions. These assumptions were proved correct for the *polyhomeotic* and *Enhancer of zeste* loci, which are both required for proper boundaries of *Scr* and *Ubx* (Dura and Ingham, 1988; Jones and Gelbart, 1990). However, the *extradenticle* gene (Wieschaus et al., 1984), which could be classified in the *Pc* group on the basis of phenotype, is not required for the proper distribution of homeotic products (Peifer and Wieschaus, 1990).

A remarkable feature of at least some *Pc* group products is that they are required simultaneously to repress different homeotic products at multiple positions along the A-P axis. For example, *Pc* is required to maintain anterior boundaries of *Antp* in parasegment (PS) 3, *Ubx* in PS5, *abdA* in PS7 and *AbdB* in PS10. It is difficult to imagine *Pc* acting as a simple transcriptional repressor in many different parasegments at the same time, especially since *Pc* protein itself appears uniformly distributed along the A-P axis (Paro and Hogness, 1991). However, it is possible that *Pc* is a ubiquitously required subunit of the repression machinery and some of the other *Pc* group products are more specifically required for the regulation of certain homeotic products. For example, *esc* mutants show abundant ectopic expression of *Antp* and *Ubx* but *Scr* is only subtly mis-expressed (Glicksman and Brower, 1990).

To characterize further the roles of *Pc* group products in regulating multiple homeotic products, we have examined the distributions of *abdA* and *AbdB* proteins in eleven of the *Pc* group mutants. We report that both *abdA* and *AbdB* are ectopically expressed in embryos mutant for ten different *Pc* group genes. We also describe the patterning and kinetics of accumulation of this ectopic expression.

Materials and methods

Mutant stocks and generation of mutant embryos

esc^{10} is a deficiency for the *esc* locus (Struhl, 1981, 1983). esc^{2} and esc^{6} are apparent null alleles, based upon severity of embryonic phenotypes (Struhl, 1981, 1983). Batches of *esc* null embryos were collected as the progeny of esc^{10}/esc^{2} parents. *esc* null embryos were also collected as the progeny of esc^{6}/esc^{6} parents. esc^{+}/esc^{-} paternally rescued embryos were generated by crossing esc^{10}/esc^{2} females to ry^{502} males.

$E(z)^{S2}$ is a temperature-sensitive allele that is null or nearly null for its homeotic function at the restrictive temperature, 29°C (Jones and Gelbart, 1990). Segmental transformations in embryos from hemizygous versus homozygous $E(z)^{S2}$ mothers are indistinguishable (R. Jones, personal communication). Batches of homozygous mutant $E(z)^{S2}$ embryos were collected at 29°C from a homozygous stock grown at 18°C. $E(z)^{S2}$/+ paternally rescued embryos were collected at 29°C as the progeny of $E(z)^{S2}$ homozygous mutant females and ry^{502} males.

Homozygous mutant embryos containing other *Pc* group alleles were collected as the progeny of heterozygous parents. The homozygous mutants constituted one-quarter of the embryos in mixed batches. Pc^{XT109} is a null allele that fails to make *Pc* protein (R. Paro, personal communication). Pc^{3} is an antimorphic allele that produces a phenotype stronger than the null (Lewis, 1978; Duncan and Lewis, 1982; Haynie, 1983). *abdA* and *AbdB* mis-expression in embryos mutant for either *Pc* allele were similar. ph^{503} is an apparent null allele affecting both coding units at the locus and *Df(1)JA52* is a deficency for the *ph* locus (Dura et al., 1987; Perrimon et al., 1985). Mis-expression was similar in both *ph* alleles. Scm^{D1} is an apparent null allele (Breen and Duncan, 1986). Scm^{H1} is a previously unreported *Scm* allele that is lethal over *Scm* null alleles (R. Jones, personal communication). Mis-expression was similar in both *Scm* alleles. Pcl^{D5} is an apparent null allele

Table 1. *List of* Pc *group mutant alleles analyzed*

Pc group gene	Alleles used	References
extra sex combs (esc)	esc^{10}	1, 2
	esc^{2}	1, 2
	esc^{6}	1, 2
Enhancer of zeste [E(z)]	$E(z)^{S2}$	3
Polycomb (Pc)	Pc^{3}	4, 5
	Pc^{XT109}	6
polyhomeotic (ph)	ph^{503}	7
	Df(1)JA52	7, 8
Sex comb on midleg (Scm)	Scm^{D1}	9
	Scm^{H1}	10
Polycomb-like (Pcl)	Pcl^{D5}	9
Sex comb extra (Sce)	Sce^{D1}	9
Additional sex combs (Asx)	Asx^{D1}	9
	Asx^{XF23}	11
	Df(2R)trix	9
Posterior sex combs (Psc)	$Psc^{14\text{-}45}$	12, 13
	Psc^{IIN48}	12, 14
	$Df(2R)vg^{D}$	13, 15
pleiohomeotic (pho)	pho^{b}	9, 16
	Df(4)G	9, 16
super sex combs (sxc)	sxc^{1}	17

Patterns of *abdA* and *AbdB* expression were examined in embryos mutant for each of the alleles listed, as described in Materials and methods, except for esc^{6} where only *AbdB* was examined. References: (1) Struhl, 1981; (2) Struhl, 1983; (3) Jones and Gelbart, 1990; (4) Lewis, 1978; (5) Duncan and Lewis, 1982; (6) R. Paro, personal communication; (7) Dura et al., 1987; (8) Perrimon et al., 1985; (9) Breen and Duncan, 1986; (10) R. Jones, personal communication; (11) G. Jürgens, personal communication; (12) Adler et al., 1989; (13) Lasko and Pardue, 1988; (14) Jürgens, 1985; (15) Brunk et al., 1991; (16) Hochman et al., 1964; (17) Ingham, 1984.

(Breen and Duncan, 1986). Sce^{D1} is the single allele for this locus (Breen and Duncan, 1986). It is a first instar larval lethal but its severity is not known. Asx^{D1} is a hypomorphic allele (Breen and Duncan, 1986). Asx^{XF23} behaves genetically as a null allele (G. Jürgens, personal communication). *Df(2R)trix* is a deficiency for the *Asx* locus (Breen and Duncan, 1986). *Df(2R)trix* and Asx^{XF23} mutant embryos showed similar levels of mis-expression that were stronger than those seen in Asx^{D1} embryos. The preferential mis-expression in the epidermis versus the CNS was seen with all three alleles. $Psc^{14\text{-}45}$ and Psc^{IIN48} are strong hypomorphs with respect to homeotic function (Jürgens, 1985; Adler et al., 1989; Lasko and Pardue, 1988; P. Adler, personal communication). $Df(2R)vg^{D}$ is a deficiency that removes most of the *Psc* transcription unit (Brunk et al., 1991; Lasko and Pardue, 1988). Mis-expression in $Psc^{14\text{-}45}$ and Psc^{IIN48} embryos was similar with slightly stronger mis-expression seen in vg^{D} embryos. pho^{b} (previously $l(4)29^{b}$) is an apparent null allele and *Df(4)G* is a deficiency for the *pho* locus (Breen and Duncan, 1986; Hochman et al., 1964). Similar subtle mis-expression was seen with both alleles. sxc^{1} is an apparent null allele (Ingham, 1984). The *Pc* group mutants analysed are listed in Table 1.

Antibody staining and dissection of embryos

Embryos were fixed and stained as described (Simon et al., 1990) using a polyclonal antibody against *abdA* (Karch et al., 1990) or a monoclonal antibody against *AbdB* (Celniker et al., 1989). Stained embryos were dissected and mounted as described (Simon et al., 1990), except for the embryo in Fig. 7A (See figure legend). Briefly, in germ band-extended (6h) embryos, the attachment of the posterior end of the germ band to the dorsal part of the head was cut and the germ band was flipped out to display the ectodermal surface in two dimensions. Later stage embryos were slit along the dorsal midline, the gut and visceral mesoderm were excised and the remaining tissues were flattened in two dimensions.

Results

Embryos mutant for *Polycomb* group alleles were stained with antibodies to *abdA* (Karch et al., 1990) or *AbdB* (Celniker et al., 1989). In most cases, two or three mutant alleles for each gene were analyzed, including null alleles or strong hypomorphs (See Methods). The mutants are described in the order of most extreme to least extreme mis-expression of these two BX-C products. In general, *esc* and *E(z)* mutant embryos showed more extreme mis-expression of *abdA* and *AbdB* than other *Pc* group mutants. This is partly because many of the *Pc* group genes are expressed maternally (Denell, 1982; Haynie, 1983; Ingham, 1984; Breen and Duncan, 1986; Dura et al., 1988) and, except for *esc* and *E(z)*, we have assayed embryos lacking only the zygotic contributions.

The wild-type expression patterns of *abdA* and *AbdB* have been described (Karch et al., 1990; Macias et al., 1990; Celniker et al., 1989; DeLorenzi and Bienz, 1990; Boulet et al., 1991). Examples of the wild-type patterns are shown here in Figs 1 and 2 for comparative purposes. *abdA* protein is first seen at about 4 hours of embryogenesis with a precise anterior boundary at the anterior edge of parasegment (PS) 7. This anterior boundary (Fig. 1A, B) persists throughout the rest of embryogenesis. *AbdB* protein is first detected at about 4 hours with an anterior boundary in PS13 which persists until about 6 hours in germ band-extended embryos (Fig. 2A). As embryogenesis proceeds, additional *AbdB* becomes detectable in parasegments 10 through 12 (Fig. 2B) with the final anterior boundary in PS10.

extra sex combs (esc)

Embryos lacking both maternal and zygotic *esc* product were generated using apparent null alleles (Struhl, 1981, 1983). When *abdA* protein is first detected in these esc^{-} embryos the pattern appears normal (Fig. 3A), but between 5 and 6 hours ectopic expression is seen just anterior to the normal boundary in PS5 and PS6 (Figs 1C, 3B). As embryogenesis proceeds, ectopic *abdA* spreads progressively into more anterior parasegments. Embryos between 6 and 7 hours often show intermediate spread, with abundant expression in PS5 and PS6 and weaker expression now detectable in PS3 and PS4 (Fig. 3C). Although there is some variability in the amount of ectopic *abdA* at this stage, the graded expression from posterior to anterior is reproducibly seen. Eventually, expression extends further anteriorly and into the head region (Fig. 3D) and by 9 hours the

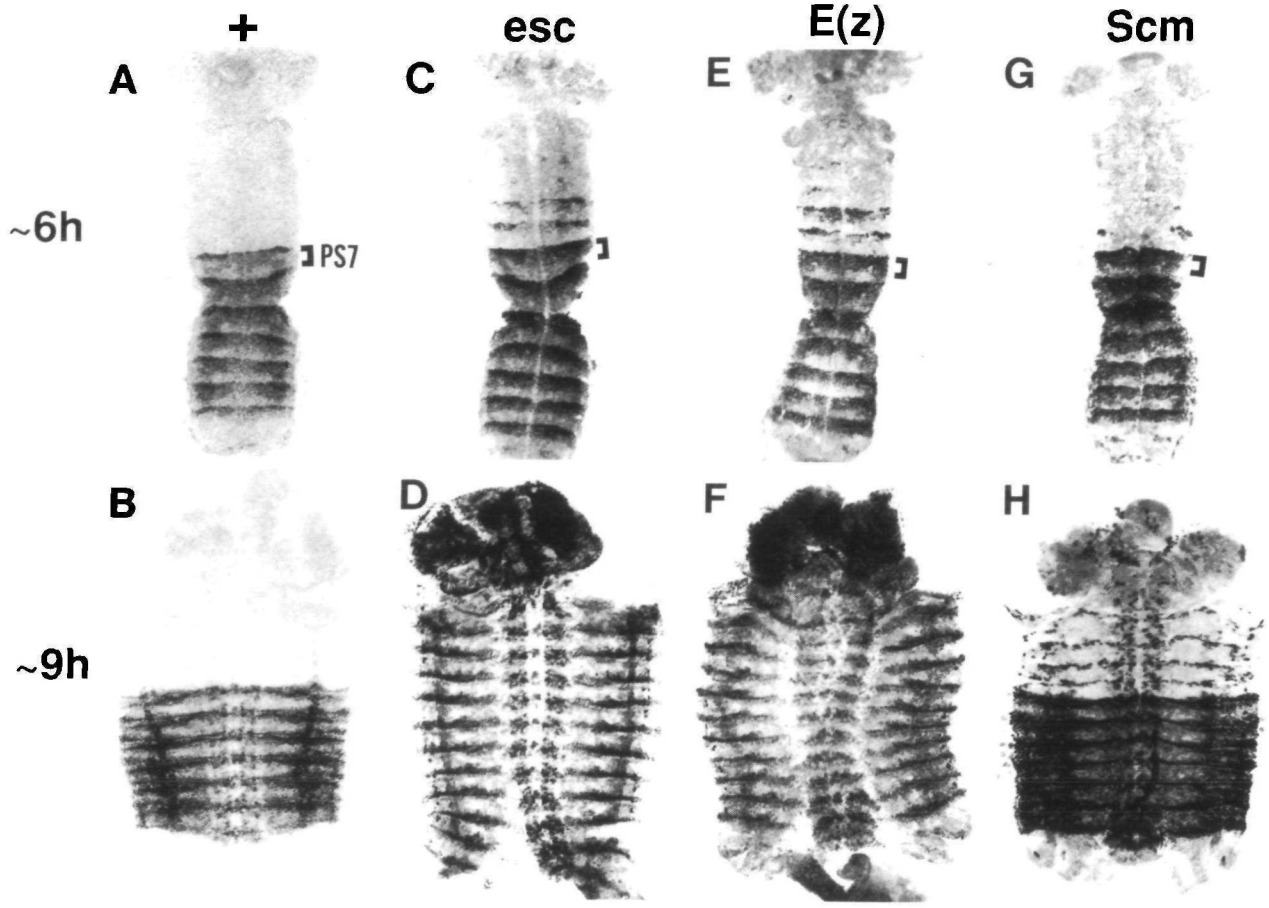

Fig. 1. *abdA* expression in *esc*, *E(z)* and *Scm* mutants. Embryos were stained with *abdA* antibody. (A, B) Wild type. The anterior boundary of *abdA* is in parasegment (PS) 7. (C, D) esc^- embryos from esc^{10}/esc^2 parents. (E, F) $E(z)^{S2}$ homozygotes at 29°C. (G, H) Scm^{D1} homozygotes. A, C, E and G show approximately 6 hour embryos at full germ-band extension. B, D, F and H show approximately 9 hour embryos after germ-band retraction. Brackets indicate PS7. Embryos here and in subsequent figures are oriented with anterior to the top and were dissected as described in Materials and methods.

entire A-P axis shows strong accumulation of *abdA* (Fig. 1D).

Although ectopic *abdA* eventually appears throughout the A-P axis, its cell-specific distribution is not uniform. Instead, ectopic *abdA* appears in the epidermis in a repeating pattern, high at the anterior margin and low at the posterior margin within each parasegment (Figs 1C, D, 3B-D). This cell-specific patterning resembles the wild-type distribution of *abdA* in parasegments 7-13 (Fig. 1A, B).

In addition to the anterior spread of *abdA* in esc^- embryos, there is also posterior spread. Normally the posterior boundary of *abdA* expression is in PS13 (Karch et al. 1990). In 6 hour esc^- embryos, *abdA* accumulates in epidermal cells in PS14. By about 8 hours ectopic *abdA* is visible in PS14 and PS15, and weakly in the hindgut rudiment (Fig: 4A). By 9 hours, the expression in the hindgut is more intense and it includes the lateral processes of the developing Malpighian tubules (Fig. 4B).

Like *abdA*, ectopic *AbdB* does not appear at the same time in all parasegments. *AbdB* appears normal in esc^- embryos between 4 and 5 hours (Fig. 3E) but, during the next hour, ectopic *AbdB* is activated as far forward as PS3 (Fig. 2C, arrow in 3F). The earliest ectopic expression is pair-rule modulated with stronger expression in the odd-numbered parasegments. Additional *AbdB* then accumulates, primarily in posterior parasegments, so that by 6 hours graded expression is seen along the A-P axis and the pair-rule distribution is still visible (Fig. 3G). By 7 hours, most embryos have fairly uniform *AbdB* expression up to PS3, with less expression anterior to this boundary (Fig. 3H). Finally, between 7 and 9 hours, the most anterior region of the embryo accumulates *AbdB*, with 9 hour embryos showing a uniform distribution in virtually all epidermal cells (Fig. 2D). This uniform epidermal distribution is similar to the pattern of *AbdB* in PS13 of wild-type embryos (Fig. 2B).

In *esc* null embryos, all the segments are transformed into copies of the normal eighth abdominal segment (posterior PS13 plus anterior PS14) (Struhl, 1981, 1983). This is explained by the uniform distribution of *AbdB* protein (Fig. 2D) since *AbdB* predominates when it appears in the same parasegment with other homeotic products (Struhl and White, 1985; Busturia

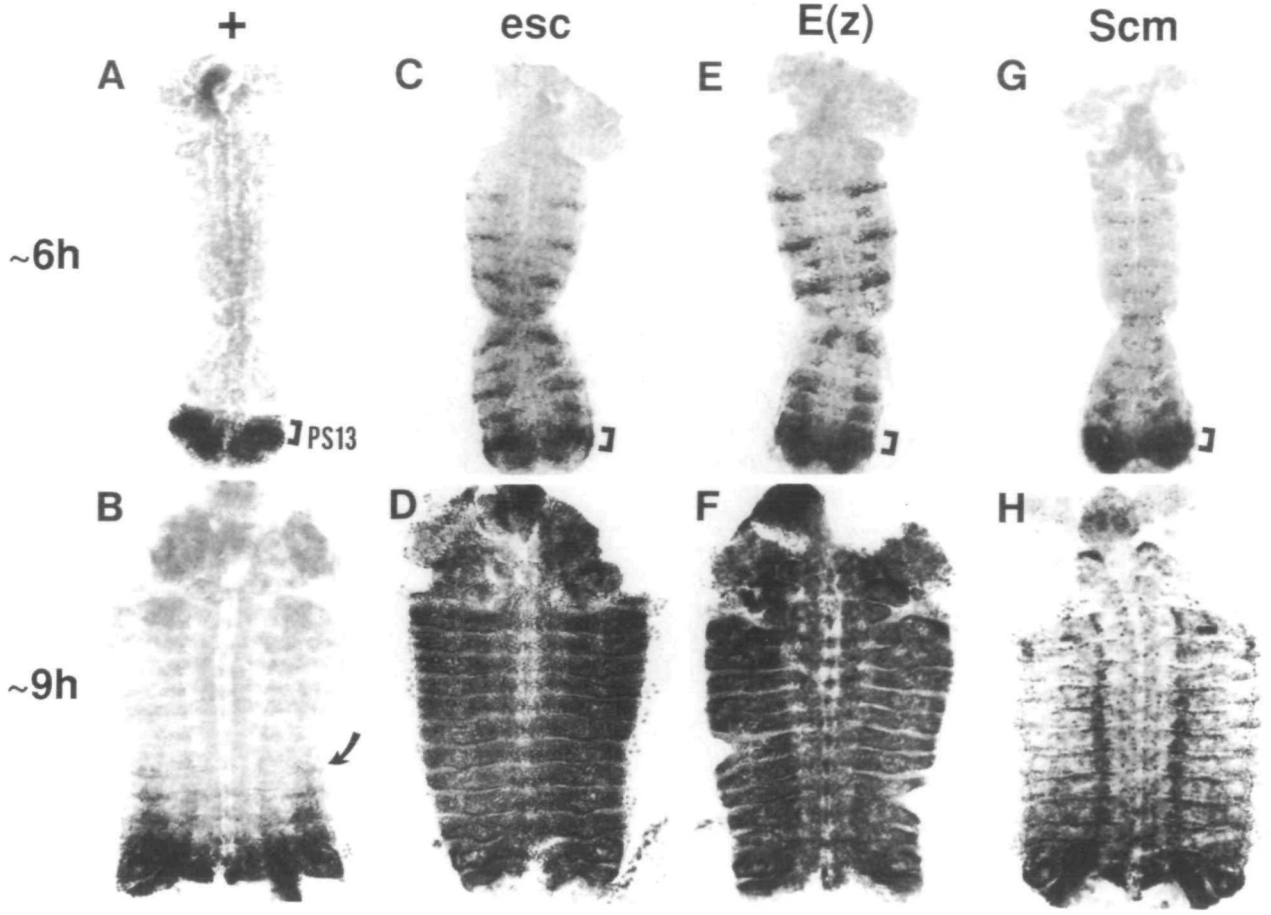

Fig. 2. *AbdB* expression in *esc, E(z)* and *Scm* mutants. Embryos were stained with *AbdB* antibody. (A, B) Wild type. The anterior boundary of *AbdB* in A is PS13 and in B it is PS10 (curved arrow). (C, D) esc^- embryos from esc^{10}/esc^2 parents. (E, F) $E(z)^{S2}$ homozygotes at 29°C. (G, H) Scm^{D1} homozygotes. A, C, E and G show approximately 6 hour embryos at full germ-band extension. B, D, F and H show approximately 9 hour embryos after germ-band retraction. Brackets indicate PS13.

and Morata, 1988). If eggs derived from esc^-/esc^- mothers are fertilized by sperm containing one copy of esc^+, then less severe transformations are seen (Struhl, 1981). Such esc^+/esc^- embryos show complete or partial transformation of thoracic and abdominal segments to eighth abdominal, but the head segments are unaffected or only partially transformed. Fig. 4D shows that *AbdB* expression in such partially paternally rescued embryos is quite uniform in much of the abdomen but much reduced relative to *esc* null embryos in the anterior thorax (PS3 and 4) and in the head (compare to Fig. 2D). The head expression appears spotty and random from embryo to embryo. The final *AbdB* distribution along the A-P axis in these esc^+/esc^- embryos now resembles that in the 6-7 hour *esc* null embryos (Fig. 3G, H). Thus, the differential severity of the phenotype along the A-P axis correlates with the differential severity of *AbdB* mis-expression along that axis.

A similar partial spread of *abdA* occurs in these esc^+/esc^- embryos. At 9 hours (Fig. 4C) and at subsequent stages, there is strong *abdA* expression in much of the thorax and abdomen but the anterior thorax and head region show weaker expression in isolated groups of cells. This contrasts to the strong *abdA* expression along the entire A-P axis in *esc* null embryos (Fig. 1D). In fact, the final *abdA* pattern has a marked PS5 anterior boundary which mirrors the transient PS5 boundary seen at earlier times in *esc* null embryos (Fig. 3B). It seems that a partial amount of esc^+ product, or its delayed appearance, causes a 'freeze' in the gradual anterior spread of *abdA* and *AbdB* in the intermediate stage.

Enhancer of zeste [E(z)]

Mutations that reduce or eliminate zygotic product of the *Enhancer of zeste* gene (Jones and Gelbart, 1990), also called *polycombeotic (pco)* (Phillips and Shearn, 1990), cause larval-to-pupal lethality. However, temperature-sensitive alleles have been used to severely reduce both maternal and zygotic $E(z)^+$ product, resulting in embryos that die with extreme transformation of all segments towards A8 (Jones and Gelbart, 1990; Phillips and Shearn, 1990).

Homozygous mutant embryos containing the temperature-sensitive allele $E(z)^{S2}$ were collected at the

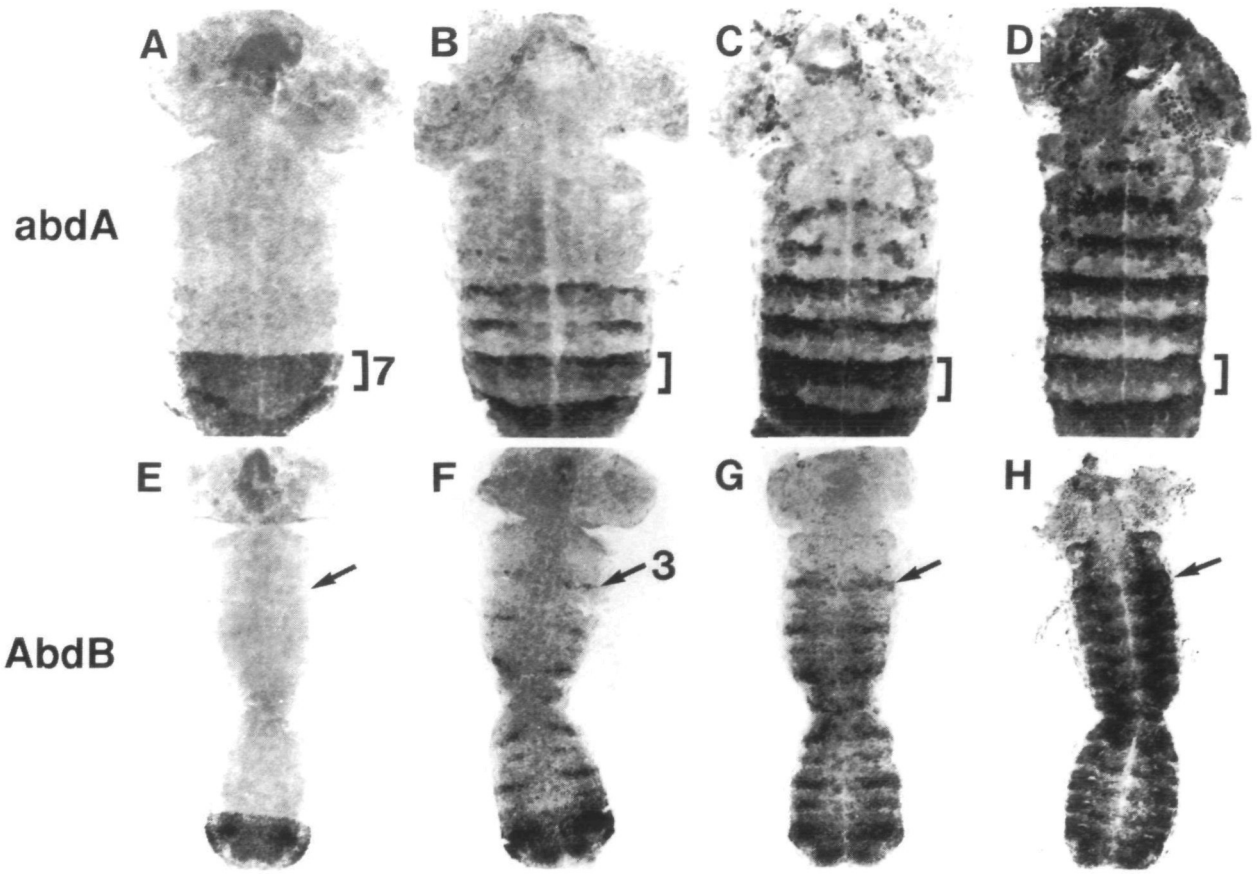

Fig. 3. Onset of *abdA* and *AbdB* mis-expression in *esc* mutant embryos. Embryos are progeny of esc^{10}/esc^{2} parents. Embryos are between 4.5 and 7 hours old and are arranged to show the progressive accumulation of ectopic homeotic products. Embryos in A-D were stained with *abdA* antibody and only their anterior halves are shown. Embryos in E-H were stained with *AbdB* antibody. Brackets indicate PS7. Arrows indicate PS3.

restrictive temperature, 29°C, and examined for *abdA* and *AbdB* distributions. Although not null for other functions of *E(z)*, the $E(z)^{S2}$ allele is null or nearly null with regard to homeotic function (Jones and Gelbart, 1990; R. Jones, personal communication, see Materials and methods). As in *esc* mutants, ectopic expression of *abdA* spreads forward gradually. At about 6 hours, ectopic *abdA* is most abundant in PS5 and PS6 (Fig. 1E). By 9 hours, *abdA* is expressed throughout the A-P axis including the head (Fig. 1F). In this same period of time, *abdA* also spreads posteriorly into PS14, PS15 and into the hindgut primordium. At all times, ectopic *abdA* is patterned within each parasegment with higher expression at the anterior margins.

The ectopic *AbdB* patterns in *E(z)* mutant embryos also resemble those seen in *esc* mutants. At 6 hours, *AbdB* is activated as far forward as PS3 in a pair-rule pattern (Fig. 2E). By 9 hours, *AbdB* is expressed throughout the A-P axis in all or nearly all epidermal cells (Fig. 2F).

If $E(z)^{S2}/+$ embryos are collected at 29°C from homozygous mutant mothers and wild-type fathers, the embryos are still extremely transformed, but there is some phenotypic rescue, primarily in the head (Jones and Gelbart, 1990). In agreement with this, both *abdA* and *AbdB* still spread throughout the A-P axis in paternally rescued $E(z)^{S2}/+$ embryos, but the accumulation of *AbdB* in the head region now appears patchy (not shown).

Polycomb (Pc)

Expression was examined in homozygous *Pc* embryos derived from heterozygous parents. Ectopic *abdA* is first seen between 5 and 6 hours of development in lateral epidermal patches in PS6. During the next 3 hours, *abdA* spreads into the more anterior parasegments. By 9-10 hours, expression is seen throughout the A-P axis and this pattern persists through 12 hours (Fig. 5B).

The earliest ectopic *AbdB* is seen in the pair-rule pattern described for *esc* and *E(z)* mutant embryos. Subsequently, the further accumulation of *AbdB* parallels that seen with these two other mutants except that the timing of the anterior spread is delayed by 2-3 hours, and *AbdB* fails to accumulate to high levels in all epidermal cells. In particular, patchy *AbdB* is seen in the head region in 9-12 hour embryos.

The patterns of ectopic *abdA* and *AbdB* in *Pc* mutants resemble those described for *esc* and *E(z)* with slightly reduced severity. However, this abundant level

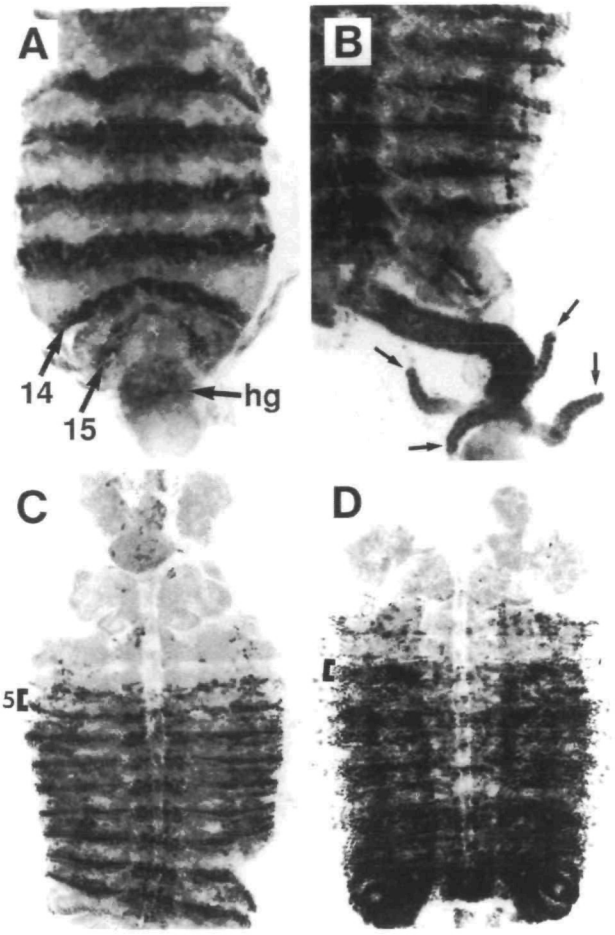

Fig. 4. Features of ectopic expression in *esc* mutant embryos. Embryos in A-C were stained with *abdA* antibody. Embryo in D was stained with *AbdB* antibody. (A, B) Progeny of esc^{10}/esc^{2} parents. (C, D) Progeny of esc^{10}/esc^{2} mothers and wild-type fathers. Arrows in A indicate ectopic expression in parasegments 14, 15 and in the developing hindgut. Arrows in B indicate developing Malpighian tubules. Brackets in C and D indicate PS5. Embryo in A is between 7 and 8 hours old. Embryos in B-D are about 9 hours old.

of mis-expression occurs with removal of zygotic *Pc* product alone. This is consistent with phenotypic analyses showing that, although *Pc* is expressed maternally (Denell, 1982; Haynie, 1983) the loss of the zygotic contribution alone is sufficient to cause a severe transformation of most segments towards A8 (Lewis, 1978; Duncan and Lewis, 1982).

polyhomeotic (ph)

Ectopic *abdA* in *ph* mutant embryos is first seen at about 6 hours primarily in medial positions in parasegments 3 through 6. By 9-10 hours, *abdA* is present throughout the A-P axis (Fig. 5C). Ectopic expression is diffcult to assay beyond this time since extensive cell death occurs in the ventral epidermis (Dura et al., 1987).

AbdB mis-expression occurs at about 6 hours in a graded pattern with most ectopic expression in the abdomen, less in the thorax and none in the head. Pair-rule modulation is not seen. By 9 hours, *AbdB* accumulates to high levels throughout the A-P axis including the head. *AbdB* appears at high levels in most epidermal cells although it fails to achieve the blanket uniform appearance seen in *esc* and *E(z)* mutants.

Sex comb on midleg (Scm)

Mis-expression in homozygous mutant *Scm* embryos is less severe than in the above mutants. Ectopic *abdA* is first seen in lateral epidermal patches in PS6 (Fig. 1G). Subsequently, *abdA* is seen in more medial positions in parasegments 3 through 6, and by 9 hours expression has extended into the head (Fig. 1H). Although expression occurs throughout the A-P axis, the cellular distribution within each parasegment is much more limited than in *esc* or *E(z)* mutants (compare to Fig. 1D, F). In the epidermis, ectopic *abdA* is preferentially expressed along both sides of the segmental grooves, marking the anterior portion of each parasegment. At later stages, about the same distribution along the A-P axis is seen, with abundant mis-expression in the CNS in a mottled pattern (Fig. 5D).

Ectopic *AbdB* is first detected at about 6 hours, extending a variable number of parasegments forward, but not anterior to PS3 (Fig. 2G). No pair-rule effect is seen. At these early times, there are more cells expressing *AbdB* ectopically in posterior abdominal segments than in thoracic segments, and *AbdB* is absent from the head. By 9 hours, *AbdB* spreads into the head in a spotty pattern, and graded expression along the A-P axis is still seen (Fig. 2H). At 12 hours, expression appears more uniform, with particularly strong mis-expression in the CNS (Fig. 6A).

Polycomb-like (Pcl)

Patterns of ectopic *abdA* and *AbdB* in *Pcl* mutant embryos are nearly identical to the patterns in *Scm* mutant embryos at all stages. This comparison is illustrated in Fig. 5E and 5D for *abdA* in 12 hour embryos. Consistent with this observation, the phenotypes in embryos lacking zygotic expression of *Pcl* or *Scm* are very similar (Jürgens, 1985; Breen and Duncan, 1986).

Sex comb extra (Sce)

Patterns of ectopic *abdA* and *AbdB* in *Sce* mutant embryos are similar to those in *Scm* and *Pcl* embryos at all stages. Ectopic expression of both products first occurs at about 6 hours and at later stages it predominates in the CNS (Fig. 5F).

Additional sex combs (Asx)

Ectopic *abdA* is first detected at about 6 hours in PS5 and PS6. Subsequently, *abdA* spreads as far forward as PS2 but little or none accumulates in the head. At 9 hours, ectopic *abdA* remains primarily in PS2 through PS6 in the epidermal cells that border the segmental grooves. Essentially the same distribution is seen at 12 hours, with widely scattered, mis-expressing cells seen also in the head (Fig. 5G). In contrast to most other *Pc*

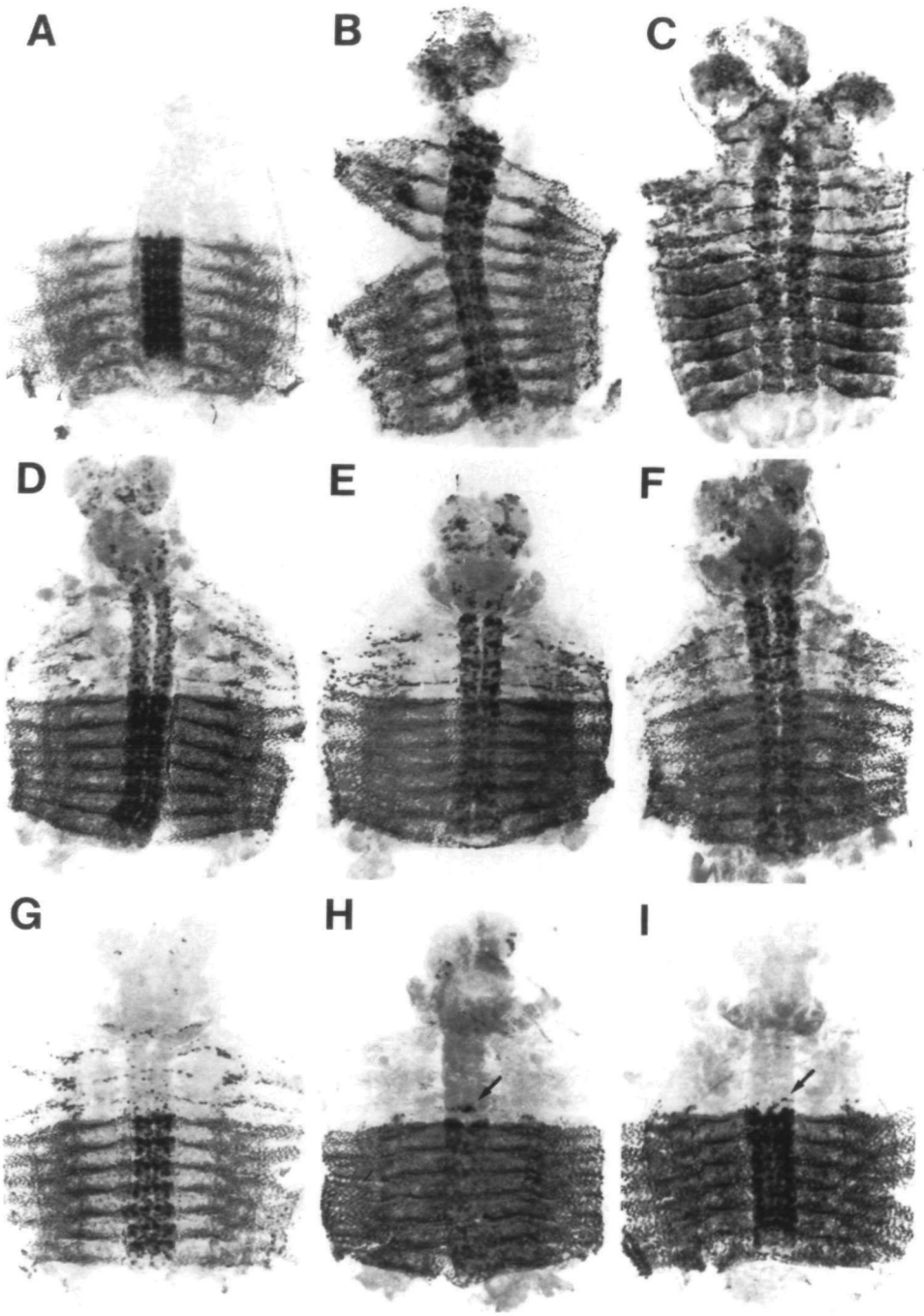

Fig. 5. *abdA* expression in *Polycomb* group mutants. Embryos are homozygous for the indicated *Polycomb* group alleles and were stained with *abdA* antibody. (A) Wild type. (B) Pc^{3}. (C) ph^{503}. (D) Scm^{D1}. (E) Pcl^{D5}. (F) Sce^{D1}. (G) Asx^{D1}. (H) Psc^{14-45}. (I) pho^{b}. The dark-staining mid-ventral structure is the CNS and the more lateral tissue is epidermis. All embryos are about 12 hours old, at the dorsal closure stage, except the embryo in C which is about 10 hours old. Arrows in H and I indicate ectopic expression in the CNS in PS6.

group mutants, ectopic *abdA* in *Asx* mutants is much more abundant in the epidermis than in the CNS.

The early stages of *AbdB* mis-expression resembles that in *Scm* mutants, with ectopic *AbdB* stronger in posterior parasegments. By 9-12 hours, this graded expression is still observed, but it is strikingly reduced in the CNS relative to that in *Scm* (compare Fig. 6B and 6A) and most other *Pc* group mutants.

Posterior sex combs (Psc)

The earliest ectopic *abdA* is seen at about 6 hours in lateral patches in PS6. By 9 hours, *abdA* spreads further forward, primarily in medial cells in PS2 through PS6, and spotty expression is seen in the head. Curiously, between 9 and 12 hours, ectopic *abdA* becomes weaker, with scattered expressing cells primarily in the brain lobes and in PS5 and PS6 in the CNS (Fig. 5H).

As in other *Pc* group mutants, the earliest ectopic *AbdB* spreads as far forward as PS3 but is largely absent from the head. By 9 hours, *AbdB* is detected throughout the A-P axis, although the number of mis-expressing cells per parasegment is less than in most other *Pc* group mutants. Between 9 and 12 hours, ectopic *AbdB* becomes concentrated in the CNS.

pleiohomeotic (pho)

pho [previously called l(4)29] mutants die as pharate

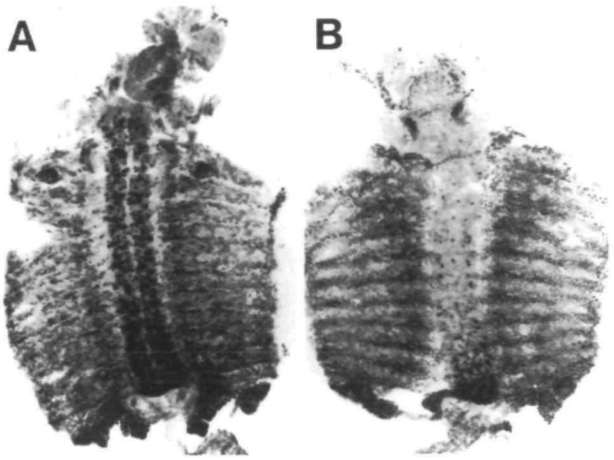

Fig. 6. *AbdB* expression in *Scm* and *Asx* mutants. Embryos at about 12 hours were stained with *AbdB* antibody. (A) Scm^{D1} homozygote. (B) *Df(2R)trix* homozygote (Asx^{-}). The dark-staining mid-ventral structure in A is the CNS. There is much less staining in the CNS in B.

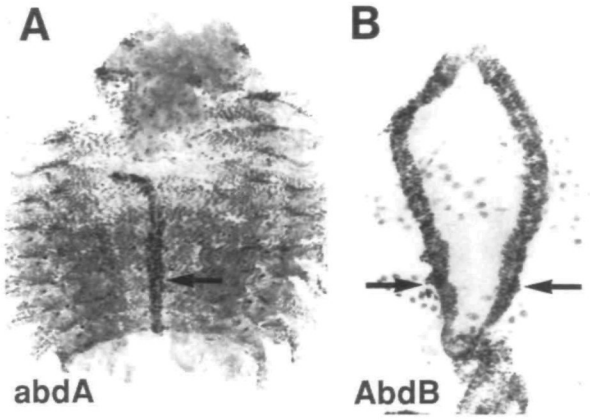

Fig. 7. Ectopic expression in mesodermal tissues. (A) *abdA* expression in the heart tube of an Scm^{D1} homozygote. The approximately 16 hour embryo was dissected by slicing along the ventral midline and removing the central nerve cord. It was then flattened with the dorsal surface up. The heart tube is the dark-staining medial structure. The arrow indicates the wild-type position of the *abdA* anterior boundary in the heart tube. (B) *AbdB* expression in the visceral mesoderm of an Asx^{D1} homozygote. The gut and associated visceral mesoderm were dissected from a 9 hour embryo. Anterior is to the top. The arrows indicate the wild-type position of the *AbdB* anterior boundary in the visceral mesoderm.

adults with segmental transformations (Gehring, 1970; Duncan, 1982). *pho* mutant embryos from heterozygous parents lack cuticle defects or transformations. However, the gene product clearly functions in embryos, since elimination of both maternal and zygotic product causes embryonic lethality and segmental transformations (Breen and Duncan, 1986).

pho mutant embryos from heterozygous parents show subtle defects in the control of *abdA* and *AbdB*. Ectopic *abdA* is detected as early as 6 hours in lateral patches in PS6, as is seen in other *Pc* group mutants (i.e. *Scm*, Fig. 1G), but the number of mis-expressing cells is much lower, usually only 5-10 cells in the entire parasegment. At later embryonic times, this very sparse mis-expression is still seen, primarily in the CNS in PS6 (Fig. 5I, arrow), but occasionally in more anterior positions.

Ectopic *AbdB* is similarly sparse. At 6 hours, up to 10 mis-expressing cells per embryo are seen, primarily in the abdominal segments. At later stages up to 20 mis-expressing cells per embryo are seen, again mostly in the abdomen.

super sex combs (sxc)

Like *pho* mutants, *sxc* mutants die as pharate adults and survive to this stage due to maternally supplied product (Ingham, 1984). In contrast to *pho*, we did not observe ectopic *abdA* or *AbdB* in homozygous mutant *sxc* embryos from heterozygous parents.

Ectopic expression in mesodermal tissues

The segmental transformation phenotypes seen in cuticles of *Pc* group mutant embryos result from mis-expression of homeotic proteins in the epidermis. Mis-expression in a number of *Pc* group mutants in another ectodermal tissue, the CNS, has been reported (Struhl and White, 1985; Wedeen et al., 1986; Dura and Ingham, 1988; Celniker et al., 1989; Jones and Gelbart, 1990) and we have extended that description here (Figs 5, 6).

Our analysis of dissected embryos shows that *Pc* group products also function in a number of other internal tissues, including mesodermal derivatives. Fig. 7A shows ectopic expression in the dorsal vessel or heart tube, which is a mesodermal tissue (Campos-Ortega and Hartenstein, 1985). *abdA* is normally expressed in the heart tube and pericardial cells with an anterior boundary in the fifth abdominal segment (PS10) (Karch et al., 1990). In the *Scm* mutant embryo shown, *abdA* spreads far forward of the normal boundary (arrow, Fig. 7A) into the thoracic segments. Fig. 7B shows anterior spread of *AbdB* in the visceral mesoderm that surrounds the gut. Normally, the *AbdB* anterior boundary in the visceral mesoderm is in PS11 (DeLorenzi and Bienz, 1990; arrows in Fig. 7B). In the *Asx* mutant shown, *AbdB* spreads at least as forward as PS3. Ectopic expression of *AbdB* in the visceral mesoderm was also seen in *esc*, *Pc*, *ph*, *Scm* and *Pcl* mutant embryos.

Discussion

Each Polycomb *group product is required for regulation of* abdA *and* AbdB

The *Pc* group genes *esc*, *E(z)*, *Pc*, *Pcl*, *ph*, *Scm*, *Sce*, *Asx*, *Psc* and *pho* are each required to confine *abdA* and *AbdB* to their proper domains along the A-P axis. The amounts of ectopic expression varied widely among the different *Pc* group mutants (Fig. 5). Much of this variation is due to differences in the maternal ex-

pression levels of the *Pc* group genes (Breen and Duncan, 1986). Most of the mutant embryos examined were null or strong hypomorphs for zygotic product but maternal product was usually unaffected. Only in the cases of *esc* and *E(z)* mutants were the maternal and zygotic products both eliminated or severely reduced. Correspondingly, *abdA* and *AbdB* mis-expression was most severe in these esc^- and $E(z)^-$ embryos, and in *Pc* mutant embryos, where the maternal contribution is small (Haynie, 1983). At the other extreme, the strong maternal components of *sxc* (Ingham, 1984) and *pho* expression (Breen and Duncan, 1986) are the likely reasons that *abdA* and *AbdB* appeared normal in *sxc* mutant embryos and were only subtly mis-expressed in *pho* mutant embryos (Fig. 5I). Embryos lacking this maternal *sxc* show segmental transformations towards eighth abdominal (Ingham, 1984), strongly suggesting that at least *AbdB* is under *sxc* control.

In the light of the large number of *Pc* group genes, it is important to determine if some products are preferentially involved in regulating certain homeotic genes but not others. In our analysis, there was little evidence for this since the general extent of *abdA* mis-expression and *AbdB* mis-expression in particular *Pc* group mutants was similar. The relative roles of *Pc* group genes may also be investigated by comparing the precise tissue patterns of mis-expression in the different mutants. As mentioned above, the interpretation of these patterns is complicated by the perdurance of maternal product, except in the cases of *esc* and *E(z)*. Figs 1 and 2 show that the timing and patterns of *abdA* and *AbdB* mis-expression in esc^- and $E(z)^-$ embryos are nearly identical. This suggests that *esc* and *E(z)* perform similar molecular functions, perhaps as components of the same machinery. The tissue distributions in the other *Pc* group mutants are also consistent with common function, with the notable exception of *Asx*. Whereas most *Pc* group mutants cause abundant mis-expression of *abdA* and *AbdB* in the CNS, *Asx* mutants show very little mis-expression in the CNS (Figs 5G, 6B). Thus, *Asx* may function primarily in the epidermis as opposed to the CNS. Alternatively, there could be differential decay of maternal *Asx* product in these two tissues.

Our analysis of *abdA* and *AbdB* shows that the *Pc* group products act as repressors in anterior parasegments. Some *Pc* group products may also act as positive regulators since *Scr, Antp* and *Ubx* appear repressed, rather than ectopically activated, in certain tissues in some *Pc* group mutants (Smouse et al., 1988; McKeon and Brock, 1991). Alternatively, these cases may be indirect consequences of repressive interactions among the multiply mis-expressed homeotic products. For example, *abdA* and *AbdB* normally act as repressors of *Ubx* in embryos (Struhl and White, 1985). Thus, a decrease in *Ubx* levels in a *Pc* group mutant might result from repression by ectopic *abdA* and *AbdB*, as has been shown in 12-14 hour *esc* mutant embryos (Struhl and White, 1985). Trans-repression of *abdA* by *AbdB* (Karch et al., 1990) may also explain why *abdA* fades in *esc* and *E(z)* mutants by 12 hours (not shown) and becomes more limited in *Psc* mutants by this time (Fig. 6H). We have attempted to minimize the complications of these secondary regulatory events in two ways. First, since patterns in late embryonic stages are more likely to be affected by the accumulation over time of other homeotic products, we have concentrated on the early patterns of ectopic expression in 5-7 hour embryos. Second, we have examined the distribution of *AbdB*, which is not known to be trans-repressed by any other homeotic product.

Molecular role of Pc *group products*

Initially, the on and off states of homeotic gene expression along the A-P axis are set by the combinatorial action of gap and pair-rule products (Duncan, 1986; Ingham and Martinez-Arias, 1986; White and Lehmann, 1986; Irish et al., 1989; Simon et al., 1990; Reinitz and Levine, 1990; Qian et al., 1991). For example, *hunchback* could set the initial *abdA* anterior boundary in PS7 by repressing *abdA* in more anterior parasegments. By 3-4 hours, the gap gene products decay (Gaul et al., 1987; Tautz, 1988) and this repression is then likely maintained by the *Pc* group products. Indeed, analysis of *esc* null embryos shows that *esc* is required for maintenance of *Ubx* expression within its proper A-P boundaries, but not for its initial activation (Struhl and Akam, 1985). Similar experiments show that *E(z)* is also required for maintenance but not initiation of *Ubx* (Jones and Gelbart, 1990). Likewise, we find that *esc* and *E(z)* are required for maintenance but not initiation of *abdA* and *AbdB* since the distribution of these two products is initially normal in esc^- and $E(z)^-$ embryos (Fig. 3A, E). The possible role of other *Pc* group products in initiation has yet to be addressed. Here we show that they are each at least involved in maintenance of *abdA* and *AbdB* during embryogenesis. A recent report also implicates many of these *Pc* group genes in the maintenance of *Ubx*, *Antp* and *Scr* in embryos (McKeon and Brock, 1991).

These data, taken together, indicate that many of the *Pc* group products act at the same time at many positions along the A-P axis. Thus, *esc, Pc, Pcl, Scm, Sce, Asx* and *ph* are each necessary to repress simultaneously *Antp, Ubx, abdA* and *AbdB* anterior to their boundaries in PS3, PS5, PS7 and PS10, respectively. Clearly, the *Pc* group products are not acting as simple repressors with limited distributions, shutting off expression wherever they happen to be located. Instead, mechanisms for transcriptional repression must be considered that account for global function of *Pc* group products along the A-P axis.

These requirements are satisfied by envisioning that the *Pc* group products act by packaging portions of homeotic loci into an inaccessible or 'closed' configuration (Paro, 1990; Peifer et al., 1987). This model suggests that the *Pc* group products sense the initial on or off state of a homeotic gene and then compact the DNA into a stably repressed, heterochromatin-like structure in cells where the DNA was originally inactive (Paro, 1990). At later times, a large number of cell-specific activators direct the intricate patterns of

homeotic expression, but this would occur only in parasegments where the factors could gain access to the DNA (Peifer et al., 1987). Thus, *abdA* remains stably off anterior to PS7 because its DNA regulatory regions would be inaccessible to positive factors in these anterior parasegments. In this way, the state of the chromatin would be fixed to 'remember' the initial positional information provided by gap and pair-rule products.

Patterns of ectopic expression in Polycomb *group mutants*

Our analysis of the kinetics of mis-expression unexpectedly revealed that ectopic *abdA* and *AbdB* do not appear simultaneously in all parasegments. Instead, in the strongest *Pc* group mutants, (*esc, E(z)* and *Pc*), *abdA* is first mis-expressed just anterior to the normal PS7 boundary in PS5 and 6 (Figs 1C and E, 3B). The more anterior thoracic and head regions accumulate *abdA* only after further time in embryogenesis (Figs 1D, F, 3C, D, 5B). Similarly, ectopic *AbdB* arises in a nonuniform pattern. At first, this involves expression in a pair-rule pattern, as far forward as PS3 (Figs 2C, E, 3F). Subsequently, the pair-rule pattern fills in and *AbdB* expression eventually spreads forward into the head (Figs 2D, F, 3H). Similar gradual anterior activation of *AbdB* transcripts in Pc^3 mutant embryos has been reported (Kuziora and McGinnis, 1988).

These early ectopic patterns can be explained in the context of the chromatin accessibility model for *Pc* group function. In wild-type embryos, *AbdB* expression in PS13 in 2 hour embryos could involve positive control by the pair-rule product *even-skipped (eve)* and repression by gap gene products, including *giant* (Reinitz and Levine, 1990), in parasegments anterior to PS13. By 3-4 hours, the gap gene products decay and the task of repression in anterior parasegments is transferred to the *Pc* group products. However, in *Pc* group mutants this transition fails and in 4 hour embryos *AbdB* can be ectopically activated by any positive factor (such as *eve*) present in anterior locations and capable of binding to the *AbdB* regulatory regions. In fact, the earliest ectopic *AbdB* pattern at 5-6 hours (Figs 2C, E, 3F) is remarkably similar to the *eve* protein pattern at slightly earlier times (Frasch et al., 1987). At about 4 hours, the wild-type pattern of *eve* protein is in alternating stripes, stronger in the odd-numbered parasegments. Like ectopic *AbdB*, the most anterior *eve* stripe is in PS3, since the *eve* stripe in PS1 decays by this time (see Fig. 8D in Frasch et al., 1987). The *eve* pattern in 4 hour $E(z)^-$ embryos resembles this wild-type pattern (J.S. and W.B., unpublished). Thus, the early pattern of ectopic *AbdB* could be explained by a pair-rule factor gaining access to and activating *AbdB* in the wrong parasegments. The apparent gradient in *AbdB* along the A-P axis could be a vestige of control by gap products, expressed at earlier times in gradients (Stanojevic et al., 1989; Pankratz et al., 1990). Similarly, mis-regulation by a combination of gap and pair-rule products could explain ectopic *abdA* appearing first in PS5 and 6.

Such a scenario may also explain two related observations. First, if a single copy of esc^+ is provided paternally, the final *abdA* and *AbdB* patterns in the resulting heterozygous embryos (Fig. 4C, D) now resemble the transient patterns seen in the null. In this situation, like in the null, the transition to *Pc* group repression at 3-4 hours would be faulty, and early segmentation products would ectopically activate *abdA* and *AbdB*. However, zygotic expression of esc^+ would eventually generate sufficient product to rescue *Pc* group repression and this would set in belatedly to fix permanently otherwise transient patterns. Second, the pair-rule distribution of *AbdB* is only seen in the strongest Pc group mutants, *esc, E(z)* and *Pc*. This is explained if the maternal contributions in the weaker mutants perdure long enough to allow the transition to *Pc* group repression to occur normally. Thus, when the maternal *Pc* group products eventually decay, pair-rule products are no longer present to influence the patterns of ectopic activation.

The cellular distribution of ectopic *abdA* within parasegments is highly patterned, with higher expression in the anterior portions of each parasegment (Fig. 1C-F). This patterning resembles the wild-type patterning of *abdA* normally restricted to PS7-13 (Fig. 1A, B). The wild-type *abdA* patterning is not due to trans-regulation by other homeotic products but rather reflects the intrinsic cell-specific controls that mediate *abdA* distribution (Karch et al., 1990). This patterning of ectopic *abdA* is most obvious in severe *Pc* group mutants, but it is also seen in the less severe mutants (Figs 1G, H, 5B-G). Thus, although control along the A-P axis has broken down in these mutants, the cell-specific controls of *abdA*, even in the ectopic parasegments, still function. Again, the patterns of ectopic expression can be explained if *abdA* regulatory DNA is not adequately compacted in *Pc* group mutants. The normal cell-specific factors might then gain inappropriate access in PS1 through PS6, resulting in ectopic *abdA* in a pattern that resembles the wild-type pattern. Although this ectopic transfer of cell-specific pattern is most obvious in embryos with *abdA*, similar effects have been noted for *Scr* and *Ubx* in imaginal discs (Jones and Gelbart, 1990; Glicksman and Brower, 1990). Similarly, the uniform blanket of ectopic *AbdB* in the strongest *Pc* group mutants (Fig. 2D, F) resembles the uniform pattern of *AbdB* normally seen only in PS13 and PS14 (Fig. 2B). In these cases, then, the defect in *Pc* group mutants involves transfer of normal homeotic pattern to inappropriate parasegments rather than indiscriminate homeotic activation. In summary, we suggest that the precise patterns of ectopic expression in *Pc* group mutants depends upon the distributions of normal activators in the ectopic parasegments.

Do multiple Polycomb *group products act together?*

Ten different *Pc* group products described here are required for both *abdA* and *AbdB* maintenance. Genetic data suggest that there may be as many as 40 members of the *Pc* group in total (Jürgens, 1985). There

are several possible explanations for the large number of *Pc* group genes. Individual *Pc* group products may interact independently with many different DNA sites within the large (>50 kb) regulatory regions of homeotic genes. The additional roles of some *Pc* group genes in diverse processes such as oogenesis, dorsal-ventral pattern formation and CNS development (Phillips and Shearn, 1990; Adler et al., 1989; Smouse et al., 1988) indicate that, at least in some instances, *Pc* group products can act independently from each other. Alternatively, some *Pc* group products may not directly affect transcription of homeotic loci but rather control transcription of other *Pc* products that are direct regulators. *Pc* protein itself is likely a direct regulator since it localizes to the ANT-C and BX-C loci on polytene chromosomes (Zink and Paro, 1989), but most of the other *Pc* group products have yet to be tested. As a third possibility, the *Pc* group products could act together in large multimeric complexes that compact ANT-C and BX-C DNA, as envisioned by the chromatin accessibility model. The recent identification of small DNA segments in the ANT-C (Zink et al., 1991) and in the BX-C (Simon et al., 1990; J.S. and W.B., unpublished) that mediate the response to *Pc* group products in vivo should help to address these issues.

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