The EMBO Journal vol.15 no.3 pp.569–580, 1996

Probes for chromatin accessibility in the *Drosophila* bithorax complex respond differently to *Polycomb*-mediated repression

Kimberly McCall[1] and Welcome Bender[2]

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA

[1]Present address: Department of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139, USA

[2]Corresponding author

The *Polycomb* group (PcG) of genes are required for maintenance of the repressed state of the homeotic genes in *Drosophila*. There are similarities between the PcG repression and mating-type silencing in yeast or heterochromatic position effect in *Drosophila*, which suggest that PcG repression may involve a highly compacted chromatin structure. To test for such a structure, heterologous DNA-binding proteins were used as probes for DNA accessibility in *Drosophila* embryos. Binding sites for the yeast transcriptional activator GAL4 and for bacteriophage T7 RNA polymerase were inserted into the *bithorax* (*bx*) regulatory region of the endogenous *Ultrabithorax* (*Ubx*) gene, which is regulated by the PcG. Ubiquitously expressed GAL4 protein directs transcription through its binding sites only in the posterior segments where the *bx* region is active. The block to GAL4 activation in the more anterior segments is dependent on *Polycomb* (*Pc*) function. In contrast, T7 RNA polymerase can transcribe from its target promoter in all segments of the embryo. Thus, Pc-mediated repression blocks activated polymerase II transcription, but does not simply exclude all proteins.

Keywords: *bithorax* complex/chromatin/*Drosophila*/*Polycomb*/gene repression

Introduction

The homeotic genes of *Drosophila* are required for the determination of proper segment identity. These genes are found in two large clusters, the Antennapedia complex and the bithorax complex (BX-C). The Antennapedia complex genes determine the identities of the head and first two thoracic segments, and the BX-C genes determine the identities of the third thoracic and the abdominal segments. For correct segmental identity, spatially restricted expression of these genes must be maintained throughout development. Mutations which alter these expression patterns produce transformations of segmental identity which in some cases lead to lethality. The domains of expression are established early in development by short-lived transcription factors encoded by the gap and pair-rule genes (Qian *et al.*, 1991; Müller and Bienz, 1992; Shimell *et al.*, 1994). These expression domains, however, are maintained throughout all stages of development, many rounds of cell division after the products of the gap and pair-rule genes have disappeared.

The genes of the *Polycomb* group (PcG) are required for this long-term repression of the homeotic genes. In embryos mutant for genes in the PcG, expression of the homeotic genes is established correctly but it subsequently spreads beyond the normal boundaries (Struhl and Akam, 1985; White and Wilcox, 1985; Wedeen *et al.*, 1986; Celniker *et al.*, 1990; McKeon and Brock, 1991; Simon *et al.*, 1992; Soto *et al.*, 1995). The frequency at which mutations are revealed by deficiency analysis indicates that there are up to 40 members of the PcG (Jürgens, 1985), although only 13 members have been genetically characterized (reviewed in Simon, 1995). The predicted amino acid sequences of the PcG genes that have been cloned {*Polycomb* (*Pc*), *polyhomeotic* (*ph*), *Posterior sex combs* (*Psc*), *Polycomblike* (*Pcl*), *extra sex combs* (*esc*) and *Enhancer of zeste* [*E*(*z*)]} do not reveal a great deal about their possible mechanisms of repression, although several PcG proteins have been shown to bind specific sites in salivary gland chromosomes (reviewed in Simon, 1995). The sequence of *Pc* revealed an intriguing homology, the 'chromodomain', to *Drosophila* HP-1, a heterochromatin-associated protein encoded by the *Su*(*var*)*205* gene, a suppressor of position effect variegation (PEV) (Paro and Hogness, 1991). Additionally, some PcG genes are modifiers of PEV and vice versa (Grigliatti, 1991), giving evidence for common molecular components shared by the two complexes. These similarities between the PcG and modifiers of PEV have led to the model in which the PcG functions as a complex in a manner similar to heterochromatin, by causing the formation of a highly compacted chromatin structure (Alberts and Sternglanz, 1990; Gaunt and Singh, 1990; Paro, 1990; Reuter and Spierer, 1992).

A compacted chromatin state might be expected to block the accessibility of trancription factors to DNA. This could explain the observation that a euchromatic gene is repressed when it is inserted into heterochromatin (PEV). Similarly, repression of several genes has been observed at the silent mating-type loci and at telomeres in *Saccharomyces cerevisiae* (reviewed by Laurenson and Rine, 1992). The PcG can also repress heterologous genes such as the *white* gene (Fauvarque and Dura, 1993; Chan *et al.*, 1994; Gindhart and Kaufman, 1995). P element enhancer traps inserted into the BX-C are regulated by the PcG, even when their insertion sites are far from known Pc response elements (McCall *et al.*, 1994; Chiang *et al.*, 1995). Additionally, enhancers internal to these P elements are inactivated in anterior segments, supporting a model of restricted chromosome accessibility.

In this study, we have examined the accessibility of DNA-binding proteins for DNA of the BX-C that is under PcG repression. We utilized probes that allow us to observe

© Oxford University Press

segment-specific differences in DNA accessibility in *Drosophila* embryos. First, we examined the ability of the PcG to block transcriptional activation by the yeast transcription factor GAL4. GAL4 is a well-characterized transcriptional activator which is able to recognize its binding site when located within nucleosomal DNA (Taylor *et al.*, 1991; Morse, 1993). To direct transcription, GAL4 requires the basic transcription machinery, including RNA polymerase II. As a second probe, we used bacteriophage T7 RNA polymerase. T7 RNA polymerase is a single subunit polymerase which, in contrast to GAL4, does not require any accessory factors. Its ability to transcribe reflects its ability to recognize its promoter (Chamberlin and Ryan, 1982). For this reason, T7 RNA polymerase has been used in yeast and mammalian cells as a probe for DNA accessibility (Chen *et al.*, 1987; Jenuwein *et al.*, 1993). We found that GAL4-dependent transcription is inhibited by Pc, but T7 RNA polymerase is not. Our results suggest that the mechanism of repression by the PcG is not achieved by the exclusion of all DNA-binding proteins, as might be expected from highly compacted chromatin. Additionally, we show that transcription early in embryonic development is not sufficient to inhibit the establishment of PcG-mediated repression.

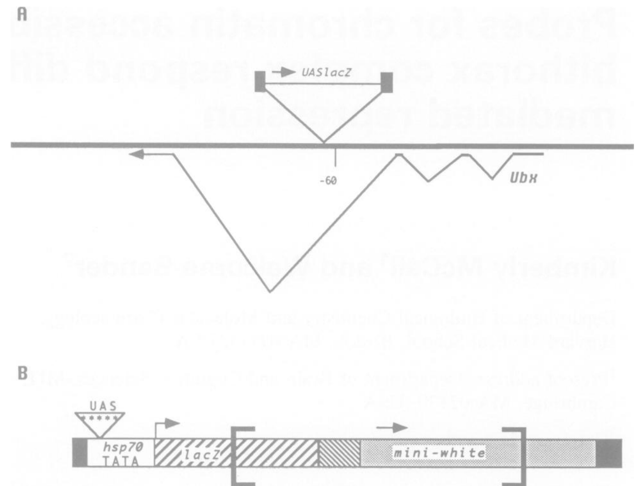

Fig. 1. The $bx^{UASlacZ}$ insertion. (**A**) The $bx^{UASlacZ}$ P element is inserted at –61kb on the BX-C map, within the large *Ubx* intron. The heavy black line represents the chromosome; arrows indicate the direction of trancription for *Ubx* and *UASlacZ*. (**B**) Larger view of the *UASlacZ* P element in (A) (from Brand and Perrimon, 1993). The large brackets indicate approximately the extent of the internal deletion. The UAS contains five GAL4-binding sites.

Results

Introduction of a GAL4-dependent promoter into the bx regulatory domain

The yeast transcription factor GAL4 can activate transcription in *Drosophila* from a promoter containing optimal GAL4-binding sites (Fischer *et al.*, 1988; Brand and Perrimon, 1993). A two component system has been developed such that one fly strain expresses the GAL4 protein in a given pattern and another strain contains the GAL4 upstream activating sequence (UAS) directing transcription of a target gene. The progeny of a cross between these two strains will express the target gene in the GAL4 pattern (Brand and Perrrimon, 1993).

We wished to see if repression of the BX-C by the PcG would block GAL4-dependent activation of a target gene. We used targeted transposition to insert *UASlacZ*, a P element which contains five GAL4-binding sites driving expression of the *lacZ* gene, into the *bx* regulatory region of the *Ubx* gene (Figure 1A). We chose the *bx* region for analysis since we had characterized a P element there which is regulated by at least two PcG members, *esc* and *Pcl* (McCall *et al.*, 1994). Targeted transposition results in the replacement of internal sequences of one P element by the sequences of another P element (Heslip *et al.*, 1992; Heslip and Hodgetts, 1994; Staveley *et al.*, 1994; Gonzy-Tréboul *et al.*, 1995). Using this method we recovered a single line with an insertion of *UASlacZ* in the *bx* region ($bx^{UASlacZ}$). The *UASlacZ* P element in this line contained an internal deletion such that the *lacZ* transcript was truncated (Figure 1B, see Materials and methods). Upon crossing this line to a GAL4-producing strain, β-gal protein could not be detected by antibody staining but *lacZ* RNA was readily detectable by *in situ* analysis. In the absence of a GAL4-producing P element, the $bx^{UASlacZ}$ P element expressed the *lacZ* RNA in early embryos. This RNA is found in a segmentally restricted domain beginning at blastoderm and is very weak by late germband extension (data not shown).

GAL4-dependent transcription from $bx^{UASlacZ}$ is inhibited in the anterior and posterior regions of the embryo

The *bx* region is contained within the parasegment 5 (PS5) regulatory region of the *Ubx* gene. PS5 corresponds to the posterior part of the second thoracic segment and the anterior part of the third thoracic segment. Mutations in the *bx* region result in the transformation of PS5 epidermal structures towards PS4, such as anterior haltere towards wing (Lewis, 1978). In the mesoderm, however, *bx* mutations primarily affect PS6 structures (Hooper, 1986). The *bx* region controls expression of *Ubx* in PS5–PS12 and is likely to be repressed directly by the PcG outside of this domain (Camprodón and Castelli-Gair, 1994; McCall *et al.*, 1994).

We examined embryos to see if the activation of transcription of $bx^{UASlacZ}$ by GAL4 was inhibited in the anterior and posterior regions of the embryo where the PcG was acting. We crossed the $bx^{UASlacZ}$ line to several GAL4-expressing lines and compared the embryonic expression patterns to a control line carrying an insertion of *UASlacZ*. Embryos resulting from a cross of *UASlacZ* to line *24B*, which expresses GAL4 primarily in the mesoderm, express *lacZ* RNA throughout the body axis (Figure 2A). In contrast, *24B/*$bx^{UASlacZ}$ embryos express *lacZ* RNA only in the mesoderm of PS6–12 (Figure 2B). This PS6 anterior boundary is consistent with the observation that *bx* mutations primarily affect PS6 but not PS5 in the mesoderm. Line *32B* expresses GAL4 in the epidermis and internal tissues. Again *lacZ* RNA is expressed throughout the body axis in *UASlacZ* embryos, but is limited to PS5–12 in $bx^{UASlacZ}$ embryos (Figure 2C and D). Surprisingly, *32B/*$bx^{UASlacZ}$ embryos express *lacZ* at high levels in the salivary glands, a structure derived from the labial segment (PS2). For a third GAL4 expres-

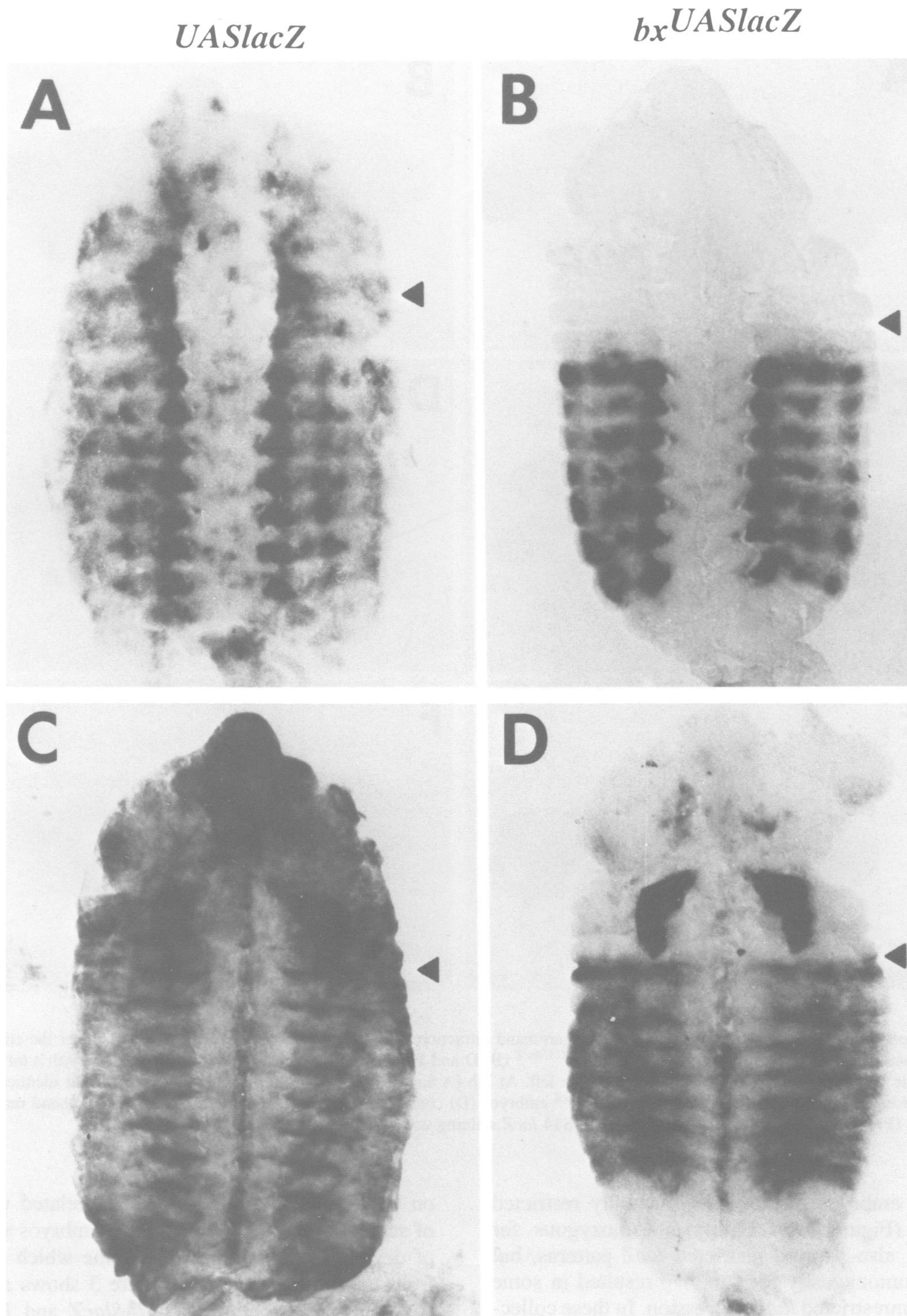

Fig. 2. GAL4-dependent transcription is segmentally restricted in $bx^{UASlacZ}$ embryos. (**A** and **B**) The mesoderm GAL4 line 24B, was crossed to *UASlacZ* (A) or $bx^{UASlacZ}$ (B) flies; embryos were collected and hybridized with a *lacZ* RNA probe. The arrowhead indicates the anterior PS5 boundary in this and all subsequent figures. Embryos in all figures are at stage 14 (10–11 h old), unless otherwise noted. *LacZ* expression is restricted to PS6-12 in $bx^{UASlacZ}$ embryos. (**C** and **D**) The ubiquitous GAL4 line 32B, was crossed to *UASlacZ* (C) or $bx^{UASlacZ}$ (D) and embryos were treated as in A and B. *lacZ* expression is restricted to PS5-12 in $bx^{UASlacZ}$ embryos with the exception of the salivary glands.

sion pattern, we crossed two lines that express GAL4 under the control of the *hsp70* heat shock promoter to *UASlacZ* and $bx^{UASlacZ}$. After heat shock, embryos displayed ubiquitous patterns similar to line *32B*, with weaker expression in the CNS (Figure 4A). Thus, with the exception of the salivary glands, GAL4-dependent transcription is limited to PS5–12. This segmentally restricted domain of expression is the same as the domain of enhancer activity for the *bx* region, suggesting that the PcG does inhibit GAL4 function.

In experiments where embryos were heterozygous for both *hsp70-GAL4* and $bx^{UASlacZ}$, 100% of the germ-

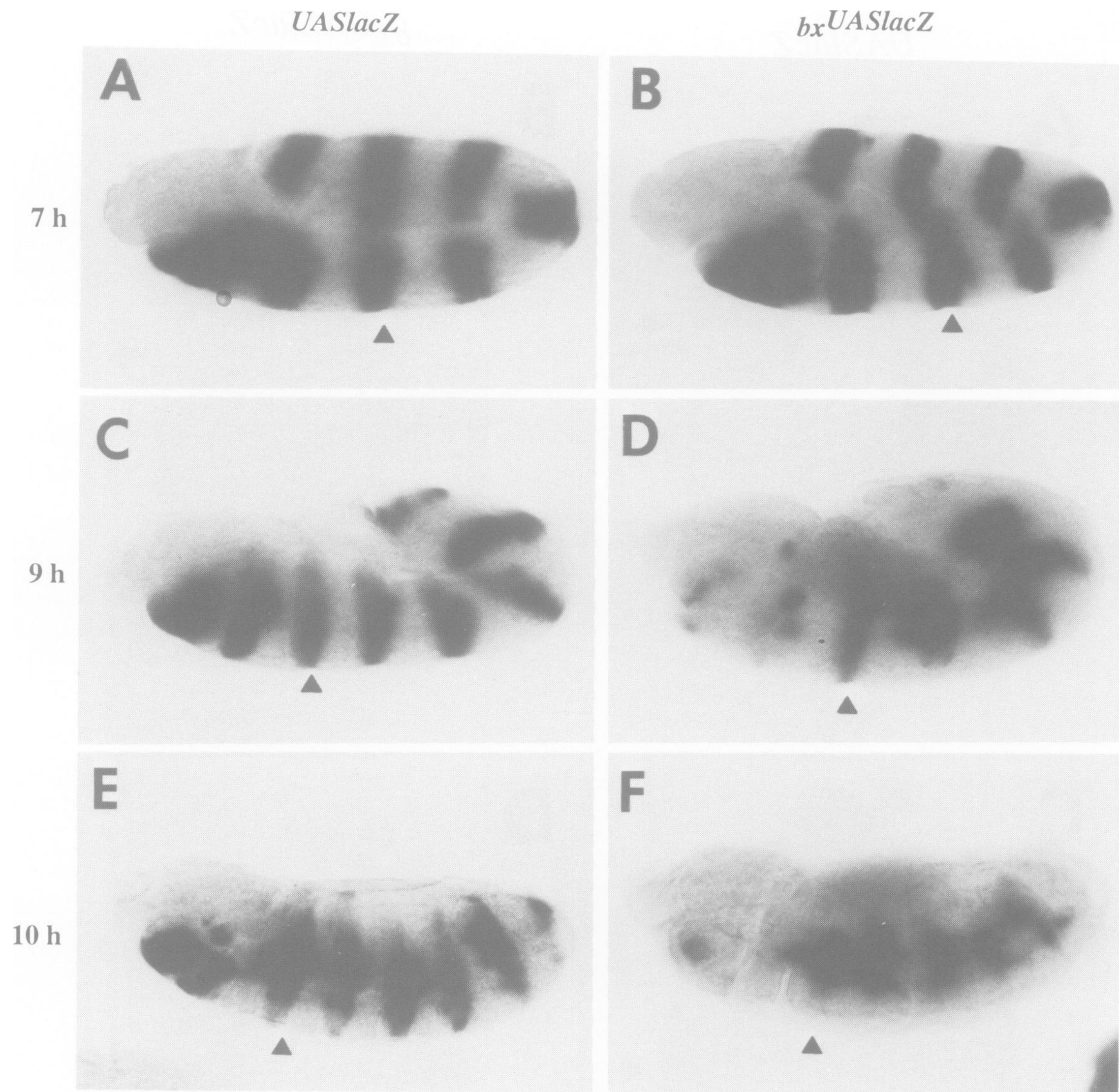

Fig. 3. Transcription mediated by GAL4 is repressed during germband retraction. The RG1 line, which expresses GAL4 under the control of the *paired* promoter, was crossed to *UASlacZ* (**A**, **C** and **E**) or $bx^{UASlacZ}$ (**B**, **D** and **F**). Embryos were collected and hybridized with a *lacZ* RNA probe. Hours of embryonic development at 25°C are indicated on the left. At 7 h (A and B), *UASlacZ* and $bx^{UASlacZ}$ embryos appear identical. By 9 h, *lacZ* staining is reduced in the head and the PS14 stripe in $bx^{UASlacZ}$ embryos (D) compared with *UAS lacZ* embryos (C). At germband retraction, $bx^{UASlacZ}$ embryos (F) show further reductions in head and PS14 *lacZ* staining compared with *UASlacZ* embryos (E).

band-retracted embryos displayed segmentally restricted *lacZ* patterns (Figure 4A). Embryos homozygous for *hsp70-GAL4*$^{7-1}$ also showed restricted *lacZ* patterns, but surprisingly, homozygosity for $bx^{UASlacZ}$ resulted in some embryos with unrestricted *lacZ* expression. In these collections, 33% of the embryos were expected to be homozygous for $bx^{UASlacZ}$ (see Materials and methods) and 14% of the germband retracted embryos displayed unrestricted *lacZ* patterns. Possible explanations for this observation are described in the Discussion.

GAL4-dependent transcription during early stages of development does not inhibit repression by the PcG

The PcG is not required for repression of the homeotic genes until germband extension (Struhl and Akam, 1985; Simon *et al.*, 1992). To determine if the repressive effects on GAL4-dependent expression correlated with the time of action of the PcG, we examined embryos at early stages of development, using the *RG1* line which drives GAL4 from the *paired* promoter. Figure 3 shows a time course of *lacZ* expression in *RG1/UASlacZ* and *RG1/*$bx^{UASlacZ}$ embryos. From blastoderm through germband extension, GAL4 is capable of directing *lacZ* expression throughout the body axis (Figure 3A and B). As the germband retracts (9 h), a restricted pattern of GAL4-dependent transcription becomes apparent in $bx^{UASlacZ}$ embryos which is not seen in *UASlacZ* embryos (compare Figure 3C and E with D and F). This demonstrates that although an active domain of GAL4-dependent transcription can be established early in embryogenesis, it becomes repressed by germband retraction. This timing is consistent with the repressive effects on GAL4-dependent transcription being mediated by the PcG and not by early acting repressors such as

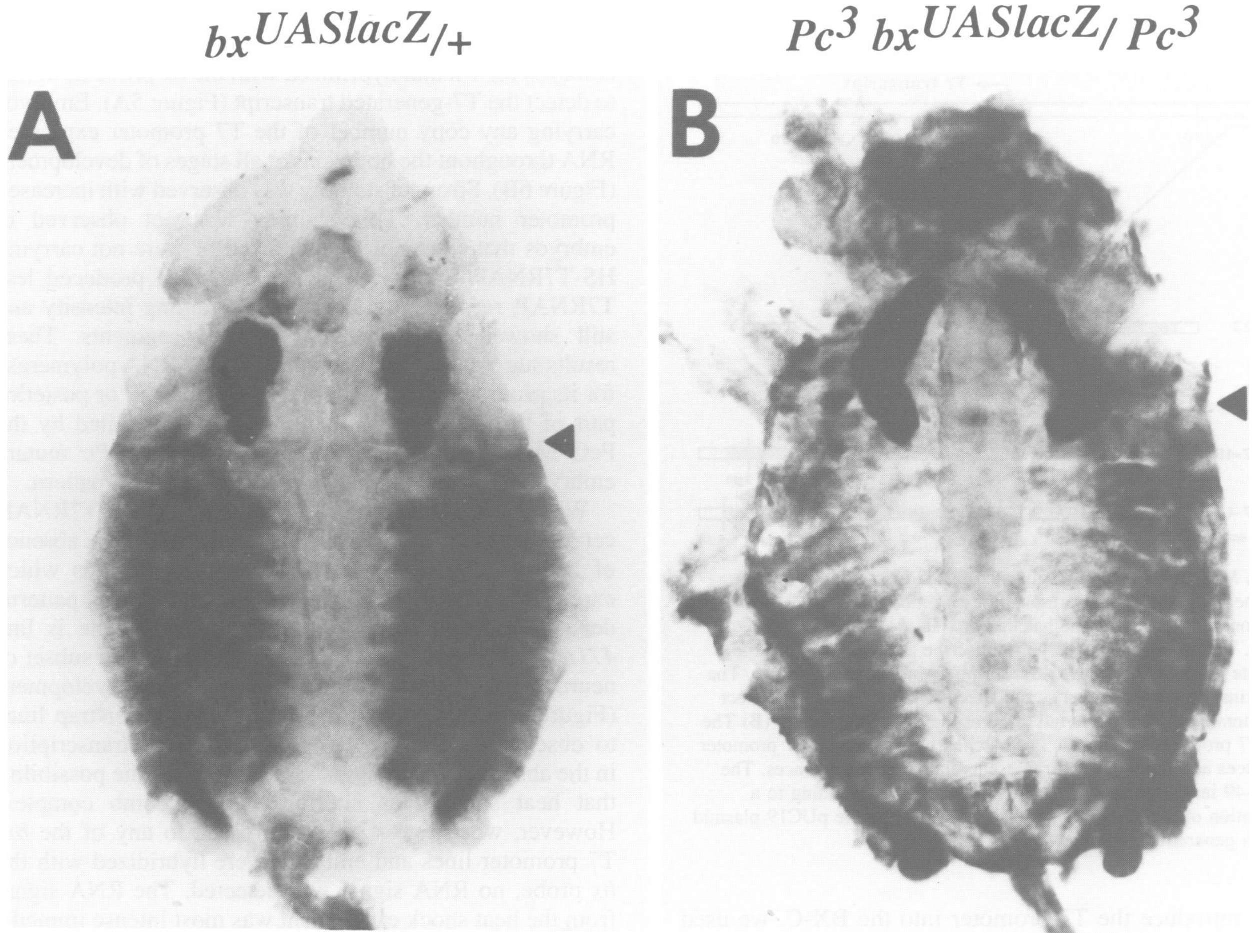

Fig. 4. The segmental restriction of GAL4-mediated transcription is dependent on *Pc*. (**A**) A HS-GAL4 line was crossed to $bx^{UASlacZ}$ and embryos were heat shocked for 1 h and hybridized with a *lacZ* RNA probe. *lacZ* expression is restricted to PS5-12, with the exception of the salivary glands. (**B**) $bx^{UASlacZ}$ embryos that are homozygous for Pc^3 do not show any segmental restriction of *lacZ* expression. This embryo is stage 15 (12–13 h old).

Hunchback. This result shows that transcription alone is insufficient to prevent PcG repression.

Pc mutants relieve the inhibition of GAL4-dependent transcription

Homozygous *Pc* mutant embryos display strong mis-expression of the homeotic gene products (White and Wilcox, 1985; Wedeen *et al.*, 1986; Celniker *et al.*, 1990; Simon *et al.*, 1992). In order to see if the repressive effects on GAL4-dependent transcription were due to action by *Pc*, we examined *lacZ* expression in homozygous Pc^3 mutant embryos. We recombined the Pc^3 mutation onto the $bx^{UASlacZ}$ chromosome and crossed the recombinant flies to the *hsp70-GAL4*$^{7\text{-}1}$ line. *hsp70-GAL4/+;*Pc^3 $bx^{UASlacZ}/+$ females were crossed with $Pc^3/TM1$ males and the embryos were heat shocked. None of the *hsp70-GAL4/+;* $bx^{UASlacZ}/+$ germband retracted embryos displayed unrestricted *lacZ* patterns (Figure 4A) but approximately half of the *lacZ*-positive progeny from the *Pc* cross expressed *lacZ* throughout the A/P axis (Figure 4B). We were able to confirm that the late stage embryos with uniform expression were the Pc^3 homozygotes by a characteristic head phenotype which is due to incomplete head involution. Thus the repression of GAL4-dependent transcription observed in $bx^{UASlacZ}$ embryos is dependent on the PcG.

Introduction of the T7 RNA polymerase system into flies

We have shown that the PcG is clearly blocking GAL4-dependent transcription, but since the GAL4 assay utilizes the *Drosophila* RNA polymerase II (pol II) machinery, it does not distinguish between the simple exclusion of transcription factors and other mechanisms of transcriptional control. Therefore we tested directly DNA accessibility using a heterologous probe which was independent of pol II. We used T7 RNA polymerase, a single subunit polymerase which is specific for its own 23 bp promoter and has been shown to function in mammalian cells (Fuerst *et al.*, 1986). In order to do this, we introduced T7 RNA polymerase into flies and inserted the T7 promoter into the BX-C.

For ubiquitous expression of T7 RNA polymerase, we obtained a modified T7 RNA polymerase gene containing the SV40 T antigen nuclear-localization signal (Dunn *et al.*, 1988). This was cloned into a P element vector under the control of the *hsp70* heat shock promoter (HS-T7RNAP) and the construct was introduced into flies by P element mediated transformation. Embryos were collected from transformant lines, heat shocked and stained using an antibody against T7 RNA polymerase. As shown in Figure 6A, a 1 h heat shock results in strong ubiquitous expression of T7 RNA polymerase.

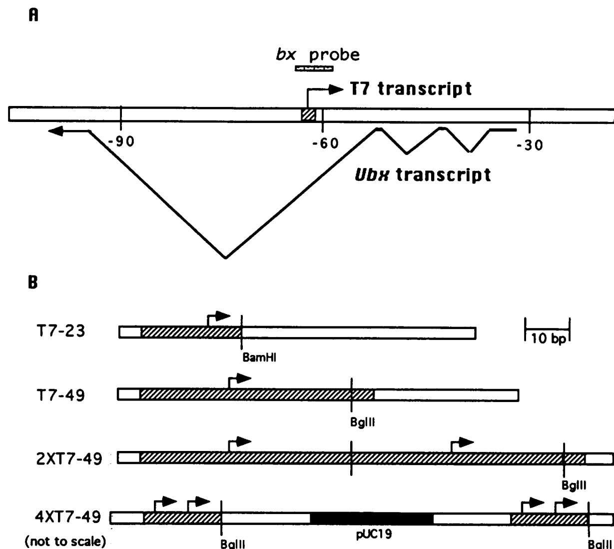

Fig. 5. Map of T7 promoters inserted in the *bx* regulatory region. (**A**) The long white bar represents the chromosome; the T7 promoter insertion is indicated by the hatched box at –61 in the *bx* regulatory region. T7 RNA polymerase will transcribe the *Ubx* intron in the opposite direction to the *Ubx* transcript, as indicated by arrows. The approximate position of the strand-specific *bx* probe used to detect T7-generated transcripts is indicated at the top of the figure. (**B**) The four T7 promoter insertions. The hatched bar represents T7 promoter sequences and the white bar represents flanking *bx* sequences. The 4×T7-49 insertion has additional sequences corresponding to a duplication of 2.3 kb of flanking *bx* sequence and the pUC19 plasmid used in generating gene convertants.

To introduce the T7 promoter into the BX-C, we used the targeted gene conversion method (Gloor *et al.*, 1991), which allows one to replace P element sequences with foreign sequences. This method takes advantage of the double strand gap DNA-repair mechanism that follows P element excision. Normally, double strand gaps are repaired by sequences on the homologous chromosome (Engels *et al.*, 1990). If these homologous sequences are removed by a chromosomal deletion, then exogenous sequences, such as homologous sequences on a P element, can be utilized. Using this method, we introduced the T7 promoter into the insertion site of $bx^{Plac(-61)}$, the same site that was used in the GAL4 experiments (Figure 5A, see Materials and methods). We obtained lines containing one, two or four copies of the T7 promoter in the *bx* region (Figure 5B). Since RNAs generated by T7 RNA polymerase are not translated efficiently in yeast or mammalian cells (Chen *et al.*, 1987; Fuerst and Moss, 1989), we designed promoters to direct transcription of the intron in the *bx* region. This RNA could then be detected using a strand-specific probe ('*bx* probe', Figure 5A). Initial results indicated that the RNAs generated by the small T7 promoter (T7-23) were unstable, and so we inserted a longer sequence from the T7 ϕ10 promoter (49 bp) which includes a 5′ RNA leader sequence which can form a hairpin. This secondary structure may stabilize the RNA (T7-49, Figure 5B) (Fuerst and Moss, 1989).

T7 RNA polymerase does not show differential accessibility to its promoter in bithorax DNA

We tested whether T7 RNA polymerase responded to PcG repression in a manner similar to GAL4, by displaying segmentally restricted transcription. We crossed the *bx*-T7 promoter lines to a HS-T7RNAP line, heat shocked embryos for 1 h and hybridized with the *bx* probe designed to detect the T7-generated transcript (Figure 5A). Embryos carrying any copy number of the T7 promoter expressed RNA throughout the body axis at all stages of development (Figure 6B). Stronger staining was observed with increased promoter number. This staining was not observed in embryos that were not heat shocked or were not carrying HS-T7RNAP. Shorter heat shocks, which produced less T7RNAP, resulted in a decrease in staining intensity and still showed no differences between segments. These results suggest that the accessibility of T7 RNA polymerase for its promoter is not inhibited in the anterior or posterior part of the embryo and therefore is not inhibited by the PcG. Moreover, we repeated this experiment in Pc^3 mutant embryos and saw no change in the expression pattern.

When we recovered transformants of HS-T7RNAP, certain lines expressed T7 RNA polymerase in the absence of heat shock. These lines were enhancer traps which expressed T7 RNA polymerase in particular patterns depending on the site of insertion. An example is line *4711*, which expresses T7 RNA polymerase in a subset of neurons in the CNS, beginning at ~9 h of development (Figure 6C). We wanted to use these enhancer trap lines to observe T7 RNA polymerase-dependent transcription in the absence of heat shock so as to rule out the possibility that heat shock was altering the Polycomb complex. However, when line *4711* was crossed to any of the *bx*-T7 promoter lines and embryos were hybridized with the *bx* probe, no RNA signal was detected. The RNA signal from the heat shock experiment was most intense immediately following heat shock (Figure 6B), so we reasoned that the heat shock state could be leading to increased RNA stability or to an increased rate of transcription by T7 RNA polymerase. Therefore, we gave brief 10 min heat shocks to embryos carrying the *bx*-T7 promoters and the *4711* T7RNAP enhancer trap. These brief heat shocks did not increase the level of T7 RNA polymerase, or change the pattern, as detected by antibody staining. After this treatment, embryos carrying 2×T7-49 or 4×T7-49 (Figure 5B) strongly expressed the *bx* RNA in a pattern which mirrored the expression pattern of T7 RNA polymerase with no segmental restriction (Figure 6D). Segmentally unrestricted expression following a 10 min heat shock was also observed using T7RNAP enhancer traps that express in the epidermis or peripheral nervous system (data not shown). It is unlikely that heat shock is affecting the Pc complex, since heat shock neither affected the restricted accessibility of GAL4 nor altered *Ubx* expression.

T7 RNA polymerase is inhibited by nucleosomal structure in Drosophila

We tried several methods to detect T7 RNA polymerase-generated RNAs in the absence of heat shock. T7 RNA polymerase has been shown to elongate poorly in mammalian cells and *in vitro*, presumably due to interference by nucleosomes (Wolffe and Drew, 1989; Kirov *et al.*, 1992; O'Neill *et al.*, 1992; Jenuwein *et al.*, 1993). We reasoned that perhaps in our system T7 RNA polymerase was also being inhibited by nucleosomes, resulting in undetectable levels of RNA at room temperature. We do not believe

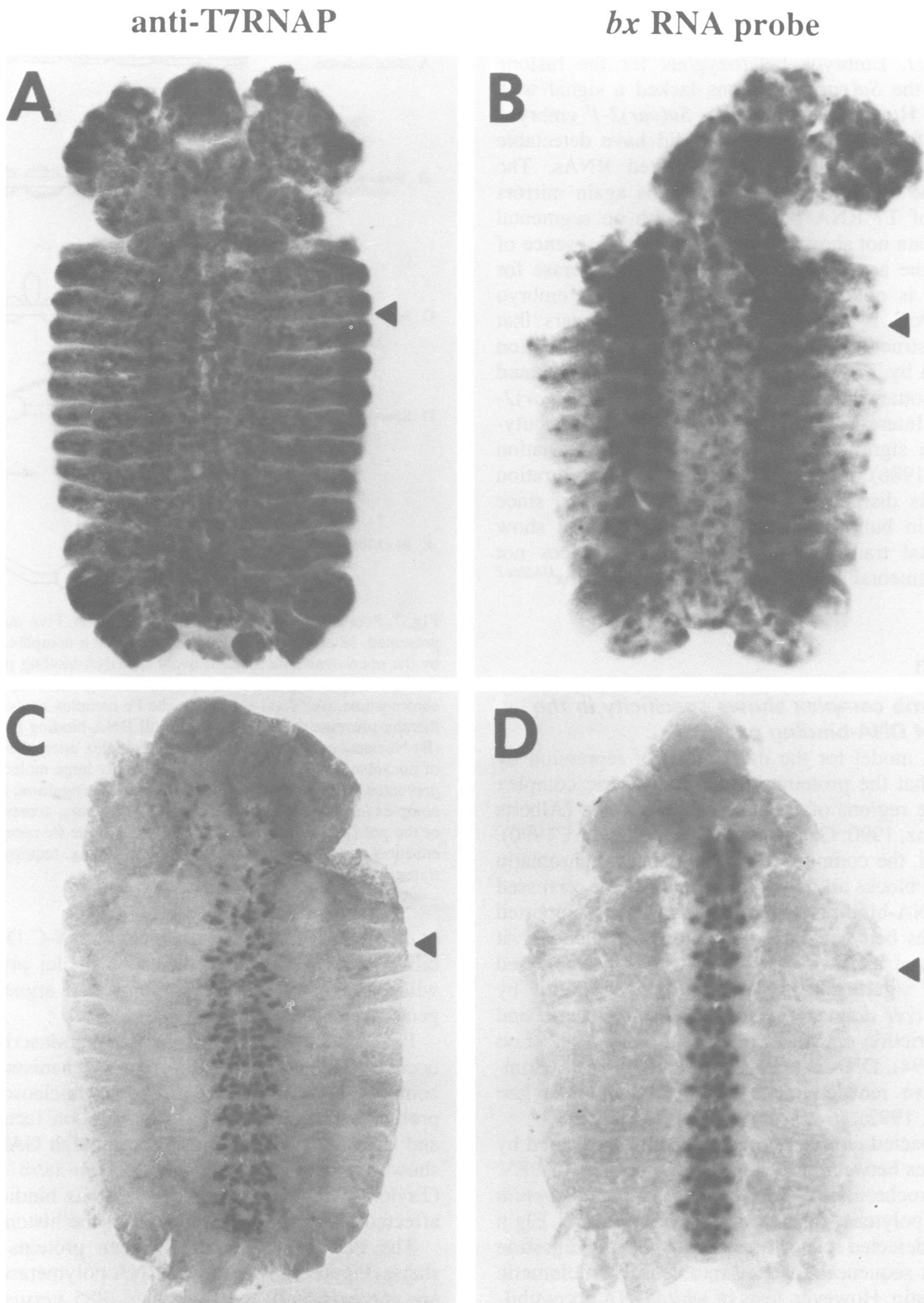

Fig. 6. T7 RNA polymerase transcribes uniformly throughout the anterior–posterior axis. (**A** and **B**) Embryos carrying the HS-T7RNAP construct and the T7 promoter insertion 4XT7-49 were heat shocked for 1 h and stained using either an antibody against T7RNAP (A) or the *bx* probe (B). In both cases the staining is ubiquitous. Certain cells, particularly those in the salivary glands, express the RNA at higher levels. (**C** and **D**) Embryos carrying the T7RNAP enhancer trap *4711* and the T7 promoter insertion 4XT7-49 were heat shocked for 10 min and stained using an antibody against T7RNAP (C) or the *bx* probe (D). Expression is observed in polymerase-expressing cells of the CNS without any segmental restriction.

that heat shock altered nucleosome structure because heat shock is not known to affect chromatin structure except at heat shock loci (Cartwright and Elgin, 1986). By disrupting nucleosomes, we hoped to detect transcripts only in segments of the embryo which were not repressed by the PcG. Therefore, we examined embryos that were heterozygous for a partial deletion of the histone cluster, heterozygous for *Su(var)3-3*^{3}, heterozygous or homozygous for *Su(var)2-1*^{1}, or embryos from flies that had been fed butyrate, an inhibitor of histone deacetylases (Candido *et al.*, 1978). All of these conditions have been shown to suppress PEV in flies (Mottus *et al.*, 1980;

Moore *et al.*, 1983; Lindsley and Zimm, 1992). The embryos examined also carried 4×T7-49 and the T7RNAP insertion *4711*. Embryos heterozygous for the histone deletions or the *Su(var)* mutations lacked a signal with the *bx* probe. However, homozygous *Su(var)2-1*^{1} embryos or embryos from flies fed butyrate did have detectable levels of T7 RNA polymerase-generated RNAs. The pattern of the *bx* RNA in these embryos again mirrors the pattern of T7 RNA polymerase, with no segmental restriction (data not shown). Thus, even in the absence of heat shock, the accessibility of T7 RNA polymerase for its promoter is not inhibited in regions of the embryo where the PcG is acting. Furthermore it appears that nucleosome structure blocks either transcription initiation or elongation by T7 RNA polymerase in *Drosophila*, and this block is somewhat relieved by butyrate or the *Su(var)2-1*^{1} mutation. Interestingly, *Su(var)2-1*^{1} mutants, like butyrate, cause a significant increase in histone acetylation (Dorn *et al.*, 1986). We do not think that this concentration of butyrate is disrupting the Polycomb complex, since flies grown in butyrate-containing media did not show any segmental transformations, and butyrate does not affect the segmental restriction of *lacZ* in GAL4/*bx*^{UASlacZ} embryos.

Discussion

The Polycomb complex shows specificity in the inhibition of DNA-binding proteins

A prominent model for the mechanism of repression by the PcG is that the proteins form a multimeric complex that coats the regions of DNA to be inactivated (Alberts and Sternglanz, 1990; Gaunt and Singh, 1990; Paro, 1990). In this model, the complex forms a compacted chromatin structure that blocks all interactions between the repressed DNA and DNA-binding proteins. The model is supported by similarities between PcG repression and silencing at the mating-type loci in *S.cerevisiae*, where the repressed genes show significant reductions in accessibility by *Escherichia coli dam* methylase, HO endonuclease and multiple restriction enzymes (Singh and Klar, 1992; Loo and Rine, 1994). DNA sequences inserted at yeast telomeres also have reduced accessibility to *dam* methylase (Gottschling, 1992).

This compacted chromatin picture is also motivated by the similarities between PcG genes and modifiers of PEV. Centric heterochromatin looks condensed in *Drosophila* diploid and polytene chromosomes. Wallrath and Elgin (1995) have detected reduced restriction enzyme digestion on P element sequences inserted into centric or telomeric heterochromatin. However, tests of *white* DNA accessibility in the *In (1)w*^{m4} rearrangement, using DNase I, endogenous endonucleases or restriction enzymes, did not show reduced DNA accessibility (Hayashi *et al.*, 1990; Locke, 1993; Schloßherr *et al.*, 1994).

A compacted chromatin model of repression, in its simplest form, predicts that all transcription factors are excluded from DNA in this repressed state (Figure 7A). We have shown that while GAL4 responds to repression by Pc, T7 RNA polymerase does not. Therefore, our results are not compatible with the compacted chromatin model for PcG repression, since the PcG complex shows specificity for the proteins which are inhibited. Schloßherr *et al.* (1994) found no differences in BX-C DNA accessibility with restriction enzymes on nuclei prepared from wild-type or *Pc*$^{-}$ embryos. They also argued against a general block to accessibility.

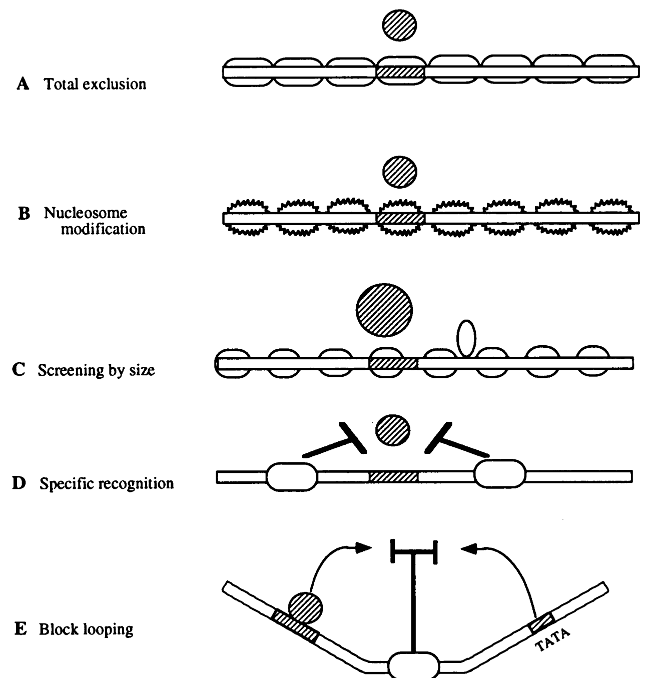

Fig. 7. Potential mechanisms of PcG repression. Five mechanisms are presented. In all cases the PcG proteins form a complex represented by the open ovals, the hatched ovals are DNA-binding proteins and the hatched rectangles are the specific binding sites within the chromosome. (**A**) Total exclusion: the Pc complex coats the DNA and thereby prevents the accessibility of all DNA-binding proteins. (**B**) Nucleosome modification: the Pc complex alters the conformation of nucleosomes. (**C**) Screening by size: only large molecules are prevented from binding DNA. (**D**) Specific recognition: the Pc complex specifically inhibits transcription factors, accessory proteins or the pol II machinery. (**E**) Blocks looping: the Pc complex prevents enhancer–promoter interactions, such as looping, required for activated transcription.

PcG inhibition of polymerase II transcription could occur via a number of different mechanisms. The PcG complex could stabilize arrays of nucleosomes which prohibit binding by certain transcription factors (Pirrotta and Rastelli, 1994; Figure 7B). Although GAL4 has been shown to bind nucleosomal DNA *in vitro* and *in vivo* (Taylor *et al.*, 1991; Morse, 1993), its binding could be affected by protein modification of the histones.

The PcG complex could screen proteins by size or shape (Figure 7C). While T7 RNA polymerase and GAL4 are approximately the same size (885 versus 881 amino acids) (Dunn and Studier, 1983; Ptashne, 1988), the PcG could function by blocking large components of the pol II transcription machinery, since these proteins are required by GAL4 but not by T7 RNA polymerase. These components include the TFIID initiation complex, the SWI/SNF complex and the polymerase itself. One form of this selective exclusion is the nuclear compartmentalization model (Paro, 1993; Schloßherr *et al.*, 1994), whereby PcG-repressed DNA is sequestered in an inactive region of the nucleus.

The PcG could interact with specific molecules required for pol II transcription (Figure 7D). Such molecules

include transcription factors, subunits of the TFIID initiation complex or RNA pol II. Alternatively, the PcG could specifically inhibit accessory factors that assist transcription factors, including members of the SWI/SNF complex or other proteins with similar functions. The SWI/SNF complex is an attractive possibility as it has been shown that mutations in SWI2/SNF2 greatly reduce transcriptional activation by GAL4 in yeast (Peterson and Herskowitz, 1992; Côté *et al.*, 1994). Furthermore, the SWI/SNF complex, from either humans or yeast, facilitates GAL4 binding to nucleosomal DNA *in vitro* (Côté *et al.*, 1994; Kwon *et al.*, 1994). Interestingly, the *Drosophila* SWI2/SNF2 homolog is encoded by the gene *brahma*, a member of the *trithorax* group. Mutations in *brahma* behave as genetic suppressors of PcG mutations (Tamkun *et al.*, 1992). Jones and Gelbart (1993) identified sequence homology between *trithorax* (*trx*) and the PcG member *E*(*z*) and they showed that *trx* mutations strongly suppress the homeotic transformations of *E*(*z*) mutant embryos. They proposed a model with competition between the *trithorax* and *Polycomb* groups.

The PcG has also been proposed to function by specifically blocking looping interactions between enhancers and promoters (Pirrotta and Rastelli, 1994; Figure 7E). Looping is likely to play a significant role in the regulation of the BX-C, as enhancers act over distances as large as 50 kb. In our experiments, the GAL4-binding sites are 28–119 bp from the TATA box in the *UASlacZ* construct (A.Brand, personal communication), yet Pc-dependent repression is still observed. This model would require that the PcG can inhibit even very small loops.

The potential mechanisms of PcG repression presented in Figure 7 can be investigated further. *In vivo* probes can be increased in size, specific interactions can be analyzed by the two-hybrid system, and additional probes can be used which do not require the pol II machinery to function.

Local transcription does not inhibit repression by the Polycomb complex

Repression of the homeotic genes is established early in development by transcription factors such as Hunchback, Krüppel and Tailless (Qian *et al.*, 1991; Müller and Bienz, 1992; Shimell *et al.*, 1994). Repression is probably fixed by the PcG before 5–6 h of development, since this is the earliest time ectopic expression of the homeotic genes is observed in PcG mutant embryos (Struhl and Akam, 1985; Simon *et al.*, 1992). We do not see the disappearance of GAL4-induced transcripts until ~9 h of development. This may be due to perdurance of the *lacZ* RNA from earlier stages of development.

The mechanism for the establishment of PcG repression is unknown. It is possible that transcription through the regulatory regions might inhibit PcG complex formation. Indeed, many if not all of the regulatory regions of the BX-C produce RNAs of unknown function early in development (Lipshitz *et al.*, 1987; Sanchez-Herrero and Akam, 1989; Cumberledge *et al.*, 1990). Our results show that transcription mediated by GAL4 in the *bx* regulatory region early in development does not inhibit PcG repression, although transcription through other regions of the BX-C could still be important. Since GAL4 can direct transcription in the anterior segments of early embryos, our results suggest that the mechanism of repression by early acting gap and pair-rule genes is distinct from the mechanism used by the PcG.

Pc repression of GAL4 fails in salivary glands

Telomeric silencing in yeast can be overcome by high levels of the transcriptional activator PPR1 (Aparicio and Gottschling, 1994). Because of that precedent, we checked the dose dependence of our two probe molecules. Increasing the concentration of GAL4 molecules 2-fold by using two copies of HS-GAL4, did not overcome PcG repression. Reducing the level of T7RNAP, by shortening heat shock time, did not affect the unrestricted pattern of the *bx* RNA. GAL4 does override repression by the Polycomb complex in the salivary glands, a PS2-derived structure. During embryogenesis, the salivary glands undergo one round of polytenization, which may provide each nucleus with two or four copies of the *UASlacZ* target (Smith and Orr-Weaver, 1991). GAL4 can also override repression in other tissues when embryos are homozygous for the $bx^{UASlacZ}$ target. The ability to inhibit PcG repression may involve the pairing of *UASlacZ* P elements, resulting in an isolated transcriptionally active domain. Alternatively, an increase in the number of bound GAL4 molecules due to co-operative binding *in trans* may be able to override PcG repression. Zink and Paro (1995) have recently studied a case of competition between GAL4 activation and PcG repression in salivary gland nuclei. They suggest that binding of GAL4 protein and Pc protein are mutually exclusive, and that Pc repression can be reverted by high concentrations of GAL4 protein.

T7 RNA polymerase is inhibited by nucleosome structure in Drosophila

Several groups have reported that the initation and elongation of transcription by T7 RNA polymerase are inhibited by nucleosomes *in vitro* (Wolffe and Drew, 1989; Kirov *et al.*, 1992; O'Neill *et al.*, 1992). Jenuwein *et al.* (1993) demonstrated that the elongation of transcription by T7 RNA polymerase in mammalian cell nuclei is blocked. T7 RNA polymerase appears to be inhibited by nucleosomes in *Drosophila*, because we cannot detect transcripts at room temperature unless the flies are fed butyrate or carry the *Su*(*var*)*2-1*^{1} mutation. Both of these conditions cause an increase in the acetylation of histones (Riggs *et al.*, 1977; Dorn *et al.*, 1986). The hyperacetylation of histones causes alterations in chromatin structure such as an increase in DNase I sensitivity and a decrease in the association with histone H1 (reviewed in Turner, 1991). Such alterations may lead to a loosening of chromatin structure that facilitates the access of DNA-binding proteins, as has been demonstrated for TFIIIA (Lee *et al.*, 1993).

Materials and methods

Drosophila strains

The expression patterns of the GAL4 and T7RNAP lines are summarized in Table I. The GAL4 lines and the *UASlacZ* line (on chromosome II) were kindly provided by Beth Noll and Norbert Perrimon; these lines are described in Brand and Perrimon (1993) or Brand *et al.* (1994). The $bx^{Plac(-61)}$ line contains a P element marked with *rosy*$^{+}$ inserted at –61 kb in the *bx* regulatory region of the BX-C (McCall *et al.*, 1994); the insertion causes a mild *bx* phenotype. The Δ2-3 chromosome contains a stably integrated source of P transposase (Robertson *et al.*, 1988).

Table I. GAL4 and T7RNAP lines used in this study

Line	Chromosome	Expression pattern	References
GAL4 lines			
24B	III	mesoderm	Brand and Perrimon (1993)
32B	III	ubiquitous	Brand and Perrimon (1993)
hsp70-GAL4$^{7-1}$	II	heat-inducible	Brand *et al.* (1994)
hsp70-GAL4$^{2-1}$	III	heat-inducible	Brand *et al.* (1994)
RG1	III	*paired* stripes	L.Fasano and C.Desplan, unpublished
UASlacZ$^{Bg4-1-2}$	II		Brand and Perrimon (1993)
T7RNAP lines			
4710E	X	heat-inducible	this study
4711	II	CNS	this study
481B	III	PNS	this study
13-1	n.d.	epidermis	this study

Fab7^1 is a dominant mutation in the *AbdB* gene (Gyurkovics *et al.*, 1990). The partial deletions for the histone clusters that were examined are Df(2L)TW65, Df(2L)TW84 and Df(2L)DS6. These and all other mutations are described in Lindsley and Zimm (1992). The butyrate experiment was done by feeding flies media containing 100 or 200 mM *n*-butyric acid (Sigma) for 3–4 days before embryo collection.

Targeted transposition

An insertion of *UASlacZ* was obtained at the site of the *bx$^{Plac(-61)}$* P element by crossing *UASlacZ/+*; *bx$^{Plac(-61)}$ Fab7^1/Sb Δ2-3* males to *rosy$^-$* females and isolating *rosy$^-$* male progeny that had lost the *bx$^{Plac(-61)}$* P element. 48 of 123 *rosy$^-$* revertants retained a *bx* phenotype and these were screened by PCR for the presence of a junction fragment between the *white* sequence on the *UASlacZ* P element and adjacent *bx* sequence. One line (*bx^{UASlacZ}*) was isolated with a correct PCR product. Further analysis by Southern blot showed that the *bx^{UASlacZ}* P element had an internal deletion of ~6 kb (see Figure 1B).

DNA manipulations

HS-T7RNAP was constructed by isolating a *Bgl*II–*Bam*HI fragment from pAR3283 which contains the T7 RNA polymerase gene fused to a nuclear localization signal from SV40 T antigen (Dunn *et al.*, 1988). This fragment was inserted into the *Bgl*II site downstream of the HSP70 promoter of pCaSpeR-hs (Thummel and Pirrotta, 1992).

The T7 promoters were cloned by PCR into *bx* DNA at the site of the *bx$^{Plac(-61)}$* P element insertion. The T7-23 promoter, which has the sequence from –17 to +6 of the T7 ϕ10 promoter followed by a *Bam*HI site, was cloned into a 10 kb *Bgl*II–*Xba*I DNA fragment from the *bx* region (coordinates –68 to –58 kb). This modified bx10 fragment was cloned into HZ50PL (Hiromi and Gehring, 1987), a P element vector marked with *rosy$^+$*. The construct is called P[bx10-T7-23].

The T7-49 promoter, which contains sequences from –23 to +26 of the T7 ϕ10 promoter (Fuerst and Moss, 1989) followed by a *Bgl*II site, was PCR amplified from pT7-7 (from Stan Tabor). The PCR product was cloned into a modified 2.3 kb *Hin*dIII fragment from the *bx* region in pUC19 (New England Biolabs) (pbx2.3*Bgl*II). This *Hin*dIII fragment was modified to optimize the frequency of gene conversion events. The sequence was recovered from the *bx$^{Plac(-61)}$* chromosome in order to reduce the frequency of base pair variations which could have a deleterious effect on the recovery of gene convertants (Gloor *et al.*, 1991). In addition, the fragment contains a unique *Bgl*II site at the location of the *bx$^{Plac(-61)}$* insertion flanked by two copies of the 8 bp duplication generated by the P element insertion. This may facilitate double strand gap repair (Johnson-Schlitz and Engels, 1993). The 2XT7-49 construct was generated by inserting two copies of the T7-49 promoter in tandem into pbx2.3*Bgl*II.

Germline transformants of HS-T7RNAP and P[bx10-T7-23] were generated as described (Simon *et al.*, 1991) by injecting into *yw;Sb Δ2-3/TM6* and *cn;ry* strains, respectively. Gene convertants for T7-49 and 2XT7-49 were obtained by directly injecting the pUC19-based plasmids into *bx$^{Plac(-61)}$* embryos as described below.

Targeted gene conversion

The T7 promoter insertions were recovered at the site of the *bx$^{Plac(-61)}$* P element using targeted gene conversion (Gloor *et al.*, 1991). All gene conversion crosses were done with a chromosomal deletion in the *bx* region, to reduce the frequency of repair from the homologous chromosome. Gene convertants for T7-23 were obtained by crossing P[bx10-T7-23] *Df(3R)Ubx^{109}* Δ2-3/*bx$^{Plac(-61)}$* males to *rosy$^-$* females. *rosy$^-$* progeny, which were reverted for the *bx$^{Plac(-61)}$* P element, were screened for the presence of the T7 promoter insertion by PCR and restriction digest of the PCR product. The PCR primers that were used hybridize to *bx* sequences at –60 and +200 bp from the starting P element insertion. One unique gene convertant from 54 *rosy$^-$* flies was isolated.

For convertants of T7-49 and 2×T7-49, donor and pUChsπΔ2-3 (Mullins *et al.*, 1989) plasmids were directly injected into *bx$^{Plac(-61)}$/Df(3R)bx^{34eprv}* embryos. After eclosion, these flies were crossed to *rosy$^-$* males or females, and again *rosy$^-$* revertant progeny were screened by PCR and restriction digest. One unique gene convertant was obtained of 78 *rosy$^-$* flies for T7-49; three lines were obtained out of 75 *rosy$^-$* flies for 2×T7-49. Two of the 2×T7-49 lines were subsequently found to have identical larger insertions containing four copies of the T7 promoter (Figure 1B, 4XT7-49). These insertions include one copy of the entire donor plasmid, including pUC19 sequence. This event probably resulted from repair using a dimer of the donor plasmid as a template, or simultaneous repair from both ends of the break using two donor plasmids as templates, as proposed by Nassif *et al.* (1994). The structure of all insertions was confirmed by Southern blot anaylsis.

Analysis of expression patterns

A digoxigenin-labeled RNA *lacZ* probe was generated as described in Gavis and Lehmann (1992). To generate a digoxigenin-labeled RNA *bx* probe, a fragment containing 2×T7-49 in the 2.3 *Hin*dIII *bx* fragment was cloned into pGEM (Promega), linearized with *Bss*HII and transcribed with SP6 RNA polymerase (New England Biolabs) following directions from the Genius kit (Boehringer Mannheim). This probe is designed to detect transcripts from –62 to –60 kb on the BX-C walk (Figure 5A). Treatment of the RNA probes and hybridization to embryos were performed as described in Gavis and Lehmann (1992). Surprisingly, the *bx* probe detects a previously unreported RNA species in CantonS embryos. This RNA, which is transcribed in the opposite direction to the *Ubx* gene, is detected between 20 and 50% egg length at blastoderm. It becomes difficult to detect by germband extension.

Embryos were heat shocked in laying vials in a 36.5°C circulating water bath for either 1 h or 10 min as described in the Results. Heat shocked embryos in GAL4 experiments were allowed to recover for 30 min; embryos expressing T7 RNA polymerase were allowed to recover for 30 min or were fixed immediately after heat shock. The *Ubx* expression patterns of *bx^{UASlacZ}/32B* and HST7RNAP; T723 embryos were indistinguishable from wild-type.

For antibody staining, embryos were fixed and stained as described (Karch *et al.*, 1990) using a rabbit polyclonal against T7 RNA polymerase kindly provided by Stan Tabor, followed by an HRP-conjugated goat anti-rabbit secondary antibody (Bio-Rad). Embryos were dissected as described, with the gut and visceral mesoderm removed (Karch *et al.*, 1990). Whole mount preparations were dehydrated and mounted in Euparol (Carolina Biological).

Acknowledgements

We are grateful to Jeff Simon for his ideas and suggestions in the early stages of this work. We thank Ophelia Papoulas for help in generating the pbx2.3*Bgl*II plasmid and Southern analysis on the *bx^{UASlacZ}* line. We

thank Kathy Matthews and the Bloomington Stock Center, Gunter Reuter, Beth Noll and Norbert Perrimon and Ophelia Papoulas for fly strains, and Stan Tabor and members of the Richardson laboratory for the T7RNAP antibody, plasmids and helpful conversations. We thank Jeff Simon, Ting Wu, Stanley Tiong and Andrew Hudson for critical reading of this manuscript. This work was supported by a grant from the National Institutes of Health to W.B.

References

Alberts,B. and Sternglanz,R. (1990) Chromatin contract to silence. *Nature*, **344**, 193–194.

Aparicio,O.M. and Gottschling,D.E. (1994) Overcoming telomeric silencing: a trans-activator competes to establish gene expression in a cell cycle-dependent way. *Genes Dev.*, **8**, 1133–1146.

Brand,A.H. and Perrrimon,N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*, **118**, 401–415.

Brand,A.H., Manoukian,A.S. and Perrimon,N. (1994) Ectopic expression in *Drosophila*. *Methods Cell Biol.*, **44**, 635–654.

Camprodón,F.J. and Castelli-Gair,J.E. (1994) Ultrabithorax protein expression in breakpoint mutants: localization of single, co-operative and redundant *cis* regulatory elements. *Roux's Arch. Dev. Biol.*, **203**, 411–421.

Candido,E.P.M., Reeves,R. and Davie,J.R. (1978) Sodium butyrate inhibits histone deacetylation in cultured cells. *Cell*, **14**, 105–113.

Cartwright,I.L. and Elgin,S.C.R. (1986) Nucleosomal instability and induction of new upstream protein–DNA associations accompany activation of four small heat shock protein genes in *Drosophila melanogaster*. *Mol. Cell. Biol.*, **6**, 779–791.

Celniker,S.E., Sharma,S., Keelan,D.J. and Lewis,E.B. (1990) The molecular genetics of the bithorax complex of *Drosophila*: *cis*-regulation in the *Abdominal-B* domain. *EMBO J.*, **9**, 4277–4286.

Chamberlin,M. and Ryan,T. (1982) Bacteriophage DNA-dependent RNA polymerases. In Boyer,P. (ed.), *The Enzymes*. Academic Press, New York, pp. 87–108.

Chan,C.-S., Rastelli,L. and Pirrotta,V. (1994) A Polycomb response element in the *Ubx* gene that determines an epigenetically inherited state of repression. *EMBO J.*, **13**, 2553–2564.

Chen,W., Tabor,S. and Struhl,K. (1987) Distinguishing between mechanisms of eucaryotic transcriptional activation with bacteriophage T7 RNA polymerase. *Cell*, **50**, 1047–1055.

Chiang,A., O'Connor,M., Paro,R., Simon,J. and Bender,W. (1995) Discrete Polycomb binding sites in each parasegmental domain of the bithorax complex. *Development*, **121**, 1681–1689.

Côté,J., Quinn,J., Workman,J.L. and Peterson,C.L. (1994) Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science*, **265**, 53–60.

Cumberledge,S., Zaratzian,A. and Sakonju,S. (1990) Characterization of two RNAs transcribed from the *cis*-regulatory region of the *abd-A* domain within the *Drosophila* bithorax complex. *Proc. Natl Acad. Sci. USA*, **87**, 3259–3263.

Dorn,R., Heymann,S., Lindigkeit,R. and Reuter,G. (1986) Suppressor mutation of position-effect variegation in *Drosophila melanogaster* affecting chromatin properties. *Chromosoma*, **93**, 398–403.

Dunn,J.J. and Studier,F.W. (1983) Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. *J. Mol. Biol.*, **166**, 477–535.

Dunn,J.J., Krippl,B., Bernstein,K.E., Westphal,H. and Studier,F.W. (1988) Targeting bacteriophage T7 RNA polymerase to the mammalian cell nucleus. *Gene*, **68**, 259–266.

Engels,W.R., Johnson-Schlitz,D.M., Eggleston,W.B. and Sved,J. (1990) High-frequency P element loss in *Drosophila* is homolog dependent. *Cell*, **62**, 515–525.

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

Fischer,J.A., Giniger,E., Maniatis,T. and Ptashne,M. (1988) GAL4 activates transcription in *Drosophila*. *Nature*, **332**, 853–856.

Fuerst,T.R. and Moss,B. (1989) Structure and stability of mRNA synthesized by vaccinia virus-encoded bacteriophage T7 RNA polymerase in mammalian cells. *J. Mol. Biol.*, **206**, 333–348.

Fuerst,T.R., Niles,E.G., Studier,F.W. and Moss,B. (1986) Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase. *Proc. Natl Acad. Sci. USA*, **83**, 8122–8126.

Gaunt,S.J. and Singh,P.B. (1990) Homeogene expression patterns and chromosomal imprinting. *Trends Genet.*, **6**, 208–212.

Gavis,E.R. and Lehmann,R. (1992) Localization of *nanos* RNA controls embryonic polarity. *Cell*, **71**, 301–313.

Gindhart,J.G.,Jr and Kaufman,T.C. (1995) Identification of Polycomb and trithorax group responsive elements in the regulatory region of the *Drosophila* homeotic gene *Sex combs reduced*. *Genetics*, **139**, 797–814.

Gloor,G.B., Nassif,N.A., Johnson-Schlitz,D.M., Preston,C.R. and Engels,W.R. (1991) Targeted gene replacement in *Drosophila* via P element-induced gap repair. *Science*, **253**, 1110–1117.

Gonzy-Tréboul,G., Lepesant,J.-A. and Deutsch,J. (1995) Enhancer-trap targeting at the *Broad-Complex* locus of *Drosophila melanogaster*. *Genes Dev.*, **9**, 1137–1148.

Gottschling,D.E. (1992) Telomere-proximal DNA in *Saccharomyces cerevisiae* is refractory to methyltransferase activity *in vivo*. *Proc. Natl Acad. Sci. USA*, **89**, 4062–4065.

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

Gyurkovics,H., Gausz,J., Kummer,J. and Karch,F. (1990) A new homeotic mutation in the *Drosophila* bithorax complex removes a boundary separating two domains of regulation. *EMBO J.*, **9**, 2579–2585.

Hayashi,S., Ruddell,A., Sinclair,D. and Grigliatti,T. (1990) Chromosomal structure is altered by mutations that suppress or enhance position effect variegation. *Chromosoma*, **99**, 391–400.

Heslip,T.R. and Hodgetts,R.B. (1994) Targeted transposition at the *vestigial* locus of *Drosophila melanogaster*. *Genetics*, **138**, 1127–1135.

Heslip,T.R., Williams,J.A., Bell,J.B. and Hodgetts,R.B. (1992) A P element chimera containing captured genomic sequences was recovered at the *vestigial* locus in *Drosophila* following tartgeted transposition. *Genetics*, **131**, 917–927.

Hiromi,Y. and Gehring,W.J. (1987) Regulation and function of the *Drosophila* segmentation gene *fushi tarazu*. *Cell*, **50**, 963–974.

Hooper,J.E. (1986) Homeotic gene function in the muscles of *Drosophila* larvae. *EMBO J.*, **5**, 2321–2329.

Jenuwein,T., Forrester,W.C., Qui,R.-G. and Grosschedl,R. (1993) The immunoglobulin μ enhancer core establishes local factor access in nuclear chromatin independent of transcriptional stimulation. *Genes Dev.*, **7**, 2016–2032.

Johnson-Schlitz,D.M. and Engels,W.R. (1993) P-element-induced interallelic gene conversion of insertions and deletions in *Drosophila melanogaster*. *Mol. Cell. Biol.*, **13**, 7006–7018.

Jones,R.S. and Gelbart,W.M. (1993) The *Drosophila Polycomb*-group gene *Enhancer of zeste* shares a domain of sequence similarity with *trithorax*. *Mol. Cell. Biol.*, **13**, 6357–6366.

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

Karch,F., Bender,W. and Weiffenbach,B. (1990) *abdA* expression in *Drosophila* embryos. *Genes Dev.*, **4**, 1573–1587.

Kirov,N., Tsaneva,I., Einbinder,E. and Tsanev,R. (1992) *In vitro* transcription through nucleosomes by T7 RNA polymerase. *EMBO J.*, **11**, 1941–1947.

Kwon,H., Imbalzano,A.N., Khavari,P.A., Kingston,R.E. and Green,M.R. (1994) Nucleosome disruption and enhancement of activator binding by a human SWI/SNF complex. *Nature*, **370**, 477–481.

Laurenson,P. and Rine,J. (1992) Silencers, silencing, and heritable transcriptional states. *Microbiol. Rev.*, **56**, 543–560.

Lee,D.Y., Hayes,J.J., Pruss,D. and Wolffe,A. (1993) A positive role for histone acetylation in transcription factor access to nucleosomal DNA. *Cell*, **72**, 73–84.

Lewis,E.B. (1978) A gene complex controlling segmentation in *Drosophila*. *Nature*, **276**, 565–570.

Lindsley,D.L. and Zimm,G.G. (1992) *The Genome of Drosophila melanogaster*. Academic Press, Inc., San Diego, CA.

Lipshitz,H.D., Peattie,D.A. and Hogness,D.S. (1987) Novel transcripts from the *Ultrabithorax* domain of the bithorax complex. *Genes Dev.*, **1**, 307–322.

Locke,J. (1993) Examination of DNA sequences undergoing chromatin conformation changes at a variegating breakpoint in *Drosophila melanogaster*. *Genetica*, **92**, 33–41.

Loo,S. and Rine,J. (1994) Silencers and domains of generalized repression. *Science*, **264**, 1768–1771.

McCall,K., O'Connor,M.B. and Bender,W. (1994) Enhancer traps in the bithorax complex mark parasegmental domains. *Genetics*, **138**, 387–399.

McKeon,J. and Brock,H.W. (1991) Interactions of the *Polycomb* group of genes with homeotic loci of *Drosophila*. *Roux's Arch. Dev. Biol.*, **199**, 387-396.

Moore,G.D., Sinclair,D.A. and Grigliatti,T.A. (1983) Histone gene multiplicity and position effect variegation in *Drosophila melanogaster*. *Genetics*, **105**, 327-344.

Morse,R.H. (1993) Nucleosome disruption by transcription factor binding in yeast. *Science*, **262**, 1563-1566.

Mottus,R., Reeves,R. and Grigliatti,T.A. (1980) Butyrate suppression of position-effect variegation in *Drosophila melanogaster*. *Mol. Gen. Genet.*, **178**, 465-469.

Müller,J. and Bienz,M. (1992) Sharp anterior boundary of homeotic gene expression conferred by the *fushi tarazu* protein. *EMBO J.*, **11**, 3653-3661.

Mullins,M.C., Rio,D.C. and Rubin,G.M. (1989) *Cis*-acting DNA sequence requirements for P-element transposition. *Genes Dev.*, **3**, 729-738.

Nassif,N., Penney,J., Pal,S., Engels,W.R. and Gloor,G.B. (1994) Efficient copying of nonhomologous sequences from ectopic sites via P-element-induced gap repair. *Mol. Cell. Biol.*, **14**, 1613-1625.

O'Neill,T.E., Roberge,M. and Bradbury,E.M. (1992) Nucleosome arrays inhibit both initiation and elongation of transcripts by bacteriophage T7 RNA polymerase. *J. Mol. Biol.*, **223**, 67-78.

Paro,R. (1990) Imprinting a determined state into the chromatin of *Drosophila*. *Trends Genet.*, **6**, 416-421.

Paro,R. (1993) Mechanisms of heritable gene repression during development of *Drosophila*. *Curr. Biol.*, **5**, 999-1005.

Paro,R. and Hogness,D.S. (1991) The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of *Drosophila*. *Proc. Natl Acad. Sci. USA*, **88**, 263-267.

Peterson,C.L. and Herskowitz,I. (1992) Characterization of the yeast SWI1, SWI2, and SWI3 genes, which encode a global activator of transcription. *Cell*, **68**, 573-583.

Pirrotta,V. and Rastelli,L. (1994) *white* gene expression, repressive chromatin domains and homeotic gene regulation in *Drosophila*. *BioEssays*, **16**, 549-556.

Ptashne,M. (1988) How eukaryotic transcriptional activators work. *Nature*, **335**, 683-689.

Qian,S., Capovilla,M. and Pirrotta,V. (1991) The *bx* region enhancer, a distant *cis*-control element of the *Drosophila Ubx* gene and its regulation by *hunchback* and other segmentation genes. *EMBO J.*, **10**, 1415-1425.

Reuter,G. and Spierer,P. (1992) Position effect variegation and chromatin proteins. *BioEssays*, **14**, 605-612.

Riggs,M.G., Whittaker,R.G., Neumann,J.R. and Ingram,V.M. (1977) n-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells. *Nature*, **268**, 462-464.

Robertson,H.M., Preston,C.R., Phillis,R.W., Johnson-Schlitz,D.M., Benz,W.K. and Engels,W.R. (1988) A stable genomic source of P element transposase in *Drosophila melanogaster*. *Genetics*, **118**, 461-470.

Sanchez-Herrero,E. and Akam,M. (1989) Spatially ordered transcription of regulatory DNA in the bithorax complex of *Drosophila*. *Development*, **107**, 321-329.

Schloßherr,J., Eggert,H., Paro,R., Cremer,S. and Jack,R.S. (1994) Gene inactivation in *Drosophila* mediated by the *Polycomb* gene product or by position-effect variegation does not involve major changes in the accessibility of the chromatin fibre. *Mol. Gen. Genet.*, **243**, 453-462.

Shimell,M.J., Simon,J., Bender,W. and O'Connor,M.B. (1994) Enhancer point mutation results in a homeotic transformation in *Drosophila*. *Science*, **264**, 968-971.

Simon,J. (1995) Locking in stable states of gene expression: transcriptional control during *Drosophila* development. *Curr. Opin. Cell Biol.*, **7**, 376-385.

Simon,J., Chiang,A. and Bender,W. (1992) Ten different *Polycomb* group genes are required for spatial control of the *abdA* and *AbdB* homeotic products. *Development*, **114**, 493-505.

Singh,J. and Klar,A.J.S. (1992) Active genes in budding yeast display enhanced *in vivo* accessibility to foreign DNA methylases: a novel *in vivo* probe for chromatin structure of yeast. *Genes Dev.*, **6**, 186-196.

Smith,A.V. and Orr-Weaver,T.L. (1991) The regulation of the cell cycle during *Drosophila* embryogenesis: the transition to polyteny. *Development*, **112**, 997-1008.

Soto,M.C., Chou,T.-B. and Bender,W. (1995) Comparison of germline mosaics of genes in the *Polycomb* group of *Drosophila melanogaster*. *Genetics*, **140**, 231-243.

Staveley,B.E., Hodgetts,R.B., O'Keefe,S.L. and Bell,J.B. (1994) Targeting of an enhancer trap to *vestigial*. *Dev. Biol.*, **165**, 290-293.

Struhl,G. and Akam,M. (1985) Altered distributions of *Ultrabithorax* transcripts in *extra sex combs* mutant embryos of *Drosophila*. *EMBO J.*, **4**, 3259-3264.

Tamkun,J.W., Deuring,R., Scott,M.P., Kissinger,M., Pattatucci,A.M., Kaufman,T.C. and Kennison,J.A. (1992) *brahma*: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell*, **68**, 561-572.

Taylor,I.C.A., Workman,J.L., Schuetz,T.J. and Kingston,R.E. (1991) Facilitated binding of GAL4 and heat shock factor to nucleosomal templates: differential function of DNA-binding domains. *Genes Dev.*, **5**, 1285-1298.

Thummel,C.S. and Pirrotta,V. (1992) New pCaSpeR P element vectors. *Drosophila Inf. Serv.*, **71**, 150.

Turner,B.M. (1991) Histone acetylation and control of gene expression. *J. Cell Sci.*, **99**, 13-20.

Wallrath,L.L. and Elgin,S.C.R. (1995) Position effect variegation in *Drosophila* is associated with an altered chromatin structure. *Genes Dev.*, **9**, 1263-1277.

Wedeen,C., Harding,K. and Levine,M. (1986) Spatial regulation of *Antennapedia* and bithorax gene expression by the *Polycomb* locus in *Drosophila*. *Cell*, **44**, 739-748.

White,R.A.H. and Wilcox,M. (1985) Regulation of the distribution of *Ultrabithorax* proteins in *Drosophila*. *Nature*, **318**, 563-567.

Wolffe,A.P. and Drew,H.R. (1989) Initiation of transcription on nucleosomal templates. *Proc. Natl Acad. Sci. USA*, **86**, 9817-9821.

Zink,D. and Paro,R. (1995) *Drosophila* Polycomb group-regulated chromatin inhibits the accessibility of a *trans*-activator to its target DNA. *EMBO J.*, **14**, 5660-5671.

Received on September 5, 1995; revised on October 10, 1995