The *polyhomeotic* gene of *Drosophila* encodes a chromatin protein that shares polytene chromosome-binding sites with *Polycomb*

Marco DeCamillis, Niansheng Cheng, Denise Pierre, and Hugh W. Brock

Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4

The *Polycomb* group (PcG) genes in *Drosophila melanogaster* are required for maintenance of correct spatial expression of homeotic genes, and their products are thought to form either a regulatory network or act as a multimeric complex. Recently, it has been suggested that because of homology between *Polycomb* (*Pc*) and *Su(var)*205, PcG genes encode chromatin proteins required for the maintenance of a determined state in chromatin. The *polyhomeotic* (*ph*) gene is a member of the PcG of genes. We present DNA sequence of a *ph* cDNA, which encodes a 169-kD protein with a single putative zinc finger, a serine/threonine-rich region, and has glutamine repeats, suggesting that *ph* is a DNA-binding protein. Polyclonal antisera directed against *ph* protein bind to ~80 sites on polytene chromosomes. Most of these sites appear to be the same as those recognized by antibodies to *Pc* protein, *ph* protein binds to insertion sites of constructs containing DNA from the *bithoraxoid* (*bxd*) region of the Bithorax complex, showing that *ph* binding to chromatin is DNA dependent. The same *bxd* constructs are recognized by *Pc* protein, strongly supporting the hypothesis that *ph* and *Pc* interact directly.

**Key Words:** *Drosophila; polyhomeotic gene; Polycomb group; chromatin protein*

Received October 18, 1991; revised version accepted December 4, 1991.

Genetic and molecular analyses have shown that spatial regulation of homeotic genes is required for correct determination of segmental identity in *Drosophila* (Lewis 1978; Akam 1987; Ingham 1988). The homeotic genes of *Drosophila* are arranged in two complexes: the Antennapedia complex (ANT-C), which contains genes that specify head and thoracic segments [Kaufman et al. 1980; Scott et al. 1983]; and the Bithorax complex (BX-C), which specifies thoracic and abdominal segments (Lewis 1978; Bender et al. 1983; Karch et al. 1985). Paro (1990) has pointed out that the early-acting segmentation genes and maternal genes that control homeotic expression pattern (for review, see Ingham 1988) are transiently expressed and that other control mechanisms must take over to maintain specific expression or repression of the homeotic genes. Genetic analysis has shown that the *Polycomb* group (PcG) of genes is required to repress homeotic gene expression (Struhl 1981; Ingham 1984).

PcG mutants exhibit homeotic transformations in embryos and adults similar to gain-of-function mutations in genes of the ANT-C and BX-C. After careful phenotypic analysis of *Polycomb* (*Pc*) mutants [Lewis 1978], Denell and Frederick [1983] suggested that *Pc* mutations upset the transmission of determined states. Molecular studies have suggested that PcG genes are not needed for correct initiation of spatially regulated homeotic gene expression but, rather, for maintenance of spatial regulation once it has been established [Struhl and Akam 1985; Wedeen et al. 1986, McKeon and Brock 1991]. *Pc* protein binds to polytene chromosome sites that contain the ANT-C and BX-C. Moreover, *Pc* protein binds to insertion sites of constructs containing regulatory DNA from *Antennapedia* (*Antp*) and *Ultrabithorax* (*Ubx*) shown to be genetically sensitive to PcG mutant backgrounds in transformed lines [Zink and Paro 1989; Zink et al. 1991; J. Simon, A. Chiang, and W. Bender, unpubl.]. These data show that *Pc* binding is DNA dependent and support a direct role for *Pc* in the regulation of homeotic genes.

Many of the PcG genes are pleiotropic, because mutations in various loci can exhibit segmentation defects [Ingham 1984; Breen and Duncan 1986], central nervous system defects [Smouse et al. 1988], cuticle defects [Dura et al. 1987], or were isolated because they interact with *zeste* [Wu et al. 1989; Jones and Gelbart 1990; Brunk et al. 1991]. Zink and Paro [1989] have shown that *Pc* protein binds to ~60 sites on polytene chromosomes, a number higher than expected for a specific regulator of the homeotic genes. These data suggest that PcG genes are involved in a common process that can affect different target genes.

Paro and Hogness [1991] showed that *Pc* has significant homology to HPl, a heterochromatinic protein that is
the product of the Su(var)205 locus (Eisenberg et al. 1990). Modifiers of position-effect variegation (PEV) are thought to be chromatin proteins (for review, see Henikoff 1990). We have shown that several PcG genes act as modifiers of PEV (D. Sinclair, N. Clegg, T. Grigliatti, and H. Brock, in prep.). These results are consistent with the idea that PcG genes are somehow required for establishment or maintenance of chromatin structure. A number of investigators have suggested that Pc regulates homeotic genes by imprinting, or promoting regional heterochromatinization, which would render cis-regulatory elements inaccessible to transcription factors, rather than by acting as transcription factors themselves (Alberts and Sternglanz 1990; Gaunt and Singh 1990; Gyurkovics et al. 1990; Paro 1990; Reuter et al. 1990). Detailed analysis of each PcG product will provide a basis for dissecting this process.

Two hypotheses have been advanced to explain why PcG genes have overlapping functions. Double and triple mutations of Polycomblike, Additional sex combs, Posterior sex combs, and Sex combs on midleg show marked enhancement of homeotic transformations, similar to strong alleles of Pc or extra sex combs (esc), compared with the relatively weak phenotypes associated with any single mutation. These interactions may reflect a regulatory hierarchy within the PcG (Jürgens 1985). Support for this idea comes from the studies of Zink and Paro (1989), who showed that Pc protein recognizes the locations of seven PcG genes on polytene chromosomes. An alternative explanation for these synergistic effects has been proposed by Locke et al. (1988), who suggested that PcG gene products may be single components of a multimeric complex. Such a complex would follow laws of mass-action kinetics for macromolecular assembly and thus be sensitive to dosage changes in PcG products. As described below, indirect evidence from analysis of polyhomeotic (ph) supports the mass-action model. However, the two models may not be mutually exclusive, as some PcG proteins could form a complex that participates in a regulatory hierarchy.

In this work we extend the molecular analysis of ph. ph is genetically complex (Dura et al. 1987), and ph mutants exhibit pleiotropic phenotypes (Dura et al. 1985, 1987; Smouse et al. 1988; Santamaria et al. 1989; Smouse and Perrimon 1990). Two genetic lesions are required on the same chromosome to create null or strong hypomorphic alleles, whereas weak hypomorphs result from any single-lesion alleles. These results show dosage sensitivity of the ph locus and support the mass-action model of PcG function. The ph locus is arranged as a tandem duplication contained within 25 kb of genomic DNA (Dura et al. 1987). Long stretches of 80–100% sequence identity exist between duplicated regions that correspond closely to long open reading frames (ORFs) (Deatrick et al. 1991). Analysis of genomic long ORFs suggests that a ph protein encoded by either repeat contains a single putative zinc finger, in addition to other domains commonly found in DNA-binding proteins. Together, these data suggest that ph contains two transcription units that encode functionally similar proteins that may interact with DNA.

Here, we characterize three cDNAs that map to the proximal repeat of the ph locus and show that the protein it encodes contains a possible zinc finger motif, a serine/threonine-rich region, and glutamine repeats, consistent with a role for ph in DNA and protein interactions. Antisera directed to ph protein shows localization of the ph protein to ~80 sites on polytene chromosomes. We find that extensive overlap exists between the binding sites of ph and Pc on polytene chromosomes. Finally, we show that the ph protein binds in vivo to the insertion site of a construct containing a 14.5-kb fragment of bithoraxoid (bxd) regulatory DNA that is genetically sensitive to ph as well as other PcG mutant backgrounds. These results are discussed in light of current models of PcG function.

Results

Isolation and sequencing of proximal ph cDNAs

As described in the introductory section, the ph locus is genetically complex. Northern analysis has identified two major embryonic transcripts that are encoded by ph (Dura et al. 1987), which map to either the proximal or distal repeat [N. Randsholt and S. Freeman, unpubl.]. Together, these data suggest that ph encodes two homologous transcription units, one located in the proximal and one in the distal repeat. To confirm that the genomic long ORFs encode ph transcripts, we have cloned and sequenced cDNAs from the proximal repeat. We screened an imaginal disc library and purified 28 clones (see Materials and methods). Three overlapping clones, c4.3, c4-6, and c4-11, that mapped to most of the proximal ph repeat were subcloned into plasmid vectors and sequenced (Fig. 1). The structure of ph will be character-
ph encodes a 169-kD chromatin protein that binds to specific sites on polytene chromosomes

To investigate the ph protein further, we raised polyclonal antisera directed against ph products. A proximal cDNA fragment (Fig. 2A), composed of sequences completely conserved between the proximal and distal repeats (Deatrick et al. 1991), was cloned into a β-galactosidase expression vector. Antibodies to this peptide will react with all known ph products (N. Cheng, unpubl.). Western blots of protein extracts from bacterial expression cultures containing the expression vector with and without the ph cDNA demonstrate that the antiserum only recognizes epitopes from the ph portion of the fusion protein (Fig. 3). The antiserum does not react with any other proteins in Western blots of proteins from embryos homozygous for a deletion that includes ph (Fig. 3, lane 6) and thus is specific for ph. The native ph protein was detected on a Western blot from salivary gland extracts prepared from third-instar larvae (Fig. 3, lane 5). A prominent band corresponding to a molecular mass of ~170 kD is evident, which is consistent with the molecular mass predicted from conceptual translation of the proximal cDNA analysis above. Other bands may be minor ph products (N. Randsholt, unpubl.) or degradation products.

The Pc protein has been found in salivary glands, and it binds to ~60 sites on polytene chromosomes (Zink and Paro 1989). The ph antiserum was used to stain polytene chromosomes and shows site-specific binding (Fig. 4A). Analysis of the binding sites shows that ph recognizes ~80 sites distributed throughout the genome (Table 1). We compared the best nuclei from many independent polytene preparations and included only those sites that were present in the majority of nuclei.

Comparison of Table 1 and the Pc-binding sites published by Zink and Paro (1989) shows a large degree of overlap. To compare ph- and Pc-binding sites more directly, we simultaneously determined the distribution of Pc antibody (kindly provided by R. Paro) and ph antibody, using chromosomes from different nuclei that had been prepared identically. In general, the strong Pc- and ph-binding sites appear to be indistinguishable (Fig. 4B,C). We have detected additional Pc-binding sites not listed in Zink and Paro (1989), and these are indicated in brackets in Table 1. In addition, we have made some revisions of the cytology where the ph and Pc sites appear indistinguishable, and these changes are indicated in parentheses in Table 1. Overall, at least 71 of 78 ph binding sites appear to overlap with Pc-binding sites.

ph protein binds to constructs containing bxd DNA

It is not clear from the above results whether ph binding to polytene chromosomes is DNA sequence dependent or whether ph associates with some chromatin regions independent of the underlying sequence. Simon et al. (1990) have constructed transformation vectors containing lacZ reporter genes under the regulation of sequences from the Box region of the BX-C. One of these, containing 14.5 kb of DNA from the bxd region at coordinates −18.5 to −4.0 on the BX-C walk, directs reporter gene expression that respects appropriate parasegmental boundaries in wild-type embryos. However, in PcG mutant embryos, including Pc− and ph− embryos, ectopic
Figure 2. (See facing page for legend.)
expression of the reporter is observed, suggesting that this bxd DNA is a potential target for PcG genes [J. Simon, A. Chiang, and W. Bender, pers. comm.]. Furthermore, experiments with antibodies to Pc show that Pc protein binds to the insertion sites of this bxd construct in two transformed lines, 85J-23 and 85-39, confirming that this DNA is a target for Pc binding [A. Chiang, W. Bender, and R. Paro, pers. comm.]. As shown in Figure 5B, a new site of ph binding is detected in the transformed line 85-39 compared with the wild-type pattern shown in Figure 5A, and the new site corresponds to the insertion site of the bxd DNA (Fig. 5C). We have also shown that ph binds to the insertion site of bxd DNA in line 85J-23 [results not shown]. These results demonstrate that ph binding to polytene chromosomes is DNA sequence dependent.

Discussion

Features of the ph protein

The putative ph zinc finger has not been shown to bind zinc and is not homologous to individual zinc fingers of the steroid receptor class [S. Henikoff, pers. comm.], even though it contains 4 cysteine residues [Evans and Hollenberg 1988; Vallee et al. 1991]. It resembles a similar unique cysteine array found in domain 3 of the adenovirus EIA protein [Moran and Mathews 1987], in which the 4 cysteines coordinate to a single zinc atom as a requirement for trans-activation [Gulp et al. 1988]. EIA does not bind DNA directly [Ferguson et al. 1985] and may interact with proteins already bound to promotor complexes (Lillie and Green 1989). It is possible that the putative ph zinc finger has a similar role for protein interaction in chromatin.

ph targets on polytene chromosomes

Consistent with deductions about ph function derived from cDNA sequence analysis, we show that ph is a chromatin protein that is present at ~80 sites on polytene chromosomes. Strong sites always stain, but there is more variability in weak sites, probably because of minor differences in the squashing technique, flatness of the preparation, or because the chromosomes are in different puffing stages. All of these differences might limit the accessibility of the ph antibody to the ph protein and increase the likelihood that weak sites may be missed. Therefore, the list presented is a minimum estimate of ph-binding sites.

Comparison of Pc- and ph-binding sites on different preparation has limitations. Some stem from the vari-
ability between preparations discussed above, which makes judging whether weak sites are present somewhat arbitrary. The resolution of cytological examination of polytene chromosomes varies with the region examined but, for many areas, is on the order of 100 kb [Spierer et al. 1983]. The experiment would be improved if double-labeling studies could be used to distinguish more reliably whether two sites overlap or are very close. Even with these reservations, comparison of ph- and Pc-binding sites on chromosomes labeled separately with ph or Pc antibodies shows extensive overlap between the binding sites (see Table 1). The degree of overlap observed cannot occur by chance and argues strongly that ph and Pc regulate the same target genes. This conclusion is not surprising, given the shared phenotypes of PcG genes. In view of the differences between ph and Pc phenotypes [Denell and Frederick 1983; Dura et al. 1987], it is not surprising that there appear to be differences between ph- and Pc-binding sites. Overall, these data strongly support the idea that PcG genes have overlapping, but not identical, functions.

The segmentation genes even-skipped and engrailed, and homeotic genes are ectopically expressed in ph mutants, suggesting that ph is a repressor of these genes [Dura and Ingham 1988; Smouse et al. 1988; McKeon and Brock 1991]. As expected, ph-binding sites have been identified in the regions known to contain these loci: 46C, 48A, 84AB, and 89E in the case of even-skipped, engrailed, ANT-C, and BX-C, respectively [Lindsley and Zimm 1985]. Positive identification of any gene as a tar-

Figure 4. Immunohistochemical localization of ph protein on polytene chromosomes. (A) Polytenie chromosome preparations from wild-type animals were reacted with ph antisera and detected using horseradish peroxidase-conjugated secondary antibody (Vector Labs). Only the autosomes are shown here. In B and C, proximal is on the left and distal is on the right. (B) Detail of ph binding to the distal part of chromosome 2R from a wild-type strain, taken under bright-field illumination. Ordered from distal to proximal, the cytological positions of ph binding are 60E, 59F, 59C, 58F, 58D, 57B, 57A, and 56C. Note that a unique ph site occurs at 58F [arrowhead]. (C) Detail of Pc binding to distal 2R, taken under phase-contrast illumination. Note that most Pc sites appear to be equivalent to ph sites but that the 58F site is absent.
Table 1. Comparison of ph and Pc protein-binding sites on polytene chromosomes

| X chromosome | Chromosome 2 | Chromosome 3 | Chromosome 4 |
|--------------|--------------|--------------|--------------|
| ph           | Pc           | ph           | Pc           |
| 2D           | 1B           | 21A          | 61A          | 102AC        |
| 4C           | 22A          | (22C)        | 61F          | [61A]        |
| 5A           | 2A           | 24A          | 62F          | [63A]        |
| 5D           | 25EF         | 65D          | 66E          | —            |
| 7B           | 26F          | 67D          | [67F]        |
| 8A           | 28A          | 67EF         | [67EF]       |
| 8B           | 29E          | 67E          | —            |
| 9A           | 31EF         | 67EF         | [67EF]       |
| 12D          | 33F          | 69C          | —            |
| 14B          | [14A]        | 69D          | —            |
| 16D          | [16D]        | 70C          | [70A]        |
| 17A          | —            | 70DE         | [70DE]       |
| 18B          | [18B]        | 76B          | —            |
| 18D          | [18D]        | 78E–79B      | —            |
| 19D          | 38F          | 82E          | —            |
| 20D          | 41CD         | 84D          | —            |
| 21D          | 43C          | 84F          | —            |
| 22D          | 44A          | 86C          | —            |
| 23D          | 45C          | 88A          | —            |
| 24D          | 46CD         | 89B          | —            |
| 25D          | 48A          | 89C          | —            |
| 26D          | 49EF         | 89E          | —            |
| 27D          | 51A          | 90E          | —            |
| 28D          | 56C          | 93E          | —            |
| 29D          | 57A          | [57A]        | 94D          | —            |
| 30D          | 57B          | [57B]        | 94E          | [94E]        |
| 31D          | 58D          | [58D]        | 96C          | [96A]        |
| 32D          | 58F          | —            | 98B          | [98A]        |
| 33D          | 59C          | [59C]        | 99AB         | [99AB]       |
| 34D          | 59F          | 99F          | —            |
| 35D          | 60E          | 100D         | [100D]       |
|              |              | 100E         | [100E]       |

For clarity, only changes to the Pc-binding sites identified by Zink and Paro (1989) are listed here. If the Pc column is blank, the observed ph-binding sites are the same as those given by Zink and Paro (1989). Pc sites that we have detected, but that are not listed in Zink and Paro (1989), are enclosed in brackets. Where we believe the ph site is equivalent to the Pc site, but disagree with the cytology of Zink and Paro (1989), the original Pc cytology is given in parenthesis. Sites not detected with ph or Pc antibody are indicated with a dash.

get of ph binding will require transformation studies such as we have carried out with the bxd DNA fragment. A systematic survey of putative ph-binding sites should eventually allow identification of most ph targets, which would greatly increase our understanding of the pleiotropic phenotypes of the PcG.

Uncertainties in the cytology of ph- and Pc-binding sites, and in the mapping of putative target genes, means that any list of potential targets of ph binding is speculative. Nevertheless, in view of the similarities between Pc and Su(var)205 (Paro and Hogness 1991) and our observation that the majority of PcG genes, but not ph itself, also modify PEV (D. Sinclair, N. Glegg, T. Grigliatti, and H. Brock, in prep.), it is interesting that ph appears to bind to four regions containing known locations of modifiers of PEV, including E(var)26A (25EF), Su(var)3-4 (84D), Su(var)3-14 (86CD), and Su(var)3-11 (94D-95A) (Lindsley and Zimm 1990). It is also interesting that ph appears to bind to many of the locations containing other PcG genes. If this speculation is correct, ph may have an activating function for some targets, because most PcG proteins are required continuously in development.

It might be expected that most of the ph-binding sites identified on polytene chromosomes represent sites of inactive genes, consistent with chromatin-imprinting models (Gaunt and Singh 1990; Paro 1990), and the observation that ph is a negative regulator of homeotic and segmentation genes (Dura and Ingham 1988; Smouse et al. 1988). Genes active in the salivary gland that are regulated by ph might not be identified.

The observation that ph binds to a bxd construct ecotopically located on polytene chromosomes demonstrates that ph recognition of the target site is DNA sequence dependent. This result makes it unlikely that ph protein recognizes general chromatin configurations found in limited positions in the genome independently of the DNA. However, this result does not ascertain
whether \(ph\) interacts directly with DNA or indirectly via proteins that recognize DNA themselves. It will be interesting to determine whether \(ph\) protein can bind DNA directly. It will also be interesting to determine the number and location of \(ph\)-binding sites within a region containing a gene.

Locke et al. (1988) proposed that the PcG proteins may form a multimeric complex. The overlap of \(ph\)- and Pc-binding sites supports this hypothesis. The observation that both \(ph\) and Pc recognize constructions containing the 14.5-kb \(bxd\) fragment provides further evidence for this idea. It will now be necessary to perform similar experiments on successively smaller pieces of target DNA to locate the target more precisely and to determine whether \(ph\) and Pc sites are separable. However, confirmation of this proposal awaits the molecular demonstration of interactions between PcG proteins. Such studies are under way in our laboratory.

Our results are consistent with the hypothesis that PcG proteins are chromatin proteins that associate to regulate the establishment or maintenance of chromatin structure. High-resolution studies of the location and distribution of PcG-binding sites on homeotic genes provide a promising opportunity to increase our understanding of chromatin regulation. These studies may lead to a wider understanding of the importance of chromatin structure to gene regulation.

### Materials and methods

#### General methods

Routine library screening, subcloning, and DNA sequencing procedures are described in Sambrook et al. (1989).

#### cDNA isolation and sequencing

A cDNA library constructed from imaginal disc mRNA (obtained from Dr. G. Rubin, University of California, Berkeley) was screened for \(ph\) cDNAs using the 4.0-kb \(SalI\) fragment from the coordinates 127.5 to 131.5 of the distal repeat as a probe (Dura et al. 1987). Twenty-eight cDNA clones were isolated and rescreened with a proximal-specific probe. Five proximal-specific cDNAs were identified and subcloned into Bluescript or pUC18 vectors. A combination of directed deletions (Henikoff 1984) and synthesis of oligonucleotide primers was used to sequence three proximal cDNAs and to confirm intra-exon splice junctions, using T7 polymerase (Pharmacia) and the dideoxynucleotide method of Sanger et al. (1977).

#### Preparation of antisera

Fusion proteins were prepared using a bacterial expression system. A 500-bp proximal cDNA fragment (see Fig. 2A) was cloned into pUR 290 (Rüther and Müller-Hill 1983). This vector, called pRE5, was transformed into bacteria and induced to express, and the fusion protein was isolated according to Rio et al. (1986) with minor modifications. Proteins were dialyzed against PBS after urea extraction, cleared by centrifugation, and used immediately for injection or to make affinity columns. Fusion protein (500 \(\mu\)g–2 mg) was used for initial and boost injections into New Zealand white rabbits. Blood was collected after two boost injections, and serum was tested for \(ph\)-binding activity.

To make \(ph\)-specific antiserum, immunogloblins were precipitated from serum in 50% ammonium sulfate, resuspended in PBS after urea extraction, cleared by centrifugation, and used immediately for injection or to make affinity columns. Fusion protein (500 \(\mu\)g–2 mg) was used for initial and boost injections into New Zealand white rabbits. Blood was collected after two boost injections, and serum was tested for \(ph\)-binding activity.

To make \(ph\)-specific antiserum, immunogloblins were precipitated from serum in 50% ammonium sulfate, resuspended in PBS, dialyzed, and passed successively over three affinity columns containing whole bacterial protein lysates, purified \(\beta\)-galactosidase, and purified fusion protein, respectively. \(ph\)-specific antibodies were eluted from the last column and used for Western blots and immunohistochemistry.

#### Western blot analysis

Bacterial proteins were prepared from expression cultures of BMH71-18 (Rüther and Müller-Hill 1983) containing pRE5 or pUR 290. Two-milliliter cultures were induced, and bacteria were pelleted by centrifugation, resuspended in protein sample buffer, and separated electrophoretically on a 7% SDS–polyacrylamide gel (Laemmli 1970). Salivary glands were dissected from 100 third-instar larvae and boiled in 200 \(\mu\)l of protein sample buffer, and the proteins were separated on SDS–polyacrylamide gels as described above. Proteins were then electroblotted onto nitrocellulose, processed using an appropriate dilution of primary antibody, and detected with secondary antibody coupled to horseradish peroxidase (Jackson Labs).

To prepare \(ph\) null embryos, \(Df(1)A52/FM7\) females were allowed to lay for 3-hr intervals. The embryos were aged for 12 hr and dechorionated in 50% bleach. Hemizygous \(Df(1)A52/Y\)
embryos were recognized by the absence of head involution at stage 14. About 50 embryos were boiled in 100 μl of sample buffer and analyzed as described above.

**Immunohistochemistry and in situ hybridization**

Staining of salivary glands and polytene chromosomes with ph antibody was performed according to Zink and Paro (1989). Chromosomes were photographed with Fuji ASA 100 slide film under bright-field illumination, although some favorable preparations were photographed under phase-contrast illumination. In situ hybridization was carried out with biotinylated Carnegie 20 DNA as a probe.

**Acknowledgments**

We thank R. Paro for the gift of Pc antibody, for communicating unpublished data, and for stimulating discussions. We are grateful to W. Bender, A. Chiang, and J. Simon for providing transformed lines, communicating unpublished data, and for helpful cooperation. We thank Anne Taylor-Smith for excellent technical assistance and for help with the computer analysis. This work was supported by a grant from the National Science and Engineering Research Council to H.W.B.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 USC section 1734 solely to indicate this fact.

**Note added in proof**

Sequence data described in this paper have been submitted to EMBL/GenBank data libraries.

**References**

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

Alberts, B. and R. Sternglanz. 1990. Chromatin contract to si­quence data described in this paper have been submitted to EMBL/GenBank data libraries.

Bender, W., M. Akam, F. Karch, P.A. Beachy, M. Peifer, P. Spierer, E.B. Lewis, and D.S. Hogness. 1983. Molecular genetics of the bithorax complex in *Drosophila melanogaster*. *Science* **221**: 23–29.

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

Brunk, B.P., E.C. Martin, and P.N. Adler. 1991. Molecular genetics of the *Posterior sex combs/Suppressor 2 of zeste* region of *Drosophila*: Aberrant expression of the suppressor 2 of zeste gene results in abnormal bristle development. *Genetics* **128**: 119–132.

Chou, P. and G. Fasman. 1978. Prediction of the secondary structure of proteins from their amino acid sequences. *Adv. Enzymol.* **47**: 145–147.

Culp, J.S., L.C. Webster, D.J. Friedman, C.L. Smith, W.-J. Huang, W.Y.-H. Felicia, M. Rosenberg, and R.P. Ricciardi. 1988. The 289-amino acid Ela protein of adenovirus binds zinc in a region that is important for trans-activation. *Proc. Natl. Acad. Sci.* **85**: 6450–6454.

Deatrich, J., M. Daly, N.B. Randsholt, and H.W. Brock. 1991. The complex genetic locus *polyhomeotic* in *Drosophila melanogaster* potentially encodes two homologous zinc-finger proteins. *Gene* **105**: 185–195.

Denell, R.E. and R.D. Fredrick. 1983. Homeosis in *Drosophila*:

A description of the Polycomb lethal syndrome. *Dev. Biol.* **97**: 34–47.

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

Dura, J.-M., H.W. Brock, and P. Santamaria. 1985. *Polyhomeotic*: A gene in *Drosophila melanogaster* required for correct expression of segment identity. *Mol. Gen. Genet.* **198**: 220–231.

Dura, J.-M., N. Randsholt, J. Deatrich, I. Erk, P. Santamaria, J.D. Freeman, S.F. Freeman, D. Weddell, and H.W. Brock. 1987. A complex genetic locus, *polyhomeotic*, is required for segmental specification and epidermal development in *D. melanogaster*. *Cell* **51**: 829–839.

Eisenberg, J.C., T.C. James, D.M. Foster-Hartnett, T. Hartnett, V. Nagan, and S.C.R. Elgin. 1990. Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **87**: 9923–9927.

Evans, R.M. and S.M. Hollenberg. 1988. Zinc fingers: Gift by association. *Cell* **52**: 1–3.

Ferguson, B., B. Kripp, O. Andrisani, N. Jones, H. Westphal, and M. Rosenberg. 1985. Ela 13s and 12s mRNA products made in *Escherichia coli* both function as nucleus-located transcription activators but do not directly bind DNA. *Mol. Cell. Biol.* **5**: 2653–2661.

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

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

Henikoff, S. 1984. Unidirectional digestion with Exonuclease III creates targeted breakpoints for DNA sequencing. *Gene* **28**: 351–359.

——. 1990. Position-effect variegation after 60 years. *Trends Genet.* **6**: 422–426.

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

——. 1988. The molecular genetics of embryonic pattern formation in *Drosophila melanogaster*. *Nature* **335**: 25–34.

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

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

Karch, F., B. Weissenbach, M. Peifer, W. Bender, I. Duncan, S. Celniker, M. Crosby, and E.B. Lewis. 1985. The abdominal region of the bithorax complex. *Cell* **43**: 81–96.

Kaufman, T.C., R. Lewis, and B. Wakimoto. 1980. Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*. *The homeotic gene complex in polytene chromosome interval 84A-B. Genetics* **94**: 115–133.

Kozak, M. 1984. Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. *Nucleic Acids Res.* **12**: 857–872.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature* **227**: 680–685.

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

Lillie, J.W. and M.R. Green. 1989. Transcription activation by the adenovirus Ela protein. *Nature* **338**: 39–44.

**ph encodes a chromatin protein**
Lindsay, D.L. and G. Zimm. 1985. The genome of Drosophila melanogaster part 1: Genes A-K. Drosophila Inf. Serv. 64: 1–227.

——. Zimm. 1990. The genome of Drosophila melanogaster part 4: Genes L-Z. Drosophila Inf. Serv. 68: 1–382.

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

McKeon, J. and H.W. Brock. 1991. Interactions of the Polycomb group of genes with homeotic loci of Drosophila. Wilhelm Roux’s Arch. Dev. Biol. 199: 387–396.

Morgan, E. and M.B. Mathews. 1987. Multiple functional domains in the adenovirus E1A gene. Cell 48: 177–178.

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

Paro, R. and D.S. Hogness. 1991. The Polycomb protein shares a homologous domain with a heterochromatin-associated protein in Drosophila. Proc. Natl. Acad. Sci. 88: 263–267.

Pearson, W.R. and D.J. Lipman. 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. 85: 2444–2448.

Reuter, G., M. Giarre, J. Farah, J. Gausz, A. Spierer, and P. Spierer. 1990. Dependence of position-effect variegation in Drosophila on dose of a gene encoding an unusual zinc-finger protein. Nature 344: 219–223.

Rio, D.C., F.A. Laski, and G.M. Rubin. 1986. Identification and immunochemical analysis of biologically active Drosophila P element transposase. Cell 44: 21–32.

Rüther, U. and B. Müller-Hill. 1983. Easy identification of cDNA clones. EMBO J. 2: 1791–1794.

Smok, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sanger, F., S. Nicklen, and A.R. Coulston. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. 74: 5463–5467.

Santamaria, P., J. Deatrick, and N.B. Randsholt. 1989. Pattern triplications following genetic ablation on the wing of Drosophila. Effect of eliminating the polyhomeotic gene. Wilhelm Roux’s Arch. Dev. Biol. 198: 65–77.

Scofield, M.P., A.J. Weiner, B.A. Polisky, T.I. Hazelrigg, V. Pirotta, F. Scalenghe, and T.C. Kaufman. 1983. The molecular organization of the Antennapedia locus of Drosophila. Cell 35: 763–776.

Simon, J., M. Peifer, W. Bender, and M. O’Connor. 1990. Regulatory elements of the bithorax complex that control expression along the anterior-posterior axis. EMBO J. 9: 3945–3956.

Smouse, D. and N. Perrimon. 1990. Genetic dissection of a complex neurological mutant, polyhomeotic, in Drosophila. Dev. Biol. 139: 169–185.

Smouse, D., C.S. Goodman, A.P. Mahowald, and N. Perrimon. 1988. Polyhomeotic: A gene required for the embryonic development of axon pathways in the central nervous system of Drosophila. Genes & Dev. 2: 830–842.

Spierer, P., A. Spierer, W. Bender, and D. Hogness. 1983. Molecular mapping of genetic and chromomeric units in Drosophila melanogaster. J. Mol. Biol. 168: 35–50.

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

Struhl, G. and M. Akam. 1985. Altered distribution of Ultrabithorax transcripts in extra sex comb mutant embryos of Drosophila. EMBO J. 4: 3259–3264.

Vallee, B.L., J.E. Coleman, and D.S. Auld. 1991. Zinc fingers, zinc clusters, and zinc twists in DNA-binding protein do-

mains. Proc. Natl. Acad. Sci. 88: 999–1003.

Villares, R. and C.V. Cabrera. 1987. The achaete-scute gene complex of D. melanogaster: Conserved domains in a subset of genes required for neurogenesis and their homology to myc. Cell 50: 415–424.

Wedge, C., K. Harding, and M. Levine. 1986. Spatial regulation of Antennapedia and bithorax gene expression by the Polycomb locus in Drosophila. Cell 44: 739–748.

Wu, C.-T., R.S. Jones, P.F. Lasko, and W.M. Gelbart. 1989. Homoeosis and the interaction of zeste and white in Drosophila. Mol. Gen. Genet. 218: 559–564.

Zink, B. and R. Paro. 1989. In vivo binding pattern of a trans-regulator of the homeotic genes in Drosophila melanogaster. Nature 337: 468–471.

Zink, B., Y. Engstrom, W.J. Gehring, and R. Paro. 1991. Direct interactions of the Polycomb protein with Antennapedia regulatory sequences in polytene chromosomes of Drosophila melanogaster. EMBO J. 10: 153–162.
The polyhomeotic gene of Drosophila encodes a chromatin protein that shares polytene chromosome-binding sites with Polycomb.

M DeCamillis, N S Cheng, D Pierre, et al.

*Genes Dev.* 1992, 6: Access the most recent version at doi:10.1101/gad.6.2.223

References

This article cites 55 articles, 15 of which can be accessed free at: [http://genesdev.cshlp.org/content/6/2/223.full.html#ref-list-1](http://genesdev.cshlp.org/content/6/2/223.full.html#ref-list-1)

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).