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

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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]

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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 expres-

sion 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 HP1, a heterochromatic protein that is

the product of the *Su(var)205* locus (Eissenberg 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* product 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-

Figure 1. Cloning of *ph* proximal cDNA. Three overlapping clones were isolated from an imaginal disc library constructed in λ gt10 obtained from G. Rubin. The sizes and relative positions of each clone, C4.3, C4-6, C4-11, are indicated under the restriction map of the proximal transcription unit (X) *Xho*I; (S) *Sal*I; (H) *Hind*III; (B) *Bam*HI).

ized elsewhere (N. Randsholt, N. Cheng, S. Freeman, and H. Brock, in prep.). Complete sequence of these clones was obtained using a combination of directed deletions (Henikoff 1984) and synthesis of oligonucleotide primers (Fig. 2A).

These data indicate that the overlapping clones together comprise a nearly full-length proximal cDNA of 5.8 kb that is missing some of the 5'-untranslated region but includes the complete coding sequence of the structural protein (Fig. 2A). There are two candidate ATG codons for the translation start, but we believe that the 5'-most codon is the probable start because there is an in-frame stop codon just upstream and reasonable agreement with the consensus start sequence (Kozak 1984). Some minor differences in the cDNA sequence compared with the genomic sequence (Deatrick et al. 1991) are detailed in the legend to Figure 2. Some occur at third positions in codons and presumably reflect DNA polymorphisms between strains, and others are revisions of the sequence. Most notably, we have detected a novel intron that splices out 63 bp of ORF sequence that is perfectly conserved between the repeats. In addition, we have identified ~200 amino acids at the amino terminus that were not identified from the genomic sequence because this identification depended on sequence conservation between the proximal and distal repeats (Deatrick et al. 1991).

Conceptual translation of the proximal cDNA is presented in Figure 2A, and the location of protein motifs is summarized in Figure 2B. This cDNA encodes a 169-kD protein that contains a single potential C4 class zinc finger domain (Evans and Hollenberg 1988). The putative zinc finger has two pairs of cysteines separated by 2 and 3 amino acids, respectively, which are, in turn, separated by 14 residues, 7 of which are basic. These basic residues could react with DNA.

Several other putative functional domains have been identified. A region is present upstream of the zinc finger from amino acid 1233 to 1290, in which 39 of 58 amino acids are either serine or threonine. Four glutamine-rich regions are found in the amino-terminal 60% of the protein. A prediction of protein structure identified a potential α -helix-forming region on the carboxy-terminal end of the *ph* protein (Chou and Fasman 1978). This putative helix contains partial similarity to the second helix of the helix-loop-helix (HLH) consensus sequence found in several classes of HLH proteins (Villares and Cabrera 1987).

A homology search for related proteins using the FASTP program (Pearson and Lipman 1988) with the PIR data bank found no significant matches to proteins sequenced previously. It is notable that *ph* protein has no "chromobox" or other sequence homology with *Pc* (Paro and Hogness 1991).

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 *Ubx* 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 *Pc*G mutant embryos, including *Pc*⁻ and *ph*⁻ embryos, ectopic

0841 GCAGCAACAGCAGCAGATTTGCCGCAAGCCAGGCCAGCATTCAACAGGCTCTCGGAAATGCCATCAGCAAACTCTTCAGG 3120
850 E Q Q Q Q V A Q A Q A Q H Q Q A L N A N A T Q G I L Q V 876

3121 TGGCGCCAAATCAATTATCATCGCTCCGTCAGCCAGCAAGCAGGACAGGCTTCCACCAACTGTATACAGCGAGCAGCTA 3200
877 A P N Q V I T S H Q O Q Q Q Q L H N Q L I Q Q O L 902

3201 CAGCAACAGCGCGAGGCACAAAGTTCAAGCCCAAGTCAGGCTCTAAGCGCAACAGCAACAGCAGCGAGAGCAGCGCA 3280
903 Q Q O A Q A Q V Q A Q V Q A Q A Q Q Q Q Q Q R E Q Q Q 929

3202 GAATATATCCAGCAGATTGTGGTGCAACAGCTGGAGGCATCTCTCAACAGCTTTCCCAAGCAGCAACAGCAACCAAT 3360
930 N I I Q Q I V V Q O S G A T Q S G T S Q Q Q Q H Q S 956

3361 CCGGCGCACTCAGCTAAGTAGCTGCCCTTCTCAGTTCTTCTGTCACAGCGCCAGCCGGAATAGCTACCTCTAGTGCT 3440
957 G Q L Q L S S V P P S V S S S T T P A G I A T S S A 982

3441 CTCGAGCAGCCCTCTCCGCTCTGGCGCATCTTTCAGACAGCTAAGCCGGTACTTGCAGTCTCTCTCCGCCCAAG 3520
983 L Q A A L S G A I F Q T A K P F S S S S P T S 1009

3521 CAGTGTGGTCAAAATACCAACACAGAGCAGCATGCTTTGGTGTACCAGAGCTACGGTGGCCAGTATACAGAGGCTCAGA 3600
1010 S V V T I T N Q S S T P L V T S S T V A S I Q Q A Q T 1036

3601 CGCAATCTGCTCAGGTCACCAACATCAGCAGCTAATCAGCGCCACAAATGCCGAGGGGACTCAACAAACAGCCACAGGA 3680
1037 Q S A Q V H Q H Q Q L I S A T I A G G T Q Q Q P Q G 1062

3681 CCGGCTACCTTACCCACAGCAAAATCAATTTGGCAATGCTCATGATGAATGCTACAGTGGTGCACCTTTCCAC 3760
1063 P P S L T P T T N P I L A N T S M M N A T V G H L S T 1089

3761 TGTCTCCGCTGTAACTGTTTCTGTGACAGCAACCGCTGTACTTCTGTGCGGGGTACGTGGTGTCTTAMGCACGGTA 3840
1090 A P P V T V S V T S T A V T S S P F Q Q L V L L S T A S 1116

3841 GTAGCGGTGGAGAGGTAGCATACCGACAGCCGCCCAAGAGACACTCTCGAAGGCGCCAGCCGCAACCTCGTCCG 3920
1117 S G G G S S I P A T P T K E T P S K G P T A T L V P 1142

3921 ATTGGTTGCCGCCAAGTCTCCTGTATCAGGAAGGACACTGCATACCCCAAAATCATCTACTCTGCCACTGTACAGCG 4000
1143 I G S P K T P V S G K D T C T T P K S S T P A T V S A 1169

4001 ATCCGTAGAGGCCAGTAGTCTCCACGGGGAAGCCCTGTCCATGGAGATGCCTCAGTAGGCTTCTCCAGCTGTCAAGG 4080
1170 S V E A S S T T G E A L S N D A C R S R S T L S K G 1196

4081 CGCCTACCTCTCCCAACAGCAAGCAAAAGCATGACAGCATGACGAGCAGCGAGTAGACCATCTCCCAACAGTGTAGTGGG 4160
1197 A T T P T S K Q S N A A V Q P P S S T T P N S V S G 1222

4161 AAAGAGCGCCAAAGCTGGCACTCGGCCAGTTAAAGCTCCCAACATCAACTTCAACACAGCAACAGCTCAACCAATG 4240
1223 K E E P K L A T C G S L T S A T S T T T T T I N G 1249

4241 GATTGGAGTAGCCAGAAGCAGCAGCCAGCAGCGGTCTCTCAACGCTAGCAGCAACACTACAGTCTTGGCAACTTTATCA 4320
1250 I G V A R T T A S T A V S T A S T T T T S S G T F I T 1276

4321 CAAGTGTGCACGACACAAACCCAGCCAGCTCAGTATCAGATATGATGATGAGAGTCTCCCAAGCGGATGATTAAG 4400
1277 S C T S T T T T T T S S I N S G S K D L P K A H I K 1302

4401 CCGAAGCTCTTAACTCAGCTATCGATGGCTTCATCATCCGAGGAGGCCAACGAGCCATTTCCGCTCACCAGCAGGATA 4480
1303 P N V L T H V I D G F I Q E A N E F P P V T R Q R Y 1329

4481 TGCAGCAAAAGAGCTCAGCATGAGCGCCAAAGAAAAGCAACATGACGAGGACATCAAGCTAAGTGGATAGCAT 4560
1330 A D K D V S D E P P F K K K A T M Q E D I K L S G I A S 1356

4561 CAGCTCCAGGCTCGGATGATGTTGCTTGGCAGCAGTGTGGAAAGATGGAGCAAAAGCTGAAGGAGAGCGCTAC 4640
1357 A P G S D H V C T G G K A A A E E E K A L K K R Y 1382

4641 TGTGCCCAGAGTGTCTCGAGGAGCGCAAGACAGGCATCGTGGAATTTGATCAGGAGAGCAAGCGGCTGGGGACAG 4720
1383 C S P G C S R Q A K N G I G G V G S G E T A A G L T G L T G 1409

4721 TGGTATGTTGGGTGGAGCCCATGGCATGGTGACAGCTGGATGAAGCCATGGCTGAGGAGGAAGTGTGCAGCAGAGG 4800
1410 G I V V D M A L V D R L D E A M E E E K M Q T R A 1436

4801 CCACCCCAAAGCTTTCAGAACTGGTTTCTCATTTTGGAGCGCTCAACAGAAGTACCTCAATGTCTACTGCCAGTCCAAGC 4880
1437 T P K L S E S F P I L G A S T E V P P M S L T A P A 1462

4881 GCGATTGTGCGCCCTGCGCTCTTGCAGTGCTGTAGGATGGCAATTTGACGTGTCACTTCCAACCTTGCACCACTGTC 4960
1463 A I S A P S A T G T T A A A A P K L S V A L P L T A P L S 1489

4961 GTGATCTACTCTGGCGCGCGCGCCCAAGTCTTCGGAAGTAAGTGAAACAGATCTCTCCGCAATCAGCAGCTGGAGTGGG 5040
1490 V V T S G A A P K S S E V N G T D R P P I S S N S V D 1516

5041 ACGATGTCCAGCACTTCATTCGGAAGACTGCCGGTGTGCAGGACTACGTGGACGACTTTATACAGCAGGAGATCGACGGC 5120
1517 D V S N F I R E L P G C Q D Y V D D F I Q Q E I D G 1542

5121 CAAGCGCTCTGTTGTTCTCAAGGACAGCAATTTGGTGAAGCTATGGGATGAAGCTGGGTCAGCTCTTAAATTTGTGGC 5200
1543 Q A L L L K E K H L V N A M G M K L G P A L K I V A 1569

5201 CAAGGTGGAGTCCATTAGGAGGTGCTCGGCACACTGGCAGGCCAAGAGTCAAGGAGCGCAGTAGGGCAGTAGAGCAGCA 5280
1570 K V E S I K E V P P P G G A C D P F A Q . 1589

5281 AAAGCGCAAAAGATGATCTCCTAACCGAGCAGTGGAACCTGGTTCACCCAGCTGTCTGCGCCAGTGATTCTGATTCAC 5360
1589 TGGGCGAGCGCAAAAGCAGGAGGAGTCTTTGCAAAATATATTAAGCATCAGGCGCATCAGGCTTTAAACCCATTGACTTT 5440

5441 GTACATCTCCCAAGTCACTTATTAAGCATATTCATTTATAAATTAACATCAGCTCACAGCTCAAAAGCAATGGAATT 5520
1552 ACTTAAATTAAGGAAGAGTATGAATTAATGGCCAGCAGTAATGGAGTAAGTCACTTTATAAATAGCATAGATT 5600

5601 TACTAGTCTAAGTAGCATATTAATTAAGCTCCCAAGCTCATGATATGTTTGCACAGTATTTAAATGTAGGAATCAATGA 5680
1568 GACATGATGATTGTCTACACACTAAGTAATTTAAGCCTTGAATTAATGATATTAATGTATGATTTTGAACCTTTTGA 5760

5761 CTAATTAATTAATGTAAGTCCTAGCACTGAAGCTTTTTTTCACATCTTTTTTTTGCATATTTGCTGAAGAAATTAATAGC 5840
1584 ACTGAATAGTGTTTAAATTAATGCTTAAAGG 5860

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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 at 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 E1A 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). E1A does not bind DNA directly (Ferguson et al. 1985) and may interact with proteins already bound to promoter 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

Figure 3. Antibodies against the *ph* protein. Antisera were raised against a *lacZ-ph* fusion. Crude bacterial lysates were prepared from expression clones containing *lacZ* (lanes 1,3) or *lacZ-ph* fusions (lanes 2,4) and separated on 7% SDS-acrylamide gels. Gels were either stained with Coomassie blue (lanes 1,2) or electroblotted onto nitrocellulose and reacted with *ph* antiserum, and binding was detected with secondary antibodies labeled with horseradish peroxidase (Vector Labs) (lanes 3,4). (Lane 5) Salivary gland lysates from 100 third-instar larvae were separated on 7% SDS-acrylamide gels and electroblotted to nitrocellulose, and the filters were reacted with *ph* antisera, as described above. (Lane 6) A Western blot of proteins prepared from 50 embryos homozygous for a deletion, *Df(1)A52*, that uncovers *ph*. *ph* null embryos were identified by their phenotype, prepared as described in Materials and methods, and proteins were separated electrophoretically and transferred to nitrocellulose. The filter was reacted with *ph* antiserum at a concentration 10× higher than that shown in lane 5. No cross-reacting antigens were detected, showing that the antibody is specific to *ph*. *Pc* antibodies reacted with similar samples did detect *Pc*, showing that the integrity of the proteins from the *ph* null embryos was maintained. Molecular mass of protein standards is shown at left (in kD).

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-

Figure 2. The nucleotide sequence of *ph* proximal cDNA. The near full-length *ph* proximal cDNA sequence, including the complete coding strand and predicted amino acid sequence, is shown in A. Two polyadenylation sequences at the 3' end of the transcript are shown in boldface type. Six ATG codons occur 5' to the ORF start but are followed by in-frame termination codons. *ph* antisera were raised against fusion proteins constructed using the cDNA fragment shown from amino acids 753 to 922 (arrows). The location of putative motifs is indicated. Blocks of glutamine-rich sequences are underscored with solid lines. A single domain in which 39 of 58 amino acids are hydroxylated serine or threonine is underscored with tildes (1233–1290). A region containing partial homology to the HLH motif is underscored with a broken line (1549–1562). A potential C4 zinc finger lies between amino acids 1365 and 1387 (boldface type). The relative positions of these features are summarized in B. The following differences between the genomic sequence reported by Deatrick et al. (1991) and the cDNA sequence reported here were noted (cDNA sequence is listed second): (nucleotide 4) G → A (change in noncoding region); (nucleotide 110) T → C (change in noncoding region); (nucleotide 1266) insertion of AGC (results in insertion of Gln); (nucleotide 2720) G → T (no change); (nucleotides 4738–4739) CA → AC (changes Ala to Asp); (nucleotide 5364) A → G (change in noncoding region).

Figure 4. Immunohistochemical localization of *ph* protein on polytene chromosomes. (A) Polytene 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.

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-

Table 1. Comparison of *ph* and *Pc* protein-binding sites on polytene chromosomes

X chromosome		Chromosome 2		Chromosome 3		Chromosome 4	
<i>ph</i>	<i>Pc</i>	<i>ph</i>	<i>Pc</i>	<i>ph</i>	<i>Pc</i>	<i>ph</i>	<i>Pc</i>
—	1B	21A		61A	[61A]	102AC	
2D		22A		61C		—	102D
4C		22B	(22C)	61F	[61F]		
5A		24A		62F	(63A)		
5D		25EF		65D			
7B		26F		66E	—		
8A		28A	[28A]	67D			
8B		29E		67E	(67F)		
9A		32EF		67EF	[67EF]		
12D		33F		69C			
14B	(14A)	35AB	—	69D			
16D	[16D]	35C		70C	(70A)		
17A	—	36AC	(36B)	70DE	[70DE]		
		37A	[37A]	76B	—		
		37B	[37B]	78E–79B	—		
		38F		82E			
		41CD		84D			
		43C		84F			
		44A		86C			
		45C		88A			
		46CD		89B			
		48A		89C			
		49EF		89E			
		51A		90E			
		56C		93E			
		57A	[57A]	94D	—		
		57B	[57B]	94E	[94E]		
		58D	[58D]	96C	(96A)		
		58F	—	98B	(98A)		
		59C	[59C]	99AB	[99AB]		
		59F		99F			
		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 ectopically 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

Figure 5. Localization of *ph* to *bxd* regulatory sequences in vivo on chromosome 3L. All chromosomes are oriented with proximal on the left and distal on the right. (A) *ph*-binding sites on wild-type chromosomes. (B) *ph* binding to the transformed line 85-39, containing 14.5 kb of DNA from the *bxd* enhancer (Simon et al. 1990). A new site of *ph* binding appears relative to wild type (arrowhead). (C) In situ hybridization of Carnegie 20 DNA, containing the *rosy* locus to polytene chromosomes of strain 85-89. Note that the site of insertion of the *bxd* transformant corresponds to the new *ph*-binding site shown in B.

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 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 *Sall* 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 intron–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 μ 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, immunoglobins 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 β -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 μ l of protein sample buffer, and the proteins were separated on SDS–acrylamide 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)JA52/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)JA52/Y*

embryos were recognized by the absence of head involution at stage 14. About 50 embryos were boiled in 100 μ l 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.

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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 metamerism in the *Drosophila* embryo. *Development* **101**: 1–22.
- Alberts, B. and R. Sternglanz. 1990. Chromatin contract to silence. *Nature* **344**: 193–194.
- 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 E1a protein of adenovirus binds zinc in a region that is important for trans-activation. *Proc. Natl. Acad. Sci.* **85**: 6450–6454.
- Deatrick, 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. Deatrick, 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.
- Eissenberg, J.C., T.C. James, D.M. Foster-Hartnett, T. Hartnett, V. Ngan, 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: Gilt by association. *Cell* **52**: 1–3.
- Ferguson, B., B. Krippel, O. Andrisani, N. Jones, H. Westphal, and M. Rosenberg. 1985. E1a 13s and 12s mRNA products made in *Escherichia coli* both function as nucleus-localized transcription activators but do not directly bind DNA. *Mol. Cell. Biol.* **5**: 2653–2661.
- Gaunt, S.J. and P.B. Singh. 1990. Homeogene 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. Weiffenbach, 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 E1a protein. *Nature* **338**: 39–44.

- Lindsley, 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.
- Moran, 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.
- Sambrook, 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. Coulson. 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.
- Scott, M.P., A.J. Weiner, B.A. Polisky, T.I. Hazelrigg, V. Pirota, 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 combs* 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 domains. *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.
- Wedeer, 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. Homeosis 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.

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