The Heterochromatin-Associated Protein HP-1 Is an Essential Protein in Drosophila With Dosage-Dependent Effects on Position-Effect Variegation

Joel C. Eissenberg,* Gary D. Morris,† Gunter Reuter‡ and Thomas Hartnett*

*Edward A. Doisy Department of Biochemistry and Molecular Biology and †Cell and Molecular Biology Program, St. Louis University School of Medicine, St. Louis, Missouri 63104, and ‡Institute of Genetics, Martin Luther University, Domplats 1, 0-4020 Halle/Saale, Germany

Manuscript received December 27, 1991
Accepted for publication February 10, 1992

ABSTRACT

Chromosome rearrangements which place euchromatic genes adjacent to a heterochromatic breakpoint frequently result in gene repression (position-effect variegation). This repression is thought to reflect the spreading of a heterochromatic structure into neighboring euchromatin. Two allelic dominant suppressors of position-effect variegation were found to contain mutations within the gene encoding the heterochromatin-specific chromosomal protein HP-1. The site of mutation for each allele is given: one converts Lys16 into a nonsense (ochre) codon, while the other is a frameshift after Ser19. In flies heterozygous for one of the mutant alleles (Su(var)2-5*), a truncated HP-1 protein was detectable by Western blot analysis. An HP-1 minigene, consisting of HP-1 cDNA under the control of an Hsp70 heat-inducible promoter, was transduced into flies by P element-mediated germ line transformation. Heat-shock driven expression of this minigene results in elevated HP-1 protein level and enhancement of position-effect variegation. Levels of variegating gene expression thus appear to depend upon the level of expression of a heterochromatin-specific protein. The implications of these observations for mechanism of heterochromatic position effects and heterochromatin function are discussed.

In many cases, genes which become translocated to a position adjacent to heterochromatin experience inappropriate inactivation (SPOFFORD 1976). This inactivation, termed heterochromatic position effect variegation (PEV), is thought to be a consequence of the abnormal spreading of a heterochromatic chromatin structure into euchromatin, mediated by chromosomal proteins (reviewed in EISSENBERG 1989). Because the proportion of cells which exhibit the position effect is extremely sensitive to the dosage of several different loci, LOCKE, KOTARSKI and TARTOF (1988) have proposed that assembly of functional heterochromatin is dependent upon a precise stoichiometry of protein subunits. In one case, a gene capable of exerting dosage-dependent modification of PEV [Su(var)3-7] has been cloned and shown to encode a protein with a zinc-finger-like motif, suggesting DNA binding function (REUTER et al. 1990). Using a series of overlapping duplications and deficiencies, WUSTMANN et al. (1989) identified a locus [Su(var)2-5] within the cytological interval 28F2-29A1 that exhibits an analogous dosage dependent effect on PEV levels. This region includes a gene encoding the heterochromatin-associated chromosomal protein HP-1 (JAMES and ELGIN 1986; JAMES et al. 1989).

The dominant suppressor of PEV Su(var)205 was recently shown to contain a point mutation in the gene encoding HP-1, leading to missplicing of the HP-1 pre-mRNA (EISSENBERG et al. 1990). Here we report that two alleles of the locus Su(var)2-5 are allelic to Su(var)205, and that these alleles are also associated with mutations in the HP-1-coding sequences. All three are lethal as homozygotes and as trans-heterozygotes. We also report that a heat-shock-activated HP-1 cDNA is capable of enhancing variegation of the white gene in the variegating rearrangement In(1)w19. Taken together, our results argue that HP-1 is an essential protein, and that it functions as part of an epigenetic mechanism capable of generating and maintaining an inactive chromatin structure. We discuss the implications of these findings for a model of mass action-driven heterochromatin assembly and for the role of heterochromatin formation in gene repression.

MATERIALS AND METHODS

Fly stocks: Flies were maintained at room temperature, on a standard cornmeal-sucrose medium containing 0.04% methylparaben as a mold inhibitor. The following chromosomes were used in these studies and are described in the references given: In(1)w19 (REUTER and WOLFF 1981); Su(var)205 and b it ri (SINCLAIR, MOTTUS and GRIGLIATTI 1983); In(2L)Cy + In(2R)Cy (LINDSLEY and GRELLE 1968). Su(var)2-5, b cn vg and Su(var)2-5+ are ethyl methanesulfonate- and X-ray-induced alleles, respectively, of a locus on 2L (WUSTMANN et al. 1989). InCyRoi = In(2L)CyRoi w + In(2R)CyRoi w is not explicitly described anywhere (to our knowledge), but is comprised of rearrangements
described in LINDSLEY and GRELLE (1968) and ASHURNER (1989).

**Nucleic acids:** DNA was prepared from whole adult flies essentially as described by JOWETT (1986) for extraction of high molecular weight DNA from embryos, but omitting the dialysis steps. Total nucleic acids were prepared from adult flies for Northern blot analysis of RNA essentially as described by MEYEROWITZ and HOGNESS (1982). Restriction endonuclease digestions were according to manufacturers recommendation (Promega). Sequencing was done by the dideoxy chain termination method of SANGER, NICKLEN and COULSON (1977) using Sequenase (United States Biochemical), according to manufacturer's recommendation. 32P-Labeling of Northern blot hybridization probe was by the random primer method (FIEBEN and VOGELSTEIN 1984), and prehybridization and hybridization was essentially as described by WAHL, STERN and STARK (1979), except that these steps were done at 65° C without formamide. The hybridized filter was washed as described, air-dried and exposed to XAR-5 X-ray film (Kodak) at -80° C using a Cronex Lightning Plus intensifying screen (Du Pont).

**Cloning of amplification products:** Reaction product was precipitated, ligated into pUC19 plasmid (YANISCH-PERRON, VIERA and MESSING 1985), transformed into host cells, and cells bearing recombinant molecules selected and plasmid DNA purified by standard procedures (MANIATIS, FRTSCH and SAMBROOK 1982).

**Protein gel electrophoresis and Western blot analysis:** Whole embryos or larvae were homogenized in sodium dodecyl sulfate (SDS) reducing buffer (60 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 350 mM β-mercaptoethanol, 0.01% bromphenol blue) in the presence of protease inhibitors (10 μM benzamidine HCl, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml phenanthroline, 10 μg/ml aprotenin, 10 μg/ml leupeptin, 10 μg/ml pepstatin A) and heated at 95° C for 4 min. Insolubles were pelleted by centrifugation prior to electrophoresis. Samples (equivalent of 15 embryos or one larva per lane) were run on a 15% SDS-polyacrylamide gel using the LAEMMLI (1970) buffer system. Proteins were then transferred electrophoretically to nitrocellulose paper in 25 mM Tris base, 192 mM glycine (free base), 20% methanol (w/v), pH 8.3 (TOWBIN, STAHELIN and GORDON 1979). After transfer, the membrane was pre-blocked by incubation with 3% bovine serum albumin (fraction V; Sigma) in TBST (10 mM Tris-HCl, pH 8.0; 150 mM NaCl; 0.05% Tween 20) for 30 min at room temperature. The primary antibody was a polyclonal rabbit serum, directed against a synthetic peptide based on amino acids 25-47 of HP-1 (a gift from R. F. CLARK and S. C. R. ELGIN), which has a specificity identical to the C1A9 antibody (JAMES and ELGIN 1986; JAMES et al. 1989) in indirect immunofluorescence staining of polytene chromosome (R. F. CLARK and S. C. R. ELGIN, unpublished observations). This antisera was diluted 1:7500 in TBST and incubated with the blot for 30 min at room temperature. Following three washes with TBST, a 1:7500 dilution (in TBST) of anti-rabbit IgG-alkaline phosphatase conjugate (Promega) was added and the blot was incubated a further 30 min at room temperature. Detection was performed using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (Promega) as described by HARLOW and LANE (1988).

**In vitro transcription and translation of CDNA clones:** A 3-μg sample of plasmid template (previously linearized by restriction endonuclease digestion) was used for each in vitro transcription reaction. The pNB40 cdna cloning vector (BROWN and KAFTOS 1988) was used in conjunction with the 5′ SP6 polymerase promoter, using the conditions described by MELTON et al. (1984). An aliquot of 1-2 μg of in vitro transcribed RNA was added to a 50-μl nucleate-treated rabbit reticuloocyte lysate reaction mix minus methionine (Promega) which had been supplemented with [35S]methionine (ICN Trans label) and incubated for 2 hr at 30° C. For gel electrophoresis, 1-5 μl of the reaction mix were added to 20 μl SDS reducing buffer, heated 4 min at 95° C and 5 μl were loaded per lane.

**Construction of the HS-HP1 plasmid:** The polymerase chain reaction (PCR) technique (SAIKI et al. 1988) was used to amplify a portion of an HP-1 cdna clone. Primers used were 5′-CTAGTTTAACTGCCTGTTCAATA-3′ (positions 130-149 in EISENBERG et al. 1990) and the M13 universal primer, which anneals to the vector sequences immediately flanking the cdna subclone. Amplification conditions were those used to amplify genomic target sequences, but using only 1 ng of plasmid DNA. The ends of the amplified DNA were blunted by the S1/T4 method described above and cloned into the Smal site of pUC18 (YANISCH-PERRON, VIERA and MESSING 1985). A clone having the 5′ end of
the cDNA close to the SalI site of the plasmid polylinker was chosen, and the insert was excised using SalI digestion (a second SalI site was co-amplified with the cDNA from the original subclone) and ligated into the SalI site of the HIC-L heat-shock expression vector (KRAUS et al. 1988). A clone with the 5’ end of the cDNA just downstream of the heat-shock promoter sequences was then digested with EcoRI and NotI, the NotI end was selectively blunted with Klenow enzyme (Promega) in the presence of dCTP and dGTP, and the cDNA-containing fragment was gel-purified. The plasmid vector pUCHSneo (STELLER and PIRROTTA 1985) was digested with EcoRI and Smal, and the cDNA was ligated into this doubly cut vector. The entire open reading frame of the cDNA was rechecked by sequencing to ensure that no PCR-induced base changes had occurred.

**Germ line transformation and selection of transformants:** Germ line transformation was performed essentially as described (RUBIN and SPRADLING 1982), using 200 µg/ml of HS-HPII DNA and 500 µg/ml ps25.7wc (KARESS and RUBIN 1984). G0 adults were mated to Canton S flies and allowed to lay eggs on standard food supplemented with live yeast. Larvae were transferred to selective media composed of instant Drosophila food (Carolina Biological) reconstituted with water containing 1 mg/ml G418 (Genticin; GIBCO-BRL). These larvae were heat shocked once a day at 37°C for 30 min to drive the neo gene on the pUCHSneo vector. Two third chromosome-linked transformant lines were used in this study. Insertion sites, determined by in situ hybridization, were at 85F and 85C.

**Eye pigment quantitation:** Crosses to evaluate transgene function were set in shell vials. Daily heat shock was performed by immersing vials halfway in a 37°C water bath for 45 min. Red eye pigments were quantitated by the acidified ethanol method of EPHRUSSI and HEROLD (1944). Heads were removed from adult flies aged 2 days or older and split mid-sagittally. Ten heads at a time were extracted in 1 ml of instant Drosophila food (Carolina Biological) reconstituted with water containing 100 mg/ml G418 (Genticin; GIBCO-BRL). These larvae were heat shocked once a day at 37°C for 30 min to drive the neo gene on the pUCHSneo vector. Two third chromosome-linked transformant lines were used in this study. Insertion sites, determined by in situ hybridization, were at 85F and 85C.

**RESULTS**

**Su(var)2-5 mutations are allelic to Su(var)205:** Su(var)2-5o4 and Su(var)2-5o5 were previously identified as dominant suppressors of PEV and recessive lethal mutations which were not complemented by deficiencies for the cytological interval 28F-29A (WUSTMANN et al. 1989). The map location of Su(var)2-5 on the second chromosome of Drosophila melanogaster at 31.1 ± 3.1 overlaps with the map position of the dominant suppressor of PEV Su(var)205 at 29.9 ± 2.0 (SINCLAIR, MOTTUS and GRIGLIATI 1983). Since it has recently been shown that the Su(var)205 mutation is associated with a point mutation in the HP-1 gene at 29A (EISENBERG et al. 1990), we tested these mutations for allelism. In crosses of Su(var)2-5 flies to Su(var)205/In(2LR)CyO flies, no transheterozygotes were recovered among the progeny (0/549 using Su(var)2-5o4/InCyRoi; 0/221 using Su(var)2-5o5/In(2LR)Cy + In(2R)Cy). Thus, the Su(var)2-5 mutations fail to complement the recessive lethality of Su(var)205.

**Su(var)2-5o4 is associated with a nonsense mutation in the HP-1 gene:** To test whether the Su(var)2-5
cDNA clone); Su(var)-5'/Su(var)-5''/InCyRoi flies. In both embryo and larval tissue; of wild-type size. This prediction was tested by West-}

dition/translation products

homogenates, a faster migrating protein was detected by an anti-HP-1 serum, in addition to the expected HP-1 band. This protein was absent in comparable homogenate; A, Wild-type (Canton S) embryo homogenate; B, Su(var)-2.5'/InCyRoi embryo homogenate; C, rabbit reticulocyte lysate-translated Su(var)-2.5'' HP-1 (mRNA from in vitro transcription of a Su(var)-2.5'' HP-1 cDNA clone); D, wild-type (Canton S) third instar larval homogenate; E, Su(var)-2.5''/InCyRoi third instar larval homogenate; G, rabbit reticulocyte lysate-translated wild-type HP-1 (mRNA from in vitro transcription of a wild-type HP-1 cDNA clone). Asterisks indicate the positions of the bands corresponding to the truncated HP-1 protein in embryo and larval extracts.

predicts a truncated HP-1 protein approximately 85% of wild-type size. This prediction was tested by Western blot analysis of embryo and larval protein from Su(var)-2.5''/InCyRoi flies. In both embryo and larval homogenates, a faster migrating protein was detected by an anti-HP-1 serum, in addition to the expected HP-1 band. This protein was absent in comparable homogenates of wild-type embryos and larvae (Figure 2, compare lanes A and B, and D and E). In vitro transcription and translation of a cDNA clone of the Su(var)-2.5'' allele gave an immunoreactive species that comigrates with the mutant species.

Both wild-type HP-1 protein and the faster-migrating, Su(var)-2.5''-specific protein have apparent molecular weights on SDS gels that are considerably larger than that predicted by conceptual translation of cDNA sequence. The fact that in vitro transcribed and translated wild-type and mutant cDNAs yield HP-1 proteins having similarly aberrant mobilities argues that this anomalous electrophoretic behavior is not likely to be the consequence of posttranslational modification. The apparent mass of the faster migrating form is approximately 85% of the apparent mass of the wild-type protein, further indicating that it is the product of the mutant allele.

Expression of a heat-shock inducible HP-1 cDNA transgene enhances variegation: The identification of mutational lesions affecting HP-1 in three independently isolated, allelic mutations strongly implicates reduced levels of functional HP-1 as the basis for the dominant suppression of PEV associated with these mutations. An independent verification would be provided if it could be shown that an HP-1 transgene enhances PEV. We constructed such a transgene, consisting of an HP-1 cDNA placed downstream of a minimal Hsp70 heat shock promoter (Kraus et al. 1988). This HP-1 “minigene” was then placed into the P element vector pUCHsneo (Steller and Pirrotta 1985), transduced into wild-type flies by P element-mediated germ line transformation (Rubin and Spradling 1982), and transformed lines were established.

To demonstrate the contribution of minigene expression to steady-state HP-1 levels, Northern and Western blot analyses were used. For the Northern blots, RNA was prepared from wild-type and transformed flies ([P(neo')] HSHP1.85F]), either as non-heat-shocked flies, immediately after a 45 min heat shock at 37 °C, or after 2- or 4-hr post-heat-shock recovery period. The blot was probed with a labeled HP-1 cDNA clone, and the relative level of HP-1 RNA in each sample was visualized autoradiographically (Figure 3). To control for differences in sample loadings, the blot was simultaneously hybridized with a clone of a housekeeping gene encoding ribosomal protein 49 (rp49). Results showed a significant excess of HP-1 RNA in transformed flies over wild-type control levels subsequent to heat shock treatment, even 4 hr after return to room temperature. Using Western blot analysis, HP-1 protein levels were found to be elevated (about 2-3-fold) in transformed larvae after heat shock as well (Figure 3), although elevated levels of HP-1 protein only appear after 2 hr post-heat shock, lagging significantly behind the RNA induction (compare top and bottom panels in Figure 3). Thus, the transgene can contribute substantially to intracellular levels of HP-1 protein in transformed flies.

A phenotypic assay for transgene function is whether it reverses, or complements, the suppression of PEV caused by mutation in the HP-1 sequences. In flies carrying the white-variegating rearrangement In(1)w~s4, this complementation would lead to a greater proportion of unpigmented eye facets. Female flies carrying In(1)w~s4 and a copy of the minigene on the third chromosome at 85F ([P(neo')] HSHP1.85F]) were crossed to In(1)w~s4 males heterozygous for one of the three Su-var alleles representing HP-1 mutations. Flies were heat-shocked daily for 45 min at 37 °C to drive expression of the transgene. Among the progeny of this cross, enhancement of variegation was apparent by inspection. To quantify levels of variegation, eye pigments were extracted from the progeny of this cross and quantitated spectrophotometrically. The results, summarized in Table 1, show that flies carrying the heat-shock driven HP-1 transgene had substantially lower levels of red eye pigment than their
Su(var)2-5+/Su(var)2-5+; P/(neoX)HSHP1.85F/TM3, Sb Ser)

...from wild-type (Canton S; wild-type (Can S; Canton S) and transformed (HS-HP1; Su(var)2-5'/Su(var)2-5'; P/(neoX)HSHP1.85F/TM3, Sb Ser) adult flies, electrophoresed in a 1.5% agarose-formaldehyde gel, and transferred to nitrocellulose. The blot was simultaneously probed with a 32P-labeled cDNA clone of HP-1 (pTH5) and a clone of the ribosomal protein 49 gene (HR0.6; WONG et al. 1981). The arrowhead marked "HP-1" indicates the position of HP-1 RNA in the accompanying autoradiograph. The arrowhead marked "rp49" indicates the position of ribosomal protein 49 gene RNA in the same autoradiograph. A, Non-heat-shocked flies; B, flies heat shocked for 45 min at 37°C; C, flies recovered for 2 hr at room temperature after a 45 min heat shock; D, flies recovered for 4 hr at room temperature after a 45 min heat shock. Lower panel, Third instar larvae were homogenized and boiled in SDS-polyacrylamide gel electrophoresis sample buffer (LAEMLI 1970), and the solubilized protein electrophoresed in a 10% SDS-polyacrylamide gel electrophoresis gel. Equal amounts (40 μg) of total protein were loaded per lane, and samples loaded correspond to the upper panel to facilitate comparison. Proteins were transferred electrophoretically to nitrocellulose paper, the blot was probed with anti-HP-1 serum, and the immunoreactive bands detected as in Figure 2. The arrowhead marked "HP-1" indicates the position of HP-1 protein. This protein sometimes appears as a doublet, depending on the level of reducing agent.

nontransgenic sibs (marked with TM3, Sb Ser). Thus, suppression of white variegation associated with each of the three Su(var) alleles was complemented by the HS-HP1 transgene. It should be noted, however, that complementation was incomplete, in that pigment values for Su(var)* sibs were 2.4% of wild type. Comparable levels of enhancement of variegation were observed for an independently derived insertion of this same transgene at 83C (data not shown).

DISCUSSION

Previous work (EISENBERG et al. 1990) showed an association between the dominant suppressor of PEV Su(var)205 (which is also recessive lethal) and mutation in the gene encoding HP-1. That association is con-

![Figure 3.](image)

**TABLE 1**

<table>
<thead>
<tr>
<th>Genotypea</th>
<th>HP-1 gene dosageb</th>
<th>Pigment valuesc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Su(var)2-504</td>
<td>TM3, Sb Ser</td>
<td>1</td>
</tr>
<tr>
<td>Su(var)2-504</td>
<td>P/(neoX)HSHP1.85F</td>
<td>2</td>
</tr>
<tr>
<td>Su(var)2-504</td>
<td>TM3, Sb Ser</td>
<td>1</td>
</tr>
<tr>
<td>Su(var)2-504</td>
<td>P/(neoX)HSHP1.85F</td>
<td>2</td>
</tr>
<tr>
<td>Su(var)205</td>
<td>TM3, Sb Ser</td>
<td>1</td>
</tr>
<tr>
<td>Su(var)205</td>
<td>P/(neoX)HSHP1.85F</td>
<td>2</td>
</tr>
</tbody>
</table>

*In each instance, male progeny were assayed. Crosses were: In(1)Pe3/Y; Su(var)Cy; +/- × In(1)Pe3+/In(1)Pe3; +/++; P/(neoX)HSHP1.85F/TM3, Sb Ser.

*Treating each Su(var)2-5 mutant allele as zero, and treating the minigene as 1 dose.

*Expressed as a percentage of wild-type red eye pigment. Mean pigment value for homozygous Su(var)2-5 sibs was 2.4 ± 1.6.

firmed and extended by the results reported here. As the three mutations—Su(var)205, Su(var)2-504 and Su(var)2-505—were independently isolated in separate screens as dominant suppressors of PEV, their inviability as trans-heterozygotes argues that the recessive lethality and dominant suppression of PEV are both likely a consequence of the same mutation, and not merely due to linked but unrelated lethal mutations.

The findings (EISENBERG et al. 1990; this report) that these mutations are all associated with different lesions in the gene encoding the heterochromatin-specific chromosomal protein HP-1 argue strongly for a common basis for the mutant phenotypes in their effects on HP-1. Enhancement of variegation by a heat-shock regulated HP-1 cDNA further supports the conclusion that PEV depends upon levels of HP-1. This enhancement by a wild-type HP-1 cDNA will provide phenotypic criteria for evaluating the function of mutant HP-1 protein generated by in vitro mutagenesis.

The mutational lesion in the Su(var)2-504 allele, which causes a frame-shift after codon 10, would lead one to expect it to be equivalent to a null allele. On the other hand, the Su(var)2-504 allele expresses detectable quantities of a protein 85% wild-type length. The reduced steady-state level of this protein compared to the wild-type gene product suggests that the mutant protein may be unstable; this instability and/or the loss of the C-terminal 15% of the protein may account for its strong phenotype. Alternatively, the identification of a nuclear localization function in the C-terminal quarter of HP-1 (J. A. POWERS and J. C. EISENBERG, manuscript in preparation) suggests a possible basis for both a loss of function and increased...
turnover in the retention of the defective protein in the cytoplasm. Genetically, these mutations heterozygous with duplications for the 29A interval show normal variegation (Wustmann et al. 1989), rather than triplo-abnormal enhancement, consistent with a null or strongly hypomorphic phenotype.

The overexpression of HP-1 resulting from heat-shock driven expression of an HP-1 cDNA led to an enhancement of PEV in flies carrying each of the three characterized Su(var)s shown here and previously (Eissenberg et al. 1990) to be associated with mutation in HP-1-coding sequences. Thus, insufficiency for HP-1, due to mutation in the HP-1 coding sequences, is associated with suppression of PEV, while overexpression of HP-1 under a heterologous promoter, results in enhancement of PEV. Taken together, these results argue strongly that HP-1 protein levels set and/or maintain the inactivated state of gene subject to heterochromatin PEV.

Role of HP-1 in heterochromatic position effects: Cytological analysis points to an altered chromatin structure imposed upon the variegating gene as the underlying basis for PEV (Henikoff 1981; Reuter, Werner and Hoffman 1982; Kornher and Kauffman 1986; Zhimulev et al. 1986). A genetic search has thus far uncovered over 20 loci which behave as modifiers of PEV (Sinclair, Mottus and Grigliatti 1983; Locke, Kotaske and Tartof 1988; Wustmann et al. 1989), suggesting a complex process underlying the genetic inactivation of genes by heterochromatin. It is likely that through the identification of such loci and the biochemical characterization of their gene products, we will come to understand how heterochromatin in particular, and developmentally programmed gene inactivation in general, comes about in metazoans (Eissenberg 1989; Paro 1990; Tartof and Bremer 1990).

Locke, Kotaske and Tartof (1988) have proposed a model for PEV that invokes (1) mass action-driven heterochromatin assembly and (2) a requirement for a precise stoichiometry of heterochromatin protein subunits. The model can account for the extraordinary sensitivity of PEV to the dosage of a relatively large number of separate genes, which may encode heterochromatin proteins or their modifiers. HP-1 would appear to fulfill the expectations of such a model, as apparent null mutations in this heterochromatin protein lead to dramatic reduction in PEV levels, while variegation is enhanced by expression of an HP-1 cDNA.

Role of HP-1 in silencing preblastoderm gene expression: Genetic evidence for preblastoderm functions associated with heterochromatin has been reported by Sandler and colleagues (Sandler 1977; Pimpinelli et al. 1985). For example, duplications for certain heterochromatic elements located on the X chromosome, the Y chromosome, and the second chromosome can complement the autosomal recessive maternal-effect mutation abnormal oocyte (abo). These duplications rescue only precellular blastoderm lethality, while paternal abo* complements the postcellular blastoderm lethality, suggesting (1) an overlapping function for these loci and (2) that the principal difference between the euchromatic and heterochromatic loci is their time of action (Pimpinelli et al. 1985). Heterochromatinization could thus represent an epigenetic regulatory switch establishing and maintaining the repression of certain preblastoderm genes. Loss of a heterochromatin protein would result in lethality due to ectopic expression of such genes. Changes in gene dosage for a heterochromatin protein might, on the other hand, have more subtle effects on timing or extent of inactivation. In this view, the report that an HP-1 mutation is functionally equivalent to one class of heterochromatic duplication in rescue of abo maternal-effect lethality (Pardue and Henning 1990) could be explained by the delayed or incomplete inactivation of heterochromatin gene expression.

Implications for mechanisms of developmental gene repression: The implication of HP-1 in the formation of heterochromatin and position-effect-mediated gene inactivation may also provide insight into other forms of developmentally programmed gene inactivation. For example, the Polycomb gene product, like HP-1, is a genetic repressor (of homeotic genes) which appears to be dose-limited (Duncan and Lewis 1982), and is a chromosomal protein (Zink and Paro 1989). Paro and Hogness (1991) noted a 37 amino acid domain of the Polycomb protein which has 65% amino acid identity to a domain within the HP-1 protein. The structural conservation and apparent functional similarities between Polycomb and HP-1 have led Paro (1990) to propose that the Polycomb protein may act by a mechanism analogous to heterochromatin formation to establish and propagate developmental inactivation of homeotic genes in appropriate body segments of the fly. Indeed, Reuter et al. (1990) have reported effects of dominant modifiers of PEV on homeotic gene expression, suggesting that the regulatory mechanisms that underlie PEV and homeotic gene repression may overlap. Similarly, the pleiotropic effects of mutations in another Polycomb-family gene, polycombotic (pec), suggest a more general function for the pec gene product in chromosome structure, beyond silencing of the homeotic loci (Phillips and Shearn 1990). Such proteins may represent the "locking molecules" proposed by Zuckerkandl (1974) to form a quaternary structure able to inhibit gene transcription. It would not be surprising to find other examples in which the modifiers of PEV and the Polycomb group share overlapping functions as
the genetic and biochemical characterization of these gene families continues.

We wish to thank R. F. CLARK and S. C. R. ELGIN for providing us with the anti-HP-1 serum, M. GREEN and M. SHIPLEY for advice on in vitro transcription/translation, S. HERRERRO for help in construction of the HS-HPI minigene, and D. COULTER, J. WALLIS, P. ZASSENHAUS and S. C. R. ELGIN for helpful advice and constructive criticism. This work was supported by National Institutes of Health grant GM40732 to J.C.E.

LITERATURE CITED


TOWBIN, H., T. STAHLHELN and J. GORDON, 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose.


Communicating editor: R. E. Denell