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, Domplatz 1, 0-4020 Halle/Saale, Germany*

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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 Lys^{169} into a nonsense (ochre) codon, while the other is a frameshift after Ser^{10}. In flies heterozygous for one of the mutant alleles (*Su(var)2-5*^{04}), 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)w*^{m4}. 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)w*^{m4h} (REUTER and WOLFF 1981); *Su(var)205* and *b lt rl* (SINCLAIR, MOTTUS and GRIGLIATTI 1983); *In(2L)Cy* + *In(2R)Cy* (LINDSLEY and GRELL 1968). *Su(var)2-5*^{04}, *b cn vg* and *Su(var)2-5*^{05} are ethyl methanesulfonate- and X-ray-induced alleles, respectively, of a locus on 2L (WUSTMANN *et al.* 1989). *InCyRoi* [= *In(2L)Cy*^{L} t^{R} + *In(2R)Cy, Cy Roi cn*^{2} *sp*^{2}] is not explicitly described anywhere (to our knowledge), but is comprised of rearrangements

described in LINDSLEY and GRELL (1968) and ASHBURNER (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. ^{32}P-Labeling of Northern blot hybridization probe was by the random primer method (FEINBERG 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° without formamide. The hybridized filter was washed as described, air-dried, and exposed to XAR-5 X-ray film (Kodak) at −80° using a Cronex Lightning Plus intensifying screen (Du Pont).

Amplification of HP-1 sequences from genomic DNA: One microgram of adult whole fly chromosomal DNA was added to 100 μl reaction mix [125 μM each, dATP, dCTP, dGTP, and TTP; 1 × Taq Polymerase Buffer (Promega); 20 pmol of each oligonucleotide primer; 2 units Taq polymerase (Promega)] in a 500-μl microcentrifuge tube, and overlayered with 50 μl light mineral oil. Amplification was performed using an Ericomp (San Diego, California) SingleBlock System microprocesser-controlled temperature cycler. Cycle conditions used were: denaturation at 94° for 1 min; annealing at 58° for 2 min; extension at 72° for 2 min. Twenty-five cycles of this sequence were performed, followed by a 7-min final extension at 72°. The double-stranded product was selectively precipitated with 2 M ammonium acetate and 2 volumes isopropanol at room temperature for 10 min. Precipitate was recovered by centrifugation and washed once with 1 ml 70% ethanol. Under these conditions the primers do not precipitate efficiently.

Asymmetric amplification of sequences for direct sequencing: An aliquot of 0.2 pmol of the selectively precipitated double-stranded amplification product was added to the standard reaction mix, except that 80 pmol of a single primer were used. The cycle conditions were: denaturation at 95° for 1 min; annealing at 65° for 2 min; extension at 72° for 2 min. This sequence was repeated for 30 cycles, followed by a 7-min final extension at 72°. The single-stranded and double-stranded amplification products were precipitated as above. The pellet was resuspended in 21 μl distilled water and 7 μl were removed for sequencing.

Cloning of amplification products: Reaction product was recovered by precipitation as above and resuspended in 17 μl distilled water. Then 2 μl of 10 × S1 nuclease digestion buffer (MANIATIS, FRITSCH and SAMBROOK 1982) and 1 μl S1 nuclease (4 units/μl; Boehringer Mannheim) were added. The digestion was at 37° for 30 min. The reaction was terminated by phenol extraction, followed by chloroform extraction and isopropanol precipitation as above. Precipitated DNA was recovered by centrifugation, dried and resuspended in 13.5 μl distilled water. To this were added 3 μl 10 × KGB buffer (KGB buffer is 100 mM potassium glutamate, 25 mM Tris-acetate (pH 7.6), 10 mM magnesium acetate, 50 μg/ml bovine serum albumin, 0.5 mM β-mercaptoethanol; MCCLELLAND *et al.* 1988), 3 μl deoxynucleotide mix (1.25 mM each of dATP, dCTP, dGTP and TTP), and 5 units T4 DNA polymerase (United States Biochemical), and the sample incubated at 37° for 20 min. Amplified, blunt-ended DNA was then recovered from an agarose gel by electroelution into a buffer-filled well cut in the gel just ahead of the desired band, and the DNA was ethanol 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, FRITSCH 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% bromophenol 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° for 4 min. Insolubles were pelleted by centrifugation prior to electrophoresis. Samples (equivalent of 15 embryos or one larvae per lane) were run on a 15% SDS-polyacrylamide (30:0.8 acrylamide:bisacrylamide) 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, STAEHELIN 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 antiserum 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-indoyl 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 KAFATOS 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 nuclease-treated rabbit reticulocyte lysate reaction mix minus methionine (Promega) which had been supplimented with [^{35}S]methionine (ICN Trans label) and incubated for 2 hr at 30°. For gel electrophoresis, 1–5 μl of the reaction mix were added to 20 μl SDS reducing buffer, heated 4 min at 95° 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′-CCAATTTAGCTGCGTGCATA-3′ (positions 130–149 in EISSENBERG *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 *Sma*I 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 *Eco*RI and *Not*I, the *Not*I 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 *Eco*RI and *Sma*I, 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-HP1 DNA and 500 μg/ml pπ25.7wc (KARESS and RUBIN 1984). G_0 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° for 30 min to drive the *neo* gene on the pUChsneo vector. Two third chromosome-linked tranformant lines were used in this study. Insertion sites, determined by *in situ* hybridization, were at 85F and 83C.

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° 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 acidified 30% ethanol, and extraction was for 24 hr in the dark.

RESULTS

***Su(var)2-5* mutations are allelic to *Su(var)205*:** *Su(var)2-5^{04}* and *Su(var)2-5^{05}* 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 GRIGLIATTI 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 (EISSENBERG *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)2-5^{04}/InCyRoi*; 0/221 using *Su(var)2-5^{05}/In(2L)Cy + In(2R)Cy*). Thus, the *Su(var)2-5* mutations fail to complement the recessive lethality of *Su(var)205*.

***Su(var)2-5^{04}* is associated with a nonsense mutation in the HP-1 gene:** To test whether the *Su(var)2-5^{04}* mutation is also associated with a lesion in the HP-1 gene, we used the PCR to amplify the HP-1 coding sequences from *Su(var)2-5^{04}/b lt rl* genomic DNA. Amplified DNA was cloned and allele specific clones were identified based upon a previously identified sequence polymorphism in the last intron of the HP-1 gene which abolishes an *Acc*I site in the *b lt rl* allele. Sequencing of two such allele-specific clones revealed an A-to-T transversion mutation (at position 1201 in EISSENBERG *et al.* 1990) which predicts a change of Lys^{169} to a nonsense (ochre) codon (Figure 1). Direct sequencing of PCR-amplified DNA confirmed heterozygosity at this position, and the same mutation was observed in PCR-generated cDNA clones. This consistency rules out the possibility that the change found in the allele-specific clone was the result of a random PCR-induced mutation.

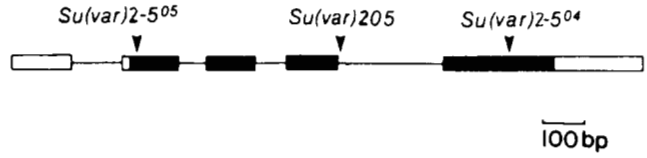

FIGURE 1.—*Su(var)2-5* alleles are associated with a point mutation in the gene encoding HP-1. Map of the HP-1 gene showing the relative positions of mutations in the *Su(var)2-5^{05}*, *Su(var)205* and *Su(var)2-5^{04}* alleles. Bar = exons, lines = introns, filled bars = protein-coding sequence.

***Su(var)2–5^{05}* is associated with a frameshift mutation in the HP-1 gene:** *Su(var)2-5^{05}* is an X-ray-induced mutation, leading us initially to expect a significant deficiency or rearrangement associated with the HP-1 locus in this stock. Southern blot analysis of genomic DNA from *Su(var)2-5^{05}* flies failed to detect any aberration in the restriction fragment pattern, suggesting a smaller lesion than we had anticipated. We next employed the direct sequencing of PCR products generated by amplification of HP-1 sequences from *Su(var)2-5^{05}/In(2L)Cy + In(2R)Cy* genomic DNA. Sequence analysis of the co-amplified alleles revealed a frameshift mutation at position 339. Since this occurs within a *Sac*I site in the wild-type sequence, we digested genomic DNA from heterozygous flies with *Sac*I to preferentially cleave the wild-type allele, allowing us to amplify across this region exclusively in the mutant allele using PCR. Results of sequence analysis of this amplification product revealed a dinucleotide deletion (positions 339–340 in EISSENBERG *et al.* 1990). The predicted consequence of this mutation is the synthesis of a nonsense peptide having only the first 10 amino acids of authentic HP-1. As we presently have no antibodies capable of detecting such a protein, we are unable to confirm that this peptide is in fact synthesized in *Su(var)2-5^{05}* flies.

***Su(var)2-5^{04}* flies express a truncated HP-1 protein:** The nonsense mutation in the *Su(var)2-5^{04}* allele

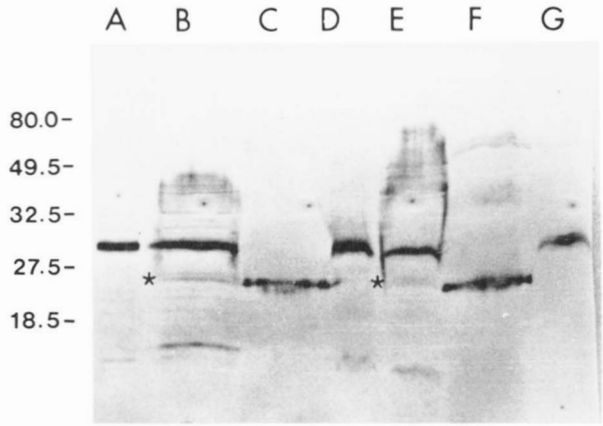

FIGURE 2.—*Su(var)2-5*^{04} flies express a truncated HP-1 protein. A Western blot of embryo homogenates (**A**, **B**), *in vitro* transcription/translation products (**C**, **F**, **G**), and larval homogenates (**D**, **E**) was probed with anti-HP-1 serum, and immunoreactive bands detected by a secondary antibody, coupled to alkaline phosphatase, followed by an alkaline phosphatase activity stain. **A**, Wild-type (Canton S) embryo homogenate; **B**, *Su(var)2-5*^{04}*/InCyRoi* embryo homogenate; **C** and **F**, rabbit reticulocyte lysate-translated *Su(var)2-5*^{04} HP-1 (mRNA from *in vitro* transcription of a *Su(var)2-5*^{04} HP-1 cDNA clone); **D**, wild-type (Canton S) third instar larval homogenate; **E**, *Su(var)2-5*^{04}*/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*^{04}*/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*^{04} 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*^{04}-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 anomolous 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*^{R}*) HSHP1.85F]*), either as non-heat-shocked flies, immediately after a 45 min heat shock at 37°, 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*^{m4}, this complementation would lead to a greater proportion of unpigmented eye facets. Female flies carrying *In(1)w*^{m4h} and a copy of the minigene on the third chromosome at 85F (*P[(neo*^{R}*) HSHP1.85F]*) were crossed to *In(1)w*^{m4h} males heterozygous for one of the three *Su-var* alleles representing HP-1 mutations. Flies were heat-shocked daily for 45 min at 37° to drive expression of the transgene. Among the progeny of this cross, enhancement of variegation was apparent by inspection. To quantitate 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

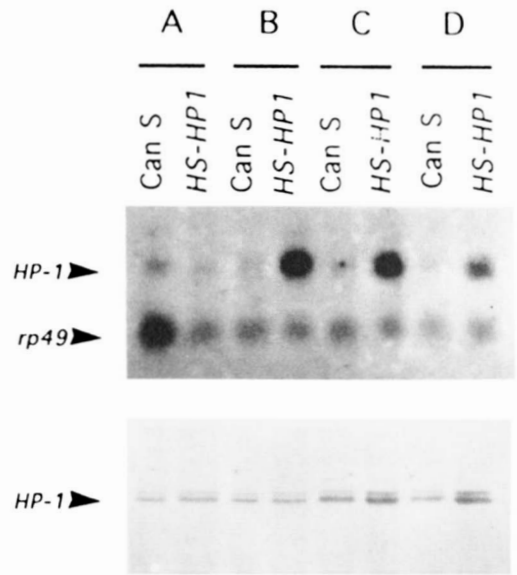

FIGURE 3.—HP-1 RNA and protein is increased after heat shock in transgenic flies. Upper panel, Total nucleic acids were prepared from wild-type (Can S; Canton S) and transformed (*HS-HP1; Su(var)2-5*$^{+}$*/Su(var)2-5*$^{+}$*; P[(neo*^{R}*) HSHP1.85F]/TM3, Sb Ser*) adult flies, electrophoresed in a 1.5% agarose-formaldehye gel, and transferred to nitrocellulose. The blot was simultaneously probed with a ^{32}P-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**, 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 (LAEMMLI 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 sample loadings 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 (EISSENBERG *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 confirmed and extended by the results reported here. As the three mutations–*Su(var)205*, *Su(var)2-5*^{04} and *Su(var)2-5*^{05}–were independenty 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 (EISSENBERG *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)2-5*^{05} 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)2-5*^{04} 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. EISSENBERG, manuscript in preparation) suggests a possible basis for both a loss of function and increased

TABLE 1

A heat-shock-driven HP-1 cDNA minigene enhances variegation in *Su(var)2-5* flies

Genotype[a]	HP-1 gene dosage[b]	Pigment values[c]
$\frac{Su(var)2\text{-}5^{04}}{+}$; $\frac{TM3,\ Sb\ Ser}{+}$	1	26 ± 4
$\frac{Su(var)2\text{-}5^{04}}{+}$; $\frac{P[(neo^{R})\ HSHP1.85F]}{+}$	2	10 ± 3
$\frac{Su(var)2\text{-}5^{05}}{+}$; $\frac{TM3,\ Sb\ Ser}{+}$	1	23 ± 2
$\frac{Su(var)2\text{-}5^{05}}{+}$; $\frac{P[(neo^{R})\ HSHP1.85F]}{+}$	2	8.7 ± 1.6
$\frac{Su(var)205}{+}$; $\frac{Tm3,\ Sb\ Ser}{+}$	1	34 ± 4
$\frac{Su(var)205}{+}$; $\frac{P[(neo^{R})HSHP1.85F]}{+}$	2	13 ± 5

[a] In each instance, male progeny were assayed. Crosses were: $In(1)w^{m4h}/Y$; *Su(var)/Cy*; +/+ × $In(1)w^{m4h}/In(1)w^{m4h}$; +/+; *P[(neo*^{R}*) HSHP1.85F]/TM3, Sb Ser.*

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

[c] 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.

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 heterochromatic 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, KOTARSKI 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, KOTARSKI 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 heterochromatic protein would result in lethality due to ectopic expression of such genes. Changes in gene dosage for a heterochromatic 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 HENNIG 1990) could be explained by the delayed or incomplete inactivation of heterochromatic 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, *polycombeotic* (*pco*), suggest a more general function for the *pco* 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.

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