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Genetics

Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophila melanogaster*

(dominant suppression of heterochromatic position effects/aberrant pre-mRNA splicing/heterochromatin-associated protein)

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ABSTRACT **We report here that a point mutation in the gene which encodes the heterochromatin-specific nonhistone chromosomal protein HP-1 in *Drosophila melanogaster* is associated with dominant suppression of position-effect variegation. The mutation, a G-to-A transition at the first nucleotide of the last intron, causes missplicing of the HP-1 mRNA. This suggests that heterochromatin-specific proteins play a central role in the gene suppression associated with heterochromatic position effects.**

The partitioning of eukaryotic chromosomes into regions which differ in their degrees of compaction has long been appreciated. Most of the transcriptionally active chromatin appears to decondense after mitotic telophase into euchromatin, but a substantial fraction of chromosomal material remains condensed as heterochromatin. Heterochromatin replicates relatively late in the cell cycle and, in tissues which undergo polytenization, the heterochromatin may be underreplicated.

The potential of heterochromatin formation to result in transcriptional inactivation is inferred from two genetic phenomena: Barr-body formation (Lyonization) in mammalian females and position-effect variegation in a variety of organisms (reviewed in ref. 1). In both cases chromosomal regions which are euchromatic under some circumstances assume the morphology of heterochromatin. The condensed structure observed in these cases is strongly correlated with transcriptional inactivity.

In *Drosophila*, the genetic dissection of heterochromatin is aided by the availability of numerous rearrangements which lead to variegated expression of euchromatic genes that have come to be relocated near the heterochromatic breakpoint. A number of loci have been identified which, when mutated, act as dominant modifiers of such variegating position effects (2–7). Many of these loci are believed to encode chromatin proteins or factors that modify chromatin structure (see refs. 8 and 9 for recent reviews).

A heterochromatin-specific chromosomal protein called HP-1 has been identified and characterized in *D. melanogaster* (10, 11). A cDNA encoding this protein has been cloned (10), and the gene has been localized to cytological position 29A on the polytene chromosome map. In this report, we provide the sequence of the gene¶, identifying exon and intron boundaries, and present molecular evidence that a point mutation at one boundary, causing missplicing of the HP-1 pre-mRNA, is associated with dominant suppression of heterochromatic position effect. This indicates a requirement for HP-1 protein in generating normal heterochromatin structure.

MATERIALS AND METHODS

Drosophila Stocks. *Su(var)205/In(2LR)CyO* and the iso-2nd line (marked with *b lt rl*) were obtained from T. Grigliatti (University of British Columbia, Vancouver). Flies were cultured in half-pint plastic bottles at room temperature, using a cornmeal-based medium supplemented with dried bakers' yeast.

Northern Blot Analysis. Total nucleic acids were purified from several flies essentially according to the method of Meyerowitz and Hogness (12). Samples were electrophoresed in agarose/formaldehyde gels (13). After electrophoresis, the gels were soaked in 20× SSC for 30 min, with one change, and blotted to nitrocellulose paper in 20× SSC. (SSC, standard saline citrate, is 0.15 M NaCl/0.015 M sodium citrate, pH 7.)

Southern Blot Analysis. Genomic DNA was prepared from a small number of adult flies by the potassium acetate/sodium dodecyl sulfate method (14). DNA samples were digested with *Hpa* II restriction endonuclease according to the supplier's recommendations and electrophoresed in an agarose gel with TAE buffer (40 mM Tris acetate, pH 7.2/1 mM EDTA) (15). The gel was then soaked in 0.5 M NaOH/1.5 M NaCl for 45 min and neutralized in 0.5 M Tris·HCl, pH 7.0/3 M NaCl for 30 min, and the separated DNA fragments were blotted to nitrocellulose paper in 20× SSC.

Probes used in the hybridization analysis were labeled to high specific activity ($1–3 \times 10^8$ cpm/μg) by the nick-translation method (16) unless otherwise specified. Filters were baked, prehybridized, and hybridized to labeled probes essentially as described (17), except that prehybridizations and hybridizations were performed at 65°C without formamide. Hybridized filters were subsequently washed as described, dried, and exposed to XAR-5 x-ray film (Kodak) at −80°C with a Cronex Lightning Plus intensifying screen (DuPont).

Cloning of the *Su(var)205* Allele of HP-1. DNA from the *Su(var)205/In(2LR)CyO* line was purified and cloned into λEMBL3 as described (18). The phage library was screened by plaque hybridization (19) using the ^{32}P-labeled insert fragment of a plasmid subclone of the wild-type HP-1 gene as a hybridization probe. Phage DNA of strongly hybridizing plaques was prepared from cleared lysates by polyethylene glycol (7%, wt/vol) precipitation of intact phage particles,

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Abbreviation: PCR, polymerase chain reaction.
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¶The genomic sequence reported in this paper has been deposited in the GenBank data base (accession no. M33749).

followed by phenol extraction and ethanol precipitation of phage DNA. Appropriate restriction fragments of phage DNA were subcloned into the plasmid vector pUC13 (20) by standard procedures (21).

Screening of cDNA Libraries. The sources of various cDNA libraries are described in the legend to Fig. 5. They were screened using HP-1 cDNA labeled to high specific activity by the random priming method (22).

Cloning of HP-1 cDNA by the Polymerase Chain Reaction (PCR). One gram of adult flies was homogenized for 2 min in a motor-driven glass/Teflon homogenizer in 5 ml of phenol plus 5 ml of RNA extraction buffer (100 mM Tris·HCl, pH 8.0/100 mM NaCl/20 mM EDTA/1% sodium *N*-lauroylsarcosine with polyvinyl sulfate at 0.04 mg/ml). The homogenate was centrifuged in a Sorvall HB-4 rotor at 10,000 rpm for 5 min. The aqueous phase was removed and repeatedly extracted with equal volumes of phenol/chloroform (1:1) until no interphase was visible. After an additional chloroform extraction, the aqueous phase was mixed with 2 volumes of absolute ethanol. Nucleic acids were recovered by centrifugation and poly(A)$^+$ mRNA was selected using 0.3 g of oligo(dT)-cellulose (New England Biolabs) by the batch method (21). Two rounds of selection were performed. Poly(A)$^+$ mRNA was recovered from the eluate by ethanol precipitation and the yield was quantitated spectrophotometrically.

First-strand cDNA was synthesized from 5 μg of poly(A)$^+$ RNA by using the Amersham cDNA Synthesis System Plus kit (Amersham). Approximately 35 ng of cDNA was subjected to 30 cycles using the Perkin–Elmer/Cetus PCR kit and oligonucleotide primers 1 and 7 shown in Fig. 2. All primers were synthesized using an Applied Biosystems oligonucleotide synthesizer. Cycle conditions used were (*i*) denaturation at 94°C, 1 min; (*ii*) annealing at 53°C, 2 min; and (*iii*) extension at 72°C, 3 min, except that the last extension step was for 7 min. Amplified DNA was recovered by ethanol precipitation and digested with *Bgl* II restriction endonuclease. The digested PCR products were purified by gel electrophoresis, ligated into the *Bam*HI site of pUC13, and transformed into competent *Escherichia coli* TG1 host cells.

DNA Sequencing. Subcloned fragments bearing regions of interest were sequenced directly from plasmid DNA. Sequencing was by the dideoxy chain-termination method with *E. coli* DNA polymerase I Klenow fragment (23), Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories; ref. 24), or Sequenase (United States Biochemical), using oligonucleotides 2–7 shown in Fig. 2.

RESULTS

The observation that a gene encoding a heterochromatin-specific chromosomal protein is located at interval 29A of the polytene chromosome map (10) led us to search for known mutations mapping to this region. Sinclair *et al.* (3) have described a number of dominant suppressors of position-effect variegation mapping to the second chromosome in *D. melanogaster*. In particular, the mutation *Su(var)205* mapped genetically to a region roughly coincident with the 29A interval. We obtained both the *Su(var)205* mutation (in a stock balanced over the *In(2LR)CyO* chromosome), and the wild-type iso-2nd chromosome line from which the mutant *Su(var)205*-bearing 2nd chromosome was derived. Restriction enzyme digests of genomic DNA from *Su(var)205/In(2LR)CyO* and iso-2nd flies were compared by Southern blot hybridization analysis. No evidence for insertions or deletions at the HP-1 locus was found (data not shown).

Northern blot analysis using HP-1 cDNA as a probe of total cellular RNA prepared from adult *Su(var)205/In(2LR)CyO* flies showed the presence of a smaller second HP-1 transcript in this stock, in addition to the wild-type HP-1 transcript (Fig. 1). The iso-2nd chromosome stock does not show this transcript (Fig. 1, lane D). When *Su(var)205/In(2LR)CyO* flies were crossed to the iso-2nd stock, the smaller RNA species cosegregated with the *Su(var)205* chromosome (Fig. 1, lanes B and C). The smaller RNA migrated in agarose/formaldehyde gel electrophoresis as though it were 300–400 bases smaller than wild-type HP-1 RNA. That no deletion of this magnitude was found in the HP-1 locus of *Su(var)205* suggested that a point mutation affecting transcriptional initiation, transcriptional termination, or posttranscriptional processing might be responsible for the smaller transcript.

Both the wild-type and aberrant transcripts were retained by oligo(dT)-cellulose (data not shown), suggesting that both species were polyadenylylated. Primer extension mapping to compare the transcription initiation site for HP-1 in wild-type and *Su(var)205/In(2LR)CyO* adult flies showed no detectable differences (initiation sites summarized in Fig. 4). These data suggest that transcriptional initiation and termination at the mutant HP-1 locus are normal.

A plausible explanation for the aberrant transcript in *Su(var)205* flies was suggested by the molecular organization of the HP-1 gene (Fig. 2). The HP-1 mRNA is processed from a larger primary transcript by RNA splicing. A point mutation within an intron sequence critical to splice-site selection could lead to missplicing, yielding a smaller, incorrectly spliced mRNA. To investigate this possibility, a genomic DNA library from *Su(var)205/In(2LR)CyO* flies was constructed in the phage vector λEMBL3 and recombinant phage containing HP-1 sequences were identified.

To distinguish clones containing the mutant allele (derived from the *Su(var)205* chromosome) from clones containing the wild-type allele (derived from the *In(2LR)CyO* chromosome), a *Hpa* II restriction polymorphism was used. When the genomic clone Charon 4HCDm7 (10) was used to probe an *Hpa* II restriction digest of genomic DNAs from *Su(var)205/In(2LR)CyO* and iso-2nd flies, an additional restriction fragment detected in the mutant line was absent from the iso-2nd

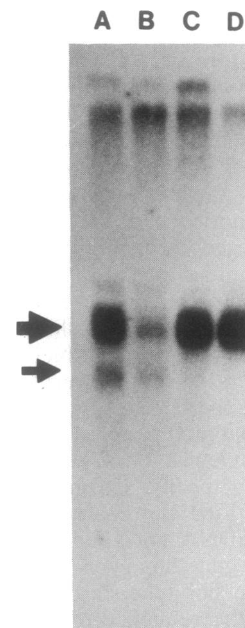

FIG. 1. Northern blot analysis of HP-1 transcripts in total nucleic acids from adult flies. Lane A, *Su(var)205/In(2LR)CyO*; lane B, *Su(var)205/b lt rl*; lane C, *In(2LR)CyO/b lt rl*; lane D, *b lt rl/b lt rl* (iso-2nd chromosome line). Total nucleic acid was electrophoresed in a 1% agarose/formaldehyde gel, blotted onto nitrocellulose, and hybridized with an HP-1 cDNA clone (10). Large arrow indicates the wild-type HP-1 mRNA; small arrow points to the aberrant, *Su(var)205*-specific RNA.

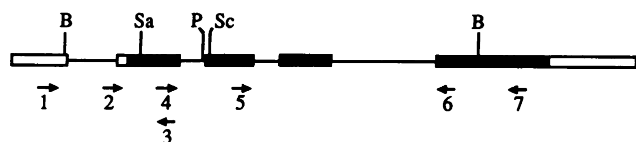

FIG. 2. Genomic map of the HP-1 gene showing the relative annealing positions of synthetic oligonucleotides used in these studies. Bars represent exon sequences and lines represent introns. Filled bars indicate the HP-1 open reading frame. Landmark restriction sites are shown above the map: B, *Bgl* II; Sa, *Sac* I; P, *Pst* I; Sc, *Sca* I. Numbered arrows below the map indicate the relative positions of oligonucleotides used in sequencing or in the PCR amplification. Arrowhead point indicates the 3' side of the oligonucleotide. Oligonucleotide sequences are as follows: 1, 5'-CCAATTTAGCTGCGTGCATA-3'; 2, 5'-TTTGTCGTGTGAAGTATT-3'; 3, 5'-GGCGGGTGCGCAAGGGAA-3'; 4, 5'-TTCCCTTGCTCACCCGCCTG-3'; 5, 5'-AGGCGAGCCGCAAGGATG-3'; 6, 5'-TTTATTGCCAGAGGG-3'; 7, 5'-GCCACTGAGGAGGGCACCAT-3'.

line (Fig. 3*A*, lanes 1 and 2). Similar analysis of the F_1 progeny from a cross of these two lines showed that the additional fragment segregated with the *In(2LR)CyO* chromosome and was thus linked to the wild-type HP-1 allele in this stock (Fig. 3*A*, lanes 3 and 4). When recombinant HP-1 phage DNAs were subjected to *Hpa* II digestion and Southern blot analysis using Charon 4HCDm7 as a probe, it was therefore possible to assign several clones to either the mutant or the wild-type class based upon whether they carried the diagnostic *Hpa* II fragment (Fig. 3*B*).

An *Eco*RI restriction fragment containing the HP-1 coding sequence of the mutant allele was subcloned from the appropriate recombinant phage DNA and the interval corresponding to the HP-1 transcription unit was sequenced. Five

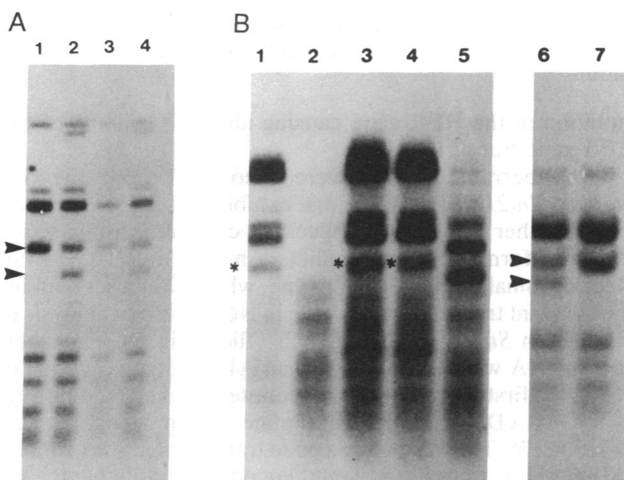

FIG. 3. An *Hpa* II restriction fragment length polymorphism distinguishes the *In(2LR)CyO* and *Su(var)205* HP-1 alleles. (*A*) Genomic DNA from adult flies, digested with *Hpa* II and subjected to Southern blot analysis using a HP-1 genomic DNA clone as a hybridization probe. Lane 1, *b lt rl*/*b lt rl* (iso-2nd chromosome line); lane 2: *Su(var)205/In(2LR)CyO*; lane 3: *Su(var)205/b lt rl*; lane 4: *In(2LR)CyO/b lt rl*. Arrowheads indicate the positions of a polymorphic *Hpa* II restriction fragment. (*B*) The *In(2LR)CyO*-linked *Hpa* II site polymorphism used to identify *Su(var)205* specific clones among λEMBL3 phage recombinants recovered from a *Su(var)205/In(2LR)CyO* genomic library. Lanes 1–5, DNA prepared from individual λEMBL3 phage recombinants and digested with *Hpa* II. Asterisks mark restriction fragments (larger of the two *Hpa* II fragments) characteristic of the *Su(var)205* allele. Lane 6, genomic DNA from *Su(var)205/In(2LR)CyO* flies, digested with *Hpa* II. Arrowheads indicate polymorphic *Hpa* II restriction fragments. Lane 7, genomic DNA from an iso-2nd chromosome line, digested with *Hpa* II. Note that all lanes in *B* were run in the same gel, to facilitate accurate comparison of DNA fragment mobilities.

base differences were observed between the wild-type (sequenced from Charon 4HcDm7; ref. 10) and *Su(var)205* alleles (Fig. 4), four of which are located in the last intron. Cloning and sequencing of the *b lt rl* parental allele showed that four of these differences are also found on the *b lt rl* parental allele; only the G-to-A transition which appears at the first nucleotide position of intron 4 is unique to the *Su(var)205* allele. The guanosine residue at the 5' end of introns appears to be a critical sequence requirement for eukaryotic mRNA splicing (25), and examples of G-to-A transition mutations found in human patients at such positions are associated with aberrant processing, resulting in the skipping of the preceding exon (26, 27). If the G-to-A mutation in the *Su(var)205* HP-1 allele leads to exon skipping in these flies, this mutation could account for the aberrant mRNA detected in the Northern blot analysis (Fig. 1). The correspondence between *Su(var)205* and HP-1 has been confirmed by the demonstration that *Su(var)2-5* (7) is allelic to *Su(var)205* by virtue of the lethality of the trans heterozygote and contains a nonsense mutation in the HP-1 sequence (T.H., G. Reuter, and J.C.E., unpublished work).

The structure of a single HP-1 cDNA clone from a λgt10 library was reported previously (ref. 10; diagrammed in Fig. 5*A*). We have since recovered several additional HP-1 cDNA clones from existing wild-type cDNA libraries. The structures of these clones (Fig. 5*B*) show a consistent splicing pattern but differ significantly from the original cDNA clone in their 5' ends. These latter clones all initiate within 62 nucleotides of one another, demarcating an interval closely linked to the HP-1 coding sequences (see Fig. 4 for precise 5' ends). This 5' heterogeneity is consistent with results of primer extension analysis (summarized in Fig. 4), and has been reported previously for "housekeeping" genes in other eukaryotes (30, 31). Whether the 5' fragment of the original cDNA clone (10) results from splicing of a message transcribed from a different (upstream) promoter or is a cloning artifact is not known.

An alternative splicing pattern was revealed (Fig. 5*C*) when wild-type cDNA clones were obtained from oligo(dT)-primed first-strand cDNA by the PCR (32). In some of the clones, the short intron immediately upstream of the AUG initiation codon was not spliced out. Failure to splice out this intron does not generate an alternative HP-1 start codon. An additional AUG triplet is present, but it is not in good translational context (33), and in any event, it is followed by a stop codon 18 nucleotides downstream. Since all of the remaining splice sites are correct in these clones, and since the cDNA was made from poly(A)$^+$ mRNA, one would expect this alternative mRNA also to translate into HP-1 protein; the significance, if any, of this alternatively spliced message is unclear.

To confirm the hypothesis of exon skipping in the HP-1 transcript from the *Su(var)205/In(2LR)CyO* stock, we prepared first-strand cDNA from poly(A)$^+$ RNA isolated from adult *Su(var)205/In(2LR)CyO* flies and used the PCR (32) to amplify HP-1 cDNA from this preparation for cloning. When cDNA clones from wild-type and *Su(var)205/In(2LR)CyO* flies were compared, two aberrant cDNA clones were found to have structures not found in wild-type cDNA (Fig. 5*E*). In one *Su(var)205/In(2LR)CyO*-specific cDNA clone, the 3' end of exon 1 was joined to the 5' end of exon 5. In the other aberrant clone, the 5' end of exon 5 was fused to the thymidine residue 18 nucleotides inside the intron 4. Note that in the latter case, an adenosine residue was found at the first nucleotide of intron 4, confirming that this aberrant cDNA was transcribed from the *Su(var)205* allele.

In the course of this analysis, several corrections were made in the previously published sequence (10) of the HP-1 cDNA and its predicted protein sequence (see Fig. 4). A reexamination of the National Biomedical Research Foun-

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cgtagtcttagcagtcgcacgcgacatttttgcacactgttcggccggcgtcatttagaacgcgcatttttttgcagatttttcatttgtggaaagtct   100
tctagcgccaacacacaggcttataaaaaccaatttagctgcgtgcataaagtgtagatcttatttaatctaggtgagccatgaaagcaaagagagtcga   200
ctaaaaatcgtactttcctttcgtactttgcgtgtgtgtgcggttgtgtgcggttgtgctaattctttccttatttttttgtcgtgtgaagtattttaata   300

         m  g  k  k  i  d  n  p  e  s  s  a  k  v  s  d  a  e  e  e  e  e  e  y  a  v  e  k  i  i
aaaacaaaaATGGCAAGAAAATCGACAACCCTGAGAGCTCGGCAAAGGTTTCCGATGCCGAAGAGGAGGAGGAGGAGTACGCCGTGGAAAAGATCATCG   400

d  r  r  v  r  k  g  k                                                                v  e  y  y  l
ACAGGCGGGTGCGCAAGGGAAAGgtgagttttccgaaccagacgtcccattatatttggtcctaaaacgccattttccacctgcagGTGGAGTACTATCT   500

 k  w  k  g  y  p  e  t  e  n  t  w  e  p  e  n  n  l  d  c  q  d  l  i  q  q  y  e  a  s  r  k  d
GAAATGGAAGGGCTATCCCGAAACTGAGAACACGTGGGAGCCGGAGAACAATCTCGACTGCCAGGATCTTATCCAGCAGTACGAGGCGAGCCGCAAGGAT   600

e                                                                              e  k  s  a  a  s  k
GAGgtaagtgaagtacaatgcgatgccaagtacagttgtgcttgctaggatttcctttacatctcgtttcggtccgtagGAGAAGTCAGCCGCCTCCAAG   700

k  d  r  p  s  s  s  a  k  a  k  e  t  q  g  r  a  s  s  s  t  s  t  a  s  k  r  k  s  e  e  p  t
AAGGATCGACCCAGCAGCAGCGCCAAGGCCAAGGAAACTCAAGGACGCGCCAGCAGTTCGACGTCCACAGCAAGCAAGCGAAAGTCCGAAGAACCAACAG   800

gtaagctatggaatgagtgtacctacaaatatttctgtatacaaattggtataacaatagtcatcacctacaatatccactggcatttatttgcccttt   900
a                                      c
tcgtcaaaattgtattcacatatgtatctcaaattttgtccttcgcaagaatggcatgcgcaagaaaaagatggtctttgcaataaaaaaatgcatctat   1000
         c                   g

                                              a  p  s  g  n  k  s  k  r  t  t  d  a  e  q  d  t  i
taaaaaattgcactgtattaattacttgttttatttttatttatttagCGCCCTCTGGCAATAAATCAAAACGTACCACAGATGCGGAGCAGGACACCAT   1100

 p  v  s  g  s  t  g  f  d  r  g  l  e  a  e  k  i  l  g  a  s  d  n  n  g  r  l  t  f  l  i  q  f
TCCCGTTTCAGGATCTACCGGATTCGATCGCGGCCTGGAGGCCGAAAAGATCTTGGGTGCCTCCGACAATAATGGCCGCCTGACATTCCTCATTCAGTTC   1200

k  g  v  d  q  a  e  m  v  p  s  s  v  a  n  e  k  i  p  r  m  v  i  h  f  y  e  e  r  l  s  w  y
AAAGGCGTGGACCAAGCAGAAATGGTGCCCTCCTCAGTGGCCAATGAAAAAATTCCACGAATGGTAATCCACTTCTACGAAGAGCGCCTATCCTGGTACT   1300
                                                     c

s  d  n  e  d
CTGATAATGAAGATtaaacagttggatcatcgaaagagcgaaaaagaacgattatacatttaacgaaaaattacccacttagaattatagctccttgcag   1400
acgctttcaaaaccacccaaccaactcttaaggcatcggactttttccataaatgtccagagtcagtgtctgtatttatgataaccatttcaataaat   1500
aaaaaaaacctttattctgctggagcctatataagtaacttttatttgcgacttgttgctcaacggtcaggatatgacgcgccatatgctga
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FIG. 4. Genomic sequence of the HP-1 locus. The predicted protein-coding DNA sequence is shown by uppercase letters. Corrections of previously published sequence information (10) are indicated by symbols below the DNA sequence (▲, nucleotide change; +, nucleotide insertion). A star indicates the position at which genomic HP-1 sequence diverges from the upstream sequence of a previously described cDNA. Lowercase letters below the sequence indicate nucleotides found to differ in the *Su(var)205* allele. Arrows indicate the first nucleotide of cDNAs recovered from various cDNA libraries; from right to left, the start sites correspond to (*i*) clones 213 and 321, (*ii*) clones 343 and 351, (*iii*) clone 431, (*iv*) clones pTH1 and pTH5, and (*v*) clone 243 (see Fig. 5*B*). Dots above the sequence indicate putative transcription start sites predicted from primer extension analysis using poly(A)$^+$ RNA from adult flies. The sequence of the intron within the 5′ untranslated region is indicated by brackets. Note the terminal AAG sequence found at the 3′ end of this intron.

dation protein sequence bank (September 1990) turned up no proteins with significant homology to the revised HP-1 protein sequence.

DISCUSSION

Both programmed X chromosome inactivation in mammals and variegating position effects in *Drosophila* can be explained in terms of gene inactivation through the imposition of a heterochromatin-like structure upon otherwise euchromatic genes. Variegating position effects in *Drosophila* provide an especially useful assay system for genetic dissection of heterochromatin function, since mutations which influence heterochromatin structure may reveal themselves by causing shifts in the extent of phenotypic variegation (8, 9). Mutations which act as either dominant enhancers or dominant suppressors of position-effect variegation have been uncovered in several screens (2–7), and examination of autosomal deficiencies and duplications points to a dosage model for an explanation of the effects of at least some of the known loci (6, 7). That one such locus encodes a protein with a reiterated zinc-finger-like motif further suggests a DNA-binding role for some of these proteins (34).

Su(var)205, a dominant suppressor of position-effect variegation, was identified as an ethyl methanesulfonate-induced modifier of white variegation in the X chromosome inversion $In(1)w^{m4}$ (3). The genetic map position of this mutation, at 30.8 ± 1.6 (35) on the second chromosome of *D. melanogaster*, roughly coincides with the locus encoding the heterochromatin-specific nonhistone chromosomal protein HP-1, at cytological map position 29A (10). In this report, we provide evidence that the *Su(var)205* mutation is a point mutation in the HP-1 gene causing aberrant splicing of the HP-1 gene transcript.

Two aberrant cDNAs were recovered from the *Su(var)205/In(2LR)Cy0* stock: one exhibiting a triple exon skip and the other exhibiting a splice to a cryptic, intronic splice site. The former species predicts a mutant mRNA 355 nucleotides smaller than the wild type, which would account for the aberrant transcript detectable in Northern blot analysis of RNA from *Su(var)205/In(2LR)Cy0* flies (Fig. 1). This aberrant mRNA would lack the normal AUG initiation codon of HP-1; the first in-frame AUG encountered when reading 5′ to 3′ in this cDNA is the methionine codon at amino acid position 176. This AUG is in a good translational start context (33) and, if it were used, would encode a peptide having the C-terminal 30 amino acids of HP-1. Note, however, that an out-of-frame AUG, also in good translational context, precedes this potential in-frame start codon. The upstream triplet would be expected to be used preferentially (33). The other aberrant transcript encoded by the *Su(var)205* allele would be translated into a protein having the amino acid sequence Asp-Lys-Leu-Trp-Asn-Glu-Ser inserted between amino acids 117 and 119, in place of Ala-118.

The defect in the *Su(var)205* HP-1 allele might be exerting its effect upon heterochromatic position-effect variegation in two different ways which are not mutually exclusive. In one model, no functional HP-1 protein product may be made from this allele, and suppression may arise through a simple dosage effect. This is consistent with the report that deficiencies that uncover 29A also act as haplo-abnormal suppressors of position-effect variegation (7). Since duplications of 29A act as triplo-abnormal enhancers of position-effect variegation (6, 7), the dosage effect model seems an attractive

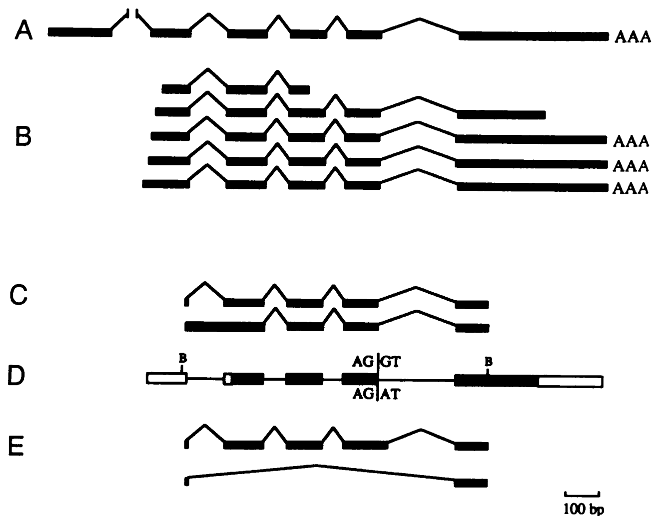

FIG. 5. Structures of cDNA clones containing HP-1 protein-coding sequence. (*A*) Structure of the λgt10 wild-type cDNA clone described by James and Elgin (10). The 5′-most 210 base pairs (bp) contained within this cDNA appear to be located elsewhere in the genome, at a minimum >40 kbp away from the sequences encoding the HP-1 protein. (*B*) Classes of cDNAs recovered from three different wild-type cDNA libraries. From top to bottom: clones 213 and 321 (same structure), isolated from a first/second-instar larval cDNA library (28); clones 343 and 351 (same structure), isolated from a first/second-instar larval cDNA library (28); clone 431, isolated from a third-instar larval cDNA library (28); clones pTH1 and pTH5 (same structure), isolated from a 0- to 4-hr embryo cDNA library (29); clone 243, isolated from a first/second-instar larval cDNA library (28). (*C*) Clones of wild-type cDNA generated from first-strand cDNA followed by PCR amplification. (*D*) Genomic organization of the HP-1 locus. Blocks represent exons and lines represent introns. Filled blocks indicate the extent of the HP-1 open reading frame. The sequence indicated above the map is found at the fourth intron 5′ splice site in wild-type clones. The sequence of the equivalent region in the *Su(var)205* allele is indicated below the map. (*E*) Aberrant cDNAs obtained from the *Su(var)205/In(2LR)Cy* stock by PCR amplification of first-strand cDNA.

one. An alternative explanation, however, is suggested by the detection of a cDNA which could encode a protein having seven amino acids inserted into the middle of HP-1. This novel protein product might participate as a defective subunit in heterochromatin formation and interfere with the integrity of heterochromatin. This would represent an "antimorph" phenotype (36). In either event, the identification of *Su(var)205* as a mutation which causes a change in the amount or structure of a heterochromatic protein confirms a much earlier speculation as to the nature of dominant suppressors of position-effect variegation. That an independently isolated, allelic dominant variegation suppressor has been found to contain a nonsense mutation in the HP-1 gene (T.H., G. Reuter, and J.C.E., unpublished work) further strengthens the association between HP-1 and modulation of heterochromatic position effects. The results reported here, coupled with the earlier characterization of HP-1 (10, 11) and of the *Su(var)205* allele (3), argue strongly that HP-1 is a necessary component of heterochromatin structure required in a precise stoichiometry for normal function of the chromosome.

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