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Heterochromatin Protein 1 Is Involved in Control of Telomere Elongation in *Drosophila melanogaster*

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Telomeres of *Drosophila melanogaster* contain arrays of the retrotransposon-like elements *HeT-A* and *TART*. Their transposition to broken chromosome ends has been implicated in chromosome healing and telomere elongation. We have developed a genetic system which enables the determination of the frequency of telomere elongation events and their mechanism. The frequency differs among lines with different genotypes, suggesting that several genes are in control. Here we show that the *Su(var)2-5* gene encoding heterochromatin protein 1 (HP1) is involved in regulation of telomere length. Different *Su(var)2-5* mutations in the heterozygous state increase the frequency of *HeT-A* and *TART* attachment to the broken chromosome end by more than a hundred times. The attachment occurs through either *HeT-A*/*TART* transposition or recombination with other telomeres. Terminal DNA elongation by gene conversion is greatly enhanced by *Su(var)2-5* mutations only if the template for DNA synthesis is on the same chromosome but not on the homologous chromosome. The *Drosophila* lines bearing the *Su(var)2-5* mutations maintain extremely long telomeres consisting of *HeT-A* and *TART* for many generations. Thus, HP1 plays an important role in the control of telomere elongation in *D. melanogaster*.

Telomeres are specialized DNA-protein complexes at the termini of linear chromosomes that ensure the stability of eukaryotic genomes (47, 64, 65). Specialized mechanisms have evolved to add DNA to the ends of eukaryotic chromosomes, balancing the loss from terminal DNA underreplication (10, 47). In most eukaryotes, a special reverse transcriptase, telomerase, adds telomeric DNA repeats to the chromosome ends, using an internal RNA template (10, 28, 29, 47). In contrast, telomeres of *Drosophila melanogaster* consist of multiple copies of *HeT-A* and *TART* elements having features of non-long-terminal-repeat retrotransposons (6, 40, 47, 48), in particular, an oligo(A) tract at the 3′ end. *HeT-A* and *TART* in telomeres are arranged head to tail (6, 36, 47, 61).

Terminal deletions in *Drosophila* have been obtained (5, 7, 27, 35, 41, 57). Again in contrast to mammalian chromosomes, broken *Drosophila* chromosomes behave as capped ones: they are stably transmitted through many generations (7). *HeT-A* and *TART* were found to be transposed to the ends of broken chromosomes (3, 4, 8, 18, 54, 57). It was shown that *Drosophila* telomeres may be elongated by the transposition of mobile elements to receding chromosome ends (6, 9, 27, 40, 47, 48), as well as by gene conversion using the homologous telomeric sequences as template and by recombination between the telomeric sequences (32, 42). The existence of different mechanisms for telomere elongation prompts one to think that telomere length may be regulated by an as-yet-uncharacterized protein complex bound thereto.

Heterochromatin protein 1 (HP1) has been reported recently to mediate normal telomere behavior in *Drosophila* (23). HP1 is a conserved nonhistone chromosomal protein that is best known for its preferential binding to pericentric heterochromatin (31, 51). The gene encoding HP1, *Su(var)2-5*, is a dosage-dependent regulator of heterochromatin silencing in *Drosophila* (20, 21). HP1 is found in all *Drosophila* telomeres, including the ends of stable terminal deletions (23). The lack of HP1 results in multiple telomere-telomere fusions producing a remarkable spectrum of abnormal chromosome configurations. Thus, HP1 is an important functional component of both heterochromatin and telomere.

In this study, we assess the role of HP1 in the regulation of telomere length. Truncated chromosomes with breaks within the *yellow* gene have been used to assess the frequency and study the mechanism of telomere shortening and elongation (2, 3, 5, 7, 8, 32, 42). The *yellow* gene is required for larval and adult cuticle pigmentation (60). The enhancers controlling *yellow* expression in the wings and body cuticle are located in the upstream region of the *yellow* gene, whereas the enhancer controlling *yellow* expression in bristles resides in the intron (5, 25, 39). Therefore, flies with terminal DNA breakpoints in the upstream region that remove the wing and body enhancers display a y^2-like phenotype: wild-type pigmentation in bristles and lack of pigmentation in the body cuticle, wing blade, and aristae (5). Here we found that additions of *HeT-A* or *TART* to the upstream *yellow* sequences activate *yellow* expression only in aristae. Terminal deficiencies with breaks at the *yellow* promoter or within the *yellow* transcription unit result in a y^1-like phenotype, i.e., complete repression of *yellow* function (5, 7, 32). Additions of *HeT-A* restore *yellow* expression in the bristles and aristae (7, 32), owing to the presence of the promoter at the *HeT-A* 3′ end (13).

Using these observations, we have developed a genetic screen system to monitor the frequency of *HeT-A*/*TART* attachment to the *yellow* receding end. Previously we found that

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transposition of *HeT-A* depended on the line genotype and ranged from less than 2×10^{-5} to 2×10^{-3} (32). Here, we show that mutations in the *Su(var)2-5* gene in the heterozygous state raise the frequency of *HeT-A*/*TART* addition to the ends of terminally truncated chromosomes by more than a hundred times. The results of Southern blot analysis and sequencing suggest that attachment takes place either by transposition to the chromosome end or by recombination between the 3′ terminus of the telomeric *HeT-A* or *TART* element and the receding end of the *yellow* regulatory region.

Previously, we observed that the ends of *yellow* terminal deficiencies could also be elongated by gene conversion if the *yellow* gene on the homologous chromosome served as template (42). *Su(var)2-5* mutations do not significantly increase the frequency of gene conversion with the homologous chromosome as the template source. In contrast, these mutations dramatically potentiate terminal DNA elongation by gene conversion if the template for DNA synthesis is on the same chromosome.

The existence of extremely large arrays of *HeT-A* and *TART* in fly lines bearing *Su(var)2-5* mutations for many generations suggests that HP1 is required for regulation of telomere length in *D. melanogaster*.

MATERIALS AND METHODS

Genetic crosses. All *Drosophila* stocks were maintained at 25°C on a standard yeast extract medium. Genetic symbols of the *yellow* alleles and their origin were described elsewhere (32, 42). Most of genetic markers used were described by Lindsley and Zimm (37). The *yac* chromosome has a deletion of the *yellow* and *achaete* genes but not of any vital genes and thus provides an opportunity to examine the behavior of the *yellow* gene on the homologue in the absence of other *yellow* sequences. The *In(I)w^{m4h}*; *Su(var)2-5^{01}/SM1* line, *In(I)w^{m4h}*; *Su(var)2-5^{04}/In(2L)^{t}In(2R)Cy Cy^{1}Roi^{1}pR^{1}cn^{1}* line, and *In(I)w^{m4h}*; *Su(var)2-5^{05}/In(2L)Cy^{1} In(2R)Cy* line were obtained from the Umeå stock center. The *Df(1)w y^{1}w^{67c23}*; *Su(var)2-5^{02}/CyO y^{+}* line, *Df(1)w y^{1}w^{67c23}*; *Su(var)2-5^{04}/CyO y^{+}* line, *Df(1)w y^{1}w^{67c23}*; *Su(var)2-5^{05}/CyO y^{+}* line, and *yw*; *Dp(2, 2)p90/CyO y^{+}* line were obtained from J. Eissenberg and described in Lu et al. (38). The *y^{tata}w* line kindly provided by C.-T. Wu is described in Morris et al. (43). The *Gaiano* line was obtained from M. Gatti.

For determination of the *yellow* phenotype, the extent of pigmentation in different tissues of adult flies was estimated visually for 3- to 5-day-old females developing at 25°C.

Molecular methods. For Southern blot hybridization, DNA from adult flies was isolated using the protocol described by Ashburner (1). Treatment of DNA with restriction endonucleases, blotting, fixation, and hybridization with radioactive probes prepared by random primer extension were performed as described in the protocols for the Hybond-N^{+} nylon membrane (Amersham, Arlington Heights, Ill.) and in the laboratory manual (52). Phages with cloned regions of the *yellow* locus were obtained from J. Modolell. The clones of *HeT-A* and *TART* were obtained from M. L. Pardue and K. L. Traverse. The probes were made from gel-isolated fragments after appropriate restriction digestion of plasmid subclones.

The junctions between newly transposed mobile elements and the DNA terminus were cloned by DNA amplification with two oligonucleotide primers. The primers used in DNA amplification were from the *yellow* gene and *HeT-A*. The numbers of nucleotide map positions are given in brackets in accordance with the *yellow* (26) and *HeT-A* (8) sequences. The primers in *yellow* are as follows: y1 (CCTGGAACATTGCAC [3053 to 3039]), y2 (AAGACGGCGTCACCAAGG TGATC [3101 to 3078]), and y3 (ACTTCCACTTACCATCACGCCAG [3293 to 3271]). The primers in *HeT-A* are as follows: h1 (TGTTGCAAGTGGCGCGC ATCC [456 to 434]) and h2 (GGTGCTTCCGTACTTCTGGCGG [359 to 338]). The primer in *TART* is t1 (CGAAACGCAACAACAAAATGG [1124 to 1144] [*TART*C]).

The products of amplification were fractionated by electrophoresis in 1.5% agarose gels in Tris-acetate-EDTA buffer. The successfully amplified products were cloned in a Bluescript plasmid (Stratagene, La Jolla, Calif.) and sequenced using the Amersham sequencing kit (Amersham).

RNA extraction and Northern hybridization were done as described by Danilevskaya et al. (15). The *HeT-A* probe, kindly provided by M. L. Pardue, was from element 23Zn-1 (GenBank accession no. U06920); the open reading frame spanned nucleotides 1746 to 4421 (46).

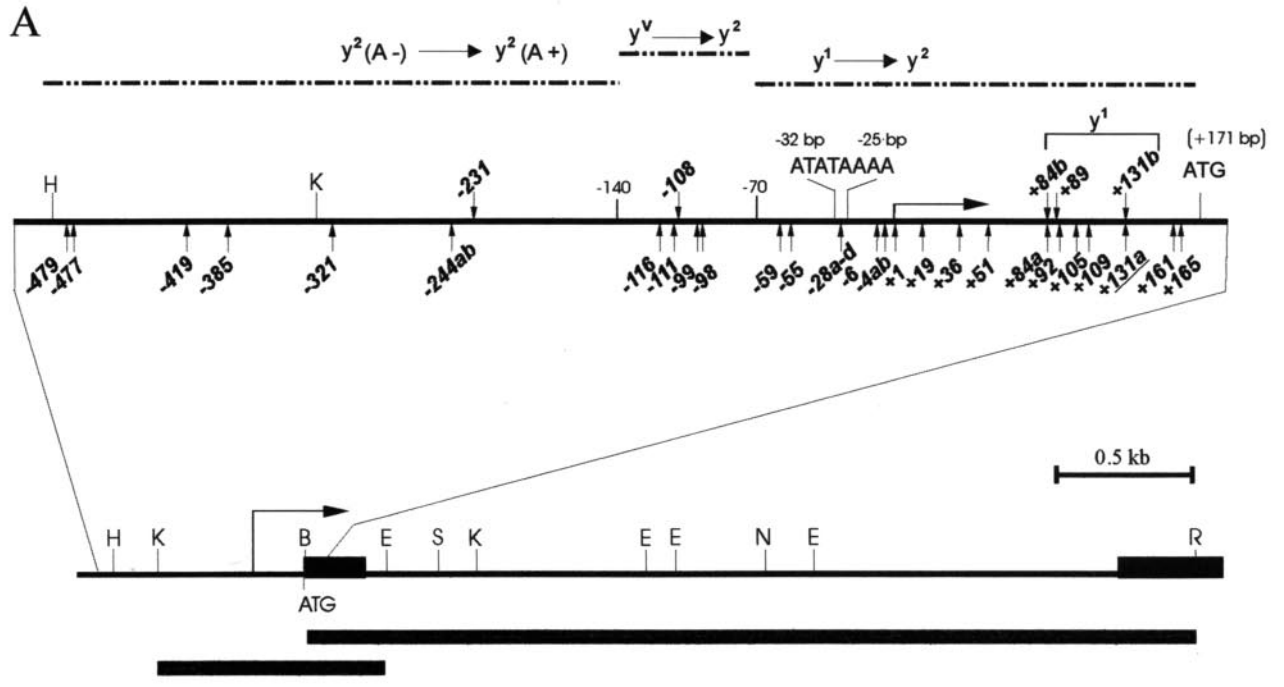
RESULTS

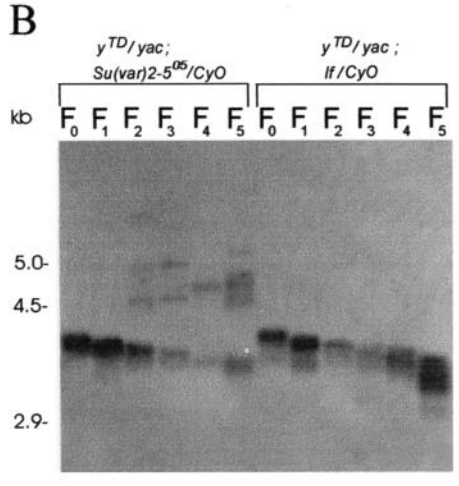
The frequency of *HeT-A* and *TART* attachment to a broken chromosome end is much higher on a *Su(var)2-5* mutant background. First, we tested whether a decrease of functional HP1 concentration induced by the *Su(var)2-5* mutations could change the frequency of *HeT-A*/*TART* attachment to the broken chromosome end. HP1 is a likely candidate for the control of telomere elongation, as it binds to telomeres, and some mutations in the *Su(var)2-5* gene encoding HP1 affect the normal behavior of telomeres (23). We have tested several available *Su(var)2-5* mutations for their influence on the frequency of the *HeT-A*/*TART* attachment to the broken chromosome end by using a modification of the previously described system (32, 42).

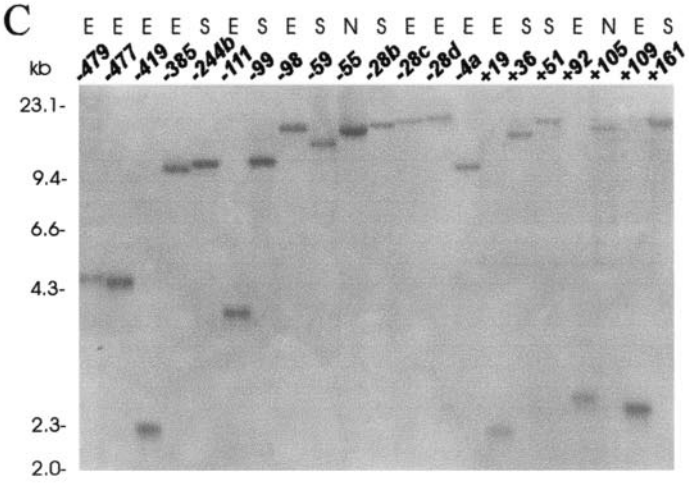
In three *Su(var)2-5* alleles, HP1 fails to interact with telomeres and terminally deficient chromosomes. These mutations are independently obtained recessive lethals (63). *Su(var)2-5^{05}* is formally equivalent to a null mutation (20, 22). *Su(var)2-5^{04}* encodes a truncated HP1 protein that lacks part of the domain required for its nuclear localization (22). *Su(var)2-5^{01}* is a point mutation at the first nucleotide of the last intron leading to formation of two aberrant transcripts (21). In contrast to these, *Su(var)2-5^{02}* is a point mutation changing just one amino acid in a conserved region of the protein (50) known as the chromo domain (CHD) (49). In the *Su(var)2-5^{02}* allele, HP1 is present in telomeres and no chromosome abnormalities are observed (23).

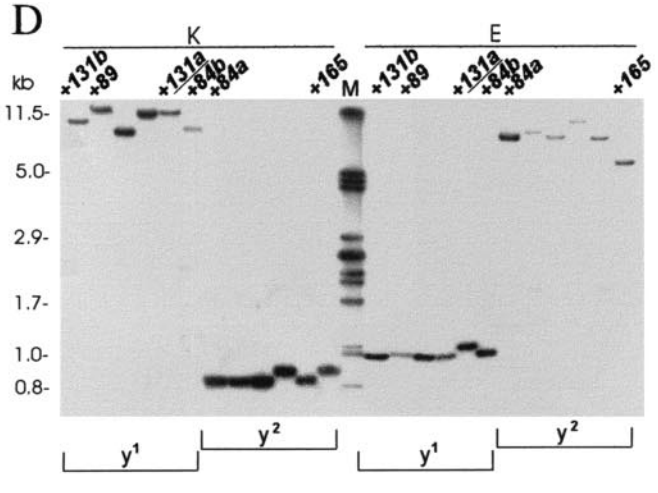
To study the frequency of *HeT-A* and *TART* attachment to broken chromosome ends, we used the terminally truncated chromosomes, with breaks in the *yellow* gene designated *yellow* terminal deficiencies (*y^{TD}*). Breaks that place the end of the chromosome at the *yellow* promoter or within the *yellow* transcription unit between positions −140 and +171 (ATG codon) relative to the *yellow* transcription start site result in a variegated-bristle (y^{v}-like) or y^{1}-like phenotype (Fig. 1A). Addition of a promoter-containing *HeT-A* to the end of the deficient chromosome (between bp −140 and +171) generates *y^{2}*-like alleles, i.e., *y^{1}* → *y^{2}* or *y^{v}* → *y^{2}* (32). Breaks between bp −1200 and −140 result in the y^{2}-like phenotype with yellow-colored aristae, y^{2}(A−) (Fig. 1A). Addition of either a *HeT-A* or a *TART* sequence restores aristal pigmentation. This observation allowed us to monitor the attachment of both *HeT-A* and *TART* to the *yellow* terminal sequences.

Five *y^{TD}/yac*; *If*/*CyO* lines carrying deficiencies terminating in the region from bp −700 to −300 were selected with the aid of Southern blot hybridization from among the flies displaying a y^{2}(A−) phenotype. The *y^{2TD}/yac*; *If*/*CyO* females were crossed to *yac*; *Su(var)2-5*/*CyO* males as shown in Fig. 2A. In the offspring, *y^{2TD}/yac*; *Su(var)2-5*/*CyO* females were crossed to *yac*; *Su(var)2-5*/*CyO* males for five successive generations to determine the appearance of flies with the y^{2}(A^{+}) phenotype. As a control, *y^{2TD}/yac*; *If*/*CyO* females were crossed to *yac*; *If*/*CyO* males. Since similar frequencies of terminal DNA ad-


A
y2 (A -) → y2 (A +)
yv → y2
y1 → y2
y1
-32 bp
-25 bp
ATATAAAA
(+171 bp)
ATG
H
K
-231
-140
-108
-70
+84b
+89
+131b
-479 -477 -419 -385 -321 -244ab -116 -111 -99 -98 -59 -55 -28a-d -6 -4ab +1 +19 +36 +51 +84a +92 +105 +109 +131a +161 +165
0.5 kb
H K B E S K E E N E R
ATG


B
yTD/yac; Su(var)2-505/CyO
yTD/yac; If/CyO
kb
F0 F1 F2 F3 F4 F5 F0 F1 F2 F3 F4 F5
5.0-
4.5-
2.9-


C
E E E E S E S E S N S E E E E S S E N E S
kb
-479 -477 -419 -385 -244b -111 -99 -98 -59 -55 -28b -28c -28d -4a +19 +36 +51 +92 +105 +109 +161
23.1-
9.4-
6.6-
4.3-
2.3-
2.0-


D
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+131b +89 +131a +84b +84a +165 M +131b +89 +131a +84b +84a +165
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5.0-
2.9-
1.7-
1.0-
0.8-
y1
y2
y1
y2

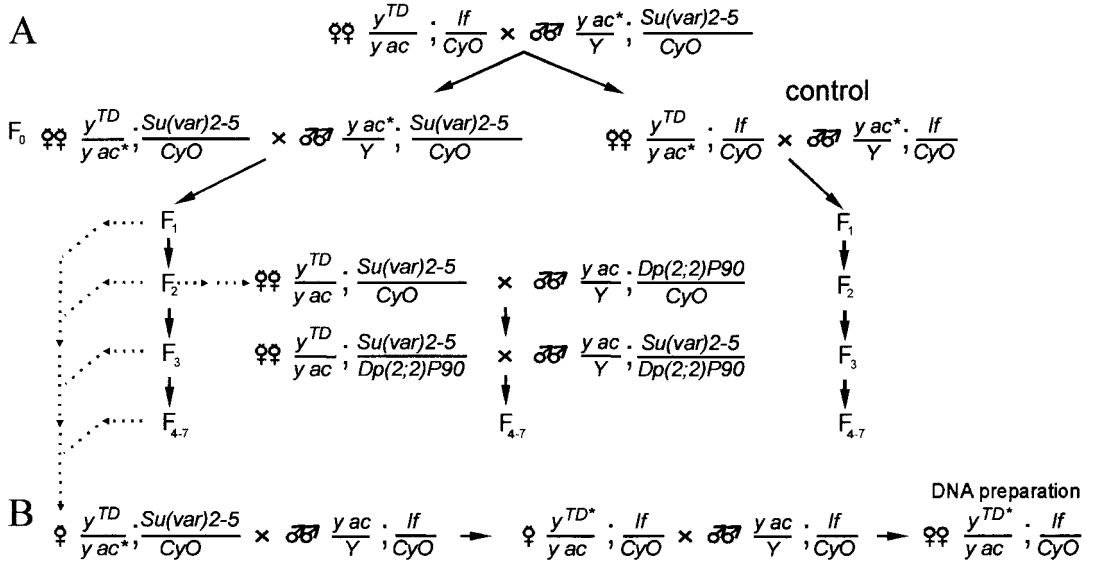


FIG. 2. The scheme used to study the role of *Su(var)* mutations in terminal DNA elongation. (A) The main scheme of crosses. The mutations of *Su(var)2-5* tested were *Su(var)2-5*^{01}, *Su(var)2-5*^{02}, *Su(var)2-5*^{04}, or *Su(var)2-5*^{05}. Depending on the purpose of the experiment, the y^{TD} chromosome contains different *y* alleles at the end of the terminally truncated chromosome. In some experiments, *yac* chromosome (*yac**) was substituted for either the *yw* or the $y^{tata}w$ chromosome. (B) The scheme for isolation of individual stable lines bearing terminally truncated chromosomes with new DNA attachments. y^{TD}* indicates a *y* allele with a new phenotype (i.e., a new DNA attachment).

dition resulting in the $y^2(A^+)$ phenotype have been observed for different y^{TD} lines, these results are combined in Table 1.

All four *Su(var)2-5* mutations tested have a strongly dominant effect on the frequency of *HeT-A*/*TART* attachment to *yellow* (Table 1). In the first generation, the frequency of *HeT-A* attachment is comparable in the y^{TD}/*yac*; *Su(var)2-5*/*CyO* and control y^{TD}/*yac*; *If*/*CyO* lines. However, in the subsequent generations, the frequency of *HeT-A*/*TART* attachment was more than 100 times higher on the *Su(var)2-5* mutant background. The lack of attachment in the first generation may be explained by a significant maternal contribution of HP1 (38). Much the same frequencies of *HeT-A*/*TART* addition have been obtained with all *Su(var)2-5* mutations, including *Su(var)2-5*^{05}, a null allele, and *Su(var)2-5*^{02}, which does not interfere with HP1 binding to telomeres or with telomere behavior (23).

The *Su(var)2-5* mutations differ in their origins, which diminishes the possible effect of a hypothetical linked but unrelated mutation. To further eliminate the possible influence of the genotype, we introduced into the y^{TD}/*yac*; *Su(var)2-5*^{02}/*CyO* and y^{TD}/*yac*; *Su(var)2-5*^{05}/*CyO* lines the *Dp(2; 2)P90* duplication covering the *Su(var)2-5* gene (Fig. 2A). *Dp(2; 2)P90*, in combination with the $In(I)w^{m4h}$ chromosome, enhances the effect of position on variegation, indicating that there is a functional *Su(var)2-5* gene in this duplication. In the presence of *Dp(2; 2)P90*, the frequency of *HeT-A*/*TART* attachment was strongly diminished (Table 1), confirming the role of mutations in the *Su(var)2-5* gene in the activation of *HeT-A*/*TART* attachment.

In terminal chromosomal deficiencies, 70 to 75 bp are lost per generation (2, 3, 5). To study the rate of DNA loss in the presence of the *Su(var)2-5* mutations, we isolated the DNAs from y^{TD}/*yac*; *Su(var)2-5*/*CyO* flies and control y^{TD}/*yac*; *If*/*CyO* flies over six consecutive generations. In every generation, the size of terminal fragments was independently measured using Southern blot analysis (Fig. 1B). In both *Su(var)2-5* mutant

FIG. 1. Molecular structure of the broken chromosome end in the *yellow* gene. (A) A schematic presentation of terminal *yellow* deficiencies associated with different y phenotypes. The promoter (ATATAAAA) and start of translation (ATG) locations are indicated relative to the transcription start site of the *yellow* gene. The coding *yellow* region is shown as a black box. The location of the start of the *yellow* transcription region is shown by a horizontal arrow on the uppermost solid horizontal line. The dotted horizontal lines show the regions of the *yellow* sequence in which the termini of the y^{TD} line that correspond to the same classes of y phenotype have been mapped. The *Kpn*I-*Eco*RV and *Bam*HI-*Eco*RI genomic fragments used as a probe for Southern blot analysis are indicated by the bottommost thick horizontal line and the line just above, respectively. The points of *HeT-A* attachment are shown by small vertical arrows below the uppermost solid horizontal line. The points of *TART* attachment are shown by small vertical arrows above the same line. Restriction enzyme abbreviations: B, *Bam*HI; K, *Kpn*I; H, *Hin*dIII; E, *Eco*RV; N, *Nru*I; S, *Spe*I; R, *Eco*RI. (B) Effect of the *Su(var)2-5*^{05} mutation on the rate of terminal DNA shortening. Southern blot analysis of DNAs prepared from 10 to 14 y^{TD}/*yac* females of the *Su(var)2-5*^{05} and control lines taken in six subsequent generations as described in the Fig. 2A legend. DNAs were digested with *Eco*RI. The filter was hybridized with the *Bam*HI-*Eco*RI probe. The additional bands correspond to new DNA attachments to the receding *yellow* sequences at the end of truncated chromosome. (C) *HeT-A* and *TART* transpositions to the broken chromosome end in the *yellow* gene. DNAs were digested with *Eco*RV (E), *Nru*I (N), and *Spe*I (S). The filter was hybridized with the *Kpn*I-*Eco*RV probe. (D) *HeT-A* and *TART* transpositions obtained in the progeny of one y^{TD}/*yac; Su(var)2-5*^{05}/*CyO* female displaying a y^1 phenotype. In the next generation, all y^{TD}/*yac*; *If*/*CyO* daughters with either a y^1- or a y^2-like phenotype were individually crossed for DNA isolation. DNAs were digested with *Kpn*I (K) and *Eco*RV (E). The filter was hybridized with the *Kpn*I-*Eco*RV probe. The junctions between *yellow* and new DNA attachments were cloned and sequenced in the cases designated with numbers (see Fig. 3).

TABLE 1. Frequencies of *HeT-A*/*TART* attachment in the presence of *Su(var)2–5* mutations[a]

Su(var) mutation	No. of flies displaying a new y phenotype/total no. in generation:							% Q (F_2-F_7)[b]
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	
If/CyO	1/1,100	2/1,320	0/1,560	1/1,820	0/1,920	2/2,440	1/1,469	0.06
Su(var)2-5^{01}	2/740	226/1,253	332/1,817	616/2,211	364/1,271	145/967	143/802	22
Su(var)2-5^{02}	6/800	140/983	303/1,122	171/791	108/620	43/273	62/353	20
Su(var)2-5^{04}	0/525	181/733	130/515	96/584	18/146			21
Su(var)2-5^{05}	0/460	98/820	128/578	70/459	61/311	67/720		14
Su(var)2-5/Dp(2;2)P90				80/806	9/1,457	2/1,365	4/1,417	0.35[c]

[a] The scheme of crosses is shown in Fig. 2. All tested lines have genotypes y^{TD}/*yac*; *Su(var)2-5/CyO* and y^{TD}/*yac*; *If/CyO*.
[b] Q, average frequency of visible events signifying a new y phenotype(s) versus total number of flies scored (as a percentage).
[c] In the case of the *Su(var)2-5/Dp(2;2)P90* combination, Q was calculated for generations F_5 to F_7 only.

and control lines, the chromosomes lost DNA sequences from the broken end at the same rate of 70 to 80 bp per generation. Thus, *Su(var)2-5* mutations do not significantly influence the stability of the terminal chromosomal deficiencies.

Possible mechanisms of *HeT-A* and *TART* attachment to the terminal *yellow* sequences. Previously, we have shown that *HeT-A* attachment to a receding chromosome end containing a *HeT-A* sequence can occur via transposition, conversion, or recombination with *HeT-A* 3′ termini of other telomeres (32). To determine the molecular nature of DNA attachment in the presence of the *Su(var)2-5* mutations, the *Su(var)2-5* mutations were crossed out from the individual y^{TD}/*yac*; *Su(var)2-5/CyO* females as shown in Fig. 2B. As a result, new *HeT-A* and *TART* additions were selected in stable y^{TD}/*yac*; *If/CyO* progeny.

To reveal new DNA attachments that could not be selected by phenotype, we also examined all progeny obtained from 10 females. In the progeny of one female, all daughters acquired new DNA attachments (Fig. 1D). A number of the derivatives obtained in the progeny of one female had identical restriction maps of the new DNA attachments, suggesting that the mutation happened at an early premeiotic stage in the germ line. However, in most cases the new DNA attachments had a different restriction map (Fig. 1C), suggesting that they arose independently at later stages in the germ line. Frequently, y^{TD} females displaying mosaic pigmentation of bristles appeared. In the progeny of such females, daughters displayed either a y^2-like (new DNA attachments) or an original y^1-like (no DNA attachments) phenotype (data not shown). This suggests that the DNA attachments were in somatic cells.

Many *HeT-A* elements have sites for *Kpn*I and *Eco*RI restriction endonucleases in the 3′ region and for *Spe*I and *Eco*RV in the central region and no sites for *Nru*I (3, 7, 8). On the other hand, all these endonucleases have sites in the *yellow* transcription unit in the vicinity of the terminal deficiency (Fig. 1A). Therefore, these enzymes were used for DNA hydrolysis in the analysis of *HeT-A* attachments (Fig. 1C). The *Kpn*I-*Eco*RV fragment subcloned from the *yellow* gene was used as a probe (Fig. 1A). The *TART* elements have a more complex and variable restriction map (15, 54).

To prove the nature of the attached elements, the junctions between terminal *yellow* sequences and new DNA attachments were cloned by PCR and sequenced (Fig. 3). The PCR primers were located in the *yellow* gene and in the conserved regions from the 3′ ends of *HeT-A* or *TART*.

Most of the DNA attachments had *Kpn*I and *Eco*RI sites at the 3′ end, as is typical of *HeT-A*. The sizes of new DNA additions differed greatly, from 1.4 to more than 20 kb (Fig. 1C and 3). In the case of large DNA additions, we could precisely estimate only the minimal size of attached DNA because of the possible existence of additional restriction sites within the attached DNA sequence.

Analysis of junctions between terminal *yellow* sequences and *HeT-A* sequences shows that *HeT-A* elements are attached to random *yellow* terminal sequences (Fig. 3). Only one of the *yellow* sequences, i.e., AAAA in the promoter region, was independently targeted four times (−28a, −28b, −28c, and −28d) (Fig. 3). In all cases, a string of adenine residues was present between the *yellow* and *HeT-A* sequences. Sequences of the *HeT-A* 3′ termini (5′ CCAGCAAAGTT 3′) were conserved in half of the *HeT-A* attachments (19 of 38). In other *HeT-A* attachments, the last T was omitted (15 cases) or last two to three nucleotides were missing (4 cases). In some of these cases, short additional sequences appeared at the junction between *yellow* and *HeT-A* (Fig. 3). These sequences are similar to the sequences located at the 3′ end (−981) or in the 5′ untranslated sequences (−470) of *HeT-A*. In two cases (−55 and −59), DNA attachments had two tandem copies of the *HeT-A* 3′ end with a string of adenine residues.

The small size of the attached DNA and the presence of oligo(A) and of a conserved *HeT-A* terminus suggest transposition of *HeT-A* to the receding *yellow*. All these criteria were met in 7 of 11 short DNA additions. Conversely, the large size of DNA additions, presence of several A bases at the end of the target *yellow* sequence, and existence of base substitutions in the normally conserved 3′ terminal GTT triplet argue in favor of recombination between the receding *yellow* and the 3′ terminus of a telomeric *HeT-A* element. In four cases, all these features are combined, and in three others, the presence of oligo(A) in the *yellow* gene is combined with the large size of attachment. These attachments may have appeared as a result

FIG. 3. Diagram of *HeT-A* and *TART* attachment to the receding *yellow* sequences. All *HeT-A* and *TART* additions are indicated as described in the legends to Fig. 1, 4, and 5. The numbers in parentheses show the approximate sizes of the attachments. The base pair locations at the junctions between *HeT-A* or *TART* and *yellow* are shown. The lowercase letters indicate substitutions in the conserved sequences at the 3′ end of *HeT-A* or *TART*. Adenine bases at the junctions may originate either from the *yellow* sequences or from the 3′ oligo(A) tail of *HeT-A* or *TART*.

		3′UTR *HeT-A*	*yellow*
-1041	(>7kb)	TACCTCCACCAGCAAAGTT***A_{1-2}	agatatatgcgatcgctttc
-981	(>10kb)	TACCTCCACCAGCAAA********→	
		→CCACCAGC*********A_{1-2}	agagcattacccacttaggg
-479	(3.6kb)	TACCTCCACCAGCAAAGTT***A_{8-9}	agaaatttggagagcaatgc
-477	(3.5kb)	TACCTCCACCAGCAAAGTgaatA_{11-14}	aaatttggagagcaatgcat
-470	(>6.5kb)	TACCTCCACCAGCAAAGTg***A_5→	
		→ttcgcttgccA_9	gagagcaatgcattctatgc
-419	(1.4kb)	TACCTCCACCAGCAAAGTT***A_3	tgaaattagatcgtcaaata
-385	(>9kb)	TACCTCCACCAGCAAAacct**A_{9-12}	aaataaatagtagtcacaac
-370	(>9kb)	TACCTCCACCAGCAAAGTT***A_{4-5}	acaactttaaaataggtctt
-363	(>9kb)	TACCTCCACCAGtAAAGTT***A_3	taaaataggtcttaatcttt
-321	(1.5kb)	TACCTCCACCAGCAAAGTT***A_6	gcacaaatcagcgcagtttt
-244a	(1.9kb)	TACCTCCACCAGCAAAGT****A_{5-6}	attaaattgggggattcgag
-244b	(>10kb)	TACCTCCACCAGCAAAGTT***A_{7-8}	attaaattgggggattcgag
-116	(>10kb)	TACCTCCACCAGCAAAGT****A_4	gaatcactaaaaccaccgaa
-111	(3.2kb)	TACCTCCACCAGCAAAGTT***A_{5-6}	actaaaaccaccgaagttgg
-99	(>10kb)	TACCTCCACCAGCAAAGTT***A_4	gaagttggcgcgcgccttcg
-98	(>17kb)	TACCTCCACCAGCAAAGT****A_{3-5}	aagttggcgcgcgccttcgt
-59	(>15kb)	TACCTCCACCAGCAAAGTT***A_{11-12}→	
		→AGCAAAGTT***A_4	tcttcgtcttcggagaaaaa
-55	(>15kb)	TACCTCCACCAGCAAAGTgaatA_6	cgtcttcggagaaaaaaacc*
-28a	(>5.5kb)	TACCTCCACCAGCAAAGT****A_{2-6}	aaaacgcggccgacatatta
-28b	(>17kb)	TACCTCCACCAGCAAAGTT***A_{1-5}	aaaacgcggccgacatatta
-28c	(>20kb)	TACCTCCACCAGCAAAGTT***A_{6-10}	aaaacgcggccgacatatta
-28d	(>20kb)	TACCTCCACCAGCAAAG*****A_{16-20}	aaaacgcggccgacatatta
-6	(>15kb)	TACCTCCACCAGCAAAGT****A_8	gccaccagtcgttaccgcgc
-4a	(>9.5kb)	TACCTCCACCAGCAAAGTT***A_3	caccagtcgttaccgcgcca
-4b	(>20kb)	TACCTCCACCAGCAAAGTT***A_9	caccagtcgttaccgcgcca
+1	(9.5kb)	TACCTCCACCAGCAAAGTT***A_{6-7}	agtcgttaccgcgccacggt
+19	(1.8kb)	TACCTCCACCAGCAAAGT****A_2	gtccacagaagaggattaaa
+25	(3.0kb)	TACCTCCACCAGCAAAGT****A_5	gccacggtccacagaagagg
+36	(>15kb)	TACCTCCACCAGCAAAGTT***A_{1-8}	aaaaaaatatcacacagccg
+48	(>20kb)	TACCTCCACCAGCAAAGaT***A_{4-5}	acacagccgaaggctagaga
+51	(>20kb)	TACCTCCACCAGCAAAGTT***A_{5-6}	agccgaaggctagagaagaa
+84a	(>5.5kb)	TACCTCCACCAGtAAAGTc****	gaacatatataaacaaatat
+92	(2.2kb)	TACCTCCACCAGCAAAGTT***A_{4-5}	ataaacaaatatatttttt
+105	(>17kb)	TACCTCCACCAGCAAAGT****A_{11-12}	attttttttattgccaaca
+109	(2.1kb)	TACCTCCACCAGCAAAGTT***A_7	ttttttattgccaacacact
+131a	(>11kb)	TACCTCCACCAGCAAAGTc***A_{17}	ggcttaagtgttaagagtga
+161	(>20kb)	TACCTCCACCAGCAAAGT****A_{4-5}	agctaagtgcaatgttccag
+165	(>7.5kb)	TACCTCCACCAGCcAAGTT***A_{0-2}	aagtgcaatgttccaggaca

		3′UTR *HeT-A*	284bp 3′UTR *HeT-A*
-55	(>15kb)	TACCTCCACCAGCAAAGTT***A_{10-14}	aaaattatttaaattgttaa*

		3′UTR *TART*	*yellow*
-231	(>10kb)	gccaaAGGCacTGAAAATATTTA$_{17-18}$	attcgagtgtatttattaaa
-108	(>6.9kb)	ttttaAGGCGTTGAAAA**TTTA$_{25-29}$	aaaccaccgaagttggcgcg
+84b	(>8.5kb)	CtATTAGGCGTTGAAAA**TTTA$_{20}$	gaacatatataaacaaatat
+89	(>12kb)	CtATTAGGCGTTGAAAA**TTTA$_{36-37}$	atatataaacaaatatattt
+131b	(>10kb)	CcATTAGGCGTTGAAAA**gTTA$_{23}$	ggcttaagtgttaagagtga

		3′UTR *TART*	126bp 3′UTR *HeT-A*
+131a	(>11kb)	CtATTAGGCGTTGAAAA**TTTA$_{20-21}$	aatgtaatgttaaactcaaa

of recombination. Still, in most cases Southern blot analysis and sequencing data do not allow one to discriminate between the two possible mechanisms of *HeT-A* attachment, transposition and recombination. It seems most probable that *Su(var)2-5* mutations induce both mechanisms.

The attachment of *TART* appears to be rarer (5 events) than that of *HeT-A* (38 events). The *TART* attachments had more variable sequences at the 3′ end and a very long string of adenine residues at the junction with *yellow* sequences than *HeT-A* attachments (Fig. 3). In one case (+131a), we found a chimeric attachment that contains 126 bp of the 3′ end of *HeT-A* followed by *TART*.

The results obtained indicate that all tested *Su(var)2-5* mutations in the heterozygous state enhance the number of *HeT-A* and *TART* attachments to the receding *yellow* sequences by at least 100 times.

Su(var)2-5 mutations do not activate terminal gene conversion from a template located on a homologous chromosome. Previously we found that the broken ends of *Drosophila* chromosomes may be extended by gene conversion by using the sequences of a homologous chromosome as template (42). As the frequency of terminal DNA elongation is strongly dependent on the genetic background of a given line, we have examined the terminal gene conversion on the *Su(var)2-5* mutant background.

In the first series of experiments, we checked how *Su(var)2-5* mutations can activate extensive DNA elongation by gene conversion. Two terminal deficiencies (y^{TD}/*yac*) were selected (Fig. 4A), terminating at about bp −550 (y^{TD-550}) and −1100 ($y^{TD-1100}$). The template for gene conversion was the y^1 allele (*yw* chromosome) generated by a single base pair change (ATG → CTG) in the *yellow* first codon (43). This y^1 allele has the intact regulatory region but a nonproductive coding region and therefore yields a null phenotype, namely, lack of pigmentation in all parts of the cuticle. The attachment of *HeT-A* or *TART* to the end of the deficient chromosome in the y^{TD} line leads to a $y^2(A^+)$ phenotype. The addition of at least the body enhancer (bp −1600) to the ends of the deficient chromosomes via gene conversion partially restores *yellow* expression in the body (*yellow* revertant, or y^r). Further addition of *yellow* sequences gradually increases the extent of pigmentation of the body cuticle and wing blades (42). Thus, it is possible to monitor (Fig. 4A) conversion tracks longer than 1,050 (y^{TD-550}) or 500 bp ($y^{TD-1100}$).

The y^{TD}/*yw*; *Su(var)2-5*^{04}/*CyO* and y^{TD}/*yw*; *Su(var)2-5*^{05}/*CyO* lines were constructed as described in the Fig. 2A legend. New y phenotypes were monitored for three subsequent generations (Table 2). Gene conversion generated y^r derivatives, while *HeT-A* or *TART* attachment to the ends of the deficient chromosomes generated $y^2(A^+)$ derivatives. In the first generation (F_1), the *Su(var)2-5* mutant and control lines had similar frequencies of gene conversion and DNA attachment. However, in the next two generations, the frequencies of *HeT-A* and *TART* attachment increased 100-fold, while the frequencies of DNA elongation by terminal gene conversion remained unchanged.

To directly show that our genetic system distinguished *HeT-A*/*TART* attachment to the receding *yellow* sequences from addition of *yellow* sequences by gene conversion, the *Su(var)2-5* mutations were crossed out from the individual y^{TD}/*yac*; *Su(var)2-5*/*CyO* females as shown in Fig. 2B. DNAs of the selected derivatives displaying new y phenotypes were studied by Southern blot analysis (data not shown). All tested y^2-like alleles yielding pigmented aristae had new DNA attachments at the ends, while y^r-like alleles displaying darker pigmentation had the *yellow* enhancers at the end of the deficient chromosomes. The addition of *HeT-A*s to the broken ends was proved by PCR cloning and sequencing of the junctions between *HeT-A* and *yellow* in five $y^2(A^+)$-like alleles (Fig. 3 and 4A).

Although the results obtained argue that the *Su(var)2-5* mutations only enhance the *HeT-A*/*TART* attachment, it is still possible that small conversion tracks do exist. To examine this possibility, we developed a special genetic system to monitor conversion tracks in the range of 100 to 200 bp (Fig. 4A). Using Southern blot analysis, we selected the $y^{TD}+250$ line carrying deficiencies terminating in the coding region of the *yellow* gene. The y^{TD+250} chromosome was balanced over the y^{tata} chromosome, in which the *yellow* TATA promoter (TATAAA) was substituted for CCCGGG (43). As a result, y^{tata} flies displayed yellow bristles. It has been shown that the *HeT-A* promoter can activate *yellow* transcription and the chimeric *HeT-A–yellow* mRNA produces a functional protein if the ATG start codon is not removed (32). Thus, the attachment of *HeT-A* restores *yellow* pigmentation in bristles if the ATG codon in the *yellow* gene has been introduced at the end of the deficient chromosome by gene conversion.

Six independent y^{TD+250}/y^{tata}*w*; *Su(var)2-5*/*CyO* lines were constructed with either *Su(var)2-5*^{04} or *Su(var)2-5*^{05} (Fig. 2A). According to Southern blot analysis, all constructed lines in F_1 carried deficiencies terminating in the region around position +250 downstream of the *yellow* transcription start site (data not shown). Thus, these lines had lost the ATG start codon of the *yellow* gene (+171). The y^{TD} lines were propagated for three generations. For each of the six lines, we examined from 600 to 1,500 flies (altogether, about 5,000 flies). As a result, only two y^2-like females were found (Fig. 4B). The junctions between *HeT-A* and the *yellow* gene were amplified and sequenced (Fig. 3 and 4A). In both cases *HeT-A* was attached to the *yellow* sequences upstream of the ATG start codon, suggesting elongation of the *yellow* sequences prior to the *HeT-A* attachment.

To directly examine the frequency of DNA attachment and gene conversion, the F_2 progeny of individual y^{TD+250}/y^{tata}*w*; *Su(var)2-5*/*CyO* flies were taken for Southern blot analysis (Fig. 4B). In 5 of 21 tested lines, i.e., in ca. 24% of cases, terminal deficiencies had new DNA attachments. In several other lines, the presence of weak bands corresponding to DNA fragments of larger size suggests that new DNA attachments were formed in the progeny of selected females.

Thus, *Su(var)2-5* mutations greatly increase the frequency of *HeT-A* and *TART* attachment to the ends of truncated chromosomes rather than that of gene conversion if the template for DNA synthesis is on a homologous chromosome.

***Su(var)2-5* mutations greatly raise the frequency of gene conversion if the template for DNA synthesis is on the same chromosome.** Thereafter, we studied the effect of *Su(var)2-5* mutations on the ratio of DNA attachment to gene conversion in the presence of a template for gene conversion on the same terminally truncated chromosome. For this, derivatives of the y^{TD2h2} line were used. This line contains a terminally truncated

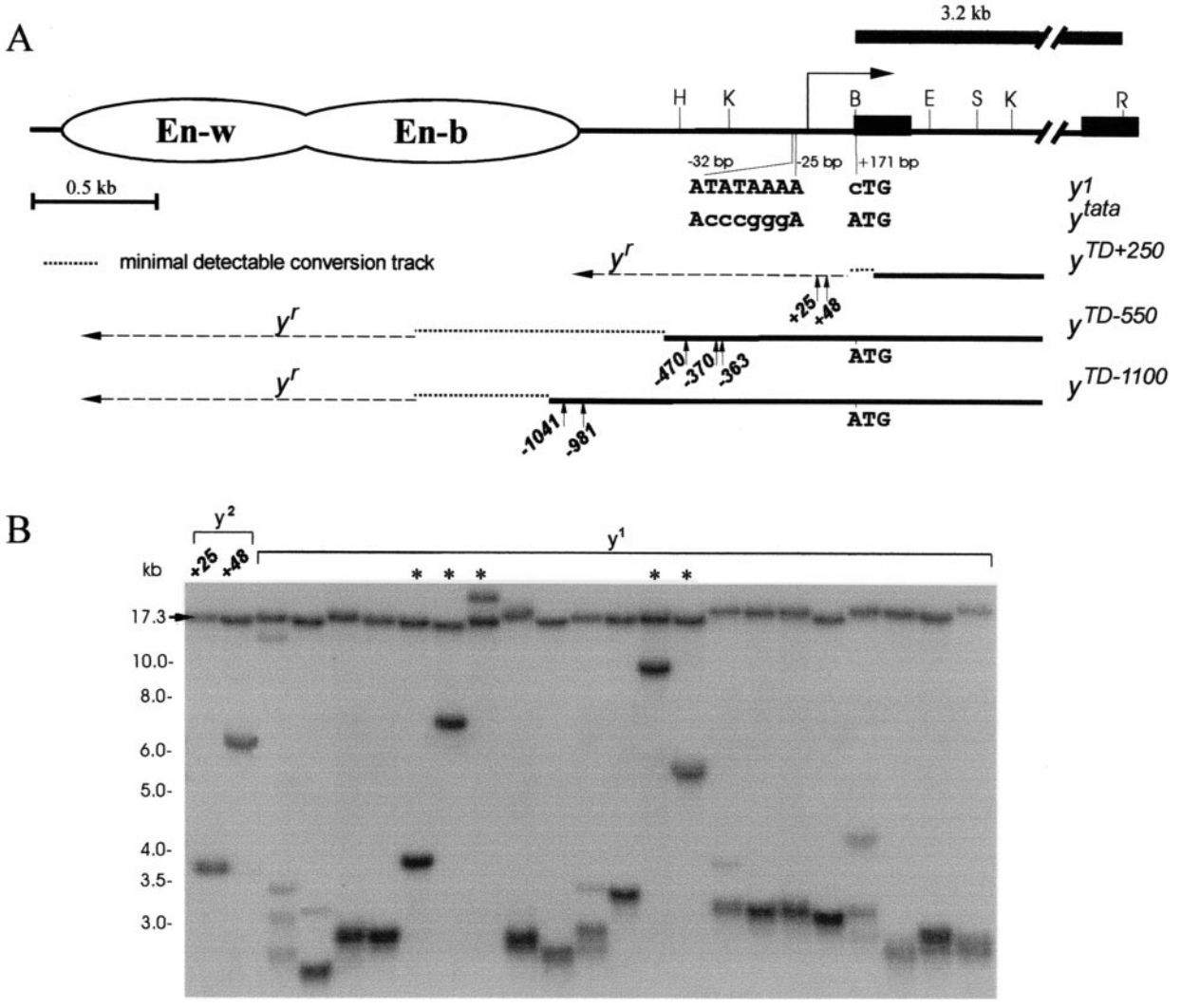


FIG. 4. The model system to study terminal DNA elongation by gene conversion on the *Su(var)2-5* mutant background in the presence of a template on the homologous chromosome. (A) Schematic presentation of the terminal *yellow* deficiencies associated with different *y* alleles. The molecular structures of the y^1 and y^{tata} mutations are shown. The wing (En-w) and body (En-b) enhancers are indicated by ovoids. The approximate regions of the ends of terminally truncated chromosomes in the y^{TD} alleles are shown by three thin horizontal black lines. The dotted horizontal lines show the regions of *yellow* sequence in which the termini of y^{TD} line with original phenotype have been mapped. The dashed horizontal lines show the regions of *yellow* sequence in which the termini of y^{TD} line acquiring a new y phenotype have been mapped. The 3.2-kb *Bam*HI-*Eco*RI genomic fragment used as a probe for Southern blot analysis is indicated by the uppermost thick line. The *HeT-A* attachment points are shown by small vertical arrows below each of the three thin horizontal black lines. The sequences at the junctions between *yellow* and *HeT-A* are shown in Fig. 3. Other designations are as defined in the Fig. 1 legend. (B) Southern blot analysis of DNAs prepared from F_2 progeny of individual $y^{TD+250}/y^{tata}w$; *Su(var)2-5*/*CyO* flies. DNAs were digested with *Eco*RI. The filter was hybridized with the 3.2-kb *Bam*HI-*Eco*RI probe. The 17.3-kb band corresponds to the DNA fragment between two *Eco*RI sites in the y^{tata} allele. Asterisks indicate y^{TD} lines displaying a y^1-like phenotype that acquired new DNA attachments. The presence of additional bands indicates the heterogeneity of the progeny, suggesting that in some sisters, terminally truncated chromosomes acquired new DNA sequences (*HeT-A* or *TART*).

X chromosome which has a duplication of the *yellow* sequences extending from bp +875 to the chromosome end (Fig. 5A). In addition to the *yellow* duplication, a *gypsy* retrotransposon is inserted between the *yellow* enhancers and promoter at position −700. The y^{TD2h2} flies have a y^2-like phenotype because the *gypsy* insulator blocks the interaction between the wing and body enhancers and the promoter (24, 26).

It has been shown that a second *gypsy* insulator placed upstream of the *yellow* enhancers neutralizes the enhancer-blocking activity of the first one (24). As a result, addition of the second *gypsy* insulator to the end of the deficient chromosome restores *yellow* expression in the body and wings (y^r). In y^{TD2h2} flies, the *gypsy* sequences may be duplicated to the end of the deficient chromosome by gene conversion using the homologous *yellow* and *gypsy* sequences located on the same chromosome as the template. Thus, the frequency of intrachromosomal gene conversion can be monitored by scoring flies according to the presence of darker pigmentation of the wing blades and body cuticle (y^r phenotype).

Two y^{TD2h2}/*yac* lines were selected by Southern blot analysis. The ends of deficient chromosomes were located at bp −500 and −600 relative to the *yellow* transcription start site (Fig.

TABLE 2. Frequencies of *HeT-A* additions and terminal gene conversions in the y^{TD}/y^{1} lines[a]

Generation	*Su(var)2–5* mutation	Total no. of flies scored	Gene conversion[b]		DNA attachment[b]	
			No.	% Q	No.	% Q
F_1	*Su(var)2-5*$^{+}$	4,522	12	0.27	2	0.04
	Su(var)2-5^{02}	3,067	14	0.45	1	0.03
	Su(var)2-5^{04}	1,290	3	0.23	0	<0.07
	Su(var)2-5^{05}	2,175	10	0.45	1	0.05
F_2	*Su(var)2-5*$^{+}$	3,821	15	0.40	1	0.03
	Su(var)2-5^{02}	1,246	3	0.24	162	13
	Su(var)2-5^{04}	1,548	1	0.06	375	24
	Su(var)2-5^{05}	1,266	2	0.16	243	20
F_3	*Su(var)2-5*$^{+}$	2,484	18	0.72	0	<0.04
	Su(var)2-5^{02}	648	2	0.31	102	15
	Su(var)2-5^{04}	984	1	0.10	162	16
	Su(var)2-5^{05}	1,690	2	0.12	281	17

[a] The scheme of crosses is shown in Fig. 2.
[b] Abbreviations: No., total number of flies displaying a new y phenotype that is generated either by DNA elongation via gene conversion or by attachment of the telomere-specific mobile elements; Q, average frequency of visible events signifying a new y phenotype versus total number of flies scored (in percent).

5A). Thus, to activate *yellow* expression in the body and wings, the minimal span of the terminal DNA elongation by gene conversion should be 700 to 800 bp. To study the role of HP1, we used the *Su(var)2-5*^{02} and *Su(var)2-5*^{05} mutations. The y^{TD2h2}/*yac*; *Su(var)2-5*^{05}/*CyO* [or y^{TD2h2}/*yac*; *Su(var)2-5*^{02}] lines were constructed as described in the Fig. 2A legend. y^{TD2h2}/*yac*; *Su(var)2-5*/*CyO* females were individually crossed to *yac*/*Y*; *Su(var)2-5*/*CyO* males over four subsequent generations. y^{TD2h2}/*yac*; *If*/*CyO* flies obtained in the same crosses were used as an internal control.

As in the experiments described above, we did not find any detectable difference between the mutant and control lines in F_1 (Table 3). However, in the subsequent generations, flies with darker wing and body pigmentation (y^{r} phenotype) appeared among y^{2}-like females with a frequency of up to 23%. To show that the y^{r} derivatives were generated by gene conversion, the progeny of individual y^{r} females was taken for DNA preparation. Southern blot analysis showed a tight correlation between the y phenotype and the size of terminal DNA elongation in the y^{r} derivatives (Fig. 5C). The point mutation in the HP1 CHD [*Su(var)2-5*^{02}] and complete inactivation of HP1 [*Su(var)2-5*^{05}] enhanced terminal gene conversion to approximately the same level. Thus, HP1 (and its CHD, in particular) is responsible for repression of terminal DNA elongation by gene conversion in y^{TD2h2} flies.

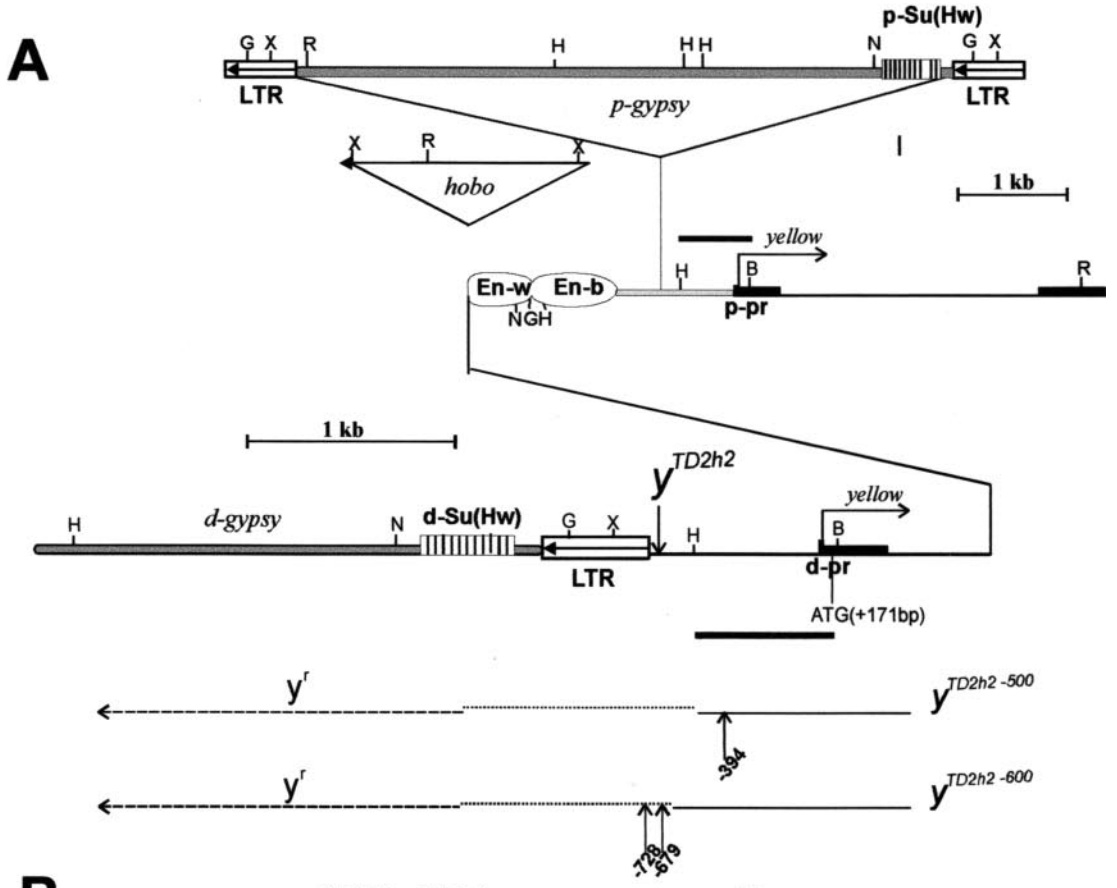
To determine the frequency of *HeT-A* and *TART* transposition, we monitored new DNA attachments between the primers in *yellow* and *HeT-A* for all females in whose progeny we found no y^{r} flies by using Southern blot analysis and PCR amplification (Table 3). The average frequency of DNA attachment was found to be 4.1%, that is, lower than in other experiments. In three cases, the junction between the attached *HeT-A* and *yellow* sequence was cloned and sequenced, confirming *HeT-A* attachment to the end of the terminally deficient chromosomes (Fig. 5B). The results obtained suggest that the presence of two tandem copies of homologous *yellow* sequences at the end of a terminally deficient chromosome significantly reduces the frequency of new DNA attachments induced by the *Su(var)2-5* mutations.

***Su(var)* mutations activate *HeT-A* transcription.** As HP1 is known to be a transcriptional repressor (19, 21), the *Su(var)2-5* mutations may derepress transcription of *HeT-A*s and thereby increase the frequency of *HeT-A* transposition via reverse transcription.

To examine this possibility, we compared *HeT-A* transcription for a wild-type line and on the *Su(var)2-5* mutant background. It was shown that when RNA from flies is probed with sequences from any part of *HeT-A*, the major species of RNA detected is a sense-strand transcript of ~6 kb (14, 15). This is the size expected for a full-length transcript of *HeT-A*. As a probe, we used a DNA fragment containing the *HeT-A* open reading frame (bp 1746 to 4421). In the *Su(var)2-5*^{01}, *Su(var)2-5*^{02}, *Su(var)2-5*^{04}, and *Su(var)2-5*^{05} mutants, as in the *Gaiano* line, *HeT-A* transcripts were 10 times as abundant as in the control lines, *Oregon* and *yac* (Fig. 6).

To exclude the possible contribution of increased copy numbers of *HeT-A* elements to the high *HeT-A* transcription rate, we also examined *HeT-A* transcription in *Su(var)2-5*/*CyO* and *If*/*CyO* females obtained in F_1 and F_2 after crosses of *yac*; *If*/*CyO* females to *Su(var)2-5* males (Fig. 6B). In F_1 females, approximately the same level of content in *HeT-A* transcripts was found in *Su(var)2-5*/*CyO* and *If*/*CyO* (Fig. 6B). This cor-

FIG. 5. Model system to study terminal DNA elongation by gene conversion on a *Su(var)2-5* mutant background in the presence of a template on the same chromosome. (A) Schematic presentation of the y^{TD2h2} allele and its derivatives associated with different y phenotypes. The approximate end of the truncated chromosome in the y^{TD2h2} allele is shown by a downward-pointing vertical arrow. The coordinates (kilobases) in the *yellow* gene and *gypsy* element are defined from the transcription start site of the distal *yellow* promoter. The *gypsy* element is inserted −700 bp upstream of the transcription start site. The Su(Hw) binding sites are indicated by the stripes in the striped boxes. The wing (En-w) and body (En-b) enhancers are indicated by ovoids. The triangle and arrowhead indicate the *hobo* element and its direction, respectively. Abbreviations: d-pr, distal *yellow* promoter; p-pr, proximal *yellow* promoter; d-Su(Hw), distal *gypsy* insulator; p-Su(Hw), proximal *gypsy* insulator; d-*gypsy*, distal *gypsy* retrotransposon; p-*gypsy*, proximal *gypsy* retrotransposon. The approximate ends of the truncated chromosomes in two y^{TD2h2} derivatives are shown by the bottommost two thin black lines. The dotted horizontal lines show the regions of the *yellow* sequence in which the termini of the y^{TD} line with the y^{2}-like phenotype have been mapped. The dashed horizontal lines show the regions of the *yellow* sequence where the termini of y^{TD} line acquiring a y^{r} (*yellow* revertant)-like phenotype have been mapped. The *Hin*dIII-*Bam*HI genomic fragment used as a probe for Southern blot analysis is indicated by thick line segments. The points of *HeT-A* attachment are shown by small arrows with numbers. The sequences at the junctions between *yellow* and *HeT-A* are shown in Fig. 3. Restriction enzyme abbreviations: B, *Bam*HI; K, *Kpn*I; H, *Hin*dIII; N, *Nco*I; G, *Bgl*II; X, *Xho*I. Other designations are defined in the Fig. 1 legend. (B) Diagram of *HeT-A* attachment to the receding *yellow* or *gypsy* sequences. All *HeT-A* additions are indicated as in Fig. 5A. Other designations are defined in the Fig. 3 legend. (C) Southern blot analysis of DNAs prepared from F_2 progeny of individual y^{TD2h2}/*yac*; *Su(var)2-5*/*CyO*^{02} flies. DNAs were digested with *Bam*HI. The filter was hybridized with the *Hin*dIII-*Bam*HI probe. The 13-kb band corresponds to the DNA fragment that hybridized with the proximal *Hin*dIII-*Bam*HI probe. The presence of additional bands indicates the heterogeneity of the progeny, suggesting that in some sisters, terminally truncated chromosomes acquired new DNA sequences.



B

	3' UTR *HeT-A*	*yellow*
-394 (>7kb)	TACCTCCACCAGCAAAGTgcat A_{4-6}	aataaaattaaataaatagt
-679 (>10kb)	TACCTCCACCAGCAAAGTT***A_4	tactctacatagtacacgtt
	3' UTR *HeT-A*	*gypsy*
-728 (>7kb)	TACCTCCACCAGCAAAGTT***A_{4-5}	agaagcaatacattgttagt

C

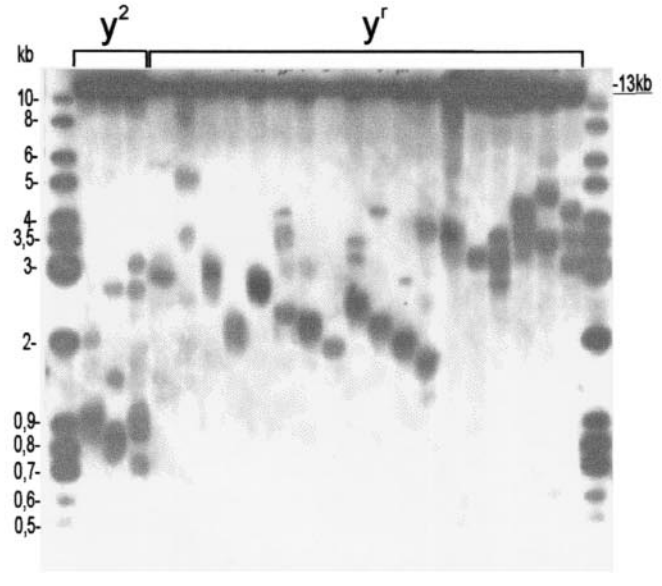


TABLE 3. Extents and frequencies of elongation of terminal sequences in y^{TD2h2} derivatives[a]

Generation	*Su(var)2-5* mutation	Gene conversion[b]			DNA attachment[b]		
		Total[c]	Events	% Q	Total[d]	Events	% Q
F_1	*Su(var)2-5*^{02}	1,400	2	0.14	54	1	2
	Su(var)2-5^{05}	1,650	3	0.18	69	0	<2
	Su(var)2-5^{+}	1,200	2	0.16	41	0	<3
F_2	*Su(var)2-5*^{02}	1,250	186	15	46	3	6.5
	Su(var)2-5^{05}	1,900	445	23	72	2	2.8
	Su(var)2-5^{+}	2,100	7	0.33	86	0	<2
F_3	*Su(var)2-5*^{02}	900	153	7.9	36	2	5.6
	Su(var)2-5^{05}	1,400	302	21.5	44	2	4.6
	Su(var)2-5^{+}	1,600	4	0.25	55	1	2
F_4	*Su(var)2-5*^{02}	750	59	20.1	34	1	3
	Su(var)2-5^{05}	1,200	168	18.2	48	2	2.1
	Su(var)2-5^{+}	1,450	4	0.28	44	0	<3

[a] The scheme of crosses is shown in Fig. 2.
[b] Events, numbers of females displaying a new y phenotype that is generated either by DNA elongation via gene conversion or by attachment of telomere-specific mobile elements; Q, average frequency of visible events signifying a new y phenotype versus number of flies scored (in percent).
[c] Total number of scored females.
[d] Total number of females examined for attachment of *HeT-A* and *TART*.

relates with a low rate of *HeT-A* attachment to the deficient chromosomes and may be explained by a significant maternal contribution of HP1. However, in F_2 the *Su(var)2-5*/*CyO* females displayed high *HeT-A* expression compared with that of the control *If*/*CyO* females. Southern blot analysis showed that samples from *Su(var)2-5*/*CyO* and *If*/*CyO* females had approximately the same levels of *HeT-A* content (data not shown).

Thus, *Su(var)2-5* mutations activate *HeT-A* transcription, and HP1 appears to repress *HeT-A* transcription in telomeres. The activation of the *HeT-A* transcription may partly contribute to the high rate of the transposition-mediated *HeT-A* additions on the *Su(var)2-5* mutant background.

Drosophila lines bearing *Su(var)2-5* mutations often have very high *HeT-A* and *TART* content. The results obtained demonstrate that the *Su(var)2-5* mutations greatly raise the frequency of DNA attachment to a terminally deficient chromosome. To study the possible effect of this phenomenon on the length of *Drosophila* telomeres, we measured the content of *HeT-A* and *TART* in different lines bearing the *Su(var)2-5* mutations.

As controls, we used *Oregon*, *yac*, and *Gaiano* lines. It was shown previously that the *Gaiano* line obtained from natural *Drosophila* populations has very long telomeres (40). As hybridization probes, we used the fragments subcloned from different parts of *HeT-A* and *TART* (Fig. 7A).

We found that DNAs isolated from the *Su(var)2-5*^{01}, *Su(var)2-5*^{04}, and *Su(var)2-5*^{05} lines hybridized with *HeT-A* and *TART* probes with approximately the same intensity as with DNA isolated from *Gaiano* line (Fig. 7B), i.e., much more strongly than with DNA from *Oregon* or *yac* strains. This means that these *Su(var)2-5* lines, like *Gaiano*, have very long telomeric arrays of *HeT-A* and *TART* elements.

However, in the *y*; *Su(var)2-5*^{02}/*CyO* y^{+}, *y*; *Su(var)2-5*^{04}/*CyO* y^{+}, and *y*; *Su(var)2-5*^{05}/*CyO* y^{+} lines obtained from Joel Eissenberg, the lengths of *HeT-A*/*TART* stretches were much smaller. The latter three lines were constructed a year and a half before our study (38), while other *Su(var)2-5* lines have been maintained for many years. One might suggest that the increase in telomere length on the *Su(var)2-5* mutant background is a slow process that takes several years.

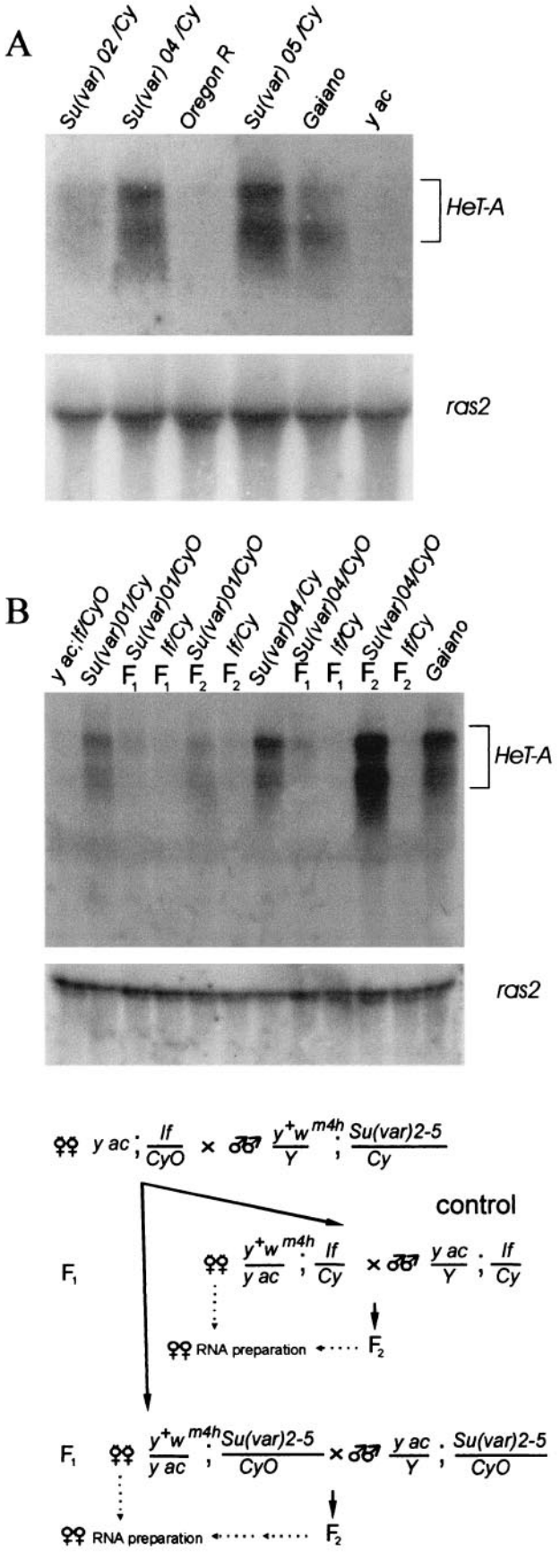
DISCUSSION

HP1 is required for control of telomere elongation in *Drosophila melanogaster*. HP1 is the first identified capping protein of *Drosophila* telomeres (23). No other component of the *Drosophila* telomeric capping complex has yet been identified, in contrast to those of mammal and yeast species (40). The bulk of HP1 is localized on the strongly heterochromatic fourth chromosome and in the pericentric heterochromatin (33, 34). In addition, it has also been clearly located at telomeres (23), where it may be a component of the telomeric end-capping complex. Mutations in the gene encoding HP1, *Su(var)2-5*, are suppressors of centromeric position-effect variegation (21). These mutations do not affect telomeric silencing (12, 23, 59), suggesting that HP1 is not a component of the telomere-associated sequence-binding protein complex, which is mainly responsible for telomeric silencing. However, a complex consisting of HP1, the origin recognition complex (ORC), and HP1/ORC-associated protein appears to bind to the telomere-associated sequence (53). HP1 mutations show phenotypes consistent with the role of HP1 in telomere capping; that is, they lead to frequent telomere-telomere attachment during mitosis and meiosis (23). Thus, HP1 may play an important role in protection of the telomere ends.

The role of HP1 in regulation of terminal DNA elongation was studied using terminal deficiencies located within the *yellow* gene. Broken chromosomes in *Drosophila* behave as capped chromosomes; they are transmitted through many generations (2, 3, 7), and HP1 is present at the ends of terminal deficiencies (23). Thus, the telomere-binding proteins can bind the ends of chromosomes in a sequence-independent manner, and the *yellow* sequences located at the end of the deficient chromosome have the properties of the real telomere.

Here we found that the *Su(var)2-5* mutations resulted in more than a 100-fold increase in the frequency of terminal DNA elongation that was realized either via mobile element attachment or via terminal gene conversion if the homologous sequence was located near the same chromosome terminus. The strong dominant effect of the *Su(var)2-5* mutations suggests that the regulation of telomere length is sensitive to HP1 concentration. The role of HP1 in regulation of telomere length is supported by the finding that the *HeT-A* and *TART* arrays are extremely long in *Su(var)2-5* lines maintained for many generations.

HP1 contains a single amino-terminal CHD motif and a single carboxy-terminal chromo shadow domain (CSD) motif separated by a linker of variable length (hinge region) (19). The CSD specifically binds nucleosomal H4 N-terminal peptides and is important for self-association of HP1 (66). This result may explain the non-sequence-specific binding of HP1 to telomeres and also the fact that CHD inactivation in the *Su(var)2-5*^{02} mutant does not influence HP1-telomere binding (23). Also, a variety of factors have been reported to interact



directly with HP1 through CSD (19). Some of these proteins may play a role in recruiting HP1 to telomeres.

The *Su(var)2-5*^{02} mutation does not induce chromosomal abnormalities as other *Su(var)2-5* mutations inactivating HP1 do (23). However, in our assays, *Su(var)2-5*^{02} had the same dominant effect on the terminal DNA elongation as *Su(var)2-5*^{05}, the null mutation in the *Su(var)2-5* gene. As CHD is not necessary for HP1 binding to telomeres and their stability, these functions can be separated from the suppression of telomere elongation, for which CHD is essential. So far, no direct interactions of HP1 that occur solely through CHD have been described (19). CHD appears to be important for interaction with ORC (45). In mammalian cells, HP1 binds to H3 through its CHD (44). CHD may also be required for modulation of CSD interaction with other proteins that may have a role in formation of the protein complex on telomeres, like the lamin B receptor and Ku-70 (19, 56). These interactions may be critical for the control of telomere elongation. A recent study showed that the *Drosophila* HP1 recognizes a "histone code" involving Lys9 (methyl-K9) in histone H3 (30). It was found that the methyl-K9 binding of HP1 occurs via its CHD. Thus, CHD may be also involved in the effective binding of HP1 to telomeres.

***Su(var)2-5* mutations activate different types of terminal DNA elongation, depending on the presence of a terminal sequence duplication on the same chromosome.** DNA addition to terminal *yellow* sequences derepressed by *Su(var)2-5* mutations may occur either via attachment of mobile element *HeT-A* or *TART* by transposition or recombination or via elongation of the *yellow* sequences by gene conversion using the homologous sequences on the same chromosome as a template. If a template is absent or located on a homologous chromosome, the *Su(var)2-5* mutations markedly increase the frequency of the attachment of both *HeT-A* and *TART* to the end of the deficient chromosome but do not affect the frequency of terminal DNA elongation by gene conversion.

We also found that *Su(var)2-5* mutations strongly enhanced *HeT-A* transcription. As most of the *HeT-A* elements are located at telomeres (47, 48), HP1 appears to be responsible for repression of the *HeT-A* transcription in telomeres. The elevated frequency of *HeT-A* transposition induced by *Su(var)2-5* mutations may be partly explained by a high content of *HeT-A* RNA, which mediates *HeT-A* transposition via reverse transcription (40, 47). However, the activation of *HeT-A* and *TART* transcription cannot influence terminal DNA elongation by recombinational mechanisms, which is also strongly elevated

FIG. 6. Analysis of *HeT-A* transcripts in the *Su(var)2-5* mutant strains. (A) Northern blot hybridization with total RNA isolated from progeny from the *In(I)w*^{m4h}; *Su(var)2-5*^{04}*/In(2L)*^{t}*In(2R)Cy[Su(var)04/Cy]*, *Df(1)w*, and *y*^{1}*w*^{67c23}; *Su(var)2-5*^{02}*/CyO y*$^{+}$*[Su(var)02/Cy]*; Oregon R lines and the *In(I)w*^{m4h}; *Su(var)2-5*^{05}*/In(2L)Cy*^{1} *In(2R)Cy[Su(var)05/Cy]*; *Gaiano* and *yac* lines. The double-strand probe from the open reading frame of *HeT-A* detects a prominent RNA of ~6 kb. The probe from the *ras2* gene gives rise to a 1.6-kb transcript that is used as a marker for the amount of RNA. (B) Northern blot hybridization with total RNA isolated from flies obtained in the crosses is depicted in the lower part of the figure. *Gaiano* and *If/CyO* lines were used as controls with high and low *HeT-A* transcription, respectively. The *In(I)w*^{m4h}; *Su(var)2-5*^{01}*/SM1 [Su(var)01/Cy]* and *In(I)w*^{m4h}; *Su(var)2-5*^{04}*/In(2L)*^{t}*In(2R)Cy Cy*^{1}*Roi*^{1}*pR*^{1}*cn*^{1}*[Su(var)04/Cy]* lines were used in the crosses.

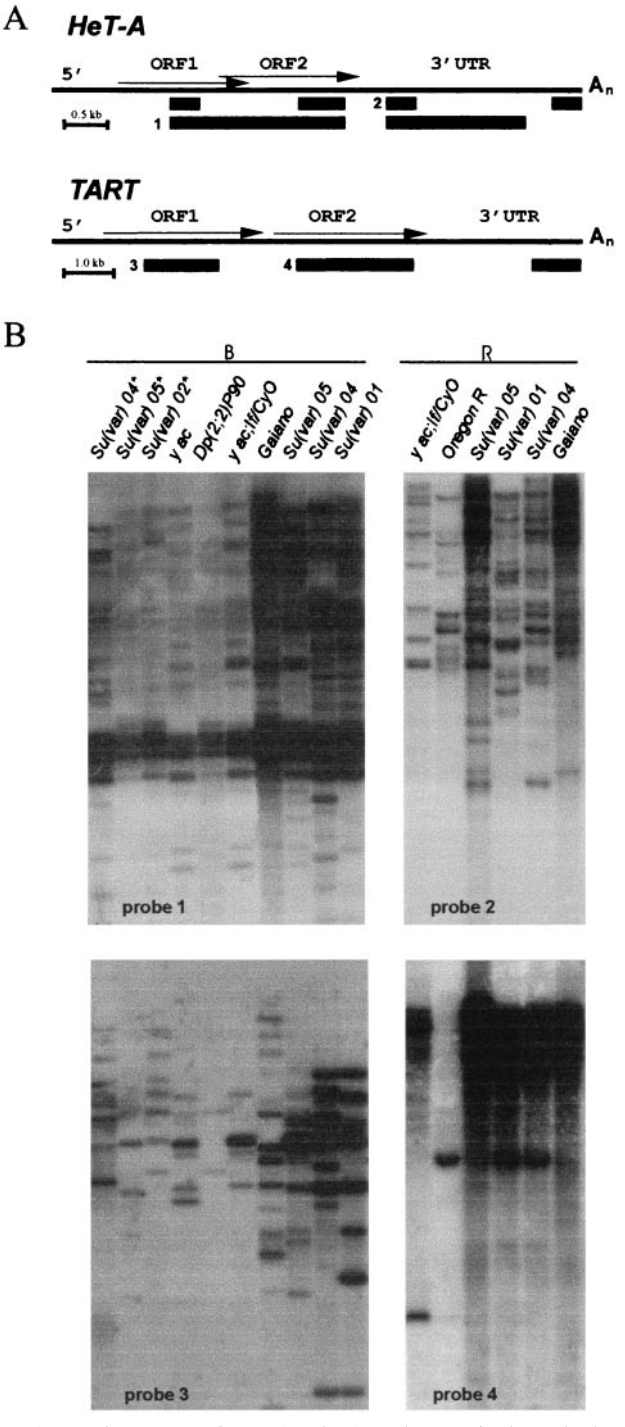


FIG. 7. The content of *HeT-A* and *TART* elements in the *Su(var)2-5* mutants. (A) Diagrams of *HeT-A* and *TART*. The bars under each diagram indicate the sequences used as probes for Southern blot analysis. (B) Southern blot analysis of *HeT-A* and *TART* copy numbers in the *Su(var)2-5* lines. Asterisks indicate new lines obtained from J. Eissenberg (38) as follows: *Su(var)02**, *Df(1)w y*^{1}*w*^{67c23}; *Su(var)2-5*^{02}*/CyO y*$^{+}$; *Su(var)04**, *Df(1)w y*^{1}*w*^{67c23}; *Su(var)2-5*^{04}*/CyO y*$^{+}$; *Su(var)05**, and *Df(1)w y*^{1}*w*^{67c23}; *Su(var)2-5*^{05}*/CyO y*$^{+}$. The controls were *Gaiano* (very long telomeres), *yac*; *If/CyO*, and *Oregon R* (normal telomeres). Results from progeny from the *Su(var)01*, *If/CyO In(1)w*^{m4h}; *Su(var)2-5*^{01}*/SM*; *Su(var)04*, *Df(1)w y*^{1}*w*^{67c23}; *Su(var)2-5*^{04}*/CyO y*$^{+}$; *Su(var)05*, *In(1)w*^{m4h}; *Su(var)2-5*^{05}*/In(2L)Cy*^{1} *In(2R)Cy*, *Dp(2, 2)P90 yw*, and *Dp (2, 2)P90/CyOy*$^{+}$ lines are shown. DNAs were digested with *Bam*HI (B) or *Eco*RI (R). The filters were probed with different fragments indicated in panel A.

on the *Su(var)2-5* mutant background. Thus, HP1 appears to be directly involved in regulation of telomere length.

Two telomere-binding proteins, TRF1 and TRF2, are engaged in telomere length control in human cells (55, 58). TRF1 and TRF2 were shown to be components of a negative feedback mechanism that restricts telomere elongation and ensures stable telomere length. TRF2 can remodel telomeric DNA into t loops in vitro, and TRF1 has several biochemical activities likely to promote t-loop formation (29). The long stretches of the double-stranded telomere DNA are looped around and the single-stranded terminus is tucked back inside the double-stranded DNA molecule, thus protecting the chromosome terminus from DNA damage response and regulating the access to telomerase (28). It was proposed that long telomeres recruit large amounts of the TRF1 and TRF2 proteins that facilitate remodeling of the telomeres into t loops (55). In the t-loop state, telomerase would no longer be able to elongate the telomere, leading to loss of sequences with cell divisions (28, 29).

Smogorzewska et al. (55) also suggested that the same mechanism is operative in telomere length control in the yeast *Saccharomyces cerevisiae*. TRF1 and TRF2 lack significant homology with the proteins Rap1p, Rif1p, and Rif2p that regulate telomere elongation in *S. cerevisiae* (11). Rif1p and Rif2p are in vivo telomere-binding proteins (12) that are brought to the telomere by virtue of their ability to interact with the carboxyl terminus of Rap1p (62). Deletion of either Rif1p or Rif2p results in telomere lengthening, and deletion of both has a synergistic effect (62). Thus, *S. cerevisiae* cells appear to assess the telomere length by monitoring the amount of telomere-bound Rap1p and Rif proteins. There are several pieces of indirect evidence for the presence of t loops in *S. cerevisiae*, which are remodeled by Rap1p and Rif proteins in the same way that TRF1 and TRF2 make t loops in human cells (16, 17). It is proposed that the *S. cerevisiae* telomere folds back onto the subtelomeric regions to form an approximately 3-kb loop (16).

It seems possible that HP1 also regulates *Drosophila* telomere length by participating in the formation of t loops. In the t-loop state, transpositions of *HeT-A* and *TART* are repressed. If homologous sequences are absent from the end of a *yellow* terminal deficiency, the HP1 concentration appears to be critical for loop formation. As a result, the *Su(var)2-5* mutations in heterozygous state can strongly activate *HeT-A* and *TART* attachment. Tandem repeats of *yellow* sequences at the end of the truncated chromosome may facilitate loop formation, which partially diminishes the role of the HP1 concentration in suppression of *HeT-A*/*TART* attachment. Thus, *Su(var)* mutations mainly enhance terminal gene conversion if a homologous sequence is located on the same chromosome. At the same time, the frequency of mobile element attachment is diminished.

Further studies of the role of HP1 and other telomere-binding proteins in the control of *Drosophila* telomere elongation are required for understanding the detailed mechanisms of the process. However, it seems possible that, despite the different organization and structures of telomeric chromatin in humans, yeast species, and *Drosophila*, they share similar basic mechanisms for the control of telomere length.

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