Enhancer of terminal gene conversion, a New Mutation in *Drosophila melanogaster* That Induces Telomere Elongation by Gene Conversion

Larisa Melnikova and Pavel Georgiev[1]

Department of Control of Genetic Processes, Institute of Gene Biology, Russian Academy of Sciences, 117334 Moscow, Russia

Manuscript received March 10, 2002
Accepted for publication August 19, 2002

ABSTRACT

Telomeres of *Drosophila melanogaster* contain arrays of the retrotransposon-like elements *HeT-A* and *TART*. Terminally deleted chromosomes can be maintained for many generations. Thus, broken chromosome ends behave as real telomeres. It was previously shown that gene conversion may extend the broken ends. Here we found that the frequency of terminal DNA elongation by gene conversion strongly depends on the genotype. A dominant *E(tc)* (*Enhancer of terminal gene conversion*) mutation markedly increases the frequency of this event but does not significantly influence the frequency of *HeT-A* and *TART* attachment to the broken chromosome end and recombination between directly repeated sequences at the end of the truncated chromosome. The *E(tc)* mutation was mapped to the 91–93 region on chromosome 3. Drosophila lines that bear the *E(tc)* mutation for many generations have telomeres, consisting of *HeT-A* and *TART* elements, that are longer than those found in wild-type lines. Thus, the *E(tc)* mutation plays a significant 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 (Zakian 1996; Pardue and DeBaryshe 1999). Specialized mechanisms have evolved to add DNA to the ends of eukaryotic chromosomes, balancing the loss from terminal DNA underreplication (Blasco *et al.* 1999; Pardue and DeBaryshe 1999). In most eukaryotes, a special reverse transcriptase, telomerase, adds telomeric DNA repeats to the chromosome ends, using an internal RNA template (Blasco *et al.* 1999; Greider 1999; Pardue and DeBaryshe 1999). In contrast, telomeres of *Drosophila melanogaster* consist of multiple copies of *HeT-A* and *TART* elements having features of non-LTR retrotransposons (Biessmann and Mason 1997; Biessmann *et al.* 1997; Pardue and DeBaryshe 1999, 2000; Mason *et al.* 2000), in particular, an oligo(A) tract at the 3′-end. *HeT-A* and *TART* in telomeres are arranged head to tail (Levis *et al.* 1993; Walter *et al.* 1995; Biessmann and Mason 1997).

Terminal deletions in Drosophila have been obtained (Mason *et al.* 1984; Biessmann and Mason 1988; Traverse and Pardue 1988; Levis 1989; Biessmann *et al.* 1990a; Golubovsky *et al.* 2001). Drosophila broken chromosomes behave as capped ones: they are stably transmitted through many generations (Levis 1989; Biessmann *et al.* 1990a). *HeT-A* and *TART* were found to be transposed to the ends of broken chromosomes (Traverse and Pardue 1988; Biessmann *et al.* 1990b, 1992a,b; Sheen and Levis 1994). *HeT-A* elements have been shown to transpose to a single chromosome end at frequencies ranging from 10^{-1} to $<10^{-4}$ (Biessmann *et al.* 1992a; Kahn *et al.* 2000; Golubovsky *et al.* 2001), although nothing is known about the control of transposition. It was shown that Drosophila terminal deficiencies might also be elongated by gene conversion using the homologous telomeric sequences as templates and by recombination between the telomeric sequences (Mikhailovsky *et al.* 1999; Kahn *et al.* 2000). However, the relative importance of transposition and conversion in telomere length maintenance is not known.

Truncated chromosomes with breaks within the *yellow* gene have been used to assess the frequency and to study the mechanism of telomere shortening and elongation (Biessmann and Mason 1988; Biessmann *et al.* 1990a,b, 1992a; Mikhailovsky *et al.* 1999; Kahn *et al.* 2000). The *yellow* gene is required for larval and adult cuticle pigmentation (Walter *et al.* 1991). 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 (Geyer and Corces 1987; Biessmann and Mason 1988; Martin *et al.* 1989). 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 (Biessmann and Mason 1988). In a previous study, we showed that gene conversion that restored the correct sequences at the chromosomal terminus took place at a frequency of $\sim 10^{-2}$/generation (Mikhailovsky *et al.* 1999). In that study, a line with a *y w* chromosome bearing a point mutation

[1]*Corresponding author:* Institute of Gene Biology, Russian Academy of Sciences, 34/5 Vavilov St., 117334 Moscow, Russia.
E-mail: georgiev_p@mail.ru

in the ATG start codon was used to balance the terminally truncated chromosomes. However, in other tested lines the frequency of terminal gene conversion was much lower.

Here we found that the *y w* line contains a dominant genetic factor, *Enhancer of terminal gene conversion*, *E(tc)*, that maps to the 91–93 region on chromosome 3 and causes strong enhancement of terminal gene conversion. On the other hand, the *E(tc)* mutation does not significantly influence the frequency of *HeT-A* and *TART* attachment to the broken chromosome end nor does it increase the frequency of recombination between directly repeated DNA sequences at the end of the truncated chromosome. The Drosophila lines bearing the homozygous *E(tc)* mutation for a long time have long telomeres consisting of *HeT-A* and *TART*, suggesting that the *E(tc)* mutation affects the function of the gene regulating the terminal gene conversion.

MATERIALS AND METHODS

Drosophila stocks and genetic crosses: All Drosophila stocks were maintained at 25° on a standard yeast medium. In this study we used the alleles with terminal deficiencies consisting of breaks in the *yellow* gene, designated *yellow* terminal deficiencies (y^{TD}). The y^{TD} alleles with a y^2-like phenotype (wild-type pigmentation in bristles and lack of pigmentation in the body and wings) were designated as y^{TD2}. The y^{TD} alleles with darker wing and body pigmentation (y^r-like phenotype) were designated as y^{TDr}. The origin of the *yellow* alleles is described elsewhere (Mikhailovsky *et al.* 1999; Kahn *et al.* 2000; Melnikova *et al.* 2002).

Most of the genetic markers used were described by Lindsley and Zimm (1992). 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 homolog in the absence of other *yellow* sequences. The *y* allele in the *y w* line is caused by a single-base-pair change (ATG → cTG) in the first codon of the *yellow* coding region (Geyer *et al.* 1990). As a result, the *y* allele has an intact regulatory region but a nonproductive coding region and therefore yields a null phenotype: lack of pigmentation in all parts of the cuticle. The Oregon-R is a standard laboratory wild-type strain. The marked stock *H Sb Gl/MKRS* [*Gl*, 3-41.4 (70C2); *Sb*, 3-58.2 (89B9-10); *H*, 3-69.5 (92D1-92F2)] was provided by the Bowling Green stock center.

The position of *E(tc)* along chromosome 3 was determined by allowing free recombination in *y^{TD}/yac; E(tc)/H Sb Gl* females. Recombinant chromosomes were collected in males over *TM6,Tb* and placed into stocks by crossing these males to *y^{TD}/y ac; TM6,Tb/MKRS* females. The presence of *E(tc)* on the recombinant chromosomes was determined after three and six generations by Southern blot analysis with probes from the *yellow* gene.

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

Molecular methods: For Southern blot hybridization, DNA from adult flies was isolated using a published protocol (Ashburner 1989). Treatment of DNA with restriction endonucleases, blotting, fixation, and hybridization with radioactive probes prepared by random primer extension was performed as described in the protocols for Hybond-N^+ nylon membrane (Amersham, Arlington Heights, IL) and in the laboratory manual (Sambrook *et al.* 1989).

High-molecular-weight DNA was prepared as described in Walter *et al.* (1995). Pulsed-field gel electrophoresis (PFGE) was carried out in a Bio-Rad (Richmond, CA) CHEF DR-II system in 0.5× TBE buffer at 14° for 18–22 hr at a voltage gradient of 6 V/cm, with the switch time ramped linearly from 10 to 90 sec. The gel was stained with ethidium bromide and photographed in UV light. For Southern transfer, the gel was incubated in 0.25 M HCl for 10 min at room temperature and washed twice in H_2O for 15 min.

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 endonuclease digestion of plasmid subclones.

RESULTS

The *y w* line contains a new mutation that increases DNA elongation by terminal gene conversion: Previously we found that the terminal DNA elongation by gene conversion occurred at a high frequency, $\sim 10^{-2}$/generation (Mikhailovsky *et al.* 1999). In Mikhailovsky *et al.* (1999), we balanced terminally truncated chromosomes over the *y w* chromosome. However, when other lines were used in crosses we found the terminal DNA elongation to be much less frequent (data not shown). To explain the dependence of terminal conversion on the genotype, we supposed that the *y w* line had a genetic factor that increased the rate of the terminal DNA elongation in crosses between lines carrying terminally truncated chromosomes and the *y w* line.

To identify this putative genetic factor, we selected one *y^{TD}/y w* line that had as high a level of terminal DNA elongation as a starting line. To examine the frequency of the terminal DNA elongation, we used derivatives of the y^{TD2h2} line (Melnikova *et al.* 2002). This line contains a terminally truncated *X* chromosome with a duplication of *yellow* sequences extending from +875 bp to the chromosome end (Figure 1A). In addition to the *yellow* duplication, a *gypsy* retrotransposon is inserted between the *yellow* enhancers and the 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 *yellow* gene promoter (Geyer and Corces 1987; Gause *et al.* 1998). It has been shown that a second *gypsy* insulator placed upstream of the *yellow* enhancers neutralizes the enhancer-blocking activity of the first one (Gause *et al.* 1998; Melnikova *et al.* 2002). As a result, addition of a 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 as template the homologous *yellow* and *gypsy* sequences located on the same chromosome. Thus, the frequency of the intrachromosomal gene conversion can be monitored by scoring flies with

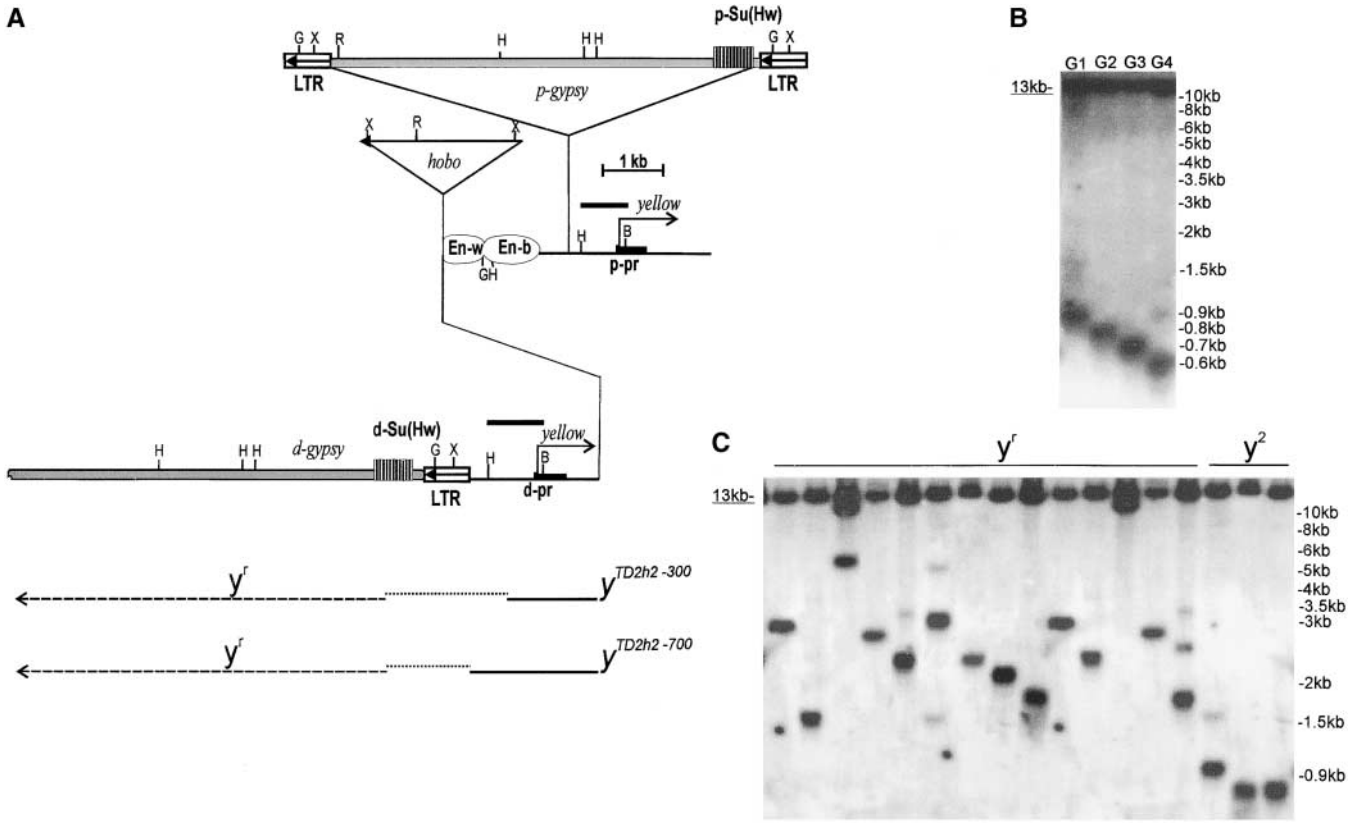


FIGURE 1.—Model system to study terminal DNA elongation by gene conversion in the presence of a template on the same chromosome. (A) A schematic presentation of the $y^{TD2h2\text{-}300}$ and $y^{TD2h2\text{-}700}$ alleles and their derivatives associated with different y phenotypes. The *gypsy* element is inserted −700 bp upstream of the *yellow* gene transcription start site. The Su(Hw) binding sites are indicated by vertical stripes. The wing (En-w) and body (En-b) enhancers are indicated by ovals. The arrow at the top of the triangle indicates the *hobo* element and its direction. d-pr and p-pr, distal and proximal *yellow* promoters; d-Su(Hw) and p-Su(Hw), distal and proximal *gypsy* insulators; *d-gypsy* and *p-gypsy*, distal and proximal *gypsy* retrotransposons. The approximate ends of the truncated chromosomes in the $y^{TD2h2\text{-}300}$ and $y^{TD2h2\text{-}700}$ derivatives are shown by thick lines at the bottom. The dotted horizontal lines show the regions of *yellow* sequence in which the termini of y^{TD} lines with y^2-like phenotype have been mapped. The dashed horizontal lines show the regions of *yellow* sequence in which the termini of the y^{TD} line acquiring a y^r-like (*yellow* revertant) phenotype have been mapped. The *Hin*dIII-*Bam*HI genomic fragment used as probe for Southern blot analysis is indicated as the thick line located above the *yellow* gene transcription start site. B, *Bam*HI; H, *Hin*dIII; G, *Bgl*II; R, *Eco*RI; X, *Xho*I. (B) The rate of terminal DNA shortening in the y^{TD2h2} line. Southern blot analysis of DNA prepared from 10–14 $y^{TD2h2}/y\ ac$ females taken in four subsequent generations. DNA was digested with *Bam*HI. The filter was hybridized with the *Hin*dIII-*Bam*HI probe. The 13-kb band (marked on the left) corresponds to the DNA fragment that hybridized with the proximal *Hin*dIII-*Bam*HI probe. (C) Southern blot analysis of DNA prepared from the F_2 of individual $y^{TD2h2\text{-}700}/y\ ac$ flies displaying either y^2-like or y^r-like phenotype. DNA was digested with *Bam*HI. The filter was hybridized with the *Hin*dIII-*Bam*HI probe. The 13-kb band (marked on the left) corresponds to the DNA fragment that hybridized with the proximal *Hin*dIII-*Bam*HI probe. The presence of additional bands indicates size heterogeneity of the progeny, suggesting that, in some sisters, terminally truncated chromosomes acquired new DNA sequences.

darker pigmentation of the wing blades and body cuticle (y^r phenotype).

Two $y^{TD2h2}/y\ ac;\ CyO/If;\ TM6,Tb/MKRS$ lines were selected by Southern blot analysis. In these lines the ends of deficient chromosomes were located at ∼−300 bp ($y^{TD2h2\text{-}300}$) and −700 bp ($y^{TD2h2\text{-}700}$) relative to the *yellow* transcription start site (Figure 1A). Thus, to activate *yellow* expression in the body and wings, the minimal span of the terminal DNA elongation by gene conversion should be 600 or 900 bp. These $y^{TD2h2}/y\ ac;\ CyO/If;\ TM6,Tb/MKRS$ lines gave y^r-like derivatives with a frequency $<2\times10^{-3}$. To study the fate of the DNA terminus in the control $y^{TD2h2}/y\ ac;\ CyO/If;\ TM6,Tb/MKRS$ lines, we isolated the DNA from flies over four consecutive generations. In every generation, the size of terminal fragments was independently measured using Southern blot analysis (Figure 1B). As found previously (BIESSMANN and MASON 1988), the chromosomes lose DNA sequences from the broken end at the same rate of 70–80 bp/generation.

To identify and map the genetic factor that might be responsible for inducing terminal gene conversion, the major chromosomes from the $y^{TD}/y\ w$ line were extracted into the $y^{TD2h2}/y\ ac;\ CyO/If;\ TM6,Tb/MKRS$ genetic background, generating four lines each containing one first chromosome, six lines each containing one second

chromosome, and eight lines each containing one third chromosome from the $y^{TD}/y\ w$ line. In the control $y^{TD2h2}/y\ ac;\ CyO/If;\ TM6,Tb/MKRS,Sb$ line, we obtained 4 y^{r}-like females among 3400 scored progeny in three subsequent generations (1.2×10^{-3}). In four $y^{TD2h2}/y\ w;\ CyO/If;\ TM6,Tb/MKRS,Sb$ lines, 14 exceptional y^{r}-like females were obtained among 4900 scored flies (2.9×10^{-3}). For the six lines carrying chromosome 2 and the four lines carrying chromosome 3 from the original $y^{TD}/y\ w$ line, we examined altogether 7400 flies and found only 10 y^{r}-like females (1.4×10^{-3}). However, in three lines carrying chromosome 3, y^{r}-like females appeared at a high frequency: we found 210 y^{r}-like females among 2700 scored females (8×10^{-2}).

To show that y^{r}-like derivatives were generated by gene conversion, the progeny of individual y^{r}-like females were taken for DNA preparation. Southern blot analysis showed a tight correlation between the y phenotype and the span of terminal DNA elongation in the y^{r}-like derivatives (Figure 1C). Frequently, DNA obtained from the progeny of a single y^{r} female hybridized with several additional bands, suggesting extensive DNA elongation. These results are evidence of a genetic factor on the original $y^{TD}/y\ w$ chromosome 3 that induces DNA elongation at the ends of the deficient chromosomes. We observed that in the progeny of heterozygous $y^{TD2h2}/y\ ac;\ CyO/If;\ 3\ chromosome/TM6,Tb$ females, y^{r}-like derivatives also appeared at a high frequency, suggesting that the genetic factor responsible for telomere elongation is dominant. This factor was named *Enhancer of terminal gene conversion*.

Three years ago, two y^{TD} alleles, y^{TDrh1} and y^{TDrh2}, which had terminal breaks in the sequences of the distal *gypsy* element at ~4.5 kb (y^{TDrh1}) and 6.0 kb (y^{TDrh2}) from the 5′-end of the chromosome, were obtained (Figure 2A). After 5, 15, 37, and 40 generations, the size of terminal fragments in both lines was independently measured using Southern blot analysis (Figure 1B). It was found that the chromosome ends had further shortened. Thus, in the absence of the *E(tc)* mutation, a terminally deficient chromosome is unable to compensate for the DNA loss that is caused by the inability of the DNA replication machinery to completely replicate the ends of linear chromosomes.

To confirm the role of the *E(tc)* mutation in induction of the terminal gene conversion, we introduced *E(tc)* into the y^{TDrh1} and y^{TDrh2} lines. After two generations, the progeny of a single $y^{TD}/y\ ac$ female were examined for the size of terminal fragments (Figure 1C). The existence of many additional bands hybridizing with the *Hin*dIII-*Bam*HI probe indicated extensive DNA elongation in the progeny of all $y^{TD}/y\ ac;\ E(tc)/E(tc)$ females taken. Thus, the *E(tc)* mutation significantly enhances DNA elongation by terminal gene conversion in the presence of two tandem copies of homologous *yellow* sequences at the end of a terminally deficient chromosome.

Genetic mapping of the *E(tc)* mutation: To check whether the effect of the *E(tc)* mutation maps as a single genetic unit, we crossed the *E(tc)* line to the line carrying three dominant markers, *Gl*, *Sb*, and *H*, which span the central part of chromosome 3 (Figure 3A). After allowing free recombination in the heterozygous progeny females, 41 recombinant third chromosomes were recovered and balanced over the *TM6,Tb* chromosome (Figure 3B). As controls, seven nonrecombinant chromosomes were also recovered, four with all the markers and three with none of these markers. After five generations, these stocks were examined for terminal DNA extension and length heterogeneity by Southern blot analysis (Figure 3C). For all recombinants, the results of Southern blot analysis (Figure 3B) are consistent with the localization of the *E(tc)* mutation in the 91–93 region close to the H marker (92D2).

The *E(tc)* mutation does not influence the frequency of recombination between direct repeats located at the end of a truncated chromosome: In the $y^{TD}/y\ ac$ lines displaying the y^{r}-like phenotype, we frequently found exceptional y^{2}-like females. Southern blot analysis showed that y^{2}-like females were generated by deletion of the duplicated *yellow* and *gypsy* sequences through recombination between homologous sequences (Figure 4). To examine the influence of the *E(tc)* mutation on the recombination between direct repeats, we compared the incidence of the y^{2}-like females in the progeny of $y^{TD}/y\ ac;\ E(tc)/E(tc)$ and $y^{TD}/y\ ac;\ TM6,Tb/MKRS$ females that had the same y^{TD} deficiency, y^{TDrh1} or y^{TDrh2}. Eleven independent y^{2}-like derivatives were found among 4200 y^{r}-like females carrying the homozygous *E(tc)* mutation (2.6×10^{-3}). In the control experiment, 7 y^{2}-like derivatives were found among 3400 scored y^{r}-like females (2.1×10^{-3}). By Southern blot analysis, all y^{2}-like derivatives lacked the 13-kb band that is diagnostic of the partial *yellow* gene duplication. Therefore these lines had a deletion of the duplicated *yellow* and *gypsy* sequences (Figure 4B). These results suggest that *E(tc)* does not influence the frequency of recombination between direct terminal repeats.

Drosophila lines bearing the *E(tc)* mutation for a long time have a high *HeT-A* and *TART* content and long arrays of repeated sequences at the end of the truncated chromosome: The results obtained demonstrate that the *E(tc)* mutation greatly raised the frequency of DNA elongation by terminal gene conversion. To study the possible effect of this phenomenon on the Drosophila telomere length, we measured the number of duplications at the end of the truncated chromosome and the content of *HeT-A* and *TART* in $y^{TDrh1};E(tc)/E(tc)$ and $y^{TDrh2};E(tc)/E(tc)$ lines over 2 years. DNA was prepared from females isolated at 3, 15, 35, and 50 generations. As hybridization probes, we used fragments subcloned from different parts of *HeT-A* and *TART* (Figure 5B). Southern blot analysis revealed a direct correlation between the increasing content of *HeT-A* and *TART* and the number of generations after the introduction of

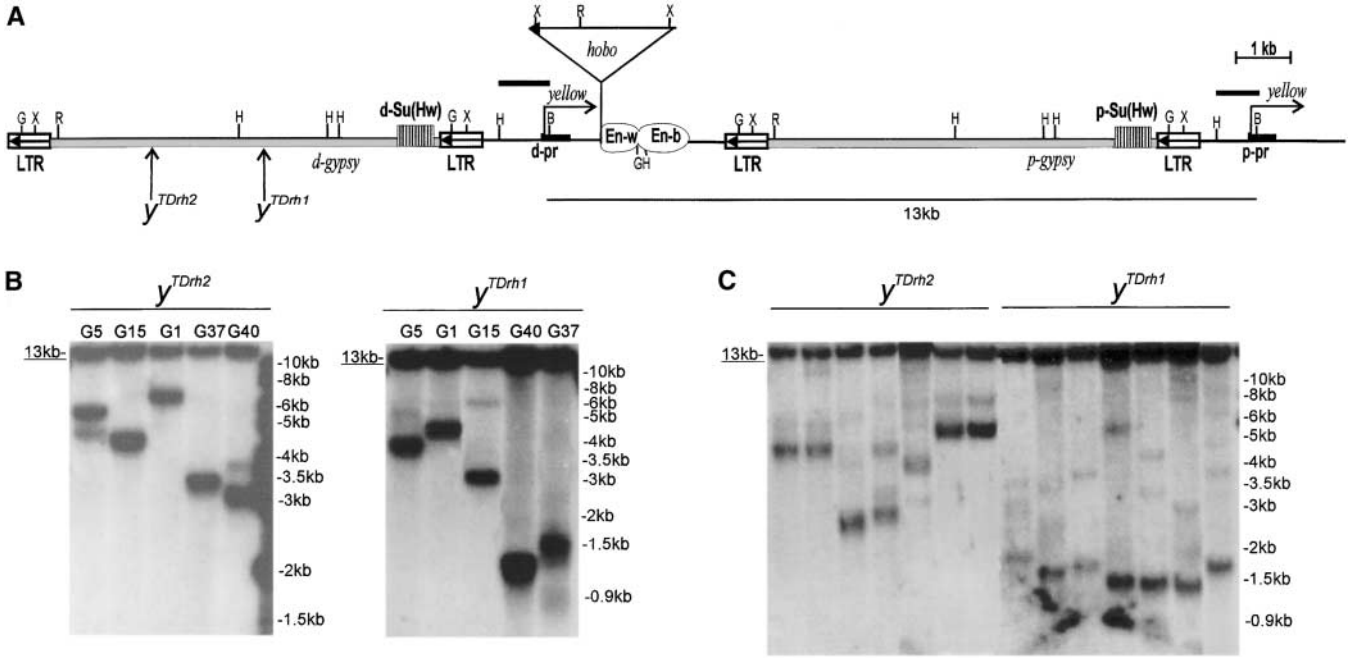


FIGURE 2.—Terminal DNA elongation in the y^{TDrh1} and y^{TDrh2} alleles. (A) A schematic presentation of the y^{TDrh1} and y^{TDrh2}. The approximate ends of the truncated chromosome in the y^{TDrh1} and y^{TDrh2} alleles are shown with upward arrows. Other designations are as in Figure 1. (B) Shortening of the DNA termini in y^{TDrh1} and y^{TDrh2} lines over 40 generations. Southern blot analysis of DNA samples prepared from 10–14 females taken from the 5th (G5), 15th (G15), 37th (G37), and 40th (G40) generations. DNA samples were digested with *Bam*HI. The filter was hybridized with the *Hin*dIII-*Bam*HI probe. (C) Southern blot analysis of the terminal DNA elongation in the y^{TDrh1}*;E(tc)/E(tc)* and y^{TDrh2}*;E(tc)/E(tc)* sublines. DNA samples were isolated from progenies of individual females and digested with *Bam*HI. The filter was hybridized with the *Hin*dIII-*Bam*HI probe.

the *E(tc)* mutation. This means that the *E(tc)* mutation induces elongation of Drosophila telomeres.

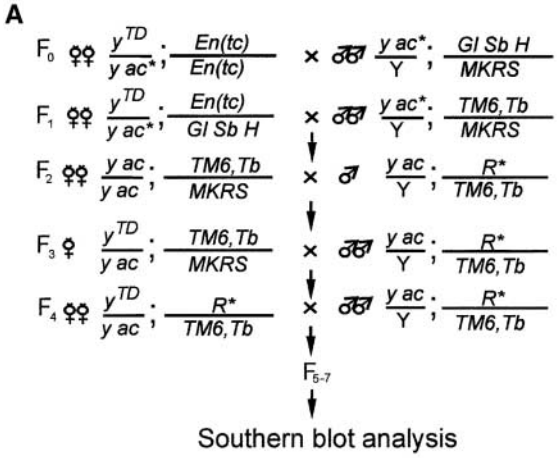
To assess the copy number of the *yellow* and *gypsy* sequences, we digested DNA with *Sac*I and probed it with the *Hin*dIII-*Bam*HI fragment (Figure 5A). The *Hin*dIII-*Bam*HI probe hybridized with two bands (Figure 5C): a 7.5-kb DNA fragment between two *Sac*I sites located in the *yellow* coding region and in *gypsy* and a 9.7-kb DNA fragment between two *Sac*I sites in two neighboring *gypsy* elements. The 7.5-kb band is unique, while the 9.7-kb band corresponds to repeated sequences. The relative intensity of the two bands was measured with a phosphorimager. As a result, direct correlation was found between the increasing number of generations and the number of duplicated copies of the *yellow* and *gypsy* sequences. After 50 generations, both y^{TDrh}*/y ac; E(tc)/E(tc)* lines had at least four copies of the duplicated *yellow* and *gypsy* sequences at the chromosome end (Figure 5C).

The size of the multiplicative region was also determined by PFGE. The *Nru*I endonuclease has a cleavage site in the *yellow* intron, but not in the duplicated *yellow* and *gypsy* sequences. Therefore, this enzyme was used to analyze the size of the DNA extension in y^{TDrh}*/y ac; E(tc)/E(tc)* lines after 50 generations. In both lines, the *Hin*dIII-*Bam*HI probe hybridized with several bands ranging from 28 to 120 kb. The major DNA band for the y^{TDrh2}*/y ac; E(tc)/E(tc)* line corresponds to the ~65-kb DNA fragment that includes five copies of the *yellow* and *gypsy* duplication. The smallest DNA fragment in the y^{TDrh1}*/y ac; E(tc)/E(tc)* line, ~28 kb, corresponds to only two copies of the duplication. The pronounced heterogeneity may be explained by a high frequency of recombination between nearby direct repeats. Southern blot analysis of DNA digested with *Bam*HI and *Eco*RI also showed extensive heterogeneity of the terminal DNA fragment (Figure 5E).

In the y^{TDrh}*/y ac; E(tc)/E(tc)* lines carrying three or four copies of the duplicated sequences, exceptional y^2-like females appeared with low frequency. We found only two y^2-like derivatives among 9700 y^{TDrh}*/y ac; E(tc)/E(tc)* flies (2×10^{-4}). We explain this result by postulating that recombination occurs preferentially between the two nearby DNA repeats located close to the end of the truncated chromosome.

The *E(tc)* mutation does not enhance the frequency of the *HeT-A* and *TART* transpositions: We could not monitor the frequency of *de novo HeT-A/TART* attachment to the broken chromosome end in the experiments described above. Therefore, we used truncated chromosomes with breaks within the *yellow* regulatory region to study the effect of the *E(tc)* mutation on the frequency and mechanisms of terminal DNA elongation.

In the first series of experiments, we examined how the *E(tc)* mutation can activate DNA elongation by gene



B

visible markers (R*)	number of lines +	number of lines −
+	3	0
Gl Sb H	0	4
Gl	8	0
Sb	7	1
Gl Sb	9	1
H	0	9
Sb H	0	6

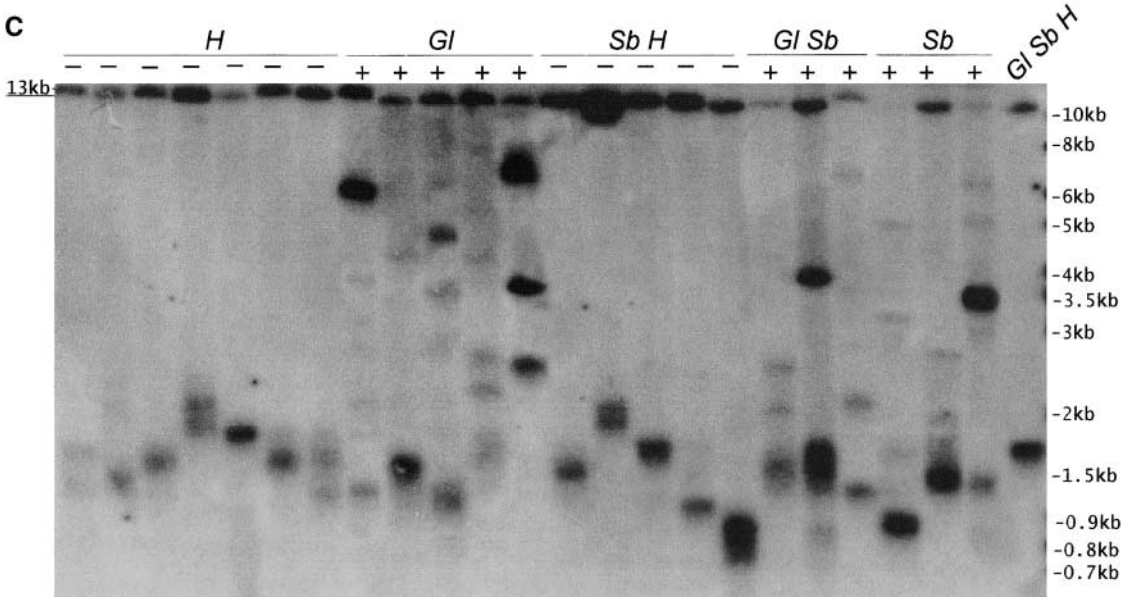


FIGURE 3.—Genetic mapping of the *E(tc)* mutation. (A) Genetic crosses made to generate recombinant chromosomes between *E(tc)* and *Gl Sb H*. *R** indicates a recombinant chromosome. (B) List of recombinants between the chromosome carrying *E(tc)* and the *Gl Sb H* chromosome. Indicated are the numbers of recombinant lines with (+) or without (−) terminal DNA elongation by gene conversion. (C) Southern blot analysis of DNA samples prepared from y^{TDrh1}*/y ac; R*/TM6* lines bearing a recombinant chromosome. The DNA samples were digested with *Bam*HI. The filter was hybridized with the proximal *Hin*dIII-*Bam*HI probe. The presence of additional bands indicates active DNA elongation by terminal gene conversion (+). The 13-kb band (marked on the left) corresponds to the DNA fragment that hybridized with the proximal *Hin*dIII-*Bam*HI probe.

conversion and *HeT-A/TART* attachment if a template for DNA replication is located on the homologous chromosome. Three terminal deficiencies were selected (Figure 6A), terminating at ~−900 bp ($y^{TD\text{-}900}$), −1000 bp ($y^{TD\text{-}1000}$), and −1200 bp ($y^{TD\text{-}1200}$) relative to the *yellow* transcription start site. The template for gene conversion was the *y* allele (*y w* chromosome). Truncated chromosomes having breaks between −1200 and −140 bp result in a y^2-like phenotype with yellow-colored aristae, y^2(A−) (Figure 6A). Addition of either a *HeT-A* or a *TART* sequence restores aristal pigmentation [y^2(A+)]. This observation allowed us to monitor the attachment of both *HeT-A* and *TART* to *yellow* terminal sequences. The addition of at least the body enhancer (−1600 bp) to the ends of the deficient chromosomes via gene conversion partially restores *yellow* expression in the body: *yellow* revertant, y^r. Further addition of *yellow* sequences gradually increases the extent of pigmentation of the body cuticle and wing blades (MIKHAILOVSKY *et al.* 1999). Thus, it is possible to monitor (Figure 6A) conversion tracts longer than 400–700 bp.

In the control y^{TD}*/y w; CyO/If; TM6,Tb/MKRS,Sb* lines we obtained four y^r-like females (1.2×10^{-3}) and two y^2(A+)-like females (0.6×10^{-3}) among 3400 scored progeny in three subsequent generations. In the experimental crosses we examined 4100 y^{TD}*/y w; E(tc)/E(tc)* flies and found 47 independent y^r-like females (1.1×10^{-2}) and only one y^2(A+) female (2×10^{-4}). To show directly

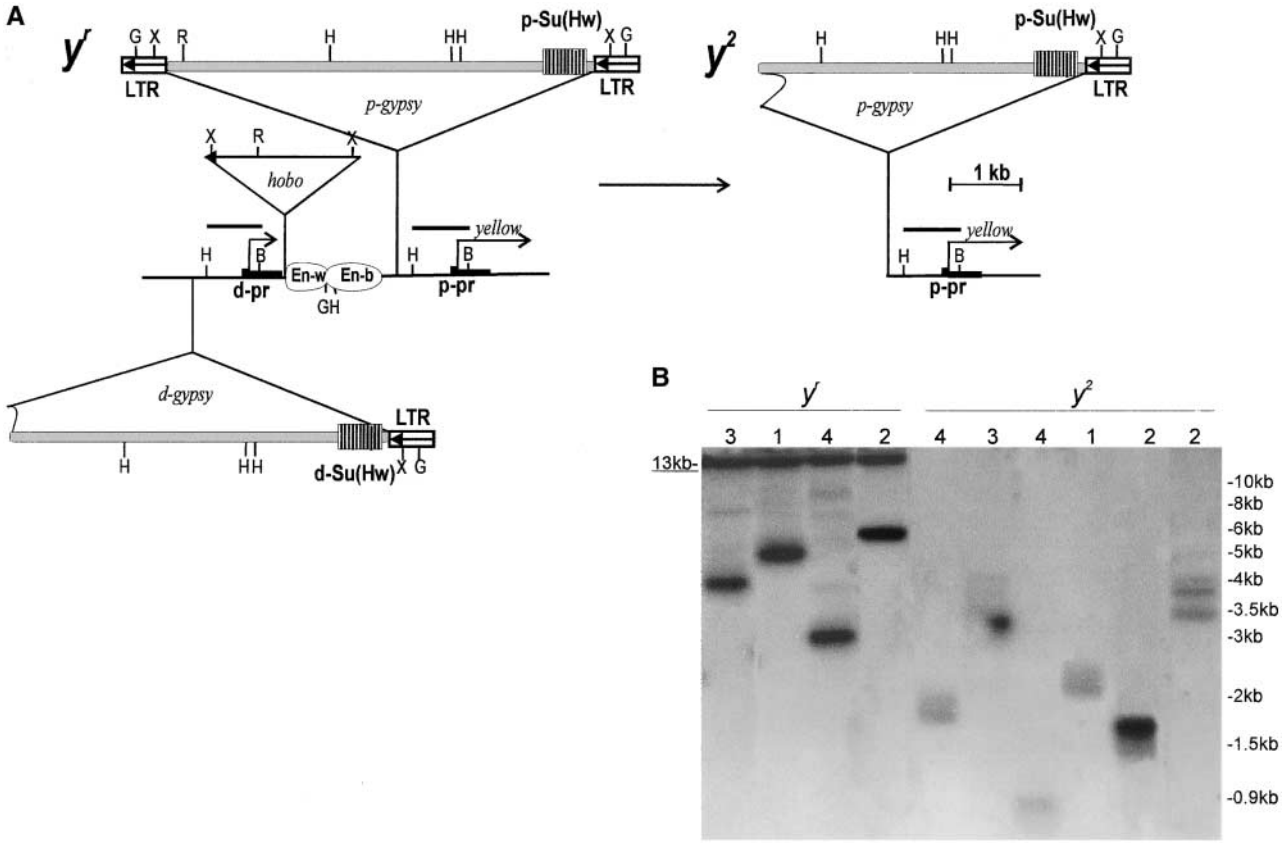


FIGURE 4.—Model system to study the frequency of recombination between direct repeats at the end of the terminal deficiency. (A) A schematic presentation of the y^{TDrh1} and y^{TDrh2} alleles and its y^2-like derivatives generated by deletion of the distal *yellow* and *gypsy* sequences. Other designations are as in Figure 1. (B) Southern blot analysis of DNA samples prepared from the y^{TDrh1}*/y ac; E(tc)/E(tc)* (1); y^{TDrh2}*/y ac; E(tc)/E(tc)* (2); y^{TDrh1}*/y ac; TM6/MKRS* (3); and y^{TDrh2}*/y ac; TM6/MKRS* (4) lines and their y^2-like derivatives. DNA samples were digested with *Bam*HI. The filters were hybridized with the *Hin*dIII-*Bam*HI probe. The 13-kb band (marked on the left) corresponds to the DNA fragment that hybridized with the proximal *Hin*dIII-*Bam*HI probe. The 13-kb DNA fragment is identical in the y^+-like alleles studied and is lacking in the y^2-like derivatives.

that our genetic system distinguished *HeT-A/TART* attachments and additions of *yellow* sequences by gene conversion, DNA samples of the derivatives displaying new y phenotypes were studied by Southern blot analysis (Figure 6B). In this experiment DNA samples generated by *HeT-A/TART* attachment did not hybridize with the probe for the distal part of the *yellow* regulatory region (*Sal*I-*Bgl*II), in contrast to those generated by gene conversion. All tested y^r derivatives were generated by addition of the *yellow* regulatory sequences (hybridization with the *Sal*I-*Bgl*II probe), while y^2(A+) derivatives had a *HeT-A/TART* attachment (no such hybridization).

Although the results obtained argue that the *E(tc)* mutation enhances only terminal DNA elongation by gene conversion, we examined the frequency of *HeT-A/TART* attachment in the absence of a homologous template for gene conversion. Two terminal deficiencies were selected (Figure 6A), terminating at ~−600 bp ($y^{TD\text{-}600}$) and −700 bp ($y^{TD\text{-}700}$). The y^{TD} chromosomes were balanced by the *y ac w* chromosome with a deficiency covering the *yellow* sequences. The addition of either a *HeT-A* or a *TART* sequence restored aristal pigmentation [y^2(A−) → y^2(A+)]. For two y^{TD}*/y ac w; E(tc)/E(tc)* lines, we examined 6700 flies and found 12 y^2(A+) females with pigmented aristae (1.8×10^{-3}). In control y^{TD}*/y ac w; TM6,Tb/MKRS* lines, 5 y^2(A+)-like females were found among 5400 scored flies (10^{-3}). The addition of *HeT-A/TART* elements to the end of the *yellow* deficiency was proved by Southern blot analysis (Figure 6B). These results confirm that the *E(tc)* mutation does not significantly influence the frequency of *HeT-A* and *TART* transposition to the end of the terminal deficiency.

DISCUSSION

Regulation of elongation of telomeres and of the ends of truncated chromosomes in *D. melanogaster*: Broken chromosomes in Drosophila behave as capped chromosomes: they are transmitted through many generations (BIESSMANN and MASON 1988; TRAVERSE and PARDUE 1988; BIESSMANN *et al.* 1990a,b, 1992a,b). Thus, the telomere-binding proteins can bind the ends of chromosomes in a sequence-independent manner, and the *yel-*

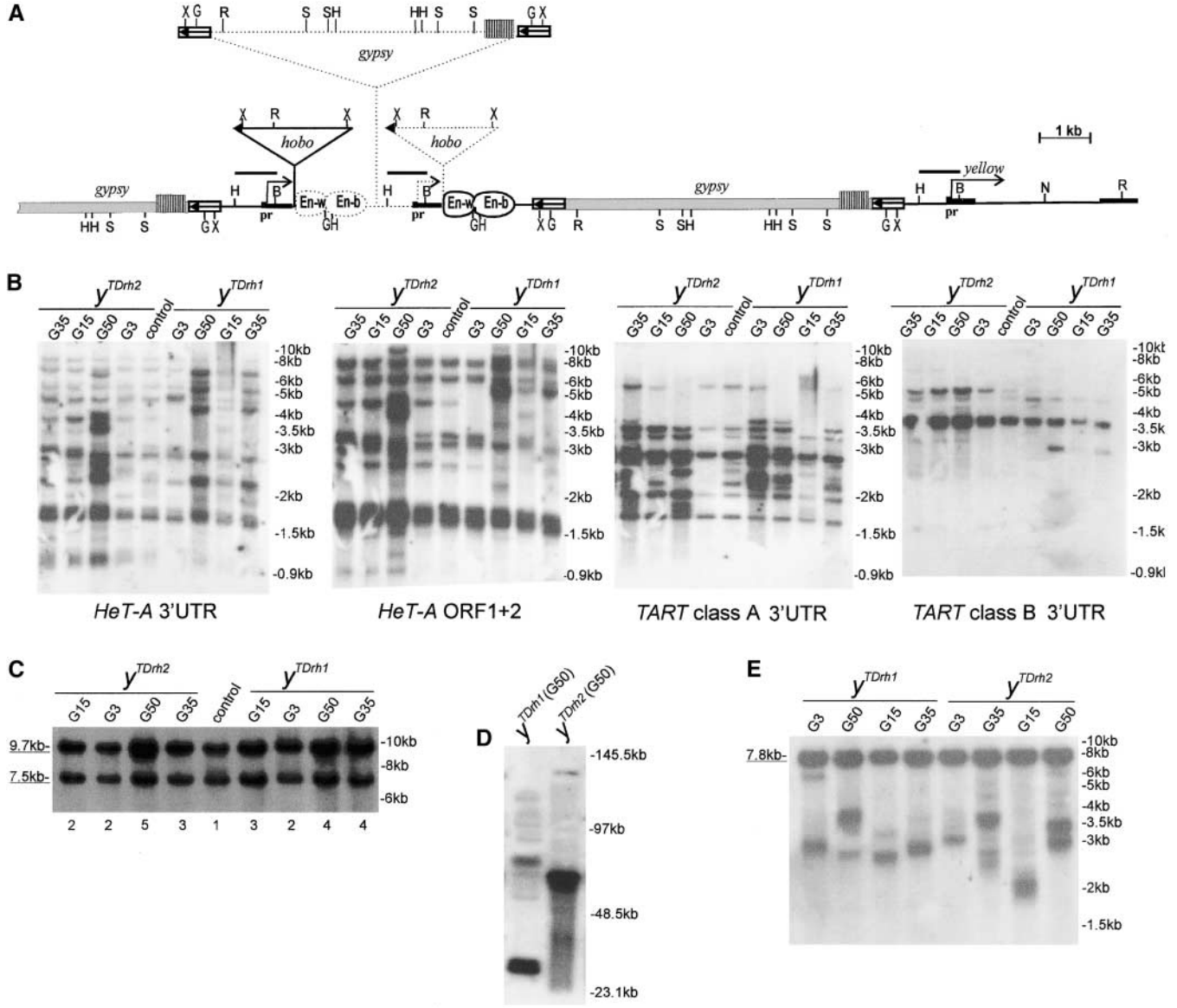


FIGURE 5.—The multiplication of the *yellow* and *gypsy* sequences at the end of the truncated chromosome and the content of *HeT-A* and *TART* elements in the *E(tc)* mutant. (A) A schematic presentation of the y^{TDrh1}*/y ac; E(tc)/E(tc)* and y^{TDrh2}*/y ac; E(tc)/E(tc)* derivatives that have more than two copies of the *yellow* and *gypsy* sequences. The additional one to three copies of duplicated sequences are indicated by dotted lines. N, *Nru*I; S, *Sac*I. Other designations are as in Figure 1. (B) Southern blot analysis of *HeT-A* and *TART* copy number in the y^{TDrh1}*/y ac; E(tc)/E(tc)* and y^{TDrh2}*/y ac; E(tc)/E(tc)* lines carrying the *E(tc)* mutation for 3, 15, 35, and 50 generations (G3, G15, G35, and G50). DNA was digested with *Bam*HI. The filters were probed with fragments from the 3′ untranslated region (UTR) of *HeT-A*, ORF1 + 2 of *HeT-A*, the 3′ UTR of *TART* class A, and the 3′ UTR of *TART* class B. These clones are described in DANILEVSKAYA *et al.* (1999). (C) Southern blot analysis of the *yellow* and *gypsy* duplication copy number. DNA was digested with *Sac*I and hybridized with the *Hin*dIII-*Bam*HI probe. The numbers below indicate the ratios of the intensities of the upper (9.7-kb) and lower (7.5-kb) bands corresponding to the *Sac*I DNA fragment. (D) Southern blot analysis of the y^{TDrh1}*/y ac; E(tc)/E(tc)* and y^{TDrh2}*/y ac; E(tc)/E(tc)* lines after 50 generations. The terminal DNA fragment cleaved with *Nru*I was examined by PFGE. A low-range pFG marker (194.0, 145.5, 97.0, 48.5, 23.1, 9.42, 6.55, 4.36, 2.32, 2.03) was used to determine the size of DNA fragments. (E) Southern blot analysis of DNA from y^{TDrh1}*/y ac; E(tc)/E(tc)* and y^{TDrh2}*/y ac; E(tc)/E(tc)* lines cleaved with *Bam*HI and *Eco*RI. The filter was hybridized with the *Hin*dIII-*Bam*HI probe.

low sequences located at the end of the deficient chromosome have the properties of a real telomere. Recently, HP1 (heterochromatin protein 1) has been reported to mediate normal telomere behavior in Drosophila (FANTI *et al.* 1998). The lack of HP1 results in multiple telomere-telomere fusions producing a remarkable spectrum of abnormal chromosome configurations. HP1 is present at the ends of terminal deficiencies (FANTI *et al.* 1998).

In the case of several tested lines bearing *yellow* terminal deficiencies, *HeT-A* elements transpose to the end of the truncated *yellow* sequences at low frequencies

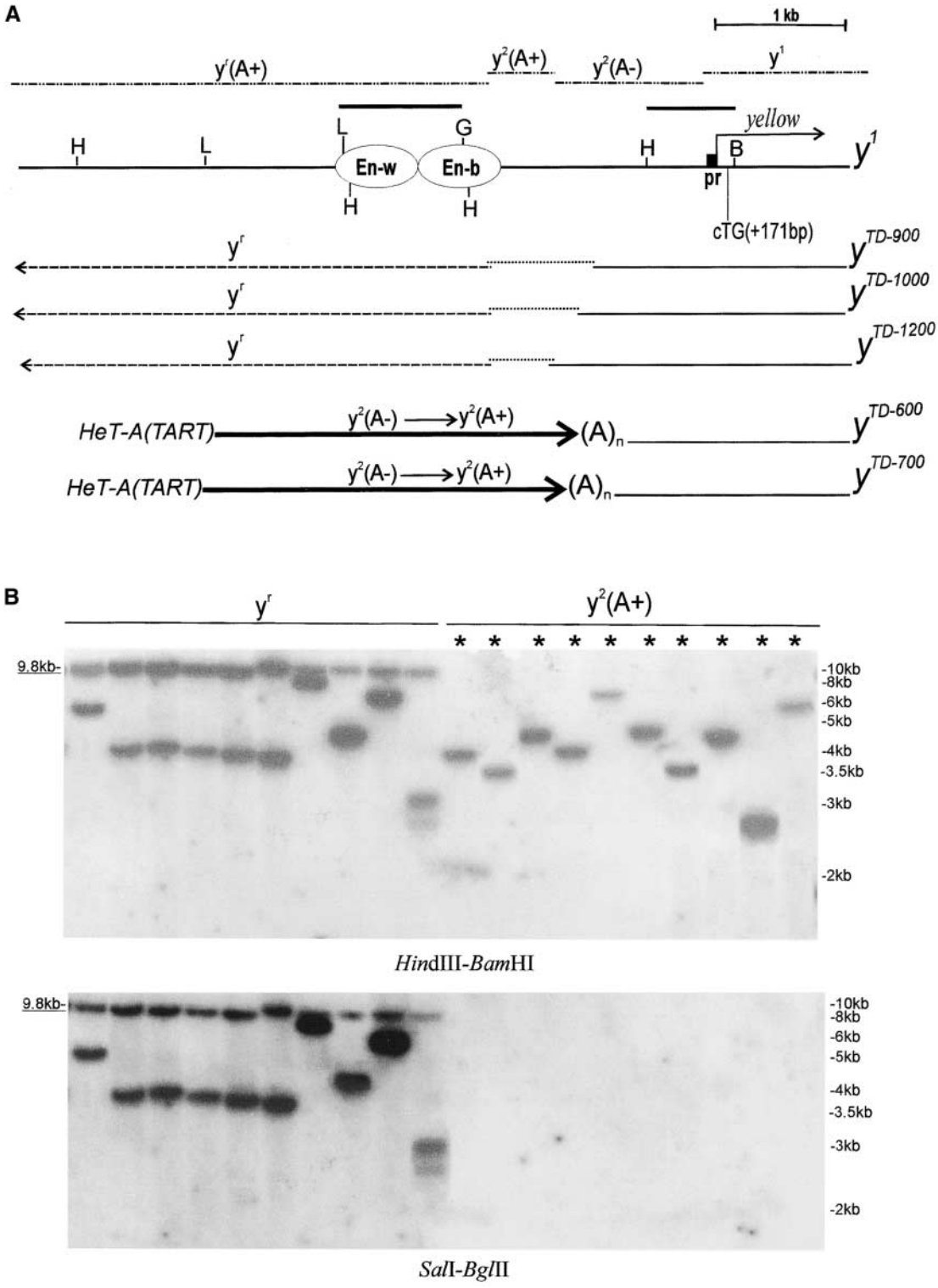


Figure 6.—The model systems to monitor the frequency of *HeT-A* and *TART* attachment to a broken end on the *E(tc)* mutant background. (A) A schematic presentation of terminal *yellow* deficiencies associated with different y^{TD} alleles. The molecular structure of the *y* mutation is shown. The approximate regions of the ends of truncated chromosomes in the y^{TD} alleles are shown by thin black lines. The dotted horizontal lines show the regions of *yellow* sequence in which the termini of the y^{TD} line with the original phenotype have been mapped. The dashed horizontal lines show the regions of *yellow* sequence in which the termini of the y^{TD} line acquiring a y^r-like phenotype have been mapped. The *Sal*I-*Bgl*II and *Hin*dIII-*Bam*HI genomic fragments used as probes for Southern blot analysis are indicated by the thick line at the top. L, *Sal*I. Other designations are as in Figure 1. (B) Southern blot analysis of DNA prepared from y^{TD} derivatives having acquired new y phenotypes. DNA was digested with *Bam*HI. The filter was consecutively hybridized with the *Hin*dIII-*Bam*HI (promoter region) and *Sal*I-*Bgl*II (upstream enhancer region) probes. Asterisks indicate y^{TD} lines that acquired new *HeT-A/TART* attachments. The presence of additional bands indicates the heterogeneity of the progeny, suggesting that in some sisters terminally truncated chromosomes acquired new DNA sequences. The 9.8-kb band (marked on the left) is the DNA fragment that hybridized with the DNA corresponding to the *y w* chromosome.

ranging from 10^{-3} to $<10^{-4}$ (Biessmann *et al.* 1992a; Kahn *et al.* 2000). In the same lines, terminal DNA elongation by gene conversion is also much less frequent than described in Mikhailovsky *et al.* (1999). As a result, the chromosomal ends recede at a rate consistent with the loss of DNA sequence by underreplication (Biessmann and Mason 1988; Levis 1989; Biessmann *et al.* 1990a). Thus, the Drosophila telomere should have an additional mechanism that lengthens short telomeres.

Recently we have shown that mutations in the *Su (var)2-5* gene encoding HP1 in the heterozygous state increase the frequency of *HeT-A* and *TART* attachment to the broken chromosome end >100-fold (Savitsky *et al.* 2002). Here we describe the *E(tc)* mutation that strongly enhances terminal DNA elongation by gene conversion. Thus, at least several proteins negatively regulate DNA elongation at the ends of the deficient chromosome. Drosophila lines bearing the *Su(Hw)2-5* mutations for a long time have extremely long telomeres consisting of *HeT-A* and *TART* (Savitsky *et al.* 2002). The *E(tc)* mutation also increases the length of the telomeres. These results suggest that both genes play an important role in the control of telomere elongation in *D. melanogaster*.

Recently, a new dominant mutation, *Tel*, which induces lengthening of telomeres, has been described (Siriaco *et al.* 2002). Interestingly, *Tel* and *E(tc)* map to the same region of the third chromosome. It was proposed that *Tel* may increase the frequency of *HeT-A* and *TART* transposition or of recombination/gene conversion events, leading to telomere elongation. *Tel* mutation was identified in the Gaiano strain isolated from the natural Drosophila population (Siriaco *et al.* 2002). Thus, *Tel* and *E(tc)* mutations have a different origin but a similar effect on telomere lengthening, leading to the supposition that they might be different alleles of the same gene.

Role of recombination/gene conversion in regulation of telomere length in *D. melanogaster*: Telomere recombination may be the primary mechanism for maintaining chromosome length in some organisms that lack telomerase (Biessmann and Mason 1997; Biessmann *et al.* 2000). There is indirect evidence that telomeres of the mosquito Anopheles (Roth *et al.* 1997; Biessmann *et al.* 1998) and the midge Chironomus (Cohn and Edstrom 1992; Lopez *et al.* 1996) are extended by recombination and gene conversion mechanisms involving long terminal repeats. As found recently, *D. virilis* has long terminal repeats at the ends of chromosomes instead of mobile elements (Biessmann *et al.* 2000), suggesting that gene conversion or unequal recombination is involved in their elongation. Even in organisms like yeast and humans, in which telomeres are extended by telomerase, recombination could be used as an efficient bypass mechanism for chromosomal length maintenance when telomerase is inactive (Lundblad and Blackburn 1993; McEachern and Hicks 1993; Bryan *et al.* 1995, 1997; McEachern and Blackburn 1996; Nakamura *et al.* 1997; Teng and Zakian 1999; Yeager *et al.* 1999; Dunham *et al.* 2000). Yeast telomere maintenance in the absence of telomerase appears to employ break-induced replication (BIR; Kraus *et al.* 2001). BIR is a nonreciprocal recombination-dependent replication process that is an effective mechanism to repair broken chromosomes. BIR begins when strand invasion creates a D-loop and sets up a replication fork. BIR can generate very long DNA elongation (Kraus *et al.* 2001). It now seems that the initial events of BIR in *Saccharomyces cerevisiae* may not be different from what occurs during gene conversion. However, the replication process in the case of gene conversion is much less processive and much more prone to dissociation than normal replication or BIR. There is a high level of dissociation of DNA polymerase from its template during gene conversion. In Drosophila, we found that the *E(tc)* mutation induces only relatively short terminal DNA tracks. Thus, we suggest that short terminal DNA attachments are generated by gene conversion using the homologous sequences as a template.

Here we have shown that the *E(tc)* mutation notably increases the frequency of terminal DNA elongation by gene conversion at the ends of truncated chromosomes, without an appreciable effect on the frequency of *HeT-A* and *TART* transposition to the chromosome end. Considering our observation that telomeres in *E(tc)* are longer than normal, our results argue that gene conversion is an important component of telomere length regulation in *D. melanogaster*. We also found that large repeated DNA fragments, including *gypsy* and part of the *yellow* gene, may function as telomere sequences. In the absence of the *E(tc)* mutation, the terminal DNA sequences were deleted at a rate of ~70 bp/generation, as calculated previously (Biessmann and Mason 1988; Biessmann *et al.* 1990a,b). However, in the presence of the *E(tc)* mutation, terminal sequences were elongated by gene conversion using homologous sequences on the same chromosome as a template. The presence of direct repeats at chromosomal ends induces frequent recombination between homologous sequences located closer to the end of the truncated chromosome, leading to deletion of repeated sequences. Such recombination events may be involved in the negative regulation of the length of telomeres consisting of repeated *HeT-A* and *TART* sequences. Interestingly, the *E(tc)* mutation does not influence the frequency of the recombination between terminal repeats. Thus, the *E(tc)* gene product appears to specifically regulate telomere elongation by gene conversion. Cloning of the *E(tc)* gene is required to understand its role in the control of telomere lengthening.

We thank Olga Yarovaia for help with the pulsed-field gel electrophoresis. We also thank M. L. Pardue, K. L. Traverse, and J. Modolell, Bloomington Center, for Drosophila stocks and plasmids. The authors are sincerely grateful to an anonymous reviewer and to A. V. Galkin

for critical reading and corrections of the manuscript. This work was supported by the Russian State Program "Frontiers in Genetics," the Russian Foundation for Basic Research, by an International Research Scholar award from the Howard Hughes Medical Institute, and by Fogarty Award (PHS-NIH) no. TW-05653 granted to H. Biessmann and P. Georgiev.

LITERATURE CITED

Ashburner, M., 1989 *Drosophila*: *A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Biessmann, H., and J. M. Mason, 1988 Progressive loss of DNA sequences from terminal chromosome deficiencies in *Drosophila melanogaster*. EMBO J. **7:** 1081–1086.

Biessmann, H., and J. M. Mason, 1997 Telomere maintenance without telomerase. Chromosoma **106:** 63–69.

Biessmann, H., S. B. Carter and J. M. Mason, 1990a Chromosome ends in *Drosophila* without telomeric DNA sequences. Proc. Natl. Acad. Sci. USA **87:** 1758–1761.

Biessmann, H., J. M. Mason, K. Ferry, M. d'Hulst, K. Valgeirsdottir *et al.*, 1990b Addition of telomere-associated HeT DNA sequences "heals" broken chromosome ends in *Drosophila*. Cell **61:** 663–673.

Biessmann, H., L. E. Champion, K. O'Hair, K. Ikenaga, B. Kasravi *et al.*, 1992a Frequent transpositions of *Drosophila melanogaster HeT-A* transposable elements to receding chromosome ends. EMBO J. **11:** 4459–4469.

Biessmann, H., K. Valgeirsdottir, A. Lofsky, C. Chin, B. Ginther *et al.*, 1992b *HeT-A*, a transposable element specifically involved in healing broken chromosome ends in *Drosophila melanogaster*. Mol. Cell. Biol. **12:** 3910–3918.

Biessmann, H., M. F. Walter and J. M. Mason, 1997 *Drosophila* telomere elongation. Ciba Found. Symp. **211:** 53–67.

Biessmann, H., F. Kobeski, M. F. Walter, A. Karsavi and C. W. Roth, 1998 DNA organization and length polymorphism at the 2L telomeric region of Anopheles gambiae. Insect Mol. Biol. **7:** 83–93.

Biessmann, H., M. Zurovcova, J. G. Yao, E. Lozovskaya and M. F. Walter, 2000 A telomeric satellite in Drosophila virilis and its sibling species. Chromosoma **109:** 372–380.

Blasco, M. A., S. M. Gasser and J. Lingner, 1999 Telomeres and telomerase. Genes Dev. **13:** 2353–2359.

Bryan, T. M., A. Englezou, J. Gupta, S. Bacchetti and R. R. Reddel, 1995 Telomere elongation in immortal human cells without detectable telomerase activity. EMBO J. **14:** 4240–4248.

Bryan, T. M., L. Marusic, S. Bacchetti, M. Namba and R. R. Reddel, 1997 The telomere lengthening mechanism in telomerase-negative immortal human cells does not involve the telomerase RNA subunit. Hum. Mol. Genet. **6:** 921–926.

Cohn, M., and J. E. Edstrom, 1992 Telomere-associated repeats in Chironomus form discrete subfamilies generated by gene conversion. J. Mol. Evol. **35:** 114–122.

Danilevskaya, O. N., K. L. Traverse, N. C. Hogan, P. G. DeBaryshe and M. L. Pardue, 1999 The two *Drosophila* telomeric transposable elements have very different patterns of transcription. Mol. Cell. Biol. **19:** 873–881.

Dunham, M. A., A. A. Neumann, C. L. Fasching and R. R. Reddel, 2000 Telomere maintenance by recombination in human cells. Nat. Genet. **26:** 447–450.

Fanti, L., G. Giovinazzo, M. Berloco and S. Pimpinelli, 1998 The heterochromatin protein 1 prevents telomere fusions in *Drosophila*. Mol. Cell **2:** 527–538.

Gause, M., H. Hovhannisyan, T. Kahn, S. Kuhfittig, V. Mogila *et al.*, 1998 *hobo* induced rearrangements in the *yellow* locus influence the insulation effect of the *gypsy* su(Hw)-binding region in *Drosophila melanogaster*. Genetics **149:** 1393–1405.

Geyer, P. K., and V. G. Corces, 1987 Separate regulatory elements are responsible for the complex pattern of tissue-specific and developmental transcription of the *yellow* locus in *Drosophila melanogaster*. Genes Dev. **1:** 996–1004.

Geyer, P. K., M. M. Green and V. G. Corces, 1990 Tissue-specific transcriptional enhancers may act in *trans* on the gene located in the homologous chromosome: the molecular basis of transvection in *Drosophila*. EMBO J. **9:** 2247–2256.

Golubovsky, M. D., A. Y. Konev, M. F. Walter, H. Biessmann and J. M. Mason, 2001 Terminal retrotransposons activate a subtelomeric *white* transgene at the 2L telomere in Drosophila. Genetics **158:** 1111–1123.

Greider, C. W., 1999 Telomeres do D-loop-T-loop. Cell **97:** 419–422.

Kahn, T., M. Savitsky and P. Georgiev, 2000 Attachment of *HeT-A* sequences to chromosome termini in *Drosophila melanogaster* may occur by different mechanisms. Mol. Cell. Biol. **20:** 7634–7642.

Kraus, E., W.-Y. Leung and J. E. Haber, 2001 Break-induced replication: a review and an example in budding yeast. Proc. Natl. Acad. Sci. USA **98:** 8255–8262.

Levis, R. W., 1989 Viable deletions of a telomere from a *Drosophila* chromosome. Cell **58:** 791–801.

Levis, R. W., R. Ganesan, K. Houtchens, L. A. Tolar and F.-M. Sheen, 1993 Transposons in place of telomere repeats at a *Drosophila* telomere. Cell **75:** 1083–1093.

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, New York.

Lopez, C. C., L. Neilsen and J.-E. Edstrom, 1996 Terminal long tandem repeats in chromosomes from *Chironomus pallidivittatus*. Mol. Cell. Biol. **16:** 3285–3290.

Lundblad, V., and E. H. Blackburn, 1993 An alternative pathway for yeast telomere maintenance rescues est-1 senescence. Cell **73:** 347–360.

Martin, M., Y. B. Meng and W. Chia, 1989 Regulatory elements involved in the tissue-specific expression of the *yellow* gene of *Drosophila*. Mol. Gen. Genet. **218:** 118–126.

Mason, J. M., E. Strobel and M. M. Green, 1984 mu-2: mutator gene in Drosophila that potentiates the induction of terminal deficiencies. Proc. Natl. Acad. Sci. USA **81:** 6090–6094.

Mason, J. M., A. Haoudi, A. Y. Konev, E. Kurenova, M. F. Walter *et al.*, 2000 Control of telomere elongation and telomeric silencing in *Drosophila melanogaster*. Genetica **109:** 61–70.

McEachern, M. J., and E. H. Blackburn, 1996 Cap-prevented recombination between terminal telomeric repeat arrays (telomere CPR) maintains telomeres in *Kluyveromyces lactis* lacking telomerase. Genes Dev. **10:** 1822–1834.

McEachern, M. J., and J. B. Hicks, 1993 Unusually large telomeric repeats in the yeast *Candida albicans*. Mol. Cell. Biol. **13:** 551–560.

Melnikova, L., M. Gause and P. Georgiev, 2002 The *gypsy* insulators flanking *yellow* enhancers do not form a separate transcriptional domain in *Drosophila melanogaster*: the enhancers can activate an isolated *yellow* promoter. Genetics **160:** 1549–1560.

Mikhailovsky, S., T. Belenkaya and P. Georgiev, 1999 Broken chromosome ends can be elongated by conversion in *Drosophila melanogaster*. Chromosoma **108:** 114–120.

Nakamura, T. M., G. B. Morin, K. B. Chapman, S. L. Weinrich, W. H. Andrews *et al.*, 1997 Telomerase catalytic subunit homologs from fission yeast and human. Science **277:** 955–959.

Pardue, M. L., and P. G. DeBaryshe, 1999 Telomeres and telomerase: more than the end of the line. Chromosoma **108:** 73–82.

Pardue, M.-L., and P. G. DeBaryshe, 2000 *Drosophila* telomere transposons: genetically active elements in heterochromatin. Genetica **109:** 45–52.

Roth, C. W., F. Kobeski, M. F. Walter and H. Biessmann, 1997 Chromosome end elongation by recombination in the mosquito *Anopheles gambiae*. Mol. Cell. Biol. **17:** 5176–5183.

Sambrook, J., E. F. Fritsch and T. Maniatis, 1989 *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Savitsky, M., O. Kravchuk, L. Melnikova and P. Georgiev, 2002 Heterochromatin protein 1 is involved in control of telomere elongation in *Drosophila melanogaster*. Mol. Cell. Biol. **22:** 3204–3218.

Sheen, F. M., and R. W. Levis, 1994 Transposition of the LINE-like retrotransposon *TART* to *Drosophila* chromosome termini. Proc. Natl. Acad. Sci. USA **91:** 12510–12514.

Siriaco, G. M., G. Cenci, A. Haoudi, L. E. Champion, C. Zhou *et al.*, 2002 Telomere elongation (Tel), a new mutation in *Drosophila melanogaster* that produces long telomeres. Genetics **160:** 235–245.

Teng, S. C., and V. A. Zakian, 1999 Telomere-telomere recombination is an efficient bypass pathway for telomere maintenance in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **19:** 8083–8093.

Traverse, K. L., and M. L. Pardue, 1988 A spontaneously opened ring chromosome of *Drosophila melanogaster* has acquired *HeT-A* DNA sequences at both new telomeres. Proc. Natl. Acad. Sci. USA **85:** 8116–8120.

Walter, M. F., B. C. Black, G. Afshar, A.-Y. Kermabon, T. R. F. Wright *et al.*, 1991 Temporal and spatial expression of the yellow gene in correlation with cuticle formation and DOPA decarboxylase activity in *Drosophila* development. Dev. Biol. **147:** 32–45.

Walter, M. F., C. Jang, B. Kasravi, J. Donath, B. M. Mechler *et al.*, 1995 DNA organization and polymorphism of a wild-type Drosophila telomere region. Chromosoma **104:** 229–241.

Yeager, T. R., A. A. Neumann, A. Englezou, L. I. Huschtscha, J. R. Noble *et al.*, 1999 Telomerase-negative immortalized human cells contain a novel type of promyelocytic leukemia (PML) body. Cancer Res. **59:** 4175–4179.

Zakian, V. A, 1996 Telomere functions: lessons from *yeast*. Trends Cell Biol. **6:** 29–33.

Communicating editor: K. Golic