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

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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 1996; Pardue et al. 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 ~10^{-2}/generation (Mikhailovsky et al. 1999). In that study, a line with a y^{w} chromosome bearing a point mutation

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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, \( \text{Enhancer of terminal gene conversion, } E(tc) \), 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 \( \text{HeT-A} \) and \( \text{TART} \) 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 \( \text{HeT-A} \) and \( \text{TART} \), 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°C on a standard yeast medium. In this study we used the alleles with terminal deficiencies consisting of breaks in the \( y \) \( w \) gene, designated \( y \) \( w \) terminal deficiencies (\( y^{10} \)). The \( y^{10} \) alleles with a \( y \)-like phenotype (wild-type pigmentation in bristles and lack of pigmentation in the body and wings) were designated as \( y^{10} \). The \( y^{10} \) alleles with darker wing and body pigmentation (\( y \)-like phenotype) were designated as \( y^{10} \). The origin of the \( y \)-like alleles is described elsewhere (Mikhailovsky et al. 1999; Kain et al. 2000; Melnikova et al. 2002).

Most of the genetic markers used were described by Lind- sley and Zimm (1992). The \( y \) \( w \) chromosome has a deletion of the \( y \) \( w \) and \( achaete \) genes, but not of any vital genes, and thus provides an opportunity to examine the behavior of the \( y \) \( w \) gene on the homolog in the absence of other \( y \) \( w \) 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 \( y \) 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 \( y \) \( w \) line is a standard laboratory wild-type strain. The marked stock \( \text{H Sh Gl/MKRS} [\text{Gl}, 3-41.4 (70C2); \text{Sh}, 3-58.2 (88B9-10)]; \text{IL, 3-69.5 (92D1-92F2)} \) was provided by the Bowling Green stock center.

The position of \( E(tc) \) along chromosome 3 was determined by allowing free recombination in \( y^{10}/y\text{ac}; E(tc)/\text{H Sh Gl} \) females. Recombinant chromosomes were collected in males over \( TM6, Tb \) and placed into stocks by crossing these males to \( y^{10}/y\text{ac}; TM6, Tb/MKRS \) 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 \( y \) \( w \) gene.

For determination of the \( y \) \( w \) phenotype, the extent of pigmentation in different tissues of adult flies was estimated visually in 3- to 5-day-old females developing at 25°C.

**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°C 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 H2O for 15 min.

Phages with cloned regions of the \( y \) \( w \) locus were obtained from J. M. Modekell. The clones of \( \text{HeT-A} \) and \( \text{TART} \) 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, \( \sim 10^{-2}/\text{generation} \) (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 \( y^{10}/y \) \( w \) 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 \( y^{10} \) \( w \) line (Melnikova et al. 2002). This line contains a terminally truncated \( X \) chromosome with a duplication of \( y \) \( w \) sequences extending from +875 bp to the chromosome end (Figure 1A). In addition to the \( y \) \( w \) duplication, a \( gypsy \) retrotransposon is inserted between the \( y \) \( w \) enhancers and the promoter at position −700. The \( y^{10} \) \( w \) flies have a \( y \)-like phenotype because the \( gypsy \) insulator blocks the interaction between the wing and body enhancers and the \( y \) \( w \) gene promoter (Geyer and Corces 1987; Gause et al. 1998). It has been shown that a second \( gypsy \) insulator placed upstream of the \( y \) \( w \) 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 \( y \) \( w \) expression in the body and wings (\( y \)'). In \( y^{10} \) \( w \) flies, the \( gypsy \) sequences may be duplicated to the end of the deficient chromosome by gene conversion using as template the homologous \( y \) \( w \) and \( gypsy \) sequences located on the same chromosome. Thus, the frequency of the intrachromosomal gene conversion can be monitored by scoring flies with
darker pigmentation of the wing blades and body cuticle (\(y^2\) phenotype).

Two \(y^{TD2}/y\) ac; CyO/IIf; TM6, Tb/MKRS lines were selected by Southern blot analysis. In these lines the ends of deficient chromosomes were located at \(\sim 300\) bp \(y^{TD22,300}\) and \(-700\) bp \(y^{TD22,700}\) 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 \(y^{TD2}/y\) ac; CyO/IIf; TM6, Tb/MKRS lines gave \(y^2\) like derivatives with a frequency \(< 2 \times 10^{-3}\). To study the fate of the DNA terminus in the control \(y^{TD2}/y\) ac; CyO/IIf; 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 \(y^D/y\) w line were extracted into the \(y^{TD2}/y\) ac; CyO/IIf; 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 \( y^{TDrh2}/y \) ac; CyO/If; TM6, Tb/MKR S,y line, we obtained four \( y \)-like females among 3400 scored progeny in three subsequent generations \((1.2 \times 10^{-3})\). In four \( y^{TDrh2}/y \) w; CyO/If; TM6, Tb/MKR S,y lines, 14 exceptional \( y \)-like females were obtained among 4900 scored flies \((2.9 \times 10^{-3})\). 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 \( y \)-like females \((1.4 \times 10^{-3})\). However, in three lines carrying chromosome 3, \( y \)-like females appeared at a high frequency: we found 210 \( y \)-like females among 2700 scored females \((8 \times 10^{-3})\).

To show that \( y \)-like derivatives were generated by gene conversion, the progeny of individual \( y \)-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 \( y \)-like derivatives (Figure 1C). Frequently, DNA obtained from the progeny of a single \( y \) 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 \( y^{TDrh2}/y \) ac; CyO/If; 3 chromosome/TM6, Tb females, \( y \)-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, \( y^{TDh1} \) and \( y^{TDh2} \), which had terminal breaks in the sequences of the distal gypsy element at \( \sim 4.5 \) kb \((y^{TDh1})\) and \( 6.0 \) kb \((y^{TDh2})\) 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 \( y^{TDh1} \) and \( y^{TDh2} \) 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 HindIII-BamHI 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, \( Gl, Sh, \) 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 \( y \)-like phenotype, we frequently found exceptional \( y \)-like females. Southern blot analysis showed that \( y \)-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 \( y \)-like females in the progeny of \( y^{TD}/y \) ac; \( E(tc) \)/\( E(tc) \) and \( y^{TD}/y \) ac; TM6, Tb/MKR S females that had the same \( y^{TD} \) deficiency, \( y^{TDh1} \) or \( y^{TDh2} \). Eleven independent \( y \)-like derivatives were found among 4200 \( y \)-like females carrying the homozygous \( E(tc) \) mutation \((2.6 \times 10^{-3})\). In the control experiment, 7 \( y \)-like derivatives were found among 3400 scored \( y \)-like females \((2.1 \times 10^{-3})\). By Southern blot analysis, all \( y \)-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 \( y^{TDh1}, E(tc)/E(tc) \) and \( y^{TDh2}, E(tc)/E(tc) \) 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
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 Sad and probed it with the HindIII-BamHI fragment (Figure 5A). The HindIII-BamHI probe hybridized with two bands (Figure 5C): a 7.5-kb DNA fragment between two Sad sites located in the yellow coding region and in gypsy and a 9.7-kb DNA fragment between two Sad 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 yTDm2/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 NruI 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 yTDm2/y ac; E(tc)/E(tc) lines after 50 generations. In both lines, the HindIII-BamHI probe hybridized with several bands ranging from 28 to 120 kb. The major DNA band for the yTDm2/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 yTDm2/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 BamHI and EcoRI also showed extensive heterogeneity of the terminal DNA fragment (Figure 5E).

In the yTDm2/y ac; E(tc)/E(tc) lines carrying three or four gypsy elements. The 7.5-kb band is unique, while the 9.7-kb band corresponds to repeated sequences. The copies of the duplicated sequences, exceptional y2-like females appeared with low frequency. We found only two y2-like derivatives among 9700 yTDm2/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 conversion.
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 $\sim$900 bp ($y^{TD900}$), $\sim$1000 bp ($y^{TD1000}$), and $\sim$1200 bp ($y^{TD1200}$) relative to the yellow transcription start site. The template for gene conversion was the y allele ($y^w$ chromosome). Truncated chromosomes having breaks between $\sim$1200 and $\sim$140 bp result in a $y^\gamma$-like phenotype with yellow-colored aristae, $y^\gamma(A^-)$ (Figure 6A). Addition of either a HeT-A or a TART sequence restores arista pigmentation [$y^\gamma(A^+)$]. 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 ($\sim$1600 bp) to the ends of the deficient chromosomes via gene conversion partially restores yellow expression in the body: yellow revertant, $y^\gamma$. 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) transcription start site. The template for gene conversion tracts longer than 400–700 bp.

In the control $y^{TM}/y^w; CyO/If; Tm6,Tb/MKRS,Sb$ lines we obtained four $y^\gamma$-like females ($1.2 \times 10^{-3}$) and two $y^\gamma(A^+)$-like females ($0.6 \times 10^{-3}$) among 3400 scored progeny in three subsequent generations. In the experimental crosses we examined 4100 $y^{TM}/y^w; E(tc)/E(tc)$ flies and found 47 independent $y^\gamma$-like females ($1.1 \times 10^{-4}$) and only one $y^\gamma(A^+)$ female ($2 \times 10^{-4}$). To show directly
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*-*BglII), in contrast to those generated by gene conversion. All tested *y* derivatives were generated by addition of the *yellow* regulatory sequences (hybridization with the *Sal*-*BglII* probe), while *y*(*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 \(\sim -600\) bp \((y^{TD}_{600})\) and \(-700\) bp \((y^{TD}_{700})\). 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 \([y^2(A\neg) \rightarrow y^2(A\neg+)]\). For two *y*\(^{TD}/y\) *ac w; E(tc)/E(tc)* lines, we examined 6700 flies and found 12 *y*\(^2(A\neg+)\) females with pigmented aristae \((1.8 \times 10^{-5})\). In control *y*\(^{TD}/y\) *ac w; TM6,Tb/MKRS* lines, 5 *y*\(^2(A\neg+)\) females were found among 5400 scored flies \((10^{-7})\). 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-
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 yD alleles. The molecular structure of the y mutation is shown. The approximate regions of the ends of truncated chromosomes in the yD alleles are shown by thin black lines. The dotted horizontal lines show the regions of yellow sequence in which the termini of the yD line with the original phenotype have been mapped. The dashed horizontal lines show the regions of yellow sequence in which the termini of the yD line acquiring a y-like phenotype have been mapped. The SalI-BglII and HindIII-BamHI genomic fragments used as probes for Southern blot analysis are indicated by the thick line at the top. L, SalI. Other designations are as in Figure 1. (B) Southern blot analysis of DNA prepared from yTD derivatives having acquired new y phenotypes. DNA was digested with BamHI. The filter was consecutively hybridized with the HindIII-BamHI (promoter region) and SalI-BglII (upstream enhancer region) probes. Asterisks indicate yD 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 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 Zarian 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 progressive 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.

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LITERATURE CITED


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


