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Attachment of *HeT-A* Sequences to Chromosomal Termini in *Drosophila melanogaster* May Occur by Different Mechanisms

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***Drosophila* telomeres contain arrays of the retrotransposonlike elements *HeT-A* and *TART*. Their transposition to broken chromosomal termini has been implicated in chromosome healing and telomere elongation. The *HeT-A* element is attached by its 3′ end, which contains the promoter. To monitor the behavior of *HeT-A* elements, we used the yellow gene with terminal deficiencies consisting of breaks in the *yellow* promoter region that result in the *y*-null phenotype. Attachment of the *HeT-A* element provides the promoterless *yellow* gene with a promoter that activates *yellow* expression in bristles. The frequency of *HeT-A* transpositions to the *yellow* terminal deficiency depends on the genotype of the line and varies from 2×10^{-3} to less than 2×10^{-5}. Loss of the attached *HeT-A* due to incomplete replication at the telomere leads to inactivation of *yellow* expression, which is restored by attachment of a new *HeT-A* element upstream of *yellow*. New *HeT-A* additions occur at a frequency of about 1.2×10^{-3}. Short DNA attachments are generated by gene conversion using the homologous telomeric sequences as templates. Longer DNA attachments are generated either by conventional transposition of an *HeT-A* element to the chromosomal terminus or by recombination between the 3′ terminus of telomeric *HeT-A* elements and the receding end of *HeT-A* attached to the *yellow* gene.**

Specialized mechanisms have evolved to add DNA to the termini of eukaryotic chromosomes, balancing the loss that occurs as a result of incomplete terminal DNA replication (11, 37). In most eukaryotes a special reverse transcriptase, telomerase, adds telomeric DNA repeats to the chromosomal ends by using an internal RNA template (11, 25, 26, 38). In contrast, *Drosophila* telomeres consist of multiple copies of *HeT-A* and *TART* elements sharing similarities with non-LTR-type retrotransposons (7, 33, 36, 38). In particular, they have an oligo(A) tract at the 3′ end. *HeT-A* and *TART* in telomeres have head-to-tail orientation (28, 33, 36, 38). Telomeres are believed to elongate by transposition of these elements to the ends of chromosomes (5, 6, 7, 36, 38, 42). All available data suggest that the *HeT-A* and *TART* elements are attached with 3′ oligo(A) tails to their target sites (4, 5, 42). The structures and functions of *HeT-A* and *TART* reveal similarities with telomeres: the *TART* reverse transcriptase is related to the catalytic subunit of telomerase (38). Still, the mechanism and the regulation of the telomere elongation by transposition remain unclear.

The terminal deficiencies that remove the chromosome end and are broken within the *yellow* gene have been used to study the mechanism of telomere recession and elongation (2, 3, 4, 5, 6, 35). The *yellow* gene is required for larval and adult cuticle pigmentation and is transcribed in the distal-to-proximal direction. The enhancers that control *yellow* expression in the wings and body cuticle are located in the 5′ upstream region of the *yellow* gene, whereas the enhancer controlling *yellow* expression in bristles resides in the intron (2, 24, 32). Therefore, flies with the terminal DNA breakpoints in the 5′ upstream region removing the wing and body enhancers display a y^2-like phenotype: wild-type pigmentation in bristles and lack of pigmentation in the body cuticle and wing blade (2). Terminal deficiencies with breaks located at the *yellow* promoter or within the *yellow* transcription unit result in the y^1-like phenotype, i.e., complete repression of *yellow* function (2, 3). Biessmann et al. (4) described the RT394 strain carrying a *HeT-A* element attached to the 5′ end of the *yellow* transcription unit. RT394 flies displayed the y^2-like phenotype in spite of deletion of the *yellow* promoter. Danilevskaya et al. (16) showed that *HeT-A* elements have a promoter element at the 3′ end. As a result, the *HeT-A* promoter initiates transcription of sequences downstream of the element. One can suggest that the *HeT-A* promoter restores *yellow* expression in bristles.

Using these observations, we have developed a genetic method to analyze the frequency of *HeT-A* transposition to the receding promoterless *yellow* terminus. Here we have found that transposition depends on the genotype of a line and varies from less than 2×10^{-5} to 2×10^{-3}. Thus, the genotype strongly affects the frequency of *HeT-A* transposition to the broken chromosomal end. Previously, we observed that the ends of the *yellow* terminal deficiencies could also be elongated by gene conversion if the *yellow* gene on the homologous chromosome served as a template (35). It was suggested that elongation of the *HeT-A* array might occur not only by virtue of transposition but also by an alternative mechanism, such as gene conversion.

To monitor the fate of the receding *HeT-A* element, we exploited the observation that less than 300 bp of the 3′ end of *HeT-A* could not activate *yellow* transcription. Addition of a new *HeT-A* element to the 5′ end of a truncated element renews *yellow* transcription. Using such a genetic screen we isolated a number of flies with elongated chromosomal termini. Southern blot analysis and sequencing showed that some *HeT-A* attachments were generated by transposition to the chromosome terminus, while others were generated by gene conversion using as a template a *HeT-A* element from the

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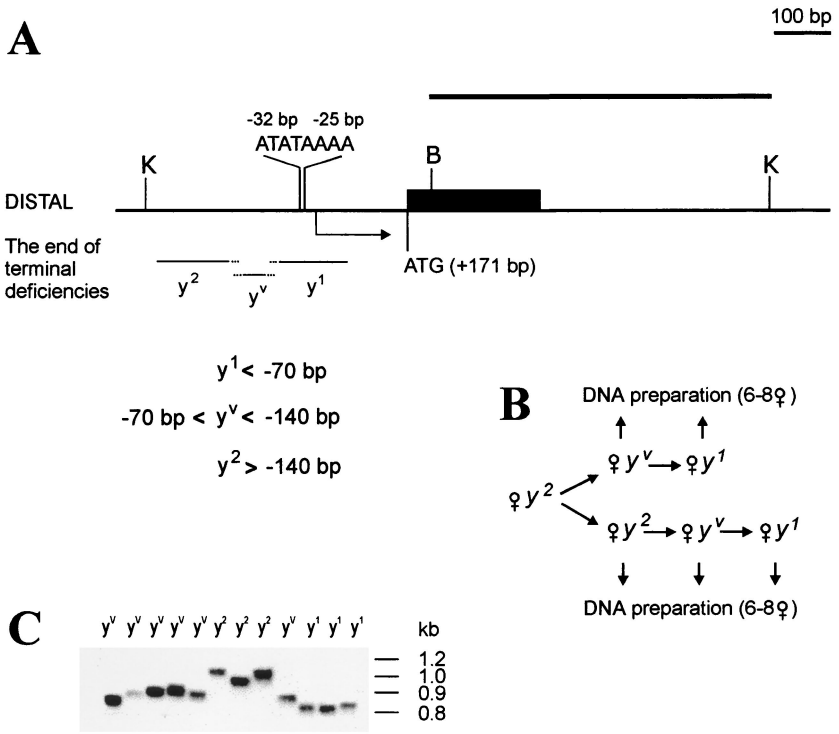

FIG. 1. Minimal promoter region responsible for *yellow* activation at the tip of the terminal deficiencies. (A) A schematic presentation of terminal *yellow* deficiencies associated with different *y* phenotypes. The localization of regulatory regions, promoter, and start of translation are indicated according to the transcription start site of the *yellow* gene. The coding *yellow* region is shown as a black box. The sequence and localization of the *yellow* promoter is presented. The start of *yellow* transcription is shown by an arrow. The translation start codon (ATG) is indicated. The thin horizontal lines show the regions of *yellow* sequence in which the termini of the y^{TD} line that correspond to the same class of y phenotype have been mapped. A *Bam*HI-*Kpn*I genomic fragment used as a probe for Southern blot analysis is indicated by a thick line in the upper part of the figure. Restriction enzyme abbreviations: B, *Bam*HI; K, *Kpn*I. (B) Scheme used to study the correlation between the DNA structure and y phenotype. In subsequent generations two or three y^{TD}/*yac* sisters displaying either the y^2-like or the y^v or y^1-like phenotype were crossed individually with *yac* males. Other females in groups of six to eight were combined according to their phenotypes and used for DNA preparation. (C) Southern blot analysis of DNAs prepared from six to eight y^{TD}/*yac* sisters displaying either y^2-like or y^v or y^1-like phenotype. DNAs were digested with *Kpn*I. The filter was hybridized with the *Bam*HI-*Kpn*I probe.

homologous chromosome. A significant fraction of *HeT-A* attachments were characterized by a large size, exceeding 20 kb. Their structure might be explained in terms of recombination between the 3′ terminus of the telomeric *HeT-A* element and the receding end of *HeT-A* attached to the *yellow* gene. As a result, the terminal deficiency carrying a single *HeT-A* element acquired a large array of telomere sequences. Our results suggest that *Drosophila HeT-A* arrays can be elongated not only by transposition but also by gene conversion and/or recombination mechanisms.

MATERIALS AND METHODS

Genetic crosses. All *Drosophila* stocks were maintained at 25°C on a standard yeast medium. Genetic symbols for the *yellow* alleles and their origin were described elsewhere (22, 35). Most of genetic markers used were described by Lindsley and Zimm (30). The *yac* chromosome contains 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 a homologue in the absence of other *yellow* sequences.

For determination of the *yellow* phenotype, the levels of pigmentation in different tissues of adult flies were estimated visually in 3- to 5-day-old males and females developing at 25°C as described in reference 22.

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 Hybond-N^+ nylon membrane (Amersham, Arlington Heights, Ill.) and in the laboratory manual (41).

The junctions between newly transposed mobile elements and the DNA terminus were cloned by DNA amplification with two oligonucleotide primers. PCR was done by standard techniques (21). The primers used in DNA amplification were from the *yellow* gene and the *HeT-A* element. The numbers of nucleotide map positions are given below in parentheses in accordance with the *yellow* sequences (23) and the *HeT-A* element (6). The primers for the *yellow* gene are as follows: y1, CCTGGAACATTGCAC (3053 to 3039); y2, AAGACGGCGTC ACCAAGGTGATC (3101 to 3078); and y3, ACTTCCACTTACCATCACGC CAG (3293 to 3271). The primers in the *HeT-A* element are as follows: h1, ATACTGCAAGTGGCGCGCATCC (455 to 434); and h2, GGTGCTTCCGT ACTTCTGGCGG (359 to 338).

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

RESULTS

Determination of the *yellow* promoter region which is sufficient for maintaining *yellow* expression at the chromosome terminus. In order to study the frequency of *HeT-A* transposition to a broken chromosomal end, we used the alleles with terminal deficiencies consisting of 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 result in the y^1-like phenotype (Fig. 1). Transposition of a promoter-containing *HeT-A* element to the end of a deficient chromosome should activate *yellow* expression in bristles (y^2-like phenotype) if the *yellow* translation start site has not been deleted. This model system provides a simple genetic screen for monitoring *HeT-A* additions.

To establish the model system we determined the minimal

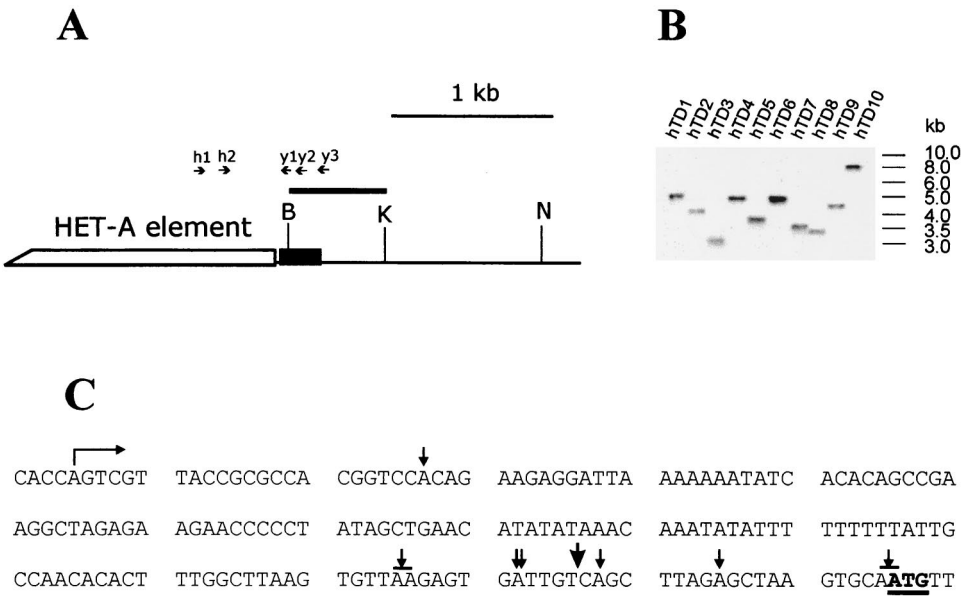

FIG. 2. *HeT-A* transpositions to the broken chromosome terminus in the *yellow* gene. (A) Partial restriction map of the wild-type *yellow* gene region, indicating the position of the *Bam*HI-*Kpn*I probe that was used for Southern blot analysis. The coding *yellow* region is shown as a black box. Restriction enzyme abbreviations: B, *Bam*HI; K, *Kpn*I; N, *Nru*I. The primers in the *HeT-A* element and the *yellow* gene used for DNA amplification are shown by arrows. (B) *HeT-A* additions as indicated by Southern blot hybridization of genomic DNA restricted with *Nru*I and probed with the *Bam*HI-*Kpn*I fragment. The *Nru*I site is at position +1835 relative to the transcription start site of the *yellow* gene. (C) Attachment points of *HeT-A* elements in the *yellow* gene. The start of *yellow* transcription is shown by a bent arrow. The translation start codon ATG is underlined. The points of *HeT-A* attachment are shown by small arrows. The attachment of *HeT-A* in the y^{hTD3} line is indicated by a large arrow. One or two A bases at the junctions may originate either from the receding *yellow* sequences or from the oligo(A) tail of the 3′ end of the attached *HeT-A* element.

regulatory region in the deficient chromosomes that allows *yellow* activation in bristles (Fig. 1). By Southern blot analysis four lines carrying deficiencies terminating in the region from −400 bp to −300 bp upstream of the *yellow* transcription start site were selected. Flies of these lines displayed a y^2-like phenotype due to *yellow* activation in bristles by the enhancer located in the *yellow* intron (2, 24, 32). The *yellow* terminal deficiencies displaying a y^2-like phenotype were designated as y^{2TD}. The y^{2TD}/*yac* females were crossed individually with *yac* males. After several generations exceptional flies with variegated bristle pigmentation (y^v phenotype) were found among y^2-like females (Fig. 1). A few of the y^2-like and y^v-like females were taken for DNA preparation; other y^v females and their y^2-like sisters were crossed individually with *yac* males (Fig. 1B). Among their progeny y^v females gave rise to y^1-like (designated y^{1TD}) females. In the next generation all progeny exhibited a y^1 phenotype. DNA was isolated from groups of six to eight sisters with the same phenotype, as shown in Fig. 1B.

Southern blot analysis (Fig. 1C) showed that the distance between the end of the chromosome and the transcription start site was 140 bp or more in y^2-like flies and less than 70 bp in y^1-like flies. It varied from 140 to 70 bp in y^v-like flies. Thus, deficiency chromosomes that terminate at position −70 bp have slightly less than the minimal sequence necessary to express the *yellow* gene in any tissue.

The model system also allowed us to identify additions of HeT-A elements into the ends of these deficient chromosomes. Transposition of a *HeT-A* element onto the end of the deficient chromosome can be detected visually in progeny of y^{1TD} females carrying a terminal deficiency chromosome broken in the interval between −70 bp and +171 bp (the position of the translation start codon of the *yellow* gene). Such transposition produces y^2-like progeny of y^1-like parents. If the *HeT-A* element is attached to the *yellow* sequence downstream of +171 bp, the bristle pigmentation is not restored. As the deficiency terminus loses about 70 bp per generation on average (2–6, 27, 42), the interval between −70 bp and +171 bp is expected to be lost over a period of three generations.

The frequency of the *HeT-A* transposition to the broken chromosome terminus in the *yellow* gene depends on the genotype. The above system was used to determine the frequency of *HeT-A* transposition. We obtained nine independent y^{2TD}/*yac* lines that had a terminally deficient chromosome broken approximately 300 bp upstream of the *yellow* transcription start site. The terminal *yellow* deficiencies in these lines originated as described previously (35) from single females after crosses with different laboratory strains, such as those with the genotype y^1w or *yacw* or $yacw^+$ or *Oregon-R*. As a result, the lines had similar but not identical chromosomal contexts.

y^{2TD}/*yac* lines were propagated for several generations, and newly arising y^1-like females were crossed individually with *yac* males for three subsequent generations. For any of the nine y^{1TD}/*yac* lines we examined 6,000 to 14,000 flies (altogether about 65,000 flies were examined). Approximately the same number of flies from each generation was scored.

The appearance of y^2-like flies among the y^1-like offspring was observed in only one of these lines. Fourteen independent $y^1 \rightarrow y^2$ transitions were found among ca. 6,900 flies scored; i.e., the frequency of y^2-phenotype appearance was about 2×10^{-3}. No y^2-like females were found in the other eight y^{1TD}/*yac* lines (58,100 flies scored). Thus, the frequency of terminal elongation in these lines was lower than 2×10^{-5}. These results suggest that the frequency of the *HeT-A* transposition strongly depends on the particular chromosomal context of the line.

To determine the molecular nature of $y^1 \rightarrow y^2$ transitions, the DNAs of the y^2-like derivatives were studied with the aid of Southern blot analysis (Fig. 2). In all y^2-like alleles, the appearance of an additional DNA sequence at the broken end was

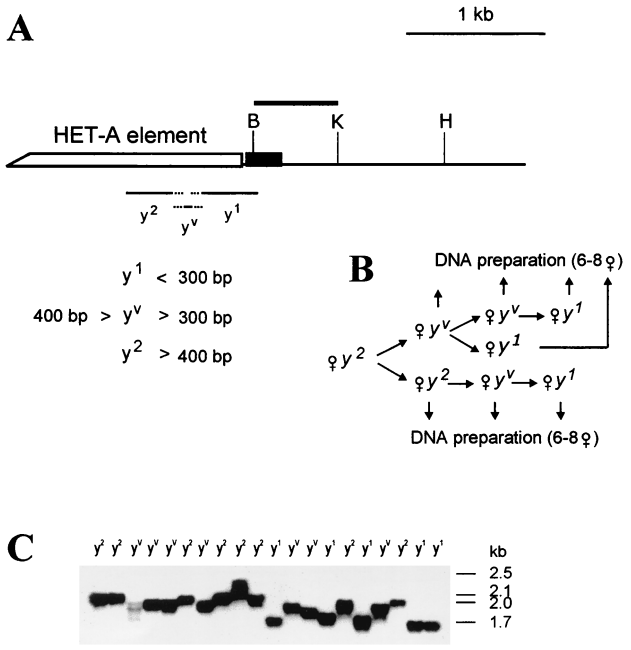

FIG. 3. Minimal region of the *HeT-A* element which is sufficient for compensation of the *yellow* promoter deletion at the tip of the X chromosome. (A) Scheme of terminal deficiencies associated with different y phenotypes. The *yellow* coding region is shown by a black box. The thin horizontal lines in the lower part of the scheme show the regions of the *HeT-A* element (numbered from the 3′ *HeT-A* end) in which termini that correspond to the y phenotype have been mapped. The figures show the distance from the first nucleotide of the 3′ end of the *HeT-A* element. Restriction enzyme abbreviation: H, *Sph*I. Other designations are as in Fig. 1. (B) The scheme used to study the relationship between the length of the terminal *HeT-A* element and the y phenotype. (C) Southern blot analysis of y^{hTD3}/*yac* sisters displaying y^2-like, y^v, or y^1-like phenotypes. DNAs were digested with *Sph*I. The filter was hybridized with the *Bam*HI-*Kpn*I fragment indicated by the thick line in panel A. The *Sph*I site is 1435 bp from the *HeT-A* attachment in y^{hTD3}.

observed. The sizes of DNA additions were measured by Southern blot hybridization of genomic DNA restricted with *Nru*I, which usually does not cleave *HeT-A* DNA (5); and the sizes varied from 1.4 to 6.6 kb. These y^2 derivatives were referred to as y^{hTD} (*HeT-A*-healed terminal deletions) and numbered from 1 to 14.

To directly show the transposition of the *HeT-A* elements to ends broken at *yellow*, we amplified by PCR the DNA between primers in the *yellow* coding region and the 3′ region of the *HeT-A* element (Fig. 2). The junctions between mobile elements and the *yellow* gene were sequenced in eight y^2-like derivatives (Fig. 2C). In all cases, a string of adenine residues was present between the *yellow* and *HeT-A* sequences. The 3′ *HeT-A* sequences (5′ CCTCCACCAGCAAAGTT 3′) were highly conserved and identical to the previously sequenced *HeT-A* elements (4, 5, 6). These results suggest that *HeT-A* addition to broken chromosomal termini occurs through transposition if the homologous sequences allowing gene conversion (35) are absent.

Estimation of a minimal *HeT-A* region that is sufficient for maintaining *yellow* expression in bristles. In order to monitor the fate of the *HeT-A* element attached previously to the *yellow* sequences, we first determined the minimal *HeT-A* region sufficient for *yellow* expression in bristles (Fig. 3). For this the y^{hTD3} line, carrying a *yellow* deficiency terminating with a 1.4-kb *HeT-A* sequence, was selected (Fig. 2B). Using Southern blot hybridization of DNA from the progeny of individual y^{hTD3}/*yac* females, two y^{hTD3} sublines carrying *yellow* terminal deficiencies with an approximately 500-bp *HeT-A* sequence attached were isolated. These y^{hTD3}/*yac* females were crossed individually with *yac* males; after several generations some females acquired variegated bristle pigmentation (y^v phenotype). Sisters displaying either y^2-like or y^v phenotypes were mated individually with *yacw* males (Fig. 3B). Six to eight y^2-like or y^v sisters were collected for DNA preparation and Southern blot analysis. In the next generation, the progeny of y^v females displayed the y^1-like phenotype, whereas progeny of y^2-like sisters acquired variegated bristle pigmentation. Again, six to eight sisters displaying either the y^1-like or y^v phenotype were collected for DNA preparation.

The results of Southern blot analysis showed that the terminal *HeT-A* element was longer than 400 bp in y^2-like flies and shorter than 300 bp in y^1-like flies (Fig. 3C). The size of the *HeT-A* element varied from 300 to 400 bp in y^v flies. Thus, 400 bp of the *HeT-A* 3′ terminus is sufficient for wild-type levels of *yellow* expression in bristles.

Detection of new *HeT-A* attachments to the terminal *HeT-A* element. As shown above, the presence of less than 300 bp from the 3′ end of *HeT-A* does not compensate for the absence of the *yellow* promoter. It may be expected that the attachment of a new *HeT-A* element should restore the *yellow* activation. If this is the case, it is possible to monitor the addition of novel *HeT-A* elements to a preexisting *HeT-A* element by visual analysis of bristle pigmentation.

To monitor the behavior of receding *HeT-A* termini, we obtained four y^{hTD3} sublines started from a single y^{hTD3} female. These lines were termed A, B, C, and D. At the beginning of the experiment the X chromosome in all four sublines carried about 400 bp of *HeT-A* sequence attached at position +152 bp of *yellow* sequence and as a result displayed a y^2-like phenotype. In the offspring of y^{hTD3}/*yac* females we selected females with y^1-like phenotype and individually crossed them with *yac* males for three successive generations. For any of four y^{hTD3} lines we examined about 14,000 flies (altogether 53,500 flies were scored). In all sublines, y^2-like females were found as single events (31 cases) or in clusters (10 cases). Sixty-four y^2-like females were found altogether. This gives an average frequency of $y^1 \rightarrow y^2$ phenotype transition of ca. 1.2×10^{-3}.

DNA was prepared from the offspring of these selected y^2-like females for Southern blot analysis (Fig. 4). Many *HeT-A* elements have sites for *Kpn*I and *Eco*RI restriction endonucleases at the 3′ end, have sites for *Spe*I and *Eco*RV in the central region, and have no sites for *Nru*I (4–6). On the other hand, all these endonucleases have sites in the *yellow* transcription unit in the vicinity of the *HeT-A* attachment (Fig. 4A). Therefore, these enzymes were used for DNA hydrolysis. The *Bam*HI-*Kpn*I fragment subcloned from the *yellow* gene was used as a probe (Fig. 4).

Usually, different individual y^2-like lines derived from the same cluster have identical restriction maps of the new *HeT-A* attachments (Fig. 4 and 5), suggesting that DNA additions happened at the premeiotic stage in the germ line. Most of the DNA attachments had *Kpn*I and *Eco*RI sites at the 3′ end, as is typical of the 3′ end of the *HeT-A* element (Fig. 5). The size of new DNA additions varied widely, from less than 1 kb to more than 20 kb. In the case of large DNA additions, we could not precisely estimate their size because of a possible existence of additional restriction sites in the new DNA sequences and also due to the low resolution of large DNA fragments by conventional Southern blot analysis. For convenience, we divided the DNA attachments into two groups according to their size: those with a size between 0.5 and 8 kb (Fig. 5A) and those with a size exceeding 10 kb (Fig. 5B).

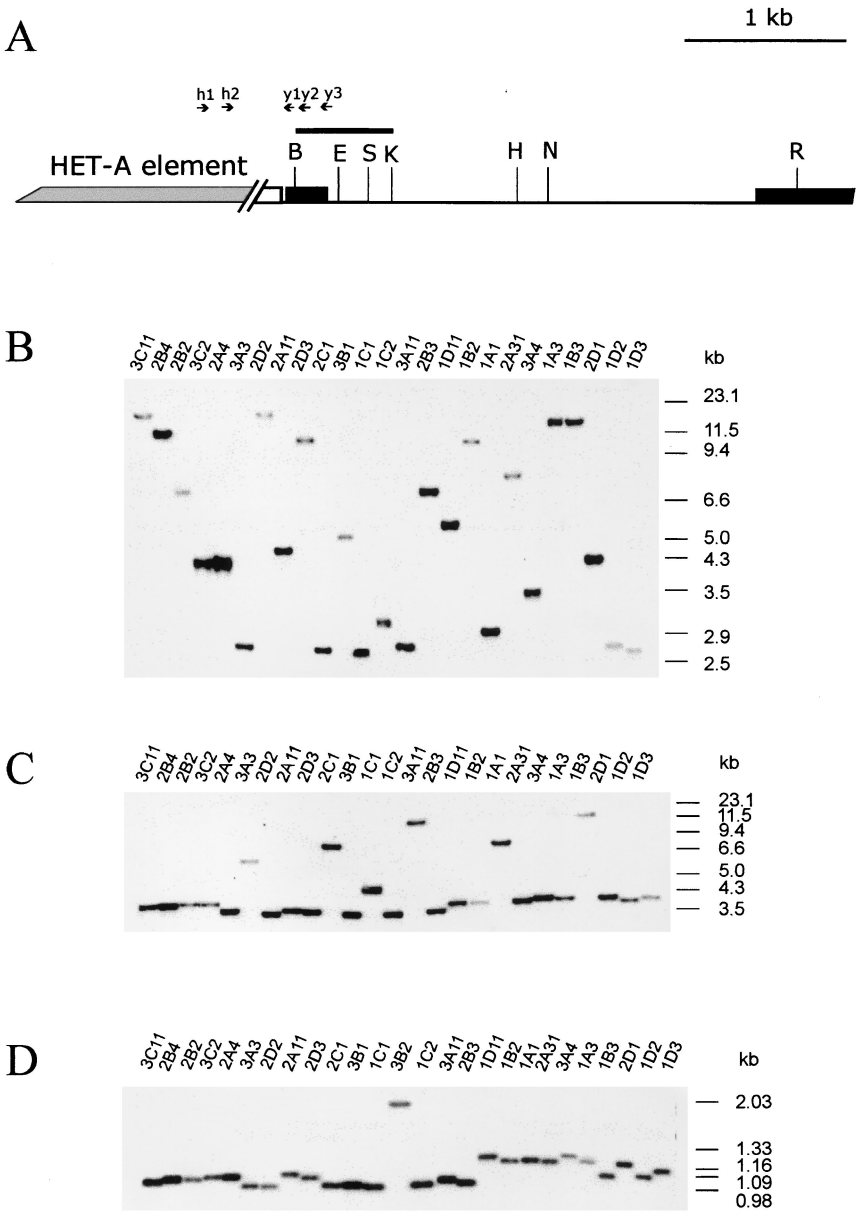

FIG. 4. Molecular additions to the tip of the X chromosome. (A) Partial restriction map of the wild-type *yellow* gene region, indicating the position of sites for restriction enzymes used for Southern blot analysis. The *HeT-A* addition is shown by a shaded box. The coding *yellow* regions are shown by black boxes. Restriction enzyme abbreviations: B, *Bam*HI; K, *Kpn*I; E, *Eco*RV; H, *Sph*I; S, *Spe*I; N, *Nru*I; R, *Eco*RI. Other designations are as in Fig. 1 and 2. (B through D) Southern blot analysis of DNAs prepared from y^2-like lines. DNAs were digested with *Nru*I (B), *Eco*RI (C), and *Kpn*I (D). The filters were hybridized with the *Bam*HI-*Kpn*I fragment.

Mechanisms of attachment of short *HeT-A* sequences: transposition of new *HeT-A* elements and terminal DNA extension by gene conversion. To study the mechanisms of attachment of *HeT-A* sequences we cloned by PCR and sequenced junctions between terminal *HeT-A* elements and new DNA attachments (Fig. 6 and 7). Two primers were used for DNA amplification, one located in the *yellow* gene and the other in the conserved region of the *HeT-A* element between 330 and 460 bp from the 3′ end (Fig. 4A). The latter was absent from the terminal *HeT-A* element in y^1-like derivatives but present in the newly attached *HeT-A* elements. The junctions were identified by comparing the relevant sequences from y^2-like derivative lines and the original y^{hTD3} line.

We analyzed a total of 23 y^2-like lines. In the cases where two y^2-like lines were obtained from the same progenitor fly, the restriction maps and nucleotide sequences were identical between the two lines (Fig. 5 and 7). Further, the structure of only independently obtained y^2-like lines will be discussed below.

Sequencing of four y^2 lines showed no oligo(A) tracts. Further, the extension on the chromosome end in these lines contained sequences internal to the *HeT-A* element immediately 5′ of the old element and not at the 3′-most end of the element (Fig. 6). The newly attached DNA of these two y^2-like lines (3B2 and 3C4) contained many nucleotide substitutions and small gaps compared to the original *HeT-A* element attached to the *yellow* terminal deficiency in the y^{hTD3} line (Fig. 6). The presence of multiple changes in the DNA sequence

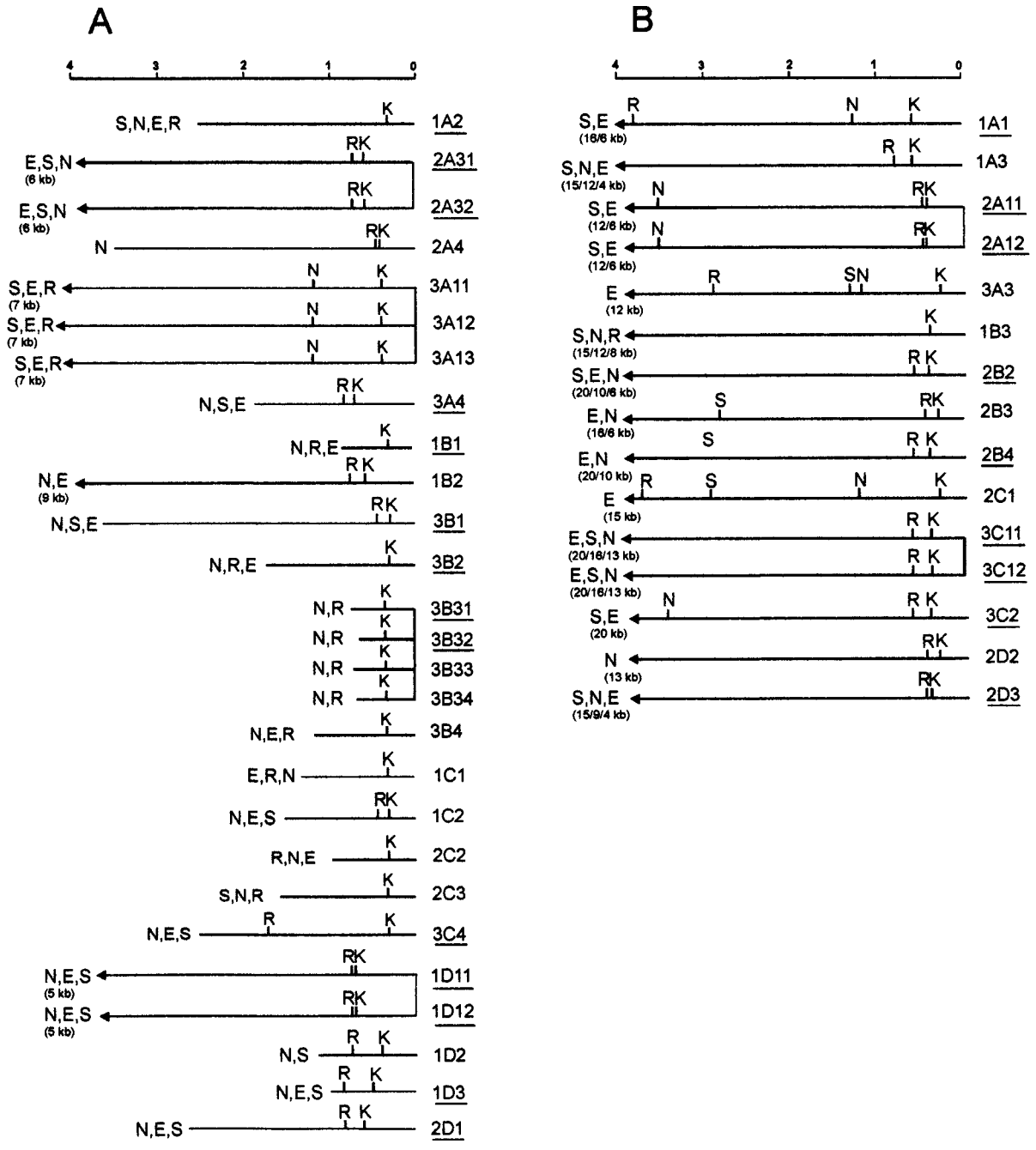

FIG. 5. Restriction map of the DNA additions to the terminal *HeT-A* element as inferred from genomic Southern blot analysis. The maps of the DNA additions obtained as clusters of identical events are linked by vertical lines. The maps start from the *Bam*HI site located in the *yellow* gene at 39 bp from the *HeT-A* attachment site in the y^{hTD3} line. (A) Restriction maps of the DNA attachments with a determined size; (B) restriction maps of the DNA attachments with undetermined size. The lines described in Fig. 6 and 7 are underlined. Abbreviations of restriction enzymes are as in Fig. 4.

suggests the DNA elongation by gene conversion on the template of a homologous, but not identical, *HeT-A* sequence located somewhere else in other telomeres. In two other lines (1A2 and 1B1), the elongated *HeT-A* element had the same structure as the original, suggesting the use of a *HeT-A* element whose structure was identical to that of receding *HeT-A* as the template (data not shown).

In two lines (2D1 and 3A4) we found an oligo(A) tract and the conservative 3′ end of a new *HeT-A* element. However, sequences proximal to the *HeT-A* 3′ terminus contained many nucleotide substitutions compared to the original *HeT-A* element. This suggests that such attachments are also generated by gene conversion. A short, truncated *HeT-A* element and the 3′ terminus of an adjacent *HeT-A* element may serve as the template in this case.

All these attachments were no longer than 2.7 kb. This is consistent with our previous observation that the average length of converted tracts as determined for terminal deficiencies in the *yellow* gene was an estimated 2.6 kb (34).

Finally, in the 3B31 line the sequence of the original *HeT-A*

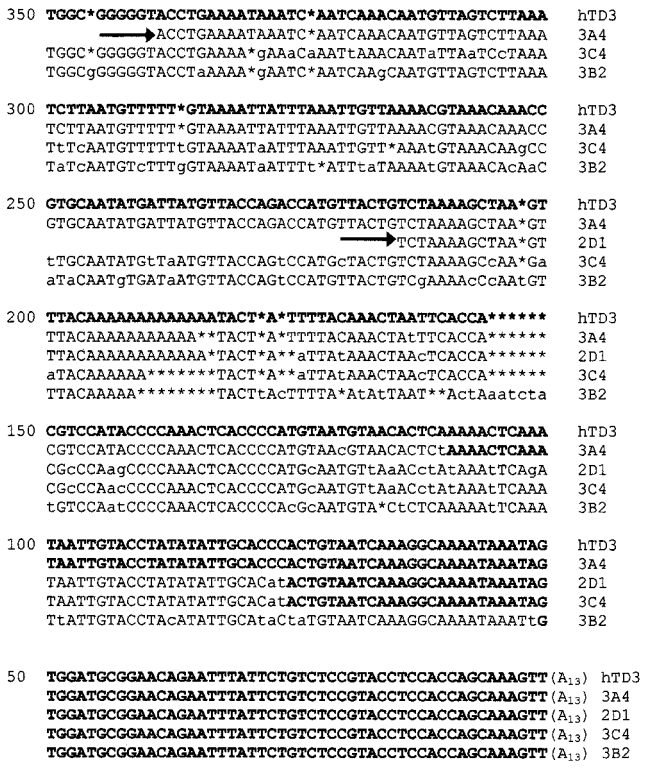

FIG. 6. Aligned sequences of the original *HeT-A* element and four y^2-like derivatives. All sequences end with the last nucleotide at the 3′ end of the original *HeT-A* element. The sequences are shown in the 5′-to-3′ orientation. Only the last 350 nucleotides of aligned sequences are shown. The small letters show the substitutions in the sequence. Asterisks indicate missing nucleotides. Arrows represent the 3′ ends of new *HeT-A* elements. The sequences that may refer to the original *HeT-A* element in the y^{hTD3} line are shown by bold letters.

element was interrupted at the position of 104 bp (numbered relative to the 3′ end of the *HeT-A* element). Attached at this point were a short, altered sequence from another *HeT-A* element (from 301 to 330 bp), two T bases, and the 3′ terminus of the third *HeT-A* element (Fig. 7). The small size of the *HeT-A* attachment (0.6 to 0.7 kb) and the presence of an additional DNA tract between the receding *HeT-A* element and the 3′ end of the new *HeT-A* addition suggest DNA elongation by gene conversion. Possibly a homologous *HeT-A* region was used as a conversion template. Thus, in 6 or 7 out of the 18 independent *HeT-A* attachments tested, gene conversion is implicated.

In two lines (2A31 and 3B1), *HeT-A* elements appear to have used their oligo(A) tails of different lengths to attach to the target sites (Fig. 7). Sequences of the 3′ *HeT-A* ends (5′C CAGCAAAGTT 3′) were conserved as in all the cases of *HeT-A* transposition to the *yellow* locus at the deficient terminus (see above). These observations suggest DNA elongation by true transposition of the *HeT-A* elements. The size of attachments (3 to 6 kb) is also consistent with these data.

Two independent lines (1D11 and 1D3) displayed a more complex structure at the junction. In the 1D11 line, the newly attached sequence begins with three A bases representing the oligo(A) tail and an additional sequence, TCAG, inserted between the oligo(A) and the conserved 3′ terminus of the new *HeT-A* element (Fig. 7). In the 1D3 line, we found a duplication of the *HeT-A* 3′ tail consisting of two tandem 3′ *HeT-A* regions (Fig. 7). The sizes of the attachments were 5 and 1 kb, respectively. The structures of the newly attached *HeT-A* sequences in the 1D11 and 1D3 lines are difficult to explain in terms of either of the two terminal DNA elongation mechanisms discussed here. Still, neither of them can be ruled out.

The large *HeT-A* attachments may be generated by an alternative mechanism. Seven independent DNA attachments (1A1, 2A11, 2B2, 2B4, 2D3, 3C11, and 3C2) belong to the second group characterized by extension exceeding 10 kb (Fig. 5B).

Apart from large size, all these DNA attachments (Fig. 7) begin with an oligo(A) tail and a conserved 3′ end typical of *HeT-A* elements. Sequence comparison revealed that the target *HeT-A* element contains several A bases at the junction. Thus, some of these A bases may belong to either the oligo(A) of the new *HeT-A* element or the target *HeT-A* element. All of these newly attached *HeT-A* elements bear different base substitutions in the normally conserved 3′ terminal GTT triplet. The generation of such DNA attachments as well as their large size may not be explained by *HeT-A* transposition or gene conversion. The presence of several A bases at the target sequence, the extremely large size of DNA attachments, and the aberrant 3′ terminal sequence suggest that they were formed as a result of recombination between the receding *HeT-A* element at the *yellow* locus and some other telomeric *HeT-A* element rather than transposition (see Discussion).

DISCUSSION

Transposition of *HeT-A* elements to terminally deficient X chromosomes. The *HeT-A* element has a promoter at the 3′ end (16). Here, we show that the 400-bp sequence at the 3′ end of the *HeT-A* element is sufficient for activation of *yellow* expression in bristles. Specific activation of *yellow* transcription by the *HeT-A* promoter in bristles only is probably due to the presence of the bristle enhancer located in the *yellow* intron. The body and wing enhancers are upstream of the *yellow* promoter and have been removed. A possible role of the *HeT-A* promoter specificity should also be considered.

The attachment of *HeT-A* or *TART* elements to the terminally deficient X chromosome may be considered real transposition events because of the absence of extended homology between the mobile elements and target site within the *yellow* locus, which is necessary for DNA elongation by gene conversion. In all cases, the oligo(A) track and the conserved 3′ end sequence of *HeT-A* necessary for transposition were found at the junction, confirming the transposition mechanism for *HeT-A* attachments (4–6; this study). The joining of the 3′ end of *TART* or *HeT-A* to the broken end is explained by a model for terminal transposition in which reverse transcription of the retrotransposon is initiated at its 3′ end by using the chromosome end as a primer (4, 5, 7, 8, 28, 33, 38, 42, 45). This model explains the invariant orientation of the *HeT-A* and *TART* copies which were isolated from native telomere.

The genetic system described here selectively visualizes only attachments of *HeT-A* elements. The *TART* element seems to contain no promoter at the 3′ terminus (19), and its attachments seem to fail to support *yellow* expression. We found that the frequency of *HeT-A* additions to the *yellow* sequences was relatively low and depended strongly on chromosomal context.

Attachment of a new *HeT-A* element to the terminal HeT-A may occur by different mechanisms. Recombination of repetitive telomeric ends has been considered an alternative reserve pathway for telomere elongation (8, 9, 11, 15, 29, 31, 34, 38, 39, 40, 46). Indirect evidence exists that telomeres of *Chironomus* and *Anopheles gambiae* are extended by recombination and gene conversion mechanisms involving long complex terminal repeats (8, 15, 31, 39). This pathway has been well documented

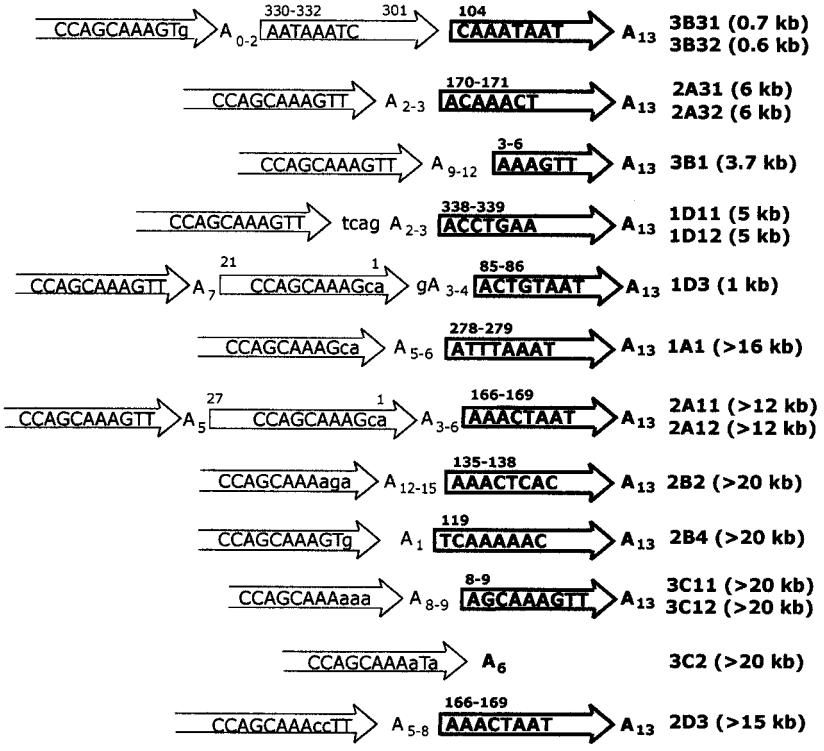

FIG. 7. Diagram of *HeT-A* additions to the receding *HeT-A* element. The numbers in parentheses show the approximate sizes of the attachments. The arrows indicate the directions of the *HeT-A* elements. The bold arrows correspond to the original *HeT-A* element. The numbers above the arrows indicate distances of either the 5′ terminus or the 5′ and 3′ termini of the *HeT-A* element from the 3′ terminus of a standard element. For this, the terminal *HeT-A* element present in the original y^{hTD3} line was used (Fig. 6). A base at the junctions may originate either from the terminal *HeT-A* element or from the 3′ oligo(A) tail of the new *HeT-A* element. The base pairs at the junction between new and old *HeT-A* elements are shown. The lowercase letters indicate substitutions in the conserved sequence at the 3′ end of the *HeT-A* element.

for yeast, where telomeres are extended by telomerase, but recombination and/or gene conversion serves as an efficient bypass mechanism for chromosome length maintenance when telomerase is inactive (11, 12, 29, 38). Recombination has also been suggested as the mechanism for telomere maintenance in several immortalized human cell lines that harbor no telomerase activity (10, 13, 14, 25, 26, 43, 44). Thus, recombination-based mechanisms are present in most organisms as an alternative mechanism of unregulated telomere elongation (37). Normally, the recombination pathway may be blocked by a special class of telomere-bound proteins and may be tightly regulated during DNA replication (38).

Previous data (4, 5) and our observations showed that the attachment of new *HeT-A* element(s) to the receding end of a terminal *HeT-A* element happened with a rather high frequency. However, in contrast to previous observations, we found that DNA elongation did not occur only by transposition of the mobile elements. Short DNA attachments were more frequently generated by DNA extension through conversion using homologous *HeT-A* sequences located on another telomere as a template. The average size of DNA extensions is approximately the same as that obtained in the experiments with terminal DNA elongation of the *yellow* sequences by conversion mechanisms (35). Sometimes the 3′ sequence of a new *HeT-A* element was found in close vicinity to the start of the conversion track. This may reflect the structure of telomeres, which often contain arrays of truncated 3′ ends of *HeT-A* elements (17, 28, 37).

The mechanism of long DNA attachments. Unexpectedly, many *HeT-A* attachments had a large size exceeding several-fold the size expected for the full-length transcript of the *HeT-A* element (17, 38). Interestingly, the large DNA attachments occur frequently. Previously, among four independent *HeT-A* attachments to a terminal *HeT-A*, two exceeded 14 kb (5). In our experiments they comprised 13 of 33 DNA elongations. The second feature of this class of attachments is the presence of substitutions in the 3′ terminal nucleotides of the *HeT-A* element. These nucleotides were conserved among all 10 studied *HeT-A* additions to the terminal *yellow* sequences, suggesting their importance for transposition. These observations argue against the transposition mechanism for the long DNA attachments.

The existence of a small amount of high-molecular-weight RNA homologous to *HeT-A* (19) still does not allow one to exclude the transposition of long *HeT-A* arrays via an RNA intermediate. This minor fraction of *HeT-A* RNA is thought to represent readthrough transcripts of tandem *HeT-A* elements (19). However, it is difficult to imagine that such long transcripts are much more efficient in transposition than the truncated or full-length *HeT-A* transcripts.

It is more likely that the large DNA attachments are generated by site-specific recombination using several A bases at the terminal *HeT-A* and the oligo(A) tail of a *HeT-A* located at another telomere. As a result, a large fragment of telomere sequence is transferred to the chromosome end.

Analysis of the sequenced *HeT-A* elements showed that the 3′ noncoding region of *HeT-A* was rather conserved, and only a few *HeT-A* subfamilies existed (17, 18). This conservation of sequences within *HeT-A* subfamilies is difficult to explain from the viewpoint that telomeres are elongated only by transposition of *HeT-A* elements via an RNA-templated step. Rapid sequence change has been reported for many elements with

an RNA-based step in replication (20). The conservation of *HeT-A* sequence was explained by postulating a limited number of replicatively active *HeT-A* elements (17). In this case, the majority of elements in the genome would be separated from a transcriptionally active *HeT-A* element by only one step of reverse transcription. Another explanation may be that the homogeneity of *HeT-A* sequences has been established by a conversion and/or recombination mechanism. The same mechanism was suggested to explain the gradient homogenization of termini in the yeast (12); the sequences closest to the ends share the highest degree of homology. More likely, both mechanisms are responsible for the *HeT-A* conservation and for telomere elongation.

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