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 retrotransposon-like 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 $2 \times 10^{-3}$ to less than $2 \times 10^{-5}$. 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 $1.2 \times 10^{-3}$. 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 y2-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 y2-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 y2-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 $2 \times 10^{-5}$ to $2 \times 10^{-3}$. 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 the receding end of HeT-A sequence. The terminus of the telomeric HeT-A element (6). The primers for the yellow gene are as follows: y1, CCTGGAACATTGCAC (3053 to 3039); y2, AAGACGGCGTGCG (3293 to 3271); and y3, ACTTCCACTTACCATACGC (359 to 338).

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. 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, AAGACGGCGTGCG (3293 to 3271); and y3, ACTTCCACTTACCATACGC (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 (yTD). Breaks that place the end of the chromosome at the yellow promoter or within the yellow transcription unit result in the y1-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 (y1-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
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 transcription start site were selected. Flies of these lines displayed a y2-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 y2-like phenotype were designated as y2TD. The y2TD/yac females were crossed individually with yac males. After several generations exceptional flies with variegated bristle pigmentation (yv phenotype) were found among y2-like females (Fig. 1). A few of the y2-like and y1-like females were taken for DNA preparation; other y1 females and their y2-like sisters were crossed individually with yac males. Among their progeny y1 females gave rise to y2-like (designated y1TD) females. In the next generation all progeny exhibited a y1 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 y2-like females and less than 70 bp in y1-like females. Thus, deficiencies 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 in 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 y1TD females carrying a terminal deficiency chromosome broken in the interval between −70 bp and +171 bp (the position of the transcription start codon of the yellow gene). Such transposition produces y2-like progeny of y1-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 y1TD/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 y1w or yacw or yacw+ or Oregon-R. As a result, the lines had similar but not identical chromosomal contexts.

y1TD/yac lines were propagated for several generations, and newly arising y2-like females were crossed individually with yac males for three subsequent generations. For any of the nine y1TD/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 y2-like flies among the y2-like offspring was observed in only one of these lines. Fourteen independent y1 → y2 transitions were found among ca. 6,900 flies scored; i.e., the frequency of y2-phenotype appearance was about 2 × 10−5. No y2-like females were found in the other eight y1TD/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 y1 → y2 transitions, the DNAs of the y2-like derivatives were studied with the aid of Southern blot analysis (Fig. 2). In all y2-like alleles, the appearance of an additional DNA sequence at the broken end was
observed. The sizes of DNA additions were measured by Southern blot hybridization of genomic DNA restricted with NruI, which usually does not cleave HeT-A DNA (5); and the sizes varied from 1.4 to 6.6 kb. These y' derivatives were referred to as y'\textsuperscript{TD} (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'\textsuperscript{TD} 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' CCTCCACCGCAAAAGTT 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'\textsuperscript{TD} 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'\textsuperscript{TD}/yac females, two y'\textsuperscript{TD} sublines carrying yellow terminal deficiencies with an approximately 500-bp HeT-A sequence attached were isolated. These y'\textsuperscript{TD}/yac females were crossed individually with yac males; after several generations some females acquired variegated bristle pigmentation (y' phenotype). Sisters displaying either y'-like or y' phenotypes were mated individually with yacw males (Fig. 3B). Six to eight y'-like or y' sisters were collected for DNA preparation and Southern blot analysis. In the next generation, the progeny of y' females displayed the y'-like phenotype, whereas progeny of y'-like sisters acquired variegated bristle pigmentation. Again, six to eight sisters displaying either the y'-like or y' 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'-like flies and shorter than 300 bp in y'-like flies (Fig. 3C). The size of the HeT-A element varied from 300 to 400 bp in y' 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'\textsuperscript{TD} sublines started from a single y'\textsuperscript{TD} 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'-like phenotype. In the offspring of y'\textsuperscript{TD}/yac females we selected females with y'-like phenotype and individually crossed them with yac males for three successive generations. For any of four y'\textsuperscript{TD} lines we examined about 14,000 flies (altogether 53,500 flies were scored). In all sublines, y'-like females were found as single events (31 cases) or in clusters (10 cases). Sixty-four y'-like females were found altogether. This gives an average frequency of y'-like phenotype transition of ca. 1.2 × 10\textsuperscript{-3}.

DNA was prepared from the offspring of these selected y'-like females for Southern blot analysis (Fig. 4). Many HeT-A elements have sites for KpnI and EcoRI restriction endonucleases at the 3' end, have sites for Spel and EcoRV in the central region, and have no sites for NruI (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 BamHI-KpnI fragment subcloned from the yellow gene was used as a probe (Fig. 4).

Usually, different individual y'-like lines derived from the same clone 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 additions had KpnI and EcoRI 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).
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 y1-like derivatives but present in the newly attached HeT-A elements. The junctions were identified by comparing the relevant sequences from y2-like derivative lines and the original y<sup>THD3</sup> line.

We analyzed a total of 23 y2-like lines. In the cases where two y2-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 y2-like lines will be discussed below.

Sequencing of four y2 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 y2-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<sup>THD3</sup> line (Fig. 6). The presence of multiple changes in the DNA sequence...
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
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 (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.

Two independent lines (1D11 and 1D3) displayed a more extended attachment (3 to 6 kb) than the second group characterized by extension exceeding 10 kb (Fig. 5B).

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 the extended DNA attachments. 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 DNA attachments contain several A bases at the junction. 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 slight 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) tract 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.
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 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 new HeT-A element added 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

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**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 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 yTdh 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.
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 sharing the highest degree of homology. More likely, both mechanisms are responsible for the HeT-A conservation and for telomere elongation.

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