Two Distinct Domains in *Drosophila melanogaster* Telomeres

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Manuscript received July 27, 2005
Accepted for publication August 16, 2005

**ABSTRACT**

Telomeres are generally considered heterochromatic. On the basis of DNA composition, the telomeric region of *Drosophila melanogaster* contains two distinct subdomains: a subtelomeric region of repetitive DNA, termed TAS, and a terminal array of retrotransposons, which perform the elongation function instead of telomerase. We have identified several Pelement insertions into this retrotransposon array and compared expression levels of transgenes with similar integrations into TAS and euchromatic regions. In contrast to insertions in TAS, which are silenced, reporter genes in the terminal HeT-A, TAHRE, or TART retroelements did not exhibit repressed expression in comparison with the same transgene construct in euchromatin. These data, in combination with cytological studies, provide evidence that the subtelomeric TAS region exhibits features resembling heterochromatin, while the terminal retrotransposon array exhibits euchromatin characteristics.

DNA sequences at the ends of eukaryotic chromosomes are the products of a telomere elongation process. In most eukaryotes, these sequences are simple repeating units that are synthesized by telomerase, but in *Drosophila melanogaster* they are tandem head-to-tail arrays of three non-long terminal repeat retrotransposons, HeT-A, TAHRE, and TART (Mason and Biessmann 1995; Pardee and DeBaryshe 2003; Abad et al. 2004b). Despite these differences, a common feature of eukaryotic chromosomes is a region of complex repeats located adjacent to the terminal sequences. These complex repeats are referred to as subtelomeric regions, or telomere-associated sequences (TAS), and differ in sequence, structure, and length among species and among telomeres within an individual (Pryde et al. 1997). The repetitive nature and the high density of transposable elements in these subtelomeric regions (Mefford and Trask 2002) are reminiscent of heterochromatin. In *D. melanogaster*, TAS consist of several kilobases of complex repeats, which exhibit similarities between the different chromosome ends. Sequences of the 2L and X TAS regions have been described in detail (Karpen and Spradling 1992; Walter et al. 1995), and *in situ* hybridizations to polytene chromosomes showed that 2L TAS share homology with 3L TAS, while X TAS share homology with 2R and 3R TAS. The 2L TAS appear to be 15 kb in length and composed of relatively simple tandem repeats of 457 bp (Walter et al. 1995; Mason et al. 2003b). While the TAS array on other chromosomes may be of similar size (Abad et al. 2004a), X TAS is more complex with a 1.8-kb and a 0.9-kb repeat, which are related to each other (Karpen and Spradling 1992).

*Drosophila* telomeric regions are able to repress gene activity, resulting in partial silencing and variegated expression of reporter genes (Karpen and Spradling 1992), referred to as telomeric position effect (TPE). These repressed transgenes have inserted into or adjacent to TAS (Levis et al. 1993; Cryderman et al. 1999; Golubovsky et al. 2001), suggesting that TAS regions of *Drosophila* telomeres are heterochromatic.

No integrations into the terminal retrotransposon array have been reported. There are several possible explanations for the absence of these integration events. First, the chromatin structure of the retrotransposon array may not allow integration of *P* elements, or if integrations do occur, they may be unstable. Second, integration events may occur in the *HeT-A/TART/TAHRE* array but are not detectable, because expression of the reporter gene is completely repressed due to the heterochromatic nature of the terminal array. Third, integrations may occur, but expression is not repressed; thus, insertion events are not distinguishable from integrations into euchromatic sites. To address this question, we took advantage of the *P* element mobilization screen performed by the Berkeley Drosophila Genome Project (BDGP) Gene Disruption Project to generate insertions at every *Drosophila* gene (Bellen et al. 2004). This screen generated >30,000 independent *P* element...
integrations using constructs SuPor-P and EPyg2. After performing inverse PCR, the BDGP sequenced the 5′- and 3′-flanking regions to determine the genomic location of each integration. We reasoned that among the large number of P-element integrations there might be some that had inserted into the HeTA/TAHRE/TART array, unless this terminal region is refractory to integration. Indeed, by screening genomic sequences flanking the P-element insertions for similarity to HeTA and TART, several such integrations were identified. Extant strains were then subjected to molecular genetic analyses, and the phenotypic expression levels of the reporter genes were determined.

The experiments described in this article were performed for several reasons: (a) to investigate the morphological characteristics of the terminal array and the TAS subterminal region in polytene chromosomes, (b) to determine if P elements can integrate into the terminal array of the Drosophila telomere, and (c) to investigate gene expression at the integration site. The results show that the terminal array is not refractory to P-element insertions. Further, expression of transgenes in the terminal transposon array is not reduced when the insertion is far from TAS. Thus, in contrast to TAS, the terminal arrays do not appear to mediate heterochromatic gene silencing. However, the silencing effect of TAS spreads into the retrotransposon array and can be detected over short distances. We, therefore, suggest that there are two distinct chromatin domains within the Drosophila telomere, one consisting of the terminal retrotransposon array, which is transcriptionally active, and the other containing TAS, which appear by genetic assays to be heterochromatic. The chromosome cap may be a third telomeric domain that does not contain a specific DNA sequence (Biessmann and Mason 1988), but requires specific capping proteins (Fant et al. 1998; Biessmann et al. 2005; Cenci et al. 2005).

MATERIALS AND METHODS

P-element inserts and Blast screening: Two P-element constructs were used in the mobilization screen by the BDGP. SuPor-P (Roseman et al. 1995) carries mini-white gene with its eye enhancer element flanked on both sides by SU(HW) binding sites that protect it from most telomeric position effects. SuPor-P also carries a yellow gene with the body and wing, but not the bristle, enhancers. P-element mobilization gave rise to the KG lines (Bellen et al. 2004). To observe the eye color in flies carrying this element, the transgene was crossed into y w<sup>122</sup>su(Hw)<sup>y</sup> background. This mutant allele of su(Hw) was caused by the insertion of 0.7 kb ofockey into the first intron, resulting in greatly reduced levels of mRNA (Parkhurst et al. 1988) and protein (Harrison et al. 1993). While Harrison et al. (1993) describe this mutation as a weak suppressor of y, we find it to be a strong suppressor. To obtain the EY lines, the EPyg2 element was mobilized. This P-element carries an unprotected mini-white gene without an enhancer and the same yellow gene with body/wing enhancers (Bellen et al. 2004). In flies carrying this element the eye color from the expression of the transgene can be observed without further crosses.

Flanking sequences were obtained from R. Levis and batch blasted (BlastN) (Altschul et al. 1997) against sequences from HeTA (GenBank U06920), TARTA (U02279), TARTB1 (U14101), TARTB2 (U14102), TARTC (U02279), 2L TAS (U35404), and I.8-kb X-TAS (L03284). To identify potential telomeric insertions when both 5′- and 3′-flanking regions of the P-element integration had been determined, we required that both identified the same region in the telomeric retrotransposon and showed the 8-bp duplication generated by the P element. In cases when only one of the flanking regions was known, sequence identity to HeTA, TART, or TAS had to be at least 80% over a substantial part of the flank. In addition to insertions identified in this manner, a SuPor-P element in 3R TAS, 316-1 (Roseman et al. 1995), was provided by P. Geyer. SuPor-P (KGO4716 at position 21A–3A1, KGO0040 at 60E1, and KGO3807 at 60D15–16) and EPyg2 (EY00630 at 59D8, KGO0487 at 62A6–7, and KGO2921 at 62A11–12) insertions into distal euchromatin were obtained from the Bloomington Stock Center (http://flystocks.bio.indiana.edu) and used as controls in gene expression studies.

Genetic isolation of P insertion chromosomes: Drosophila stocks were maintained and crossed were performed at 25° on cornmeal, molasses medium with dry yeast added to the surface. Genetic markers and special chromosomes are described by FlyBase (FlyBase Consortium 2003). To identify the chromosome carrying the insertion, males carrying the transgene were crossed to y w<sup>122</sup>; Sco/SML or y w<sup>122</sup>; Sb/TM6, Ubx females. F1 males were then crossed to y w<sup>122</sup> females, and segregation of the reporter genes was noted. Simultaneous backcrosses of F1 males to balancer females allowed new stocks to be formed with y w<sup>122</sup> on the X chromosome and the marked element on an autosome. Insertions on the X and fourth chromosomes were not considered further.

In a similar fashion, su(Hw)<sup>y</sup> was crossed into KG lines to render the SU(HW) binding sites nonfunctional as insulators. When the SuPor-P element was carried on chromosome 2, y w<sup>122</sup>, homozygous transgene females were crossed to y w<sup>122</sup>; Sb/TM6 males, and F1 transgene+/−; Sb/+ females crossed with y<sup>sc</sup> v; su(Hw)<sup>y</sup>/TM6 males. F2 transgene+/−; su(Hw)<sup>y</sup>/Sb females were then crossed with y w<sup>122</sup>; Sb/TM6 females. Transgene+/−; su(Hw)<sup>y</sup>/TM6 progeny from this cross were mated together in single pairs, and transgene homozygotes were selected by monitoring segregation in subsequent generations.

When the SuPor-P element was on chromosome 3, y w<sup>122</sup>, homozygous transgene males were crossed to y<sup>sc</sup> v; su(Hw)<sup>y</sup>/TM6 females, and Ubx<sup>y</sup> F1 females crossed to y w<sup>122</sup>; Sb/TM6, Ubx males. Ten F2 sc<sup>y</sup> v<sup>v</sup> Ubx males were mated singly to y w<sup>122</sup>; Sb/TM6 females and stocks produced by brother-sister matings, as above. The resulting stocks were then tested for the presence of su(Hw) by crossing males to y<sup>sc</sup> v; su(Hw)<sup>y</sup>/TM6 females and examining y<sup>sc</sup> v<sup>v</sup> Ubx<sup>y</sup> sons for expression of sc.

In situ hybridizations, genomic DNA libraries, and Southern blotting: These were done as described previously (Golubovsky et al. 2001). The P-element vectors EPyg2 or Casper were used to generate digoxigenin-labeled hybridization probes for in situ hybridization.

Polymerase chain reaction amplifications: Polymerase chain reactions (PCR) to amplify <1-kb fragments were done in 50-μl reactions containing ~1 μg of genomic DNA with 2.5 units Taq polymerase (Shuoz, Otsu, Japan) at an annealing temperature of 5–10° below the melting temperature of the primers, with 2-min. synthesis at 72°. Longer DNA fragments were amplified using the Herculase PCR system from Stratagene (La Jolla, CA) or the Phusion polymerase (Finzymes, Espoo, Finland), allowing longer times for extension. Amplified DNA products were tested on agarose gels and sequenced.
and subjected to PCR with primers from either the 3′ or the 5′ ends of the digested DNA was circularized by ligation overnight at 4°C. Digested DNA was precipitated, dissolved in 10 μl of 1× reaction volumes with 2 μl of T4 DNA polymerase (New England Biolabs, Beverly, MA). Enzymes were inactivated by heating for 20 min at 65°C, and 1–2 μl of the digested DNA was used as template for PCR with either the 3′ or the 5′ primers designed to amplify restriction fragments. Restriction enzymes (MboI, HpaII) or six-cutter enzymes (CiaI, SfiI, SalI) (New England Biolabs) were used:

3′P-ry-1: 5′-CCATCGATCCCTGGGTTGAAAT 3′ (Tm 67.2°C), 3′P-ry-4: 5′-CAATCTAGCTGACTCAGAACGCT 3′ (Tm 64.0°C), 5′Plac-R: 5′-GGCCTAGCTGCAGAGGCACCC 3′ (Tm 68.9°C), 5′Plac-1: 5′-CAACCAAGGCTGTCACTCCCATATTTA 3′ (Tm 74.7°C), 5′Plac-4: 5′-GTGACTGTGCGTTAGGTCCTGTTCATTG 3′ (Tm 67.5°C).

2R TAS-dist (TAS 2R/3R/X): 5′-CCTGTTTGGTGTGAAA GCATTCG 3′ (Tm 67.1°C), 2L SAT-R1 (TAS 2L/3L): 5′-CATCTCGTTCATCCGCCACC 3′ (Tm 70.0°C), 3′ roo-F: 5′-GCGATCTGATGTCGTTCATTAACCG 3′ (Tm 70.1°C), allTART-R: 5′-ATTCCTGGGAGGAGGTGTG 3′ (Tm 67.1°C), TART-R2: 5′-GAGCGCGCCGAAAGGGCTG 3′ (Tm 63.3°C), TART-R1: 5′-GGGATCTCCTGGGGTTTGAAT 3′ (Tm 67.5°C), TART-roo-F: 5′-GTGGGCGGAAATGAGTGAGATGG 3′ (Tm 72.5°C).

DNA sequencing and sequence analysis: Sequences were determined in an ABI 3700 sequencer using the Prism Ready Reaction DyeDeoxy terminators v.3.1 from Applied Biosystems (Foster City, CA).

Determination of gene expression: Expression of the white reporter gene was determined in both hemizygous and homozygous males and females. Homozygotes were taken directly from stock. Hemizygotes were produced by crossing males from the insertion stock to y su(+) females that were devoid of suppressors of TPE. When SuPor-P lines were tested, the tester females also carried su(Hw)+ and homozygous su(Hw)+ progeny were examined. To determine expression of the white reporter gene, individuals were collected within a few hours of eclosion and aged 3 days. Expression of the yellow reporter gene was determined in males hemizygous for the transgene. Photographs were taken using a Nikon SMZ-U stereomicroscope with the diaphragm half open.

Electron microscopy of polytene chromosomes: Oregon-R and Tel individuals, as well as their hybrids, were used. Chromosomes were prepared for electron microscopy (EM) observations as described (Semeshin et al. 1989, 2001). Larval salivary glands were dissected in Ephrussi and Beadle solution (Ephrussi and Beadle 1936) and squashed in 45% acetic acid after purification through Zymoclean columns (Zymo Research, Orange, CA) or after cloning into pGem-T-easy (Promega, Madison, WI).

Inverse PCR was done to confirm the flanking sequences provided by the BDGP and in some cases to obtain larger DNA fragments from the flanking regions. Briefly, 5 μg of genomic DNA was digested with restriction enzymes (MboII, HpaII) or six-cutter enzymes (CiaI, Sfi, SalI) (New England Biolabs, Beverly, MA). Enzymes were inactivated by heating for 20 min at 65°C, and 1–2 μg of the digested DNA was circularized by ligation overnight at 4°C in 400-μl reaction volumes with 2 μl of T4 DNA polymerase (New England Biolabs). DNA was precipitated, dissolved in 10 μl of TE, and subjected to PCR with primers from either the 3′ or the 5′ end of the P elements, using either Taq polymerase (for short fragments) or Phusion polymerase (for long fragments) allowing extension times at 72°C between 2 and 15 min, depending on the expected fragment sizes. The following primers (synthesized by Genosys Biotechnologies, The Woodlands, TX) were used:

3′Placb-F: 5′-CATACGGTTAATGGATGTTCTCTTGCC 3′ (Tm 67.2°C), 3′P-py-1: 5′-CCTTAGCATGCTGGGTTTGAAT 3′ (Tm 67.3°C), 3′P-py-4: 5′-CAATCTAGCTGACTCAGAACGCT 3′ (Tm 64.0°C), 5′Plac-R: 5′-GGCCTAGCTGCAGAGGCACCC 3′ (Tm 68.9°C), 5′Plac-1: 5′-CAACCAAGGCTGTCACTCCCATATTTA 3′ (Tm 74.7°C), 5′Plac-4: 5′-GTGACTGTGCGTTAGGTCCTGTTCATTG 3′ (Tm 67.5°C).

2R TAS-dist (TAS 2R/3R/X): 5′-CCTGTTTGGTGTGAAA GCATTCG 3′ (Tm 67.1°C), 2L SAT-R1 (TAS 2L/3L): 5′-CATCTCGTTCATCCGCCACC 3′ (Tm 70.0°C), 3′ roo-F: 5′-GCGATCTGATGTCGTTCATTAACCG 3′ (Tm 70.1°C), allTART-R: 5′-ATTCCTGGGAGGAGGTGTG 3′ (Tm 67.1°C), TART-R2: 5′-GACAGCGCGAAAGGGCTG 3′ (Tm 63.3°C), TART-R1: 5′-GGGATCTCCTGGGGTTTGAAT 3′ (Tm 67.5°C), TART-roo-F: 5′-GTGGGCGGAAATGAGTGAGATGG 3′ (Tm 72.5°C).

RESULTS

Morphology of the 2L telomere in polytene chromosomes: EM was used to obtain detailed cytological information about the 2L telomere of salivary gland chromosomes. This telomere has been studied extensively by molecular techniques. Junctions of the oligo(A) tails of HeT-A to the distal end of TAS, as well as the proximal junction of TAS to single-copy regions, have been defined (Walter et al. 1995). We took advantage of the very long retrotransposon arrays at the chromosome ends of the Tel strain (Sriraco et al. 2002). TAS were localized at the tip of 2L in a Tel/Oregon-R hybrid by in situ hybridization (Figure 1), in bands 21B1 and 21B2. All material distal to this band must consist of the terminal retrotransposon array (Walter et al. 1995).

Subsection 21A, which contains the terminal retrotransposon array, is very short in Oregon-R (Figure 2A). It appears as loose reticulate material, and we could not detect any bands in this region, although single bands are indicated on the cytological map (Lindsley and Zimm 1992). Two single and distinct bands are situated proximal of this reticulate material, which we classify as 21B1 and 21B2. These two bands are easily identified in a Tel 2L chromosome (Figure 2B). In this strain, however, the 21B1 band appears slightly decondensed. Consistent with the extended retrotransposon array in Tel, the 21A region is considerably longer than that in Oregon-R (Figure 2C) and as in Oregon-R has no banding pattern. This enlarged subsection consists of two morphologically distinguished parts: a light proximal zone and denser distal zone, which sometimes has a granular appearance (Figure 2B). In some cases, the distal zone appears to have a banded structure. However, it is impossible to count these structures in serial sections because of their varying number and irregular shape. In any case, both of the zones in 21A are...
obviously decompacted. Chromosome regions with decondensed bands are characteristic of active euchromatic regions, for example, the middle and proximal part of the 21B subsection (Figure 2). By contrast, intercalary heterochromatin regions are densely packed; the 22A1–2 band is a typical region of intercalary heterochromatin (Zhimulev and Belyaeva 2003). Euchromatic bands are intermediate between these extremes. Regions 21C and D show several euchromatic bands. Our EM data can thus distinguish differently compacted chromatin morphologies in the telomere region: the distalmost retrotransposon array appears to be similar to interband regions, while TAS resembles banded material.

**Molecular analysis of integrations into the retrotransposon array:** Comparing the flanking regions of ~20,000 independent P-element integrations provided by the BDGP with HeT-A and TART-A, B, C sequences by BLASTN, we identified 6 integrations in HeT-A, 1 in TAHRE, and 24 in TART (Figure 3). The integration sites in HeT-A and TART were nonrandomly distributed and showed a strong bias for the last 1.5 kb of the TART 3’-UTR. The factors influencing insertion site preference are not well understood, but correlate well with physical properties of chromatin rather than with nucleotide sequence, except for an increased GC content (Liao et al. 2000). A hotspot was observed 656 bp upstream of the oligo(A) tail of TART-B1, with 12 independent EPgy2 integrations, all with the 3’ end of P facing toward the oligo(A) tail. No SuPor-P insertions were observed at this site. As our effort to identify these insertions began some time after the start of the BDGP mobilization screen, only 13 of the 31 lines were available to us. We characterized only three of the seven insertions into the TART hotspot; thus, nine insertions in the retrotransposon array were analyzed (Table 1).

We first mapped the insertion sites to chromosome by segregation analysis and determined the cytological positions by *in situ* hybridization to polytene chromosomes. Seven insertions were located in the retrotransposon array (Table 1), indicating that the terminal array

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**Figure 2.**—Electron microscopy of 2L telomeres. Telomeres of the 2L chromosome in Oregon-R (A), Tel (B), and an Oregon-R/Tel hybrid (C) are shown. Identities of the cytological bands are indicated. In B, the two zones of subsection 21A1–4 are indicated with brackets labeled with one or two asterisks. The bracket labeled TAS in C indicates the position of TAS DNA according to *in situ* hybridization data shown in Figure 1. Scale bar, 1 μm.

**Figure 3.**—Integration sites of P elements into telomeric retrotransposons HeT-A, TAHRE, and TART. Schematic of HeT-A, TAHRE, and TART elements with the position of P-element insertions identified. Boldface type identifies the insertions that were characterized.
is not refractory to \(P\)-element integrations. Two others, with flanking regions similar to \(TART\), were not located at a telomere and were not considered further.

Because the oligo(A) tails of the telomeric retrotransposons always face proximally (Bießmann et al. 1993), the sequences flanking the \(P\) element can be used to determine its orientation. With this information, PCR amplifications were attempted with extension times of up to 15 min to amplify the DNA between the \(P\) element and TAS using one primer specific for the region of the \(P\) element facing the centromere and another primer located in the \(2L\) or \(3R\) X TAS regions facing the chromosome end. This strategy was successful with KG01591 and KG10047, but failed with the other lines. For the latter insertion lines, libraries in \(\lambda\) phage were established and screened.

The transgene in KG10047 was mapped by chromosome segregation and in situ hybridization to the \(2R\) telomere. Primers \(3'\)-Parb-F and \(2R\) TAS-dist amplified a 0.4-kb fragment from genomic DNA, which was sequenced from both ends, showing the \(P\) element at nucleotide position 5521 in its RT-ORF. After the 3′-UTR of this \(P\) element are two \(9\)-\(UTRs\), which terminate in their ORF with the \(3'\)-tail, followed by a 48-bp sequence identical to \(2R\) TAS (Figure 4). The distance between the \(P\) element and TAS is only 359 bp.

The transgene in KG01591 was mapped to the tip of \(3R\). The same primer set as above amplified a 5-kb DNA fragment from genomic KG01591 DNA. We also cloned the region between the \(P\) element and TAS in \(\lambda\) phage and determined its sequence. The \(SuPor\)-\(P\)-element was integrated into the GAG-ORF at a site equivalent to nucleotide 1340 of the \(HeT-A\) element 9D4 (GenBank accession no. X68130). The \(HeT-A\) element sequences continue to its oligo(A)\(_{2}\)-tail, which is attached to \(3R\) TAS. The \(HeT-A\)/TAS junction is located within the 173-bp repeat unit that is also in the 1.8-kb repeat of the \(XTAS\) (Karpen and Spradling 1992).

\(\lambda\) libraries were established from three integrations in the hotspot in the 3′-UTR of \(TART\)-\(B\) 656 bp upstream of the oligo(A) tail (Figure 3) and screened with a yellow gene probe to clone the DNA proximal to the insertions. Thus, 14 kb of flanking region was obtained from 656 bp from \(A\)-tail, the composition of the retrotransposon array diverges. In KG00453, the \(A\)-tail is attached to a \(3R\) TAS element at nucleotide position 5521 in its RT-ORF. After the 3′-UTR of this \(TART\) element are two \(HeT-A\) 3′-UTRs, the first of which contains the last 358 bp of the ORF.

In KG00453, the 3′-UTR of the \(TART\)-\(B\) 656 bp upstream of the oligo(A) tail (Figure 3) and screened with a yellow gene probe to clone the DNA proximal to the insertions. Thus, 14 kb of flanking region was obtained from KG00453, 10 kb from KG00802, and 8 kb from KG01387. Although all three \(P\)-elements were located at the \(3R\) telomere, their environments were very different (Figure 4).

In all three integrations, sequences immediately flanking the \(3'\)-end of \(P\) were identical and continued to the oligo(A) tail of a \(TART\)-\(B\) element. However, past this A-tail, the composition of the retrotransposon array diverges. In KG00453, the A-tail is attached to a \(TART\)-\(B\) element at nucleotide position 5521 in its RT-ORF. After the 3′-UTR of this \(TART\) element are two \(HeT-A\) 3′-UTRs, the first of which contains the last 358 bp of the ORF. In KG00802, a second 550-bp-long \(TART\)-\(B\) 3′-UTR follows the oligo(A) tail of the \(TART\)-\(B\) that harbors the insertion. The oligo(A) tail of this second \(TART\) element is attached to a 3-kb \(HeT-A\) fragment truncated in its ORF. The nucleotide position of the junction between the \(TART\) and the ORF of \(HeT-A\) is identical to that in KG00453. This \(HeT-A\) element terminates in a double oligo(A) tail attached to the 5′-UTR of a \(HeT-A\) element, which terminates in its ORF with the \(BamHI\) site used for cloning.

The oligo(A) tail of the \(TART\)-\(B\) element that harbors the KG01387 insertion is followed by three short

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### Table 1

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<th>Distance to TAS</th>
<th>Variegation</th>
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* † SuPor-P insertions are labeled “KG,” except for 316-1 (Roseman et al. 1995). EPy2 insertions are labeled “EY.”


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<td>2R telomere</td>
<td>&gt;12 kb</td>
<td>No</td>
</tr>
<tr>
<td>EY00453</td>
<td>TART-B1, 656 bp from A-tail</td>
<td>3L telomere</td>
<td>&gt;25 kb</td>
<td>No</td>
</tr>
<tr>
<td>EY00802</td>
<td>TART-B1, 656 bp from A-tail</td>
<td>3L telomere</td>
<td>&gt;19 kb</td>
<td>No</td>
</tr>
<tr>
<td>EY01387</td>
<td>TART-B1, 656 bp from A-tail</td>
<td>3L telomere</td>
<td>&gt;25 kb</td>
<td>No</td>
</tr>
<tr>
<td>EY09966†</td>
<td>TART-C, 614 bp from A-tail</td>
<td>4 telomere</td>
<td>&lt;14 kb</td>
<td>Yes</td>
</tr>
<tr>
<td>EY09589</td>
<td>TART, 5′ and 3′ repeat; stalkerm</td>
<td>2R at 46/47</td>
<td>ND†</td>
<td>Yes</td>
</tr>
<tr>
<td>EY10037</td>
<td>TART, 5′ and 3′ repeat; stalkerm</td>
<td>2L base</td>
<td>ND†</td>
<td>Yes</td>
</tr>
<tr>
<td>KG01031</td>
<td>XTAS</td>
<td>2R telomere</td>
<td>0</td>
<td>Yes</td>
</tr>
<tr>
<td>KG01528</td>
<td>XTAS</td>
<td>2R telomere</td>
<td>0</td>
<td>Yes</td>
</tr>
<tr>
<td>EY03383</td>
<td>XTAS</td>
<td>2R telomere</td>
<td>0</td>
<td>Yes</td>
</tr>
<tr>
<td>316-1</td>
<td>XTAS</td>
<td>2R telomere</td>
<td>0</td>
<td>Yes</td>
</tr>
<tr>
<td>EY10337</td>
<td>XTAS related</td>
<td>2L base</td>
<td>ND†</td>
<td>Yes</td>
</tr>
<tr>
<td>EY09260</td>
<td>2L-TAS related</td>
<td>2L base</td>
<td>ND†</td>
<td>Yes</td>
</tr>
</tbody>
</table>

** Notes:**
- † Not done.
- ‡ y wI225; su(Hw)/su(Hw) flies carrying this element died.
(30- to 500-bp) TART 3' UTRs. Proximal to this arrangement is the 5' UTR of another TART-B1 element, which continues into the GAG-ORF. Surprisingly, sequences at the proximal XbaI site that was used for cloning originated from a roo/B104 retrotransposon. The distal and proximal junctions were amplified using primers TART-roo-F, TART-roo-R, and a primer from the roo inverted repeats. Sequencing showed the precise continuation of TART GAG sequences at the proximal side of the roo insertion and a 5-bp duplication characteristic of the roo transposable element. Moreover, PCR with TART-roo-F and TART-roo-R primers amplified a 9-kb fragment from genomic EY01387 DNA, which proved by partial sequencing to be a full-length roo element.

Finally, using a primer from the proximal side of roo and one in the TART RT (allTART-R), we amplified a fragment from genomic EY01387 DNA, whose predicted identity was confirmed by sequencing. There are about 80 copies of roo in the genome, spread out over all the chromosome arms (Scherer et al. 1982).

Due to the limits of fragment size clonable into λ phage, we were unable to isolate the entire stretch of DNA between P and TAS in any of these three EY strains. Long-range PCR amplifications from genomic DNA with one primer that recognizes 3L TAS (3L SAT-R1) and the other primer from 3' P (3' P-ry-4 or 3' Plarb-F) or, in the case of EY01387, 3' roo-F, failed to produce any fragments.

The integration in strain EY08176 was in the GAG ORF of TAHRE with the 5' end of P facing proximally. Sequencing of the 3'- and 5'-flanking regions obtained by inverse PCR showed that the TAHRE element that harbors the insertion is full length. The presence of a characteristic TAHRE RT ORF was confirmed by PCR and sequencing. Proximal to this element and two very short 3' UTRs of 20 and 9 bp lies the 5' UTR of a HeT-A element, which extends into the ORF at the end of the cloned fragment.

Identification of P-element insertions into TAS: We screened P-element flanks obtained from the BDGP with the 2L TAS repeat and the 1.8-kb repeat of XTAS, which together represent almost all of the known TAS sequences. One insertion flanked by 2L TAS and 109 insertions in the 1.8-kb repeat of XTAS were identified, but only six stocks were available for our analyses (Table 1). Sequences related to TAS, however, also occur in locations other than the telomeres. After genetic isolation of P insertion chromosomes, the flanking sequences were obtained by inverse PCR and sequenced. The EY10337 and EY09260 elements had inserted into TAS-related sequences near the centromere of chromosome 2 and were excluded from further analysis. In situ hybridizations
located the insertions of KG01031, KG01528, and EY03383 at telomere 2R. Their flanking regions, as isolated by inverse PCR, had similarity to the 173-bp repeats within the 1.8-kb repeat sequence defined by X TAS. A stock of one other insertion of the SuPor-P element into the 3R TAS, 316-1, was also included in our studies (ROSEMANN et al. 1995).

Expression of reporter genes at telomeric sites: Expression of reporter genes inserted into the terminal retrotransposon array was compared to gene expression when the same P-element construct was integrated into TAS or euchromatin. To examine the expression of the reporter genes, null mutations y$^t$ and w$^7$-23 were used at the normal genomic locations of these genes. Insertions of the SuPor-P and EPgy2 elements produced different phenotypes and will be considered separately. Individuals in the KG lines, carrying the SuPor-P element, exhibited relatively high levels of white and yellow expression (Figure 5). As noted previously (GOLUBOVSKY et al. 2001), males consistently show darker eye color than females of the same genotype. We, therefore, present only females in the discussion of SuPor-P expression.

The w gene in the SuPor-P element is adjacent to an eye-specific enhancer and surrounded by SU(HW) binding sites, which act as insulators. Integrants with SuPor-P thus show a strong red eye-color phenotype in a wild-type background, independent of the integration site. The su(Hw)$^9$ mutation, therefore, was introduced into the SuPor-P lines to observe the effects of the surrounding sequence on w gene expression. Females hemizygous for the transgene and homozygous for su(Hw)$^9$ were obtained by crossing KG males to y$^t$ sc w; su(Hw)$^9$ females. The transgene inserted into 2R and 3R TAS shows a strongly repressed and variegated eye-color phenotype, especially when homozygous (Figure 5). KG10047 also shows weak w gene expression and strong variegation, suggesting some spreading of silencing from TAS into HeT-A. All three of the insertions into or close to TAS exhibit more repression and variegation in transgene homozygotes than in hemizygotes. KG01591 females, however, show very little suppression or variegation of the transgene and resemble females with a euchromatic insertion. Weak repression is observed in hemizygous females, but this is not seen in males or homozygous females. Thus, the w reporter genes in three SuPor-P integrations in TAS, or in HeT-A very close to TAS, are strongly repressed and variegated, while a similar w reporter gene in HeT-A farther from TAS is only weakly repressed.

The EPgy2 insertion lines show orange to pale eye color, depending on the location of the insertion. Given the relatively light eye colors and the greater w expression in males, we discuss expression in males here. The mini-white gene in EPgy2 lacks the eye-specific enhancer. Thus, integrations into euchromatin exhibit a light orange eye color in hemizygotes and a darker orange eye color in homozygotes (Figure 5). Three EPgy2 insertions into TART, all at least 19 kb distal from 3R TAS, exhibit essentially the same eye-color phenotype as euchromatic EPgy2 elements, while EY03383, which is in 2R TAS, shows very little or no w expression. Unlike the other EPgy2 insertions into the terminal array, EY08176 exhibits an eye color that is slightly lighter than males with euchromatic insertions (Figure 5). The reason for this difference is not known. Variegation of the w reporter gene is not seen for any of these insertion lines. Expression of w in EY03383 is virtually indistinguishable from a null. Thus, expression of the w reporter in the EPgy2 transgene resembles that in SuPor-P,
because both reporters are strongly repressed when in TAS, but only weakly, or not visibly, repressed when in the terminal array.

Both the EPgy2 and the Supor-P constructs have a yellow gene that is not surrounded by SU(HW) binding sites. Expression of this y reporter, which is reflected in abdomen pigmentation, especially in the last two male tergites, is independent of the nature of the P-element construct, but reflects the nature of chromatin surrounding the transgene. Males carrying a marked P element in TAS show considerable repression and variegation of the y reporter (Figure 5). Most males with an insertion in the retrotransposon array exhibited no discernable repression of y and resembled control males with a euchromatic insertion. The y gene in KG10047 males, however, exhibited minor variegation, possibly due to close vicinity to TAS. Thus, y expression in the abdomens of these flies reflects w expression in their eyes.

It is possible that at least some of the KG lines, and possibly others, were discarded because they exhibited variegation (Bellen et al. 2004). This might have prevented us from identifying variegating elements in the terminal retrotransposon array. Only a small subset of the insertions analyzed by the GDGP, however, appear to have been subject to this treatment; there is no mention that the collections as a whole were culled of variegating elements (Bellen et al. 2004). In any case, we asked what proportion of the insertions we examined exhibited variegation (Table 1). Of the four KG elements, two variegated, one did not, and one could not be tested because yw65C2; su(Hw)/su(Hw) flies carrying this insertion did not survive. In addition, five of the nine EPgy2 insertions exhibited variegation. As expected, all three of the inserts in or within 1 kb of TAS variegate. Thus, we see no evidence of a bias against variegating insertions.

White expression in many of the insertion lines, especially those carrying the EPgy2 element, is less than that seen in wild type. We, therefore, asked to what extent expression of the EPgy2 element might be increased by the presence of a dominant suppressor of telomeric silencing. As most suppressors of TPE are deficiencies of 2L TAS (Golubovsky et al. 2001; Mason et al. 2004), we examined the effects of three such deficiencies in different genetic backgrounds, Df(2L)met62, l(2)g65C, and Su(TS)M1. None of these affected w expression in any of the EPgy2 elements in the terminal array. This is as expected, as the EPgy2 element in the terminal array expresses to the same extent as in euchromatin (Figure 5). The extreme repression of w seen in EY03383 (Figure 5) shows only slight suppression in the presence of these TAS deficiencies, but the moderate repression of y in EY03383 is strongly suppressed to give an abdomen with very little variegation. Thus, this element in TAS behaves similarly to other TAS insertions.

Mutations in gbp, a gene that encodes the histone H3 lysine 79 methyltransferase, also act as dominant sup-

pressors of TPE (Shanower et al. 2005). We, therefore, asked what effect the gbpXXV mutation has on expression of the EPgy2 insertions in the terminal array. As with the 2L deficiencies, we found that gbpXXV did not change the level of expression of the inserts in the terminal array.

DISCUSSION

Noncoding repetitive sequences make up a large portion of eukaryotic genomes. Large blocks of repetitive DNA are mostly packaged into heterochromatin around centromeres, but their organization and structure has been difficult to analyze. By contrast, the smaller regions of heterochromatin at the telomeres provide an opportunity to study their DNA and protein composition. Our EM data provide the first clear evidence that two distinct chromatin subdomains exist within a telomeric region: the terminal retrotransposon array is diffuse and morphologically resembles interband regions or puffs, while the subterminal TAS region resembles regular bands.

Our results show that the terminal retrotransposon array at Drosophila telomeres is not refractory to the integration of P elements, but reporter genes inserted into these two domains of the Drosophila telomere are affected differently. Except for the P-element integrations described here, the insertion of a full-length roo element into the otherwise stereotypical HeT-A/TAHRE/TART array is the only documented insertion of a transposon into the telomeric retrotransposon region and demonstrates that transposable elements are capable of inserting into the telomeric array.

Configuration of otherwise undisturbed retrotransposon arrays at telomeres: Analyzing the structure and composition of the telomeric retrotransposon array can provide information about the dynamic events of new transpositions and terminal erosion that shape the organization of chromosome ends in Drosophila. HeT-A, TAHRE, and TART sequences are predominantly found at telomeres, but tandem arrays of relatively short HeT-A segments also occur in autosomal centromeric heterochromatin and in interstitial regions of the heterochromatic Y chromosome (Young et al. 1983; Traverse and Pardue 1989; Agudo et al. 1999). Thus, isolation of HeT-A sequences from genomic DNA libraries does not ensure that the cloned fragments originated from a telomere (Biessmann et al. 1993). By walking from TAS into the terminal retrotransposon array, two normal telomeres have been analyzed, defining the junction between the proximalmost HeT-A element and the subtelomeric TAS (Karp et al. 1992; Walter et al. 1995). Directional cloning of chromosome ends (Biessmann et al. 1993) demonstrated that the oligo(A) tails of HeT-A elements face toward the centromere.

Results presented here confirm and extend these observations. The telomeric retrotransposon arrays are
highly polymorphic. HeT-A, TAHex and TART elements are intermingled, and the elements are often truncated at the 5′ end, although full-length elements were also found. Our results are consistent with recent analyses of BACs that span the TAS regions and extend into the terminal arrays (Abad et al. 2004a) and support the idea of a dynamic Drosophila telomere. The abundance of 5′-truncated retroelements is striking. While these incomplete elements will not produce full-length transcripts of the element, they may provide additional promoters for transcription of proximally located elements (Danilevskaya et al. 1997).

The nature of Drosophila telomeres: Heterochromatin in Drosophila is distinct from euchromatin by several criteria, including cytological staining, timing of replication, a propensity for ectopic pairing, under-replication in polytene chromosomes, and ability to silence gene activity (Weiler and Wakimoto 1995; Zhimulev 1998; Grewal and Elgin 2002). Most heterochromatin is found around the centromeres, but smaller regions are present at the telomeres and scattered around the genome as intercalary heterochromatin (Zhimulev and Belyaeva 2003). It has been inferred that telomeres exist in a heterochromatin configuration. While this may be true in most studies, earlier observations showed that some but not all telomeres are replicated late; however, they are not among the last sequences to be replicated during S phase (Berendes and Meyer 1968; Zhimulev and Belyaeva 2003).

Ectopic pairing is a feature often associated with heterochromatin. Telomere-telomere interactions have been well documented and shown to vary widely between strains and over time (Berendes and Meyer 1968). While the nature of these ectopic contacts is not known, threads connecting the telomeres, at least in some cases, hybridize with HeT-A (Rubin 1978; Young et al. 1983; Traverse and Purdue 1989) and TAS probes (Karpen and Spradling 1992). The observation that telomere interactions are dramatically increased in the Tel strain, which has extremely long telomeric retrotransposon arrays, suggests that these interactions are mediated by the retrotransposons or proteins associated with them. These interactions are resolved in diploid brain cells in mitosis, arguing against covalent DNA-DNA bonds (Siriaco et al. 2002).

Direct comparison of copy number of TAS and HeT-A sequences in diploid vs. polytene tissues to determine possible underreplication is not possible, because these sequences are also found in other genomic locations. Therefore, Pelement insertions into the subtelomeric TAS and the pericentric heterochromatin have been used as tags to address this question (Karpen and Spradling 1992; Zhang and Spradling 1995; Wallrath et al. 1996). These measurements reflect vast differences according to the insertion locations, but telomeric insertions into TAS exhibit very modest, if any, underrepresentation in polytene chromosomes.

Gene silencing at telomeres: Transcriptional silencing is a sensitive criterion for defining heterochromatin. TAS is likely to be directly involved in silencing telomeric transgenes, suggesting a heterochromatin character (Levin et al. 1993; Cryderman et al. 1999; Golubovsky et al. 2001; Karpen and Spradling 1992). Indeed, a 6-kb TAS array exhibited array-length-dependent and orientation-dependent repression of a w reporter gene (Kurenova et al. 1998), and a single 1.2-kb region derived from the 1.8-kb X TAS repeat induced pairing-sensitive repression of a reporter gene (Boivin et al. 2003). Telomeric silencing is different from silencing that occurs in closely linked copies of mini-white genes (Dorer and Henikoff 1994), because TPE on the major autosomes does not respond to mutations in Su(var)205, the gene that encodes HP1 (Wallrath and Elgin 1995; Cryderman et al. 1999).

Pelement insertions allowed us to detect telomeric subdomains by their different ability to silence integrated transgenes. In agreement with the previous studies, we find that reporter genes surrounded by TAS are repressed. The same Pelement constructs inserted into the terminal retrotransposon array, however, generally resemble euchromatic insertions in their level of reporter gene expression, except when they are located close to TAS. These observations support our model for TPE, which proposes that variegated expression of reporter genes at telomeres is the result of competition between the repressive effects of TAS and the stimulating effects of the HeT-A promoters (Mason et al. 2000). This interaction between HeT-A and TAS might constitute a mechanism by which TAS regulate telomere elongation by controlling HeT-A promoter activity (Mason et al. 2003a).

The HP1 protein has been reported to play a role in telomere capping, elongation, and HeT-A transcription (Savitsky et al. 2002; Perrini et al. 2004). The mechanism by which HP1 might act to promote HeT-A transcription and elongation is unclear; as it is not possible to estimate the number of transcripts per genomic HeT-A copy number, because both increase in the presence of a mutation in Su(var)205. Further, mutations in Su(var)205 do not affect TPE (Wallrath and Elgin 1995; Cryderman et al. 1999), and HP1 does not bind to the long terminal retrotransposon arrays carried by Tel mutants, except at the cap region (Siriaco et al. 2002).

The relative position of heterochromatic telomeric domains in Drosophila appears to be reversed from that in telomeres of other eukaryotes. In yeast, the terminal-most telomeric repeats are heterochromatic by virtue of their nonnucleosomal chromatin packaging and their gene silencing ability even in nontelomeric locations (Tham and Zakian 2002), while insertions of reporters
into the subtelomeric Y’ elements are generally subject to very little, if any, repression (Przyde and Louis 1999). This difference between Drosophila and yeast may reflect the fundamental difference in how the terminal DNA structures are generated. In yeast and most other eukaryotes, simple repeats are added by telomerase onto the chromosome end, where they bind a number of proteins and assume a heterochromatin-like state called the telosome (Wright et al. 1992). In contrast, the terminal retrotransposon arrays in Drosophila are themselves the source of RNA transcripts that are essential components in telomere elongation by serving as mRNA for the synthesis of proteins necessary for transposition and as templates for reverse transcription. The fact that HeT-A and TART elements are actively transcribed would not necessarily require that they be embedded in a euchromatic structure, because a number of active genes transcribed from Pol II promoters are known to be located in centric heterochromatin (Weiler and Warimoto 1995). These promoters appear well adapted to their euchromatic environment and display PEV when moved to euchromatic locations. It has been proposed that the HeT-A promoter may belong to this category (Pardue and DeBaryshe 2000; George and Pardue 2003). However, our findings suggest that the HeT-A promoter is more likely a euchromatic promoter, consistent with the observation that it functions normally when moved to other euchromatic positions (George and Pardue 2003) and that HeT-A elements placed upstream of a telomeric white (Golubovsky et al. 2001; Mason et al. 2003b) or yellow (Kahn et al. 2000) gene have an activating, not a repressing, influence on gene expression.

We thank Bob Levis for providing TART sequences and fly stocks. Other stocks were provided by Pam Geyer, the Berkeley Drosophila Genome Project, and the Bloomington Drosophila Stock Center. The BDGP also provided Pelement flanking sequences. We also thank Igor Zimin for discussions, Alfredo Villasante for unpublished information, and Janine Santos, Marilyn Diaz, and members of the Mason lab for critically reading the manuscript. Oscar Ramirez, Patrick Williams, Diana Le, Jonathan Cheah, and Max Biessmann performed excellent lab work. This project was supported by the U.S. Public Health Service grant GM-56729 to H.B. Work in Novosibirsk was supported by a grant from the program of the Russian Academy of Sciences in Physico-Chemical Biology N 10.1. This research was supported, in part, by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences.

LITERATURE CITED


Domains in Drosophila Telomeres


Perrini, B., L. Piacentini, L. Fantz, F. Alteri, S. Chiugiarelli et al., 2004 HP1 controls telomere capping, telomere elongation, and telomere silencing by two different mechanisms in Drosophila. Molecular Cell 15: 467–476.


Communicating editor: K. G. Golic