Transcriptional Activity of the Telomeric Retrotransposon *HeT-A* in *Drosophila melanogaster* Is Stimulated as a Consequence of Subterminal Deficiencies at Homologous and Nonhomologous Telomeres[▽]

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Drosophila melanogaster telomeres have two DNA domains: a terminal array of retrotransposons and a subterminal repetitive telomere-associated sequence (TAS), a source of telomere position effect (TPE). We reported previously that deletion of the 2L TAS array leads to dominant suppression of TPE by stimulating in *trans* expression of a telomeric transgene. Here, we compared the transcript activities of a *w* transgene inserted between the retrotransposon and TAS arrays at the 2L telomere in genotypes with different lengths of the 2L TAS. In contrast to individuals bearing a wild-type 2L homologue, flies with a TAS deficiency showed a significant increase in the level of telomeric *w* transcript during development, especially in pupae. Moreover, we identified a read-through *w* transcript initiated from a retrotransposon promoter in the terminal array. Read-through transcript levels also significantly increased with the presence of a 2L TAS deficiency in *trans*, indicating a stimulating force of the TAS deficiency on retrotransposon promoter activity. The read-through transcript contributes to total *w* transcript, although most *w* transcript originates at the *w* promoter. While silencing of transgenes in nonhomologous telomeres is suppressed by 2L TAS deficiencies, suggesting a global effect, the overall level of *HeT-A* transcripts is not increased under similar conditions.

Telomeres, nucleoprotein structures at chromosome ends, play two crucial roles in chromosome biology: they provide end protection by “capping” chromosome ends, and they maintain chromosome length by adding telomeric sequence and thus solve the end replication problem (8). There are several known mechanisms of telomere maintenance, including telomerase and homologous recombination extending short canonical telomere repeats in humans, homologous recombination extending tandem arrays of more complex repeats in lower Diptera and alliaceous plants, and targeted transposition in *Drosophila melanogaster* (5, 6, 43). Three non-long-terminal-repeat retrotransposable elements, *HeT-A*, *TART*, and *TAHRE*, which maintain *Drosophila* telomere length by transposition onto chromosome ends, are the only known retroelements with such a cellular function. The telomeric retroelements contain unusually long 3′ untranslated regions (UTRs), which carry a promoter (14, 27, 40) to transcribe the elements downstream in the terminal array. Occasional long read-through transcripts

encompassing more than one retroelement or homologous recombination may give rise to simultaneous addition of multiple elements (4). Specific targeting of the elements by their 3′ oligo(A) tails to chromosome ends probably occurs via target-primed reverse transcription (34) and does not depend on the DNA sequence at the terminus (4, 5). Incomplete replication, combined with transposition, leads to mixed tandem arrays of complete and 5′-truncated elements on the ends of *Drosophila* chromosomes (1, 21, 35, 44). The level of retrotransposon transcription is correlated with cell proliferation, with the highest level of transcript being found in proliferating imaginal disc cells (20, 53) and in adult gonads, the primary location of cell proliferation during adult life. Recent evidence (48) showed a role for the RNA interference (RNAi) machinery in the transposition of telomeric retroelements, especially of *TART*, to chromosome ends and thus a possible role for RNAi in the control of *Drosophila* telomere length.

Immediately proximal to the terminal *HeT-A*, *TART*, *TAHRE* (HTT) array, *Drosophila* telomeres contain 15 to 26 kb of complex subterminal telomere-associated sequence (TAS) (1, 28, 33, 54). TAS arrays in *Drosophila* vary in sequence among telomeres but share sequence motifs among chromosome ends (28, 54). Similar structural motifs are also found in TAS regions of other eukaryotes (45). Genetically marked transposable elements that have inserted into TASs show repressed and variegated expression of the reporter gene (11, 19, 22, 24, 28, 32), referred to as telomeric silencing or telomere position effect (TPE). Evidence that the TAS is responsible for TPE

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comes from studies showing that a TAS adjacent to a reporter gene at nontelomeric positions also reduces reporter expression (9, 30). These observations are consistent with the immunostaining results showing that TASs, either at telomeres or in ectopic locations, bind *Polycomb* group proteins (2, 9). HTT arrays, however, bind proteins associated with open chromatin (2), and reporter genes inserted into the terminal HTT array are not repressed (7).

Molecular and genetic analysis of eye color variants derived from a *white* (*w*) reporter transgene inserted between the HTT array and TAS region of the left arm of chromosome 2 (2L) revealed several components that affect TPE (22, 36, 37, 38). Higher *w* expression was associated with increased numbers of *HeT-A* elements in the HTT array in *cis*, indicating an activating influence of terminal retrotransposons on the repressed *w* reporter. These genetic studies, however, could not distinguish between indirect activation through a change in the chromatin structure of the telomeric *w* gene and direct activation via read-through transcription from a more distal *HeT-A* promoter. Altered expression of the transgene also allowed the detection of interactions between homologous telomeres, because expression of the telomeric *w* reporter gene is increased in response to deletion of some or all of the TAS on the homologous chromosome (22, 31, 38). Our model of *HeT-A* activation based on these observations postulates that telomere communication may serve to assess telomere integrity and to stimulate enhancer/promoter activity of retrotransposons. This, in turn, may result in chromosome elongation when a deleted or interrupted TAS is present (22, 37).

Here we extended the genetic studies of TPE by measuring transcription from promoters of telomeric retrotransposons. This allowed us to test several predictions of the model. First, using three different telomeric insertions, we found that transcription of telomeric transgenes can be initiated at a promoter of an upstream *HeT-A* element. We then used the level of this *HeT-A*-transgene read-through transcript as an indicator of the activity of a specific *HeT-A* element in a *P*{*w*^{var}} *11-5* strain carrying a *white* (*w*) reporter transgene between the HTT array and a truncated TAS region of the left arm of chromosome 2. The *HeT-A/w* read-through transcript in *P*{*w*^{var}} *11-5* shows a significant correlation with total *w* transcript, although a majority of the total *w* transcript originates from the *w* promoter. Second, a 2L TAS deficiency leads to a release in silencing of both the *w* and *HeT-A* promoters. Third, silencing of *w* reporter genes in nonhomologous telomeres is suppressed by 2L TAS deficiencies, suggesting a global effect of these deficiencies on TPE. 2L TAS deficiencies, however, affect the activities only of retrotransposons and transgenes located within the TAS or in the proximal end of the HTT array, close to the TAS. Finally, total *HeT-A* transcript does not significantly correlate with *HeT-A/w* read-through transcript, nor does it respond to the presence of 2L TAS deficiencies, indicating that 2L TAS deficiencies do not play a significant role in telomere maintenance in *Drosophila*.

MATERIALS AND METHODS

***Drosophila* strains.** *Drosophila* stocks were maintained and crosses performed at 25°C on cornmeal-molasses medium with dry yeast added to the surface. Except for Oregon R, which has its *w* gene in the normal nontelomeric position on the *X* chromosome, all strains carried *y*¹ and the *w*^{67c23} deficiency for the

endogenous *w* gene. Insertion strains *P*{*w*^{var}} *11-5* (referred to herein as *11-5*), with a copy of the genomic *w* gene inserted between the HTT array and the TAS (22), and *P*{*w*⁺} *39C-5* (referred to herein as *39C-5*), with a mini-*w* gene in the middle of the TAS array (52), have reporter genes that are read in a distal-to-proximal direction (11). *P*{*w*^{var}} *11-10* is a variant of *P*{*w*^{var}} that has lost some or all of the *white* coding sequence (22). *P*{*w*⁺} *39C-27* (referred to herein as *39C-27*) and *P*{*w*⁺} *39C-62* (referred to herein as *39C-62*) carry mini-*w* insertions in 2R and 3R, respectively (52). TPE suppressor strain *Df*(2L)*M26* (referred to herein as *M26*) has a complete 2L TAS deficiency and a *TART* element attached directly to unique sequence DNA at the 2L telomere, leaving *CG11023* and *l*(2)*gl* intact (unpublished data). *Df*(2L)*net62* is an interstitial deficiency for *l*(2)*gl* (29) that extends into the TAS but leaves the HTT array intact. *l*(2)*gl*^{GB26} is a terminal deficiency that removes the *l*(2)*gl* gene (41), while *l*(2)*gl*^{GB52} carries a *roo* insertion in *l*(2)*gl*, and *l*(2)*gl*^{DPV275} carries a *roo* element in place of much of the *l*(2)*gl* coding sequence (41). We also used two strains carrying insertions of *EPgy2* (*EY08176*) or *SuPorP* (*KG01591*) elements in the HTT array (7) and four strains carrying euchromatic insertions of a mini-*w* gene with a 2L TAS inserted between the eye enhancer and the promoter: *P*{*w*⁺} *836I/U1* (A6-514) and *P*{*w*⁺} *836I/U1* (A8-2A) have <1 kb of the TAS (referred to herein as A6-514 and A8-2A), while *P*{*w*⁺} *836I/U1* (A8-511) and *P*{*w*⁺} *836I/U1* (B8-13Y) have 6 kb of the TAS (referred to herein as A8-511 and B8-13Y) (30).

Quantitative measurement of red-eye pigments. Five adult heads were homogenized in a 1:1 mixture of chloroform and ammonium hydroxide (0.1%); the homogenate was centrifuged, and the optical density of the upper aqueous layer was measured at 485 nm (3). Pigment analysis was performed in five samples for each strain.

RNA isolation and cDNA synthesis. RNA samples of Oregon R and *11-5* were isolated from whole wandering larvae, late-stage pupae, and 1-day-old adults using an RNeasy minikit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. RNA from ovaries was extracted using the TRIzol reagent (Gibco BRL Life Technologies, Rockville, MD). For each sample we used 30 dissected ovaries. RNA was converted to cDNA using either oligo(dT) or primers specific for the expected read-through transcripts (*P*-ry-4, 5'Plac-1, or white D) and Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA).

PCR primers used here were as follows: *P*-ry-4, 5'-CAATCATATCGCTGTCTCACTCA-3'; 3'larB (forward), 5'-CATACGTTAAGTGGATGTCTCTTG C-3'; *TAHRE* gag (forward), 5'-CCCCTCGATCGACAATACAG-3'; *TAHRE* gag (reverse), 5'-GTTAGTGTGGTCTGTGCTG-3'; *HeT-F1* (forward), 5'-CTGTCTCCGTACCTCCACCAGC-3'; *HeT-F2* (forward), 5'-CCCCAACTCA CCCATGAATG-3'; *HeT-F3* (forward), 5'-GCTTCCAGCGACTCGGTGCTTC CG-3'; lacI, 5'-CACCAAGGCTCTGCTCCACAAT-3'; 3'larB (forward), 5'-GGCTATCGACGGGACCACC-3'; 1591 upstream P, 5'-CGTCCCGCGCTTA CCAATAC-3'; white D, 5'-CCACCGTTTGTAGCGTTACCTAGC-3'; wCS (forward), 5'-CCTCTTTATCGGCTCCCTAACG-3'; wCS (reverse), 5'-TCGT GTGCTGACATTTGCTGAG-3'; set 1 (forward), 5'-CACATCGTCCGTGAA AATGG-3'; set 1 (reverse), 5'-AGCACCCGTACTTCGGACAC-3'; set 2A (forward), 5'-CCGAACCCATAACACATTCCTCTC-3'; set 2A (reverse), 5'-T AAATCAGCGCTGCCATTG-3'; set 2B (forward), 5'-CTGTTACCATC GTCCGTG-3'; set 2B (reverse), 5'-TGTTGTCGTCCCGTTTCG-3'; set 3 (forward), 5'-GCCACCGTTTGTAGCGTTAC-3'; set 3 (reverse), 5'-AAGAAGC GAGAGGAGTTTGG-3'; w-1 (reverse), 5'-AAGGAAGTAACCTGAACGT AGGCG-3'; HCS (forward), 5'-ATTGTCTCTCTCCGTCCACC-3'; HCS (reverse), 5'-TTCTCTATGCTATTGTGCTGCTGTC-3'; *RpL32* (forward), 5'-G GACAGTATCTGATGCCAAC-3'; *RpL32* (reverse), 5'-ATCTCGCCGCGAG TAAACGC-3'; *RpS17* (forward), 5'-AAGCGCATCTGCGAGGAG-3'; *RpS17* (reverse), 5'-CCTCCTCTGCAACTTGATG-3'.

Qualitative RT-PCR. Qualitative RT-PCR was used to detect read-through transcripts. The PCR mixture contained 200 nM of forward and reverse primers with about 500 ng of cDNA. PCRs were denatured at 94°C for 3 min, followed by a 30-s denaturation at 94°C, annealing at 55°C for 30 s, and a 1-min extension at 72°C for 35 cycles. Products were loaded and electrophoresed in 2.5% agarose gel in TAE.

Quantitative real-time PCR (qRT-PCR). qRT-PCR was performed using an Mx3000P real-time PCR system with SYBR Green PCR Master Mix (Stratagene, La Jolla, CA). Threshold cycle was used to assess relative levels of target transcripts versus reference *RpL32* transcripts. Quantification was performed in two independent experiments with five samples for each strain set up in triplicates.

Northern hybridization. RNA samples (20 µg/lane) were separated on an 0.8% agarose gel, and transferred to Hybond-N nylon membrane. Hybridization was performed with ³²P-labeled RNA probes according to the method of Sambrook et al. (46).

The M26 deficiency is a dominant suppressor of TPE. The *11-5* strain, which carries a w^+ transgene inserted between the retrotransposon array and a partially deleted TAS at the tip of 2L (Fig. 1A), was characterized previously (22), but after re-isolation of the chromosome carrying the transgene, we re-analyzed the sequence of the w^+ insertion and found that the junction between *HeT-A* and the w^+ transgene had moved closer to the eye enhancer (Fig. 1B and C). To determine whether silencing of the transgene was suppressed by a 2L TAS deficiency in the same way as the original isolate (22), we crossed the newly reisolated *11-5/11-5* flies with *M26* flies, which carry a complete deletion of the 2L TAS (Fig. 2A).

Consistent with previous results, *11-5/M26* flies exhibited red eyes, in contrast to *11-5/+*, which showed yellow eyes. *11-5* homozygotes had red eyes (Fig. 2B). This confirmed that the *M26* deficiency acts as a dominant suppressor of TPE on the homologue. We performed a spectrophotometric determination of red eye pigment levels in these genotypes, including wild-type Oregon R (Fig. 2C). Differences in pigment levels correlated with observed eye color phenotypes. A slightly brighter red eye color in *11-5/11-5* than Oregon R is confirmed by a slightly higher level of red pigments. To obtain a more direct measurement of TPE, we quantified *w* transcript levels by qRT-PCR with the primer set wCS (Fig. 1A) on RNA from late-stage pupae, because pigment deposition into eyes occurs

FIG. 2. Activation of the $P\{w^{var}\}11-5$ transgene by a 2L TAS deficiency. (A) Schematic diagram showing the 2L telomere structure in homozygous $11-5$ with a partially deleted TAS, in hemizygous $11-5/M26$ with a complete TAS deficiency on the 2L homologue, and in hemizygous $11-5/+$ with a wild-type homologue carrying a complete TAS. (B) Eyes of the wild-type Oregon R, homozygous $11-5$, and hemizygous $11-5/M26$ and $11-5/+$. (C) Levels of red eye pigments in flies of the same genotypes.

at this stage (16). Transcript levels in pupae (Fig. 3) are in good correlation with the observed pigment levels (Fig. 2B and C). Thus, the perceived eye color in strains in which w is variegated due to TPE is a good approximation of quantitative measures of late pupal w transcription and further indicates that $M26$ suppresses TPE by increasing transcript levels of telomeric inserts.

Telomeric silencing is stage specific. Due to a lack of published information about telomeric silencing during *Drosophila* development, we asked if the effects of TPE on telomeric w expression may vary over time and if suppression of TPE by 2L TAS deficiencies also has a developmental component. We measured by qRT-PCR the level of w transcript in RNA ex-

tracted from wandering larvae, early-, mid-, and late-stage pupae, and 1-day-old adults (Fig. 3). During development there were significant changes in w transcript levels in both $11-5/11-5$ and the Oregon R wild type, although they did not vary in concert. Transcript levels of the telomeric w transgene in $11-5/11-5$ showed a tendency to increase with development, while transcript levels of the endogenous w gene showed the reverse trend (Fig. 3) (16). Levels of telomeric w transcript in larvae and early pupae are strongly repressed compared to the endogenous w of Oregon R and are not proportional to eye pigment deposition in adults (Fig. 2B and C). Considering that the level of w transcript in $11-5$ is based on two copies of w , compared to one w copy in both $11-5/M26$ and $11-5/+$, we can see that transcript levels of w per copy in all three genotypes are comparable in larvae and early pupae. Both $11-5/11-5$ and $11-5/M26$ show a release in telomeric silencing during late pupal and adult stages, in contrast to $11-5/+$, which shows relatively steady levels of w during development. Transcript levels from $11-5/M26$ and $11-5/+$ in late pupae and adults suggest that suppression of TPE by $M26$ leads to a roughly twofold increase in w expression. As $11-5$ is associated with a partial 2L TAS deficiency (22), transcript levels of $11-5/11-5$ and $11-5/M26$ indicate that the effects of partial and complete TAS deficiencies on TPE are approximately equal. Thus, the developmental expression patterns of genomic w and a telomeric w are different, suggesting that telomeric silencing has a distinct developmental profile.

Read-through transcript from a *HeT-A* promoter into downstream sequences. It has been postulated that *HeT-A* transcription occurs in a distal-to-proximal direction starting at a promoter in the 3' UTR of an upstream *HeT-A* element (14). It also was suggested that the stimulating effect on $P\{w^{var}\}$ expression by a TAS deficiency might be by direct read-through transcription driven from the promoters of distal retroelements through the w transgene (22). To test the possibility of *HeT-A/w* read-through, we examined single-copy w^+ P elements inserted into an HTT array using two insertions, *EY08176* and *KG01591*, of a P element inserted into a *TAHRE* element in the 2R telomere and a *HeT-A* element at the 3R telomere, respectively (7). In both lines, the retrotransposons with the P element insertions are flanked by upstream *HeT-A* elements. Maps of the insertions, which we characterized by sequencing of genomic lambda phages, are shown in Fig. 4A.

Because strong *HeT-A* expression has been found in adult male and female gonads (53), we tested for transcription of the inserted elements from a retrotransposon promoter by producing cDNA from RNA samples taken from ovaries using the P-ry-4 primer anchored in *EY08176* or the lac1 primer anchored in *KG01591* (Fig. 4A). Transcripts were identified by PCR with primers (*TAHRE*-gag) specific to *TAHRE* and primers (5'larB and 1591 upstream P) specific to *HeT-A*, respectively. In parallel, RNAs were reverse transcribed with an oligo(dT) primer to test for the ribosomal *RpS17* transcript as a positive control. Ethidium bromide-stained gels (Fig. 4B) clearly identified read-through transcripts in both insertion lines. To identify an initiation site for the read-through transcription, cDNA of *EY08176* was subjected to PCR amplification with primer 3'larB, in the *EY08176* element, combined with three primers located in the 3' UTR of *HeT-A* at distances of 20 bp (HeT-F1), 120 bp (HeT-F2), and 350 bp (HeT-F3)

FIG. 3. Levels of *white* transcripts in Oregon R, homozygous *11-5*, *11-5/M26*, and *11-5/+* relative to transcripts of *RpL32* at various stages of development. The RT-PCR products were derived using the wCS primers after the production of cDNA using oligo(dT).

upstream from its oligo(A) tail. Although combinations of 3'larB primer with primers HeT-F2 and HeT-F3 gave no PCR product, amplification with HeT-F1 produced an ~950-bp fragment (Fig. 4C), which suggests that transcription initiated in the *HeT-A* element between 120 and 20 bp from its oligo(A) tail, supporting earlier hybridization experiments (14).

For all cDNA samples tested by RT PCR we also included controls for DNA contamination that had been prepared without reverse transcriptase (RT-). None of these control samples showed a PCR signal. These PCR experiments provided direct evidence of *HeT-A* transcriptional activity in a distal-to-proximal direction and raised the possibility of read-through transcription from a *HeT-A* promoter into adjacent transgenes.

To identify a read-through transcript from a distal *HeT-A* promoter into the adjacent *w* sequence in *11-5*, we used PCR with three sets of primers (1, 2A, and 2B) specific to *w* sequence upstream of the promoter and *11-5* cDNA as a template (Fig. 1A and B). cDNA was synthesized from total adult RNA using oligo(dT) or the white D primer. No PCR product was found in Oregon R cDNA, confirming that *w* upstream regions are not transcribed in the normal genomic location of this gene. Neither was a product found in the RT- samples. However, a product was detected from amplifying *11-5* cDNA, indicating that the normally untranscribed, upstream region is included in a transcript of the telomeric transgene in *11-5*. We infer that this transcript initiated at the promoter of an upstream telomeric retroelement. Next we used the primers from *HeT-A* (HeT-F1, HeT-F2, and HeT-F3) in conjunction with the primer set 3 (reverse) from the upstream *w* region (Fig. 1A) to identify the origin of the read-through transcript. Unlike with EY08176, amplification was obtained with all three *HeT-A* primers (Fig. 4E), indicating that the read-through transcript not only originates from the most proximal *HeT-A* promoter but also is created by a contribution of at least two retrotransposons. Further, we attempted to detect the read-through transcripts on Northern blots. We tested RNA extracted from whole larvae of *11-5* and Oregon R with an RNA probe specific to coding sequence of *w*. In both Oregon R and *11-5* we found only one hybridization signal, with a size corresponding to the normal, processed *w* transcript from a *w* promoter (Fig. 4F). In *11-5*, the absence of a band with a size of

at least 4.1 kb (2.3 kb of normal *w* transcript plus 1.8 kb of upstream of *w* to a promoter of the first *HeT-A* element) probably indicates a very low abundance of a *HeT-A-white* read-through transcript. As *HeT-A* elements have shown strong expression in gonads (53), we attempted to detect the read-through transcript in ovarian *11-5* RNA on a Northern blot with a probe specific to upstream *w* sequence but were unsuccessful. Even though *HeT-A* transcripts can be detected on Northern blots (20) and by whole-mount in situ hybridization (53), these transcript levels reflect the cumulative activity of all *HeT-A* elements in the genome, while our efforts are aimed at detecting the activity of a single *HeT-A* promoter or a small subset of *HeT-A* promoters, which might produce low levels of variably sized transcripts that are undetectable by Northern blots.

Although PCR data clearly showed the presence of *HeT-A-w* read-through transcript in *11-5*, we conclude that this read-through transcript is only a minor component of a total *w* transcript and that the majority of *w* transcript is transcribed from the *w* promoter. This suggests that the stimulating effect of a TAS deficiency on telomeric *w* expression by read-through transcription driven from the *HeT-A* promoter through *w*, as was proposed to occur in *11-5* (22), may not be the primary component in the suppression of TPE.

A TAS deficiency stimulates promoter activity of the *HeT-A* element(s). The *HeT-A/w* read-through transcript in *11-5* we identified by PCR allowed us to test the effects of a TAS deficiency in *trans* on *HeT-A* promoters distal to the telomeric *w* transgene. Using qRT-PCR with the primer set 2B from the upstream region of *w* and the primer set HeT-F1/w-1, encompassing the 3' end of *HeT-A* and the upstream region of *w* (Fig. 1A and B), we quantified the levels of *HeT-A/w* read-through product in the same genotypes that were tested for *w* expression (Fig. 3). Quantitation was performed with larvae, late-stage pupae, and adults. Both sets of primers gave comparable data showing an increase of *HeT-A/w* read-through transcript in the presence of complete or partial TAS deficiencies in a manner similar to that observed with primers for the *w* coding sequence (Fig. 3). The data shown in Fig. 5 are for the primer set HeT-F1-w-1. The levels of read-through transcript varied in concert with the levels of total *w* transcript in *11-5* and

FIG. 5. Relative levels of *HeT-A/white* read-through transcripts in *11-5/11-5*, *11-5/M26*, and *11-5/+* at various stages of development. cDNA was generated using an oligo(dT) primer, and transcripts were identified using the primer set *HeT-1F-w-1*.

increased from larvae through adults. The levels of read-through transcript also correlated with eye color and pigment levels (Fig. 2), although the measured pigment levels in *11-5/+* appear to be somewhat low compared with the quantity of transcripts. There was no amplification at any stage in Oregon R (data not shown). A comparison of *w* transcript levels (Fig. 3) with read-through transcript levels relative to *RpL* transcript (Fig. 5) suggests that a 2L TAS deficiency increases expression from both telomeric *w* and telomeric retrotransposon promoters but that the expression from the *w* promoter exceeds expression from the retrotransposon promoters.

The TAS modulates activity of nontelomeric but TAS-adjacent *w* transgenes. Although a comparison of transcript levels of telomeric and nontelomeric *w* showed significantly different developmental profiles (Fig. 3), levels of telomeric *w* and *HeT-A/w* read-through transcripts varied in concert. To ask whether developmental changes in *w* transcription at the 2L telomere are induced by the TAS and whether these changes are independent of telomeric position and/or presence of adjacent HTT, we compared mini-*w* transcript levels in larvae and late pupae that carry insertions of TAS-mini-*w* fusion constructs in different euchromatic positions. The constructs are bounded

FIG. 6. Expression of TAS-mini-*w* fusion transgenes. (A) Eye color phenotypes of the flies bearing nontelomeric TAS-mini-*w* insertions. (B) Levels of mini-*white* transcript in nontelomeric TAS-mini-*w* insertions relative to *RpL* 32 transcript.

by SU(HW) binding sites that act as insulators and prevent position effects at insertion sites, thus allowing us to test a direct effect of an adjacent TAS on mini-*w* independently of their chromosomal position. The A6-514 and A8-2A constructs have <1 kb of the TAS between the *w* eye enhancer and the promoter (30), and flies carrying these constructs exhibit red eyes. A6-511 and B8-13Y have 6 kb of the TAS between the eye enhancer and the promoter, and adults carrying these inserts exhibit yellow eyes. Eye color phenotypes of A6-514 and A6-511 are shown in Fig. 6A. Similar to observations on telomeric *w* (Fig. 2), larval transcript levels of TAS-mini-*w* fusions (Fig. 6B) were inconsistent with the adult eye color phenotypes and did not reflect different levels of heterochro-

FIG. 4. Read-through transcripts from *HeT-A* promoters into *P* elements residing within the HTT array. (A) Schematic maps of the HTT regions immediately distal to the *P* element insertions are shown. EY08176 has a single *EPgy2* element inserted into the GAG open reading frame of a *TAHRE* element in the 2R telomere with a *HeT-A* element immediately upstream of this *TAHRE*. KG01591 carries a *SuPor-P* element inserted into the open reading frame of a *HeT-A* element at the 3R telomere, such that the *w* reporter gene is read in the opposite direction that of the HTT array. Directly upstream of this *HeT-A* lies a short (168-bp) *HeT-A* fragment with an oligo(A) tail followed by a 3' *HeT-A* UTR with another oligo(A) tail. Dashed arrows indicate potential read-through transcripts from the upstream *HeT-A* promoters; short arrows indicate the primers used for cDNA synthesis (P-ry-4, lac1), and arrowheads indicate the primers used for RT-PCR detection of read-through transcripts. (B) An ethidium bromide-stained gel to identify read-through transcripts in both insertion lines is shown. Primer combinations are indicated below the gel. Genomic DNA served as a positive control and ovarian RNA without cDNA conversion as a negative control. The Oregon R lanes serve as a size control for the other lanes. The ribosomal gene, *RpS17*, served as a positive control. (C) An ethidium bromide-stained gel to determine the position of initiation of read-through transcription in EY08176 is shown. cDNA obtained with the P-ry-4 primer was subjected to PCR amplification with one primer anchored in *P* (3'larB) and the other in the *HeT-A* 3' UTR. Three primers in *HeT-A* (*HeT-F1*, -2, and -3) were used for this purpose. (D) Read-through transcript detected in *11-5*. Products of RT-PCR with primer sets 1 and 2 and primers to *RpS17*. *11-5* and Oregon R cDNA synthesized using oligo(dT) or white D primer was used for the PCR. Samples without reverse transcriptase provided a negative control (RT-), and genomic DNA (G) provided a positive control. For positions of primers, see Fig. 1A and B. (E) Read-through transcript detected in *11-5* with primer set 3 (reverse), specific to upstream sequence of *w*, combined with primers *HeT-F1*, 2, and 3 (forward), specific to the 3' UTR of *HeT-A*. Specific PCR products are noted by arrows. (F) Northern hybridization of RNA extracted from larvae of Oregon R and *11-5* with a RNA probe specific to coding sequence of *w*.

FIG. 7. (A and B) Long-range regulation of telomeric silencing by a 2L TAS deficiency. (A) Females carrying a mini-*w* reporter gene in a TAS region were mated to *M26/SM1* males, and the resulting males were examined for eye color. *M26* is a 2L TAS deficiency; *39C-27* is in the 2R telomere; *39C-62* is in the 3R telomere. *SM1* is a control that does not suppress telomeric silencing. (B) Diagram of the stimulating effect of 2L TAS deficiency on expression of mini-*w* inserted into the 2R TAS of line *39C-27* and into the 3R TAS of line *39C-62*. (C) Eyes and red eye pigments in insertion lines *39C-5*, bearing *w* in the 2L TAS as hemizygous *39C-5/M26*, and *39C-5/+*.

matic silencing by the TAS. In strains with short TASs (A6-514 and A8-2A), pupal mini-*w* transcript was increased, whereas strains with a longer TAS array (A6-511 and B8-13Y) showed relatively steady transcript levels between larval and pupal stages. Due to the lack of a control without any TAS, we could not ask whether the relatively short TAS array in A6-514 and A8-2A might induce silenced levels of *w* transcript. However, the data clearly indicate that, independent of telomeric position or an adjacent HTT array, a long TAS array in *cis* keeps levels of *w* expression low during development, as we observed in both euchromatic TAS-mini-*w* and telomeric *11-5* insertions.

Long-range effects of suppressors of TPE. To test whether 2L TAS deficiencies can suppress silencing on nonhomologous telomeres as well as on homologues, we crossed females carrying a mini-*w* transgene in the TAS of 2R (*39C-27*) or 3R (*39C-62*) (52) to *M26/SM1* males. The resulting *39C-27/M26* and *M26/+;39C-62/+* males had darker eyes than their *39C-27/SM1* and *SM1/+;39C-62/+* brothers (Fig. 7A), indicating that suppression of TPE by 2L TAS deficiencies extends to other telomeres (Fig. 7B). To confirm that the observed TPE suppression is not due to another factor in the genetic background, we tested several other deficiencies of the 2L TAS: *Df(2L)net62*, *l(2)gl^{GB26}*, and *P{w^{var}}11-10*, a variant of *P{w^{var}}* that is deficient for some or all of the *w* reporter but has an intact *l(2)gl* gene (22). These deficiencies gave similar results to those observed for *M26*. Also, to confirm that *l(2)gl*, which is a locus adjacent to the TAS array, is not involved in TPE and that telomeric silencing is affected only by the TAS, we tested

l(2)gl^{DV275}, which has an intact TAS array and a deficiency restricted to the *l(2)gl* locus, and *l(2)gl^{GB52}*, which has an insertion mutation in the *l(2)gl* locus. These mutations did not affect expression of telomeric inserts (data not shown), confirming that the 2L TAS, not the neighboring *l(2)gl* gene, plays a role in telomeric silencing. These data indicate that the effect of 2L TAS deficiencies on TPE are global, rather than restricted to homologues.

Expression of transgenes residing within the TAS. As TAS deficiencies elevate expression of *w* inserted into the TAS, we examined the possibility of read-through transcription from a retroelement promoter into the adjacent TAS region and a contribution of such read-through transcription on expression of *w* residing within the TAS. For this purpose we tested *39C-5*, which carries a *white* insertion in the 2L TAS with its transcription occurring in a distal-to-proximal manner (11). A 2L TAS deficiency on the homologue leads to a minor but discernible increase of *w* expression in flies with this insertion, which we confirmed by pigment analysis (Fig. 7C). We further tested *39C-27* and *39C-62* individuals for the presence of retroelement read-through transcript, although the exact positions of these two *w* insertions within the TAS array are not known. For all three lines the presence of read-through transcript was tested in homozygotes and hemizygotes bearing the *M26* deficiency using primer set 3 (Fig. 1A). However, in none of these cases did we observe any transcription product (data not shown). This suggests that the increase of *w* transcription in these TAS-deficient flies is due to direct release of TAS silenc-

FIG. 8. Overall *HeT-A* transcript activity. (A) Levels of total *HeT-A* transcripts in 11-5/11-5, 11-5/M26, 11-5/+ relative to *Rpl32* transcript at various stages of development are shown. (B) Relative *HeT-A* copy numbers in the genomes tested. (C) Relative levels of *HeT-A* transcripts standardized to relative copy number of genomic *HeT-A*.

ing on the *w* promoter with little or no contribution of direct retroelement read-through transcription.

Influence of 2L TAS deficiency on overall *HeT-A* transcription. Because 2L TAS deficiencies have an effect on TAS-repressed transgenes elsewhere in the genome and on *HeT-A* promoter activity, it was of interest to investigate the overall impact of 2L TAS deficiencies on general *HeT-A* transcription. For this task, we used HCS primers specific to *HeT-A* coding sequence and quantified overall *HeT-A* transcript levels in larvae, late pupae, and adults (Fig. 8A). To calculate the relative *HeT-A* expression per genomic *HeT-A* copy number, we measured relative numbers of *HeT-A* copies in each genotype (Fig. 8B) using the same primer set and standardized the *HeT-A* expression to the relative copy number in each stock (Fig. 8C). Total *HeT-A* transcripts did not show consistency with *HeT-A/w* read-through transcript levels (Fig. 5). These observations indicate that overall *HeT-A* transcription is independent of TAS influence and suggests that TAS influence is restricted to the proximal telomeric retroelements. Thus, the level of total *HeT-A* transcript is not significantly dependent on control by the TAS.

DISCUSSION

This study extends our earlier observations relating to a role for the TAS in telomeric silencing, telomere maintenance, and other aspects of telomere behavior in *Drosophila*. Based on our previous studies, we proposed the *HeT-A* activation model of TPE (22, 37) to explain TPE in *Drosophila*; this model states that the silencing effects of heterochromatin-like chromatin spread from the TAS into the HTT array, while transcriptional

activity from *HeT-A* promoters exerts an activating influence on more proximal regions of the HTT array. TAS deficiencies lead to a stimulation of telomeric *w* transgenes and retrotransposons on the homologous chromosome, which suggests that homologous TAS-TAS associations may modulate interactions between telomeres. We further speculated that these interactions modulate overall HTT activity and subsequent transposition and telomere elongation. Here, we report several results that address predictions based on the model.

Telomeric gene expression. One major aspect of TPE incorporated into the *HeT-A* activation model is that disruption or deletion of the TAS causes suppression of telomeric silencing in *trans* (22, 39). Here we used the M26 deficiency, which completely deletes the 2L TAS while leaving the adjacent *l(2)gl* gene intact. Consistent with results on deficiencies that remove both the 2L TAS and *l(2)gl*, this deficiency acts as a dominant suppressor of TPE, while mutations in *l(2)gl* do not. Thus, the TAS, rather than *l(2)gl*, seems to be the relevant agent in regulating TPE. The elevated expression of the telomeric *w* transgene is correlated with an increase in the read-through transcript from a *HeT-A* promoter into *w*. This is the first direct evidence that the TAS can modulate *HeT-A* promoter activity in *trans*.

As shown collectively by Northern hybridization and PCR, total *w* expression in the 11-5 insertion between the HTT array and the TAS is produced by contributions from the telomeric *w* promoter and promoters of at least two telomeric retroelements. This is consistent with the observation that expression of the 11-5 reporter increases with increasing HTT array length in *cis* (22). Although the read-through transcript is only a minor component of total *w* transcript levels in 11-5, and a

majority of the *w* transcript is produced from the *w* promoter, we can conclude that a 2L TAS deficiency leads to a release of silencing on both telomeric *w* and telomeric retroelement promoters. The relative intensities of these two promoters as seen here are consistent with the observation that the *HeT-A* promoter is relatively weak (53). A 2L TAS deficiency may stimulate the *w* promoter directly; however, transcription initiated in the HTT array may also stimulate the *w* promoter, even in the absence of read-through by change of chromatin structure. These two hypotheses are not mutually exclusive. Read-through transcripts from telomeric retrotransposons into other downstream sequences were detected here by RT-PCR and have also been found in expressed sequence tag libraries as cDNAs containing sequences from more than one telomeric retrotransposon (40).

Using insertion elements in TASs, we tested the possibilities that transcription from the most proximal retroelement might continue into TASs and that such transcription might be elevated in 2L TAS-deficient flies, in which TPE is suppressed. Although *w* transcript levels increased in 2L TAS-deficient flies, we did not see any PCR product indicating read-through transcript from the retroelement. This suggests that retroelement transcripts do not extend far into the TAS, if at all, and the elevation of *w* transcription in these lines may be due instead to a release of TAS silencing on *w*.

Telomere interactions. We demonstrate here for the first time that deficiencies of the 2L TAS can suppress TPE at nonhomologous telomeres, indicating that nonhomologues may interact. Deficiencies of the 2L TAS suppress silencing of *w* transgenes inserted into the 2L, 2R, and 3R telomeres. Thus, these deficiencies appear to be global suppressors of TPE, consistent with the observations that expression of a gene depends on its position within the interphase nucleus and on somatic pairing of chromosomes (12, 13, 15, 23, 25, 51). While there is no direct evidence pertaining to the question of whether physical associations between telomeres influence the genetic interactions we report here, cytological observations made with a number of organisms suggest that eukaryotic nuclei maintain reproducible long-range chromosomal organization during interphase, such as telomere interactions with other telomeres and with the nuclear lamina, and occupancy by individual chromosomes of distinct nuclear territories (10, 17, 18, 26). In addition, Polycomb group proteins bind TAS regions (2, 9) and may mediate long-range interactions. *Mcp*, for example, a *Drosophila* Polycomb response element, can mediate physical interactions between remote chromosomal sites (50). Similar long-range interactions have been described for the mouse *HoxD* cluster (49).

One prediction of the *HeT-A* activation model, that telomere-telomere interactions modulate overall HTT transcription, subsequent transposition, and chromosome elongation (22, 37), is not supported by the present data. Although 2L TAS deficiencies suppress TPE on all telomeres tested and suppress silencing of proximal telomeric retroelements, we did not see a concomitant response of overall *HeT-A* transcript level. Our results agree with recent evidence (7) that *w* genes located in the HTT array close to the TAS are under TAS control and show repression and variegation, while *w* genes in the HTT array far from the TAS are not repressed and behave as if they were in euchromatin independent of TAS influence.

Thus, the effect of TAS deficiencies on TPE and retroelement transcription may only extend a limited distance from the TAS, and the effect of TAS deficiencies on TPE do not indicate a significant control mechanism over telomere length. More important in controlling chromosome elongation appears to be the cap structure at the telomere ends, possibly because it controls accessibility of the chromosome ends to new transpositions (42, 47). A possible role for RNAi in telomere length control and *TART* transposition in *Drosophila* was also reported (48).

Developmental changes in telomeric silencing. A novel process discovered in this study is a developmental modulation of telomeric silencing. The developmental expression profile of the read-through transcript in the upstream region of a telomeric *w* gene resembles the expression profile of the telomeric *w* itself, as well as the profile of TAS–mini-*w* fusion constructs in euchromatic positions, and differs from that of the *w* gene in its normal genomic location. It also differs from the expression profile for total *HeT-A*, suggesting that the TAS specifically modulates transcription in the adjacent region, i.e., telomeric *w* and the proximal *HeT-A* elements in 11-5. Overall *HeT-A* transcript levels reflect the sum of all *HeT-A* promoters, most of which are outside the range of TAS influence.

Collectively, the present results indicate that changes in expression of telomeric transgenes that occur in the presence of a shortened 2L TAS during late stages of development imply a similar modulation of the activity of telomeric retrotransposons as a result of long-range telomere-telomere interactions.

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REFERENCES

1. Abad, J. P., B. de Pablos, K. Osogawa, P. J. de Jong, A. Martin-Gallardo, and A. Villasante. 2004. Genomic analysis of *Drosophila melanogaster* telomeres: Full-length copies of *HeT-A* and *TART* elements at telomeres. *Mol. Biol. Evol.* **21**:1613–1619.
2. Andreyeva, E. N., E. S. Belyaeva, V. F. Semeshin, G. V. Polkhokova, and I. F. Zhimulev. 2005. Three distinct chromatin domains in telomere ends of polytene chromosomes in *Drosophila melanogaster* Tel mutants. *J. Cell Sci.* **118**:5465–5477.
3. Ashburner, M. 1989. *Drosophila: a laboratory manual*. Cold Spring Harbor Press, Cold Spring Harbor, NY.
4. Biessmann, H., L. E. Champion, M. O'Hair, K. Ikenaga, B. Kasravi, and J. M. Mason. 1992. Frequent transpositions of *Drosophila melanogaster* *HeT-A* transposable elements to receding chromosome ends. *EMBO J.* **11**:4459–4469.
5. Biessmann, H., and J. M. Mason. 2003. Telomerase-independent mechanisms of telomere elongation. *Cell. Mol. Life Sci.* **60**:2325–2333.
6. Biessmann, H., and J. M. Mason. 1997. Telomere maintenance without telomerase. *Chromosoma* **106**:63–69.
7. Biessmann, H., S. Prasad, V. E. Semeshin, E. N. Andreyeva, Q. Nguyen, M. F. Walter, and J. M. Mason. 2005. Two distinct domains in *Drosophila melanogaster* telomeres. *Genetics* **171**:1767–1777.
8. Blackburn, E. H. 1991. Telomeres. *Trends Biochem. Sci.* **16**:378–381.
9. Boivin, A., C. Gally, S. Netter, D. Anxolabehere, and S. Ronsseray. 2003. Telomeric associated sequences of *Drosophila* recruit Polycomb-group proteins in vivo and can induce pairing-sensitive repression. *Genetics* **164**:195–208.
10. Bystricky, K., T. Laroche, G. van Houwe, M. Blaszyk, and S. M. Gasser.

2005. Chromosome looping in yeast: telomere pairing and coordinated movement reflect anchoring efficiency and territorial organization. *J. Cell Biol.* **168**:375–387.
11. Cryderman, D. E., E. J. Morris, H. Biessmann, S. C. R. Elgin, and L. L. Wallrath. 1999. Silencing at *Drosophila* telomeres: nuclear organization and chromatin structure play critical roles. *EMBO J.* **18**:3724–3735.
12. Csink, A. K., and S. Henikoff. 1996. Genetic modification of heterochromatic association and nuclear organization in *Drosophila*. *Nature* **381**:529–531.
13. Csink, A. K., and S. Henikoff. 1998. Large-scale chromosomal movements during interphase progression in *Drosophila*. *J. Cell Biol.* **143**:13–22.
14. Danilevskaya, O. N., I. R. Arkhipova, K. L. Traverse, and M. L. Pardue. 1997. Promoting in tandem: the promoter for telomere transposon *HeT-A* and implications for the evolution of retroviral LTRs. *Cell* **88**:647–655.
15. Donaldson, K. M., and G. H. Karpen. 1997. *trans*-suppression of terminal deficiency-associated position effect variegation in a *Drosophila* minichromosome. *Genetics* **145**:325–337.
16. Fjose, A., L. C. Polito, U. Weber, and W. J. Gehring. 1984. Developmental expression of the white locus of *Drosophila melanogaster*. *EMBO J.* **3**:2087–2094.
17. Foe, V. E., and B. M. Alberts. 1985. Reversible chromosome condensation induced in *Drosophila* embryos by anoxia: visualization of interphase nuclear organization. *J. Cell Biol.* **100**:1623–1636.
18. Funabiki, H. I., I. Hagan, S. Uzawa, and M. Yanagida. 1993. Cell cycle-dependent specific positioning and clustering of centromeres and telomeres in fission yeast. *J. Cell Biol.* **121**:961–976.
19. Gehring, W. J., R. Klemenz, U. Weber, and U. Kloter. 1984. Functional analysis of the *white*⁺ gene of *Drosophila* by P-factor-mediated transformation. *EMBO J.* **3**:2077–2085.
20. George, J. A., and M. L. Pardue. 2003. The promoter of the heterochromatic *Drosophila* telomeric retrotransposon, HeT-A, is active when moved into euchromatic locations. *Genetics* **163**:625–635.
21. George, J. A., P. G. DeBaryshe, K. L. Traverse, S. E. Celniker, and M. L. Pardue. 2006. Genomic organization of the *Drosophila* telomeric retrotransposable elements. *Genome Res.* **16**:1231–1240.
22. Golubovsky, M. D., A. Y. Konev, M. F. Walter, H. Biessmann, and J. M. Mason. 2001. Terminal retrotransposons activate a subtelomeric *white* transgene at the 2L telomere in *Drosophila*. *Genetics* **158**:1111–1123.
23. Hari, K. M., K. R. Cook, and G. H. Karpen. 2001. The *Drosophila Su(var)*2–10 locus regulates chromosome structure and function and encodes a member of the PIAS protein family. *Genes Dev.* **15**:1334–1348.
24. Hazelrigg, T., R. Levis, and G. M. Rubin. 1984. Transformation of *white* locus DNA in *Drosophila*: dosage compensation, *zeste* interaction, and position effects. *Cell* **36**:469–481.
25. Henikoff, S., J. M. Jackson, and P. B. Talbert. 1995. Distance and pairing effects on the *brown* dominant heterochromatic element in *Drosophila*. *Genetics* **140**:1007–1017.
26. Hochstrasser, M., D. Mathog, Y. Gruenbaum, H. Saumweber, and J. W. Sedat. 1986. Spatial organization of chromosomes in the salivary gland nuclei of *Drosophila melanogaster*. *J. Cell Biol.* **102**:112–123.
27. Kahn, T., M. Savitsky, and P. Georgiev. 2000. Attachment of *HeT-A* sequences to chromosomal termini in *Drosophila melanogaster* may occur by different mechanisms. *Mol. Cell Biol.* **20**:7634–7642.
28. Karpen, G. H., and A. C. Spradling. 1992. Analysis of subtelomeric heterochromatin in the *Drosophila* minichromosome *Dp1187* by single P element insertional mutagenesis. *Genetics* **132**:737–753.
29. Korochkina, L. S., and M. D. Golubovsky. 1978. Cytogenetic analysis of induced mutations on the left end of the second chromosome of *D. melanogaster*. *Drosophila Information Service* **53**:197–200.
30. Kurenova, E., L. Champion, H. Biessmann, and J. M. Mason. 1998. Directional gene silencing induced by a complex subtelomeric satellite from *Drosophila*. *Chromosoma* **107**:311–320.
31. Laurenti, P., Y. Graba, R. Rosset, and J. Pradel. 1995. Genetic and molecular analysis of terminal deletions of chromosome 3R of *Drosophila melanogaster*. *Gene* **154**:177–181.
32. Levis, R., T. Hazelrigg, and G. M. Rubin. 1985. Effects of genome position on the expression of transduced copies of the *white* gene of *Drosophila*. *Science* **229**:558–561.
33. Levis, R. W., R. Ganesan, K. Houtchens, L. A. Tolar, and F. M. Sheen. 1993. Transposons in place of telomeric repeats at a *Drosophila* telomere. *Cell* **75**:1083–1093.
34. Luan, D. D., M. H. Korman, J. L. Jakubczak, and T. H. Eickbush. 1993. Reverse transcription of R2Bm RNA is primed by a nick at the chromosomal target site: a mechanism for non-LTR retrotransposition. *Cell* **72**:595–605.
35. Mason, J. M., and H. Biessmann. 1995. The unusual telomeres of *Drosophila*. *Trends Genet.* **11**:58–62.
36. Mason, J. M., A. Haoudi, A. Y. Konev, E. Kurenova, M. F. Walter, and H. Biessmann. 2000. Control of telomere elongation and telomeric silencing in *Drosophila melanogaster*. *Genetica* **109**:61–70.
37. Mason, J. M., A. Y. Konev, and H. Biessmann. 2003. Telomeric position effect in *Drosophila melanogaster* reflects a telomere length control mechanism. *Genetica* **117**:319–325.
38. Mason, J. M., A. Y. Konev, M. D. Golubovsky, and H. Biessmann. 2003. Cis- and trans-acting influences on telomeric position effect in *Drosophila melanogaster* detected with a subterminal transgene. *Genetics* **163**:917–930.
39. Mason, J. M., J. Ransom, and A. Y. Konev. 2004. A deficiency screen for dominant modifiers of telomeric silencing in *Drosophila*. *Genetics* **168**:1353–1370.
40. Maxwell, P. H., J. M. Belote, and R. W. Levis. 2006. Identification of multiple transcription initiation, polyadenylation, and splice sites in the *Drosophila melanogaster* TART family of telomeric retrotransposons. *Nucleic Acids Res.* **34**:5498–5507.
41. Mechler, B. M., W. McGinnis, and W. J. Gehring. 1985. Molecular cloning of *lethal(2)giant larvae*, a recessive oncogene of *Drosophila melanogaster*. *EMBO J.* **4**:1551–1557.
42. Melnikova, L., H. Biessmann, and P. Georgiev. 2005. The Ku protein complex is involved in length regulation of *Drosophila* telomeres. *Genetics* **170**:221–235.
43. Pardue, M. L., and P. G. DeBaryshe. 2003. Retrotransposons provide an evolutionarily robust non-telomerase mechanism to maintain telomeres. *Annu. Rev. Genet.* **37**:485–511.
44. Pardue, M. L., S. Rashkova, E. Casacuberta, P. G. DeBaryshe, J. A. George, and K. L. Traverse. 2005. Two retrotransposons maintain telomeres in *Drosophila*. *Chromosome Res.* **13**:443–453.
45. Pryde, F. E., H. C. Gorham, and E. J. Louis. 1997. Chromosome ends: all the same under their caps. *Curr. Opin. Genet. Dev.* **7**:822–828.
46. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
47. Savitsky, M., O. Kravchuk, L. Melnikova, and P. Georgiev. 2002. Heterochromatin protein 1 is involved in control of telomere elongation in *Drosophila melanogaster*. *Mol. Cell Biol.* **22**:3204–3218.
48. Savitsky, M., D. Kwon, P. Georgiev, A. Kalmykova, and V. Gvozdev. 2006. Telomere elongation is under the control of the RNAi-based mechanism in the *Drosophila* germline. *Genes Dev.* **20**:324–354.
49. Spitz, F., F. Gonzales, and D. Duboule. 2003. A global control region defines a chromosomal regulatory landscape containing the HoxD cluster. *Cell* **113**:405–417.
50. Vazquez, J., M. Muller, V. Pirrotta, and J. W. Sedat. 2006. The Mcp element mediates stable long-range chromosome-chromosome interactions in *Drosophila*. *Mol. Biol. Cell* **17**:2158–2165.
51. Wakimoto, B. T., and M. G. Hearn. 1990. The effects of chromosome rearrangements on the expression of heterochromatic genes in chromosome 2L of *Drosophila melanogaster*. *Genetics* **125**:141–154.
52. Wallrath, L. L., and S. C. R. Elgin. 1995. Position effect variegation in *Drosophila* is associated with an altered chromatin structure. *Genes Dev.* **9**:1263–1277.
53. Walter, M. F., and H. Biessmann. 2004. Expression of the telomeric retrotransposon HeT-A in *Drosophila melanogaster* is correlated with cell proliferation. *Dev. Genes Evol.* **214**:211–219.
54. Walter, M. F., C. Jang, B. Kasravi, J. Donath, B. M. Mechler, J. M. Mason, and H. Biessmann. 1995. DNA organization and polymorphism of a wild-type *Drosophila* telomere region. *Chromosoma* **104**:229–241.