Cis- and *trans*-acting Influences on Telomeric Position Effect in *Drosophila melanogaster* Detected With a Subterminal Transgene

James M. Mason,* Alexander Y. Konev,*,[1] Mikhail D. Golubovsky*,† and Harald Biessmann‡,[2]

**Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709-2233,* ‡*Developmental Biology Center, University of California, Irvine, California 92697 and* †*Division of Evolutionary Theory, Institute of Science and Technology History, Russian Academy of Sciences, Saint Petersburg 199034, Russia*

Manuscript received August 2, 2002
Accepted for publication December 11, 2002

ABSTRACT

One model of telomeric position effect (TPE) in *Drosophila melanogaster* proposes that reporter genes in the vicinity of telomeres are repressed by subterminal telomere-associated sequences (TAS) and that variegation of these genes is the result of competition between the repressive effects of TAS and the stimulating effects of promoters in the terminal *HeT-A* transposon array. The data presented here support this model, but also suggest that TPE is more complex. Activity of a telomeric *white* reporter gene increases in response to deletion of some or all of the TAS on the homolog. Only transgenes next to fairly long *HeT-A* arrays respond to this *trans*-interaction. *HeT-A* arrays of 6–18 kb respond by increasing the number of dark spots on the eye, while longer arrays increase the background eye color or increase the number of spots sufficiently to cause them to merge. Thus, expression of a subtelomeric reporter gene is influenced by the telomere structure in *cis* and *trans*. We propose that the forces involved in telomere length regulation in Drosophila are the underlying forces that manifest themselves as TPE. In the wild-type telomere TAS may play an important role in controlling telomere elongation by repressing *HeT-A* promoter activity. Modulation of this repression by the homolog may thus regulate telomere elongation.

THE structure of the terminal DNA array at telomeres in *Drosophila melanogaster* differs significantly from that of yeast and most other eukaryotes (Mason and Biessmann 1995). Drosophila telomeres do not possess arrays of simple repeats that are generated by telomerase. Instead, Drosophila maintains its telomeres by transposition of specific non-long-terminal-repeat retrotransposons, *i.e.*, *HeT-A* and *TART*, to chromosome ends. Both of these elements have unusually long 3′ untranslated regions (UTRs), which encompass approximately one-half of the element. At least 600 bp of *HeT-A* near the oligo(A) tail contain promoter activity when tested with a *lacZ* reporter in a transient expression assay using tissue culture cells (Danilevskaya *et al.* 1997), and, *in vivo*, 400 bp of the 3′ end of *HeT-A* are sufficient to increase *yellow* expression at the tip of a terminally deficient chromosome (Kahn *et al.* 2000). Proximal to the terminal retrotransposon array Drosophila telomeres carry several kilobases of complex satellites, referred to as telomere-associated sequences (TAS), which vary in sequence among telomeres, but have sequence similarities between different chromosome ends (Karpen and Spradling 1992; Walter *et al.* 1995).

Drosophila telomeric regions are able to repress gene activity. When inserted into a telomere, reporter genes exhibit repressed and variegated expression, referred to as telomeric position effect (TPE; Gehring *et al.* 1984; Hazelrigg *et al.* 1984; Levis *et al.* 1985; Karpen and Spradling 1992; Tower *et al.* 1993; Roseman *et al.* 1995; Wallrath and Elgin 1995). Molecular analyses showed that these repressed transgenes have inserted within, or adjacent to, a TAS array (Karpen and Spradling 1992; Levis *et al.* 1993; Cryderman *et al.* 1999; Golubovsky *et al.* 2001), suggesting that the TAS regions of Drosophila telomeres are heterochromatic and that TAS plays a role in telomeric silencing (Kurenova *et al.* 1998). To investigate *cis*- and *trans*-acting effects on TPE we used a variegating *white* (*w*) transgene in the *2L* telomere, termed *P{w^{var}}* (Gehring *et al.* 1984). The insertion carries a genomic *w* gene with its eye-specific enhancer, flanked distally by two truncated *HeT-A* elements and proximally by a shortened *2L* TAS (Golubovsky *et al.* 2001), and is transcribed from distal to proximal. It has lost all of the distal 5′ *P*-element sequences, and the 3′ end of the first *HeT-A* element is attached upstream of the eye-testis enhancer. While expression of this reporter in nontelomeric positions generally gives red eyes (Gehring *et al.* 1984), the eye color in the *y^{1} w^{67c23}*; *P{w^{var}}* strain was originally orange, similar to *w^{apricot}*, with a few small red spots. *w* gene expression is sensitive to molecular changes occurring distally

[1]*Present address:* Postgenomics Corporation, San Diego, CA 92121.

[2]*Corresponding author:* Developmental Biology Center, University of California, Irvine, CA 92697. E-mail: hbiessma@uci.edu

on the same chromosome, and some variants are sensitive to alterations at the homologous telomere (Golubovsky *et al.* 2001).

Taking into account the general structure of the Drosophila telomere and the genetic behavior of variant *P{w^{var}}* chromosomes with altered *HeT-A/TART* arrays, we proposed a new model of TPE, the *HeT-A* activation model (Mason *et al.* 2000; Golubovsky *et al.* 2001), which postulates that TAS represses transcription toward the *HeT-A/TART* array, while the terminal *HeT-A* elements promote transcription toward the centromere from promoters located in their 3′ noncoding regions (Danilevskaya *et al.* 1997). Transcription from *HeT-A* promoters may partially alleviate the silencing of a downstream reporter gene, or *HeT-A* element that is caused by the subtelomeric satellite. A reporter gene inserted between the two arrays, or within TAS, is subject to these competing influences. Variegated expression may thus be caused by a competition between the centromere-directed "activating force" of *HeT-A* transcription and the "repressive force" of the TAS. Transcription is a necessary first step in transposition of a retrotransposon and for many elements is a limiting step in this process (Chaboissier *et al.* 1990; McLean *et al.* 1993; Pasyukova *et al.* 1997). Thus, modulation of *HeT-A* transcription by TAS may be important for the regulation of telomere length.

It is formally possible that a nontelomeric master *HeT-A* or *TART* element exists, but the only full-length elements known are in telomere regions. Sequences from the *HeT-A* 3′ UTR are found at telomeres and in centric heterochromatin, but not in euchromatin (Rubin 1978; Young *et al.* 1983), while sequences from the open reading frame (ORF) of *HeT-A* (Danilevskaya *et al.* 1992) and *TART* (Levis *et al.* 1993) are found exclusively at chromosome tips. *HeT-A* elements that have been analyzed soon after transposition vary in sequence (Biessmann *et al.* 1994), indicating that a single master element does not exist. It is, thus, likely that many *HeT-A* elements are capable of retrotransposition, and these are in arrays adjacent to TAS.

The level of expression of the subtelomeric *w* transgene also depends on the structure of the homologous telomere. We have proposed that homologous chromosomes assess the status of their telomeres by transient interactions mediated by their TAS regions (Golubovsky *et al.* 2001). When the homologous TAS is long, the interaction is strong and silencing occurs (Figure 1A). In some ways this interaction resembles pairing-dependent silencing (Kassis *et al.* 1991; Henikoff 1997). If this interaction is compromised by shortened or absent TAS arrays, promoter activity of the *HeT-A* elements is increased. Telomeric regions with intact TAS regions repress, or fail to activate, the *HeT-A* promoters located on the homolog. We cannot currently distinguish *trans*-inactivation of *HeT-A* by a homologous wild-type TAS array, which is alleviated when the homologous TAS is

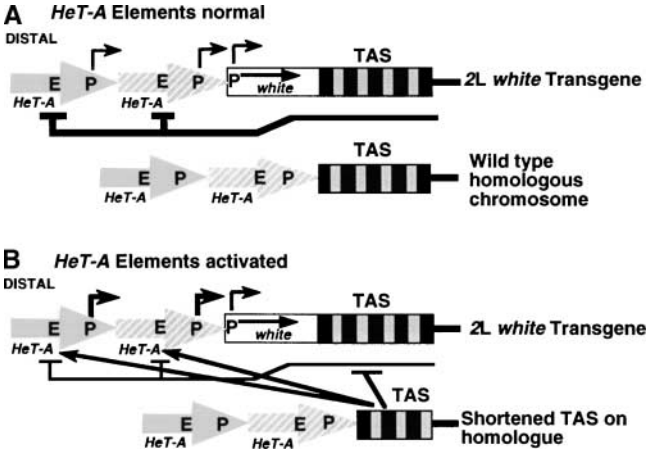

Figure 1.—The *HeT-A* activation model of telomeric position effect. (A) The model proposes that the variegation exhibited by a *white* gene inserted into a telomere is due to competition between the activating influence of *HeT-A* and the repressive influence of TAS. Telomeres associate by some means other than euchromatic homology and assess the integrity of TAS elements. Silencing is strong when the TAS array on the homologous telomere is long. The *HeT-A/TART* array is shown as two large arrows. Transcription is indicated by small bent arrows. (B) When a short or interrupted TAS element is identified, expression of *HeT-A* increases. A signal indicating a short or missing TAS element may either activate enhancers directly or suppress the *cis*-acting TAS repression, either of which would result in increased promoter activity of *HeT-A*. E, enhancers on *HeT-A*; P, promoters on *white* and *HeT-A*.

incomplete, from *trans*-activation by a short or absent TAS array. Either of these mechanisms may be mediated by direct interactions between the *HeT-A* promoter/enhancer region and TAS on the homolog or by indirect effects resulting from TAS-TAS interactions (Figure 1B). The molecular mechanisms mediating integrity assessment of paired TAS regions are not known, but a more detailed discussion is presented elsewhere (Mason *et al.* 2003).

Here we report on an extension of these studies using secondary variants of *P{w^{var}}*. Separate fly lines (families) were established from isolated chromosomes derived from *P{w^{var}}*, and eye color changes were monitored within each family. Telomeres are dynamic, and as expected, elongation and shortening events of the terminal *HeT-A/TART* array occurred fairly frequently. By monitoring the ancestry of a given eye color variant and isolating the affected chromosome, we were able to trace the molecular events responsible for the eye color change. Using this regimen, several new eye color variants were isolated and characterized at the molecular level, which offered new insights into the contributions of telomeric sequences acting on TPE *in cis*. First, consistent with our previous results (Golubovsky *et al.* 2001), in a transgene hemizygote with a wild-type *2L* telomere on the homolog, the eye color is correlated with the length of *white* sequence remaining in the transgene.

Second, in a heterozygote with a defective or deficient *2L* telomere on the homolog, a single *HeT-A* 3′ UTR upstream of *P{w^var}* increases the number of dark spots, and multiple *HeT-A* elements appear to increase mottling to the point where the spots merge to give the eye a more uniform brown-red appearance. Third, the level of expression induced by the homologous chromosome depends on the nature of TAS in the inducing chromosome. Derivatives of *P{w^var}* with short TAS arrays, especially those with little or no white expression of their own, have strong effects on *P{w^var}* expression *in trans*, while chromosomes that have lost all of the TAS array have relatively weak effects on transgene expression on the homolog. Deficiencies or disruptions of TAS on the homologous telomere might disrupt a pairing-dependent repression mediated by TAS or, alternatively, induce promoter activity of a full-length *HeT-A* 3′ UTR or inhibit the *cis*-silencing of this promoter.

MATERIALS AND METHODS

Drosophila crosses: Drosophila stocks were maintained and crosses were performed at 25° on cornmeal-molasses medium with dry yeast added to the surface. The *y^1 w^67c23; P{w^var}* stock has been described recently (Golubovsky *et al.* 2001). Other genetic markers and special chromosomes are described by Lindsley and Zimm (1992) and in FlyBase (http://flybase.bio.indiana.edu/). Unless otherwise noted, the *X* chromosome in all stocks and crosses carries *y^1 w^67c23*. As noted previously, several *2L* terminal deficiencies have the same effect on expression of *P{w^var}* variants (Golubovsky *et al.* 2001). We therefore used only one of these deficiencies, *l(2)gl^26*, which has been described previously as *l(2)gl^GB26* (Walter *et al.* 1995). Eye colors were determined in young individuals, and males were aged for 3 days past eclosion for photography.

Families were established and new variant chromosomes were isolated by crossing a single male with the chromosome of interest to *Sco/SM1, Cy* females, backcrossing F_1 males to balancer females, and interbreeding *Cy* F_2 progeny. As the new variants were first identified in heterozygotes, ≥10 F_1 males were backcrossed independently, and stocks were selected in the F_3 generation on the basis of the phenotype of the homozygotes.

DNA amplification by PCR: Polymerase chain reactions to amplify <1-kb fragments from genomic DNA were done in 50-μl reactions containing 0.5 mg of genomic DNA with 2.5 units Taq polymerase (Shuzo, 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 Taq-Plus long PCR system from Stratagene (La Jolla, CA), allowing appropriately longer times for extension. Amplified DNA products were tested on agarose gels and sequenced either directly after purification with Geneclean (Bio 101, Carlsbad, CA) or after cloning into pGem-T-easy (Promega, Madison, WI). The following primers (synthesized by Genosys Biotechnologies, The Woodlands, TX) were used. Primers HeT-R2, HeT-ZK1, and HeT-5′ were modeled after consensus sequences from *HeT-A* elements using the following GenBank accession nos. M84200, M84201, U06947, U06920, and X77049. HeT-5′ is positioned around the presumed ATG start codon of the ORF, HeT-ZK1 is located in the first zinc-knuckle motif of the gag-like protein. Primer HeT-L3 was synthesized according to the sequences in *HeT-A* elements that are located at the distal end of the *P{w^var}* chromosome. Nucleotide positions of the *white* gene primer, white-D, are indicated according to the accession number of the *w* gene (X02974). For approximate positions and directions of these primers, see Figures 2 and 6.

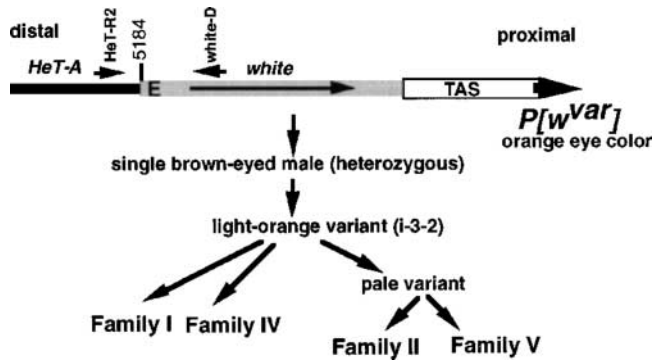

Figure 2.—Flowchart of the events involved in establishing individual families from *y w^67c23; P{w^var} al*, which carries a genomic *w* transgene at the telomere of *2L*. (Top) A representation of the *P{w^var}* transgene, which is flanked proximally by TAS and distally by a short array of *HeT-A* elements (Golubovsky *et al.* 2001). The direction of *w* transcription is indicated by an arrow. E, the eye enhancer on the genomic *w* transgene. The single founder chromosome (i-3-2) from which all families were derived had a light-orange eye color with slight variegation. The positions and orientation of the primers HeT-R2 and white-D, which were used to amplify the DNA fragments containing the *HeT-A/w* junctions, are shown above the map.

HeT-R2: 5′-CCCCAAACTCACCMCATGYAATG-3′
HeT-L3: 5′-CATTCTTATTGAATTTTCCTTTCATTGCAGG-3′
HeT-ZK1: 5′-GGCTTCAGGCATGCCAAAAACTCTTGC-3′
HeT-5′: 5′-CAACATGTCCAYGTCCGACAACCTWTTTTCTG-3′
white-D: 5′-GCTAGGTAACGCTACAAACGGTGG-3′ (nucleotides 7493–7470).

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

Genomic DNA isolation, field inversion gel electrophoresis, Southern blotting, and hybridizations: These were done as described previously (Golubovsky *et al.* 2001). The 2.2-kb DNA fragment from the *w* transgene extending from the *Xho*I site at nucleotide 8925 distally to the *Bam*HI site at nucleotide 6759 was used as hybridization probe.

RESULTS

Telomere dynamics in individual families: To study the dynamics at the *2L* telomere that is marked with the *P{w^var}* transgene, we established four individual lines (families) from a single founder second chromosome. This chromosome was derived from a single heterozygous *P{w^var}* male isolated in March 1996, as shown in Figure 2. One chromosome carried a brown-red variant, which was discarded; the other, termed i-3-2, carried a light-orange variant of the transgene and was placed into homozygous stock after crossing to *y w^67c23; Sco/SM1*. From this stock we established families I and IV, and

TABLE 1

Eye color variants analyzed

Family	Variant class				
	White	Pale	Light orange	Highly variegating	Brown-red
None	white-1-f				
I		I-7-2 I-10-1 I-12-1 I-14-2	I-7-1 I-11-1 I-12-4 I-20-1 I-25	I-6-2-1 I-17-1-1 I-18-5 I-19-3 I-21-2-2-3	
II	II-6-2	II-17-3-2		II-5-3-1-2 II-3-1	II-1-2-5-2 II-3-2-4 II-5-5-5 II-5-3-1 II-8 II-13-2 II-14-4-6
IV			IV-10-4 IV-13 IV-16-2	IV-9-1	IV-16-4-3
V					V-4-1

For each variant the roman numeral designates the family from which the variant was isolated, the first Arabic number is the serial number of the variant, and the numbers following represent subsequent variant isolation steps within a subline. The variant white-1f was not isolated from any of the four families, but arose directly in *P{w^{var}}*.

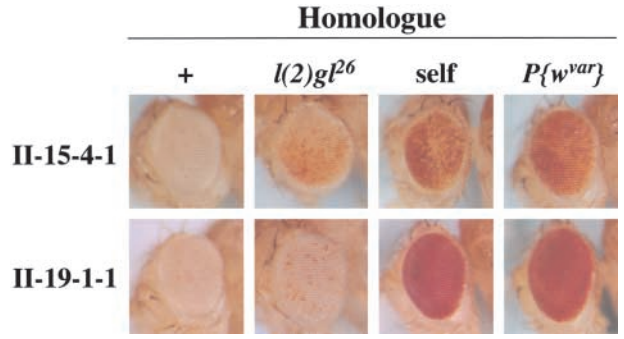

FIGURE 3.—Eye color phenotypes of representative *P{w^{var}}* variants. II-15-4-1 is a typical highly variegated variant. II-19-1-1 falls into the brown-red class I category. The phenotype of a brown-red class II is shown in GOLUBOVSKY *et al.* (2001). "+" indicates that the homolog has a wild-type telomere. In crosses to *y w^{67c23}; Sco/SM1*, both Sco and Cy flies show the same pale eye color. *l(2)gl^{26}* is a terminal deficiency that removes all of the *HeT-A* and TAS arrays from *2L* as well as the adjacent transcribed gene. "self" indicates that the variant is homozygous. *P{w^{var}}* is a typical orange variant that has been under continual selection to maintain a phenotype as close to the original as possible.

from a pale derivative that arose spontaneously in the founder stock, families II and V (Figure 2).

These families were maintained over a period of 32 months, during which time ~100 new variants arose spontaneously and independently. As before (GOLUBOVSKY *et al.* 2001), their eye colors ranged from pure white to red. Second chromosomes from flies with eye color different from that of the founder chromosome (*i.e.*, light orange in families I and IV and pale in families II and IV) were isolated and homozygous stocks were established. Upon further observation, it was obvious that these sublines were also unstable, producing new eye color variants, which were isolated and kept as separate stocks. Thus, the origin of a given variant can be traced back to the family founder. This is reflected in our nomenclature. The roman numeral designates the family in which the variant arose, the first Arabic number is the serial number of the original variant isolated from each family, and the following numbers represent subsequent isolation steps, with new numbers added when a new variant appeared in the purified subline. Because similar eye color variants appeared independently multiple times within each family, and because only a limited repertoire of eye colors could consistently be identified, we chose 32 representative variants for molecular analyses; these are listed in Table 1.

A previous report described variant telomeres derived directly from the *P{w^{var}}* telomeric transgene and classified them into five categories according to the eye color of a homozygous male (GOLUBOVSKY *et al.* 2001). Here we describe secondary variants of the same telomeric insert and follow the convention established previously for identifying them. In addition to the five categories used previously (white, pale, yellow/light orange, orange, and brown-red) we found two new categories, highly variegated (abbreviated var!!) and a second class of brown-red. The phenotype of II-15-4-1, a representative var!! variant, is shown in Figure 3. When heterozygous with a wild-type chromosome, the eyes are pale, with few if any spots. When the homologous telomere is deficient, the eyes are yellow with a fairly large number of spots. The homozygote has eyes that appear under low magnification as if they were darker than eyes of the deficiency heterozygote. Under high power it is evident that the background eye color is approximately the same, but that the homozygote has an increased number (and possibly increased intensity) of dark spots. The heterozygote of II-15-4-1 with *P{w^{var}}* has orange eyes, as might be expected from previous results (GOLUBOVSKY *et al.* 2001), but with increased spot number. Another new category of *P{w^{var}}* variants, exemplified in Figure 3 by II-19-1-1, appears to be a typical brown-red variant when homozygous or heterozygous with *P{w^{var}}* (GOLUBOVSKY *et al.* 2001), but has pale eyes when hemizygous with a wild-type chromosome and does not respond strongly to the *l(2)gl^{26}* deficiency.

Molecular structure of variants: *Transgene junctions:* With primers from TAS and *P*-element sequences at the proximal side of the *white* transgene we verified by PCR that the *w*/TAS junction was unchanged from that de-

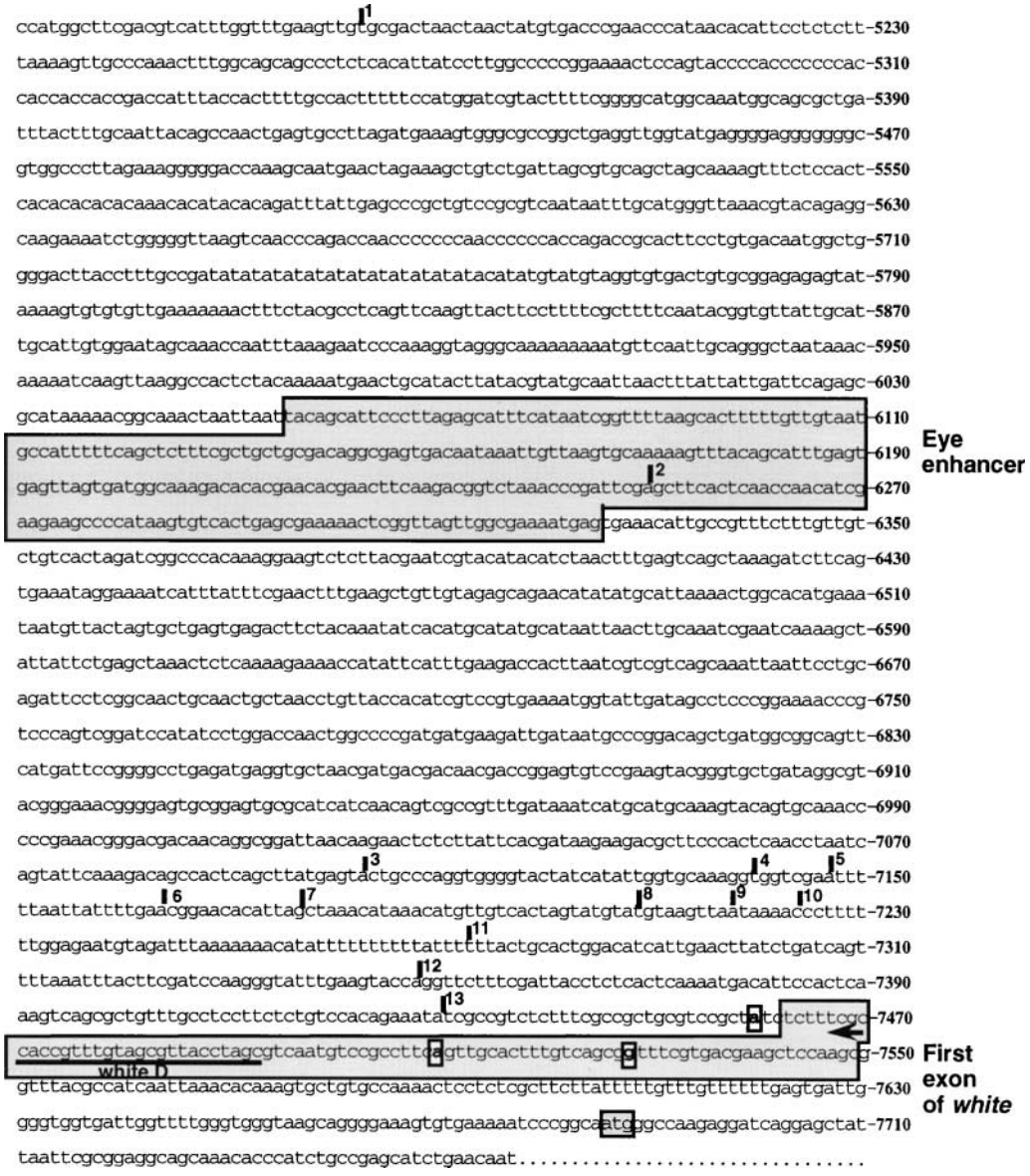

FIGURE 4.—Positions of junctions between *HeT-A* elements and the 5′ upstream region of the *w* transgene in different variant chromosomes. The vertical lines indicate the nucleotide in which the *HeT-A/w* junction occurs in a specific variant. Sequences above and to the left of the vertical line are deleted and replaced by *HeT-A*; sequences to the right are retained. The number by each vertical line notes the junction for a particular variant class as shown below. Some variants have the same *HeT-A/w* junctions, especially those in the same family. Some pale variants and the brown-red variant II-8 were heterogeneous, with different junctions identified in the same stock. Phenotypes are indicated as in Table 1 in parentheses. **1**, *P{w^{var}}* (orange); **2**, I-7-1 (light orange), I-19-3 (var!!), IV-9-1 (var!!), IV-13 (light orange); **3**, I-14-2#2 (pale); **4**, II-5-3-1-2 (var!!), II-5-5-5 (brown-red), II-14-4-6 (brown-red), II-8#1 (brown-red), II-13-2 (brown-red); **5**, IV-16-4-3 (brown-red); **6**, I-12-1#3 (pale); **7**, I-14-2#1 (pale); V-4-1(brown-red); **8**, I-14-2#4 (pale); **9**, II-8#4 (brown-red); **10,** I-14-2#15 (pale); **11,** I-6-2-1 (var!!); **12,** II-17-3-2 (pale); **13,** I-12-1#7 (pale). The region of the eye enhancer is shaded (QIAN *et al.* 1992). The minimal, downstream region with *w* promoter activity (KUTACH and KADONAGA 2000) and the start codon are also boxed and shaded. The three possible transcription start sites are boxed; they are A7460 (O'HARE *et al.* 1984), A7510 (PIRROTTA *et al.* 1985), and G7528 (FlyBase, Fban0002759). An arrow shows the primer white-D. Nucleotide positions of the *w* gene are according to GenBank accession no. X02974.

termined in the original *P{w^{var}}* transgene and its derivatives (GOLUBOVSKY *et al.* 2001). We then determined the nucleotide position of *HeT-A* attachment at the distal end of the *white* transgene in these new variants. This was done by PCR amplification of genomic DNA using a primer (HeT-R2) located ~90 bp upstream of the oligo(A) tail in the 3′ end of *HeT-A* and another (white-D) from the beginning of the first exon of the subtelomeric *white* gene (see Figure 4). Amplified fragments were cloned in pGEM-T and sequenced. In all eye color variants analyzed here, with the exception of the white variants, there was a *HeT-A* element attached with its oligo(A) tail to the *white* transgene. A *TART* element was never observed to be directly attached to *w*. Attachments of *HeT-A* occurred at many different points of the transgene, all of which are located much closer to the start of *w* gene transcription than in the original *P{w^{var}}* (Figure 4).

Most variant stocks were homogeneous, with only a single junction site. However, from some pale stocks (*e.g.*, I-14-2 and I-12-1) multiple PCR fragments with distinct *HeT-A/w* junctions could be subcloned (designated I-14-2#1, #2, #4, #15, and I-12-1#3 and #7). These junction sites represent independent *HeT-A* transposition events, because the oligo(A) tails of the elements differ in length. The junction sites are clustered within ~100 bp of the start of *w* transcription, and the terminally attached *HeT-A* elements in these strains are very short (<400 bp). Thus, the heterogeneity in the pale stocks could not be distinguished phenotypically. One

brown-red stock (II-8) was also heterogeneous and gave two different junction sites, designated II-8#1 and II-8#4 (positions 4 and 9 in Figure 4). Even though the two brown-red chromosomes had distinguishable length *HeT-A* arrays (data not shown), we made no attempt to isolate them from the stock because, as in the above-mentioned pale stocks, they did not cause distinguishable eye phenotypes.

The *HeT-A/w* junction of our original *P{w^{var}}* stock (Golubovsky *et al.* 2001) is shown in Figure 4 as position 1. It occurs at nucleotide 5184 in the *w* sequence and leaves the eye-testis enhancer region intact (Figure 4). In many of the light-orange, brown-red, and highly variegated variants in families I and IV a *HeT-A* element is attached with an $(A)_{15}$ tail to nucleotide 6251 of *w* (position 2 in Figure 4), suggesting that the stocks retained the junction of the light-orange founder chromosome of these two families. Likewise, in most variants from family II the *HeT-A/w* junction occurs at nucleotide 7141 (position 4 in Figure 4) with an invariant $(A)_2$ tail, suggesting that this is the original junction of the pale founder chromosome from which families II and V were derived. In these variants, the *HeT-A/w* junction was conserved, and changes at the *2L* telomere that alter expression of the transgene occur distal to this junction. While most highly variegated stocks in family I had the typical breakpoint at nucleotide 6251, one exception (I-6-2-1) arose from a pale intermediate and consequently had a breakpoint very close to the *w* gene at nucleotide 7274.

Many derivatives from all four families in which the *HeT-A/w* junction differed from that of the original founder chromosome and became located closer to *w* were found (Figure 4). These probably arose from terminal deficiencies that deleted the entire *HeT-A* array at the *2L* telomere, followed by the attachment of a new *HeT-A* to a different position in the upstream region of *w*. Although these junctions are all located within a small region upstream of the *w* transcription start site, the homozygous eye color phenotypes of these stocks differ significantly and vary from pale to brown-red with no apparent correlation with the position of the junction point. As will become evident below, the expression level of the *w* transgene is correlated with the length of the new *HeT-A* array attached at the distal end of the chromosome.

Mapping of the *HeT-A/w* junctions reveals three important features of TPE in Drosophila that were not fully described before. First, all of these eye color variants, even the pale variants, show variegation, although in pale strains the spots may be only slightly darker than the background color of the eye. Thus, the presence of the eye enhancer in the upstream region of the subtelomeric transgene (Qian *et al.* 1992) appears not to be required for variegated expression.

Second, in all variants that show at least some eye color, a basal *w* promoter activity remains with the transgene (see Figure 4), since the TATA-less *w* gene features a downstream promoter element (Kutach and Kadonaga 2000). Thus, for instance in the pale stock I-12-1#7 with very short (<400 bp) *HeT-A* sequences attached to the transgene (junction 13 in Figure 4), low levels of *w* transcription appear to be driven by this basic *w* promoter with little, if any, contribution from the *HeT-A* element promoter activity. A similar argument can be made for the other pale variants with junctions that are close to the transcription start site.

Third, there is little, if any, stimulating *HeT-A* activity when the homologous telomere is wild type. As reported earlier (Golubovsky *et al.* 2001), in the absence of a distal *HeT-A* element there is a correlation between the length of the *w* sequence upstream of the ORF and that of *w* expression, provided that the transgene is opposite a wild-type telomere. Comparison of the present variants that do have *HeT-A* elements attached to the *w* transgene with those reported previously indicates that *HeT-A* addition distal to the transgene has no effect on expression in such hemizygotes.

HeT-A array length: The contribution of distally attached *HeT-A* elements to *w* gene expression at the telomere is clearly exemplified by comparing variants with identical *HeT-A/w* junctions but very different eye colors in the homozygotes. For instance, the presence of <400 bp of *HeT-A* attached to position 7 (Figure 4) in variant I-14-2#1 results in pale eye color, while attachment of >30 kb of *HeT-A* to the same position in variant V-4-1 results in brown-red eye color (see also Figure 5). Moreover, very closely positioned *HeT-A/w* junctions (compare junctions at 8, 9, 10, and 11) can give rise to very different eye colors, depending on the length of the *HeT-A* addition in the strains (Figure 5).

The total lengths of the terminal *HeT-A* additions were determined by genomic Southern blots using four restriction enzymes (*Hin*dIII, *Xho*I, *Sst*I, *Nru*I) with known sites in the *w* transgene (Figure 5). These experiments showed that the white variants were caused by terminal deficiencies that deleted parts of the *w* coding region. The majority of pale variants had very short terminal *HeT-A* additions (<400 bp) that could not be distinguished from the position of the *HeT-A/w* junction as determined by PCR (Figure 4). Only one had an addition as long as 2 kb (II-17-3-2). Light-orange variants carried between 3 and 5 kb of terminal array. The highly variegating variants had intermediate-sized (6–18 kb) *HeT-A* arrays. All brown-red variants had long terminal additions >30 kb. These data confirm our previous conclusion that the longer the terminal *HeT-A* array length, the higher the level of expression of the subterminal *w* reporter gene (Golubovsky *et al.* 2001).

HeT-A array composition: We next determined the composition and arrangement of transposable elements in several variants up to 6 kb distal to *w*. We performed long-range PCR reactions on genomic DNA using one primer (white-D) from the *w* transgene and another

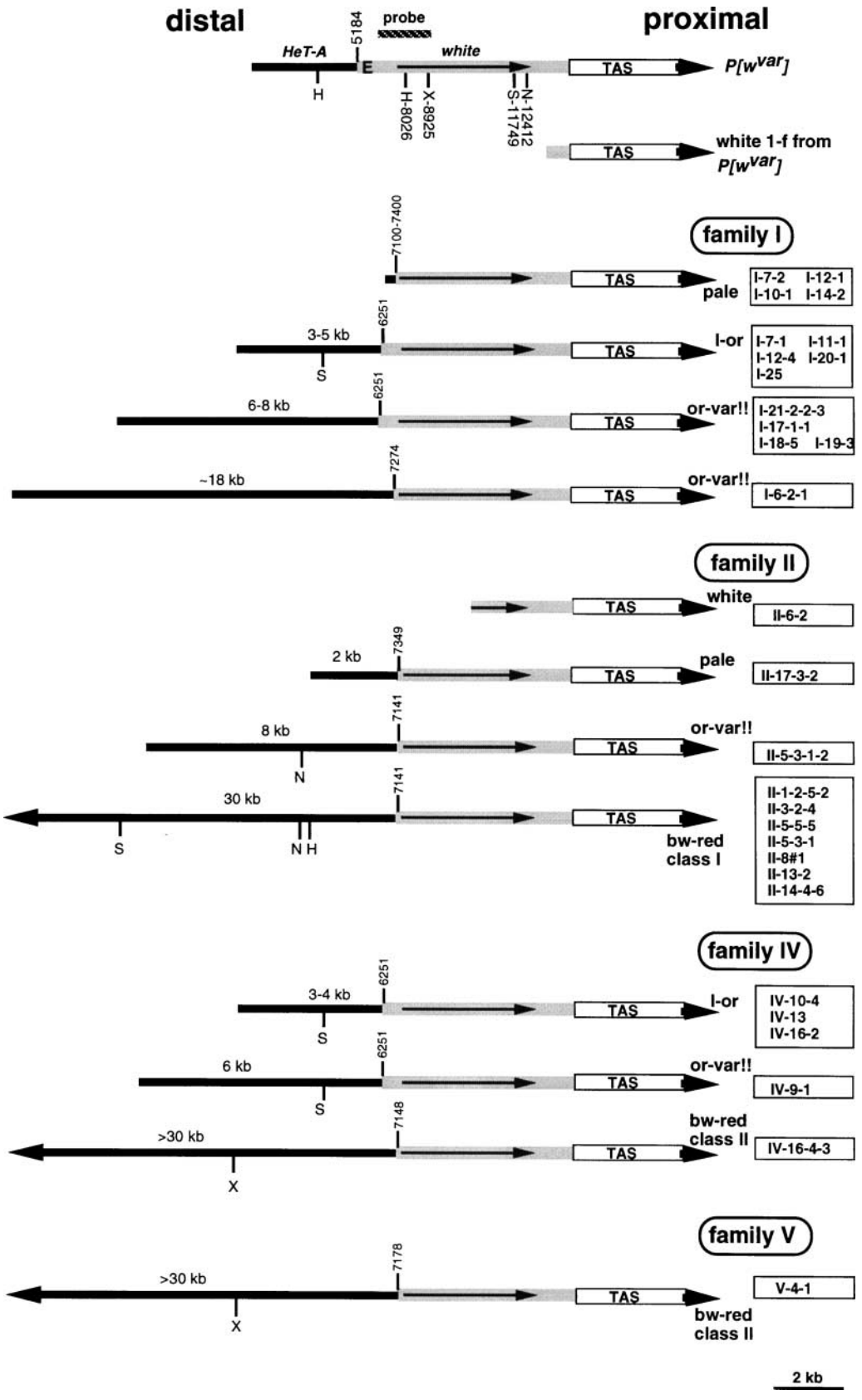

FIGURE 5.—Terminal structures of variant chromosomes. DNA was digested separately with four restriction enzymes that have known sites in the *white* transgene (*Hin*dIII, *Xho*I, *Sst*I, *Nru*I). The nucleotide positions of the cutting sites are shown in the map for *P{w^var}* at the top. Also shown are the positions of the *HeT-A/w* junction in *P{w^var}* at nucleotide 5184 and the 2.2-kb DNA fragment (probe) from the *w* transgene that was used as a hybridization probe on Southern blots. The length of the terminal *HeT-A* addition is shown for each variant, as well as the nucleotide position of the *HeT-A/w* junction (see Figure 4). The variants are grouped by family and eye color phenotype as homozygotes. The white-1f variant arose directly in the *P{w^var}* stock and not in any of the families.

(HeT-ZK1) modeled after the most-conserved sequence in the first zinc-knuckle of the gag-like polypeptide (Figure 6A). In all cases, amplified DNA fragments were sequenced at both ends to verify their identity and to obtain their location within *HeT-A* (see Figure 6B). The PCR reactions amplified fragments of 6 kb (I-21-2-2-3,

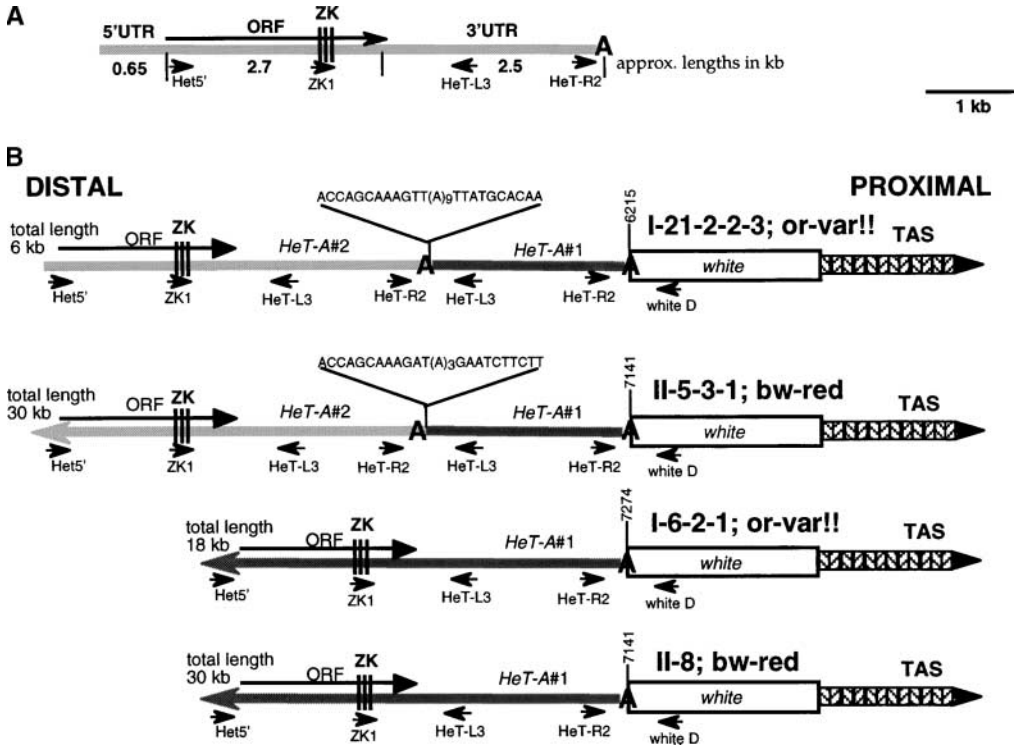

FIGURE 6.—Molecular structure of the proximal 5 kb of the terminal *HeT-A* addition in four variants. A map of a complete 5.8-kb *HeT-A* element is shown in A. The shaded line indicates the *HeT-A* element itself. An arrow above the element indicates the ORF encoding a gag-like protein containing three zinc-knuckle motifs (ZK). Arrowheads below the element indicate the positions and the orientation of the primers used in the amplification of the junction fragments from the variants. Numbers below the element indicate the length of constituent parts of the element. The "A" at the 3′ end of the element indicates the oligo(A) tail that is used to attach to the chromosome. The arrangement of *HeT-A* elements at the chromosome ends of two representative highly variegated and two brown-red variants was determined by sequencing and is shown in B. The *white* transgene is shown as an open rectangle. The vertical number at the left end of the *w* gene indicates the nucleotide of the *w* sequence that forms the junction with *HeT-A*. Arrowheads at either end of the structures indicate that the sequence continues. The total length of the *HeT-A* addition is given on the distal end. Separate *HeT-A* elements in the array are indicated by changes in shading, and the sequences of the junctions are shown above the lines.

orange var!!), 5.5 kb (II-5-3-1, brown-red), and 3.5 kb (I-6-2-1, orange var!! and II-8, brown-red). Variants II-5-3-1-2 (orange var!!) and II-1-2-5-2, II-3-2-4, II-14-4-6, II-5-5-5, and II-13-2 (brown-red) amplified the same size fragment as II-5-3-1 did (data not shown), suggesting a similar *HeT-A* array structure near the *w* transgene. Since the zinc-knuckle region of the ORF is located ~3.5 kb upstream of the *HeT-A* oligo(A) tail (Figure 6A), the two variants, I-6-2-1 and II-8, may have a full-length *HeT-A* element attached directly to the *w* transgene. This was confirmed by PCR using primers HeT-5′ (Figure 6A) and white-D, which amplified a 5-kb DNA fragment in these two variants, consistent with the position of primer HeT-5′ 1.5 kb farther upstream on *HeT-A*. This analysis, however, did not allow us to determine the structure distal to the first full-length *HeT-A* element. Variants I-21-2-2-3 and II-5-3-1 amplified 6- and 5.5-kb fragments, respectively, with primers white-D and HeT-ZF1, which is ~2 kb longer than that predicted from the position of the HeT-ZK1 primer. Indeed, a nested PCR reaction with primers HeT-R2 and HeT-L3 performed on the previously amplified fragment resulted in a small fragment, which contained the junction of two *HeT-A* elements as determined by sequencing. Element 2 has an oligo$(A)_9$ tail in I-21-2-2-3 and an oligo$(A)_3$ tail in II-5-3-1. In both cases, element 1 terminated at a position ~300 bp downstream of the stop codon of the ORF, although the junction between elements 1 and 2 is at a slightly different position (22 bp difference). Again, the difference in homozygous eye color phenotype is consistent with the difference in total length of the terminal addition (see Figure 5).

Genetic interactions between homologous telomeres: Brown-red variants of the $P\{w^{var}\}$ transgene express the dark eye color when the homologous telomere carries a variant of $P\{w^{var}\}$ or a terminal deficiency, but not when the homologous telomere is wild type. To investigate this *trans*-interaction in more detail and to determine which sequences at the telomere might be required, we tested the variants described here and other similar variants that were not characterized molecularly, in pairwise combinations. Specifically, we tested the variants for their ability to respond to or to induce a transgene on the homolog as evidenced by eye color changes.

Results of these experiments are summarized in Table 2 (for chromosome structures see Figure 5). When the homolog carried a transgene or a deficiency for the telomere, white variants were indifferent to any changes in the homolog, pale and light-orange variants showed only modest responses to the homolog, and highly variegated and brown-red variants responded by increased mottling or eye color darkening. Interestingly, there are different levels of activation depending on the homolog (see below). Moreover, we could distinguish two classes

TABLE 2

Eye color phenotypes of variants in various heteroallelic combinations

	Allelic combination				
Variant class	Variant/+[a]	Homozygous	Variant/*P{w^{var}}*	Variant/white[b]	Variant/*l(2)gl^{26}*
P{w^{var}}	Orange, few spots	Orange, few spots	Orange, few spots	Dark orange, very many spots	Orange, few spots
White[b]	White	White	Dark orange, very many spots	White	White
Pale	Pale, few spots	Pale yellow, few spots	Mottled brown	Pale, few spots	Pale, few spots
Light orange	Yellow, few spots	Light orange, few spots	Orange, few spots	Light orange, few spots	Light orange, few spots
Highly variegated	Pale, few spots	Orange, very many spots	Orange, many spots	Orange, very many spots	Orange, many spots
Brown-red, class I	Pale, few spots	Brown-red	Brown-red	Brown-red	Light orange, some spots
Brown-red, class II	Pale, few spots	Brown-red	Brown-red	Brown-red	Brown-red

[a] "+" indicates a wild-type telomere without a transgene.

[b] The white class includes II-6-2 and variants described previously (Golubovsky *et al.* 2001). White-1f resembles *l(2)gl^{26}* in *trans*-activating phenotype.

of brown-red variants when they were tested over the terminal deficiency *l(2)gl^{26}*. Class I did not respond well to *l(2)gl^{26}* (*e.g.*, variant II-19-1-1 in Figure 3) or to other *P*-element insertions (such as *P{w^{+}}39C-5*; Wallrath and Elgin 1995) into the *2L* telomere (see Figure 3), while class II responded strongly to all alterations at the *2L* homolog.

The molecular structure of the brown-red variants described here differs from those analyzed previously (Golubovsky *et al.* 2001). The latter have an ~50-kb-long terminal array attached to the *w* transgene upstream of the eye-testis enhancer (junction 1 in Figure 4), leaving the enhancer intact. These brown-red variants had orange eye color over a wild-type homolog and brown-red eye color over any other variant. By contrast, the new brown-red variants of both class I and II had ~30-kb-long terminal *HeT-A* arrays attached very close to the initiation of transcription of the *white* transgene, deleting the eye-testis enhancer (junctions at 4, 5, 7, and 9 in Figure 4). They had pale eye color over either a wild-type homolog or the *SM1* balancer, but brown-red-to-red eye color when the homologous telomere carried any *P{w^{var}}* variant transgene.

As brown-red variants of both classes had similar *HeT-A* array lengths and *HeT-A/w* junctions at similar positions, neither of these can explain the differences in phenotype. The two classes, however, can be distinguished by the restriction maps of the first attached *HeT-A* elements (Figure 5). Class I brown-red variants have a *Nru*I and a *Hin*dIII site 2 kb distal to the junction, while class II variants of both families IV and V have a *Xho*I site 3–4 kb from the junction. The class I variants all arose in family II and all share the same *HeT-A/w* junction. Therefore, they cannot be considered as independent events. Even so, it is possible that the nature or sequence of individual *HeT-A* elements may play a role in the ability of the *HeT-A* array to respond to changes on the homolog.

We tested a variety of variant transgenes to determine what makes a chromosome activate a dark-eye responsive variant (Table 2 and Figure 7). As stated above, a wild-type *2L* telomere had no inducing ability for any variant; it always caused a weaker or the same expression as in the homozygous condition. Light-orange variants did not activate the original *P{w^{var}}* variant, but had some effect on brown-red and highly variegated variants. All pale and most white variants strongly enhanced the expression of the original *P{w^{var}}*, class I and class II brown-red, as well as highly variegated variants. Two different white derivatives of *P{w^{var}}* exhibited different strengths in the ability to activate a homologous transgene (Figure 7). Both white variants retain the same proximal *w*/TAS junction, as confirmed by PCR; the only difference between them is the position of the chromosome end within the *w* transgene (see Figure 5). In II-6-2, ~6–7 kb of *w* sequence remains, but white-1f has only ~1 kb of the transgene left. While variant II-6-2 and other white variants broken in the ORF (Golubovsky *et al.* 2001) strongly induce a responding brown-red variant, the white-1f variant is much weaker and resembles in its inducing ability the terminal deficiency *l(2)gl^{26}*, which induced class II brown-red variants strongly, class I brown-red variants weakly, but the original *P{w^{var}}* very little, if at all (see Figure 3 and Table 2). The terminal deficiency *l(2)gl^{258}* also resembles *l(2)gl^{26}* in its ability to increase expression of a brown-red variant on its homolog, even though it has regained *HeT-A* sequences at its terminus. Both *l(2)gl* terminal deficien-

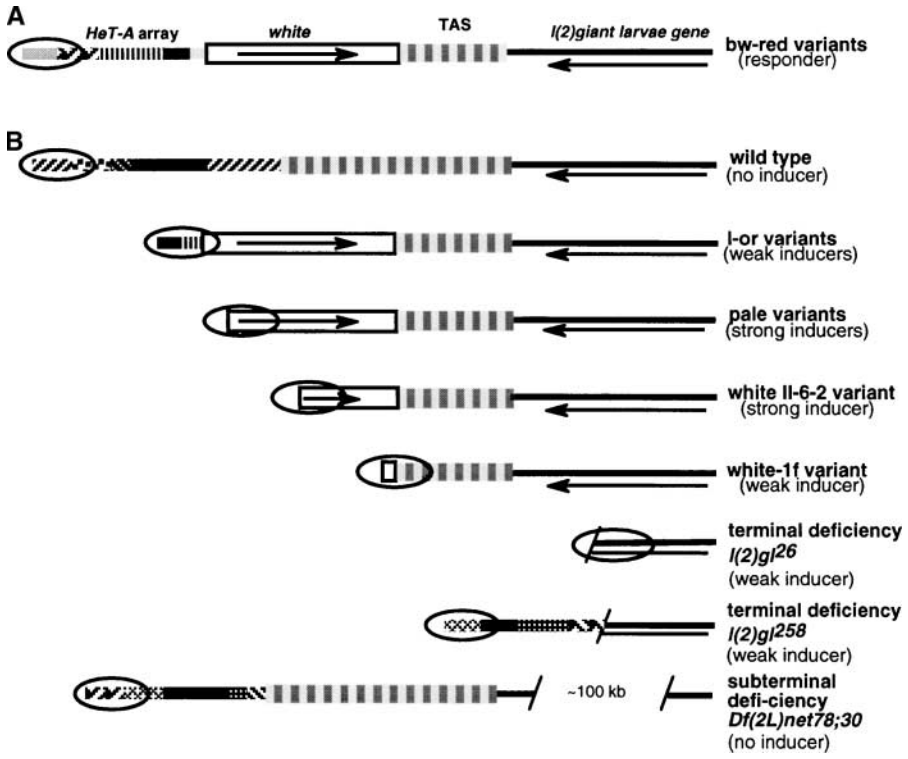

FIGURE 7.—Comparison of the structure of variants with their ability to induce a brown-red variant located at the homologous telomere. (A) The map of the brown-red variant, which responds by increased *w* expression. The proposed end-capping complex is shown as an oval. (B) A schematic representation of the structure at the tip of *2L* is given for each of the variants and deficiencies tested. The ability to induce a darker eye color in the brown-red homolog (A) is given in parentheses for each of the inducer chromosomes tested (B).

cies lack TAS, as determined by *in situ* hybridization to polytene chromosomes (WALTER *et al.* 1995). The interstitial deficiency *Df(2L)net78;30*, which removes the *l(2)gl* gene and ~100 kb to the right (GREEN and SHEPHERD 1979; MECHLER *et al.* 1985), resembles a wild-type chromosome in its effect on a *w* transgene on the homolog.

These observations suggest that the TAS region at the homologous telomere may play a role in modulating the *w* transgene. To determine the length of TAS on various chromosomes with different effects on the homologous transgene, genomic DNA was digested to completion with three different four-cutter restriction enzymes (*Hae*III, *Hha*I, *Hpa*II) that have no site in the *2L* TAS repeat (WALTER *et al.* 1995) and hybridized to a *2L* TAS repeat probe. As these enzymes will cut outside of TAS, this experiment will give a maximal estimated length for TAS. Figure 8 shows that the *2L* TAS region in the *y* w^{67c23} stock is ~15 kb in length and that it is shortened to ~10 kb in *P{w^{var}}* and in eye color variants derived from it. Variants V-4-1 and KR-3-2, a brown-red derivative described in GOLUBOVSKY *et al.* (2001), are shown here. To determine the length of its TAS, the *Df(2L)net78;30* deficiency was analyzed as a heterozygote with KR-3-2. Hybridization with TAS distinguishes the two chromosomes and shows the shortened KR-3-2 TAS and the apparently full-length TAS of *Df(2L)net78;30* in the same lanes. The TAS regions of *Df(2L)net78;30* and the *Cy* balancer are full length and indistinguishable in size. This result suggests that a deficiency for *l(2)gl* itself, or adjacent proximal sequence as in *Df(2L)net78;30*, does not induce brown-red *P{w^{var}}* variants to higher levels of expression and supports the idea that the length of TAS plays a role in interactions between homologs.

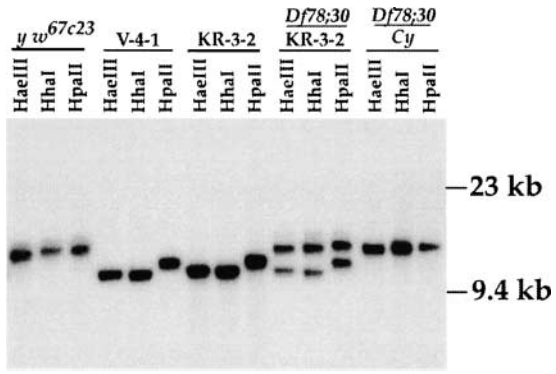

FIGURE 8.—Autoradiograph of a Southern blot containing genomic DNA cut with three different four-cutter restriction enzymes (*Hae*II, *Hha*I, *Hpa*II) that do not cut the TAS region, hybridized to a 6-kb *Eco*RI-*Sac*I fragment consisting entirely of *2L* TAS repeats (21). The size of the hybridizing genomic band represents the maximal length of TAS for each of the chromosomes tested. The size of TAS in y^1 w^{67c23} is ~15 kb, but only ~10 kb in the *P{w^{var}}* derivatives V-4-1 and KR-3-2. In the heterozygote *Df(2L)net78;30*/KR-3-2 both chromosomes are distinguishable, showing that the subterminal deficiency *Df(2L)net78;30* carries a full-length TAS.

Telomere interactions may be even more complex. All of the eye color variants have the same shortened TAS array, but the pale variants, which carry very little *HeT-A* on the chromosome end, strongly affect a trans-

gene on the homolog. On the other hand, orange and light-orange variants with longer *HeT-A* arrays exhibit relatively weak effects on a homologous transgene. Moreover, the *l(2)gl* terminal deficiencies, which lack TAS, have only weak effects. White variants with >4 kb of *w* gene sequence remaining distal to TAS are strong inducers, but the white-1f variant, which resembles other white variants except that it has a very short (1 kb) *w* sequence, is only a weak inducer and phenotypically resembles the *l(2)gl* terminal deficiencies.

DISCUSSION

The stimulating role of *HeT-A* elements in TPE: The present studies reveal a new class of highly variegating eye color variants that allow us to discriminate between two components of TPE: the level of background *w* expression in the eye and the extent of variegation. Highly variegated variants have intermediate-length *HeT-A* arrays, suggesting that these variants represent an intermediate step in eye color darkening between the light-orange variants with little or no *HeT-A* and the brown-red variants with ~50 kb of *HeT-A* and *TART* reported previously (Golubovsky *et al.* 2001). Moreover, the structures associated with different variegation phenotypes suggest either that a long *HeT-A* promoter or two tandem *HeT-A* promoters can counteract the repressive effect of TAS more effectively than a single truncated *HeT-A* promoter or that *HeT-A* promoters distant from the suppressive effects of TAS may be more effective at stimulating *w* transcription. This increased *w* expression in more ommatid clusters results in more mottling, rather than in an increase in the background eye color, suggesting that expression increases as a change in the probability of a stochastic event, as might be expected with the addition of discrete elements, rather than as a result of ratcheting up of a rheostat.

The brown-red variants isolated in previous experiments had long *HeT-A/TART* terminal additions attached to the *w* transgene at position 5184, leaving the eye enhancer intact (Golubovsky *et al.* 2001) and leaving open the possibility that the eye color generated by the subtelomeric transgene or the extent of variegation might be controlled by the enhancer. Variants with breakpoints very close to the transcription start site, even the pale and yellow ones, show variegation, indicating that the *w* enhancer is not required for mottled expression. Further, many new brown-red variants that had *HeT-A/w* junctions very close to the *w* promoter exhibit pale eyes opposite a wild-type telomere. The transition from highly variegated to brown-red eye color coincides with terminal addition, while the switch from brown-red to highly variegated coincides with the shortening of the terminal *HeT-A* array. Thus, the ability to increase *w* expression does not depend on the eye-specific enhancer. These conclusions are supported by the fact that subtelomeric insertions of a mini-*white* gene that lacks the enhancer also variegate (Wallrath and Elgin 1995) and are inducible (M. D. Golubovsky, H. Biessmann and J. M. Mason, unpublished data).

Further, variants must have a full-length, or at least a relatively long, *HeT-A* element to be responsive, suggesting that the sequences that *cis*-activate the subtelomeric w^+ transgene in response to the homologous telomere are not located in the transgene itself, but on *HeT-A*. Since short fragments from the *HeT-A* 3′ UTR, including the promoter, are not sufficient to respond to the homolog, we propose that the responsive element is a discrete region within *HeT-A*, not located in the 3′ end of the UTR, that acts as an enhancer of *HeT-A* transcription. This putative enhancer appears to be sensitive either directly or indirectly to the nature of the homologous telomere.

The basic *w* promoter is contained in a short downstream promoter element (DPE) located around the proposed transcription start sites (Kutach and Kadonaga 2000). Among chromosomes in which *HeT-A* elements are attached very close to this promoter region, the eye color of homozygotes correlates strongly with the nature and length of the attached *HeT-A* elements. While we cannot formally distinguish activation of the reporter gene by the addition of *HeT-A* promoter/enhancer activity from activation of the reporter by removal of the chromosome end from the vicinity of the *w* promoter, we favor the former for the following reasons. First, in studies of a *yellow* gene that was placed at the *X* chromosome terminus by terminal deletion, inactivation of enhancers that are within ~4 kb of the chromosome end was observed (Mikhailovsky *et al.* 1999). Removal of the chromosome end by >4 kb had no further effect, independent of the length or the sequence of the intervening DNA. The changes in *w* gene expression that we see are correlated with much longer stretches of DNA.

Second, very short pieces of *HeT-A* elements, less than the length of the *HeT-A* promoter, are insufficient to increase expression. Apparently, these severely truncated *HeT-A* elements cannot provide significant promoter or enhancer activity for the *w* transgene. While the chromosome end and its presumed capping complex are known to interfere with enhancer function (Mikhailovsky *et al.* 1999), they do not inhibit promoters. The *yellow* TATA-containing promoter is active if >140 bp of DNA is located between the end of the chromosome and the *yellow* transcription start site, and other promoter types, such as the DPE from the *w* gene, the TATA-containing promoter from the *eve* gene, and the TATA + DPE-containing promoter from the *hsp70* gene, are also functional at the end of the deficient chromosome (L. Melnikova, H. Biessmann and P. Georgiev, unpublished results).

Third, the content of the *HeT-A* array appears to be more important than its length. One long *HeT-A* element has a stronger effect on *w* reporter expression than

an array of similar length containing multiple truncated copies of the 3′ UTR, suggesting that specific sequences upstream of the promoter are needed to activate the transgene. Further, the two classes of brown-red variants that react to homologs differently, described here, are distinguished by a restriction map of the *HeT-A* element closest to the reporter gene, rather than by the length of the array itself.

Fourth, all of the eye color changes associated with the addition of *HeT-A* elements to the chromosome end depend on the condition of the homologous telomere (or, more likely, TAS). It is easier to understand changes in expression caused by disruptions or deletions of the telomere on the homolog in terms of (direct or indirect) interactions of a signal with specific sites (enhancers?) upstream of the reporter gene, rather than in terms of the homologous signal measuring the length of the *HeT-A* array over several kilobases.

Effect of the homologous telomere: White variant II-6-2 is a stronger inducer than white-1f, even though their TAS arrays have the same length. The only detectable difference between these two variants is the position of the terminal break within the *w* transgene. In II-6-2, ~6–7 kb of *w* sequence remains, while white-1f has only ~1 kb of the transgene, which brings the end of the chromosome very near TAS. Interference of the physical end of a chromosome with the accessibility of DNA sequences may be due to a chromosome end-capping protein complex, which may be able to inactivate protein-DNA interactions at enhancers up to 4 kb away from the chromosome end (Mikhailovsky *et al.* 1999). The TAS region in white-1f may be compromised in its ability to interact with the TAS on the homolog by its proximity to the chromosome terminus, which may explain why white-1f phenotypically resembles the terminal deficiencies that lack TAS altogether. This putative inactivation of TAS by a capping complex does not occur in variant II-6-2 or in other white variants (Golubovsky *et al.* 2001) in which >4 kb of *w* sequence separates the chromosome end from TAS.

The difference between the strong *trans*-effects of the white and pale variants, on the one hand, and the weak effects of the light-orange variants, on the other hand, is more difficult to understand. The presence of the *w* sequence distal to the shortened TAS array in white and pale variants seems to stimulate activity on the homolog. Light-orange variants, which have a longer *w* segment, exhibit less ability to stimulate the homolog. If the distal *w* sequence allows the two homologous TAS arrays to pair, the short TAS on a *P{w^{var}}* variant chromosome may be insufficient to cause *trans*-inactivation. Longer *w* sequences, possibly including the ZESTE-binding sites upstream of the ORF, may reconstitute a repressive domain. These speculations require further investigation.

The mechanisms responsible for heterochromatic gene silencing and variegated expression are not fully understood. Two primary models, the heterochromatic spreading model and the intranuclear compartmentalization model, have been proposed to explain these phenomena (Henikoff 1995). Either of these might be responsible for the proposed repressive effect of TAS. Interphase nuclei in some species exhibit a reproducible organization (Dernburg *et al.* 1995; Lamond and Earnshaw 1998). In Drosophila, chromosomes in embryonic and salivary gland cells are arranged with telomeres clustered at one side and centromeres at the other side of the nucleus (Foe and Alberts 1985; Hochstrasser *et al.* 1986), and individual chromosome arms occupy specific regions of the nucleus (Mathog *et al.* 1984; Foe and Alberts 1985). The nuclear position of a specific locus on a minichromosome relative to a heterochromatin domain may be determined by the structure of the chromosome itself, including the presence of an intact telomere region, or by the presence of an intact telomere on the homolog (Donaldson and Karpen 1997). Loss of the telomere region from the left end of *Dp(1;f)1187* increased the variegation of a nearby *yellow* gene. This was termed terminal-deficiency-associated position-effect variegation (TDA-PEV). The observation that many of the suppressors of TDA-PEV are also suppressors of PEV suggests that this phenomenon is associated with centric heterochromatin, rather than with telomeric silencing, and that the *trans*-suppression induced by the homolog is due to a change in nuclear position (Donaldson *et al.* 2002). The *trans*-suppression of telomeric silencing that we see may also result from changes in nuclear position that depend on the integrity of the homologous TAS region.

Role of the capping complex: The heterochromatin protein, HP1, product of the *Su(var)205* gene, is located primarily in centric heterochromatin and on the fourth chromosome; it is also found at chromosome ends in Drosophila, independent of the presence of either the *HeT-A/TART* or the TAS arrays (Fanti *et al.* 1998; Siriaco *et al.* 2002). Frequent telomere-telomere attachments during mitosis and meiosis in homozygous *Su(var)205* mutants suggest a role in telomere capping (Fanti *et al.* 1998). As HP1 has no direct DNA-binding ability itself, we may postulate that an unidentified telomere-binding protein recruits HP1 to the chromosome end. One candidate for this recruitment may be HOAP (Shareef *et al.* 2001), as in *caravaggio* mutants that lack full-length HOAP, HP1 levels are reduced at diploid chromosome ends (Cenci *et al.* 2003), and HP1 appears absent from polytene chromosome tips (M. Gatti, personal communication). In heterozygous HP1 mutants the transposition frequency of *HeT-A* and *TART* elements to broken chromosome ends is increased 100-fold (Savitsky *et al.* 2002), but it is unclear whether the increased transposition rate is due to a failure in capping efficacy, making the chromosome end more accessible to retrotransposons, or to a derepression of *HeT-A* promoters. Nor is it clear whether the increase in *HeT-A* transcript level observed in these mutants is a

cause or a result of the long *HeT-A* array. However, while *Su(var)205* mutations are suppressors of centromeric position-effect variegation (Eissenberg *et al.* 1990), they do not affect telomeric silencing (Wallrath and Elgin 1995), suggesting that HP1 is not a component of a potential TAS-binding protein complex responsible for silencing at telomeres (Kurenova *et al.* 1998; Mason *et al.* 2000, 2003), and are not responsible for *HeT-A* transcription. Similarly, a mutation in the gene *caravaggio*, encoding HOAP, has no effect on TPE using a 2L mini-*white* transgene (M. Gatti, personal communication). It is possible, therefore, to hypothesize two independent telomere-binding complexes in Drosophila: a capping complex including the HP1 and HOAP proteins and a heterochromatic complex initiated at TAS. Whether or not these two complexes interact with each other needs to be investigated.

Conclusion: Our model of TPE is based on competition between activation by the modulation of the *HeT-A* promoters and repression by the subtelomeric TAS. In this aspect it is similar to other PEV systems, for which a balance may exist between negative factors that promote the formation of condensed heterochromatin and positively acting enhancers and promoters that antagonize heterochromatic silencing (Aparicio and Gottschling 1994; Walters *et al.* 1996; Zhuma *et al.* 1999; Ahmad and Henikoff 2001; Martin 2001; Dillon and Festenstein 2002). Our model emphasizes the interactions between *HeT-A* and TAS *in cis* as well as *in trans*. These interactions are detectable by the presence of a subtelomeric reporter gene that responds to the activating and repressing forces that occur normally in Drosophila telomeres. It is likely that in the absence of a reporter the same mechanisms are at work. Thus, the reporter acts as a surrogate for *HeT-A* activity, and the model describes a mechanism for the genetic regulation of *HeT-A* transcription and, probably as a result, *HeT-A* transposition and telomere elongation. The present data support the model in two respects. First, the *white* gene enhancer and ZESTE-binding sites that overlap the enhancer are not necessary for variegation of the telomeric transgene, since they can be deleted with no effect on variegation. Second, the data suggest the presence of an element that responds to the homologous TAS array, possibly indirectly, and that maps to *HeT-A* elements *in cis* to the transgene. This responsive element appears to be separable from and 5′ to the *HeT-A* promoter. A key point of the model is the link between the quality and quantity of subtelomeric repeats and the telomere elongation mechanism. A similar influence of subtelomeric repeats on telomere elongation by telomerase has been documented in *Saccharomyces cerevisiae* (Craven and Petes 1999).

We thank Leila Bozorgnia, Aneeta Maheshwari, and Hoang Nguyen for help with the molecular analyses and Dr. Marika Walter for many discussions. Part of the work was supported by the U.S. Public Health Service grant GM-56729 to H.B.

LITERATURE CITED

Ahmad, K., and S. Henikoff, 2001 Modulation of a transcription factor counteracts heterochromatic gene silencing in Drosophila. Cell **104:** 839–847.

Aparicio, O. M., and D. E. Gottschling, 1994 Overcoming telomeric silencing: a trans-activator competes to establish gene expression in a cell cycle-dependent way. Gene Dev. **8:** 1133–1146.

Biessmann, H., B. Kasravi, T. Bui, G. Fujiwara, L. E. Champion *et al.*, 1994 Comparison of two active HeT-A retroposons of *Drosophila melanogaster*. Chromosoma **103:** 90–98.

Cenci, G., G. Siriaco, G. D. Raffa, R. Kellum and M. Gatti, 2003 The Drosophila HOAP protein is required for telomere capping. Nat. Cell Biol. **5:** 82–84.

Chaboissier, M.-C., I. Busseau, J. Prosser, D. J. Finnegan and A. Bucheton, 1990 Identification of a potential intermediate for transposition of the LINE-like I factor in *Drosophila melanogaster*. EMBO J. **9:** 3557–3563.

Craven, R. J., and T. D. Petes, 1999 Dependence of the regulation of telomere length on the type of subtelomeric repeat in the yeast *Saccharomyces cerevisiae*. Genetics **152:** 1531–1541.

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.

Danilevskaya, O. N., D. A. Petrov, M. N. Pavlova, A. Koga, E. V. Kurenova *et al.*, 1992 A repetitive DNA element, associated with telomeric sequences in *Drosophila melanogaster*, contains open reading frames. Chromosoma **102:** 32–40.

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.

Dernburg, A. F., J. W. Sedat, W. Z. Cande and H. W. Bass, 1995 Cytology of telomeres, pp. 295–338 in *Telomeres*, edited by E. H. Blackburn and C. W. Greider. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Dillon, N., and R. Festenstein, 2002 Unravelling heterochromatin: competition between positive and negative factors regulates accessibility. Trends Genet. **18:** 252–258.

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.

Donaldson, K. M., A. Lui and G. H. Karpen, 2002 Modifiers of terminal deficiency-associated position effect variegation in Drosophila. Genetics **160:** 995–1009.

Eissenberg, J. C., T. C. James, D. M. Foster-Hartnett, T. Hartnett, V. Ngan *et al.*, 1990 Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **87:** 9923–9927.

Fanti, L., G. Giovinazzo, M. Berloco and S. Pimpinelli, 1998 The heterochromatin protein 1 prevents telomere fusions in Drosophila. Mol. Cell **2:** 527–538.

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.

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.

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.

Green, M. M., and S. H. Shepherd, 1979 Genetic instability in *Drosophila melanogaster*. the induction of specific chromosome 2 deletions by MR elements. Genetics **92:** 823–832.

Hazelrigg, T., R. W. Levis and G. M. Rubin, 1984 Transformation of *white* locus DNA in *Drosophila*: dosage compensation, *zeste* interaction, and position effects. Cell **36:** 469–481.

Henikoff, S., 1995 Gene silencing in Drosophila. Curr. Top. Microbiol. Immunol. **197:** 193–208.

Henikoff, S., 1997 Nuclear organization and gene expression: homologous pairing and long-range interactions. Curr. Opin. Cell Biol. **9:** 388–395.

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.

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.

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.

Kassis, J. A., E. P. VanSickle and S. M. Sensabaugh, 1991 A fragment of engrailed regulatory DNA can mediate transvection of the *white* gene in Drosophila. Genetics **128:** 751–761.

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.

Kutach, A. K., and J. T. Kadonaga, 2000 The downstream promoter element DPE appears to be as widely used as the TATA box in *Drosophila* core promoters. Mol. Cell. Biol. **20:** 4754–4764.

Lamond, A. I., and W. C. Earnshaw, 1998 Structure and function in the nucleus. Science **280:** 547–553.

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.

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.

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.

Martin, D. I. K., 2001 Transcriptional enhancers—on/off gene regulation as an adaptation to silencing in higher eukaryotic nuclei. Trends Genet. **17:** 444–448.

Mason, J. M., and H. Biessmann, 1995 The unusual telomeres of Drosophila. Trends Genet. **11:** 58–62.

Mason, J. M., A. Haoudi, A. Y. Konev, E. Kurenova, M. F. Walter *et al.*, 2000 Control of telomere elongation and telomeric silencing in *Drosophila melanogaster*. Genetica **109:** 61–70.

Mason, J. M., A. Y. Konev and H. Biessmann, 2003 Telomeric position effect in *Drosophila melanogaster* reflects a telomere length control mechanism. Genetica (in press).

Mathog, D., M. Hochstrasser, Y. Gruenbaum, H. Saumweber and J. Sedat, 1984 Characteristic folding pattern of polytene chromosomes in *Drosophila* salivary gland nuclei. Nature **308:** 414–421.

McLean, C., A. Bucheton and D. J. Finnegan, 1993 The 5′ untranslated region of the *I* factor, a long interspersed nuclear element-like retrotransposon of *Drosophila melanogaster*, contains an internal promoter and sequences that regulate expression. Mol. Cell. Biol. **13:** 1042–1050.

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.

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

O'Hare, K., C. Murphy, R. Levis and G. M. Rubin, 1984 DNA sequence of the *white* locus of *Drosophila melanogaster*. J. Mol. Biol. **180:** 437–455.

Pasyukova, E., S. Nuzhdin, W. Li and A. J. Flavell, 1997 Germ line transposition of the *copia* retrotransposon in *Drosophila melanogaster* is restricted to males by tissue-specific control of *copia* RNA levels. Mol. Gen. Genet. **255:** 115–124.

Pirrotta, V., H. Steller and M. P. Bozzetti, 1985 Multiple upstream regulatory elements control the expression of the *Drosophila white* gene. EMBO J. **4:** 3501–3508.

Qian, S., B. Varjavand and V. Pirrotta, 1992 Molecular analysis of the *zeste-white* interaction reveals a promoter-proximal element essential for distant enhancer-promoter communication. Genetics **131:** 79–90.

Roseman, R. R., E. A. Johnson, C. K. Rodesch, M. Bjerke, R. N. Nagoshi *et al.*, 1995 A *P* element containing suppressor of hairy-wing binding regions has novel properties for mutagenesis in *Drosophila melanogaster*. Genetics **141:** 1061–1074.

Rubin, G. M., 1978 Isolation of a telomeric DNA sequence from *Drosophila melanogaster*. Cold Spring Harbor Symp. Quant. Biol. **42:** 1041–1046.

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.

Shareef, M. M., C. King, M. Damaj, R. Badagu, D. W. Huang *et al.*, 2001 Drosophila heterochromatin protein 1 (HPI)/origin recognition complex (ORC) protein is associated with HP1 and ORC and functions in heterochromatin-induced silencing. Mol. Biol. Cell **12:** 1671–1685.

Siriaco, G. M., G. Cenci, A. Haoudi, L. E. Champion, C. Zhou *et al.*, 2002 *Telomere elongation* (*Tel*), a new mutation in *Drosophila melanogaster* that produces long telomeres. Genetics **160:** 235–245.

Tower, J., G. H. Karpen, N. Craig and A. C. Spradling, 1993 Preferential transposition of Drosophila *P* elements to nearby chromosomal sites. Genetics **133:** 347–359.

Wallrath, L. L., and S. C. R. Elgin, 1995 Position effect variegation in Drosophila is associated with an altered chromatin structure. Gene Dev. **9:** 1263–1277.

Walter, M. F., C. Jang, B. Kasravi, J. Donath, B. M. Mechler *et al.*, 1995 DNA organization and polymorphism of a wild-type Drosophila telomere region. Chromosoma **104:** 229–241.

Walters, M. C., W. Magis, S. Fiering, T. Eidemiller, D. Scalzo *et al.*, 1996 Transcriptional enhancers act in cis to suppress position-effect variegation. Gene Dev. **10:** 185–195.

Young, B. S., A. Pession, K. L. Traverse, C. French and M. L. Pardue, 1983 Telomere regions in *Drosophila* share complex DNA sequences with pericentric heterochromatin. Cell **34:** 85–94.

Zhuma, T., R. Tyrrell, B. Sekkali, G. Skavdis, A. Saveliev *et al.*, 1999 Human HMG box transcription factor HBP1: a role in hCD2 LCR function. EMBO J. **18:** 6396–6406.

Communicating editor: K. Golic