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Control of telomere elongation and telomeric silencing in *Drosophila melanogaster*

James M. Mason[1], Abdelali Haoudi[1], Alexander Y. Konev[1], Elena Kurenova[2], Marika F. Walter[3] & Harald Biessmann[3]

[1]*Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709-2233, USA (Phone: (919) 541-4483, Fax: (919) 541-7593; E-mail: masonj@niehs.nih.gov)* [2]*Thurston Arthritis Research Center, University of North Carolina, Chapel Hill, NC 27599, USA;* [3]*Developmental Biology Center, University of California, Irvine, CA 92697, USA*

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Abstract

Chromosome length in *Drosophila* is maintained by the targeted transposition of two families of non-LTR retrotransposons, *HeT-A* and *TART*. Although the rate of transposition to telomeres is sufficient to counterbalance loss from the chromosome ends due to incomplete DNA replication, transposition as a mechanism for elongating chromosome ends raises the possibility of damaged or deleted telomeres, because of its stochastic nature. Recent evidence suggests that *HeT-A* transposition is controlled at the levels of transcription and reverse transcription. *HeT-A* transcription is found primarily in mitotically active cells, and transcription of a w^+ reporter gene inserted into the 2L telomere increases when the homologous telomere is partially or completely deleted. The terminal *HeT-A* array may be important as a positive regulator of this activity in *cis*, and the subterminal satellite appears to be an important negative regulator in *cis*. A third chromosome modifier has been identified that increases the level of reverse transcriptase activity on a HeT-A RNA template and greatly increases the transposition of *HeT-A*. Thus, the host appears to play a role in transposition of these elements. Taken together, these results suggest that control of *HeT-A* transposition is more complex than previously thought.

Introduction

Unlike telomeres in most other organisms, which terminate in a long array of simple repeats that are maintained by telomerase, chromosome ends in *Drosophila* contain long terminal arrays of non-LTR retrotransposons of the *HeT-A* and *TART* families (Figure 1). As these transposon arrays are maintained by targeted transposition of these elements (Mason & Biessmann, 1993; Mason & Biessmann, 1995), *Drosophila* provides a unique opportunity to investigate telomere structure-function relationships. *Drosophila* telomeres are found to contain three distinct DNA components (Karpen & Spradling, 1992; Levis et al., 1993; Walter et al., 1995): a terminal array of retroposons; several kilobases of a complex subterminal satellite, termed Telomere Associated Sequence (TAS); and a single copy region between TAS and the distal-most single copy gene (Figure 2). TAS from different chromosomes exhibit sequence similarities (H. B., unpublished) and some limited cross-reactivity by *in situ* hybridization (Karpen & Spradling, 1992).

Telomere specific proteins have not yet been identified in *Drosophila*. HP1, for example, binds to telomere regions, but also to centromeric heterochromatin and many euchromatic sites (Fanti et al., 1998). Similarly, the deficiency, $Su(z)2^5$, affects the expression of reporter genes in telomeric regions, but both of the genes uncovered by this deficiency are known to bind to many sites in euchromatin (Rastelli, Chan & Pirrotta, 1993). Other mutations have been isolated in *Drosophila* that affect the expression of telomeric reporter genes (Konev & Mason, unpublished) and may identify genes encoding telomeric proteins.

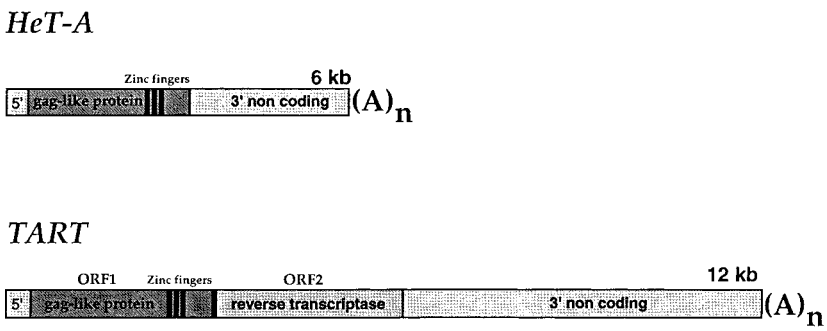

Figure 1. *HeT-A* and *TART*. Two non-LTR retrotransposons are proposed to target chromosome ends. Open reading frames with presumed protein products, and 5′ and 3′ non-coding regions are indicated. '(A)n' indicates the (dA/dT)n region joining each element to the more proximal region of the chromosome.

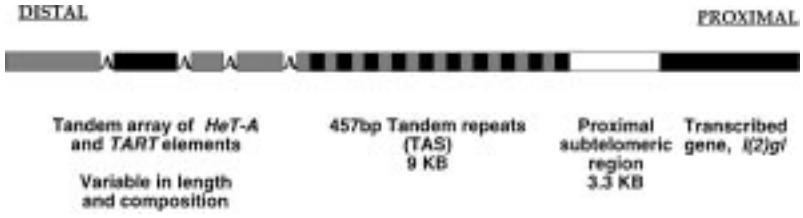

Figure 2. Telomere structures at the left end of chromosome 2. At the extreme end of the chromosome, individual *HeT-A* and *TART* elements of variable lengths are shown. 'A' indicates the (dA/dT)n array at the 3′ end of each transposon by which it is attached to the next proximal component of the chromosome. The subterminal satellite, or telomere associated sequence (TAS) is indicated by small, alternating light and dark gray boxes. The white box indicates the subterminal region between TAS and the nearest transcribed gene, *l*(2)*gl*. This subterminal region has no open reading frame, and its function is not known.

These, however, have not been characterized in detail.

We suggest the possibility that the two major DNA structural components of *Drosophila* telomeres, *HeT-A* elements and TAS interact to control chromosome length. Transcriptional activity of *HeT-A* elements in the terminal array may be necessary for telomere elongation. Recent evidence on the nature of variegating P element insertions into *Drosophila* telomeres, however, suggests that the adjacent TAS arrays may cause transcriptional silencing (Karpen & Spradling, 1992; Roseman et al., 1995; Kurenova et al., 1998; Cryderman et al., 1999). We summarize here recent data on the molecular structure of telomeric reporter genes and their potential use as surrogates for *HeT-A* transcription.

HeT-A transposition and its regulation

Drosophila chromosome ends lose 50–100 base pairs per sexual generation (Biessmann & Mason, 1988; Levis, 1989; Biessmann, Carter & Mason, 1990). This loss is balanced by the addition of the telomere-specific retrotransposons, *HeT-A* and *TART* (Figure 1). There are about 30–50 *HeT-A* elements and 5–10 *TART* elements in the genome. *HeT-A* elements are approximately 6 kb in length, and the single 2.8 kb open reading frame (ORF) encodes a retroviral-related gag-like protein with three zinc finger nucleic acid binding motifs (Biessmann et al., 1992). This protein may be tightly bound to the RNA intermediate (Danilevskaya et al., 1994b), and target the RNA to chromosome ends. An ORF encoding a reverse transcriptase (RT) is absent. The 12 kb *TART* element belongs in a subclass of non-Long Terminal Repeat (LTR) retrotransposons with unequal terminal repeats (Danilevskaya et al., 1999), and carrying two ORFs, that encode a gag-like protein and a reverse transcriptase (Levis et al., 1993; Sheen & Levis, 1994). Natural chromosome ends in *Drosophila* consist of unequal tandem arrays of *HeT-A* and *TART* elements with the oligo(A) tails of the elements facing towards the centromere (Figure 2). These retroelements are thought to transpose between chromosome ends (Mason & Biessmann, 1995), but very little is known about the regulation of telomere elongation in *Drosophila*. The diagram in Figure 3 shows the proposed 'transposition cycle' of a *HeT-A* element. A transcriptional promoter exists in the 3′ end of *HeT-A* (Danilevskaya et al., 1997). Thus, telo-

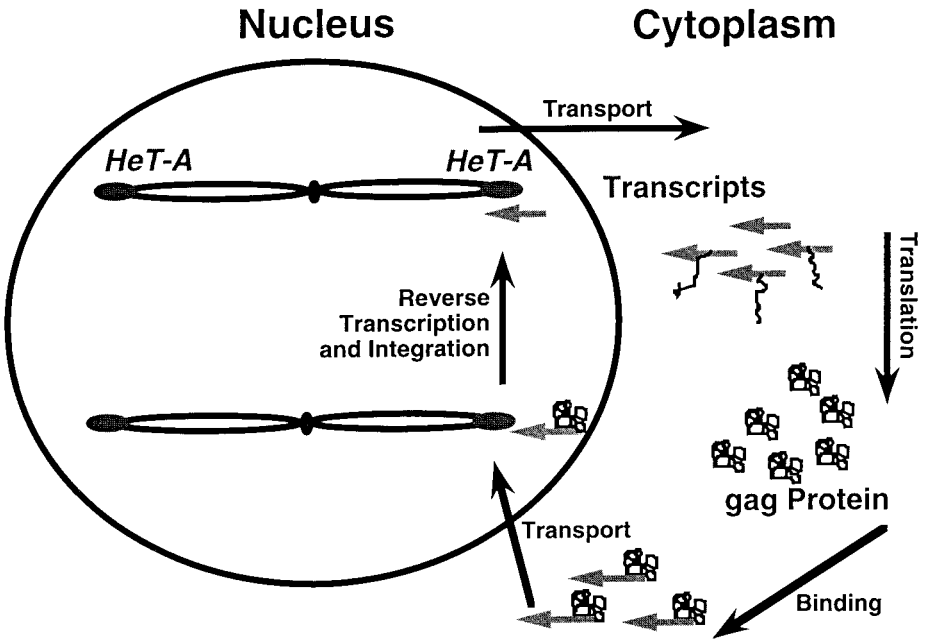

Figure 3. Proposed *HeT-A* transposition cycle. As indicated by gray ovals, retrotransposable *HeT-A* elements reside at the ends of chromosome arms. To initiate the transposition cycle, *HeT-A* is transcribed, and the RNA transcripts, indicated as short gray arrows, are transported to the cytoplasm. Translation produces a gag-like protein, indicated as dark squiggles, which bind to the transcripts. The resulting ribonucleoprotein particles are then transported back into the nucleus, where reverse transcription and integration occur simultaneously.

meric elements are likely transcribed by read-through from the promoter of the next distal element. The transcripts leave the nucleus to serve as mRNA for the translation of the *HeT-A* element-encoded gag-polypeptide, and in the case of *TART*, also for the translation of the reverse transcriptase. The function of the gag-like protein is not clear, but by analogy with the retroviral protein, it likely forms a ribonucleoprotein complex with the RNA transposition intermediate to direct it to the chromosome ends. As with other non-LTR retrotransposons, integration probably occurs by reverse transcription of the element primed by the chromosome end (Hutchinson III et al., 1989).

Since *HeT-A* lacks the ORF for reverse transcriptase, its movement must rely on an RT from another source, either another retroelement, or the host itself. If a host-specific RT gene exists, it has yet to be found. *HeT-A* transposition to a broken X chromosome end has been measured at roughly 1% by screening for terminal fragment length polymorphisms over several generations (Biessmann et al., 1992). This rate, if it remains constant, is sufficient to counterbalance the erosion at chromosome termini. A number of recent observations, however, suggest that the situation is more complex. A. Haoudi, G.M. Siriaco, G. Cenci, M. Gatti, L. Champion and J.M. Mason (in preparation) identified a dominant genetic factors from a wild type line of *Drosophila* that increases RT activity and induces the *HeT-A* array at chromosome ends to grow to enormous size. The mechanism by which this factors exerts its effect is not known. It may encode an RT protein, a regulator of an RT, or a factor that controls the access of *HeT-A* RNA to an RT enzyme. A second genetic factor has been found that greatly reduces or eliminates *HeT-A* transposition (Golubovsky, Konev, Biessmann & Mason, in preparation). This factor has not yet been characterized in any detail. In addition, transcriptional activity of *HeT-A* elements may be regulated by the structure of the homologous telomere as will be discussed in more detail below.

Another way to investigate the potential regulation of *Drosophila* telomere elongation by retroelements is to determine the tissue-specific expression of *HeT-A* elements by whole-mount *in situ* hybridization in a variety of adult and larval tissues during normal development and after growth stimulation (Walter & Biessmann, in preparation). Although *HeT-A* RNA expression is only the first step leading to telomere elongation, there is precedence from other retrotransposons that the level of the RNA transcript may serve as a reasonable indicator of transpositional activity

(Chaboissier et al., 1990; McLean, Bucheton & Finnegan, 1993; Pasyukova et al., 1997). High levels of *HeT-A* RNA transcript are present in the female and male germ lines throughout all stages of oogenesis and spermiogenesis. Although transposition in somatic cells may occur, only transposition in the germ line will assure that the newly extended telomere is passed on to the next generation. The high levels of *HeT-A* RNA transcript in ovaries and testes suggest that telomere elongation by *HeT-A* can occur in the germ line. In larval tissues, a good correlation of strong *HeT-A* expression with the pattern of cell proliferation was detected in the larval brain and in the cycling cells at the morphogenetic furrow in the eye-antennal disc. No *HeT-A* RNA, however, was detected in the terminally differentiated polytene cells of the larval salivary glands. After selectively increasing proliferation in specific larval tissues by the *Drosophila* tumor suppresser mutants, *lethal*(1)*discs large* (Woods & Bryant, 1989) and *warts* (Justice et al., 1995), strongly increased *HeT-A* expression in the overgrown larval tissues such as the optic lobes of the brain and the imaginal discs was detected. These preliminary data suggest that transcriptional activity of *HeT-A* in *Drosophila* is correlated with cell proliferation.

Telomeric silencing

Evidence for telomeric silencing

Silencing and variegated expression of reporter genes occurs when *P* elements insert into either of the two regions of *Drosophila* chromosomes, the centromeric regions causing position effect variegation (PEV), and the telomeric regions causing telomeric position effect (TPE). Chromatin in both of these regions is considered to exist in a heterochromatic configuration, which is believed to be responsible for the observed silencing effects. In order to understand TPE, two components need to be considered: trans-acting repressors of TPE, and *cis*-acting DNA sequences, that might be binding sites for such *trans*-acting repressors. To date, little is known about the proteins that function in *Drosophila* TPE, although they appear to be different from those that play a role in PEV.

PEV and TPE appear to be qualitatively different. In general, pericentric insertions are much more repressed than telomeric insertions (Wallrath & Elgin, 1995). More importantly, none of the about 25 genes tested so far that influence *in trans* the severity of centromeric variegation, termed suppressers and enhancers of variegation (Reuter & Spierer, 1992), has been found to have an effect on TPE (Talbert, Leciel & Henikoff, 1994; Roseman et al., 1995; Wallrath & Elgin, 1995). Only the small deficiency, $Su(z)2^5$, which deletes at least three genes, appears to affect TPE (Cryderman et al., 1999). Two of the three genes known to be disrupted by this deficiency encode proteins that are members of the Polycomb group of proteins responsible for long-term developmental silencing in euchromatin. In addition, an extra Y chromosome, which suppresses PEV, has no effect on TPE. These differences suggest the contributions of different *cis*-and *trans*-acting components.

Since *Drosophila* telomeres do not possess arrays of telomerase-generated telomeric repeats, which could bind proteins like the *S. cerevisiae* silencing protein Rap1p (Longtine et al., 1989) or its human homologue, TRF1p (Chong et al., 1995), it is unclear how and where the heterochromatic structure is generated at the *Drosophila* telomere. However, all telomeric w^+ transgenes analyzed to date that are repressed and variegate are embedded in or adjacent to TAS (Karpen & Spradling, 1992; Levis et al., 1993; Wallrath & Elgin, 1995; Cryderman et al., 1999; Golubovsky, Konev, Biessmann & Mason, in preparation). This suggests a direct involvement of TAS in telomeric silencing. Their structures are summarized in Figure 4. To our knowledge, no insertion has yet been obtained into the terminal *HeT-A*/*TART* array, even though its target size is bigger than that of most TASs, nor into the single copy region that lies proximal to TAS at 2L (Walter et al., 1995) and at other telomeres (Levis et al., 1993). It is possible that insertions into these regions are either completely silenced or they are not silenced at all; both cases would prevent detection of the transgene in a screen for variegating inserts. Alternatively, the *HeT-A*/*TART* array may be packaged in such a way that *P* element insertion frequency is greatly reduced in this region. There is no evidence, however, that *HeT-A* elements are packaged as heterochromatin or cause heterochromatic spreading. In fact, *HeT-A* transpositions onto a terminally deficient *X* chromosome broken within the $5'$ upstream region of the *yellow* gene do not cause silencing or variegation of this gene (Biessmann et al., 1990).

Moreover, the TAS from the 2L telomere may be able to nucleate higher order chromatin structure (Kurenova et al., 1998). In *P*-element constructs,

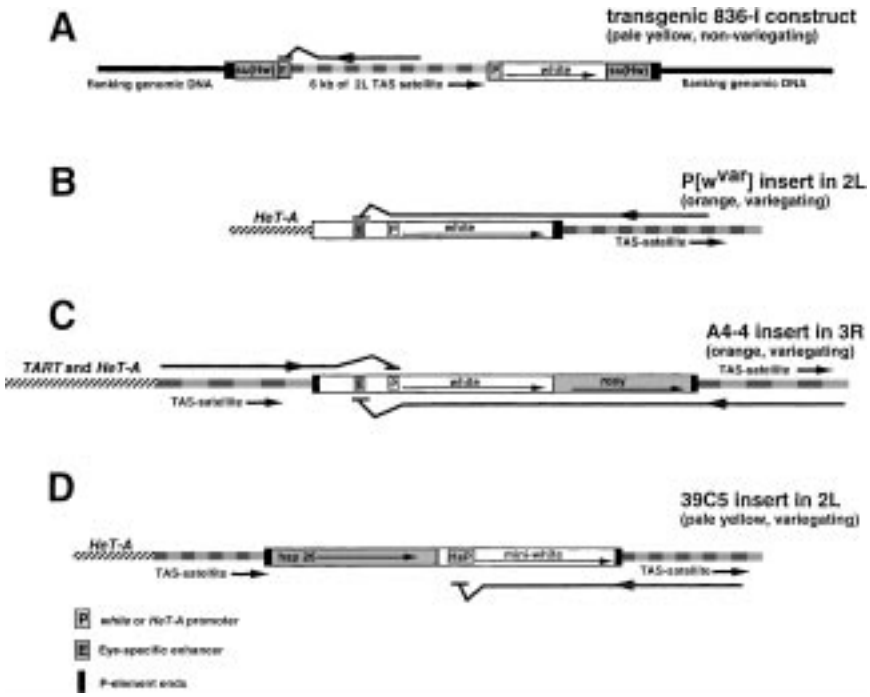

Figure 4. A w^+ transgene with telomere associated sequences and three w^+ telomeric inserts. TAS sequences are indicated by alternating light and dark boxes. The black arrow points toward the proximal end of TAS, as it occurs in its natural, chromosomal position. The direction of transcription is indicated by the long arrows. Presumed silencing effects of TAS are indicated by the gray lines with an arrowhead in the middle. A. The 836 I construct (Kurenova et al., 1998) contains a mini-*white* reporter gene and 2L TAS sequences inserted between the *white* gene promoter and the eye-specific enhancer. This transgene is bordered by SU(HW) binding sites to reduced position effects and inserted into the genome by P-mediated transformation. Hemizygous transformants that retained the full, 6 kb TAS fragment exhibited yellow or lighter eye color when TAS was inserted in the orientation shown, but dark orange eye color when TAS was in the opposite orientation. Homozygotes were slightly darker. B. The $P\{w^{var}\}$ element contains a 12 kb genomic *white* fragment inserted between a short *HeT-A* array comprised of two truncated *HeT-A* elements distally, and TAS proximally. The 2L TAS is about 3 kb shorter than in Oregon R (Walter et al., 1995), and the distal *P*-element end and 600 bps of *white* sequence have been lost. Homozygotes and hemizygotes have orange eyes with small red spots. C. The $P\{ry^+w^+\}A4-4$ element (Levis, Hazelrigg & Rubin, 1985) contains a 12 kb genomic *white* fragment inserted into the TAS array on 3R. The *white* gene fragment in this element was similar to that used in $P\{w^{var}\}$ before the latter was truncated, and the eye color phenotype is similar. D. $P\{w^+\}39C-5$ (Wallrath & Elgin, 1995) contains a mini-*white* gene driven by an hsp70 promoter inserted into the TAS array at 2L. Hemizygotes have very pale eyes with a few spots, but homozygotes have much darker eyes.

TAS repeats were positioned between the eye-specific enhancer and the promoter of the *w* reporter gene (Figure 4A). Silencing was array-length dependent, occurred only in one orientation, and was suppressed by mutations in $Su(z)2$, but not by mutations in suppressers of variegation. The directionality of silencing caused by 2L TAS may be based on cooperative binding of proteins to the satellite repeats, possibly binding directly to DNA or interacting with each other to modify the structure of the satellite chromatin. In these experiments, we could not distinguish between a 'centromere-directed' repression of the *w* promoter and a 'telomere-directed' repression of the eye-enhancer, or the formation of a boundary that prevented the enhancer from interacting with the promoter. The suppression by *Su(z)2* suggests that TAS acts as a silencer, and the position of the variegating $P\{w^{var}\}$ insert between the *HeT-A* array and TAS in the 2L tip (Figure 4B) suggests that TAS silences in the distal direction. Silencing by the 2L TAS, however, is different from silencing that occurs by closely linked copies of mini-*white* genes. Such arrays may produce folded structures by somatic pairing (Dorer & Henikoff, 1994), which are recognized and perhaps stabilized by the heterochromatin-specific protein HP1 (Fanti et al., 1998). However, a tandemly repeated DNA organization array alone cannot be the cause for silencing by the 2L subtelomeric satellite in these constructs, because the satellite in the opposite orientation, or the insertion of an *Anopheles gambiae* subtelomeric satellite array did not repress the *white* reporter gene (Kurenova et al., 1998). Moreover, in

contrast to tandemly arranged mini-*white* genes, silencing by 2L TAS does not respond to mutations in the gene that encodes HP1.

Models of telomeric silencing

To explain the variegation of telomeric insertions, we need to consider the following observations. First, TPE does not appear to depend on the orientation of the transgene in relation to the chromosome end (Karpen & Spradling, 1992). Second, the variegating spots in the eye caused by TPE are always of darker color than the background of the eye, for example, red spots in an orange eye, suggesting that the transgene is less repressed in these spots than in the rest of the eye. Third, the chromosome end itself does not appear to cause silencing because terminal deficiencies with broken chromosome ends immediately upstream of the yellow gene do not show variegated *yellow* expression (Biessmann & Mason, 1988; Biessmann et al., 1992). Similarly, the vicinity of the chromosome end *per se* in terminal deletions of 3R does not result in variegated expression of the integrated *white* reporter gene (Levis, 1989; Sheen & Levis, 1994). Fourth, a 6 kb fragment of 2L TAS represses *w* gene activity in nontelomeric positions but does not cause variegation, indicating that silencing is separable from variegation (Kurenova et al., 1998). By analogy with proposed models for PEV, we can suggest models to explain TPE.

The spreading model

In many chromosomal rearrangements, inactivation of euchromatic genes by heterochromatin occurs over long distances of up to 1 MB or more, with a gradient of gene inactivation inversely related to distance. There is a coincident alteration of chromosome morphology as the affected euchromatic region changes to a more heterochromatic state (Henikoff, 1981; Hayashi et al., 1990; Belyaeva et al., 1993). The visible compaction of juxtaposed euchromatic bands in polytene chromosomes has been viewed as evidence for the spreading of chromatin condensation during the formation of heterochromatin (Tartof, Hobbs & Jones, 1984). According to this model, large multimeric complexes of heterochromatic proteins assemble onto the adjoining euchromatin, thus forming a compact structure that is inaccessible to the transcriptional machinery. It has been proposed that chromatin condensation initiates at specific sequence elements and spreads along the chromosome to a termination site by cooperative attachment of limited amounts of proteins into multimeric complexes (Locke, Kotarski & Tartof, 1988; Tartof & Bremer, 1990). Applied to TPE, this model emphasizes the hypothetical heterochromatic configuration of TAS and its spreading influence on the enhancer/promoter of the adjacent transgene (Figure 4). Variegation may then be caused by a loss of this silencing in some cells, giving rise to darker pigmented clones in the eye. The silencing effect of TAS would weaken over distance in a binomial fashion, and more intense variegation would occur when the enhancer/promoter region is separated by other sequences from TAS. Consequently, the transgenic constructs with 2L TAS next to the reporter gene (Kurenova et al., 1998) would not exhibit variegation, because the TAS is directly adjacent to the enhancer and promoter, thus causing strong and non-reversible repression.

The nuclear compartmentalization model

There is cytological evidence that *Drosophila* telomeres occupy a special region in the nucleus and can transiently associate with the nuclear envelope (Dernburg et al., 1995) and with other telomeres, at least in polytene nuclei (Hinton & Atwood, 1941; Hinton, 1945; Kaufmann & Gay, 1969). Furthermore, telomeres may form tight interactions at interphase that must be disrupted by protein ubiquitination and degradation before mitosis (Cenci et al., 1997). This situation is reminiscent of yeast, where telomeres interact with each other and are clustered in foci near the nuclear periphery (Klein et al., 1992; Gilson, Laroche & Gasser, 1993). Mutations in SIR genes that are required for TPE in yeast disrupt telomere clustering (Palladino et al., 1993; Cockell et al., 1995; Gotta et al., 1996; Maillet et al., 1996), suggesting that a telomeric nucleoprotein complex is required for this clustering. The yeast complex must also be disrupted by ubiquitination and destruction of some of its components (Huang et al., 1997). Similarly, the human telomeric DNA binding protein, TRF1 (Chong et al., 1995), is involved in nuclear matrix association of the telomeric complex (Luderus et al., 1996).

The nuclear compartmentalization model proposes that silencing occurs by the sequestration of genes to certain chromosomal locations (Talbert, Leciel & Henikoff, 1994; Henikoff, Jackson & Talbert, 1995). One heterochromatic region is occupied by the chromocenter, the clustered arrangement of centromeres. Another may at times be occupied by

the telomeres. Thus, nuclear domains exist that are distinct from the euchromatic domains. Such physical separation of centromeric and telomeric domains is achieved in the Rabl configuration of chromosomes (Rabl, 1885), which is observed in some somatic nuclei, including the embryonic (Foe & Alberts, 1985; Hiraoka, Agard & Sedat, 1990) and salivary gland nuclei of *Drosophila* (Mathog et al., 1984; Hochstrasser et al., 1986).

Interactions with other nuclear components may also play a role in silencing. In yeast, associations between telomeres and other nuclear structures such as the nuclear envelope or nuclear matrix correlate with repression (Stavenhagen & Zakian, 1994). Similar interactions may be responsible for TPE in *Drosophila*. These interactions would require the cooperative functions of several telomeric DNA and protein components. This model emphasizes localization of a gene within a certain nuclear domain, and since telomeric and centromeric domains are physically separated, they could contain distinct complements of silencing proteins (Singh & Huskisson, 1998; Cryderman et al., 1999). Variegation might arise because telomeric *P*-element insertions may occasionally dislocate the telomere from the silencing domain, weaken telomeric interactions, or destabilize telomeric structures. The observation that TAS in ectopic positions causes silencing of the w^+ reporter gene without variegation (Kurenova et al., 1998) is difficult to interpret in the context of the nuclear domain model. While these data do not exclude a role for compartments in silencing, it is also possible that telomeres are not compartmentalized to the same extent as centromeres.

The HeT-A *activation model*

Based on our recent observations with a telomeric transgene at 2L, we propose here a third model of TPE, which combines features of the two models discussed above, and adds a new, telomere-specific factor, the *HeT-A* element. The variegating *P* element insert in the 2L telomere, termed $P\{w^{var}\}$ (Gehring et al., 1984) carries as a reporter the complete genomic *white* gene with its eye-specific enhancer. The eye color phenotype of the w^1; $P\{w^{var}\}$ strain is orange with a few small red spots. We have determined the molecular structure of this transgene (Golubovsky, Konev, Biessmann & Mason, in preparation). The w^+ transgene is transcribed from distal to proximal, and is flanked distally by two truncated *HeT-A* elements, and proximally by the 2L TAS (Figure 4B). It has lost all of the distal, 5′ *P*-element sequences, and the 3′ end of the first *HeT-A* element is attached directly upstream of the eye enhancer. Upon outcrossing, flies with variegated brown-red eyes appeared at a frequency of 0.6% per gamete. These brown-red variants are due to new terminal *HeT-A* additions. Thus, the terminal addition of a new *HeT-A* element to $P\{w^{var}\}$ causes higher activity of the telomeric *w* transgene (Golubovsky, Konev, Biessmann & Mason, in preparation). The eyes in these variants are of darker color than in the original $P\{w^{var}\}$ strain, but they still variegate.

To explain this observation, we suggest a contribution of the transcriptional activity of the terminal *HeT-A* elements from their promoters, which are located in the 3′ noncoding region (Danilevskaya et al., 1997). Thus, transcription from *HeT-A* promoters may partially alleviate silencing of a downstream reporter gene caused by the subtelomeric satellite. A similar effect of transcription on TPE has been demonstrated in yeast (Renauld et al., 1993; Sandell, Gottschling & Zakian, 1994). Variegation may, thus, be caused by a competition between the centromere-directed 'opening force' of *HeT-A* transcription and the 'repressive force' of the TAS. This model is also consistent with the previously reported observation (Levis, 1989; Sheen & Levis, 1994) that deletions of the distally located TAS and the terminal *HeT-A*/*TART* array from the 3R tip of the chromosome carrying $P\{ry^+w^+\}$ *A4-4* (Figure 4C) relieve repression on the *white* reporter gene. Levis (1989) proposed that deletion of the distal 3R TAS is important for release of the repression on the reporter in $P\{ry^+w^+\}$ *A4-4*.

Trans-effects on HeT-A *promoter activity*

Very little is known about the regulation of *HeT-A* and *TART* transposition, and consequently about telomere length regulation in *Drosophila*. However, we have recently discovered a very suitable system for studying *HeT-A* transposition and telomere dynamics, by using a conveniently integrated transgene at the 2L telomere (Golubovsky, Konev, Biessmann & Mason, in preparation). The brown-red variants of $P\{w^{var}\}$, which have three *HeT-A* elements attached to the 2L telomere (described above), respond to disturbances at the telomere of the homologous chromosome. While they show repressed, orange eye color when the homologue contains an undisturbed (wild type) telomere, an alteration or deletion of the 2L telomere of the homologous chromosome results in darkening of the eye color. This effect is specific to alterations of the

telomere itself, because insertions into and deletions of the $l(2)gl$ gene that do not affect the TAS array have no effect. We propose a model that the telomeres of the two homologues interact with each other to assess their integrity. Consequently, a 'disturbed' telomere will stimulate promoter activity of the *HeT-A* elements on the telomere of the homologue, thus resulting in increased transcription from the *white* reporter gene and darker eye color. Thus, changes at one telomere affect the expression of a reporter gene at the homologous telomere in *trans* (Laurenti et al., 1995; Golubovsky, Konev, Biessmann & Mason, in preparation), and the nature of the *HeT-A* array in *cis* also plays a role in determining the level of expression. Overcoming TAS repression may depend on homologous pairing, as do transvection (Gelbart & Wu, 1982; Wu & Morris, 1999) and euchromatic gene silencing at PRE sites (Kassis, VanSickle & Sensabaugh, 1991; Kassis, 1994; Gindhart & Kaufman, 1995), or it may depend on telomere-telomere interactions of a more global nature.

If we assume that the $P\{w^{var}\}$ reporter gene at the tip of 2L is a surrogate for *HeT-A*, then TAS represses *HeT-A* transcription, but incompletely and transiently. Furthermore, the degree of repression seems to be related to the number of *HeT-A* elements at the tip of the repressed chromosome. There are many possible explanations of this observation:

(1) TAS may present a set level of repressive force that can be overcome with additional *HeT-A* elements with their 3′ promoters.
(2) TAS may only repress over a limited distance, and additional *HeT-A* elements are too far from TAS to be affected.
(3) The 3′ UTR of *HeT-A* distal to the promoter may contain a boundary that prevents TAS repression from affecting more distal *HeT-A* elements.

Conclusions

Given that *HeT-A* performs an essential cellular function, we expect that *HeT-A* transposition will turn out to be under cellular control. There is increasing evidence that regulation may occur at one or more steps in the process. (a) *HeT-A* transcription may be determined in part by the integrity of the homologous telomere. Short terminal deficiencies that remove the HeT-A array and part of the reporter gene from the homologue increase expression on the repressed chromosome (Laurenti et al., 1995; Golubovsky, Konev, Biessmann & Mason, in preparation). (b) The *trans* effects of the homologous telomere depend on the nature of the *HeT-A* array and possibly other DNA elements (TAS) in *cis*. Such interactions might be used to assess telomere integrity, and if telomeres are 'disturbed', could result in stimulation of *HeT-A* promoter activity. (c) Transcription of *HeT-A* may also be developmentally regulated, as shown by the correlation of its RNA levels with cell proliferation (Walter and Biessmann, in preparation). (d) Some *HeT-A* elements have a translational frameshift (Danilevskaya et al., 1992; Danilevskaya et al., 1994a), which may be used to control the level of one or more protein products. (e) Control of reverse transcriptase may provide important regulation of *HeT-A*, a retrotransposon that does not encode an RT enzyme. Indeed, a genetic factor on chromosome 3 has been found that modulates the activity or accessibility of an RT that may be used during *HeT-A* transposition (Golubovsky, Konev, Biessmann & Mason, in preparation). (f) We have discovered a potential suppresser of *HeT-A* transposition on chromosome 2 (Haoudi, Siriaco, Cenci, Champion, Gatti & Mason, in preparation). The nature of this genetic factor is still unknown, but it suggests that there may be at least two host genes involved in *HeT-A* transposition. Taken together, these observations suggest a complex regulatory system to control *HeT-A* transposition. We are only beginning to understand how these regulatory systems operate. Future work will have to address these interesting newissues.

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