Three distinct chromatin domains in telomere ends of polytene chromosomes in *Drosophila melanogaster Tel* mutants

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Summary

Drosophila melanogaster **telomeric DNA is known to comprise two domains: the terminal tract of retrotransposons (*HeT-A*, *TART* and *TAHRE*) and telomere-associated sequences (TAS). Chromosome tips are capped by a protein complex, which is assembled on the chromosome ends independently of the underlying terminal DNA sequences. To investigate the properties of these domains in salivary gland polytene chromosomes, we made use of *Tel* mutants. Telomeres in this background are elongated owing to the amplification of a block of terminal retroelements. Supercompact heterochromatin is absent from the telomeres of polytene chromosomes: electron microscopy analysis identifies the telomeric cap and the tract of retroelements as a reticular material, having no discernible banding pattern, whereas TAS repeats appear as faint bands. According to the pattern of bound proteins, the cap, tract of retroelements and TAS constitute distinct and non-overlapping domains in telomeres. SUUR, HP2, SU(VAR)3-7 and H3Me3K27 localize to the cap region, as has been demonstrated for HP1. All these proteins are also found in pericentric heterochromatin. The tract of retroelements is associated with proteins characteristic for both heterochromatin (H3Me3K9) and euchromatin (H3Me3K4, JIL-1, Z4). The TAS region is enriched for H3Me3K27. PC and E(Z) are detected both in TAS and many intercalary heterochromatin regions. Telomeres complete replication earlier than heterochromatic regions. The frequency of telomeric associations in salivary gland polytene chromosomes does not depend on the *SuUR* gene dosage, rather it appears to be defined by the telomere length.**

Key words: Telomeres, Heterochromatin, Silencing, Telomeric Associations, *Drosophila*

Introduction

Telomeres are specialized DNA-protein complexes that cap the termini of linear chromosomes. They compensate for incomplete DNA replication and contribute to the stability of chromosomes and karyotype (Muller, 1932; Pardue and Debaryshe, 1999; Biessmann and Mason, 2003). Telomeres also participate in nuclear architecture maintenance, and are known to associate with nuclear lamina (Hochstrasser et al., 1986; Marshall et al., 1996; Hari et al., 2001) or with nuclear matrix (de Lange, 1992; Luderus et al., 1996). However, the factors that underlie these phenomena, as well as the mechanisms of telomere functioning, remain poorly understood in *Drosophila melanogaster*.

In eukaryotes, such as budding yeast, fission yeast and humans, telomeres are considered to be essentially heterochromatic (Perrod and Gasser, 2003). Heterochromatin is characterized by a high degree of DNA compaction, late replication in S phase, and by association with specific silencing proteins (Richards and Elgin, 2002). Intercalary (IH) and pericentric heterochromatin regions in many *Diptera* species are characterized by DNA underreplication and non-homologous (ectopic) pairing of chromosomal regions in polytene chromosomes (Zhimulev, 1998). The question whether *D. melanogaster* telomeres are heterochromatic in nature remains open, as cytological analysis failed to reveal substantial heterochromatic blocks on the chromosome ends (Gall et al., 1971; Pimpinelli et al., 1976), and in salivary gland polytene chromosomes the morphology of most distal chromosome regions varies in different stocks from decompacted reticular material to dark dense bands (Zhimulev, 1998).

In *D. melanogaster*, distal chromosome regions are capped by a protein complex, which binds the chromosome ends in a DNA sequence-independent manner (Biessmann et al., 1990). In contrast to most eukaryotes, terminal DNA elongation in *D. melanogaster* does not require telomerase activity, and is mainly provided by the attachment of non-long terminal repeat retrotransposons, *HeT-A* and *TART*, to chromosome ends (Biessmann et al., 1992; Sheen and Levis, 1994). Less frequently, recombination or conversion events contribute to the maintenance of telomere length (Kahn et al., 2000; Melnikova and Georgiev, 2002). Recently another specific telomeric retrotransposon, *TAHRE*, has been described (Abad et al., 2004a). Thus, the terminal DNA sequences in *D. melanogaster* chromosomes are composed of head-to-tail repeats of retroelements *HeT-A*, *TAHRE* and *TART*; subtelomeric TAS (telomere-associated sequences) repeats are situated proximal and border euchromatic genes (Levis et al.,

1993; Walter et al., 1995; Biessmann and Mason, 2003; Abad et al., 2004a).

It remains unclear whether telomeric domains display the properties of heterochromatin, because data on their organization are controversial. HP1 protein is a structural component of the telomeric cap (Fanti et al., 1998) and pericentric heterochromatin (Maison and Almouzni, 2004). However, the *Su(var)205* gene, coding for HP1, does not appear as a modifier of telomeric position effect (TPE) (Wallrath and Elgin, 1995). Additionally, Danilevskaya et al. (Danilevskaya, 1998) suggested that *HeT-A* elements could form heterochromatic structure in telomeres, based on the observation that in *D. melanogaster* the localization of relatively conserved *HeT-A* 3′UTR is restricted to the telomeres, pericentric heterochromatin, and Y-chromosome (Traverse and Pardue, 1989; Abad et al., 2004b). However, subsequent experiments showed the *Het-A* promoter to be capable of activating transcription of the promoterless gene *yellow* (Kahn et al., 2000). Also, in an euchromatic context, *Het-A* sequences failed to repress w^+ reporter (George and Pardue, 2003).

There are data supporting the idea that TAS repeats form heterochromatic domains. Namely, TAS repeats induce w^+ repression in transgenic constructs (Kurenova et al., 1998). Also, integration of a reporter gene in TAS repeats or in close proximity to TAS is accompanied by TPE (Karpen and Spradling, 1992; Cryderman et al., 1999; Golubovsky et al., 2001; Mason et al., 2003).

One of the approaches to address the question of structural and functional organization of telomeres is to perform precise localization of chromatin proteins in these regions. However, the available data preclude unambiguous identification of a telomere as a distinct morphological structure on the chromosome tip, even when salivary gland polytene chromosomes are used. Small sizes of telomeres in most *Drosophila* laboratory stocks make interpretation of protein immunolocalization data problematic. In *Tel* mutants, the copy number of *HeT-A* and *TART* elements is augmented in telomeres (Siriaco et al., 2002), so the distal most chromosome regions are significantly extended. This allows the cap, *HeT-A/TAHRE/TART* and TAS repeats to be spatially resolved via cytological analysis. We have used this peculiar feature of *Tel* mutants to investigate the ultrastructure of telomeres, telomere replication timing in S phase and distribution of various proteins along the telomere in salivary gland polytene chromosomes. We conclude that three telomeric domains are distinct from heterochromatin in their morphology. All of them are not late replicating and show unique patterns of bound proteins. We also demonstrate that the frequency of telomeric associations (TA) in polytene chromosomes does not depend on the amount of HP1 and SUUR proteins. We report that ectopic pairing of telomeric regions is dependent on telomere length in polytene chromosomes.

Materials and Methods

Fly stocks and constructs

The following mutant stocks were used in this work: *y w*, *Tel* (*Telomere elongation*), *SuUR* (*Suppressor of Underreplication*), *ru h SuUR*, *D*^{3}*/TM6C*. *Su(var)205* alleles used were *Su(var)205*^{01}, *Su(var)205*^{03} and *Su(var)205*^{05}. Oregon R was used as a wild-type stock. Female *Su(var)205*-null mutants were identified in a progeny of a cross *Su(var)205*^{03}*;+/T(2;3)TSTL* × *Su(var)205*^{05}*;+/T(2;3)TSTL* by the absence of *Tubby* marker. Flies double mutant for *Su(var)205*^{03} [or *Su(var)205*^{05}] and *SuUR* genes were generated as follows: +/+; *Su(var)205; +/T(2,3) TSTL* females were first crossed to *Binsc/Y*; *+/Gla Bc; ru h SuUR* males. In their progeny *+/Binsc*; *Gla Bc*; *ru h SuUR/T(2,3) TSTL* and *+/Y*; *Su(var)205/Gla Bc*; *ru h SuUR/+* flies were mated to obtain *+/Y; Su(var)205/Gla Bc; ru h SuUR* sons. These were crossed with females +/+; *Su(var)205; +/T(2,3) TSTL*. Thus, *Su(var)205*^{03}; *ru h SuUR/T(2,3) TSTL* and *Su(var)205*^{05}*; ru h SuUR/T(2,3) TSTL* stocks were established. Description of balancers and mutants is given in FlyBase (Drysdale et al., 2005). Larvae were reared on standard cornmeal medium at 18°C.

The H7 construct (Makunin et al., 2002) contains the entire open reading frame and part of the 3′ untranslated region of the *SuUR* gene, placed under the control of the *hsp70* promoter. The H7 stock is homozygous for two insertion sites, located on the X and on the third chromosome, and has the *SuUR*$^-$ background. Starting from the 5-6 hour embryos, the cultures were daily heat-shocked at 37°C for 40 minutes until third instar larvae.

The swellings develop as the result of ectopic SUUR overexpression in UAS-GAL4 system (Zhimulev et al., 2003a). We generated the UAS-*SuUR*$^+$; *ru h SuUR Sgs3*-GAL4 stock by recombining the *Sgs3*-GAL4 transgene onto *SuUR* mutant background. The progeny from a cross *+; Tel* × UAS-*SuUR*$^+$*/Y; ru h SuUR Sgs3*-GAL4 were cultured at 29°C.

Cytology

Electron microscopy was carried out as described (Semeshin et al., 2004). Sections 120-150 nm thick were cut with an LKB-IV ultratome and examined under the JEM-100C electron microscope at 80 kV.

Fluorescence in situ hybridization (FISH) on polytene chromosomes (Ashburner, 1989) was performed using digoxigenin-labeled 2L TAS DNA (6 kb *Eco*RI-*Sac*I fragment) (Walter et al., 1995) and X TAS (PCR-amplified 1.2 kb fragment, as described earlier) (Boivin et al., 2003).

Immunostaining was performed as described in Czermin et al. (Czermin et al., 2002). The primary antibody dilutions used were as follows: rabbit polyclonal anti-SUUR (E-45), 1:50; rabbit polyclonal anti-PC, 1:600; rabbit polyclonal anti-E(Z), 1:800; mouse monoclonal anti-HP1 (CA19), 1:80; rabbit polyclonal HP2, 1:600; rabbit polyclonal H3Me3K9, 1:50; rabbit polyclonal H3Me3K27, 1:175; rabbit polyclonal SU(VAR)3-7, 1:400; rabbit polyclonal anti-H4AcK12 (Serotec), 1:100; mouse monoclonal Z4, 1:30; rabbit polyclonal JIL-1, 1:100; rabbit polyclonal H3Me3K4 (Abcam), 1:100; monoclonal mouse antibodies against the Ser5-phosphorylated CTD of RNA polymerase II-PolIIo (IgM, Covance), 1:50. The squashes were incubated with secondary FITC- or Rhodamine-labeled goat anti-rabbit and anti-mouse IgG-specific conjugates (Abcam, 1:200) or with anti-mouse IgM-FITC conjugates (Sigma, 1:250).

To trace replication via 5′-iodo-2′-deoxyuridine (IdU, Sigma) incorporation, salivary glands from *Tel/+* larvae were dissected in Ephrussi-Beadle solution (EB: 129 mM NaCl, 4.7 mM KCl, 1.9 mM $CaCl_2$) and cultivated for 20-30 minutes in 10 μM IdU solution in EB. Salivary glands were washed three times with large volumes of EB and were left in this solution before fixation for an additional 20-30 minutes. Subsequent manipulations were as described above for the immunostaining procedure, with the exception that before applying primary antibodies the squashes were incubated in 2 N HCl for 30 minutes to denature DNA, then neutralized in 100 mM $Na_2B_4O_7$, and washed in PBS-T. We used 1:150 diluted mouse anti-BrdU antibodies (Becton Dickinson) that also recognized IdU. To ensure reproducibility and to control for the possible impact of different chromatin configurations in neighboring cells (variegation) on the results of our analysis, immunostainings and IdU incorporation were performed three to five times with 8-10 slides in each experiment. No

influence of asynapsis in polytene chromosomes on the immunostaining pattern was detected.

Preparations of salivary gland chromosomes stained with acetic orcein were made by standard methods. TAs were counted in eight to ten larvae (with at least 80 nuclei scored for each experimental condition). Only the nuclei where all five telomeric regions of large chromosome arms could be seen were used for the analysis.

Results

Comparison of molecular and cytological maps of telomeres in polytene chromosomes

To investigate the morphology of telomeres we carried out electron microscopy analysis of polytene chromosomes from salivary glands of *Tel/+* larvae. The use of hybrids enables comparative analysis of both homologs in a single cell. Assignment of a homolog to the genotype was based on the length of the telomere. We noticed significant variation in telomere length in *Tel* mutants. Variation was seen between different telomeres in one nucleus and between the same telomeres in different larvae. The longest telomeres tended to form in chromosome arms X, 2L and 2R, with shorter ones found in 3L and 3R.

The most distal region of the chromosome, which was operationally defined as the cap, does not display any banding, and is represented by decompacted reticular material (Fig. 1A, Fig. 2A). Proximal to the cap lies a diffuse region, which is substantially elongated in *Tel* chromosomes, and which corresponds to the *HeT-A/TAHRE/TART* repeats according to FISH data (Siriaco et al., 2002). These regions are not uniformly decondensed; however, no reproducible banding pattern could be seen in the *HeT-A/TAHRE/TART* repeat region (Fig. 1A, Fig. 2A). Thus, the cap and *HeT-A/TAHRE/TART* repeats do not differ significantly in terms of morphology. The fibrous decompacted zone in all telomeric regions is proximally limited by two to three very faint bands, depicted on the Bridges polytene map (Bridges, 1935). These are the most distal chromosomal regions where polytene chromosome banding pattern is discernible. However, the presence and morphology of these faint bands is variable even under electron microscopy. We investigated whether this banded structure belongs to the telomeres, and if so, to which zone it maps.

TAS repeats represent the proximal domain of the telomere, but they may be absent from chromosome arms in different *D. melanogaster* stocks (Mechler et al., 1985; Boivin et al., 2003; Abad et al., 2004c). Using FISH, we tested for the presence of TAS repeats in our stocks: Oregon R, *Tel*, *SuUR,* and UAS-*SuUR*$^{+}$*; ru h SuUR Sgs3*-GAL4. TAS DNA sequences from chromosome X and 2L telomeres were used as probes. The 1.8 kb × TAS DNA sequence is homologous to the 2R and 3R TAS repeats (Karpen and Spradling, 1992), whereas 2L TAS DNA was observed to cross-hybridize with the 3L telomere (Mechler et al., 1985; Walter et al., 1995). According to our FISH data, TAS repeats were present in all chromosome arms in Tel stock only (Table 1, Fig. 1B, Fig. 2B). In Oregon R, TAS repeats were absent from the X chromosome and in *SuUR* stock there were no signals in chromosome arms X, 2L and 3L. In the stock UAS-*SuUR*$^{+}$*; ru h SuUR Sgs3*-GAL4, TAS repeats were absent from the X chromosome, and chromosome arms 2L and 3R were found to be polymorphic for the presence of TAS (Table 1).

Table 1. Mapping of TAS repeats to telomeres

	Chromosome				
Stock	X	2L	2R	3L	3R
Tel	+	+	+	+	+
Oregon R	–	+	+	+	+
SuUR	–	–	+	–	+
UAS-*SuUR*$^{+}$*; ru h SuUR Sgs3*-GAL4	–	+/–	+	+	+/–

+, signal is present; –, no signal; +/–, polymorphism in the stock.

FISH data localize TAS repeats to the region of the first faint bands as identified by electron microscopy (Fig. 1B, Fig. 2B), but we were unable to determine whether they map to a specific band. The number of faint bands within distal chromosome regions does not vary, irrespective of the presence or absence of TAS repeats. We used TAS hybridization signals as the markers to set the proximal telomere border. Faint telomeric bands lie adjacent to the well-defined and discrete bands, which are reliably identified under the light microscope. It was only in chromosome arms X (1B1-2) and 2R (60F2-3) that the subtelomeric bands corresponded to the typical IH regions, as we previously demonstrated (Zhimulev et al., 2003b). All telomeric domains are morphologically distinct from both the intercalary and the pericentric heterochromatin (Zhimulev, 1998) in that they do not contain supercompacted material. The cap and *HeT-A/TAHRE/TART* domains are also very different from typical euchromatic regions, as they do not display reproducible banding patterns.

Localization of euchromatin- and heterochromatin-specific proteins in telomeres

Modified histones and non-histone proteins serve as epigenetic markers of eu- and heterochromatin (Grewal and Elgin, 2002; Lachner et al., 2003). We sought to compare the localization of such proteins in the three telomeric domains. We performed indirect immunofluorescence analysis, using antibodies specific for heterochromatin associated proteins SUUR, H4AcK12, PC, E(Z), H3Me3K9, H3Me3K27, HP1, HP2 and SU(VAR)3-7. We also used antibodies against euchromatic proteins H3Me3K4, JIL-1, Z4 and PolIIo.

SUUR is a marker of heterochromatin, as it localizes exclusively to the late replication regions of polytene chromosomes, and is involved in the control of proper replication completion in these regions (Zhimulev et al., 2003b). SUUR-specific signals are found in the cap regions of all *Tel* chromosome arms, and are not detectable in the *HeT-A/TAHRE/TART* regions. In the TAS domain, the SUUR signal is absent from the chromosome arms X, 2L, 3L and 3R, whereas in 2R the signal, if present, cannot be reliably distinguished from the subtelomeric IH band (Fig. 1C, Fig. 2C).

We have previously described the formation of specific chromosome structures, termed swellings, which arise in intercalary and pericentric heterochromatin regions upon SUUR overexpression in the transgenic system *Sgs3*-GAL4>UAS-*SuUR*$^{+}$ (Zhimulev et al., 2003a). Upon SUUR overexpression in UAS-*SuUR*$^{+/+}$*; Tel/ru h SuUR Sgs3*-GAL4 hybrids, the most distal swellings were consistently observed only in subtelomeric IH regions in X and 2R chromosomes

(Fig. 1D, Fig. 2D). To confirm this observation we used TAS probes in FISH analysis of chromosomes with well-developed swellings (Fig. 1E, Fig. 2E). We noted that the width and degree of compaction of the TAS region remained unchanged. In the 2R chromosome arm, TAS and the IH region in 60F2-3 are so close to each other that TAS appears to be on the immediate border of the swelling, which might explain the more diffuse signal seen here (Fig. 1E). Therefore, we conclude that swellings are not formed by any of the telomere domains.

Previous studies showed that TAS repeats were able to recruit some Pc-G proteins (Boivin et al., 2003). For our analysis we chose the PC protein, a component of PRC1 complex (Shao et al., 1999; Saurin et al., 2001), and the E(Z),

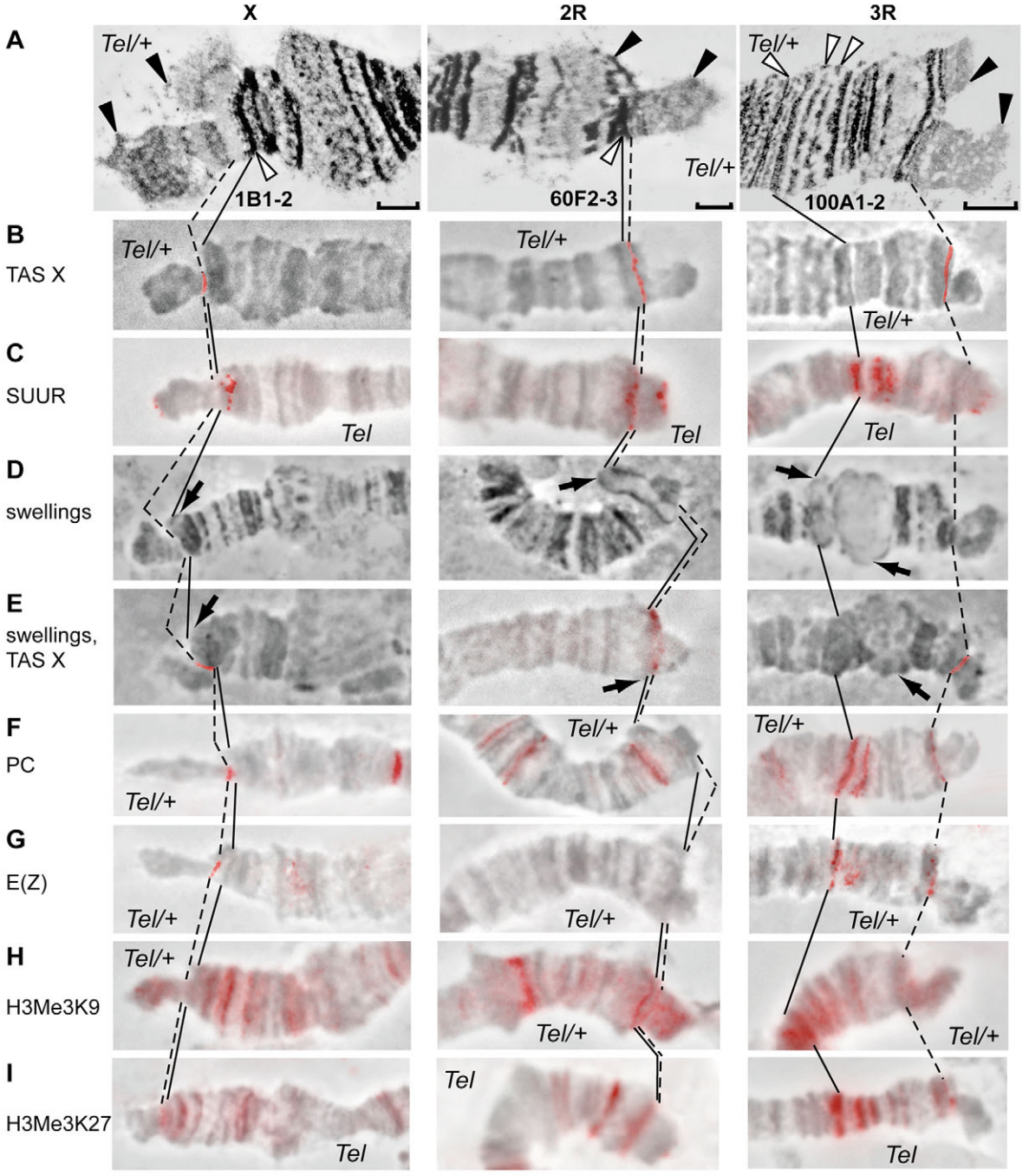

Fig. 1. Telomere structure and distribution of proteins in polytene chromosomes X, 2R and 3R. (A) Electron microscopy. (B,E) FISH of TAS X probe. (C,F-I) Chromosome localization of chromatin proteins in telomeric regions: SUUR (C), PC (F), E(Z) (G), H3Me3K9 (H), H3Me3K27 (I). (D,E) Induction of swellings in UAS-*SuUR*$^{+/+}$; *Tel/ru h SuUR Sgs3*-GAL4. (D) Orcein staining. (B,C,E-I) Merged image of the phase contrast (black and white) and immunofluorescence (red). Black arrowheads indicate the cap regions, white arrowheads show IH regions. Swellings are marked by arrows. Dashed lines, TAS repeats; solid lines, marker regions. Bars, 1 μm.

which is a subunit of ESC/E(Z) (Müller et al., 2002). PC and E(Z) labeling sites were correlated with the presence of TAS repeats in the proximal telomeric regions of all chromosome arms in Oregon R, *Tel*, and *SuUR* (Fig. 1F,G, Fig. 2F-I). In the X chromosomes of *Tel*/+ hybrids, the signal was detected only in the *Tel* homolog, but not in the wild-type counterpart. In TAS regions of 2L and 3L chromosomes arms the signal is consistently present on both homologs in *Tel*/+ hybrids and is absent from *SuUR*. The 3R TAS region binds PC and E(Z) in all the three genotypes (data not shown). An exception is the 2R telomere, where PC and E(Z) are not detected, despite the presence of TAS.

Modified histones H3Me3K9 and H3Me3K27 are well-established markers of heterochromatin (Lachner et al., 2003). In all *Tel* chromosomes, the regions corresponding to the *HeT-A/TAHRE/TART* repeats display bright, discrete H3Me3K9 signals (Fig. 1H, Fig. 2J). Their number and location varied between the same chromosomes from different larvae, which could reflect the heterogeneity of *HeT-A/TAHRE/TART* arrays in the *Tel* stock. Intense H3Me3K27 labeling was found in TAS regions. Sometimes weak H3Me3K27 labeling was observed also in the cap (Fig. 1I, Fig. 2K). In the 2R chromosome, H3Me3K9 and H3Me3K27 signals around TAS cannot be resolved from the neighboring IH region.

We have localized SU(VAR)3-7 [detected in telomeric regions and pericentric heterochromatin (Delattre et al., 2000)] and HP2 [the HP1 partner (Shaffer et al., 2002)] to the very tips of *Tel*/+ chromosomes, in addition to HP1 (Siriaco et al., 2002) and SUUR (Fig. 1C, Fig. 2C). The signals appeared as a faint band or a chain of small granules within the cap region (Fig. 3A-C). In *Tel*/+ chromosomes, the immunofluorescence signals corresponding to the cap complex components were most frequently observed in the longer homolog, but not in the shorter one. We believe this was an artifact of the preparation procedure, resulting from unequal breakage of TAs typical to *Tel* chromosomes. Less frequently, the opposite situation was also observed.

Turner et al. (Turner et al., 1992) reported the pericentric heterochromatin and certain bands of *D. melanogaster* salivary gland polytene chromosomes to be enriched in histone H4 acetylated at lysine 12 (H4AcK12). We mapped the H4AcK12-specific signal in

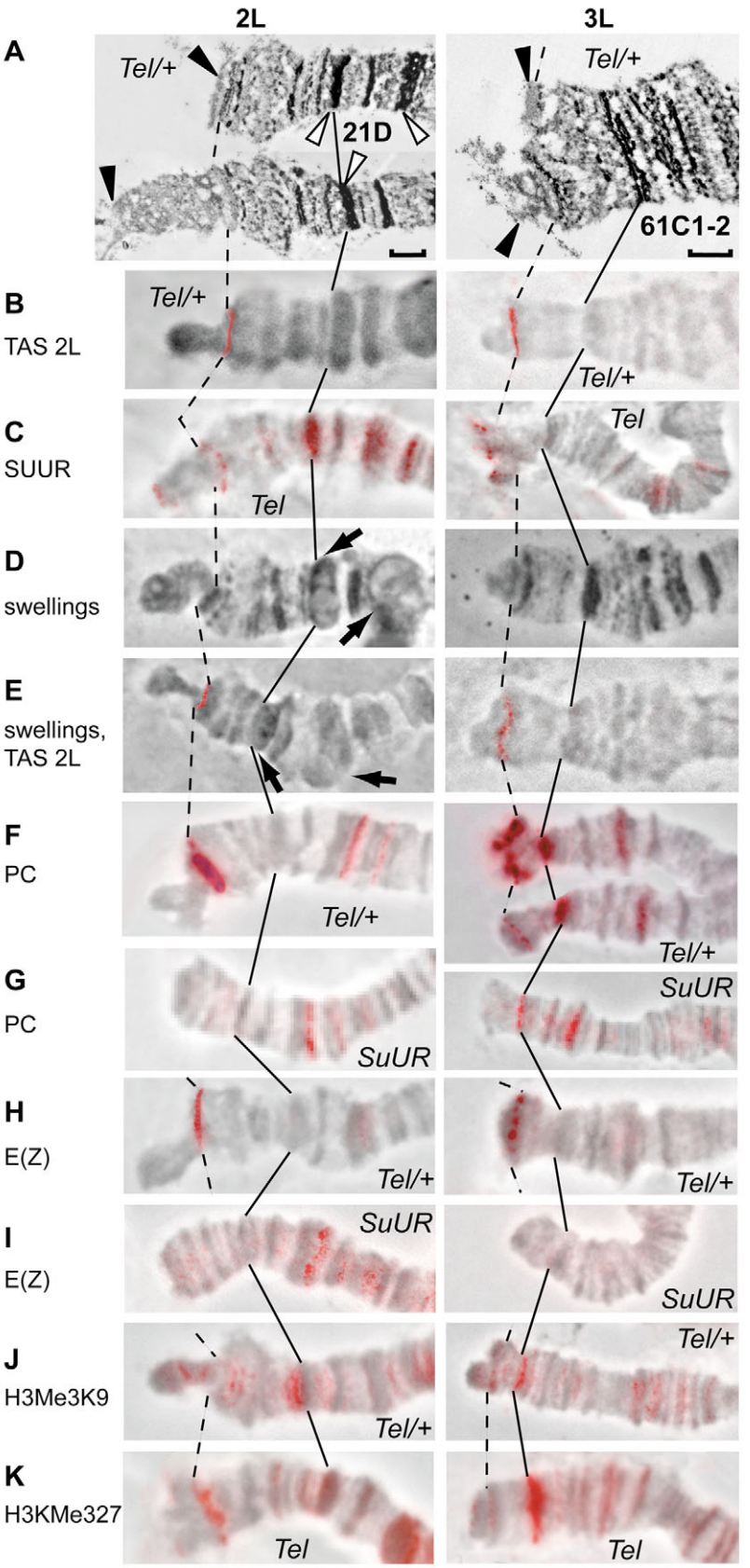

Fig. 2. Telomere structure and protein localization in polytene chromosome arms 2L and 3L. (A) electron microscopy. (B,E) FISH of the 2L TAS probe. (C,F-K) Localization of antibodies to the chromatin proteins in telomeric regions: SUUR (C), PC (F,G), E(Z) (H,I), H3Me3K9 (J) and H3Me3K27 (K). (D and E) Induction of swellings in hybrids UAS-*SuUR*$^{+/+}$*; Tel/ru h SuUR Sgs3*-GAL4; (D) orcein staining. Swellings are marked by arrows. Dashed lines, TAS repeats; solid lines, marker regions. Bars, 1 μm.

the regions of intercalary and pericentric heterochromatin, and in many euchromatic bands, but not in the telomeric regions (Fig. 3D); this is in agreement with studies by Swaminathan et al. (Swaminathan et al., 2005). We therefore conclude that H4AcK12 does not represent a specific marker of either telomere or heterochromatin in general.

Since the telomeric *HeT-A/TAHRE/TART* domain does not appear heterochromatic according to our cytology analysis, we immunolocalized several euchromatic proteins in this region. The phosphokinase JIL-1 (Jin et al., 1999) phosphorylates histone H3 at serine 10, and is required to maintain the 'open' chromatin state (Wang et al., 2001). In our experiments, *HeT-A/TAHRE/TART* repeats were found to recruit JIL-1 (Fig. 3E). Z4 protein, which is present in most, if not all, decompacted interband regions (Eggert et al., 2004), binds the region of *HeT-A/TAHRE/TART* repeats co-localizing with H3Me3K4 (Fig. 3F,G), which is present in actively transcribed genes (Schübeler et al., 2004). We also immunolocalized an isoform of PolIIo in which the C-terminal domain is phosphorylated at serine 5. This isoform is found in actively elongating transcription complexes (Palancade and Bensaude, 2003). We found that PolIIo signals were not present in the telomeric region (Fig. 3H), and that the distally located signal on the X chromosome corresponded to the subtelomeric region.

Indirect immunofluorescence data on HP1 co-localization with H3Me3K9 in telomeres have been reported (Perrini et al., 2004). To test whether the proteins used in our experiments and localizing within the *Het-A/TART/TAHRE* domain also associate with the cap, we co-localized the HP1 protein found in the cap complex with the proteins H3Me3K4, H3Me3K9 and JIL-1. No overlap between HP1 and the other signals was observed; this was also found for HP2 and Z4 proteins (Fig. 3I-L). H3Me3K9 localization pattern is not limited to the *Tel* mutant background, as we obtained similar results in *y w* stock (Fig. 3M). Therefore, our data do not support JIL-1, H3Me3K9, H3Me3K4 and Z4 as components of telomere cap complex, but suggest that these proteins are recruited by the *HeT-A/TAHRE/TART* array, which is devoid of capping proteins.

Our data on localization of antibodies to H3Me3K9 in telomeres of HP1-null mutants provide evidence of the independence of distribution of HP1 and H3Me3K9 (Fig. 3N), which is in line with the findings of Ebert et al. (Ebert et al., 2004).

Likewise, Z4 and PC localization did not show a perfect merge of signal (Fig. 3O). Thus, the distribution of Z4 in telomeres is restricted to the *HeT-A/TAHRE/TART* array. These protein localization data argue that cap, *HeT-A/TAHRE/TART* and TAS repeats form distinct, non-overlapping domains.

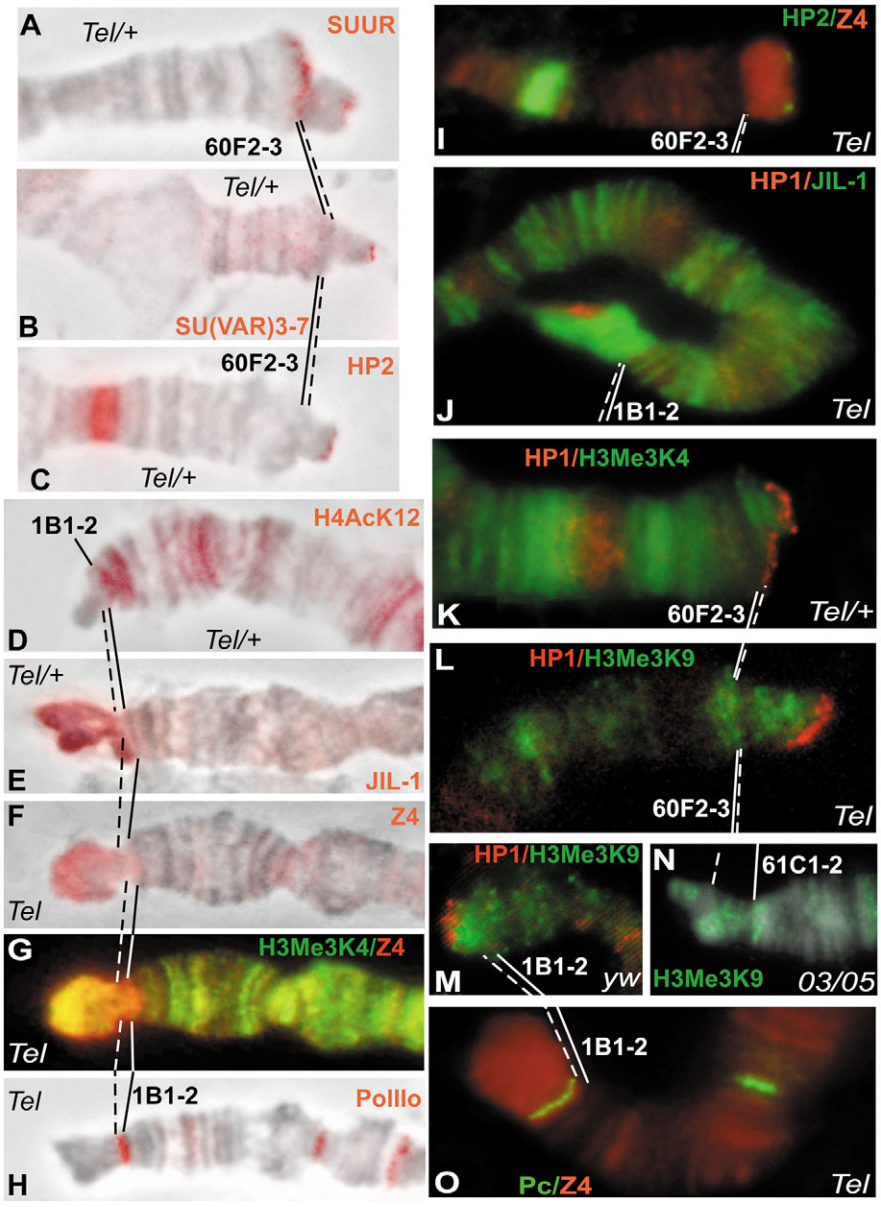

Fig. 3. Localization of antibodies to the chromatin proteins in the telomeric regions of polytene chromosomes 2R (A-C,I,K,L), X (D-H,J,M,O) and 3L (N). Capping proteins: (A) SUUR, (B) SU(VAR)3-7 and (C) HP2 localize to the chromosome tip. (D) H4AcK12 is not detected in telomeres. *HeT-A/TAHRE/TART* repeat recruits typical euchromatic proteins: (E) JIL-1, (F) Z4, (G) H3Me3K4 and Z4, both of which co-localize within this region. No productive transcription is detected within the *HeT-A/TAHRE/TART* region, since no signal for phospho-serine5 PolIIo is seen (H). Upon simultaneous immunodetection of cap- and *HeT-A/TAHRE/TART*-array-specific proteins, no overlap of the two signals is observed: HP2 and Z4 (I), HP1 and JIL-1 (J), HP1 and H3Me3K4 (K), HP1 and H3Me3K9 (L,M). (N) H3Me3K9 is detected within the *HeT-A/TAHRE/TART* region of *Su(var)205*^{03}/*Su(var)205*^{05} larvae. (O) PC protein, recruited to the TAS repeats, and Z4 do not merge perfectly within the region of TAS repeats. Dashed lines, TAS repeats; solid lines, marker regions.

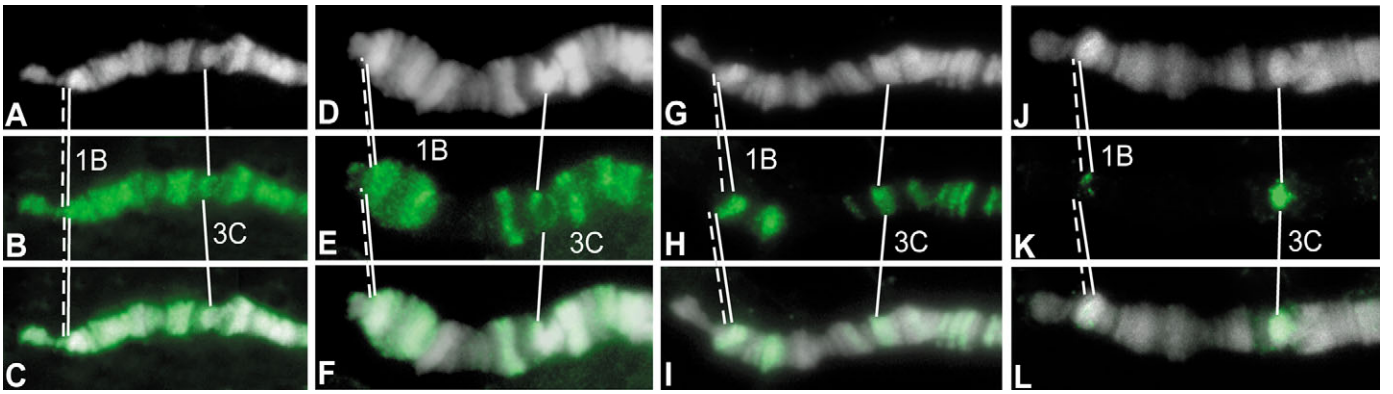

Fig. 4. IdU incorporation dynamics in the telomere of *Tel*/+ polytene chromosome X. (Top) Hoechst staining of DNA; (Middle) IdU incorporation pattern; (Bottom) merged image. IdU incorporation at the discontinuous labeling phase: (A-C) all over the telomere; (D-F) the cap region still replicates; (G-I) telomeric regions no longer incorporate the label by the beginning of the late S phase; (J-L) replication of the chromosome tip is completed prior to the replication termination in the IH regions. Dashed lines, TAS repeats; solid lines, marker regions.

Telomere replication timing

One of the most important diagnostic features of heterochromatin is its late replication and underreplication in polytene chromosomes. Based on the kinetics of incorporation of [^{3}H]thymidine, all telomeric regions were described as late replicating (Zhimulev et al., 2003b). However, in these experiments the *SuUR* and Oregon R stocks used had short telomeres, and so it was not possible to determine unambiguously whether it was the telomere or the adjacent subtelomeric bands that replicated late. To gain better resolution in replication timing of distal regions of polytene chromosomes we used the *Tel*/+ larvae and IdU incorporation technique. After a pulse of IdU, the polytene chromosomes show different labeling patterns, depending on the stage of the S-phase, as described previously (Zhimulev et al., 2003b). In telomeres, IdU incorporation is observed over all three domains during the phase of discontinuous labeling, when the puffs and the interbands completed replication (Fig. 4A-C). A little later, when the label is present in the chromocenter, IH and in many euchromatic bands, the signal is no longer detectable in the region of *HeT-A/TAHRE/TART* repeats, with cap regions still replicating at this time point (Fig. 4D-F). By the beginning of the late S-phase, when the replication progresses through the chromocenter and roughly 120 IH sites, all telomeric domains no longer incorporate the label (Fig. 4G-I). Replication of the most distal, terminal DNA completes far sooner than the replication of IH regions (Fig. 4J-L). Thus, telomeres cannot be classified as late replicating regions.

Frequency of telomeric associations is dependent on the telomere length in polytene chromosomes

Ectopic pairing of non-homologous chromosome regions is a characteristic feature of polytene chromosomes in many dipteran species. The contacts that are formed between the pericentric heterochromatin regions eventually form the common chromocenter. IH regions are also known to frequently contact each other and with pericentric heterochromatin (Zhimulev, 1998). Telomeres are often cited as being paired with intercalary and pericentric heterochromatin, but it is not known whether it is the telomere that contacts the heterochromatin, or the subtelomeric sequences, which in chromosome arms X and 2R correspond to the IH. Better characterized is ectopic pairing between the telomeres, when end-to-end joining of telomeres is observed. In diploid cells this is manifested as TA, which are resolved in mitotic or meiotic anaphase [e.g. in *Tel* mutants (Siriaco et al., 2002)], and as telomeric fusions that disrupt chromosome segregation in mitosis and meiosis and lead to cell lethality [e.g. in *Su(var)205* mutants (Fanti et al., 1998)]. These types of telomeric contacts cannot be distinguished in polytene chromosomes, therefore we use here only one term, TA. Morphologically, the TA appears as an intimate contact between individual chromatids or bundles of chromatids from different chromosomes (Fig. 5). In TA, stretches of material joining the chromosome ends hybridize with *HeT-A* (Rubin, 1978; Siriaco et al., 2002) or TAS probe (Karpen and Spradling, 1992). We questioned whether telomeric association and ectopic pairing of heterochromatic regions could be analogous. The nature of ectopic pairing is not well established; however, strong correlation of underreplication

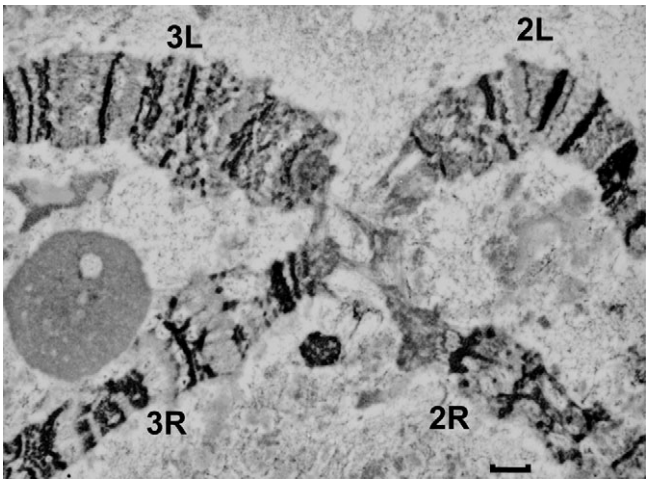

Fig. 5. Telomeric associations in *Tel* are formed by the cap or tract of *HeT-A/TAHRE/TART* as revealed by electron microscopy. Bar, 1 μm.

Table 2. Frequency of telomeric associations in different stocks*

Genotype	Number of telomeres observed (percentage of telomeres found in associations)	Percentage of associated telomeres					Share of pairwise TA of the total TA (%)	
		X	2L	2R	3L	3R	2L-2R	3L-3R
Su(var)205^{03}/Su(var)205^{05};SuUR^{+}	422 (36)	11	44	40	44	38	16	37
Su(var)205^{03}/Su(var)205^{05};SuUR (no doses of *SuUR^{+}*)	562 (32)	32	48	47	23	3	48	1
Su(var)205^{01}/CyO	665 (17)	3	22	29	12	17	42	12
+/+ (F1 derivative of the *Su(var)205^{01}/CyO* stock with the second chromosome replaced for the wild-type)	650 (8)	7	1	6	18	24	0	42
Su(var)205^{03}/Gla Bc	575 (29)	12	31	32	7	16	41	5
+/+ (F1 derivative of the *Su(var)205^{03}/Gla Bc* stock with the second chromosome replaced for the wild-type)	720 (10)	2	4	21	22	43	0	42
H7, SuUR (daily heat shocks, SUUR overexpression)	565 (20)	0	23	39	3	35	–	–
H7, SuUR (development at 18°C, control)	500 (17)	2	18	27	7	37	–	–

*Frequency of telomeric associations was calculated as the percentage of telomeres forming associations out of all the telomeres observed.

extent and the frequency of ectopic contacts of heterochromatic regions has been reported (Zhimulev, 1998).

SuUR mutation completely suppresses underreplication of intercalary heterochromatin, with complete disappearance of ectopic contacts (Belyaeva et al., 1998). In laboratory fly stocks with two wild-type *SuUR* alleles, the involvement of each of the IH regions in ectopic pairing, as well as the individual underreplication extent in a given IH region, has a consistent frequency. By contrast, the frequency of TAs in polytene nuclei of these strains varies significantly from 1-3% to 60-70%, and it is positively correlated with the length of telomeres (Zhimulev, 1998). To analyze the possible effect of *SuUR* gene product on the frequency of TAs, we made use of the stocks bearing *Su(var)205^{03}* and *Su(var)205^{05}* alleles, which are known to have long *HeT-A/TAHRE/TART* tracts and display high frequency of TAs (Fanti et al., 1998; Savitsky et al., 2002). We calculated the TA frequency for the salivary gland nuclei of third instar larvae that were trans-heterozygous for *Su(var)205^{03}* and *Su(var)205^{05}* alleles and had either *SuUR^{+}* or *SuUR$^{–}$* background. We observed no statistically significant difference in the percentage of associated telomeres in these backgrounds (Table 2). However, we did notice that the telomeres of the introduced *SuUR*-bearing third chromosome retained low TA frequencies, in contrast to the original *SuUR^{+}* chromosome. In order to verify whether TAs were dependent on the individual properties of telomeres, we replaced the second chromosomes bearing *Su(var)205* mutant alleles by wild-type homologs from the *+/+; D^{3}/TM6C* stock. The newly introduced second chromosome also independently participated in TA: the frequency of TAs of this chromosome was reduced, with the 2R-2L combination being completely absent (Table 2). The telomeres of other chromosomes continued to demonstrate high frequencies of TA, with notable changes in the frequencies of pairing between them, most probably due to the absence of competition with the telomeres 2L and 2R. Thus, the TA frequency in polytene chromosomes did not depend on the presence of *Su(var)205* mutations in the genome. The situation described was seen in F1 following the replacement of the second chromosome, and was maintained afterwards for at least seven subsequent generations.

We also measured the TA frequency in a transgenic stock H7, where SUUR overexpression has been previously demonstrated to increase the heterochromatin underreplication extent (Makunin et al., 2002). Daily heat shocks to induce

Table 3. Indirect immunofluorescence localization of proteins in telomeric domains of chromosomes

Protein	Cap					*HeT-A/TART/TAHRE*					TAS				
	X	2L	2R	3L	3R	X	2L	2R	3L	3R	X	2L	2R	3L	3R
SUUR	+	+	+	+	+	–	–	–	–	–	–	–	?	–	–
HP1*	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
HP2	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
SU(VAR)3-7	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
H4AcK12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PC	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+
E(Z)	–	–	–	–	–	–	–	–	–	–	+	+	–	+	+
H3Me3K9	–	–	–	–	–	+	+	+	+	+	–	–	?	–	–
H3Me3K27	–	+	+	+	–	–	–	–	–	–	+	+	?	+	+
Z4	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–
H3Me3K4	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–
JIL-1	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–
PolIIo	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

*Siriaco et al., 2002 and our data

+, signal is present; –, no signal; ?, indistinguishable from IH region 60F2-3.

SUUR overexpression in H7 do not alter the overall frequency of TA, neither do they modify the frequencies of TAs between individual telomeres of different chromosomes (Table 2). These values remain the same as the control. The behavior of TAs described in this experiment is remarkably different from that of the IH regions, which participate in ectopic pairing more frequently the stronger underreplication is. Extra dosage of *SuUR*$^{+}$ does not influence the TA frequency. In stocks having 0, 2, 4 doses of the *SuUR*$^{+}$ gene the TA frequency totaled 2-3% (data not shown), whereas the frequency of ectopic pairing in IH regions was strikingly *SuUR*-dependent (Zhimulev et al., 2003b). Interestingly, HP1 overexpression throughout development, did not modify the TA frequency in polytene chromosomes (not shown). Therefore, telomeric associations differ from IH ectopic pairing in polytene chromosomes by displaying no correlation with the amount of SUUR protein.

We did not detect the association of telomeres with the regions of intercalary and pericentric heterochromatin. Upon investigation of 200 slightly squashed nuclei of *Tel* salivary glands we did not score a single case of telomere-IH association, with all associations involving the telomeres exclusively. This observation also supports the view of telomeric association and ectopic pairing as distinct phenomena in telomeric and heterochromatic regions of polytene chromosomes.

Discussion

Ultrastructure of telomeres

Molecular and genetic analyses provide the evidence for existence of three distinct domains in distal regions of chromosomes: cap complex, which is assembled on the terminal DNA in a sequence-independent manner, the array of *HeT-A/TAHRE/TART* elements, and TAS repeats (Biessmann and Mason, 2003). The size of the *HeT-A/TAHRE/TART* tract varies in different chromosome arms, totaling up to 147 kb in the X, 0-50 kb in 2L, 90 kb in 2R, 26 kb in 3L, and 43 kb in 3R (Mason et al., 2003; Abad et al., 2004c). The *HeT-A/TAHRE/TART* array length is significantly increased on *Su(var)205* (Savitsky et al., 2002), *Tel* (Siriaco et al., 2002) and *E(tc)* (Melnikova and Georgiev, 2002) backgrounds. Electron microscopy analysis demonstrates that distal regions of chromosomes in *Tel* mutants appear as a decompacted reticular-like material which, according to the FISH data, corresponds to the amplified *HeT-A/TAHRE/TART* repeats (Siriaco et al., 2002). Cap complex is estimated to span 4-6 kb of terminal DNA (Savitsky et al., 2002), and in *Tel* chromosomes cap region cannot be distinguished from the neighboring domain by morphology. In general, both the cap and the chromatin comprising *HeT-A/TAHRE/TART* arrays do not resemble typical intercalary and pericentric heterochromatin, and look more similar to the decompacted β-heterochromatin, which also displays reticular structure. Reticular morphology probably originates from the repeated nature of the DNA in this region, which leads to homologous pairing between the fragments of the same strand.

As visualized by electron microscopy, the *HeT-A/TAHRE/TART* array is bordered with faint bands, which correspond to the localization sites of TAS repeats, according to our FISH analysis. The total length of the TAS domain in telomeric regions is known to be small, approximately 10-25 kb (Karpen and Spradling, 1992; Mason et al., 2003; Abad et al., 2004c). A middle-sized band normally contains about 30 kb DNA. Analysis of bands formed from the DNA of transposons having a known amount of DNA showed that 5 kb is the minimal size necessary for creating a band discernible under the electron microscope (Zhimulev, 1998). The size of TAS repeats in *D. melanogaster* telomeres is at the resolution threshold at the electron microscopy level, in contrast to the IH regions, which often form very large and dense bands spanning up to 200-300 kb (Moshkin et al., 2001). Thus, at the level of cytology, faint bands formed by TAS are distinct from typical heterochromatin.

Cap, *HeT-A/TAHRE/TART* and TAS repeats bind non-overlapping and specific sets of proteins

We demonstrate that the cap region binds a number of proteins that are known to be localized to the silenced pericentric heterochromatin regions. These are HP2 (Shaffer et al., 2002), SU(VAR)3-7 (Delattre et al., 2000), SUUR (Makunin et al., 2002) and H3Me3K27 (Perrini et al., 2004; Ebert et al., 2004) (Table 3). Association of HP1 and HOAP with the cap region has been demonstrated (Fanti et al., 1998; Siriaco et al., 2002; Cenci et al., 2003).

It is possible that HP1 targeting to the cap region occurs via interactions with other proteins. One candidate is HOAP, which forms a complex with HP1 (Shareef et al., 2001) and is present in cap regions of *Su(var)205* null mutants (Cenci et al., 2003). A number of additional proteins appear to contribute to the stability of the HOAP/HP1 complex since, in *tefu (ATM)* (Oikemus et al., 2004), *mre11* and *rad50* mutants (Ciapponi et al., 2004), HP1 and HOAP fail to accumulate in cap regions in polytene chromosomes.

In polytene chromosomes, the region of *HeT-A/TAHRE/TART* repeats also associates with a striking combination of proteins: H3Me3K9, characteristic of heterochromatin, and a euchromatin-specific histone isoform H3Me3K4 (Schübeler et al., 2004), Z4 (Eggert et al., 2004) and JIL-1 (Jin et al., 1999). None of these proteins localizes to the cap region (Table 3).

There are several lines of evidence indicating that the chromatin in the *HeT-A/TAHRE/TART* region in polytene chromosomes might exist in a state that is poised for activation. First, according to our electron microscopy data, in salivary gland cells the *HeT-A/TAHRE/TART* domain does not show a high degree of DNA compaction. Second, this domain has a histone H3 lysine 4 tri-methylation mark, which is associated with actively transcribed genes (Schübeler et al., 2004). However, no actively elongating RNA polymerase isoform (with CTD phosphorylated at serine 5) is detected in this region, nor are the transcripts of *HeT-A* and *TART* transposons produced in salivary glands (George and Pardue, 2003; Walter and Biessmann, 2004).

TAS region is distinct from other telomeric domains, recruiting specific proteins, such as PC and E(Z) (Table 3), that are known to be subunits of the PRC1 (Saurin et al., 2001) and ESC/E(Z) (Müller et al., 2002) complexes respectively. In vitro E(z) displays histonemethyltransferase activity towards histone H3 lysine residues 9 and 27 (Czermin et al., 2002). We demonstrate strong enrichment of H3Me3K27 isoform in TAS repeat regions.

Further support comes from the correlation of TAS presence and localization of PC and E(Z) proteins, which was demonstrated in our work for all but one telomere. Boivin et al. found no significant correlation between the TAS repeats and localization of the Pc-G (PC, PH, PSC, and SCM) proteins (Boivin et al., 2003), which might be attributable to the polymorphism for TAS repeats in the stocks used. SCM was reported to be recruited to the 2R telomere in some cases (Boivin et al., 2003). It is possible that the 2R telomere recruits a third silencing complex, distinct from PRC1 and ESC/E(Z), which contains the SCM protein (Roseman et al., 2001; Hodgson et al., 2001). Why different TAS might recruit distinct complexes of Pc-G proteins is currently unknown and this requires further investigation.

When the X-chromosome TAS 1.8 kb fragment is placed in a transgenic construct, it displays properties analogous to those of Polycomb response elements (PRE): it contributes to pairing sensitive repression of the adjacent reporter gene and mediates targeting of Pc-G proteins to the transposon insertion site (Boivin et al., 2003). Strong correlation of PC and E(Z) localization sites with the presence of TAS repeats in the telomeres of chromosome arms 2L and 3L thus suggests that these TAS elements should also possess PRE-like properties.

As mentioned above, similarly to PRE, TAS repeats cause reporter gene inactivation in transgenic assays. When the reporter is integrated within TAS or immediately adjacent in the context of telomere, the same effect is also observed, which is generally referred to as TPE (Karpen and Spradling, 1992; Crydermann et al., 1999; Mason et al., 2003). Taking into account the parallels between PRE and TAS, and the fact that both PRE and TAS bind repressive Pc-G complexes of proteins, TAS appear to represent the regions of Pc-G-mediated silencing (Boivin et al., 2003). Recent evidence further supports this idea: the only established TPE modifier, *gpp*, codes for a protein with an H3Me2K79 histonemethyltransferase activity, and shows genetic interactions with the *Pc-G* genes (Shanower et al., 2005). However, no data are available to prove a direct effect, as H3Me2K79 is not present at the telomeres (Shanower et al., 2005), whereas the tri-methyl isoform is absent from *Drosophila* (McKittrick et al., 2004). The effects of many other described TPE modifiers require thorough reassessment (Mason et al., 2004), as the early screenings for TPE modifiers did not account for the possible influence of the genetic background (Cryderman et al., 1999; Boivin et al., 2003). To summarize, the only feature that appears common for TAS regions and IH is that both of them appear to be subject to Pc-G-dependent silencing (Zhimulev et al., 2003b).

The distinct localization pattern observed for a number of chromatin proteins in the most distal regions of polytene chromosomes in the *Tel* stock is not unique to this mutant background. Thus far, HP1 and Pc-G proteins were localized to the distinct telomere domains in a stock with short *HeT-A/TAHRE/TART* tracts (Boivin et al., 2003). According to our data, HP1 did not co-localize with H3Me3K9 in *Tel* and *y w* stocks, which differ in *HeT-A/TAHRE/TART* array length. Finally, very similar protein localization patterns (most notably JIL-1 and Z4) were independently described in chromosomes of wild-type stocks (Wang et al., 2001; Eggert et al., 2004).

Nature of telomeric associations in polytene chromosomes

Telomeres in polytene chromosomes, as well as intercalary and pericentric heterochromatin regions, are capable of forming contacts with each other. Nevertheless, the nature of TAs and the mechanism of ectopic pairing of heterochromatic regions are obviously different, because the TA frequency is independent of the amount of SUUR protein, remaining unchanged whether *SuUR* gene is mutant or overexpressed. This contrasts with the observation that ectopic pairing of heterochromatic regions is completely undetectable in *SuUR* mutants and increases greatly with higher SUUR protein levels, concomitant with the increase in underreplication extent. As DNA underreplication is a prerequisite for ectopic pairing (Zhimulev, 1998), then either the telomeres are not underreplicated, or underreplication in telomeres is *SuUR*-independent. We demonstrate that there is no late replication in the region of cap and of the *HeT-A/TAHRE/TART* array in telomeres of *Tel* mutants, and therefore these regions might be undergoing complete replication. By contrast, underreplication was demonstrated for the TAS repeats in the minichromosome *Dp1187* (Karpen and Spradling, 1992) and for the w^+ reporter inserted into the TAS clusters of 2R and 3R chromosomes (Wallrath et al., 1996), ranging from 1.4- to 2.6-fold in extent. Nevertheless, TAs and ectopic pairing of heterochromatic regions in polytene chromosomes of salivary glands represent fundamentally distinct phenomena, because TAs appear to be mainly dependent on the size of the *HeT-A/TAHRE/TART* array. The removal of *Tel* (Siriaco et al., 2002) and *Su(var)205* mutant alleles (our data) from the genome did not modify the frequencies of TAs of chromosomes that were elongated in the mutant stock, whereas the newly introduced chromosomes with short telomeres displayed consistently low frequency of forming TAs in polytene tissue. Therefore, in both *Su(var)205* and *Tel* mutants, the TA frequency in polytene chromosomes largely depends on the length of the *HeT-A/TAHRE/TART* arrays, independently of whether associations of telomeres are resolved in diploid tissue (Fanti et al., 1998; Siriaco et al., 2002). In mutants, the lack of proteins encoded by the genes *Su(var)205, tefu (ATM), mre11* and *rad50* leads to a dramatic increase in frequency of telomeric fusions in diploid dividing cells. Since these associations of telomeres do not break in mitotic anaphase, this observation suggests that these proteins play an important role in protecting the telomeres from fusions (Fanti et al., 1998; Oikemus et al., 2004; Ciapponi et al., 2004). The important differences observed between the polytene and the mitotically dividing cells are most probably due to the fact that salivary gland differentiation takes place in early embryogenesis. Transition of mitotic divisions to endocycles occurs in 8-9-hour-old embryos (Orr-Weaver, 1994). At this time, the maternally contributed HP1 obtained from heterozygous *Su(var)205*/Balancer mothers is still sufficient to suppress telomeric fusions. If formed in the interphase of the last mitosis, associations of telomeres persist through the endocycles, and the polytene nucleus represents a relic of the pre-formed telomeric associations. In this situation, the key factor is the length of the *HeT-A/TAHRE/TART* array, whereas the deficit of maternal HP1 in mutant third instar larvae provides the explanation for the dependence of telomeric fusion frequency on HP1 level in mitotically dividing neuroblasts and imaginal disks cells.

Conclusion

We established that the three telomeric regions – cap, *HeT-A/TAHRE/TART* and TAS repeats – target specific sets of proteins and thus form distinct non-overlapping domains. We also demonstrated that the heterochromatin characteristics widely attributed to telomeres in salivary gland polytene chromosomes, such as formation of dense bands, late completion of replication, formation of swellings upon SUUR overexpression, ectopic contacts with intercalary and pericentric heterochromatin regions, involve not the telomeres but the subtelomeric regions, which in the chromosome arms X and 2R are typical IH regions. In chromosomes with normal, short telomeres, these regions appear to be located on the chromosome tips, and are misidentified as telomeric heterochromatin. Although cap and TAS regions resemble intercalary and pericentric heterochromatin in the protein repertoires bound, neither displays the abovementioned features of heterochromatin. This can be partly explained by the small sizes of cap and TAS regions: they are significantly smaller than the huge IH blocks that encompass hundreds of kilobase pairs of DNA. The short DNA sequences that form TAS repeats and cap complex can complete replication early and, therefore, replicate completely. As mentioned above, ectopic contacts in the IH largely depend on the degree of underreplication in these regions. Absence of detectable underreplication appears to lead to the inability of the telomeric regions to associate with other regions in heterochromatin. Formation of telomeric associations is possibly based on homologous pairing, which would be dependent on the copy number of *HeT-A/TAHRE/TART* and TAS repeats.

However, the small size of telomeric DNA is not the only factor that makes these regions unique. Although cap and TAS appear similar to heterochromatic regions, these domains are nevertheless distinct from heterochromatin, since they lack a typical heterochromatic protein marker, H3Me3K9. More striking is the overlapping localization of H3Me3K9 and of a number of typical euchromatic proteins within *HeT-A/TAHRE/TART* arrays. These findings argue that telomeric domains in polytene chromosomes should not be viewed as classic heterochromatin. The organization of telomeric domains is probably defined by the specific functions of these structures and requires further investigation, especially in diploid tissues and in the wild-type background.

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