2

Control of Telomere Length in *Drosophila*

Sergey Shpiz and Alla Kalmykova
Institute of Molecular Genetics, Russian Academy of Sciences, Moscow
Russia

1. Introduction

The problem of incomplete end replication of DNA was originally raised by the Russian scientist Aleksey Olovnikov (Olovnikov, 1971, 1973). The main function of telomeric DNA is the compensation of end degradation. In most eukaryotes, telomeric DNA is maintained by the action of telomerase, which is responsible for the synthesis of short 6-9 nucleotide repeats using an RNA component as a template (Greider and Blackburn, 1985). In contrast, telomeres of *Drosophila* are maintained as a result of retrotransposition of specialized telomeric non-long terminal repeat retrotransposons (Biessmann et al., 1990a; Biessmann et al., 1992a; Levis et al., 1993; Abad et al., 2004b).

Retrotransposons are also found in telomeric regions of such organisms as the silkworm *Bombix mori* (Okazaki et al., 1995; Takahashi et al., 1997), the green alga *Chlorella vulgaris* (Higashiyama et al., 1997) and a flagellated protozoan parasite *Giardia lamblia* (Arkhipova and Morrison, 2001). In *Bombyx mori* and *Chlorella*, there is a mixed type of telomere elongation: telomeric retrotransposons are inserted into telomerase-generated sequences (Fig.1). In *Drosophila* genomes, no telomerase orthologs have been found (Osanai et al., 2006). Elongation of *Drosophila* telomeres is mediated by specialized telomeric retroelement transpositions onto chromosome ends (Biessmann et al. 1992a; Levis et al. 1993). Recombination represents a bypass mechanism for chromosome length maintenance (Mikhailovsky et al. 1999; Kahn et al. 2000).

This review is focused on the mechanism of telomeric transposition control, which is a crucial step in *Drosophila* telomere elongation. Telomeric retroelements are arranged in *Drosophila* telomeres in mixed tandem head-to-tail arrays, with their 3′ ends orientated toward the centromere. Telomeric element transcripts serve as a template for transposition according to target-primed reverse transcription. In this case, as well as in telomerase encoding organisms, telomere elongation utilizes reverse transcription, i.e., synthesis of DNA from an RNA template. *Drosophila* represents a unique model system with an alternative mechanism of telomere maintenance. A characteristic feature of *Drosophila* telomeres is that the RNA template for telomere elongation is encoded by the telomeric sequences themselves, in contrast to a telomerase RNA component encoded by a separate cellular gene. Regulation of the activity of genes encoding telomerase and the RNA template as well as changes in concentration of the telomere repeat binding proteins play a crucial role in telomere length control in organisms that use telomerase. To understand the mechanism of length control of *Drosophila* telomeres, it is important to know how the addition of retrotransposon elements onto chromosome ends is regulated. This process is

directly associated with the transcriptional regulation of telomeric retrotransposons and chromatin structure in the telomeric region. Components of the telomere capping protein complex are involved in *Drosophila* telomere length control. Recent studies of *Drosophila* telomeres have demonstrated the importance of the RNA interference (RNAi) pathway in *Drosophila* telomere homeostasis. In the *Drosophila* germline, retrotransposons are silenced by the PIWI-interacting RNA (piRNA) pathway (Aravin et al. 2007). The telomeric retroelements *HeT-A, TART* and *TAHRE,* which are involved in telomere maintenance in *Drosophila*, are also the targets of the piRNA-mediated silencing. The abundance of telomeric retroelement transcripts, both sense and antisense, as well as the frequency of their transpositions onto chromosome ends, are controlled by a piRNA-mediated mechanism (Savitsky et al., 2006; Shpiz et al., 2007). piRNAs induce transcriptional silencing of the telomeric retrotransposons (Shpiz et al., 2011), suggesting a putative role for the piRNA pathway in the formation of the telomeric chromatin that protects chromosome ends from fusion and is involved in meiotic and mitotic telomere behavior.

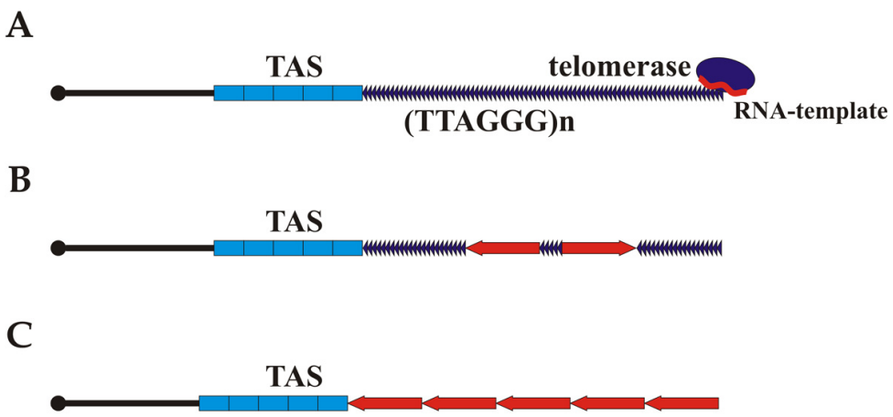

Fig. 1. Different modes of telomere elongation. (A) Telomeric DNA is maintained by telomerase, which synthesizes short 6-9 nucleotide arrays using an RNA component as a template. The human-specific telomeric repeat is shown. (B) A mixed type of telomere elongation: the telomeric retrotransposons are inserted into the short repeats formed by telomerase (*Bombyx mori, Chlorella*). (C) *Drosophila* telomeres are formed as a result of retrotransposition of specialized telomeric retrotransposons. Telomere-associated sequences (TASs) are indicated.

2. Structure of *Drosophila* telomeres

Telomeres are nucleoprotein complexes localized at the ends of linear chromosomes. Based on this, *Drosophila* telomeres can be subdivided into three domains (Andreyeva et al., 2005; Biessmann et al., 2005; Frydrychova et al., 2008) (Fig.2). The very end of the chromosome is protected by a special protein complex, the so-called telomeric cap. This structure prevents chromosome ends from end-to-end fusions and degradation by DNA repair mechanisms. The second domain accommodates the telomeric retrotransposon arrays that replace telomerase-generated repeats and supports chromosome end elongation. Lastly, proximally located repetitive complex sequences form subtelomeric or telomere associated sequences (TASs). These domains are characterized by specific chromatin structures that ensure proper telomere functioning.

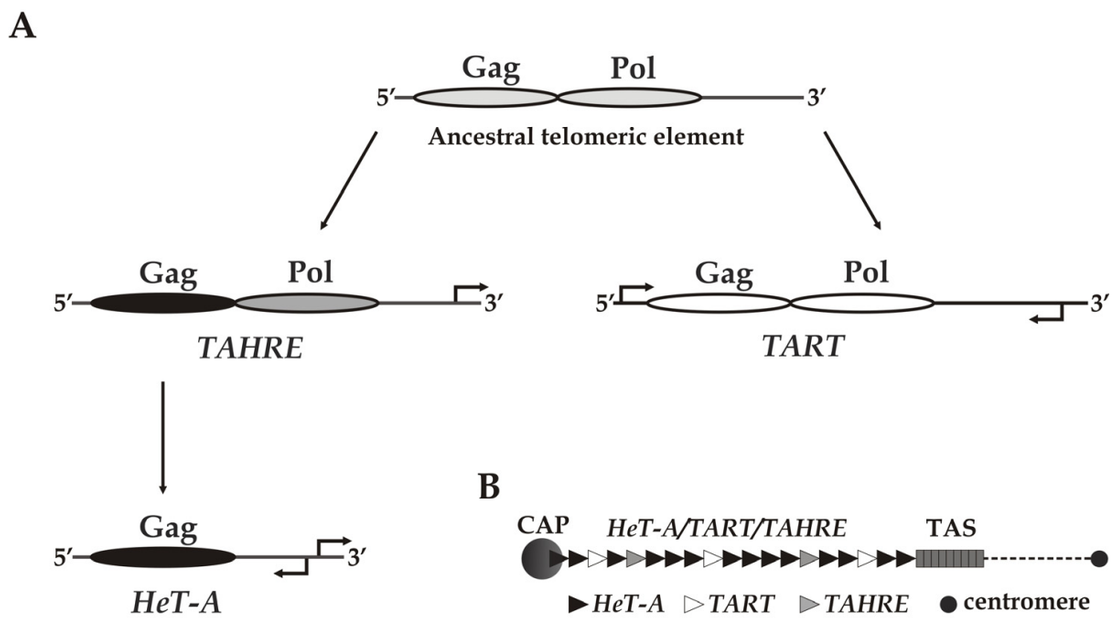

Fig. 2. *Drosophila* telomeres. (A) Putative evolutionary relationship between *D. melanogaster* telomeric retrotransposons. Transcription start sites are indicated by arrows. Ovals correspond to ORFs for Gag and Pol proteins. (B) *Drosophila* telomere region. The head-to-tail array of *HeT-A*, *TART*, and *TAHRE* retrotransposons is shown. The satellite-like TASs are located proximally. The protective protein cap complex is formed at the chromosome end.

2.1 *Drosophila* telomeric retrotransposons

The telomeres of *Drosophila melanogaster* consist of the specialized telomeric retrotransposons *HeT-A, TART* and *TAHRE* (Biessmann et al., 1992a; Levis et al., 1993; Abad et al., 2004b). They are LINE (long interspersed nucleic elements) or non-LTR (long terminal repeat)-type retroelements. Spontaneous transpositions of *HeT-A, TART* and *TAHRE* to telomeres have been observed, indicating that all three retrotransposon families participate in *Drosophila* telomere maintenance (Biessmann et al., 1992b; Sheen and Levis, 1994; Kahn et al., 2000; Golubovsky et al., 2001; Shpiz et al., 2007). *HeT-A, TART* and *TAHRE* are present at *Drosophila* telomeres in mixed tandem head-to-tail arrays; their oligo(A) tails always face proximally, towards the centromere. LINE elements use a target-primed reverse transcription mechanism for their transposition (Luan et al., 1993). It has been suggested that *Drosophila* telomeric retrotransposons can use the 3′ protruding end of the chromosome as a primer, but the mechanism of site-specific transpositions of telomeric retroelements to the chromosome end remains unclear. Telomere targeting of retrotransposon mRNA is the most crucial stage in telomere elongation. It has been proposed that Gag proteins target the telomeric retroelement mRNA to chromosome termini in cultured *Drosophila* cells (Rashkova et al., 2002; Rashkova et al., 2003; Fuller et al., 2010).

HeT-A, the most abundant *Drosophila* telomeric element, contains a single open reading frame (ORF) encoding a Gag-like RNA-binding protein but lacks reverse transcriptase (RT). *TART* has two ORFs, encoding the Gag and Pol proteins. The ORF2 has both endonuclease and RT domains. Both elements have unusually long 3′ and 5′ untranslated regions (UTR).

TAHRE as well as *TART* has two ORFs ; ORF2 of *TAHRE* is similar to that of *TART*. The 5′ UTR, ORF1 and 3′ UTR of *TAHRE* are similar to the corresponding regions of *HeT-A,* which prompted the designation of a newly discovered telomeric element *TAHRE* (Telomere-Associated and *HeT-A*-Related Element) (Abad et al., 2004b). It was proposed that a putative ancestral element evolved to provide telomere maintenance in *Drosophila* (Fig.2A). *TART* and *TAHRE* diverged from a common ancestor. *HeT-A* lacks ORF2 and may have derived from a processed copy of *TAHRE* (Abad et al., 2004b). All of the analyzed *D. melanogaster* stocks have both *HeT-A* and *TART* elements, and the copy numbers are approximately 30 *HeT-A* and 10 *TART* per genome (Abad et al., 2004a; George et al., 2006). A single complete and three truncated *TAHRE* were identified in the genome of the *Drosophila* stock sequenced by the Genome Project (Abad et al., 2004b). Obviously, the structural role is not a primary function for *TART* and *TAHRE*, as some telomeres contain neither *TART* nor *TAHRE* elements (Levis et al., 1993; Abad et al., 2004a; Shpiz et al., 2007). It has been proposed that the RT activity necessary for *HeT-A* transposition might be provided by *TART* or *TAHRE* (Levis et al., 1993; Rashkova et al., 2002; Abad et al., 2004b). It is noteworthy that autonomous and nonautonomous telomere-specific retrotransposons were described in the genomes of evolutionary distant *Drosophila* species (Villasante et al., 2007). One of the reasons for the cooperation of several telomeric elements throughout evolution may be the distribution of different roles among elements.

Most of the non-LTR retrotransposons utilize an internal 5′UTR promoter to transcribe a full-length RNA that serves as a template for transposition. An unusual feature of the telomeric retroelements is that the promoters of *HeT-A* and *TAHRE* are localized in the 3′ UTR and drive transcription of a downstream element (Danilevskaya et al., 1997; Shpiz et al., 2007). An antisense promoter was detected in close proximity to the *HeT-A* promoter, which drives sense expression (Shpiz et al., 2009). It appears as if the common promoter drives bidirectional expression of *HeT-A*. The *TART* element was shown to also be transcribed bidirectionally from internal sense and antisense promoters that are localized within non-terminal direct repeats in the *TART* 5′ and 3′ regions (Danilevskaya et al., 1999; Maxwell et al., 2006). An unusual feature of non-coding *HeT-A* and *TART* antisense transcripts is that they are spliced (Maxwell et al., 2006; Shpiz et al., 2009). The role of antisense transcripts in the RNA silencing of the telomeric retrotransposons will be discussed below.

According to cytological and genetic data, chromatin in the region of telomeric retrotransposon arrays exhibits euchromatic characteristics. There are several examples of *white* transgene integration into coding and promoter regions of *HeT-A*, *TART* and *TAHRE*. In most cases, when the insertion is far from a TAS, the normal activity of a reporter gene is observed (Biessmann et al., 2005). The longer the *HeT-A* array is upstream of the telomeric *white* transgene located between the TAS and telomeric retroelements, the higher the expression of the reporter gene (Golubovsky et al., 2001). This indicates that telomeric retrotransposon arrays may activate the expression of nearby genes; however, the mechanism of this *trans*-activation remains unknown. Cytological studies have also characterized the region of telomeric retroelements as a zone of decondensed chromatin similar to euchromatin (Andreyeva et al., 2005; Biessmann et al., 2005). It is noteworthy that in organisms encoding telomerase, proteins binding to the telomerase-generated repeats form a heterochromatic silencing complex (Schoeftner and Blasco, 2009).

2.2 Structure of the *Drosophila* capping complex

The protein complex at the chromosome end forms a cap that protects DNA ends against the repair system and prevents telomere fusions. The formation of the *Drosophila* cap does not require specific telomeric sequences at the chromosome ends. Terminally deleted chromosomes in the absence of telomeric and subtelomeric sequences may form a cap as well as natural telomeres (Fanti et al., 1998; Perrini et al., 2004). Several proteins that protect *Drosophila* telomeres from end-to-end fusion events have been identified (Cenci et al., 2005). Among them are HOAP (Heterochromatin Protein 1/origin recognition complex-associated protein), HipHop, Moi (Modigliani) and Ver (Verrocchio), which are the founding components of a *Drosophila* capping complex (Cenci et al., 2003; Raffa et al., 2009; Gao et al., 2010; Raffa et al., 2010). These proteins are highly enriched at telomeres. The HOAP/Ver/Moi complex is a functional analog of shelterin, a protein complex that protects human chromosome ends (Palm and de Lange, 2008). This complex was named "terminin" (Raffa et al., 2009). Terminin accumulation at chromosome ends prevents telomere fusion and helps in recruiting nonterminin components of the *Drosophila* capping complex. It was proposed that *Drosophila* lost the shelterin that binds telomeric DNA in a sequence-specific fashion and evolved terminin to bind chromosome ends independent of the terminal DNA sequence. HP1 (heterochromatic protein 1) is another important structural component of the *Drosophila* chromosome cap (Fanti et al., 1998). In addition to the chromosome cap, this protein is also associated with centromeric regions and many euchromatic sites. Mutations in the HP1 coding gene cause aberrant chromosome associations and telomeric fusions in neuroblast cells, imaginal disks, early embryos and male meiotic cells, providing evidence that HP1 mediates normal telomere behavior in different *Drosophila* cells and tissues (Fanti et al., 1998). Mutations in genes encoding ATM and ATR kinases, which are the main enzymes of the cell response to DNA damage, and components of the MRN repair complex also cause telomere fusions (Bi et al., 2004; Ciapponi et al., 2004; Oikemus et al., 2004; Silva et al., 2004; Song et al., 2004; Bi et al., 2005). However, these proteins are not stable cap components and, most likely, they mediate HP1/HOAP/Moi recruitment at chromosome ends (Fig.3).

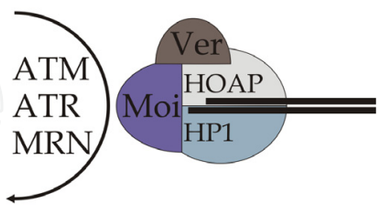

Fig. 3. *Drosophila* telomeric cap structure. The main structural cap components HOAP, Moi, Ver and HP1 are recruited to the chromosome ends by the assistance of the ATM, ATR and MRN components of the DNA damage response.

It has been suggested that transient interactions between DNA repair factors and terminal DNA sequences facilitate association of HOAP-HP1 with chromosome termini, which in turn would recruit Moi. A specific feature of the cap assembly is the redundancy of the pathways that affect capping protein recruitment to the telomere. For example, telomeric

localization of both HP1 and HOAP requires functional Mre11/Rad50 components of the MRN complex. However, even in the absence of this complex, mitotic chromosomes retain the ability to recruit low levels of HOAP (Ciapponi et al., 2004). ATM and ATR kinases act redundantly in telomere protection (Bi et al., 2005). ATM may fully compensate ATR's absence in telomere capping while ATR may partially compensate for the loss of ATM (Bi et al., 2005). The mutations in the *effete* and *woc* genes, which encode an ubiquitin conjugating enzyme and a putative transcriptional factor, respectively, result in *Drosophila* telomere fusions; however, their role in telomere capping is still unclear (Cenci et al., 1997; Raffa et al., 2005). Heterochromatic proteins such as HP2 (a partner of HP1), Su(var)3-7 (a protein of pericentromere chromatin containing zinc fingers) and SUUR (a protein localized in late replicated regions, which is typical for heterochromatin) bind to polythene chromosome ends in the salivary glands (Andreyeva et al., 2005), suggesting that cap represents condensed chromatin structure. The repressive effect of the cap on the expression of terminally located genes extends as far as 4 kb (Mikhailovsky et al., 1999; Melnikova et al., 2004). Thus, in natural telomeres, only very distal telomeric retroelement may be affected by the capping complex.

Capping complexes might control telomere length by affecting the accessibility of the chromosome termini for telomeric element transposition. This process is of great importance in germinal cells. However, the majority of available data regarding cap structure was obtained by inspecting somatic cells, such as interphase salivary gland cells and mitotic larval brain cells. It should be noted that cap structure and the mechanisms of its assembly appear to differ in distinct cells and/or during the cell cycle. For example, a mutation in the gene *tefu* encoding ATM did not change the localization of HOAP at the telomeres of mitotic chromosomes, but it did decrease the amount of HOAP and HP1 at the telomeres of salivary gland polytene chromosomes (Bi et al., 2004; Oikemus et al., 2004). No detailed information about the cap structure of the germ cell chromosomes has been obtained. HP1 has been found at the ends of normal chromosomes as well as of terminally deleted chromosomes in the nuclei of nurse cells in *Drosophila* ovaries by immunohistochemistry (Shpiz et al., 2011) . Chromatin immunoprecipitation (ChIP) analysis revealed binding of both HP1 and HOAP with *HeT-A* in ovaries (Khurana et al., 2010). These data indicate that at least HP1 and HOAP provide chromosome capping in ovarian cells. Recent studies revealed a role of the piRNA pathway components in the assembly of the telomere capping complex in early embryogenesis (Khurana et al., 2010). piRNAs are essential for the retrotransposon silencing in the germline (Vagin et al., 2006; Brennecke et al., 2007; Aravin et al. 2007). *armi* and *aub* piRNA pathway components, which encode a putative RNA helicase and a piRNA binding Argonaute protein, respectively, are needed for telomere resolution during mitotic divisions in early embryos (Khurana et al., 2010). Maternally deposited piRNA pathway proteins likely function at this developmental stage (Brennecke et al., 2008). ChIP analysis on mutant *armi* and *aub* embryos revealed reduction (but not elimination) of the HOAP and HP1 binding to *HeT-A*, indicating the role of these components in telomere cap assembly. It is noteworthy that neither *ago3* nor *rhi* piRNA components affect HOAP or HP1 binding to *HeT-A*. A subpopulation of *HeT-A*-specific piRNAs was proposed to direct assembly of the telomere cap (Khurana et al., 2010). However, in *Drosophila*, chromosome capping is a sequence-independent process. *aub* and *armi* likely provide a redundant pathway for the recruitment of telomere capping proteins in early embryogenesis.

2.3 Subtelomeric region

The TAS is an extended heterochromatic chromosome region that is proximally adjacent to telomeric repeats in different organisms, the function of which remains unclear. In *Drosophila*, TASs consist of 15-25 kb of satellite-like repeats or fragments of mobile elements. TASs differ in length and sequence among chromosome arms, although some repeats share similarity. 2L TAS has homology with 3L TAS, while X TAS shares homology with 2R and 3R TAS. For example, TASs of the 2L and 3L chromosome arms are similar 460 bp repeats. X TASs are more complex repeats that are 0.9 and 1.8 kb in size (Karpen and Spradling, 1992; Wallrath and Elgin, 1995). The small fourth chromosome of *Drosophila* has a special TAS structure that is made of fragments of various mobile elements (Cryderman et al., 1999). *Drosophila* salivary gland polytene chromosome subtelomeric regions look cytologically more condensed when compared with a region resembling euchromatin and containing telomeric retrotransposons (Andreyeva et al., 2005; Biessmann et al., 2005). The eye color reporter gene *white* becomes silenced after insertion into a TAS (Karpen and Spradling, 1992; Cryderman et al., 1999; Golubovsky et al., 2001). Inactivation of a reporter gene inserted into a subtelomeric region is referred to as telomeric position effect (TPE). Transgenes in a TAS or close to a TAS demonstrate repressed and variegated expression. The silencing effect spreads to the limited distance from the TAS into the telomeric retrotransposon array (Frydrychova et al., 2007). As mentioned above, the reporter genes in the terminal *HeT-A, TAHRE,* or *TART* retroelements do not exhibit repressed expression (Biessmann et al., 2005).

In contrast to the classic position effect related to the suppression of a gene integrated into pericentric heterochromatin, *Drosophila* TPE does not depend on HP1 and known modifiers of the position effect. An exception is the fourth chromosome, where TPE depends on HP1 (Cryderman et al., 1999). This is not surprising because the fourth chromosome is cytologically located in the chromocenter, a region enriched with heterochromatic proteins. The TAS regions of most chromosome arms contain motifs recognized by the repressors of the Polycomb (PcG) protein family (Cryderman et al., 1999; Boivin et al., 2003; Andreyeva et al., 2005). The chromatin of the TAS region is enriched with methylated Lys27 of histone H3 (H3Me3K27) due to the activity of the histone methyltransferase E(z) (Czermin et al., 2002; Andreyeva et al., 2005). E(z) is a member of the Polycomb group proteins and exhibits chromomethylase activity toward Lys9 and 27 of histone H3 (Czermin et al., 2002). Methylation at these positions is a label that attracts PcG repressor proteins, which results in gene silencing. Interestingly, translocations of the second chromosome TAS to the fourth chromosome and vice versa retained the structure of the chromatin and TPE features in the translocated regions (Cryderman et al., 1999). The translocation of the fourth chromosome TAS to the second chromosome caused a sharp decrease in TPE-induced transgene silencing while dependence of TPE on HP1 within the translocated fragment was retained (Cryderman et al., 1999). This phenomenon may be attributed to changes in nuclear localization of the translocated fragment. Indeed, the fourth chromosome is usually positioned in the chromocenter that is enriched with heterochromatic proteins, whereas translocation transfers it to the periphery of the nucleus. These results suggest that TASs provide sequence-specific assembly of protein complexes involved in larger protein nuclear compartments. In addition, flies with a TAS deficiency on chromosome 2L show an increase in the expression level of telomeric *white* transgenes located both at homologous and non-homologous chromosomes, suggesting long-range telomere communication in the nucleus (Golubovsky et al., 2001; Mason et al., 2003; Frydrychova et al., 2007). Based on these data, it

is tempting to propose that TASs and associated proteins play a key role in processes related to telomere positioning inside the nucleus.

3. Mechanisms of *Drosophila* telomere length control

Telomeres formed by telomerase consist of short repeats like TTAGGG in vertebrates. These repeats form a double-stranded DNA sequence of several thousand base pairs and a single strand 3′ overhang of several hundred nucleotides in length. Embedding of the single strand end into the telomeric DNA duplex forms a telomeric t-loop (de Lange, 2004). The telomeric repeats as well as the t-loop are recognized by specific DNA-binding telomeric proteins, representing a platform for assembly of the telomere–protein complex. This complex stabilizes the structure of the t-loop, protects the chromosome end against degradation and controls telomere length by regulating the accessibility of the chromosome end for telomerase (Smogorzewska and de Lange, 2004). In vertebrates, conservative DNA-binding telomeric proteins TRF1 and TRF2 directly bind the duplex of telomeric repeats and recruit other proteins to the telomere (such as the tankyrases and RAP1). TRF1 and TRF2 also associate with proteins of the DNA repair system including the heterodimer Ku70/Ku80, the MRN complex (MRE11, RAD50, NBS1) and ATM-kinase (Chan and Blackburn, 2002; Goytisolo and Blasco, 2002; Smogorzewska and de Lange, 2004). Several dozen proteins are involved in the formation of the telomere complex in mammals. The mode of the complex formation is similar for different organisms and includes sequence-specific recognition of telomeric repeats.

The heterogeneity of terminal sequences distinguishes *Drosophila* telomeres from the telomeres formed by short repeats. The transposition of three various retroelements to the chromosome end and the process of end degradation results in varied terminal nucleotide sequences, even between chromosomes of the same individual. It remains unclear whether *Drosophila* telomeres terminate with the long 3′ overhang and form the t-loop like structure. Indirect evidence for this possibility is that *Drosophila* telomeres can be elongated as a result of the recombination of tandem repeats located on the same chromosome (Kahn et al., 2000; Savitsky et al., 2002; Melnikova et al., 2005). It is possible that recombination is facilitated by integration of the chromosome end into the internal region of terminal DNA. Nevertheless, no evidence for the existence of a stable t-loop in *Drosophila* has been obtained by molecular or cytological methods. Thus, *Drosophila* telomeres contain neither specific terminal sequences nor t-loop configuration. Although regulation of telomere length in telomerase-expressing organisms is a complex process that depends on numerous factors, it may be described as protein titration on telomeric DNA: a decrease in telomeric proteins on a shortened telomere increases its susceptibility to telomerase in germinal cells or represents a signal for arrest of somatic cell division (Chan and Blackburn, 2002; Smogorzewska and de Lange, 2004).

The activity of genes encoding telomerase and the RNA template are also important factors regulating telomere length (Nugent and Lundblad, 1998). In *Drosophila,* no specific signaling pathway responsible for the retrotransposon addition to the shortened telomere has been discovered so far. Taking into account the peculiarities of *Drosophila* telomere structure, one may suggest that two steps are crucial for its telomere elongation: accessibility of the chromosome end for transpositions and control of the transposition frequency through the regulation of the telomeric retrotransposon expression. Table 1 represents a list of genes or genomic loci that affect *Drosophila* telomere length that confirm this hypothesis and demonstrate a striking crosstalk between the mechanisms of telomere protection and regulation of telomeric retrotransposon expression.

Protein/ Function	Mutant allele	Mutant phenotype				Reference
		Increased transpositions to TD	Increased length of natural chromosomes	Increased HeT-A/TART expression	Telomere fusions	
HP1/hetero chromatic protein	*Su(var)2-5*^{02} *Su(var)2-5*^{04} *Su(var)2-5*^{05}	+ + +	+ + +	+ (s) + (s) + (s)	- + +	Savitsky et al. 2002 Fanti et al. 1998 Perrini et al. 2004
Ku70/Ku80/ DNA repair system	deficiencies	+	+	-	-	Melnikova et al. 2005, Cenci et al. 2005
Tel/genomic locus controlling telomere length	*Tel* (from natural *Gaiano* stock)	ND	+	+ * (s)	-	Siriaco et al. 2002
SpnE/piRNA pathway, RNA helicase	*spnE*^{1}/ *spnE*^{hls3987}	+	-	+ (ov)	ND	Savitsky et al. 2006
Aub/ piRNA pathway, PIWI subfamily protein	*aub*^{QC42}/ *aub*^{HN}	+	-	+ (ov)	+	Savitsky et al. 2006, Khurana et al. 2010
Armi/ piRNA pathway, RNA helicase	*armi*^{1}/ *armi*$^{72.1}$	ND	+	+ (ov)	+	Khurana et al. 2010, Malone et al. 2009
Rhi/piRNA pathway, germline-specific homologue of HP1	*rhi*^{02086}/ *rhi*^{KG00910}	ND	+	+ (ov)	-	Khurana et al. 2010, Klattenhoff et al. 2009
Ago3/ piRNA pathway, PIWI subfamily protein	*ago3*^{4931}/ *ago3*^{3658}	ND	+	+ (ov)	-	Khurana et al. 2010, Li et al. 2009, Malone et al. 2009

TD terminally deleted chromosome; *ND* not determined; *s* somatic tissues; *ov* ovaries
* The increased level of *HeT-A* transcripts in the *Gaiano* stock is likely a consequence of high *HeT-A* copy number in the *Gaiano* telomeres (Savitsky et al. 2002).

Table 1. Negative regulators of *Drosophila* telomere length.

3.1 Role of the capping complex in *Drosophila* telomere length control

The frequency of spontaneous *HeT-A* transpositions to the chromosome end has been estimated to be in the range of 10^{-5} to 10^{-3} (Biessmann et al., 1992a; Kahn et al., 2000). Mutations in the *Su(var)2-5* gene encoding the major heterochromatic protein and cap component HP1 increase the addition of *HeT-A/TART* to the ends of terminally truncated chromosomes by more than a hundred times (Savitsky et al., 2002). HP1 mutations cause significant increases in *HeT-A* and *TART* expression. *Drosophila* lines harboring *Su(var)2-5* mutations maintain extremely long *HeT-A/TART* arrays at the natural chromosome termini (Savitsky et al., 2002; Perrini et al., 2004). Telomere elongation and *HeT-A/TART* derepression are observed in all studied *Su(var)2-5* mutants, but one of these mutations that disrupts the chromodomain does not influence HP1 capping capacity (Fanti et al., 1998; Perrini et al., 2004). It appears that the HP1 silencing effect on telomeric retrotransposon expression is more crucial for the negative control of telomere length than its role in the telomere capping. It should be noted here that the expression of *HeT-A/TART* and cap formation was studied in the somatic tissues of *Su(var)2-5* mutants, while terminal attachments causing telomere elongation that are detectable in the progeny take place in the germ cells. Thus, to elucidate the specific role of HP1 in the control of telomere length, its impact on the telomere biology in the germline should be investigated.

The Ku70/Ku80 heterodimer, a component of the DNA repair system, is an essential component of the human telomeric protein complex (Song et al., 2000; d'Adda di Fagagna et al., 2004; Jaco et al., 2004; Myung et al., 2004). A decrease in *Ku70* or *Ku80* gene dosage in *Drosophila* causes a sharp increase in the frequency of *HeT-A* and *TART* attachments to broken chromosome ends and in terminal DNA elongation by gene conversion (Melnikova et al., 2005). Ku70 mutant flies have elongated telomeres that contain an increased number of *HeT-A* and *TART* elements (Cenci et al., 2005). At the same time, a reduced concentration of Ku70 or Ku80 does not affect *HeT-A* transcript abundance in flies, and Ku70 null mutation does not cause telomeric fusions (Cenci et al., 2005; Melnikova et al., 2005). A role of the Ku complex in the accessibility of *Drosophila* chromosome termini for transpositions has been suggested (Melnikova et al., 2005).

In the HOAP mutants, as well as in the double ATM/ATR mutants, *HeT-A* expression is increased (Bi et al., 2005). However, it is currently unknown whether mutations of other than HP1 cap components lead to excessive telomere elongation.

The dominant factor *Telomere elongation (Tel)* was genetically identified in the natural *Drosophila Gaiano* stock that has unusually long telomeres (Siriaco et al., 2002). *E(tc)* locus, which affects terminal gene conversion, was also mapped to the same chromosome region (Melnikova and Georgiev, 2002). These factors might be different alleles of the same, but as yet unidentified, gene involved in *Drosophila* telomere length control.

3.2 Mechanisms of regulation of telomeric retrotransposon expression in somatic tissues

Telomeric retrotransposon transcripts serve as templates for the synthesis of proteins necessary for transposition as well as for reverse transcription primed by the 3′ end of the telomeric DNA. As a result of end underreplication, *Drosophila* telomeres shorten by 75 bp per generation (Biessmann and Mason, 1988; Levis, 1989; Biessmann et al., 1990b), whereas

transposition of full-size retrotransposon results in chromosome elongation by 6-12 kb. Evidently, maintenance of normal length of telomeres requires strict control of transposition frequency and transcriptional activity of telomeric retroelements. In spite that the telomeric array exhibits euchromatic features telomeric retrotransposon expression is repressed in somatic tissues. Several negative regulators of telomeric element expression have been shown, such as HP1, ATM and ATR kinases, and PROD protein (Savitsky et al., 2002; Bi et al., 2005; Torok et al., 2007). It was proposed that a special chromatin structure forms along telomeric retrotransposon array providing retrotransposon silencing (Frydrychova et al., 2008). The mechanism of the telomeric element silencing in the somatic tissues is unknown. The role of RNA interference system in this process is still not clear. Twenty-one nucleotide long endogenous siRNAs (endo-siRNAs) have been identified in the somatic cells of *D. melanogaster* (Czech et al. 2008; Ghildiyal et al. 2008; Kawamura et al. 2008). This class of short RNAs is produced in a Dicer-2-dependent manner and can direct AGO2 to cleave target RNAs. A subset of endo-siRNAs that are homologous to retrotransposons including *HeT-A* was identified. However, *HeT-A* expression was not significantly affected by the RNAi pathway disruption in cell culture or imago tissues (Ghildiyal et al. 2008). It should be noted that the *HeT-A* promoter is active in replicating diploid larval tissues (George and Pardue, 2003). Evidently, endo-siRNAs control the steady-state abundance of *HeT-A* RNA only in those somatic tissues where the *HeT-A* promoter is active. Detailed histological analysis is needed to learn more about the contribution of the endo-siRNA pathway to telomeric retrotransposon silencing in the fly soma.

Importantly, we found that the expression of telomeric retrotransposons and the frequency of their transpositions onto chromosome ends are specifically regulated by an RNAi-based mechanism in the germline where heritable transpositions occur (Savitsky et al., 2006). These data are significant for the understanding of the control of *Drosophila* telomere length.

3.3 Role of the piRNA pathway in *Drosophila* telomeric retrotransposon expression in the germline

The piRNA pathway is directed by a distinct class of 24-30-nucleotide-long RNAs called PIWI-interacting RNAs (piRNAs), which are produced by a Dicer-independent mechanism and associated with Argonaute proteins from the PIWI subfamily (Brennecke et al., 2007; Aravin et al., 2008) (Fig.4). The piRNA pathway protects the genome in germ cells from transposable element activity. Piwi, Aubergine (Aub) and Argonaute 3 (AGO3) bind piRNAs and serve as core components of the piRNA machinery in *Drosophila* ovaries (Saito et al. 2006; Brennecke et al. 2007; Gunawardane et al. 2007; Li et al. 2009; Malone et al. 2009). These proteins are engaged in an amplification loop to mediate the generation of sense and antisense piRNAs from the transposon transcripts (Brennecke et al., 2007). Other proteins such as RNA helicases Spindle-E (Spn-E) and Armitage (Armi), nucleases Zucchini (Zuc) and Squash (Squ), the germline-specific homologue of HP1 Rhino and the product of the *vasa* locus are involved in transposon silencing in the germline and are required for piRNA production/stabilization (Cook et al. 2004; Vagin et al. 2004; Vagin et al. 2006; Pane et al. 2007; Klattenhoff and Theurkauf 2008; Klattenhoff et al. 2009; Malone et al. 2009). Transposon derepression and transpositions are observed in piRNA pathway mutants, pointing to the primary role of this system in the silencing of parasitic elements (Aravin et al. 2001; Sarot et al. 2004; Vagin et al. 2004; Kalmykova et al. 2005).

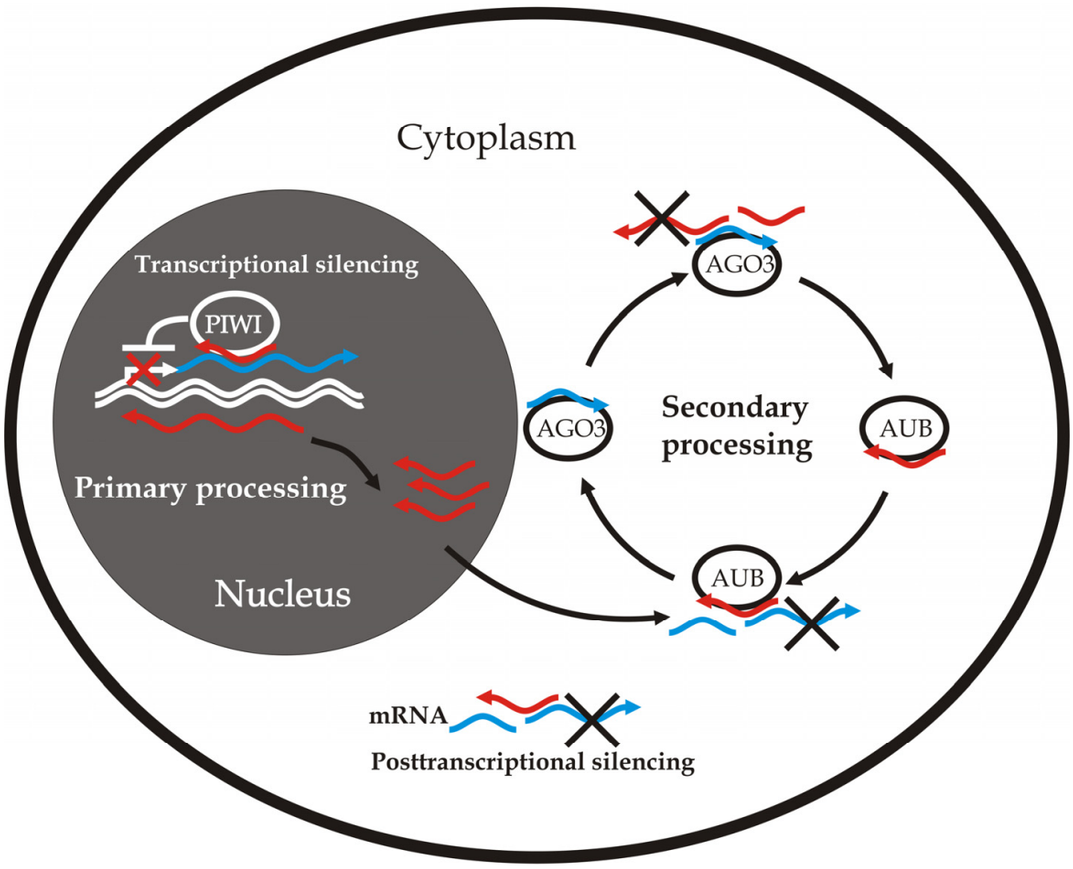

Fig. 4. piRNA pathway. The diagram shows the principal steps of the production and actions of piRNAs. piRNA-mediated protein complexes cleave complementary mRNAs in the cytoplasm or silence homologous loci in the nucleus.

In spite of their vital genomic function, *Drosophila* telomeric retroelements as well as other parasitic transposons were shown to be targets of the piRNA-mediated silencing pathway (Vagin et al., 2004; Savitsky et al., 2006; Shpiz et al., 2007; Shpiz et al., 2009). Mutations in the *spn-E, aub, piwi, squ* and *zuc* genes and *vasa* locus result in accumulation of telomeric element transcripts in ovaries (Vagin et al. 2004; Savitsky et al. 2006; Pane et al. 2007; Shpiz et al. 2007; Shpiz et al. 2009), but the same mutations do not affect telomeric element expression in *Drosophila* testes (A.K., unpublished data). *In situ* RNA hybridization analysis has revealed different patterns of accumulation of *HeT-A, TART* and *TAHRE* transcripts in the ovaries of piRNA mutants (Savitsky et al., 2006; Shpiz et al., 2007). *TART* transcripts accumulate in supporting nurse cells predominantly at the late stages of oogenesis, whereas *HeT-A* and *TAHRE* transcripts are detected in a growing oocyte from the earlier stages of oogenesis. This finding suggests that *TAHRE* rather than *TART* is a source of reverse transcriptase for the transpositions of non-autonomous *HeT-A* elements. Sense and antisense piRNAs specific to telomeric retrotransposons have been revealed in libraries of short RNAs and by Northern analysis (Saito et al. 2006; Savitsky et al. 2006; Brennecke et al. 2007; Shpiz et al. 2007; Malone et al. 2009; Shpiz et al. 2009). Their levels are dramatically lower in the ovaries of piRNA mutants, which correlate with increased expression.

Antisense transcripts of transposable elements are important intermediates in the piRNA pathway because they serve as templates for piRNA generation (Brennecke et al., 2007; Gunawardane et al., 2007). Interestingly, both *HeT-A* and *TART* produce long non-coding processed antisense transcripts from their internal promoters (Maxwell et al., 2006; Shpiz et al., 2009). The *HeT-A* antisense transcription start site was mapped to the 3′ UTR of this element 150 bp upstream of the sense transcription start site. *HeT-A* and *TART* antisense transcripts are targets of the piRNA pathway and accumulate in the germ cell nuclei of the piRNA pathway mutants. Thus, steady-state expression of *HeT-A* and *TART* retrotransposons could be a result of an intricate piRNA-mediated interplay of their sense and antisense transcripts (Shpiz et al., 2009). Thus, the piRNA system suppresses excessive retrotransposon activity and maintains transcripts at low levels because this system requires the presence of sense and antisense transcripts to act as triggers of this mechanism.

3.4 Role of the piRNA pathway in *Drosophila* telomere length control and telomeric chromatin assembly

We have shown that increased expression of *HeT-A/TART* in the piRNA pathway mutants results in an increased rate of their transpositions onto chromosome ends, i.e., telomere elongation (Savitsky et al., 2006). To screen for new transpositions on chromosome ends in the piRNA pathway mutants, we used truncated X chromosomes (designated y^{TD}) with a break in the *yellow* locus that controls body and bristle pigmentation. The break is located in the upstream regulatory region and results in the y^2-like fly phenotype with yellow aristae (a bristle-like part of the antenna). The addition of *HeT-A* or *TART* retroelements can be monitored by a yellow-to-black change in aristae pigmentation (Savitsky et al., 2002; Savitsky et al., 2006) (Fig.5).

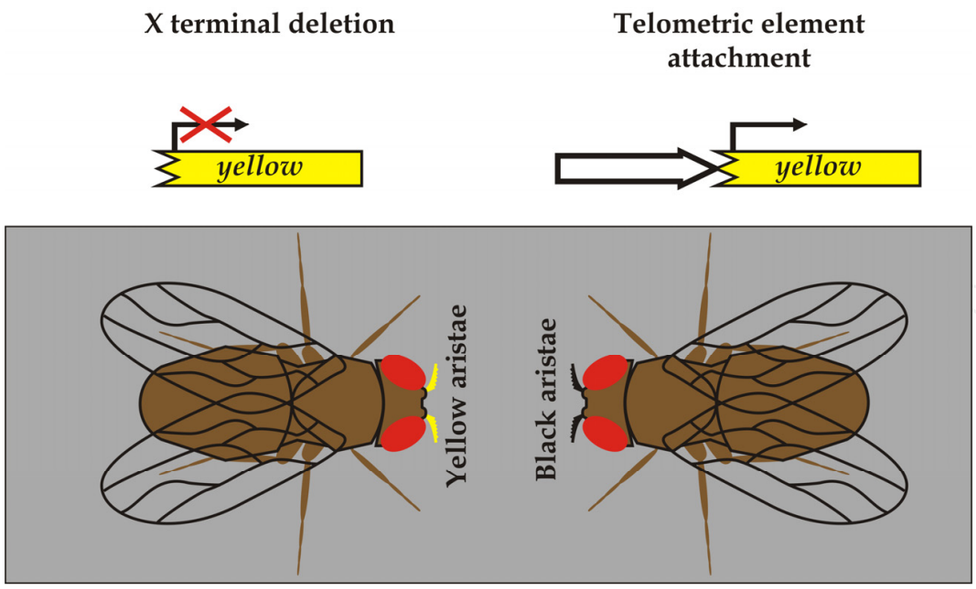

Fig. 5. Genetic assay to measure the frequency of telomeric element attachments to the broken chromosome end. Schematic representation of the telomeric retrotransposon attachments to the broken *yellow* gene located at the end of the terminally deleted X chromosome. As a result of terminal attachment, *yellow* is activated, causing yellow-to-black change in aristae pigmentation.

Both *spn-E* and *aub* mutations have a strong dominant effect on the frequency of attachments, resulting in a 20- to 100-fold increase in the appearance of flies with black-colored aristae. In flies carrying a single copy of the *spn-E* or *aub* mutant allele, the majority of the new transpositions were *TART*, whereas in the ovaries of sterile homozygous *spn-E* females, *HeT-A* transpositions were more frequent. This difference might be explained by peculiarities of the dosage effect of the piRNA mutations on *HeT-A/TART* expression. Clusters of flies with identical *TART* attachments were isolated in the progeny of *spn-E* and *aub* mutants, indicating that piRNA-mediated control of terminal transpositions occurs in premeiotic cells. It is noteworthy that despite the greatly increased frequency of *HeT-A* and *TART* attachments to the broken ends observed in this assay, *spn-E* or *aub* mutant lines do not have detectably longer telomeres on their native chromosomes (Savitsky et al., 2006; Khurana et al., 2010). This indicates that the truncated chromosome end is more sensitive to telomeric element attachments than the native telomere. Despite the fact that the protein cap can be formed on the broken chromosome ends, they lack both telomeric retrotransposons and subtelomeric repeats, which results in considerably different chromatin structure. The attachment of retrotransposons to native telomeres is likely impeded compared to the truncated chromosome. A recent study detected an increase in *HeT-A* copy number in *rhi*, *armi* and *ago3* piRNA pathway mutant stocks (Khurana et al., 2010). Among them, only the *armi* mutation affects HOAP and HP1 capping protein recruitment in early embryogenesis, whereas all of them cause telomeric element derepression in the germline. It seems that transcript accumulation is a main reason for telomere elongation in piRNA pathway mutants. However, piRNAs may affect not only transcript abundance but also chromatin structure.

Distinct short RNA-mediated silencing mechanisms have been described. Short RNAs have been shown to target the associated protein complex to degrade complementary mRNAs that mediate post-transcriptional silencing (Elbashir et al., 2001). Short-RNA-mediated heterochromatin assembly was described in fission yeast, plants and ciliates (Hamilton et al., 2002; Volpe et al., 2002; Liu et al., 2004). In this case, heterochromatinization diminishes the transcriptional capacity of the target locus, resulting in transcriptional silencing. In mice, transposon-specific piRNAs drive transposon promoter methylation in the male germline (Aravin et al., 2008; Kuramochi-Miyagawa et al., 2008). In the *Drosophila* model, the mechanism of the piRNA-mediated gene silencing has remained obscure. The three *Drosophila* PIWI proteins PIWI, Aub and Ago3 cleave complementary RNA *in vitro,* suggesting their involvement in the post-transcriptional degradation of mRNA (Saito et al., 2006; Brennecke et al., 2007; Gunawardane et al., 2007). piRNAs were shown to mediate post-transcriptional retrotransposon mRNA degradation into cytoplasmic bodies in the *Drosophila* germline (Lim et al., 2009). There is also evidence for the influence of piRNAs on the chromatin state (Josse et al., 2007; Klenov et al., 2007). In our recent study, we addressed the mechanism of piRNA-mediated silencing of telomeric retrotransposons (Shpiz et al., 2011). This problem is of great interest because, in the case of transcriptional silencing, it might be related to the formation of the telomeric chromatin. Using different approaches, we have shown that transcriptional activity of the telomeric retroelements substantially increased in the piRNA pathway mutants. Nuclear run-on assay (Jackson et al., 1998; Core et al., 2008) on ovarian tissues has been used to estimate the density of transcriptionally active RNA-polymerase complexes at telomeric loci. An increase in the nascent transcripts emerging from telomeric loci as well as from some other retrotransposons has been shown.

This observation was confirmed by the observation of enrichment of retrotransposon sequences in piRNA pathway mutants with two histone H3 modifications known to be linked to the RNA polymerase II activity (dimethylation of lysines 4 and 79). These data provided strong evidence for piRNA-mediated transcriptional silencing of the telomeric retrotransposon loci in the *Drosophila* germline. Most likely, transposon defense in the germline is a combination of the piRNA-mediated post-transcriptional and transcriptional silencing. This suggests that piRNAs are important participants in telomeric chromatin assembly.

Telomeric retrotransposon arrays as mentioned above display the features of open chromatin, however, no actively elongating RNA polymerase isoforms have been detected in this region (Andreyeva et al., 2005; Biessmann et al., 2005). We suggest that piRNAs mediate sequence-specific binding of the inhibition protein complex locally at the *HeT-A* promoter in the germ cells rather than heterochromatinization along telomeric arrays. Components of the transcription initiation complex of the telomeric retrotransposons may be considered as a putative link between piRNAs and inhibition of the transcription (Fig.6). However, little is known about the transcription factors that regulate telomeric element expression. The PROD protein, which is involved in heterochromatin formation, represses *HeT-A* expression (Torok et al., 2007). JIL-1 and Z4, which are associated with decompacted chromatin regions, are recruited to the telomeric retrotransposon array and colocalize with euchromatin-specific histone H3 trimethylated at lysine 4 in somatic tissues (Andreyeva et al., 2005).

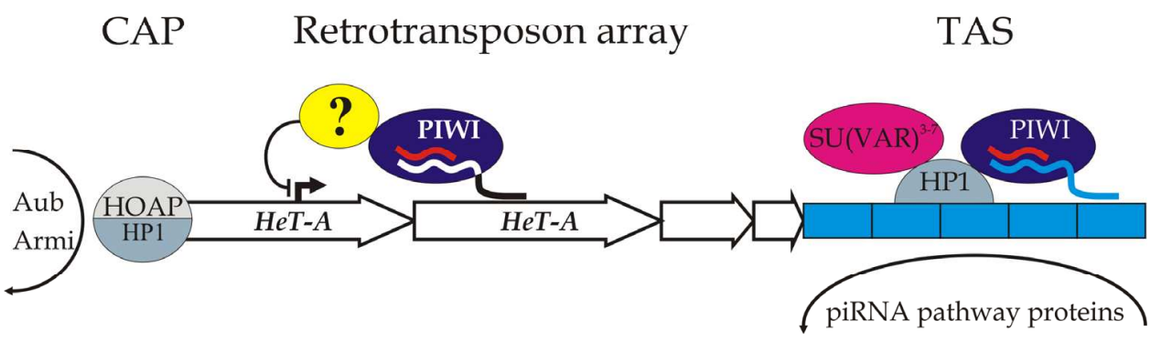

Fig. 6. Putative role of the piRNA pathway in *Drosophila* telomere functions in the germline. The piRNA/PIWI complex is proposed to mediate binding of the transcriptional inhibitors at the *HeT-A* promoter (yellow filled circle). The piRNA pathway components *aub* and *armi* are involved in telomere cap protein recruitment. TAS-specific piRNAs mediate chromatin assembly in this region.

HeT-A/TART derepression was recently reported to be a result of loss of histone H3 lysine 9 trimethylation by the methyltransferase dSETDB1 in the germline (Rangan et al., 2011). dSETDB1 was proposed to be required for piRNA production. This result underlined the importance of chromatin structure for piRNA-mediated expression of telomeric

retrotransposons. Thus, piRNAs affect not only transcript abundance but changes in the chromatin state of telomeric retroelements as well. We believe that both factors cause the excessive telomere length detected in piRNA pathway mutants (Table 1). These data underscore the importance of the piRNA pathway in *Drosophila* telomere homeostasis.

However, there are still more open questions than clear answers in this field. Telomere fusions in early embryos are observed only in *aub* and *armi* piRNA mutants, which affect cap formation (Khurana et al., 2010), whereas an increase in the *HeT-A* copy number was detected in most investigated piRNA pathway mutants. Moreover, TAS regions also produce piRNAs (Brennecke et al., 2007; Yin and Lin, 2007; Todeschini et al., 2010). The study of transgenes inserted in TASs led to the discovery of a phenomenon called a telomeric trans-silencing effect (TSE) (Ronsseray et al., 2003). A transgene inserted in a TAS can *in trans* repress the expression of a homologous transgene in the germline. In recent studies, TSE was found to depend on the piRNA silencing pathway and heterochromatin components (Josse et al., 2007; Todeschini et al., 2010), suggesting that TAS may be considered as a platform for piRNA-mediated chromatin assembly.

As mentioned above, the telomeric region of *D. melanogaster* is subdivided into three distinct subdomains based on DNA composition and chromatin structure: the cap, the retrotransposon array and the TAS region. Interestingly, in spite of the distinct features of these domains, the chromatin structure of each is under the control of the piRNA silencing pathway. This may suggest that there are several levels of *Drosophila* telomere length regulation and that the piRNA pathway is one of the important participants in this complex process (Fig. 6).

4. Role of RNAi in the telomere function in different organisms

In the fission yeast *Schizosaccharomyces pombe*, RNAi is required for heterochromatin assembly (Hall et al., 2002; Volpe et al., 2002). Short RNAs guide histone methyltransferase to the target locus to methylate lysine 9 of histone H3 (H3K9) with subsequent binding of the heterochromatic protein Swi6. Specific repeated elements (dg and dh) that are present at all major heterochromatic regions, including pericentromeric regions, subtelomeres and the mating-type locus, have been shown to act as targets of RNAi-mediated silencing (Hall et al., 2002; Volpe et al., 2002). Disruption of the RNAi system leads to defects in telomere clustering during mitosis and meiosis, although the silencing of transgenes inserted within subtelomeric loci, telomere length and telomeric Swi6 localization are not affected (Hall et al., 2003; Sugiyama et al., 2005). It was shown that fission yeast employs two independent mechanisms to maintain gene silencing at telomeres (Fig. 7). A chromatin-remodeling complex is recruited to yeast telomeres via interaction with the telomeric repeat binding proteins Ccq1 and Taz1 or the RNAi machinery that acts through dg and dh repeats embedded within subtelomeric regions (Kanoh et al., 2005; Hansen et al., 2006; Sugiyama et al., 2007). Thus, the maintenance of telomeric chromatin depends on redundant RNAi-dependent and RNAi-independent mechanisms. The removal of genes encoding RNAi components has little impact on the maintenance of the silencing of reporters or on telomere length; however, it affects telomere dynamics during mitosis and meiosis. The RNAi machinery was proposed to be essential for higher-order chromatin organization at telomeres (Sugiyama et al., 2005).

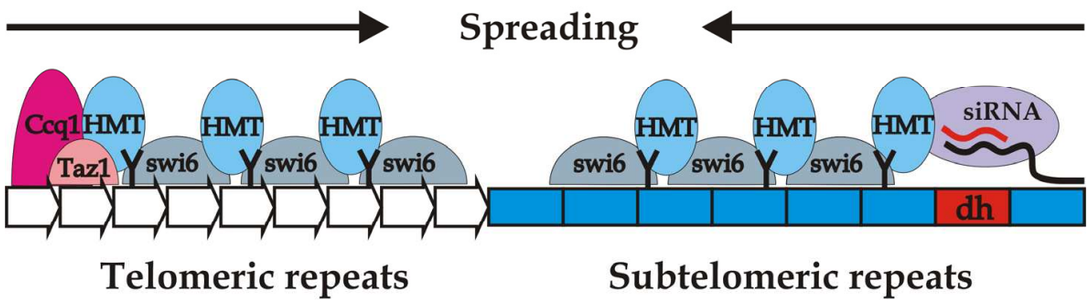

Fig. 7. Telomeric chromatin assembly in *S. pombe.* A chromatin-remodeling complex is recruited to yeast telomeres via redundant RNAi-dependent and RNAi-independent mechanisms. Subtelomeric repeats generate siRNAs that guide histone methyltransferase to methylate lysine 9 of histone H3 (H3K9me) with subsequent binding of the heterochromatic protein Swi6. Alternatively, telomere binding proteins Ccq1 and Taz1 induce the methylation of histone H3-K9 to recruit Swi6, which results in establishment of telomere heterochromatin.

In *Tetrahymena,* mutation of the *Dcl1p* gene that encodes the germline-specific ortholog of the RNase III enzyme Dicer causes serious impairments in meiotic chromosome behavior (Mochizuki and Gorovsky, 2005). The chromosome "bouquet" (i.e., telomere clustering in one region of the nuclear periphery) formation preceding homologous pairing did not occur in meiotic prophase, which resulted in impairments of chromosome segregation and higher lethality of progeny. This observation points to an important role of the RNAi component Dcl1 in chromosome segregation and telomere dynamics during conjugation in *Tetrahymena* (Mochizuki and Gorovsky, 2005). Taken together, these data suggest that a requirement for RNAi machinery in telomere function is probably conserved among eukaryotes. Telomere and/or subtelomeric arrays in different organisms irrespective of the mode of telomere elongation contain repetitive sequences, which are a potential source of short RNAs and the putative targets for RNAi.

5. Conclusion

Despite the different mode of chromosome end elongation (telomerase or transpositions), eukaryotic telomeres are functionally equal. They restore receding chromosome ends, protect them from the cell repair system, and are involved in the processes of chromosome positioning in the nucleus and chromosome condensation in meiosis and mitosis. The role of piRNAs in the expression of *Drosophila* telomeric retrotransposons, control of telomere length and assembly of the telomeric chromatin clearly indicates the importance of the piRNA pathway in *Drosophila* telomere homeostasis. Mutations in the RNAi machinery have been shown to disrupt telomere function in both *S. pombe* and *Tetrahymena*. RNAi likely mediates telomeric chromatin assembly, which plays a crucial role in telomere dynamics. It is tempting to speculate that RNA silencing plays a universal role in telomere function in various organisms irrespective of the mode of telomere elongation.

6. Acknowledgements

This work was supported by grants to A.K from the Russian Academy of Sciences program for Molecular and Cell Biology and the Russian Foundation for Basic Researches (09-04-00305).

7. References

Abad, J.P.; De Pablos, B.; Osoegawa, K.; De Jong, P.J.; Martin-Gallardo, A. & Villasante, A. (2004a). Genomic analysis of Drosophila melanogaster telomeres: full-length copies of HeT-A and TART elements at telomeres. *Mol Biol Evol*, Vol.21, No.9, Sep, pp. 1613-1619

Abad, J.P.; De Pablos, B.; Osoegawa, K.; De Jong, P.J.; Martin-Gallardo, A. & Villasante, A. (2004b). TAHRE, a novel telomeric retrotransposon from Drosophila melanogaster, reveals the origin of Drosophila telomeres. *Mol Biol Evol*, Vol.21, No.9, Sep, pp. 1620-1624

Andreyeva, E.N.; Belyaeva, E.S.; Semeshin, V.F.; Pokholkova, G.V. & Zhimulev, I.F. (2005). Three distinct chromatin domains in telomere ends of polytene chromosomes in Drosophila melanogaster Tel mutants. *J Cell Sci*, Vol.118, No.Pt 23, Dec 1, pp. 5465-5477

Aravin, A.A.; Naumova, N.M.; Tulin, A.V.; Vagin, V.V.; Rozovsky, Y.M. & Gvozdev, V.A. (2001). Double-stranded RNA-mediated silencing of genomic tandem repeats and transposable elements in the D. melanogaster germline. *Curr Biol*, Vol.11, No.13, Jul 10, pp. 1017-1027

Aravin, A.A.; Hannon G.J. & Brennecke J. 2007. The Piwi-piRNA pathway provides an adaptive defense in the transposon arms race. *Science*, Vol.318, No.5851, Nov 2, pp. 761-764

Aravin, A.A.; Sachidanandam, R.; Bourc'his, D.; Schaefer, C.; Pezic, D.; Toth, K.F.; Bestor, T. & Hannon, G.J. (2008). A piRNA pathway primed by individual transposons is linked to de novo DNA methylation in mice. *Mol Cell*, Vol.31, No.6, Sep 26, pp. 785-799

Arkhipova, I.R. & Morrison, H.G. (2001). Three retrotransposon families in the genome of Giardia lamblia: two telomeric, one dead. *Proc Natl Acad Sci U S A*, Vol.98, No.25, Dec 4, pp. 14497-14502

Bi, X.; Wei, S.C. & Rong, Y.S. (2004). Telomere protection without a telomerase; the role of ATM and Mre11 in Drosophila telomere maintenance. *Curr Biol*, Vol.14, No.15, Aug 10, pp. 1348-1353

Bi, X.; Srikanta, D.; Fanti, L.; Pimpinelli, S.; Badugu, R.; Kellum, R. & Rong, Y.S. (2005). Drosophila ATM and ATR checkpoint kinases control partially redundant pathways for telomere maintenance. *Proc Natl Acad Sci U S A*, Vol.102, No.42, Oct 18, pp. 15167-15172

Biessmann, H. & Mason, J.M. (1988). Progressive loss of DNA sequences from terminal chromosome deficiencies in Drosophila melanogaster. *Embo J*, Vol.7, No.4, Apr, pp. 1081-1086

Biessmann, H.; Mason, J.M.; Ferry, K.; d'Hulst, M.; Valgeirsdottir, K.; Traverse, K.L. & Pardue, M.L. (1990a). Addition of telomere-associated HeT DNA sequences "heals" broken chromosome ends in Drosophila. *Cell*, Vol.61, No.4, May 18, pp. 663-673

Biessmann, H.; Carter, S.B. & Mason, J.M. (1990b). Chromosome ends in Drosophila without telomeric DNA sequences. *Proc Natl Acad Sci U S A*, Vol.87, No.5, Mar, pp. 1758-1761

Biessmann, H.; Champion, L.E.; O'Hair, M.; Ikenaga, K.; Kasravi, B. & Mason, J.M. (1992a). Frequent transpositions of Drosophila melanogaster HeT-A transposable elements to receding chromosome ends. *Embo J*, Vol.11, No.12, Dec, pp. 4459-4469

Biessmann, H.; Valgeirsdottir, K.; Lofsky, A.; Chin, C.; Ginther, B.; Levis, R.W. & Pardue, M.L. (1992b). HeT-A, a transposable element specifically involved in "healing" broken chromosome ends in Drosophila melanogaster. *Mol Cell Biol*, Vol.12, No.9, Sep, pp. 3910-3918

Biessmann, H.; Prasad, S.; Semeshin, V.F.; Andreyeva, E.N.; Nguyen, Q.; Walter, M.F. & Mason, J.M. (2005). Two distinct domains in Drosophila melanogaster telomeres. *Genetics*, Vol.171, No.4, Dec, pp. 1767-1777

Boivin, A.; Gally, C.; Netter, S.; Anxolabehere, D. & Ronsseray, S. (2003). Telomeric associated sequences of Drosophila recruit polycomb-group proteins in vivo and can induce pairing-sensitive repression. *Genetics*, Vol.164, No.1, May, pp. 195-208

Brennecke, J.; Aravin, A.A.; Stark, A.; Dus, M.; Kellis, M.; Sachidanandam, R. & Hannon, G.J. (2007). Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila. *Cell*, Vol.128, No.6, Mar 23, pp. 1089-1103

Brennecke, J.; Malone, C.D.; Aravin, A.A.; Sachidanandam, R.; Stark, A. & Hannon, G.J. (2008). An epigenetic role for maternally inherited piRNAs in transposon silencing. *Science*, Vol.322, No.5906, Nov 28, pp. 1387-1392

Cenci, G.; Rawson, R.B.; Belloni, G.; Castrillon, D.H.; Tudor, M.; Petrucci, R.; Goldberg, M.L.; Wasserman, S.A. & Gatti, M. (1997). UbcD1, a Drosophila ubiquitin-conjugating enzyme required for proper telomere behavior. *Genes Dev*, Vol.11, No.7, Apr 1, pp. 863-875

Cenci, G.; Siriaco, G.; Raffa, G.D.; Kellum, R. & Gatti, M. (2003). The Drosophila HOAP protein is required for telomere capping. *Nat Cell Biol*, Vol.5, No.1, Jan, pp. 82-84

Cenci, G.; Ciapponi, L. & Gatti, M. (2005). The mechanism of telomere protection: a comparison between Drosophila and humans. *Chromosoma*, Vol.114, No.3, Aug, pp. 135-145

Chan, S.W. & Blackburn, E.H. (2002). New ways not to make ends meet: telomerase, DNA damage proteins and heterochromatin. *Oncogene*, Vol.21, No.4, Jan 21, pp. 553-563

Ciapponi, L.; Cenci, G.; Ducau, J.; Flores, C.; Johnson-Schlitz, D.; Gorski, M.M.; Engels, W.R. & Gatti, M. (2004). The Drosophila Mre11/Rad50 complex is required to prevent both telomeric fusion and chromosome breakage. *Curr Biol*, Vol.14, No.15, Aug 10, pp. 1360-1366

Cook, H.A.; Koppetsch, B.S.; Wu, J. & Theurkauf, W.E. (2004). The Drosophila SDE3 homolog armitage is required for oskar mRNA silencing and embryonic axis specification. *Cell*, Vol.116, No.6, Mar 19, pp. 817-829

Core, L.J.; Waterfall, J.J. & Lis, J.T. (2008). Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. *Science*, Vol.322, No.5909, Dec 19, pp. 1845-1848

Cryderman, D.E.; Morris, E.J.; Biessmann, H.; Elgin, S.C. & Wallrath, L.L. (1999). Silencing at Drosophila telomeres: nuclear organization and chromatin structure play critical roles. *Embo J*, Vol.18, No.13, Jul 1, pp. 3724-3735

Czermin, B.; Melfi, R.; McCabe, D.; Seitz, V.; Imhof, A. & Pirrotta, V. (2002). Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell*, Vol.111, No.2, Oct 18, pp. 185-196

Czech, B.; Malone C.D.; Zhou R.; Stark A.; Schlingeheyde C.; Dus M.; Perrimon N.; Kellis M.; Wohlschlegel J.A.; Sachidanandam R. et al. 2008. An endogenous small interfering RNA pathway in Drosophila. *Nature*, Vol.453, No.7196, Jun 5, pp. 798-802

d'Adda di Fagagna, F.; Teo, S.H. & Jackson, S.P. (2004). Functional links between telomeres and proteins of the DNA-damage response. *Genes Dev*, Vol.18, No.15, Aug 1, pp. 1781-1799

Danilevskaya, O.N.; Arkhipova, I.R.; Traverse, K.L. & Pardue, M.L. (1997). Promoting in tandem: the promoter for telomere transposon HeT-A and implications for the evolution of retroviral LTRs. *Cell*, Vol.88, No.5, Mar 7, pp. 647-655

Danilevskaya, O.N.; Traverse, K.L.; Hogan, N.C.; DeBaryshe, P.G. & Pardue, M.L. (1999). The two Drosophila telomeric transposable elements have very different patterns of transcription. *Mol Cell Biol*, Vol.19, No.1, Jan, pp. 873-881

de Lange, T. (2004). T-loops and the origin of telomeres. *Nat Rev Mol Cell Biol*, Vol.5, No.4, Apr, pp. 323-329

Elbashir, S.M.; Lendeckel, W. & Tuschl, T. (2001). RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes Dev*, Vol.15, No.2, Jan 15, pp. 188-200

Fanti, L.; Dorer, D.R.; Berloco, M.; Henikoff, S. & Pimpinelli, S. (1998). Heterochromatin protein 1 binds transgene arrays. *Chromosoma*, Vol.107, No.5, Nov, pp. 286-292

Frydrychova, R.C.; Biessmann, H.; Konev, A.Y.; Golubovsky, M.D.; Johnson, J.; Archer, T.K. & Mason, J.M. (2007). Transcriptional activity of the telomeric retrotransposon HeT-A in Drosophila melanogaster is stimulated as a consequence of subterminal deficiencies at homologous and nonhomologous telomeres. *Mol Cell Biol*, Vol.27, No.13, Jul, pp. 4991-5001

Frydrychova, R.C.; Mason, J.M. & Archer, T.K. (2008). HP1 is distributed within distinct chromatin domains at Drosophila telomeres. *Genetics*, Vol.180, No.1, Sep, pp. 121-131

Fuller, A.M.; Cook, E.G.; Kelley, K.J. & Pardue, M.L. (2010). Gag proteins of Drosophila telomeric retrotransposons: collaborative targeting to chromosome ends. *Genetics*, Vol.184, No.3, Mar, pp. 629-636

Gao, G.; Walser, J.C.; Beaucher, M.L.; Morciano, P.; Wesolowska, N.; Chen, J. & Rong, Y.S. (2010). HipHop interacts with HOAP and HP1 to protect Drosophila telomeres in a sequence-independent manner. *Embo J*, Vol.29, No.4, Feb 17, pp. 819-829

George, J.A. & Pardue, M.L. (2003). The promoter of the heterochromatic Drosophila telomeric retrotransposon, HeT-A, is active when moved into euchromatic locations. *Genetics*, Vol.163, No.2, Feb, pp. 625-635

George, J.A.; DeBaryshe, P.G.; Traverse, K.L.; Celniker, S.E. & Pardue, M.L. (2006). Genomic organization of the Drosophila telomere retrotransposable elements. *Genome Res*, Vol.16, No.10, Oct, pp. 1231-1240

Golubovsky, M.D.; Konev, A.Y.; Walter, M.F.; Biessmann, H. & Mason, J.M. (2001). Terminal retrotransposons activate a subtelomeric white transgene at the 2L telomere in Drosophila. *Genetics*, Vol.158, No.3, Jul, pp. 1111-1123

Ghildiyal, M.; Seitz H.; Horwich M.D.; Li C.; Du T.; Lee S.; Xu J.; Kittler E.L.; Zapp M.L.; Weng Z. et al. 2008. Endogenous siRNAs derived from transposons and mRNAs in Drosophila somatic cells. *Science*, Vol.320, No.5879, May 23, pp. 1077-1081

Goytisolo, F.A. & Blasco, M.A. (2002). Many ways to telomere dysfunction: in vivo studies using mouse models. *Oncogene*, Vol.21, No.4, Jan 21, pp. 584-591

Greider, C.W. & Blackburn, E.H. (1985). Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. *Cell*, Vol.43, No.2 Pt 1, Dec, pp. 405-413

Gunawardane, L.S.; Saito, K.; Nishida, K.M.; Miyoshi, K.; Kawamura, Y.; Nagami, T.; Siomi, H. & Siomi, M.C. (2007). A slicer-mediated mechanism for repeat-associated siRNA 5' end formation in Drosophila. *Science*, Vol.315, No.5818, Mar 16, pp. 1587-1590

Hall, I.M.; Shankaranarayana, G.D.; Noma, K.; Ayoub, N.; Cohen, A. & Grewal, S.I. (2002). Establishment and maintenance of a heterochromatin domain. *Science*, Vol.297, No.5590, Sep 27, pp. 2232-2237

Hall, I.M.; Noma, K. & Grewal, S.I. (2003). RNA interference machinery regulates chromosome dynamics during mitosis and meiosis in fission yeast. *Proc Natl Acad Sci U S A*, Vol.100, No.1, Jan 7, pp. 193-198

Hamilton, A.; Voinnet, O.; Chappell, L. & Baulcombe, D. (2002). Two classes of short interfering RNA in RNA silencing. *Embo J*, Vol.21, No.17, Sep 2, pp. 4671-4679

Hansen, K.R.; Ibarra, P.T. & Thon, G. (2006). Evolutionary-conserved telomere-linked helicase genes of fission yeast are repressed by silencing factors, RNAi components and the telomere-binding protein Taz1. *Nucleic Acids Res*, Vol.34, No.1, pp. 78-88

Higashiyama, T.; Noutoshi, Y.; Fujie, M. & Yamada, T. (1997). Zepp, a LINE-like retrotransposon accumulated in the Chlorella telomeric region. *Embo J*, Vol.16, No.12, Jun 16, pp. 3715-3723

Jackson, D.A.; Iborra, F.J.; Manders, E.M. & Cook, P.R. (1998). Numbers and organization of RNA polymerases, nascent transcripts, and transcription units in HeLa nuclei. *Mol Biol Cell*, Vol.9, No.6, Jun, pp. 1523-1536

Jaco, I.; Munoz, P. & Blasco, M.A. (2004). Role of human Ku86 in telomere length maintenance and telomere capping. *Cancer Res*, Vol.64, No.20, Oct 15, pp. 7271-7278

Josse, T.; Teysset, L.; Todeschini, A.L.; Sidor, C.M.; Anxolabehere, D. & Ronsseray, S. (2007). Telomeric trans-silencing: an epigenetic repression combining RNA silencing and heterochromatin formation. *PLoS Genet*, Vol.3, No.9, Sep, pp. 1633-1643

Kahn, T.; Savitsky, M. & Georgiev, P. (2000). Attachment of HeT-A sequences to chromosomal termini in Drosophila melanogaster may occur by different mechanisms. *Mol Cell Biol*, Vol.20, No.20, Oct, pp. 7634-7642

Kalmykova, A.I.; Klenov, M.S. & Gvozdev, V.A. (2005). Argonaute protein PIWI controls mobilization of retrotransposons in the Drosophila male germline. *Nucleic Acids Res*, Vol.33, No.6, pp. 2052-2059

Kanoh, J.; Sadaie, M.; Urano, T. & Ishikawa, F. (2005). Telomere binding protein Taz1 establishes Swi6 heterochromatin independently of RNAi at telomeres. *Curr Biol*, Vol.15, No.20, Oct 25, pp. 1808-1819

Karpen, G.H. & Spradling, A.C. (1992). Analysis of subtelomeric heterochromatin in the Drosophila minichromosome Dp1187 by single P element insertional mutagenesis. *Genetics*, Vol.132, No.3, Nov, pp. 737-753

Kawamura, Y.; Saito, K.; Kin, T.; Ono, Y.; Asai, K.; Sunohara, T.; Okada, T.N.; Siomi, M.C. & Siomi, H. (2008). Drosophila endogenous small RNAs bind to Argonaute 2 in somatic cells. *Nature*, Vol.453, No.7196, Jun 5, pp. 793-797

Khurana, J.S.; Xu, J.; Weng, Z. & Theurkauf, W.E. (2010). Distinct functions for the Drosophila piRNA pathway in genome maintenance and telomere protection. *PLoS Genet*, Vol.6, No.12, pp. e1001246

Klattenhoff, C. & Theurkauf, W. (2008). Biogenesis and germline functions of piRNAs. *Development*, Vol.135, No.1, Jan, pp. 3-9

Klattenhoff, C.; Xi, H.; Li, C.; Lee, S.; Xu, J.; Khurana, J.S.; Zhang, F.; Schultz, N.; Koppetsch, B.S.; Nowosielska, A. et al. (2009). The Drosophila HP1 homolog Rhino is required for transposon silencing and piRNA production by dual-strand clusters. *Cell*, Vol.138, No.6, Sep 18, pp. 1137-1149

Klenov, M.S.; Lavrov, S.A.; Stolyarenko, A.D.; Ryazansky, S.S.; Aravin, A.A.; Tuschl, T. & Gvozdev, V.A. (2007). Repeat-associated siRNAs cause chromatin silencing of retrotransposons in the Drosophila melanogaster germline. *Nucleic Acids Res*, Vol.35, No.16, pp. 5430-5438

Kuramochi-Miyagawa, S.; Watanabe, T.; Gotoh, K.; Totoki, Y.; Toyoda, A.; Ikawa, M.; Asada, N.; Kojima, K.; Yamaguchi, Y.; Ijiri, T.W. et al. (2008). DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. *Genes Dev*, Vol.22, No.7, Apr 1, pp. 908-917

Levis, R.W. (1989). Viable deletions of a telomere from a Drosophila chromosome. *Cell*, Vol.58, No.4, Aug 25, pp. 791-801

Levis, R.W.; Ganesan, R.; Houtchens, K.; Tolar, L.A. & Sheen, F.M. (1993). Transposons in place of telomeric repeats at a Drosophila telomere. *Cell*, Vol.75, No.6, Dec 17, pp. 1083-1093

Li, C.; Vagin V.V.; Lee S.; Xu J.; Ma S.; Xi H.; Seitz H.; Horwich M.D.; Syrzycka M.; Honda B.M. et al. 2009. Collapse of germline piRNAs in the absence of Argonaute3 reveals somatic piRNAs in flies. *Cell*, Vol.137, No.3, May 1, pp. 509-521

Lim, A.K.; Tao, L. & Kai, T. (2009). piRNAs mediate posttranscriptional retroelement silencing and localization to pi-bodies in the Drosophila germline. *J Cell Biol*, Vol.186, No.3, Aug 10, pp. 333-342

Liu, Y.; Mochizuki, K. & Gorovsky, M.A. (2004). Histone H3 lysine 9 methylation is required for DNA elimination in developing macronuclei in Tetrahymena. *Proc Natl Acad Sci U S A*, Vol.101, No.6, Feb 10, pp. 1679-1684

Luan, D.D.; Korman, M.H.; Jakubczak, J.L. & Eickbush, T.H. (1993). Reverse transcription of R2Bm RNA is primed by a nick at the chromosomal target site: a mechanism for non-LTR retrotransposition. *Cell*, Vol.72, No.4, Feb 26, pp. 595-605

Malone, C.D.; Brennecke J.; Dus M.; Stark A.; McCombie W.R.; Sachidanandam R. & Hannon G.J. 2009. Specialized piRNA Pathways Act in Germline and Somatic Tissues of the Drosophila Ovary. *Cell*, May 1, pp. 522-535

Mason, J.M.; Konev, A.Y.; Golubovsky, M.D. & Biessmann, H. (2003). Cis- and trans-acting influences on telomeric position effect in Drosophila melanogaster detected with a subterminal transgene. *Genetics*, Vol.163, No.3, Mar, pp. 917-930

Maxwell, P.H.; Belote, J.M. & Levis, R.W. (2006). Identification of multiple transcription initiation, polyadenylation, and splice sites in the Drosophila melanogaster TART family of telomeric retrotransposons. *Nucleic Acids Res*, Vol.34, No.19, pp. 5498-5507

Melnikova, L. & Georgiev, P. (2002). Enhancer of terminal gene conversion, a new mutation in Drosophila melanogaster that induces telomere elongation by gene conversion. *Genetics*, Vol.162, No.3, Nov, pp. 1301-1312

Melnikova, L.; Biessmann, H. & Georgiev, P. (2004). The vicinity of a broken chromosome end affects P element mobilization in Drosophila melanogaster. *Mol Genet Genomics*, Vol.272, No.5, Dec, pp. 512-518

Melnikova, L.; Biessmann, H. & Georgiev, P. (2005). The Ku protein complex is involved in length regulation of Drosophila telomeres. *Genetics*, Vol.170, No.1, May, pp. 221-235

Mikhailovsky, S.; Belenkaya, T. & Georgiev, P. (1999). Broken chromosomal ends can be elongated by conversion in Drosophila melanogaster. *Chromosoma*, Vol.108, No.2, May, pp. 114-120

Mochizuki, K. & Gorovsky, M.A. (2005). A Dicer-like protein in Tetrahymena has distinct functions in genome rearrangement, chromosome segregation, and meiotic prophase. *Genes Dev*, Vol.19, No.1, Jan 1, pp. 77-89

Myung, K.; Ghosh, G.; Fattah, F.J.; Li, G.; Kim, H.; Dutia, A.; Pak, E.; Smith, S. & Hendrickson, E.A. (2004). Regulation of telomere length and suppression of genomic instability in human somatic cells by Ku86. *Mol Cell Biol*, Vol.24, No.11, Jun, pp. 5050-5059

Nugent, C.I. & Lundblad, V. (1998). The telomerase reverse transcriptase: components and regulation. *Genes Dev*, Vol.12, No.8, Apr 15, pp. 1073-1085

Oikemus, S.R.; McGinnis, N.; Queiroz-Machado, J.; Tukachinsky, H.; Takada, S.; Sunkel, C.E. & Brodsky, M.H. (2004). Drosophila atm/telomere fusion is required for telomeric localization of HP1 and telomere position effect. *Genes Dev*, Vol.18, No.15, Aug 1, pp. 1850-1861

Okazaki, S.; Ishikawa, H. & Fujiwara, H. (1995). Structural analysis of TRAS1, a novel family of telomeric repeat-associated retrotransposons in the silkworm, Bombyx mori. *Mol Cell Biol*, Vol.15, No.8, Aug, pp. 4545-4552

Olovnikov, A.M. (1971). [Principle of marginotomy in template synthesis of polynucleotides]. *Dokl Akad Nauk SSSR*, Vol.201, No.6, pp. 1496-1499

Olovnikov, A.M. (1973). A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J Theor Biol*, Vol.41, No.1, Sep 14, pp. 181-190

Osanai, M.; Kojima, K.K.; Futahashi, R.; Yaguchi, S. & Fujiwara, H. (2006). Identification and characterization of the telomerase reverse transcriptase of Bombyx mori (silkworm) and Tribolium castaneum (flour beetle). *Gene*, Vol.376, No.2, Jul 19, pp. 281-289

Palm, W. & de Lange, T. (2008). How shelterin protects mammalian telomeres. *Annu Rev Genet*, Vol.42, pp. 301-334

Pane, A.; Wehr K. & Schupbach T. 2007. zucchini and squash encode two putative nucleases required for rasiRNA production in the Drosophila germline. *Dev Cell*, Vol.12, No.6, Jun, pp. 851-862

Perrini, B.; Piacentini, L.; Fanti, L.; Altieri, F.; Chichiarelli, S.; Berloco, M.; Turano, C.; Ferraro, A. & Pimpinelli, S. (2004). HP1 controls telomere capping, telomere elongation, and telomere silencing by two different mechanisms in Drosophila. *Mol Cell*, Vol.15, No.3, Aug 13, pp. 467-476

Raffa, G.D.; Cenci, G.; Siriaco, G.; Goldberg, M.L. & Gatti, M. (2005). The putative Drosophila transcription factor woc is required to prevent telomeric fusions. *Mol Cell*, Vol.20, No.6, Dec 22, pp. 821-831

Raffa, G.D.; Siriaco, G.; Cugusi, S.; Ciapponi, L.; Cenci, G.; Wojcik, E. & Gatti, M. (2009). The Drosophila modigliani (moi) gene encodes a HOAP-interacting protein required for telomere protection. *Proc Natl Acad Sci U S A*, Vol.106, No.7, Feb 17, pp. 2271-2276

Raffa, G.D.; Raimondo, D.; Sorino, C.; Cugusi, S.; Cenci, G.; Cacchione, S.; Gatti, M. & Ciapponi, L. (2010). Verrocchio, a Drosophila OB fold-containing protein, is a component of the terminin telomere-capping complex. *Genes Dev*, Vol.24, No.15, Aug 1, pp. 1596-1601

Rangan, P.; Malone, C.D.; Navarro, C.; Newbold, S.P.; Hayes, P.S.; Sachidanandam, R.; Hannon, G.J. & Lehmann, R. (2011). piRNA Production Requires Heterochromatin Formation in Drosophila. *Curr Biol*, Vol.21, No.16, Aug 23, pp. 1373-1379

Rashkova, S.; Karam, S.E.; Kellum, R. & Pardue, M.L. (2002). Gag proteins of the two Drosophila telomeric retrotransposons are targeted to chromosome ends. *J Cell Biol*, Vol.159, No.3, Nov 11, pp. 397-402

Rashkova, S.; Athanasiadis, A. & Pardue, M.L. (2003). Intracellular targeting of Gag proteins of the Drosophila telomeric retrotransposons. *J Virol*, Vol.77, No.11, Jun, pp. 6376-6384

Ronsseray, S.; Josse, T.; Boivin, A. & Anxolabehere, D. (2003). Telomeric transgenes and trans-silencing in Drosophila. *Genetica*, Vol.117, No.2-3, Mar, pp. 327-335

Saito, K.; Nishida, K.M.; Mori, T.; Kawamura, Y.; Miyoshi, K.; Nagami, T.; Siomi, H. & Siomi, M.C. (2006). Specific association of Piwi with rasiRNAs derived from retrotransposon and heterochromatic regions in the Drosophila genome. *Genes Dev*, Vol.20, No.16, Aug 15, pp. 2214-2222

Sarot, E.; Payen-Groschene G.; Bucheton A. & Pelisson A. 2004. Evidence for a piwi-dependent RNA silencing of the gypsy endogenous retrovirus by the Drosophila melanogaster flamenco gene. *Genetics*, Vol.166, No.3, Mar, pp. 1313-1321

Savitsky, M.; Kravchuk, O.; Melnikova, L. & Georgiev, P. (2002). Heterochromatin protein 1 is involved in control of telomere elongation in Drosophila melanogaster. *Mol Cell Biol*, Vol.22, No.9, May, pp. 3204-3218

Savitsky, M.; Kwon, D.; Georgiev, P.; Kalmykova, A. & Gvozdev, V. (2006). Telomere elongation is under the control of the RNAi-based mechanism in the Drosophila germline. *Genes Dev*, Vol.20, No.3, Feb 1, pp. 345-354

Schoeftner, S. & Blasco, M.A. (2009). A 'higher order' of telomere regulation: telomere heterochromatin and telomeric RNAs. *Embo J*, Vol.28, No.16, Aug 19, pp. 2323-2336

Sheen, F.M. & Levis, R.W. (1994). Transposition of the LINE-like retrotransposon TART to Drosophila chromosome termini. *Proc Natl Acad Sci U S A*, Vol.91, No.26, Dec 20, pp. 12510-12514

Shpiz, S.; Kwon, D.; Uneva, A.; Kim, M.; Klenov, M.; Rozovsky, Y.; Georgiev, P.; Savitsky, M. & Kalmykova, A. (2007). Characterization of Drosophila telomeric retroelement TAHRE: transcription, transpositions, and RNAi-based regulation of expression. *Mol Biol Evol*, Vol.24, No.11, Nov, pp. 2535-2545

Shpiz, S.; Kwon, D.; Rozovsky, Y. & Kalmykova, A. (2009). rasiRNA pathway controls antisense expression of Drosophila telomeric retrotransposons in the nucleus. *Nucleic Acids Res*, Vol.37, No.1, Jan, pp. 268-278

Shpiz, S.; Olovnikov, I.; Sergeeva, A.; Lavrov, S.; Abramov, Y.; Savitsky, M. & Kalmykova, A. (2011). Mechanism of the piRNA-mediated silencing of Drosophila telomeric retrotransposons. *Nucleic Acids Res*, Vol.39, No.20, Nov 1, pp. 8703-8711

Silva, E.; Tiong, S.; Pedersen, M.; Homola, E.; Royou, A.; Fasulo, B.; Siriaco, G. & Campbell, S.D. (2004). ATM is required for telomere maintenance and chromosome stability during Drosophila development. *Curr Biol*, Vol.14, No.15, Aug 10, pp. 1341-1347

Siriaco, G.M.; Cenci, G.; Haoudi, A.; Champion, L.E.; Zhou, C.; Gatti, M. & Mason, J.M. (2002). Telomere elongation (Tel), a new mutation in Drosophila melanogaster that produces long telomeres. *Genetics*, Vol.160, No.1, Jan, pp. 235-245

Smogorzewska, A. & de Lange, T. (2004). Regulation of telomerase by telomeric proteins. *Annu Rev Biochem*, Vol.73, pp. 177-208

Song, K.; Jung, D.; Jung, Y.; Lee, S.G. & Lee, I. (2000). Interaction of human Ku70 with TRF2. *FEBS Lett*, Vol.481, No.1, Sep 8, pp. 81-85

Song, Y.H.; Mirey, G.; Betson, M.; Haber, D.A. & Settleman, J. (2004). The Drosophila ATM ortholog, dATM, mediates the response to ionizing radiation and to spontaneous DNA damage during development. *Curr Biol*, Vol.14, No.15, Aug 10, pp. 1354-1359

Sugiyama, T.; Cam, H.; Verdel, A.; Moazed, D. & Grewal, S.I. (2005). RNA-dependent RNA polymerase is an essential component of a self-enforcing loop coupling heterochromatin assembly to siRNA production. *Proc Natl Acad Sci U S A*, Vol.102, No.1, Jan 4, pp. 152-157

Sugiyama, T.; Cam, H.P.; Sugiyama, R.; Noma, K.; Zofall, M.; Kobayashi, R. & Grewal, S.I. (2007). SHREC, an effector complex for heterochromatic transcriptional silencing. *Cell*, Vol.128, No.3, Feb 9, pp. 491-504

Takahashi, H.; Okazaki, S. & Fujiwara, H. (1997). A new family of site-specific retrotransposons, SART1, is inserted into telomeric repeats of the silkworm, Bombyx mori. *Nucleic Acids Res*, Vol.25, No.8, Apr 15, pp. 1578-1584

Todeschini, A.L.; Teysset, L.; Delmarre, V. & Ronsseray, S. (2010). The epigenetic trans-silencing effect in Drosophila involves maternally-transmitted small RNAs whose production depends on the piRNA pathway and HP1. *PLoS ONE*, Vol.5, No.6, pp. e11032

Torok, T.; Benitez, C.; Takacs, S. & Biessmann, H. (2007). The protein encoded by the gene proliferation disrupter (prod) is associated with the telomeric retrotransposon array in Drosophila melanogaster. *Chromosoma*, Vol.116, No.2, Apr, pp. 185-195

Vagin, V.V.; Klenov, M.S.; Kalmykova, A.I.; Stolyarenko, A.D.; Kotelnikov, R.N. & Gvozdev, V.A. (2004). The RNA interference proteins and vasa locus are involved in the silencing of retrotransposons in the female germline of Drosophila melanogaster. *RNA Biol*, Vol.1, No.1, May, pp. 54-58

Vagin, V.V.; Sigova, A.; Li, C.; Seitz, H.; Gvozdev, V. & Zamore, P.D. (2006). A distinct small RNA pathway silences selfish genetic elements in the germline. *Science*, Vol.313, No.5785, Jul 21, pp. 320-324

Villasante, A.; Abad, J.P.; Planello, R.; Mendez-Lago, M.; Celniker, S.E. & de Pablos, B. (2007). Drosophila telomeric retrotransposons derived from an ancestral element that was recruited to replace telomerase. *Genome Res*, Vol.17, No.12, Dec, pp. 1909-1918

Volpe, T.A.; Kidner, C.; Hall, I.M.; Teng, G.; Grewal, S.I. & Martienssen, R.A. (2002). Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science*, Vol.297, No.5588, Sep 13, pp. 1833-1837

Wallrath, L.L. & Elgin, S.C. (1995). Position effect variegation in Drosophila is associated with an altered chromatin structure. *Genes Dev*, Vol.9, No.10, May 15, pp. 1263-1277

Yin, H. & Lin, H. (2007). An epigenetic activation role of Piwi and a Piwi-associated piRNA in Drosophila melanogaster. *Nature*, Vol.450, No.7167, Nov 8, pp. 304-308

IntechOpen

IntechOpen