Chapter 17

Telomeres: Their Structure and Maintenance

Radmila Capkova Frydrychova and James M. Mason

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/51356

1. Introduction

Telomeres are essential nucleoprotein structures at the ends of eukaryotic chromosomes. They play several essential roles preserving genome stability and function, including distinguishing chromosome ends from DNA double stranded breaks (DSBs) and maintenance of chromosome length. Due to the inability of conventional DNA polymerases to replicate the very end of a chromosome, sometimes known as the end replication problem, chromosome ends shorten with every round of DNA replication. In the absence of special telomere maintenance mechanisms this telomere shortening leads to replicative senescence and apoptosis. Several telomere maintenance mechanisms have been identified; these are reflected in several known types of telomeres. In most eukaryotes telomeres comprise a tandem array of a short, 5-8 bp, well conserved repeat unit, and telomere length is maintained by telomerase, a specialized reverse transcriptase that carries its own RNA template and adds telomeric sequences onto chromosome ends [1]. Nevertheless, in some organisms the array of short telomeric sequence motifs has been replaced with less conventional sequences, such as satellite sequences or transposable elements. The telomeres of such organisms are maintained through homologous recombination or through transposition of the mobile elements [2,3]. These different telomere types present distinct difficulties for chromosome end protection. Telomeres maintained by telomerase are protected by a proteinaceous telomere cap, termed shelterin, that recognizes chromosome ends in a DNA sequence specific manner, while telomeres with long terminal repeat units are protected by a cap, termed terminin, that binds to chromosome ends independently of DNA sequence.

2. The structure of telomeric DNA: "usual" and "unusual" telomeres

The most common telomere structure found across the whole eukaryotic tree is a simple telomeric repeat of the form $(T_xA_yG_z)_n$ generated by telomerase. For example, the sequence in

unikonts generally, including animals, fungi and amoebozoa, is T_2AG_3, while in most plants and green algae it is T_3AG_3. Within these broad generalizations, however, there are exceptions. Some species seem to have lost the canonical telomeric motif altogether. We will mention a few examples here, then describe one of these examples in more detail.

2.1. Chromalveolata

The terminal sequence motif seems to be quite variable among the Chromalveolates, while still adhering to the consensus telomeric motif (Figure 1). Apicomplexa species use three different motifs [4-6], and ciliates use two [4,7]. Dinoflagellates use T_3AG_3 [8], similar to plants and green algae, while diatoms use T_2AG_3 [9], similar to unikonts. Photosynthetic species in the Chromalveolates are derived from the engulfment of a red alga. The resulting nucleomorphs retain the algal linear chromosomes and telomeres that are very different. The cryptomonad, *Guillardia theta*, for example, uses T_3AG_3 in its nucleus and $(AG)_7A_2G_6A$ in its nucleomorph [11,12].

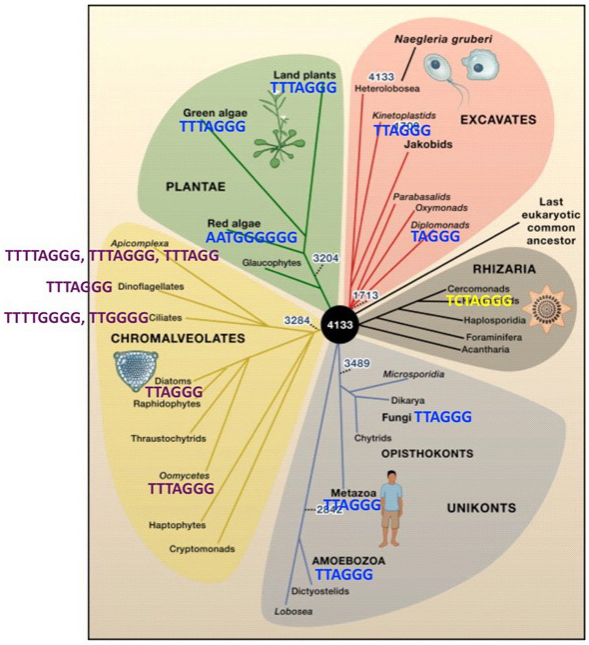

Figure 1. Diagram showing five major eukaryotic supergroups and representative telomeric motifs. These groups are shown to have diverged from a single latest common ancestor, because the evolutionary relationships are not known. Trees connecting the major taxa within these supergroups are shown, but the branch lengths are arbitrary. Representative telomeric motifs are shown for the major subtaxa. In some cases, two or three representative motifs are known for one of these taxa, as shown. Exceptions to these representations are discussed in the text. The figure was modified from [10] with permission.

http://dx.doi.org/10.5772/51356

It seems likely that the telomere binding proteins in these organisms are either different in the two intracellular bodies, or do not bind in a DNA sequence specific manner.

2.2. Plantae

Among the Plantae, land plants and green algae mostly use T_3AG_3 as a telomeric motif, while the red algae have a very different sequence at their chromosome ends. The red alga *Cyanidioschyzon merolae*, for example, uses A_2TG_6 [13]. While telomeres in most green algae conform to the telomeric motif of this kingdom, the order Chlamydomonadales includes species that carry the telomeric motifs T_4AG_3, T_3AG_3 and T_2AG_3, apparently independently of phylogeny as determined by the 18S rDNA sequence [14]. Some species of this order lack all three of these telomeric motifs and carry unknown DNA sequences at their chromosome ends. It is possible that the 18S rDNA sequence does not represent an accurate reflection of phylogeny or the telomeric motif is quite variable in this order. In either case, it seems that sequence specific binding by telomeric proteins may have eased in this order.

Similarly, while most land plants retain the canonical T_3AG_3 telomeric motif, telomeres in a few orders differ from this structure. Within the monocot order Asparagales some species of Alliaceae have switched to the sequence T_2AG_3, and others appear to have lost the canonical telomeric sequence completely. It has been proposed that the telomeres of these latter Alliaceae species are maintained through transposition of mobile elements or through homologous recombination between the satellite sequences [15,16]. In the eudicot order Solanales the canonical telomeric motif as well as telomerase are absent from several genera of the family Solanaceae [17-20]. The actual telomeric sequence and compensation mechanism in this group of plants, however, remain unknown.

2.3. Unikonta

The T_2AG_3 telomeric sequence is found widely among the unikonts (Figure 1). While this is generally true within the fungi, representatives of two classes, Schizosaccharomycetes and Saccharomycetes, use variable, degenerate telomeric sequences that may result from replication infidelity or slippage [12]. In *Saccharomyces cerevisiae*, for example, the repeat motif is $TG_{1\text{-}3}$.

Similarly, T_2AG_3 is found widely at chromosome ends among metazoans. The animal phylum Arthropoda, however, uses the sequence T_2AG_2 at telomeres, and its sister phylum Tardigrada lacks both of these telomeric motifs [21]. Insects are the largest class of arthropods, and even here individual insect taxa may have different forms of the canonical sequence or even unrelated telomeric sequences. Insects seem to have lost the canonical arthropod telomeric motif several times [22,23]. In some cases, such as the coleopteran superfamily Tenebrionoidea, the arthropod repeat has been replaced by a similar motif, in this case $TCAG_2$ [24], while in many other instances the new telomeric DNA sequence has not been identified.

Insects of the orders Diptera, Mecoptera and Siphonaptera (superorder Antliophora) do not carry a canonical telomeric DNA sequence at their chromosome ends [23,25]; nor do they have a telomerase gene [26], indicating that telomerase was lost some 260-280 Mya. Even so, Diptera is one of the most successful insect orders, with some 152,000 species [27]. This

suggests that telomerase and the canonical telomeric DNA sequences generated by telomerase, *per se*, are not critical for evolutionary survival. It is possible telomerase is expendable, as long as the telomere capping complex is compatible with whatever terminal DNA sequence is present on chromosome ends. When the primary pathway for telomere replication is defective, an alternative backup mechanism can restore telomere function. It was documented in yeast. Yeast mutants lacking telomerase showed the progressive telomere loss and, although the majority of the cells died, a minor subpopulation survived via homologous recombination [28].

Long satellite sequences have been reported in nematoceran species. Chromosome tips of several *Chironomus* species (infraorder Culicomorpha) consist of large, 50-200 kb, blocks of complex, tandemly repeated sequences that have been classified into subfamilies based on sequence similarities. Different telomeres display different sets of subfamilies, and the distribution of subfamilies differs between individuals within a species. The variation of the satellite sequences supports the proposal that telomeres in *Chironomus* are elongated by a gene conversion mechanism involving these long blocks of complex repeat units [29-32]. A similar situation has been found in *Anopheles gambiae* (infraorder Culicomorpha) using a plasmid fortuitously inserted into the complex telomeric sequences at the tip of chromosome 2L. The telomere carrying the plasmid was found to engage in frequent recombination events that resulted in extension of the terminal array [33,34]. Recently, a similar case was reported in *Rhynchosciara americana* (infraorder Bibionomorpha). Tandem arrays of short repeats, 16 and 22 bp in length, were found to extend to chromosome ends [35]. Although telomere elongation could not be assayed in this case, it seems likely that the mechanism is similar to that seen in other nematoceran species. In many respects, these complex arrays resemble subtelomeric sequences [36], suggesting a possible mechanism for telomere formation upon the loss of telomerase.

Telomere structures have only been examined in a single brachyceran genus, *Drosophila* (infraorder Muscomorpha). Telomeric DNA sequences consist of long arrays of non-long terminal repeat (LTR) retrotransposons and are thus very different from those found in Nematocera. These telomeric transposons resemble long interspersed elements (LINEs) found in mammals, but have some differences that may reflect their telomere-specific 'lifestyle.' Three families of telomeric elements have been described in *Drosophila melanogaster* (subgenus Sophophora), *HeT-A*, *TART* and *TAHRE* [2]; in all cases these elements are attached to the chromosome by their 3' oligo(A) tails. Many of the elements are truncated at the 5' end, possibly due to the end replication problem. *HeT-A* transposons are about 6 kb in length and make up about 80-90% of the elements found at chromosome ends. They are atypical LINE-like elements in three respects: the 3' untranslated region (UTR) comprises about 3 kb or half the length of the element; the transcriptional promoter is at the 3' end of the element to prevent loss when the element is present at the chromosome terminus with its 5' end exposed to incomplete DNA replication of linear DNA; and an open reading frame (ORF) coding for a reverse transcriptase is absent. *TART* elements are about 10 kb in length and make up about 10-20% of the telomeric retrotransposons. They are also unusual elements, but in some ways that differ from *HeT-A*: they also have an unusually long 3' UTR; they have a relatively strong antisense promoter of unknown function and a pair of perfect

http://dx.doi.org/10.5772/51356

long non-terminal repeats that may be important for replication [37,38]; they make a reverse transcriptase, but the encoded Gag-like protein is unable to target telomeres in the absence of the *HeT-A* Gag [39]. *TAHRE* elements closely resemble *HeT-A*, except they encode their own reverse transcriptase. Thus, while *TAHRE* seems to be the only one of the three elements capable of independent transposition, it is by far the least abundant, comprising only 1% of the telomeric retrotransposons.

HeT-A and *TART* elements have also been found in *Drosophila virilis* (subgenus Drosophila) Although there is little sequence homology across species, the two types of retrotransposons can be recognized by their telomeric locations and unusual structures, as described above [40,41]. Given the difficulty in finding homology between evolutionarily related telomeric elements within the *Drosophila* genus, finding similar elements in other brachyceran species based on homology alone is unlikely. Thus, it is not known when these targeted transpositions took over the role of telomere maintenance from homologous recombination.

Human telomeres have been shown to form a large terminal loop dependent on the presence of a 3' G strand overhang at the telomeric end. This 3' end is tucked back into the double-stranded DNA as a loop, termed a t-loop [42]. Similar t-loops may also be formed in yeast [43].

3. Proteins associated with telomeres

The telomere cap, a multiprotein structure at chromosome end ensuring stability and integrity of the genome, was revealed by early cytological observations of chromosomal rearrangements after exposure to ionizing radiation [44]. The telomere cap allows cells to distinguish their natural chromosome ends from DSBs, thus protecting the chromosome termini from inadvertent DNA damage response (DDR) activities. Defects in the cap, or DSBs elsewhere in the genome, lead to activation of cell cycle checkpoints followed by DDR mechanisms. A consequence of inappropriate DSB repair are end-to-end fusions of chromosomes, i.e. formation of ring chromosomes or dicentric linear chromosomes, followed by chromosome breakage, which results in genomic instability and loss of cellular viability [45,46]. Although, in this context, telomeres perform the same essential function across phyla, cap proteins of diverse organisms are less conserved that one might expect. Even within a single taxonomic class, such as mammals, telomeric proteins display less conservation than other chromosomal proteins [47]. In mammals the telomere-specific cap complex has been termed 'shelterin' (Figure 2). The six-protein complex is formed by double-stranded TTAGG repeat-binding proteins TRF1 and TRF2, which recruit TIN2 and TPP1. The latter proteins make a bridge between the TRF proteins and G-overhang-binding protein, POT1. The sixth protein is the TRF2-interacting protein RAP1 [46,48,49]. A characteristic of shelterin proteins is specific and exclusive association with telomeric DNA, where they are permanently present throughout the cell cycle and serve as platform for a transient and dynamic recruitment of a number of telomere-associated factors, referred to as non-shelterin telomeric proteins. These non-shelterin proteins are required for telomere protection and replication but also have nu-

merous nontelomeric functions. Examples include DDR proteins that are commonly involved in DSB repair through nonhomologous end joining (NHEJ) or homologous recombination (HR), such as ATM, ATR and Ku70/80, which associate with TRF1 and TRF2, and the MRN complex, composed of the MRE11, RAD50 and NBS1 (MRN) proteins, which associates with TRF2 [50-55]. Another protein associated with TRF2 is Apollo, an exonuclease important for recreating the 3' overhang [51,56]. The binding of shelterin proteins and formation of a functional cap require a terminal DNA array of specific sequence and of satisfactory length.

Analysis of deleterious events at shelterin-free telomeres revealed six pathways for end protection [57]. The primery protection by shelterin is against classical NHEJ and unwanted activation of ATM and ATR signaling. Additionally, shelterin provides a defense against alternate NHEJ, HR and 5' end resection. Another protective layer is achieved through the Ku70/80 heterodimer or 53BP1. 53BP1 minimizes resection but only at telomeres eliciting a DNA damage signal. Ku70/80 blocks alternate NHEJ and HR at telomeres independent of a DNA damage signal [57].

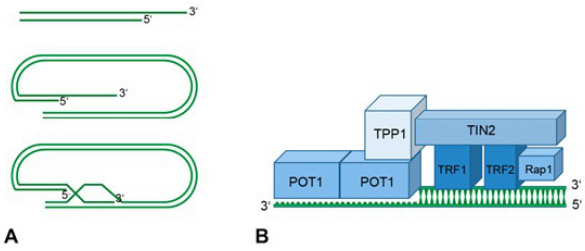

Figure 2. A. The telomere forms a t-loop structure characterized by invasion of the 3' overhang into a double stranded telomeric DNA. B. Six proteins, TRF1, TRF2, TPP1, POT1, TIN2, and RAP1 form a dedicated telomere-protection protein complex in humans [48,49,58].

Telomeres in *Saccharomyces cerevisiae* are protected by two separate protein complexes. One is the Rap1/Rif1/Rif2 complex, which localizes to double-stranded telomeric DNA. The other is the Cdc13/Stn1/Ten1 (CST) complex, which is targeted to the single-stranded G-overhangs through sequence-specific binding of Cdc13. Defects in the CST complex result in degradation of the C-stand and activation of DDR mechanisms [47]. As with shelterin, CST interacts with numerous proteins required for telomere function. Some evolutionary conservation in the protein composition of the cap is expected, for instance similarities to CST and shelterin are observed in telomeric proteins of numerous organisms. This is documented for mammalian CST, which, although not involved in telomere capping, facilitates telomere replication and, if impaired, leads to catastrophic telomeric defects [59]. Another example is Ver, a component of the *Drosophila* cap with weak structural similarities to Stn1 [60,61].

A multiprotein capping complex in *Drosophila*, termed 'terminin,' is an analog of mammalian shelterin [61]. One major difference between these two complexes is that terminin does

http://dx.doi.org/10.5772/51356

not bind to a specific telomeric DNA sequence. Rather limited information is available about the structure and function of four known terminin proteins, HOAP, Moi, Ver, and HipHop. As with shelterin, terminin proteins localize specifically to telomeres and appear to function only at telomeres. HOAP is encoded by the *cav* gene [62]; Moi is a HOAP-binding protein encoded by *moi* [63,64]; Ver is structurally homologous to STN1 and is encoded by *ver* [60]; and HipHop is a HP1-HOAP interacting protein [65]. Assembly of the terminin complex requires strict dependencies. For example, the binding of HOAP and HipHoP to telomeres is interdependent, loss of one protein reduces binding of the other [65]; HOAP is required for Ver and Moi localization [61]. The terminin complex seems to occupy a broad region covering a more than 10 kb from the chromosome termini [65]. As with shelterin proteins, defects in terminin proteins lead to frequent telomeric fusions.

As there is no specific telomeric DNA sequence in Drosophila, terminin binding to telomeric DNA is sequence-independent, which makes a substantial difference between mammalian and Drosophila telomeres. In contrast to mammals, the complete loss of a Drosophila telomere does not definitely mean inescapable damage to genome stability and cell death, because under the right circumstances the telomere cap can be formed *de novo* as on any broken chromosome end and perform there the same protective functions as the regular telomere. This demonstrates that the telomeric retrotransposons, although important for telomere elongation, are not required as an unique assembly platform for cap formation [2,66,67].

Similar to shelterin, terminin presents a docking site for binding of additional proteins, called non-terminin capping proteins. Although not exclusively located at telomeres and having some telomere-unrelated functions, these proteins are required for the capping function and, in many cases, facilitate terminin assembly. There are several known non-terminin proteins; most of them were identified because their mutants display frequent telomeric fusions [61]. The best characterized is HP1a that is encoded by *Su(var)205*. The presence of HP1 at telomeres is required for HOAP binding, which reveals the importance of HP1 for terminin assembly. As in mammals, other non-terminin proteins are DNA repair factors: the *Drosophila* homologs of the ATM kinase and proteins of the MRN complex. Defects in the MRN complex lead to reduction of HOAP and HP1 at telomeres and frequent telomeric fusions. Through its effects on the binding of HOAP and possibly other terminin components, the MRN complex seems to be essential for the terminin formation [61,68,69]. ATM prevents telomeric fusions, and defects in this protein partially affect HP1/HOAP localization [70-72]. Woc is a zinc-finger protein preventing telomeric fusions, but acting independently of HP1, HOAP, and RAD50 [73]. UbcD1 is an E2 ubiquitin conjugating enzyme. It has been suggested that UbcD1-mediated ubiquitination of telomeric proteins is an essential post-translational modification ensuring their proper function [61,74]. In contrast to non-terminin and non-shelterin proteins that are largely conserved, a comparison between shelterin and terminin reveals no obvious homology in protein composition. Loss of conservation between shelterin and terminin proteins may correspond the evolutionary stage when a Antliophoran ancestor lost telomerase-based telomere elongation and had to evolve a sequence-independent protection of chromosome ends and acquire a new elongation system.

A highly condensed chromatin structure is a common characteristic of telomeres from yeast to man. Usually telomeres are heterochromatic, and the heterochromatic properties are thought to play an important role in telomeric function [75-77]. Telomeric chromatin is the source of telomeric position effect (TPE), a silencing of transgenes inserted into telomeres or their vicinity [78]. Besides the cap region, *Drosophila* telomeres contain two distinct chromatin domains: a subtelomeric region of repetitive DNA, termed TAS (telomere associated sequence), exhibiting features that resemble heterochromatin, and a terminal array of retrotransposons with euchromatic characteristics [79]. The *Drosophila* TAS region is, in contrast to retrotransposon array, the source of TPE [79,80]. Although organized into a heterochromatic structure, the vertebrate TTAGGG sequence remains unmethylated due to the lack of a appropriate cytosine substrate. The subtelomeric region is, in contrast, heavily methylated by DNA methyltranferases DNMT1, DNMT3a and DNMT3b [81]. Both in vertebrates and *Drosophila*, telomeric and subtelomeric regions are enriched in histone H3 methylated at lysine 9 (H3K9me), mediated by a H3K9-specific histone methyltransferase and HP1.

4. Telomeric replication and its difficulties

Based on DNA and protein composition, telomeres are typical heterochromatin, so their replication should correspond with a common paradigm of late heterochromatin replication. Based on early microscopic studies, it is generally accepted that DNA replication at early stages of S phase is associated with expressed genes, whereas repressed tissue-specific genes or heterochromatic regions are replicated during the late stages of replication [82-84]. The late replication seems to be common, but definitely is not universal [85]. Replication of human telomeres takes place throughout S phase, and specific telomeres tend to replicate at defined stages, some replicating early and others late [86]. The pattern of replication timing seems to be conserved between homologous chromosomes and does not vary between cells of different individuals. Although no correlation was found with telomere length or telomerase activity, a strong association was observed with nuclear localization. Late-replicating telomeres show a preferential association with the nuclear periphery, while early-replicating telomeres are preferentially located near the nuclear center [86]. A different situation was found in budding yeast, *Saccharomyces cerevisiae*, where early telomere replication correlates with short telomeric length and telomerase activity [87,88]. In fission yeast, *Schizosaccharomyces pombe*, telomere replication corresponds to S/G2 phase [85,89].

Because of the repetitive nature of telomeric DNA, telomeres present a significant problem for their replication. Spontaneous replication fork regression in telomeric DNA *in vitro* was determined to be 41% higher than seen in non-repeated DNA [90]. The obstacles during replication may lead to formation of cruciform intermediates, resulting in unwanted recombination events, amplifications or deletions [90,91]. Most of the telomere is replicated by a standard replication fork, however, to achieve efficient telomere replication a number of additional steps are needed. The process requires cooperation between standard replication factors and telomeric proteins, DDR proteins and numerous additional fac-

http://dx.doi.org/10.5772/51356

tors [47]. Examples of additional proteins are RecQ-type helicases that are present at replication forks in addition to standard helicases and are shown to unwind structures similar to chickenfoot intermediates [90,92]. Cooperation of replication factors with shelterin proteins is also documented. TRF1 mutants showed a reduction in replication efficiency, suggesting that TRF1 promotes efficient replication of telomeric DNA by preventing fork stalling [93]. Similarly, Taz1, a TRF1 homolog in fission yeast, has been shown to prevent fork stalling [94]. Another example is mammalian CTC1; deletion of CTC1 results in increased loss of leading C-strand telomeres, dramatic telomere loss and accumulation of excessive single-stranded telomeric DNA [95].

In yeast, the replication of telomeres is initiated in subtelomeric regions, and the replication fork moves towards the chromosome termini [96]. In mammalian cells, the origin of telomeric replication and direction is ambiguous.

After the replication fork reaches the chromosome terminus, the lagging strand gains a 3' overhang due to the removal of the primer for the terminal Okazaki fragment. At the same time C-strand specific resection occurs by nucleases Exo1 and/or Dna2 to produce a G-overhang on the leading strand [89]. If active, telomerase elongates the G-overhangs by addition of new telomeric repeats. Telomerase action is followed by complementary C-strand synthesis by DNA pol α. The process is terminated by additional processing to remove the RNA primer and to leave a 40-400 nucleotide G-overhang. The timing of the events differs between species. In human cells, telomere replication occurs at the same time as telomerase-mediated extension, and fill-in synthesis of C- strand is delayed until S/G2. Budding yeast shows tight coupling between G-strand extension and C-strand synthesis [89,91].

5. The mechanisms of telomeric elongation and their regulation

Telomerase is a ribonucleoprotein reverse transcriptase that utilizes its protein subunit (TERT in mammals, Est2p in *S. cerevisiae*) to elongate the 3' end of telomeric DNA using an internal RNA subunit (TR) as a template [97-99]. Telomerase activity is related to cell proliferation status: it is high in actively cycling cultures and low in quiescent differentiated cells [100]. Telomerase is not detected in human mature sperm or unfertilized eggs, but after fertilization telomerase is rapidly activated. A dramatic increase is observed in blastocysts, but during later stages of gestation telomerase activity declines. In the 16-week fetus Wright [101] showed high levels of telomerase in liver and intestine; detectable activity in lung, skin, muscle, adrenal glands, and kidney; and very weak or no activity in brain, bone or placental extracts. Most somatic cells in adults show no telomerase activity, as enzyme activity is limited to specific types of proliferative cells, such as embryonic, stem and epithelial cells, the germline, or cells of the hematopoetic system [102,103]. Telomerase activity is highly regulated. Reactivation of telomerase is associated with tumor development, and conversely, insufficient telomerase activity is linked to stem cell diseases, such as dyskeratosis congenita and aplastic anemia [104-106].

Telomerase is regulated through genetic, epigenetic and environmental factors: TERT and TR transcription, posttranscriptional and posttranslational modifications of TERT, and telomerase recruitment and processivity [104]. TERT promoter activity has been studied extensively, and numerous transcription factors have been found to interact with TERT. TERT transcription is, for instance, activated by the oncogene c-Myc and suppressed by the tumor-suppressor WT1 (Wilm's tumor suppresor). Misregulation of TERT through the c-Myc or WT1 pathways is associated with telomerase reactivation in cancer cells [107,108]. Although transcription of TERT is the major determinant of telomerase activity, TERT transcript levels do not always correlate with enzyme activity. Posttranslational phosphorylation may regulate telomerase activity, as may telomerase degradation through ubiquitination, as the half-life of telomerase activity was approximately 24 hours [109]. In human cells the POT1-TPP1 complex was found to be a key regulator of telomerase processivity [110-113].

Little is known about the regulation of telomere length in *Drosophila*, where two modes of telomere elongation have been described: transposition of telomeric elements and gene conversion. The process of telomeric transposition is composed of several steps: 1. transcription of the telomeric elements, 2. export of retroelement transcripts from the nucleus to the cytoplasm, 3. translation, 4. nuclear re-import of the retroelement transcripts together the retroelement proteins, 5. recognition of chromosome ends, and 6. target-primed reverse transcription, which attaches the 3' oligo(A) tails of the elements to chromosome termini [2]. Transposition of these elements to chromosome termini does not depend on a specific DNA sequence at the target site and together with the loss of telomeric DNA results in tandem arrays of mixed complete and 5' truncated elements [2]. The regulation of telomere elongation may be on the level of retroelement transcription and/or accessibility of the chromosome ends for new retroelement attachments. A variety of proteins have been identified to play a role in *Drosophila* telomere capping, however, only a few proteins are known to function in telomere elongation. HP1 was found to have a dual role in telomere protection and telomere length control. Compared to wild-type, heterozygotous *Su(var)205* mutants displayed much longer telomeres associated with a dramatic increases in retroelement transcription and transposition [114-116]. The regulation of retroelement transcription by HP1 was observed along the terminal retrotransposon array, thus this HP1 function is not limited to the telomere cap [117]. No, or only minor, changes were observed in telomere length or retroelement transcription in mutants of genes involved in telomere capping, such as *cav*, *moi*, *ver* or *atm* [61,117], which may indicate that terminin does not control telomere length. Another gene regulating telomere length is *prod*. Although *prod* mutants showed elevated levels of *HeT-A* transcripts, no change in telomere length was observed, suggesting that elevated retroelement transcription is not sufficient for telomere length growth [118]. Similar data were observed for members of rasiRNA (repeat-associated small interfering RNA) pathway *aub* (*aubergine*) and *Spn-E*. Their mutants displayed higher *HeT-A* transcript levels [119], albeit without any significant increase in telomere length (our unpublished data). In parallel with telomerase activity, transcription of telomeric elements is observed only in proliferating cells, such as embryonic cells, cells of imaginal discs, testis and ovaries [120,121].

http://dx.doi.org/10.5772/51356

Telomere length is maintained through an interplay between telomere maintenance mechanisms and telomere shortening events. Based on human research it has been proposed that telomerase activity and telomere length are modulated by different endogenous and exogenous factors, such as emotional or physical stress, health conditions and aging [102]. However, the prime factor in telomeric shortening may well be oxidative stress. Due to a high content of guanines, telomeres are particularly vulnerable to oxidative damage, and the impact of oxidative stress on telomeric length has been proposed to be much larger than the end-replication problem [122]. Endogenous oxidative stress is associated with several cellular processes, such as the mitochondrial OXPHOS system and inflammation. Mitochondrial dysfunction-induced reactive oxygen species and hyperoxia *in vitro* lead to accelerated telomere shortening and reduced proliferative lifespan of cultured somatic cells [123]. Thus, short telomeric length in humans appears to be linked to the limited proliferative capacity of normal somatic cells, and it is likely that telomeric shortening is one of the key events related to cellular senescence and organismal aging. As telomeres shorten with age, telomere length is considered as a biomarker of aging and a forecaster of longevity [102].

6. Conclusion

The ends of all linear chromosomes face the same difficulties regardless of their structures. Chromosome ends are not replicated completely by the standard replication machinery, resulting in loss of sequence and a 3' overhang on half of the replication products. Early eukaryotes may have solved the end replication problem by co-opting a reverse transcriptase that had arisen in a retrotransposable element [124] and using it to generate arrays of a simple repeat unit. They then solved the end protection problem by engineering long 3' overhangs on all termini, which could then loop around and tuck into the double stranded telomeric region and coating the terminal arrays with proteins that recognize the product of the reverse transcriptase. This combination of telomere maintenance by telomerase and protection by CST/shelterin served eukaryotes well and has been amazingly stable for more than a billion years.

Depending on how strictly shelterin recognizes the telomeric motif, the sequence may be conserved over long expanses of time, as in unikonts. If shelterin is less strict in recognizing the telomerase-generated motif, this sequence may have more latitude to vary, as in chromalveolates. If the protective telomere cap completely loses its ability to recognize the telomeric sequence, telomerase and the canonical telomeric motif may be lost. Many eukaryotes, including yeast and humans use unequal homologous recombination/gene conversion as a backup telomere maintenance system. It appears that in some species of plants and animals telomerase has been lost, and gene conversion has taken over as the primary mechanism to maintain chromosome length, with the eventual loss of telomeric motif. Chromosome length maintenance and end protection must be maintained through all of this. The evolution of new telomere structures, therefore, requires a delicate interplay between these two functions, as well as other telomeric functions that may be less well understood, such as heterochromatin formation and meiotic chromosome pairing.

Acknowledgements

The authors were supported in part by the Intramural Research Program, NIEHS, U. S. National Institutes of Health and by grant no. GAČR P501/10/1215 (Grant Agency of the Czech Republic, Prague).

Author details

Radmila Capkova Frydrychova[1*] and James M. Mason[2*]

*Address all correspondence to: radmila.frydrychova@hotmail.com

1 Institute of Entomology, Czech Republic

2 National Institute of Environmental Health Sciences, USA

References

[1] Greider, C.W., & Blackburn, E. H. (1996). Telomeres, telomerase and cancer. *Sci Am*, 274(2), 92-97.

[2] Mason, J. M., Frydrychova, R. C., & Biessmann, H. (2008). Drosophila telomeres: an exception providing new insights. *Bioessays*, 30(1), 25-37.

[3] Biessmann, H., & Mason, J. M. (2003). Telomerase-independent mechanisms of telomere elongation. *Cell Mol Life Sci*, 60(11), 2325-2333.

[4] Zakian, V. A. (2012). Telomeres: The beginnings and ends of eukaryotic chromosomes. *Exp Cell Res*, 318(12), 1456-1460.

[5] Sohanpal, B. K., Morzaria, S. P., Gobright, E. I., & Bishop, R. P. (1995). Characterisation of the telomeres at opposite ends of a 3 Mb Theileria parva chromosome. *Nucleic Acids Res*, 23(11), 1942-1947.

[6] Liu, C., Schroeder, A. A., Kapur, V., & Abrahamsen, M. S. (1998). Telomeric sequences of Cryptosporidium parvum. *Mol Biochem Parasitol*, 94(2), 291-296.

[7] Klobutcher, L. A., Swanton, M. T., Donini, P., & Prescott, D. M. (1981). All gene-sized DNA molecules in four species of hypotrichs have the same terminal sequence and an unusual 3′ terminus. *Proc Natl Acad Sci U S A*, 78(5), 3015-3019.

[8] Alverca, E., Cuadrado, A., Jouve, N., Franca, S., & Moreno Diaz., de la Espina. (2007). Telomeric DNA localization on dinoflagellate chromosomes: structural and evolutionary implications. *Cytogenet Genome Res*, 116(3), 224-231.

[9] Bowler, C., Allen, A. E., Badger, J. H., Grimwood, J., Jabbari, K., Kuo, A., et al. (2008). The Phaeodactylum genome reveals the evolutionary history of diatom genomes. *Nature*, 456(7219), 239-244.

[10] Koonin, E. V. (2010). Preview .The incredible expanding ancestor of eukaryotes. *Cell*, 140(5), 606-608.

[11] Gilson, P. R., & McFadden, G. I. (2002). Jam packed genomes--a preliminary, comparative analysis of nucleomorphs. *Genetica*, 115(1), 13-28.

[12] Wellinger, R. J., & Sen, D. (1997). The DNA structures at the ends of eukaryotic chromosomes. *Eur J Cancer*, 33(5), 735-749.

[13] Nozaki, H., Takano, H., Misumi, O., Terasawa, K., Matsuzaki, M., & Maruyama, S. (2007). A 100%-complete sequence reveals unusually simple genomic features in the hot-spring red alga Cyanidioschyzon merolae. *BMC Biol*, 5, 28.

[14] Fulneckova, J., Hasikova, T., Fajkus, J., Lukesova, A., Elias, M., & Sykorova, E. (2012). Dynamic evolution of telomeric sequences in the green algal order Chlamydomonadales. *Genome Biol Evol*, 4(3), 248-264.

[15] Pich, U., Fuchs, J., & Schubert, I. (1996). How do Alliaceae stabilize their chromosome ends in the absence of TTTAGGG sequences? *Chromosome Res*, 4(3), 207-213.

[16] Schubert, I., Rieger, R., Fuchs, J., & Pich, U. (1994). Sequence organization and the mechanism of interstitial deletion clustering in a plant genome (Vicia faba). *Mutat Res*, 325(1), 1-5.

[17] Fajkus, J., Kovarik, A., Kralovics, R., & Bezdek, M. (1995). Organization of telomeric and subtelomeric chromatin in the higher plant Nicotiana tabacum. *Mol Gen Genet*, 247(5), 633-638.

[18] Peska, V., Sykorova, E., & Fajkus, J. (2008). Two faces of Solanaceae telomeres: a comparison between Nicotiana and Cestrum telomeres and telomere-binding proteins. *Cytogenet Genome Res*, 122(3-4), 380-387.

[19] Sykorova, E., Lim, K. Y., Fajkus, J., & Leitch, A. R. (2003). The signature of the Cestrum genome suggests an evolutionary response to the loss of (TTTAGGG)n telomeres. *Chromosoma*, 112(4), 164-172.

[20] Watson, J. M., & Riha, K. (2010). Comparative biology of telomeres: where plants stand. *FEBS Lett*, 584(17), 3752-3759.

[21] Vitkova, M., Kral, J., Traut, W., Zrzavy, J., & Marec, F. (2005). The evolutionary origin of insect telomeric repeats, (TTAGG)n. *Chromosome Res*, 13(2), 145-156.

[22] Frydrychova, R., & Marec, F. (2002). Repeated losses of TTAGG telomere repeats in evolution of beetles (Coleoptera). *Genetica*, 115, 179-187.

[23] Frydrychova, R., Grossmann, P., Trubac, P., Vitkova, M., & Marec, F. (2004). Phylogenetic distribution of TTAGG telomeric repeats in insects. *Genome*, 47(1), 163-178.

[24] Mravinac, B., Mestrovic, N., Cavrak, V. V., & Plohl, M. (2011). TCAGG, an alternative telomeric sequence in insects. *Chromosoma*, 120(4), 367-376.

[25] Mason, J. M., & Biessmann, H. (1995). The unusual telomeres of Drosophila. *Trends Genet*, 11(2), 58-62.

[26] Sasaki, T., & Fujiwara, H. (2000). Detection and distribution patterns of telomerase activity in insects. *Eur J Biochem*, 267(10), 3025-3031.

[27] Wiegmann, B. M., Trautwein, M. D., Winkler, I. S., Barr, N. B., Kim, J. W., Lambkin, C., et al. (2011). Episodic radiations in the fly tree of life. *Proc Natl Acad Sci U S A*, 108(14), 5690-5695.

[28] Lundblad, V., & Blackburn, E. H. (1993). An alternative pathway for yeast telomere maintenance rescues est1- senescence. *Cell.*, 73(2), 347-360.

[29] Nielsen, L., & Edstrom, J. E. (1993). Complex telomere-associated repeat units in members of the genus Chironomus evolve from sequences similar to simple telomeric repeats. *Mol Cell Biol*, 13(3), 1583-1589.

[30] Nielsen, L., Schmidt, E. R., & Edstrom, J. E. (1990). Subrepeats result from regional DNA sequence conservation in tandem repeats in Chironomus telomeres. *J Mol Biol*, 216(3), 577-584.

[31] Cohn, M., & Edstrom, J. E. (1992). Chromosome ends in Chironomus pallidivittatus contain different subfamilies of telomere-associated repeats. *Chromosoma*, 101(10), 634-640.

[32] Biessmann, H., Walter, M. F., & Mason, J. M. (1997). Drosophila telomere elongation. *Ciba Found Symp*, 211, 53-67.

[33] Biessmann, H., Kobeski, F., Walter, M. F., Kasravi, A., & Roth, C. W. (1998). DNA organization and length polymorphism at the 2L telomeric region of Anopheles gambiae. *Insect Mol Biol*, 7(1), 83-93.

[34] Roth, C. W., Kobeski, F., Walter, M. F., & Biessmann, H. (1997). Chromosome end elongation by recombination in the mosquito Anopheles gambiae. *Mol Cell Biol*, 17(9), 5176-5183.

[35] Madalena, C. R., Amabis, J. M., & Gorab, E. (2010). Unusually short tandem repeats appear to reach chromosome ends of Rhynchosciara americana (Diptera: Sciaridae). *Chromosoma*, 119(6), 613-623.

[36] Pryde, F. E., Gorham, H. C., & Louis, E. J. (1997). Chromosome ends: all the same under their caps. *Curr Opin Genet Dev*, 7(6), 822-828.

[37] George, J. A., Traverse, K. L., DeBaryshe, P. G., Kelley, K. J., & Pardue, M. L. (2010). Evolution of diverse mechanisms for protecting chromosome ends by Drosophila TART telomere retrotransposons. *Proc Natl Acad Sci U S A*, 107(49), 21052-21057.

[38] 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*, 91(26), 12510-12514.

[39] Rashkova, S., Karam, S. E., Kellum, R., & Pardue, M. L. Gag proteins of the two Drosophila telomeric retrotransposons are targeted to chromosome ends. *J Cell Biol*, 159(3), 397-402.

[40] Casacuberta, E., & Pardue, M. L. (2003). HeT-A elements in Drosophila virilis: retrotransposon telomeres are conserved across the Drosophila genus. *Proc Natl Acad Sci U S A*, 100(24), 14091-14096.

[41] Casacuberta, E., & Pardue, M. L. (2003). Transposon telomeres are widely distributed in the Drosophila genus: TART elements in the virilis group. *Proc Natl Acad Sci U S A*, 100(6), 3363-3368.

[42] Greider, C. W. (1999). Telomeres do D-loop-T-loop. *Cell*, 97(4), 419-422.

[43] Tomaska, L., Willcox, S., Slezakova, J., Nosek, J., & Griffith, J. D. (2004). Taz1 binding to a fission yeast model telomere: formation of telomeric loops and higher order structures. *J Biol Chem*, 279(49), 50764-50772.

[44] Muller, H. J., Herskowitz, I. H., Abrahamson, S., & Oster, I. I. (1954). A Nonlinear Relation between X-Ray Dose and Recovered Lethal Mutations in Drosophila. *Genetics*, 39(5), 741-749.

[45] Riha, K., Heacock, M. L., & Shippen, D. E. (2006). The role of the nonhomologous end-joining DNA double-strand break repair pathway in telomere biology. *Annu Rev Genet*, 40, 237-277.

[46] Capper, R, Britt-Compton, B, Tankimanova, M, Rowson, J, Letsolo, B, & Man, S. (2007). The nature of telomere fusion and a definition of the critical telomere length in human cells. *Genes Dev*, 21(19), 2495-2508.

[47] Linger, B. R., & Price, C. M. (2009). Conservation of telomere protein complexes: shuffling through evolution. *Crit Rev Biochem Mol Biol*, 44(6), 434-446.

[48] Palm, W., & de , L. T. (2008). How shelterin protects mammalian telomeres. *Annu Rev Genet*, 42, 301-334.

[49] Chen, L. Y., Liu, D., & Songyang, Z. (2007). Telomere maintenance through spatial control of telomeric proteins. *Mol Cell Biol*, 27(16), 5898-909.

[50] d'Adda di, F. F., Teo, S. H., & Jackson, S. P. (2004). Functional links between telomeres and proteins of the DNA-damage response. *Genes Dev*, 18(15), 1781-1799.

[51] Diotti, R., & Loayza, D. (2011). Shelterin complex and associated factors at human telomeres. *Nucleus*, 2(2), 119-135.

[52] Longhese, M. P. (2008). DNA damage response at functional and dysfunctional telomeres. *Genes Dev*, 22(2), 125-140.

[53] Celli, G. B., Denchi, E. L., & de, L. T. (2006). Ku70 stimulates fusion of dysfunctional telomeres yet protects chromosome ends from homologous recombination. *Nat Cell Biol*, 8(8), 885-890.

[54] Denchi, E. L., & de, L. T. (2007). Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. *Nature*, 448(7157), 1068-1071.

[55] Lamarche, B. J., Orazio, N. I., & Weitzman, M. D. (2010). The MRN complex in double-strand break repair and telomere maintenance. *FEBS Lett*, 584(17), 3682-3695.

[56] Wu, P., van Rooney, O. M., & de , S. L. T. (2010). Apollo contributes to G overhang maintenance and protects leading-end telomeres. *Mol Cell*, 39(4), 606-617.

[57] Sfeir, A., & de , L. T. (2012). Removal of shelterin reveals the telomere end-protection problem. *Science*, 336(6081), 593-597.

[58] Greider, C. W. (1999). Telomeres do D-loop-T-loop. *Cell*, 97(4), 419-422.

[59] Gu, P, Min, J. N., Wang, Y, Huang, C, Peng, T, & Chai, W. (2012). CTC1 deletion results in defective telomere replication, leading to catastrophic telomere loss and stem cell exhaustion. *EMBO J*, 31(10), 2309-2321.

[60] Raffa, G. D., Raimondo, D., Sorino, C., Cugusi, S., Cenci, G., Cacchione, S., et al. (2010). Verrocchio, a Drosophila OB fold-containing protein, is a component of the terminin telomere-capping complex. *Genes Dev*, 24(15), 1596-1601.

[61] Raffa, G. D., Ciapponi, L., Cenci, G., & Gatti, M. (2011). Terminin: a protein complex that mediates epigenetic maintenance of Drosophila telomeres. *Nucleus*, 2(5), 383-391.

[62] Cenci, G., Siriaco, G., Raffa, G. D., Kellum, R., & Gatti, M. (2003, Jan). The Drosophila HOAP protein is required for telomere capping. *Nat Cell Biol*, 5(1), 82-84.

[63] Raffa, G. D., Siriaco, G, Cugusi, S, Ciapponi, L, Cenci, G, & Wojcik, E. (2009). The Drosophila modigliani (moi) gene encodes a HOAP-interacting protein required for telomere protection. *Proc Natl Acad Sci U S A*, 106(7), 2271-2276.

[64] Komonyi, O., Schauer, T., Papai, G., Deak, P., & Boros, I. M. (2009). A product of the bicistronic Drosophila melanogaster gene CG31241, which also encodes a trimethylguanosine synthase, plays a role in telomere protection. *J Cell Sci*, Mar 15, 122(Pt 6), 769-774.

[65] Gao, G., Walser, J. C., Beaucher, M. L., Morciano, P., Wesolowska, N., Chen, J., et al. HipHop interacts with HOAP and HP1 to protect Drosophila telomeres in a sequence-independent manner. *EMBO J*, 29(4), 819-829.

[66] Biessmann, H., Kasravi, B., Bui, T., Fujiwara, G., Champion, L. E., & Mason, J. M. (1994, Apr). Comparison of two active HeT-A retroposons of Drosophila melanogaster. *Chromosoma*, 103(2), 90-98.

[67] Capkova, F. R., Biessmann, H., & Mason, J. M. (2008). Regulation of telomere length in Drosophila. *Cytogenet Genome Res*, 122(3-4), 356-364.

[68] Ciapponi, L., Cenci, G., & Gatti, M. (2006, Jul). The Drosophila Nbs protein functions in multiple pathways for the maintenance of genome stability. *Genetics*, 173(3), 1447-1454.

[69] Ciapponi, L., Cenci, G., Ducau, J., Flores, C., Johnson-Schlitz, D., & Gorski, M. M. (2004). The Drosophila Mre11/Rad50 complex is required to prevent both telomeric fusion and chromosome breakage. Curr Biol Aug 10; , 14(15), 1360-1366.

[70] Oikemus, S. R., McGinnis, N, Queiroz-Machado, J, Tukachinsky, H, Takada, S, Sunkel, C. E., et al. (2004, Aug). Drosophila atm/telomere fusion is required for telomeric localization of HP1 and telomere position effect. *Genes Dev*, 18(15), 1850-1861.

[71] Oikemus, S. R., Queiroz-Machado, J., Lai, K., Mc Ginnis, N., Sunkel, C., & Brodsky, M. H. (2006, May). Epigenetic telomere protection by Drosophila DNA damage response pathways. *PLoS Genet*, 2(5), e71.

[72] Bi, X., Wei, S. C., & Rong, Y. S. Telomere protection without a telomerase; the role of ATM and Mre11 in Drosophila telomere maintenance. *Curr Biol*, 14(15), 1348-1353.

[73] Raffa, G. D., Cenci, G., Siriaco, G., Goldberg, M. L., & Gatti, M. The putative Drosophila transcription factor woc is required to prevent telomeric fusions. *Mol Cell*, 20(6), 821-831.

[74] Cenci, G., Rawson, R. B., Belloni, G., Castrillon, D. H., Tudor, M., Petrucci, R., et al. UbcD1, a Drosophila ubiquitin-conjugating enzyme required for proper telomere behavior. *Genes Dev*, 11(7), 863-875.

[75] Canudas, S, Houghtaling, B. R., Bhanot, M, Sasa, G, Savage, S. A., Bertuch, A. A., et al. A role for heterochromatin protein 1gamma at human telomeres. *Genes Dev*, 25(17), 1807-19.

[76] Iglesias, N., Redon, S., Pfeiffer, V., Dees, M., Lingner, J., & Luke, B. (2011, Jun). Subtelomeric repetitive elements determine TERRA regulation by Rap1/Rif and Rap1/Sir complexes in yeast. *EMBO Rep*, 12(6), 587-593.

[77] Luke, B., & Lingner, J. TERRA: telomeric repeat-containing RNA. *EMBO J*, 28(17), 2503-2510.

[78] Schoeftner, S, & Blasco, M. A. A 'higher order' of telomere regulation: telomere heterochromatin and telomeric RNAs. *EMBO J*, 28(16), 2323-2336.

[79] Biessmann, H., Prasad, S., Semeshin, V. F., Andreyeva, E. N., Nguyen, Q., Walter, M. F., et al. (2005, Dec). Two distinct domains in Drosophila melanogaster telomeres. *Genetics*, 171(4), 1767-1777.

[80] Kurenova, E., Champion, L., Biessmann, H., & Mason, J. M. (1998, Nov). Directional gene silencing induced by a complex subtelomeric satellite from Drosophila. *Chromosoma*, 107(5), 311-320.

[81] Schoeftner, S., & Blasco, M. A. (2010, Apr). Chromatin regulation and non-coding RNAs at mammalian telomeres. *Semin Cell Dev Biol*, 21(2), 186-193.

[82] Su, T. T. Heterochromatin replication: better late than ever. *Curr Biol*, 20(23), R 1018-R1020.

[83] Leach, T. J., Chotkowski, H. L., Wotring, M. G., Dilwith, R. L., & Glaser, R. L. (2000, Sep). Replication of heterochromatin and structure of polytene chromosomes. *Mol Cell Biol*, 20(17), 6308-16.

[84] Gilbert, D. M., Takebayashi, S. I., Ryba, T., Lu, J., Pope, B. D. , Wilson, K. A., et al. (2010). Space and time in the nucleus: developmental control of replication timing and chromosome architecture. *Cold Spring Harb Symp Quant Biol*, 75, 143-153.

[85] Kim, S. M., Dubey, D. D., & Huberman, J. A. Early-replicating heterochromatin. *Genes Dev*, 17(3), 330-335.

[86] Arnoult, N., Schluth-Bolard, C., Letessier, A., Drascovic, I., Bouarich-Bourimi, R., Campisi, J., et al. (2010, Apr). Replication timing of human telomeres is chromosome arm-specific, influenced by subtelomeric structures and connected to nuclear localization. *PLoS Genet*, 6(4), e1000920.

[87] Bianchi, A., & Shore, D. Molecular biology Refined view of the ends. *Science*, 320(5881), 1301-1302.

[88] Bianchi, A., & Shore, D. Early replication of short telomeres in budding yeast. *Cell*, 128(6), 1051-1062.

[89] Stewart, J. A., Chaiken, M. F., Wang, F., & Price, C. M. (2012). Maintaining the end: roles of telomere proteins in end-protection, telomere replication and length regulation. Mutat Res Feb 1 , 730(1-2), 12-19.

[90] Fouche, N., Ozgur, S., Roy, D., & Griffith, J. D. (2006). Replication fork regression in repetitive DNAs. *Nucleic Acids Res*, 34(20), 6044-6050.

[91] Sampathi, S., & Chai, W. (2011, Jan). Telomere replication: poised but puzzling. *J Cell Mol Med*, 15(1), 3-13.

[92] Yang, Q., Zhang, R., Wang, X. W., Spillare, E. A., Linke, S. P., Subramanian, D., et al. (2002). The processing of Holliday junctions by BLM and WRN helicases is regulated by. J Biol Chem Aug 30 , 277(35), 31980-31987.

[93] Sfeir, A., Kosiyatrakul, S. T., Hockemeyer, D., Mac Rae, S. L., Karlseder, J., Schildkraut, C. L., et al. (2009). Mammalian telomeres resemble fragile sites and require TRF1 for efficient replication. CellJul 10 , 138(1), 90-103.

[94] Miller, K. M., Rog, O., & Cooper, J. P. (2006). Semi-conservative DNA replication through telomeres requires Taz1. NatureApr 6 , 440(7085), 824-828.

[95] Gu, P, Min, J. N., Wang, Y, Huang, C, Peng, T, Chai, W, et al. CTC1 deletion results in defective telomere replication, leading to catastrophic telomere loss and stem cell exhaustion. *EMBO J*, 31(10), 2309-2321.

[96] Raghuraman, M. K., Winzeler, E. A., Collingwood, D., Hunt, S., Wodicka, L., Conway, A., et al. (2001). Replication dynamics of the yeast genome. Science Oct 5 , 294(5540), 115-121.

[97] Greider, C. W., & Blackburn, E. H. The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. Cell (1987). Dec 24 , 51(6), 887-98.

[98] Greider, C. W. (2006). Telomerase RNA levels limit the telomere length equilibrium. *Cold Spring Harb Symp Quant Biol*, 71, 225-229.

[99] Greider, C. W., & Blackburn, E. H. (2004). Tracking telomerase. CellJan 23 2 , 116, S 83-S 86.

[100] Greider, C. W. (1994). Mammalian telomere dynamics: healing, fragmentation shortening and stabilization. Curr Opin Genet Dev Apr; , 4(2), 203-211.

[101] Wright, W. E., Piatyszek, M. A., Rainey, W. E., Byrd, W., & Shay, J. W. (1996). Telomerase activity in human germline and embryonic tissues and cells. *Dev Genet*, 18(2), 173-179.

[102] Zhu, H., Belcher, M., & van der Harst, P. (2011, May). Healthy aging and disease: role for telomere biology? *Clin Sci (Lond)*, 120(10), 427-440.

[103] Le , S., Zhu, J. J., Anthony, D. C., Greider, C. W., & Black, P. M. (1998, May). Telomerase activity in human gliomas. *Neurosurgery*, 42(5), 1120-1124.

[104] Cifuentes-Rojas, C., & Shippen, D. E. (2012). Telomerase regulation. Mutat Res Feb 1 , 730(1-2), 20-7.

[105] Nelson, N. D., & Bertuch, A. A. (2012). Dyskeratosis congenita as a disorder of telomere maintenance. Mutat Res Feb 1 , 730(1-2), 43-51.

[106] Armanios, M. Y., Chen, J. J., Cogan, J. D., Alder, J. K., Ingersoll, R. G., Markin, C., et al. (2007). Telomerase mutations in families with idiopathic pulmonary fibrosis. N Engl J Med Mar 29 , 356(13), 1317-1326.

[107] Greenberg, R. A., O'Hagan, R. C., Deng, H., Xiao, Q., Hann, S. R., Adams, R. R., et al. (1999). Telomerase reverse transcriptase gene is a direct target of c-Myc but is not functionally equivalent in cellular transformation. Oncogene Feb 4 , 18(5), 1219-1226.

[108] Kyo, S., Takakura, M., Fujiwara, T., & Inoue, M. (2008, Aug). Understanding and exploiting hTERT promoter regulation for diagnosis and treatment of human cancers. *Cancer Sci*, 99(8), 1528-1538.

[109] Holt, S. E., Wright, W. E., & Shay, J. W. (1997, Apr). Multiple pathways for the regulation of telomerase activity. *Eur J Cancer*, 33(5), 761-766.

[110] Loayza, D., & de, LT. (2003). POT1 as a terminal transducer of TRF1 telomere length control. Nature Jun 26 , 423(6943), 1013-1018.

[111] Wang, F., Podell, E. R., Zaug, A. J., Yang, Y., Baciu, P., Cech, T. R., et al. (2007). The POT1-TPP1 telomere complex is a telomerase processivity factor. Nature Feb 1 , 445(7127), 506-510.

[112] Zaug, A. J., Podell, E. R., Nandakumar, J., & Cech, T. R. (2010). Functional interaction between telomere protein TPP1 and telomerase. Genes Dev Mar 15 , 24(6), 613-22.

[113] Zaug, A. J., Podell, E. R., & Cech, T. R. (2005). Human POT1 disrupts telomeric G-quadruplexes allowing telomerase extension in vitro. Proc Natl Acad Sci U S A Aug 2 , 102(31), 10864-10869.

[114] Savitsky, M., Kravchuk, O., Melnikova, L., & Georgiev, P. (2002, May). Heterochromatin protein 1 is involved in control of telomere elongation in Drosophila melanogaster. *Mol Cell Biol*, 22(9), 3204-3218.

[115] Perrini, B., Piacentini, L., Fanti, L., Altieri, F., Chichiarelli, S., & Berloco, M. (2004). HP1 controls telomere capping, telomere elongation, and telomere silencing by two different mechanisms in Drosophila. Mol Cell Aug 13 , 15(3), 467-476.

[116] Frydrychova, R. C., Biessmann, H., Konev, A. Y., Golubovsky, M. D., Johnson, J., Archer, T. K., et al. (2007, Jul). 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*, 27(13), 4991-5001.

[117] Frydrychova, R. C., Mason, J. M., & Archer, T. K. (2008, Sep). HP1 is distributed within distinct chromatin domains at Drosophila telomeres. *Genetics*, 180(1), 121-131.

[118] Torok, T., Benitez, C., Takacs, S., & Biessmann, H. (2007, Apr). The protein encoded by the gene proliferation disrupter (prod) is associated with the telomeric retrotransposon array in Drosophila melanogaster. *Chromosoma*, 116(2), 185-195.

[119] 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 Feb 1 , 20(3), 345-354.

[120] George, J. A., & Pardue, M. L. (2003, Feb). The promoter of the heterochromatic Drosophila telomeric retrotransposon, HeT-A, is active when moved into euchromatic locations. *Genetics*, 163(2), 625-635.

[121] Walter, M. F., & Biessmann, H. (2004, May). Expression of the telomeric retrotransposon HeT-A in Drosophila melanogaster is correlated with cell proliferation. *Dev Genes Evol*, 214(5), 211-9.

[122] Oikawa, S., Tada-Oikawa, S., & Kawanishi, S. (2001). Site-specific DNA damage at the GGG sequence by UVA involves acceleration of telomere shortening. Biochemistry Apr 17 , 40(15), 4763-8.

[123] Barja, G. (2002). Rate of generation of oxidative stress-related damage and animal longevity. Free Radic Biol Med Nov 1 , 33(9), 1167-1172.

[124] Belfort, M., Curcio, M. J., & Lue, N. F. (2011). Telomerase and retrotransposons: reverse transcriptases that shaped genomes. Proc Natl Acad Sci U S A Dec 20 , 108(51), 20304-20310.