Trends in Genetics

CellPress

Review

Genome stability from the perspective of telomere length

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Telomeres and their associated proteins protect the ends of chromosomes to maintain genome stability. Telomeres undergo progressive shortening with each cell division in mammalian somatic cells without telomerase, resulting in genome instability. When telomeres reach a critically short length or are recognized as a damage signal, cells enter a state of senescence, followed by cell cycle arrest, programmed cell death, or immortalization. This review provides an overview of recent advances in the intricate relationship between telomeres and genome instability. Alongside well-established mechanisms such as chromosomal fusion and telomere fusion, we will delve into the perspective on genome stability by examining the role of retrotransposons. Retrotransposons represent an emerging pathway to regulate genome stability through their interactions with telomeres.

Highlights

Telomeres and associated proteins safeguard chromosome ends to preserve genome stability.

Destabilization of telomeres leads to fusion of telomeres and accumulation of DNA damage.

Telomeres and retrotransposons exhibit reciprocal regulation of each other.

Shortest telomeres can cause genome instability by epigenetic activation of retrotransposons.

Telomeres and associated components

Linear chromosomes are grouped in pairs within the nucleus of mammalian cells. To maintain genome stability, the natural ends of linear chromosomes need to be protected from exonucleolytic degradation and fusion events. This protection is carried out by telomeres and associated proteins. Telomeres are repetitive DNA sequences (TTAGGG in vertebrates) that span thousands of nucleotides as double-stranded DNA (dsDNA) and terminate as 130–210-nucleotide, G-rich, single-stranded DNA (ssDNA) [1]. Telomere ssDNA has the ability to invade dsDNA and form distinct telomere structures known as displacement loops (D-loops) and telomere loops (T-loops) [2].

In addition to DNA repeats, functional telomeres consist of multiple proteins. The shelterin complex, comprising Trf1, Trf2, Pot1, Rap1, Tin2, and Tpp1, is responsible for safeguarding telomere DNA [3]. Trf1 and Trf2 directly bind to telomere dsDNA [4–7], while Pot1 recognizes telomere ssDNA and forms a heterodimer with Tpp1 [8–10]. Tin2 interacts with DNA-binding proteins within the complex to stabilize the shelterin structure [11,12], while Rap1 is recruited to telomeres by Trf2 [13]. Another crucial complex for telomere maintenance is the CST (CTC1, STN1, and TEN1) complex [3]. The CST complex competes with Tpp1-Pot1 for binding to telomere ssDNA, obstructing telomerase access to telomere G overhangs. This termination of telomere extension leads to the recruitment of DNA polymerase α primase, which fills the C-strand [14–16]. Various accessory factors are also present at telomeres. Rtel1 dissembles G-quadruplex (G4) structures formed on telomere ssDNA, facilitating DNA replication and removing T-loops to prevent overprocessing of telomeres by the SLX1/4 nuclease complex [17]. Telomeric repeat-containing RNA (*Terra*), a long noncoding RNA (lncRNA) transcribed from telomeric DNA, forms an R-loop with telomeric DNA and aids in the establishment of heterochromatin at telomeres [18,19].

Telomere length varies across different species, but it is not directly correlated with the lifespan of those species. For example, humans have an average lifespan of 73.2 years and are born with

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telomeres ranging from 8 to 15 kb, while mice are born with telomeres ranging from 50 to 60 kb but have an average lifespan of about 2 years [20,21]. This difference in lifespan may be attributed to variations in the rate of telomere shortening, as studies have found that the rate of telomere shortening can predict the lifespan of a species [22]. In the absence of maintenance mechanisms, telomeres naturally shorten with each cell division due to internal damage and replication issues that prevent the synthesis of the lagging-strand sequence at the ends of chromosomes during DNA replication [23,24]. When telomeres become too short, cells can enter replicative senescence, a permanent cell cycle arrest, ensuring that short telomeres do not become shorter. When telomeres become excessively damaged or critically short in proliferating cells, they gradually lose their ability to protect chromosomes from end-to-end fusion, leading to genomic instability. Most cells cannot survive such instability. However, if other genetic changes, such as the silencing of tumor suppressor genes or the activation of oncogenes, occur along with genomic instability, the telomere maintenance pathways can be reactivated [25].

Telomere shortening can be counteracted by the enzyme telomerase or through alternative lengthening of telomeres (ALT). Telomerase is composed of several components, including the reverse transcriptase Tert, the template lncRNA *Terc*, and accessory proteins such as Dkc1, Nop10, Tcab1, Nhp2, and Gar1 [26]. Telomerase uses the RNA template to extend the telomeres. In the absence of telomerase, telomeres can also be lengthened through ALT, which relies on homologous recombination-based DNA synthesis at telomeres [27]. The majority of cancers rely on telomerase to extend their telomeres, while approximately 10–15% of cancers use ALT as a means of maintaining telomere length [28,29]. The elongation of telomeres allows cells carrying mutations to continue proliferating and eventually become cancerous.

In view of the important roles of telomeres and associated components, revisiting telomere function will provide further insights into the regulation of genome stability. This review aims to summarize recent findings on the relationship between telomere length and genomic changes.

Role of telomere-associated proteins and RNAs in regulating telomere length and chromosomal stability

Telomere-associated components are intricately involved in maintaining the integrity and functionality of telomeres, as well as ensuring the stability of the chromosomes. Chromosomal fusion and telomere fusion can occur during DNA replication as a result of various factors (Figure 1). The unique structure and heterochromatin nature of telomeres, including G4 and T-loop structures, can hinder the progress of replication forks [30,31]. When replication forks are stalled, they may collapse, leading to the formation of DNA double-strand breaks (DSBs) [32]. There are two primary pathways for repairing DSBs, homology-directed repair (HDR) and nonhomologous end joining (NHEJ). Both NHEJ and HDR can repair DSBs at telomeres, with HDR being an error-free repair mechanism, while NHEJ-mediated repair increases the risk of chromosomal fusion at telomeres [33]. The fusion events caused by dysfunctional telomeres require the assistance of DNA ligases. In the absence of DNA ligase IV (LigIV), the frequency of interchromosomal translocations is greatly reduced [34], indicating the critical role of LigIV-dependent classical nonhomologous end joining (C-NHEJ) in driving interchromosomal telomere fusion. By contrast, alternative nonhomologous end joining (A-NHEJ) is necessary for telomere fusion between intrachromosomal sister chromatids. DNA ligase I (LigI) participates in both inter- and intrachromosomal telomere fusion [35].

In the absence of factors that facilitate the replication of telomeric DNA, telomeres tend to shorten and exhibit an increased propensity for chromatid fusion. For example, the loss of *Rtel1* results in telomere shortening and an elevation in genomic rearrangements [36]. Deletion of *Rtel1* in

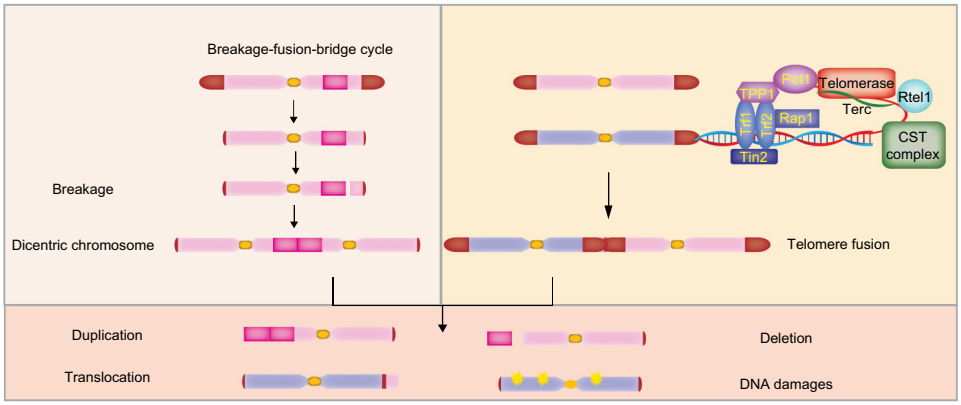

Figure 1. Effect of the breakage–fusion–bridge cycle on genome stability and role of telomere fusion and disruption of telomere proteins on genome instability. As a consequence, the genome becomes unstable, showing translocation, deletion, duplication, and DNA damage.

embryonic stem cells (ESCs) also increases sensitivity to DNA damage without impacting telomere length [37]. Depletion of the G4-processing exonuclease *Exo1* leads to telomere shortening and chromosome fusion. *Terra* also plays a role in facilitating telomere DNA replication [38]. Deletion of *Terra* impairs telomere DNA replication and is associated with decreased telomere length, increased telomere DNA damage, and events of telomere fusion [39,40].

The shelterin complex components Trf1 and Trf2 play crucial roles in regulating telomere length and stability. Loss of *Trf1* or *Trf2* leads to distinct phenotypes in terms of telomere fusion and telomere length. *Trf2* deletion in ESCs does not impact telomere length, and knockout of *Trf2* or *Trf1* alone does not induce telomere fusion [41]. However, *Trf22* depletion results in end-to-end chromosome fusions in all other cell types [42,43]. The function of Trf1 is conserved in both ESCs and somatic cells [44]. Loss of *Trf1* induces telomere replication stress and telomere loss [44]. Deletion of *Trf1* specifically in germ cells also causes chromosome end-to-end fusion [45]. Conversely, overexpression of Trf1 or Trf2 leads to progressive telomere shortening. Additionally, Trf2 overexpression, along with XPF nuclease-dependent telomere shortening, triggers increased chromosomal instability in mouse skin [46]. Overexpression of TRF1 also results in subtelomere recombination in senescent cells [47].

The shelterin complex components Pot1a and Pot1b appear to have distinct functions in regulating telomere length and chromosomal stability. Depletion of *Pot1a* in mice elongates telomeres and activates HDR-mediated chromosome fusions [48,49]. By contrast, loss of *Pot1b* or *Tpp1* results in telomere shortening and chromosomal fusion [50]. The shelterin complex component Tin2 is also implicated in telomere length regulation. *Tin2* mutation causes severe short-telomere syndromes [51,52]. Similarly, *Tin2* depletion elicits unstable chromosomal ends and chromosomal fusions [53]. As an accessory subunit of Trf2, *Rap1* loss does not cause mean telomere length shortening but does induce telomere fusion [54].

Chromosomal stability is also regulated by telomere proteins involved in DNA damages. For instance, an important protein complex associated with telomeres, known as the CST complex, plays a dual role in both preserving telomere length and managing DNA damage. The CST complex promotes C-strand fill-in and maintains the length of 3′ polyG ssDNA [14–16]. Loss of the key component of the CST complex *CTC1* results in extension of telomere G-overhang and telomeric DNA damage, while the loss of *STN* leads to progressive telomere shortening

[55]. Apart from its role at telomeres, the CST complex is also involved in genome-wide replication recovery. CTC1 mutation causes telomere loss, reduces CST interaction with RAD51, and disrupts CTC1 binding to GC-rich genomic fragile sites, thereby increasing spontaneous chromosome breakage and chromosome fragmentation under replication stress [56,57]. CST dysfunction can also cause diverse forms of DNA damage [58], indirectly linking telomere length maintenance to DNA damage. These findings highlight the important role of telomere-associated components in regulating telomere length and genome stability.

Impact of telomere length on genome stability

Senescent or cancer cells containing critically short telomeres often exhibit telomere fusion. Intra-sister chromosomal fusion is most commonly observed, while interchromosomal telomere fusion and fusion of telomeres with nontelomeric DNA DSB sites are less frequent [34]. Telomere loss and fusion have been linked to extensive genomic rearrangements [59–61] (Figure 1). Upon chromosome fusion, dicentric chromosomes form, and the fused chromatids create a bridge during anaphase, which breaks when the centromeres move toward opposite poles [62]. Since the breakage sites often do not coincide with the fusion region, one daughter cell carries a chromosome with terminal deletion, which allows another cycle of breakage–fusion–bridge to occur [62]. The other daughter cell carries a chromosome with inverted duplication [62]. As chromothripsis accumulates, DNA replication is accompanied by extensive DNA damage [63]. Consequently, gene amplification and chromosome translocations emerge during cell replication [63]. As a result, cells accumulate subclonal heterogeneity, similar to that found in cancer cells [63].

Telomere length changes with defects in telomere DNA replication, the CST complex, or the shelterin complex. However, the mean telomere length is not directly inversely correlated with chromosomal fusion. Chromosomal fusion can still occur during telomere maintenance/elongation, such as in the case of *Pot1a* or *Trf2* depletion. In instances of telomere shortening, chromosomal fusion is associated with an increased incidence of critically short telomeres resulting from genetic disruption of the shelterin complex. This is consistent with the finding that the shortest telomeres in a cell are responsible for chromosomal instability [64]. Telomere fusions frequently occur in mice lacking telomerase [65]. Our recent study also demonstrates that only cells with critically short telomeres experience an increase in the number of chromosomal fusion events [66]. Cells with short telomeres can still maintain their chromosomal stability. Therefore, it is important to determine whether the number of chromosomes with critically short telomeres increases, in addition to the maintenance or elongation of average telomere length, especially in cases of *Pot1a* or *Trf2* depletion.

Long telomeres may also promote genome stability by exerting control over DNA damage. Notably, mice with hyperlong telomeres accumulate less DNA damage in tissues during aging. By contrast, critical short telomeres induce persistent DNA damage, as well as aging and aging-associated diseases (Figure 1). Cells with critically short telomeres demonstrate a significantly increased amount of DNA damage [66]. This finding is consistent with the observation that DNA repair genes are downregulated only in cells with critically short telomeres but not in wild-type cells or cells with short telomeres [66]. The increased genome-wide DNA damage events are supported by the higher number of single nucleotide variants (SNVs), indels, and single base substitutions (SBSs) in cells with critically short telomeres [66]. Dysfunctional DNA repair ability adds to increased DNA damage and mutations. Another possible source of mutations caused by critically short telomeres is replication stress, which is induced by oncogene overexpression [67]. Replication stress is a major cause of genome instability, as the collapse of DNA replication forks results in the induction of DSBs [32]. Due to the deficiency of NHEJs near telomeres, DSBs are vulnerable to mutations and rearrangements once they occur at

subtelomeric regions [24]. The remaining question is how critically short telomeres suppress the expression of DNA repair genes while activating oncogenes.

Retrotransposons and telomere length

Reciprocal regulation between retrotransposons and telomere length

Retrotransposons, crucial constituents of the genome, play integral roles in genome architecture, stability, and transcriptional regulation [68–70]. Telomere length is initially associated with retrotransposon activity in yeast [71]. Upon inactivation of telomerase, telomere length progressively shortens, accompanied by Ty1 long terminal repeat (LTR)-retrotransposon activation through the telomere checkpoint pathway in yeast [71]. Subsequently, the DNA damage checkpoint is activated, leading to the arrest of yeast cells in G2/M phase [71]. However, those cells that manage to escape cell cycle arrest develop alternative telomere structures, partially repressing the transcriptional activation of Ty1 LTR retrotransposons [71]. The activation of the Ty1 LTR retrotransposon can promote the stabilization of yeast telomeres. The reverse transcriptase of Ty1 utilizes Ty1 cDNA intermediates as primers to reverse transcribe Y′ RNA into Ty1 cDNA, which then recombines with subtelomeric Y′ elements to maintain telomeres in surviving yeast cells with critically short telomeres [72]. A similar phenomenon is observed in the *Drosophila* genome. The *Drosophila* genome lacks a gene encoding telomerase and canonical telomere repeats. *Drosophila* does not rely on conserved short DNA repeats as telomeres for end protection and instead have domesticated retrotransposons, which are inserted specifically at chromosome ends to protect chromosomes from erosion [73]. In *Bombyx mori* (silkworm), telomeres comprise canonical telomere repeats and retrotransposons. These findings suggest reciprocal regulation between retrotransposons and telomere length.

An analogous regulatory strategy has also been observed in mammalian cells. Loss of telomerase in a variety of mouse cells and tissues leads to shortened telomeres and concurrent activation of retrotransposons [66]. Upon passaging, telomeres become critically short, which is associated with extensive activation of retrotransposons, including LINE1s, IAPs, and MERVL [66]. These activated retrotransposons are enriched at subtelomeric regions [66]. The activation of retrotransposons can be attributed to the decrease in the heterochromatin mark H3K9me3 and increased chromatin accessibility [66], since retrotransposons are known to be silenced by H3K9me3-marked heterochromatin in both human and mouse cells [68,69,74].

Aging cells with shorter telomeres demonstrate reduced H3K9me3 levels (Figure 2) [75]. Consistently, premature aged cells from Werner syndrome patients also display shorter telomeres and reduced H3K9me3 levels [76]. Similarly, Hutchinson–Gilford progeria syndrome (HGPS) cells demonstrated accelerated telomere erosion and a reduction in H3K9me3 [77]. As telomeres shorten and H3K9me3 decreases, retrotransposons such as LINE1 and HERVK become activated in both aging cells and premature aged cells (Figure 2) [78–81]. Proteins involved in establishing H3K9me3 and maintaining telomeres also contribute to the suppression of retrotransposons (Figure 2). For instance, the Daxx and Atrx complex safeguards the genome by silencing repetitive elements, including telomeres and retrotransposons, through Suv39h recruitment and H3K9me3 [82]. Hdac5, which facilitates H3K9me3 deposition and telomere maintenance, also has the ability to repress retrotransposons [83]. Several other proteins indirectly participate in regulating telomere length and H3K9me3 deposition on retrotransposons, such as CLOCK, SIRT7, and DGCR8. Interestingly, CLOCK, SIRT7, and DGCR8 are downregulated in senescent cells, which often have shorter telomeres [79–81]. CLOCK regulates telomeres by controlling the expression of Tert mRNA while simultaneously repressing LINE1 through H3K9me3 [81,84]. SIRT7 promotes the expression of TR4, which is involved in the ALT process [85,86]. SIRT7 represses LINE1 and counteracts cellular aging [80,87]. DGCR8 controls the

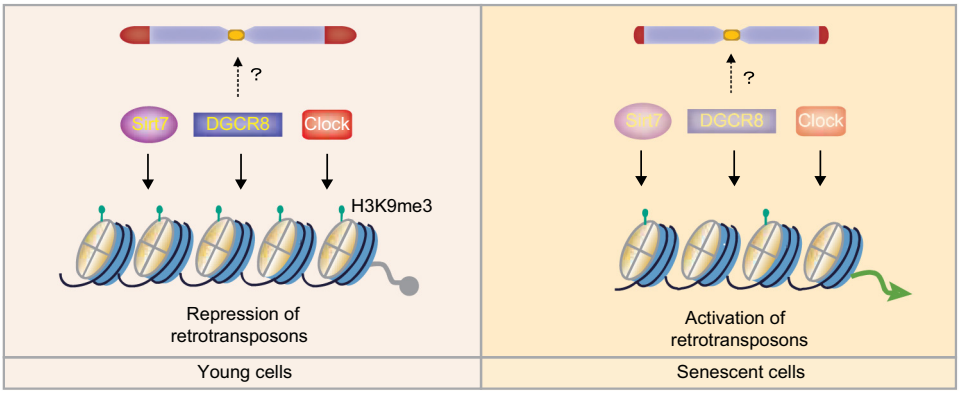

Figure 2. Regulation of telomere length and retrotransposons by H3K9me3-marked heterochromatin in young and senescent cells. In young cells, retrotransposons are repressed by H3K9me3-marked heterochromatin. In senescent cells, retrotransposons are activated upon reduced expression of Sirt7, DGCR8, and CLOCK. It remains to be determined whether Sirt7, DGCR8, and CLOCK contribute to telomere maintenance and telomeric heterochromatin in senescent and young cells. Broken arrow represents uncertain regulation.

stability of human telomerase RNA and is essential for maintaining H3K9me3-marked heterochromatin, including LINE1 loci [79,88]. Considering that telomeres are also part of H3K9me3-marked heterochromatin, it would be intriguing to investigate whether CLOCK, SIRT7, or DGCR8 directly regulate telomere length and stability in young and senescent cells. These findings highlight the intricate interconnection between telomeres, H3K9me3, and the restriction of retrotransposons.

As an important part of heterochromatin, telomere length not only affects the number of telomeric H3 histones but also regulates telomeric H3K9me3 frequency [89]. Short telomeres tend to have a lower H3K9me3 density per H3 compared with longer telomeres [89]. This may further contribute to chromatin openness at telomeric regions and affect retrotransposon expression at subtelomere regions through telomere position effects. In this mechanism, subtelomeric genes are repressed by telomeric heterochromatin, while telomere shortening leads to the activation of subtelomeric genes [90]. Additionally, distant retrotransposons may also be influenced by telomeres through long-distance looping, which becomes more pronounced in the absence of functional telomeres [66]. Reintroduction of telomerase represses activated retrotransposons, highlighting the critical role of telomere length in restricting retrotransposon expression in mammals [66]. ESCs with critically short telomeres exhibit increased chromatin interactions at subtelomeric regions, which is associated with enhanced transcription [66]. The activation of MERVL in ESCs with critically short telomeres can be explained by the upregulation of genes located at subtelomeres, such as *Zscan4*, which is known to promote MERVL expression [91].

In certain instances, retrotransposon activation may facilitate telomere elongation (Figure 3). In early mouse blastocysts, the expression activation of the non-LTR retrotransposon LINE1 coincides with telomere extension [92]. In agreement, a decrease in relative telomere length and LINE1 repression by methylation has been observed in patients with autism [93]. However, unlike yeast and silkworm, retrotransposons in mammals do not directly elongate telomeres through insertion. Instead, LINE1 activates the expression of cMyc and Klf4, which in turn trigger *Tert* transcription [94]. Inhibition of LINE1 reverse transcriptase with azidothymidine (AZT) impedes telomere elongation and the expression of two-cell genes in mouse early embryos [95]. AZT also inhibits telomere elongation and increases LINE1 copy number in early mouse

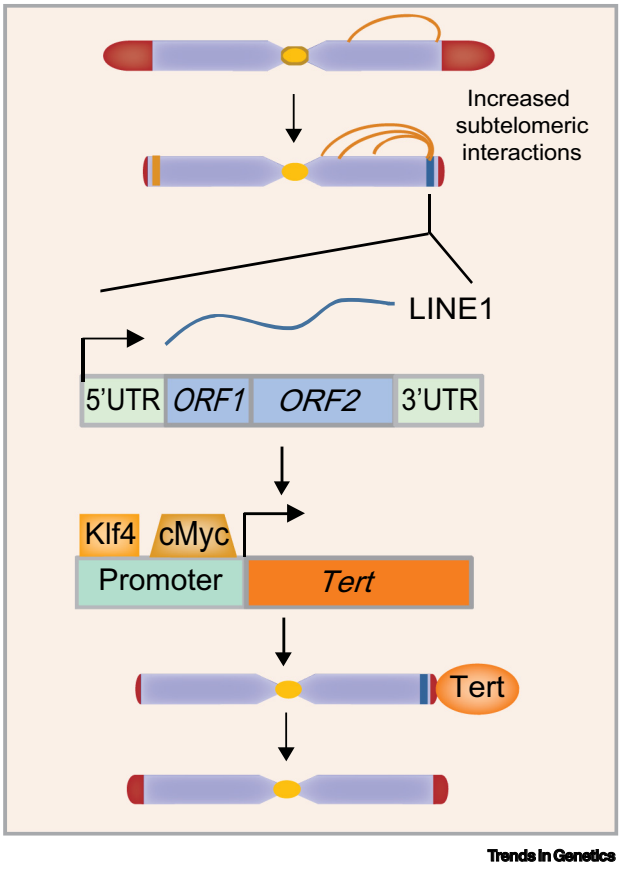

Figure 3. Inter-regulation between telomere length and retrotransposons in mammalian cells. Critically short telomeres activate subtelomeric LINE1, which promotes the expression of Klf4 and cMyc, subsequently activating the expression of Tert, which is responsible for extending telomere length. Abbreviation: UTR, untranslated region.

embryos [96]. It is worth noting that telomerase in nature is a reverse transcriptase, and AZT has been used as a telomerase inhibitor [97]. Thus, using AZT as a LINE1 reverse transcriptase inhibitor may inhibit telomerase. The development of more specific inhibitors targeting LINE1 reverse transcriptase will allow for a specific investigation of the role of LINE1 in telomere length regulation.

Impact of retrotransposons on genome stability

Both telomeres and retrotransposons are repetitive elements that are hotspots for recombination and represent a serious challenge for genome integrity. Maintaining these repeated elements in a compact heterochromatic structure suppresses recombination and unwanted mutagenic transposition and is therefore indispensable for genomic stability [98]. Activation of retrotransposons due to critically short telomeres can induce genome instability in mammals through various routes (Figure 4).

Evolutionarily young members of the ERVK family, endogenous retroviruses, display strong retrotransposition potential [99]. In laboratory mice, approximately 10% of germline mutations are caused by IAPs and ETn; both of which belong to the ERVK family [100]. Additionally, IAPs, MMETn, and MTA can retrotranspose to other genomic locations when activated in response to telomere shortening [66]. Somatic insertions of IAPs or MMETn have been linked to changes in splicing sites and gene expression [101]. However, HERVK lacks the ability to retrotranspose, despite detectable HERVK insertional polymorphisms within the human population [102]. These findings imply that HERVs may contribute to homologous recombination, which can result in

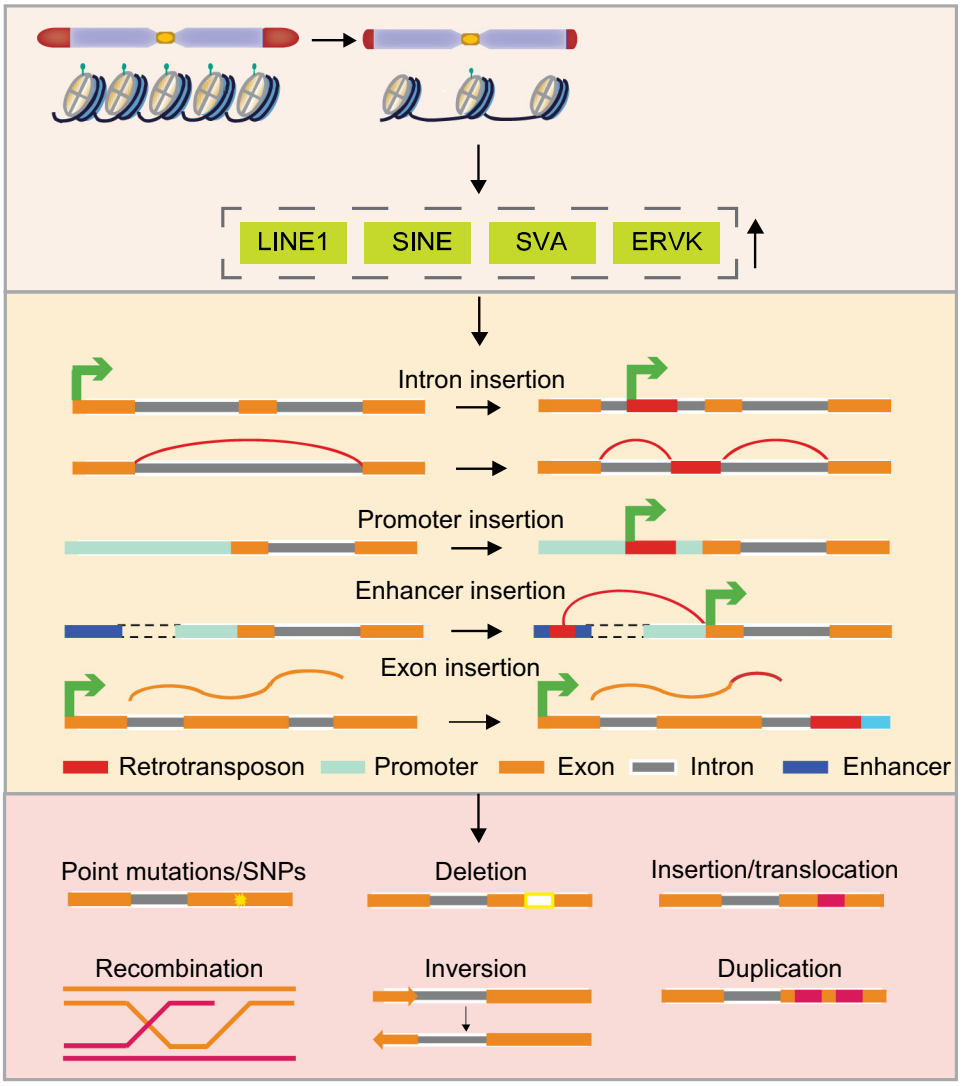

Figure 4. Activation of retrotransposons upon telomere shortening destabilizes the host genome by insertion into various genomic loci. Telomere shortening leads to chromatin openness and subsequent activation of retrotransposons such as LINE1. Retrotransposons such as LINE1 may serve as an alternative promoter (intron insertion), a donor of a splicing site (intron insertion), an alternative enhancer (enhancer insertion), or an exon (exon insertion). As a result of retrotransposon activation, point mutations/SNPs, deletion, insertion/translocation, recombination, inversion, and duplication may occur.

chromosomal rearrangements. In addition, HERVK may affect genome stability indirectly by assembling into virus-like particles and spreading to other cells while inducing cellular senescence [78]. The HERVK encoded env protein can promote cellular proliferation and oncogenesis by interacting with various oncoproteins [103]. HERVW derived proteins can mediate cell fusion, thereby eventually causing polyploidy or aneuploidy [104]. The aforementioned findings highlight an important role of ERVs in regulating genome stability.

When LINE1 is activated upon telomere shortening, it may express the nucleic acid chaperone ORF1, along with ORF2P, which possesses endonuclease and reverse transcriptase activities.

LINE1, SVA, and SINE elements exhibit retrotransposition capabilities both in embryonic stem cells with critically short telomeres and in somatic cells [66,105–107]. LINE1 elements harboring disabled endonucleases can exploit dysfunctional telomeres as integration sites [108]. When retrotransposons insert themselves near genes, they have the potential to disrupt gene expression, including the induction of oncogene expression, such as Ostf1 and Ret [66]. If the insertion occurs within the coding region of a gene, it can cause frameshift mutations and premature termination, resulting in the loss of gene function. Retrotransposon insertions within introns can induce alternative splicing of genes [109,110]. Retrotransposons can also contribute to structural variations [111] and impact neighboring chromatin environments by attracting epigenetic modifiers [112].

In addition to retrotransposition-induced genome instability, LINE1 can induce genome instability by encoding endonucleases. During LINE1 insertion, the LINE1 endonuclease can recognize genomic DNA and generate DSBs [113]. Consequently, cells overexpressing LINE1 exhibit increased DNA damage and G2/M cell cycle arrest [113]. Additionally, DSBs near retrotransposons may facilitate invasion of the 3′ end of the broken DNA strand into the template strand of a nonallelic homologous retrotransposon [114]. This leads to the joining of the synthesized strand carrying retrotransposons with the other strand via NHEJ, resulting in the insertion of part of the retrotransposon at the DSB site [114]. Another mechanism by which retrotransposons can induce genome instability is through TE-mediated nonallelic homologous recombination (NAHR). Retrotransposons, such as Alu, can provide nonallelic homology to the invading strand for annealing [115]. As a result of NAHR, genomic deletion, duplication, or inversion can occur [116]. These studies also explain the observed mutations and genomic rearrangements at nonsubtelomeric regions in cells with critically short telomeres [66].

Mice have long telomeres, such that several generations of telomere shortening without telomerase are able to reach the shortest telomeres [64]. The shortest telomeres, not the average telomere length, lead to cell senescence [64]. Human telomeres are shorter than mouse telomeres [20,21]. The fact that the shortest telomeres in the mouse ESC model cause genetic instability via retrotransposons could also occur in humans, but further experiments are needed to validate this notion.

Concluding remarks

Our understanding of the role of telomeres in regulating genome stability has significantly advanced in recent years due to the development of genomic technologies. These studies have unveiled new routes through which telomeres regulate genome stability. Retrotransposons have emerged as crucial partners of telomeres in maintaining genome stability. Telomeres and transposons share the characteristic of being repeat sequences, which posed challenges for study in the past. However, with the advent of long-read sequencing technology and improvements in sequencing accuracy, it is now possible to precisely locate transposons and decipher the structure of the genome [117]. Moreover, the progress in modern genetic tools enables the mapping of the epigenetic landscape of highly repetitive regions such as centromeres and telomeres [118,119]. Current strategies also allow for the direct determination of their complete sequence and exact length of telomeres [120]. However, numerous questions regarding the relationship between telomeres and genome stability still await elucidation (see Outstanding questions). As we surpass these constraints and discover solutions to these questions in the future, novel insights will be unveiled regarding the role of mammalian telomeres in the maintenance of genome stability. Telomere shortening and dysfunction represent one of major hallmarks of aging [121]. Retrotransposon derepression is linked to cell senescence, tumorigenesis, and aging [78,122,123]. Short telomeres also can promote cell senescence, tumorigenesis, and

Outstanding questions

It remains to be explored whether the shortest telomeres regulate retrotransposons to influence genome stability in human senescent cells and cancer cells.

Human cells generally have shorter telomeres than mouse cells. It will be interesting to check whether human cells are more sensitive to telomere attrition-induced retrotransposon activation and genomic instability.

Different retrotransposons can respond differently to various telomere lengths, and the underlying mechanism remains to be determined.

Do the shortest telomeres derepress retrotransposons through DNA damage signals, in addition to heterochromatic regulation?

The shortest or dysfunctional telomeres have been known to induce DNA damage signaling at the telomere. It is unclear how the shortest telomeres also impair the DNA repair pathways found in the study [66].

Retrotransposon activation is accompanied by compromised DNA damage repair pathways and activated cancer-related pathways following telomere attrition. It remains to be understood whether and how retrotransposons influence the DNA damage repair and cancer pathways.

Further experiments are needed to understand how telomere length regulates 3D chromatin structure, chromatic accessibility, epigenetics, and transcription and their association with genome stability.

It will be interesting to identify the factors that govern the length and rate of telomere shortening in various species.

The mechanism by which the epigenetic state of telomeres is intricately linked to the maintenance of genome stability remains to be discovered.

Telomeric variant sequences exist within telomeres. Their roles in regulating telomere structure and ensuring genome stability are still to be unveiled.

aging through aberrant activation of retrotransposon-induced genomic instability. Gaining a comprehensive understanding of telomere sequences and their relationship with retrotransposon repression and genome stability will significantly contribute to combating diseases and aging.

Short telomeres are hallmarks of aging and tumors. Recently, retrotransposons and inflammation have been recognized as important mechanisms in the processes of aging and tumorigenesis. Future experiments will seek to investigate whether and how short telomeres influence retrotransposons and inflammation in the context of aging and cancer.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2022YFA1103800; 2018YFA0107000) and the National Natural Science Foundation of China (32070858 to X.L.; 32030033, 82230052, 31970667 to L.L.).

Declaration of interests

The authors declare no conflicts of interest.

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