Review
TRENDS in Biochemical Sciences Vol.30 No.7 July 2005
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# Major cutbacks at chromosome ends

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**To distinguish a telomere from a double-strand break, a minimum number of telomere repeats must 'cap' each chromosome end. The length of each repeat array will reflect a unique history of addition and losses. Telomere losses are predicted to occur slowly but surely with every replication cycle (referred to as 'typical' telomere loss) in addition to intermittently and, potentially, rapidly ('sporadic'). Recent studies have shown that sporadic telomere losses can result from failure to properly repair (oxidative) damage to telomeric DNA, from failure to properly process higher-order structures of G-rich DNA and from homologous recombination reactions. Differences in telomere-erosion pathways between normal and malignant cells provide novel targets for the prevention and therapy of disease.**

## Introduction

Based on extensive studies by many different groups, it has become clear that telomeres and telomerase have crucial roles in proliferation of normal and malignant cells. The loss of telomere repeats with replication and with age in human cells was first proposed as a molecular mechanism of replicative senescence in 1990 [1–3]. Further data supporting this idea were provided in studies showing that transfer of the telomerase reverse transcriptase gene could extend the replicative lifespan of apparently normal diploid human cells [4,5]. Since then, many papers have reported data compatible with the notion that telomere shortening limits the number of times most normal human somatic cells can divide *in vitro* and *in vivo*. In humans, the telomere length in human vascular endothelium is inversely correlated with age [6,7], and the rate of telomere loss with age in T lymphocytes exceeds that in other cell types [8]. Limitations in the proliferative potential of cells of the cardiovascular and immune system are consistent with the recent finding that the telomere length in human blood cells correlates with the onset of age-dependent mortality [9]. In healthy women, sustained psychological stress was found to be associated with indicators of accelerated cellular and organismal aging including reduced telomere length in blood leukocytes [10]. Interest in telomere biology is also derived from studies of human tumors. Numerous studies have shown that tumor cells and immortal cell lines typically express high levels of telomerase [11] and are required to do so to sustain their proliferative activity [12].

The main focus of telomere biology research over the past two decades has been the mechanisms that elongate telomeres (mainly the action of telomerase) and various proteins such as telomere-repeat-binding factor (TRF)-1 and TRF2 that regulate telomerase action [13]. Much less attention has been paid to the mechanisms that lead to loss of telomere repeats. Indeed, telomere loss is typically explained as resulting from incomplete DNA replication (the 'end-replication problem') [14,15], and errors in chromosome-end processing following replication [16,17] are sometimes mentioned. Studies reporting that (oxidative) damage to telomere repeats could be the major cause of telomere shortening in human cells [18,19] are less frequently cited, perhaps because these findings complicate notions about telomere loss acting as a simple 'mitotic clock'.

Recently, two other mechanisms of telomere shortening were proposed: (i) the failure to unwind or correctly process higher-order structures of G-rich telomeric DNA [20,21] and (ii) the deletion of T loops by homologous recombination [22]. Ding *et al.* [20] reported that RTEL (regulator of telomere length), a DNA helicase-like molecule predicted to recognize or unwind G quadruplex DNA, is required to maintain long tracts of telomere repeats in the mouse. Crabbe *et al.* [21] proposed that the helicase protein deficient in patients with Werner's syndrome (WRN) is necessary for efficient replication of G-rich telomeric DNA, preventing telomere dysfunction and consequent genomic instability. Wang *et al.* [22], showed that a mutant allele of TRF2 can induce deletions of telomeric DNA and it was proposed that such deletions could also occur in normal cells. Ideally, the importance of each of the various telomere-attrition pathways that have been proposed should be established for different cell types (Table 1). This is a particularly difficult challenge not only because telomerase could compensate for the loss of telomeric DNA to different degrees in different cell types, but also because the importance of various telomere-erosion pathways might vary between cells, for example, as a function of levels of telomerase expression (see later), the length of telomere tracts, the amount of damage to telomeric DNA and the efficiency of various pathways involved in the repair of such damage. Nevertheless, studies in this general area should be encouraged because knowledge of the pathways that cause telomere attrition might result in effective strategies to minimize telomere loss. It is possible that such strategies are more easily implemented than strategies aimed at elongation of telomeres that target telomerase.

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Available online 4 June 2005

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**Table 1. Typical and sporadic loss of telomeric DNA**

| Mechanism of telomere loss | Occurrence at individual chromosome ends | Molecules involved | Refs |
|---|---|---|---|
| The 'end-replication problem' | Typical, occurring at ends replicated by lagging-strand-DNA synthesis | Replication proteins | [14,15] |
| Nucleolytic processing of 5′ ends | Typical, occurring at ends replicated by leading-strand-DNA synthesis | Mre11–Rad50–Nbs1(Xrs2) complex; the Ku70–Ku80 heterodimer; Exo1[a] | [16,17,34,36,50] |
| Oxidative damage, failed repair or recombination | Sporadic, dependent on signaling pathways and redox state in cells, local oxygen radical production and detoxification, mitochondrial function | | [18,19,54] |
| T-loop deletion, rapid telomere deletion | Sporadic | XRCC3 | [22,52] |
| Recombination following G-rich DNA folding into stable higher-order structures such as G-quadruplex DNA[b] | Sporadic | RTEL[a]<br>WRN[a]<br>BACH1[a]<br>DDX11[a] | [20]<br>[21]<br>[79]<br>[80] |

[a]The involvement of these molecules in the processing of telomeric DNA remains to be proven or clarified.

[b]Repair of certain telomeric DNA lesions, T-loop deletion and resolution of telomeric G4 DNA could involve similar recombination reactions.

## Structure and function of telomeres in mammals

Telomeres, or the ends of linear chromosomes, consist in all vertebrates of tandem repeats of $(TTAGGG/CCCTAA)_n$ and associated proteins [13,23]. Telomeres typically end in a single-strand overhang at the 3′ end, which folds back onto duplex telomeric DNA to form a 'T-loop' structure [24]. The length of the repeats varies between chromosomes and between species. In humans and mice, the length of telomere repeats at individual chromosome ends in individual cells is strikingly variable [25–27]. Human chromosome ends are typically capped with 2–10 kilobase pairs of detectable telomere repeats depending on the type of tissue, the age of the donor and the replicative history of the cells. Individual human chromosome ends show marked variation in average telomere length, chromosome 17p, for example, typically has one of the shortest array of repeats [28]. Telomeres prevent the ends of linear chromosomes from appearing as DNA breaks and, thus, protect chromosome ends from degradation and fusion. It has been proposed that telomeres can switch between an open state (in principle, enabling elongation by telomerase) and a closed state (inaccessible to telomerase) and that the open state is inversely related to the length of the repeat tract [29]. This model is supported by recent data in yeast [30]. In most human cells, telomerase seems to be present at limiting levels, thereby enabling elongation of only a limited number of critically short telomeres. Recent data showing a link between telomeres and DNA-damage-response proteins in senescent cells have provided further support for the involvement of telomeres in the replicative senescence of human cells [31,32].

In summary, a minimum number of repeats is required at every chromosome end to recruit sufficient telomere-specific proteins for the formation of a fully functional telomere that distinguishes a normal end from a double-strand break. Telomerase is present in limiting amounts in most human cells and cannot prevent the progressive accumulation of short telomeres. Apoptosis or replicative senescence typically results when the number of critically short telomeres exceeds the limited number that telomerase can maintain.

## Regulation of telomere-length

In immortal cells, the average telomere length reflects the equilibrium between telomere loss and telomere elongation. The situation is more complicated at individual chromosome ends because elongation and loss events could differ in degree and frequency, and do not have to occur during every cell cycle. Most of our knowledge about the molecules involved in telomere-length regulation has derived from studies of the average telomere length in unicellular organisms such as *Tetrahymena*, *Saccharomyces cerevisiae* and *S. pombe*. *Tetrahymena* was used in the discovery of telomerase [33] and *S. cerevisiae* has been instrumental in elucidating pathways of telomere replication [34,35] and checkpoint activation [36]. Interestingly, recent studies of *S. cerevisiae* have shown that telomerase does not act on every telomere in each cell cycle and seems to prefer short telomeres [30]. A large number (>200) of genes are known to affect telomere metabolism in *S. cerevisiae* [37]. Yet, telomere-length regulation in this species is expected to be simple compared with that in higher eukaryotes. The major complicating factor is that cells in multicellular organisms belong to either reproductive or somatic tissues and that, within those tissues, cells are typically organized into stem cells, progenitor cells and end cells [38]. Maintenance of genome integrity (and telomeres) is most important for the cells of the germline and the early embryo, but is probably less efficient in various somatic cells, especially fully differentiated cells. This is most dramatically illustrated by the complete lack of nuclei in human red blood cells. Differences in the efficiency of DNA-repair pathways between (stem) cell types are increasingly being recognized [39–42] and could play an important part in the aging of cells and tissues. Such differences add complexity to generalizations about telomere-length regulation in mammals. To complicate things further, the situation seems to differ between mammalian species. This is illustrated by the different phenotypes of telomerase deficiency in humans and mice. In laboratory mice, complete lack of telomerase activity is tolerated for up to several generations [43]. By contrast, a modest twofold reduction in telomerase levels in humans (resulting, for

example, from haplo-insufficiency for the telomerase RNA-template gene [23,44,45] or the human telomerase reverse transcriptase gene [46]) can result in premature death, typically, from complications of aplastic anemia or immune deficiency. Together with the age-related decline in telomere length observed in humans (but not in mice), these observations have provided support for the idea that telomerase levels are extremely tightly regulated and limiting in most human somatic cells. One possibility is that telomere loss evolved as a mechanism to suppress the growth of tumors in long-lived species, such as humans, but not in small and short-lived animals, such as rodents [47,48]. Most studies of telomere-length regulation have focused on telomerase. From such studies, it is known that regulation of telomerase occurs at the level of synthesis of the enzyme and via the state of its substrate – the telomere itself [13,23]. The regulation of telomerase remains an active research area, but a detailed discussion of the studies in this area is beyond the scope of this review.

## Mechanisms of telomere loss

The loss of telomeric DNA can be broadly separated into two distinct classes: typical and sporadic (Table 1). The typical loss of telomeric DNA is expected to occur at every chromosome end at every replication cycle (unless the expected loss is countered by elongation events) (Figure 1). A basic assumption in models of typical telomere loss is that telomeric DNA is replicated from internal origins and that DNA replication is not initiated from the very end of chromosomes. This assumption is supported by data in yeast [49] but not (yet) in vertebrates. The typical 'end-replication problem' (Figure 1a) results from the failure of conventional DNA polymerases to replicate the very 3′ end of the chromosome [14,15]. The problem is caused by limitations imposed by lagging-strand-DNA replication of the G-rich template sequences (Figure 1a) and has two components. First, the removal of the RNA primer used for initiation of DNA replication will result in a gap of a few nucleotides in the newly formed strand. Second, assumed failure by the replisome to deposit the last RNA primer at the very 3′ end will result in a further extension of the gap (Figure 1a).

With the finding that chromosomes typically end with a single 3′ overhang came the realization that some form of exonucleolytic processing must take place on the C-rich template strand to create a new 3′ overhang [16,17]. According to a current model, the blunt end arising from leading-strand replication of the C template strand (Figure 1b) must be processed to yield a 3′ overhang. The nature of the molecules involved in the recruitment, activation and arrest of the processing steps at the 'leading-strand' telomere are topics of active research. Various molecules, including Mre11–Rad50–Nbs1(Xrs2)

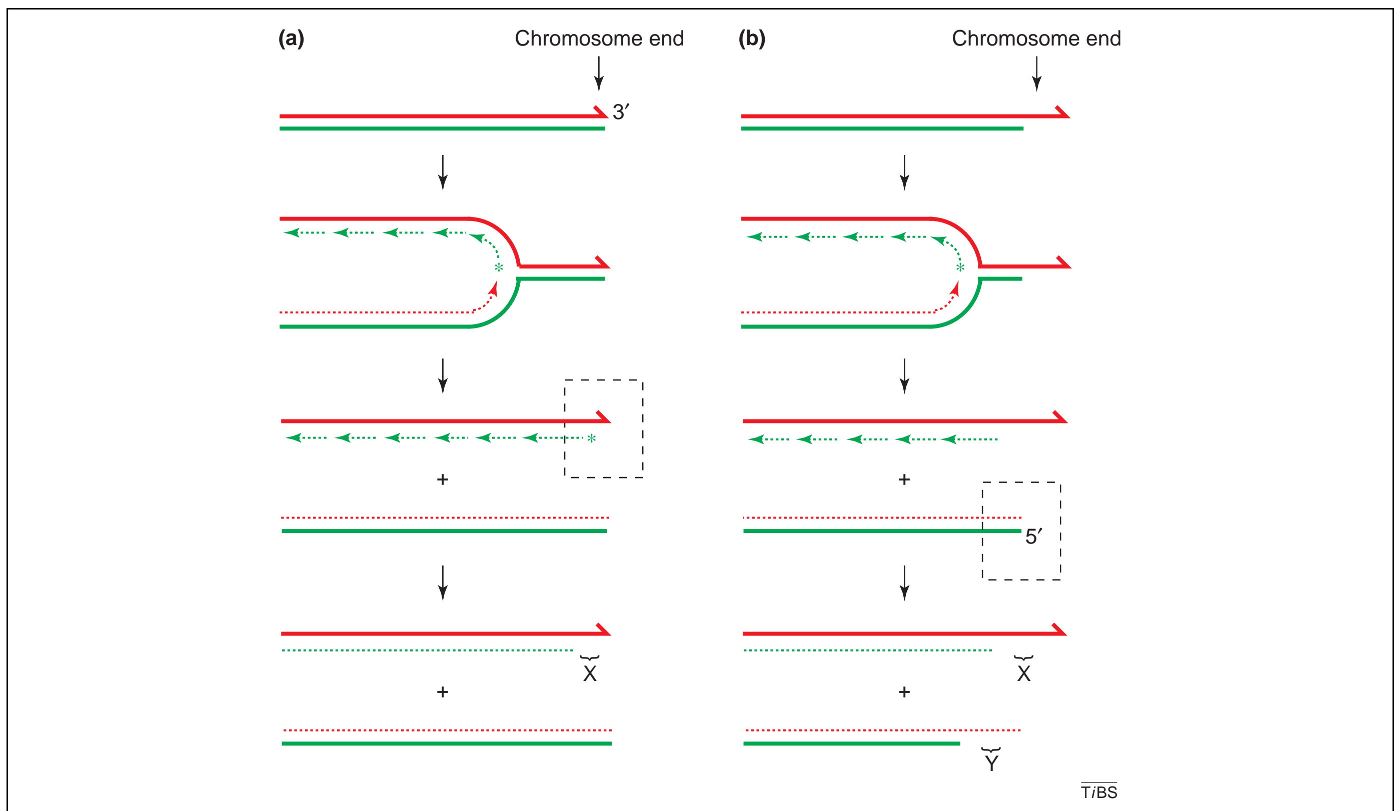


**Figure 1.** Loss of telomeric DNA with replication. Replication from internal origins results in two typical problems. **(a)** According to the classical 'end-replication problem', the 3′ end of chromosomes (half arrow) on the G-rich strand (red) is not fully replicated because lagging-strand replication of the 3′ strand requires a RNA primer (asterisks). Following removal of the primer, a gap of a few nucleotides on the newly formed strand (broken lines) will emerge. Furthermore, because it is not expected that the RNA primer is deposited at the very end of the chromosome terminus (box), a gap of X number of nucleotides is formed in the newly formed strand. **(b)** Because all telomeres end in a 3′ single-strand overhang, loss of telomeric DNA with replication must also result from exonucleolytic processing of a number of nucleotides (Y) of the 5′ end of the C-rich (green) template strand (box). The actual amount of telomeric DNA (X plus Y) lost from these typical replication problems is not known but could be small relative to the losses encountered from sporadic losses that result from DNA damage and errors in replication, repair and recombination.

complex, the Ku70–Ku80 heterodimer, DNA-PK and Exo1 have been implicated in these processes [34,36,50].

It is interesting to note that the loss of telomeric DNA in the absence of mechanisms to extend telomeres has been estimated to be <10 base pairs (bp) per division per chromosome end in various organisms [31]. The much higher rate of telomere attrition observed with most human cells (typically 50–200 bp per division) supports the idea that telomeres in human cells could be actively degraded [31]. At present, it is not known if the 'extra' DNA is lost primarily from the typical processing steps in the current model of telomere replication (Figure 1b) or from sporadic loss of telomeric DNA. If sporadic losses were to contribute significantly to the overall loss of telomeric DNA, (tumor) cells that can avoid or counter such sporadic loss events are predicted to have a greatly increased replicative potential.

### Sporadic loss of telomeric DNA

Telomere loss can also result from sporadic events such as failed repair of damage to telomeric DNA or from problems arising occasionally during replication, repair or recombination. The study of sporadic-loss events represents a challenge because typical telomere-length measurements are biased towards measuring the average telomere length. Interestingly, using single telomere-length analysis (STELA; a PCR-based approach that accurately measures the full spectrum of telomere lengths from individual chromosomes [27]), a significant proportion of variably shortened telomeres next to a major population of average-sized telomeres have been observed in DNA from humans [27] and *Caenorhabditis elegans* [51]. These observations support the idea that sporadic telomere losses could contribute significantly to telomere-length homeostasis in a manner that is, perhaps, similar to be the rapid telomere deletion described in yeast [52].

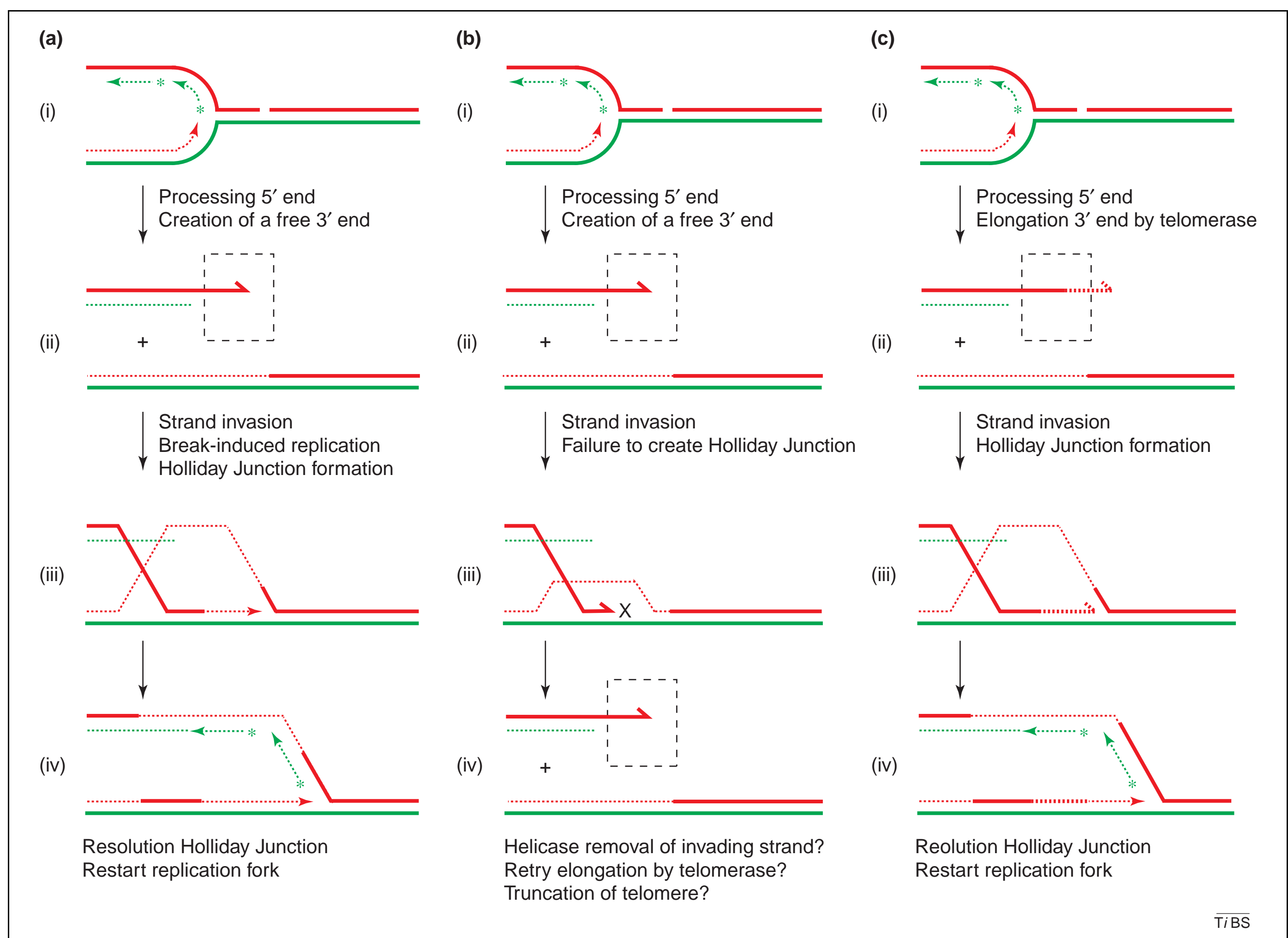


**Figure 2.** Speculative models of repair events following collapse of a replication fork encountering a nick in the template for lagging-strand-DNA synthesis. **(a)** Nick at an internal genomic site. **(b)** Nick in telomeric DNA, no telomerase present. **(c)** Nick in telomeric DNA, telomerase present. (i) It is assumed that telomeres are replicated from internal replication origins resulting in lagging-strand replication of the G-rich strand (red) and leading-strand replication of the C-rich strand (green), and that differences between replicated and non-replicated DNA dictate that replication can only resume at DNA that has not yet been replicated. (ii) Effective repair of lesions in telomeric DNA could require extension of the 3′ end by telomerase [exemplified by the box in (c)] before strand invasion (iii) because telomere-end-binding proteins such as Pot1 [X in b(iii)] might prevent extension of the invading 3′ strand that could occur by break-induced replication [a(iii)]. In the absence of telomerase, repair by recombination [b(iv)] could fail because a Holliday Junction cannot form. In this case, replication can only resume if the free end invades out of register beyond the original lesion into non-replicated template sequences. The likelihood of such a recombination event is predicted to depend on the length of the repeat array. With or without recombination, rapid telomere loss could result. DNA, solid lines; G-rich DNA, red; C-rich DNA, green; newly synthesized DNA, red and green broken lines; 3′ end, half arrow; RNA primer for initiation of lagging-strand-DNA synthesis, asterisks; replication direction (5′→3′ newly formed strand), full arrows.

## Oxidative damage to telomeric DNA

DNA is a relatively unstable molecule that can accumulate a perplexing diversity of lesions arising from environmental agents (ranging from UV light and ionizing radiation to tobacco smoke), normal cellular metabolism (in particular, reactive oxygen species derived from oxidative respiration and lipid peroxidation) and spontaneous disintegration [53]. Free radicals play an important part in the normal function of cells [54] and it has been shown that telomeric G-rich DNA is 5–10-fold more vulnerable to oxidative damage than non-telomeric genomic DNA [55,56]. It is not clear, however, if oxidative lesions to telomeric DNA are actively repaired independently of DNA replication. Repair of oxidative damage to nucleotides is typically achieved using nucleotide-excision repair [53], but this pathway is known to be less efficient for lesions in non-transcribed sequences [57]. Repair of UV-light-induced pyrimidine dimers in telomeric DNA declined with age, and was less efficient than repair of similar lesions at endogenous genes but more efficient than that of inactive, non-coding regions [58].

Relative to genomic DNA, the repair of lesions in telomeric DNA could be complicated by several factors. First, the repeat nature of the DNA-target sequences is expected to complicate the correct alignment of sequences during repair by homologous recombination [59]. Second, during typical recombinational repair, a 3′ end invades the homologous duplex and is extended by DNA synthesis using the homologous template; telomere-binding proteins could actively prevent such extension. Third, because it is not known if replication is initiated from telomere ends, replication forks that have stalled or collapsed during replication of telomeric DNA, presumably, cannot be rescued by convergence of replication forks [60]. The consequences of a replication-fork collapse at telomeres have not been studied in mammalian cells. A diversity of reactions have been found in prokaryotes [60,61], indicating a complex interplay between DNA replication, recombination and repair, and the situation at telomeres in eukaryotes is currently unclear.

## Is telomerase involved in repair of (telomeric) DNA?

What are the molecular mechanisms involved in the repair of (oxidative) lesions in telomeric-DNA lesions or the repair of replication forks stalled in telomeric DNA? Some examples of hypothetical (and speculative) DNA-repair reactions following collapse of a replication fork at a nick in the DNA template for lagging-strand-DNA synthesis are shown in Figure 2. One potentially important difference between repair events at telomeric and genomic sequences could be the presence of proteins that bind to telomere ends, such as Pot1 [62], that could interfere with a crucial step in recombinational repair – break-induced replication [63]. At telomeres (and perhaps selected sites in genomic DNA), repair by recombination could require the extension of a 3′ end by telomerase before strand invasion (Figure 2). A role for telomerase in the repair of telomeric DNA by recombination is consistent with observations that support a telomere-length-independent role for telomerase [64]. Alternatively, telomerase could be required to elongate telomeres that are critically short owing to errors in telomere repair, replication or recombination. This mechanism was proposed to explain the survival of murine embryonic stem cells deficient in RTEL before differentiation [20] and the survival of normal cells following T-loop deletion [22]. Because sporadic losses from any cause do not have to result in detectable changes in the average telomere length, the repair of such lesions (and the survival of cells) could require telomerase in a manner independent of telomere length. Furthermore, telomerase could also be required for the repair of lesions in genomic DNA by providing limited extension of 3′ G-rich sequences that cannot be extended by break-induced replication. Such a role for telomerase in DNA repair is in agreement with the observations that loss of the PIF1 DNA helicase in *S. cerevisiae* results in *de novo* telomere formation and gross chromosomal rearrangements [65,66]. PIF1 is postulated to dissociate telomerase from DNA [67] and could aid in the repair of DNA double-strand breaks by enabling telomerase to provide limited extension of 3′ ends while preventing *de novo* telomere formation. Subsequent processing by mismatch-repair pathways could ensure correct base pairing. Notably, limited extension of 3′ ends, which is required for repair of telomeric and genomic DNA, could possibly be accomplished by telomerase variants that are incapable of net telomere elongation [64].

In summary, sporadic loss of telomeric DNA resulting from replication errors or from failure to properly repair damage to telomeric DNA could, in theory, contribute significantly to the overall loss of telomeres observed with age and with replication. Telomerase could have a role in the repair of telomeric DNA by elongating telomeres that have become critically short. In addition, telomerase could play a similar part in the repair of genomic G-rich sequences. Application of novel techniques, such as chromosome orientation fluorescence *in situ* hybridization (CO-FISH) [68,69], have revealed high frequencies of 'telomere' sister-chromatid exchange in human and murine cells, which is compatible with the idea that repair of damage to telomeric DNA could use different pathways than those of the repair of lesions within genomic DNA. Further studies on the replication, recombination and repair of telomeric DNA using techniques, such as STELA [27] and CO-FISH [68,69], are needed to delineate the role of various DNA-repair pathways in countering sporadic telomere loss in different cell types.

## Deletion of T loops

Telomeres are known hot-spots for recombination events [70] including exchanges between sister chromatids [69]. Recombination between telomere sister chromatids could possibly enable cells to bypass a requirement for telomerase in repair of telomeric lesions (Figure 2b) and has been proposed as a mechanism that could delay senescence in telomerase-negative cells [69,71]. High rates of recombination have, indeed, been observed in telomerase-negative tumor cells [72] including high rates of sister-telomere exchange [73]. The T-loop structure of mammalian telomeres is thought to provide a protective cap to chromosome ends and repress non-homologous end

joining (NHEJ) at natural chromosome ends. Recently, a mutant allele of TRF2 was described that suppressed NHEJ but induced catastrophic deletions of telomeric DNA [22]. The deletion events were stochastic and occurred rapidly to generate dramatically shortened telomeres that were accompanied by a DNA-damage response and induction of senescence. T-loop-sized deletions were shown to require XRCC3, a protein implicated in Holliday junction resolution, and created T-loop-sized telomeric circles. Telomeric circles were also detected in unperturbed cells and it was suggested that T-loop deletion by homologous recombination (HR) could contribute to telomere attrition in normal cells. T-loop deletions were shown to have great preference for telomeric sequences replicated by leading-strand-DNA synthesis. The reason for this preference remains to be elucidated but it could be related to the differential processing of leading- and lagging-strand telomeres following replication (Figure 1b). It was suggested that the T-loop deletions observed in TRF2 mutant cells are similar to the rapid telomere deletions described in yeast [52].

## Deletions of G-rich DNA

Recent observations in *C. elegans* [51] and mice [20] suggest that certain G-rich-DNA sequences can cause specific problems during replication, and it was proposed that loss of telomeric DNA resulting from such problems is a dominant factor in the regulation of telomere length in mice [20]. *C. elegans* with a mutation in the *dog-1* (deletion of G-rich DNA-1) gene have a highly unusual mutator phenotype characterized by short (typically 100–200 nucleotides) deletions throughout their genomic DNA [51]. Strikingly, deletions invariably originate around the 3′ end of polyguanine tracts and are observed in ~50% of genomic G-tracts above a threshold length of >18 consecutive guanine residues. Because only half of the G-tracts show these uni-directional deletions, it was proposed that deletions could result from failure to resolve higher-order structures of G-rich DNA such as guanine quadruplex (G4) DNA [74], which arise stochastically during lagging-strand replication of G-rich template sequences [51] (Figure 3).

Following the observations in *dog-1*-deficient *C. elegans*, the murine *Rtel* gene (for regulator of telomere length) was cloned and characterized [20]. *Rtel* encodes a helicase-like protein similar to the DOG-1 protein. It was found that $Rtel^{-/-}$ mice died at day 10 or 11 of gestation with severe proliferative defects of cells in the central nervous and cardiovascular system. *Rtel*-deficient embryonic stem cells were viable, but displayed telomere loss and genetic

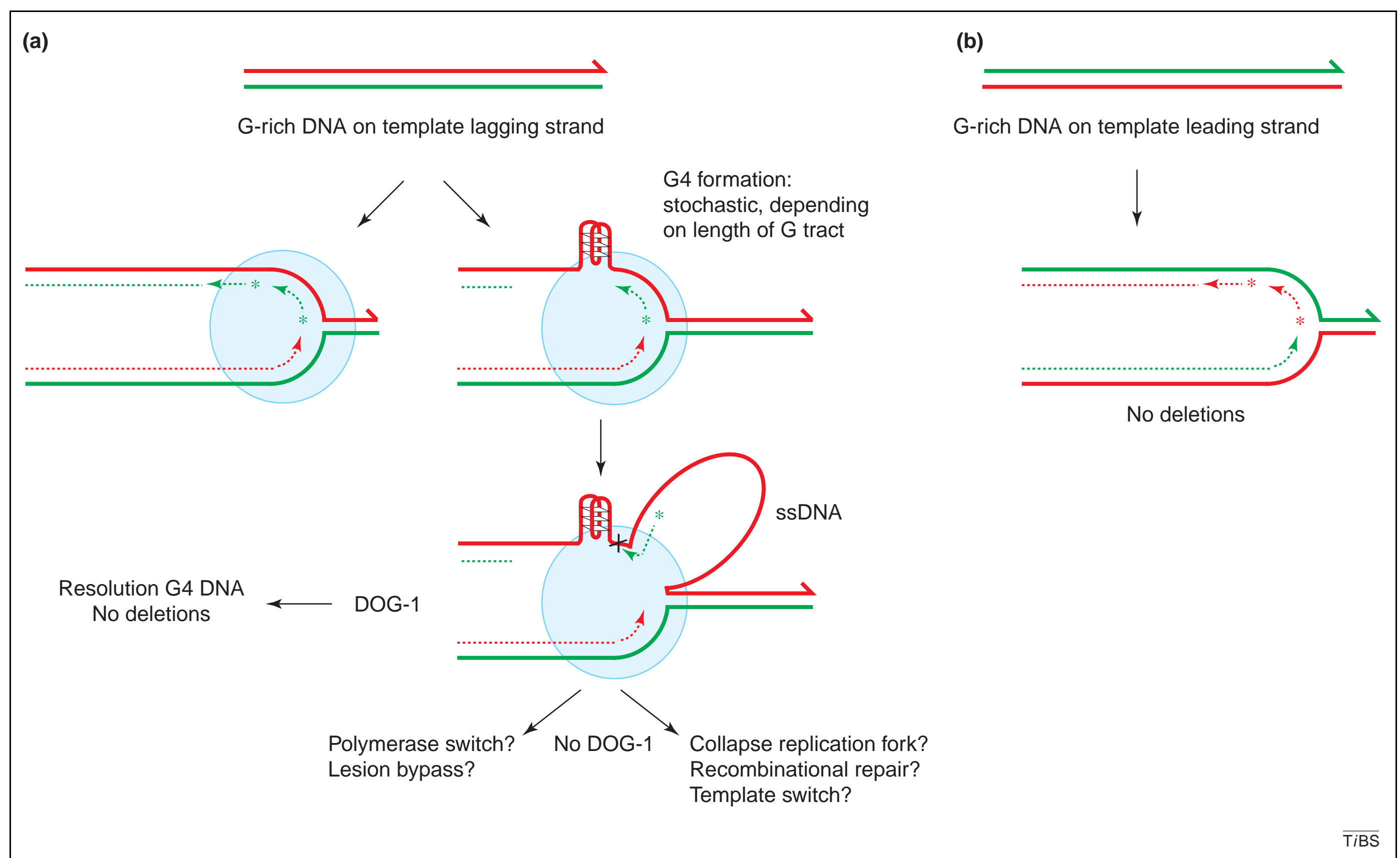


**Figure 3.** Model of the replication problem in *Caenorhabditis elegans* lacking the *dog-1* gene (based on [51]). **(a)** Polyguanine tracks of >18 consecutive guanine residues replicated by lagging-strand synthesis occasionally form a stable G4-DNA structure that requires the DOG-1 protein for resolution. G4 formation is predicted to be stochastic with the major variable being the length of the G tract. In the presence of DOG-1, the G4 DNA is resolved. Without DOG-1, several scenarios are possible. A polymerase switch could result in replication past the lesion. Alternatively, the replication fork could collapse and resume downstream. The exposed single-stranded DNA could trigger recombinational repair using the G-rich DNA from the sister chromatid as a template. Various molecules, including the WRN helicase, could be involved in repair reactions and the G4 lesion could be excised later, for example, by the nucleotide-excision-repair pathway. **(b)** If the G tract is replicated by leading-strand synthesis higher-order structures of G-rich DNA are not expected to form, which explains why deletions occur in only ~50% of poly-G tracts in *dog-1* mutants. Template DNA, solid lines; G-rich DNA, red; C-rich DNA, green; newly synthesized DNA, red and green broken lines; 3′ end, half arrow; RNA primer for initiation of lagging-strand DNA synthesis, asterisks; replication direction (5′→3′ newly formed strand), full arrows.

instability upon differentiation *in vitro*. Based on these observations, it was proposed that RTEL, similar to DOG-1, is required to recognize or resolve higher-order structures of G-rich DNA such as G4 DNA. Further studies of the DOG-1 and RTEL proteins are required to determine whether these proteins are indeed helicases as predicted, and to study the type of structures that are recognized and/or resolved by these proteins.

## G4 DNA and telomere-length regulation

A variety of G4-DNA structures obtained from single-stranded G-rich telomeric DNA *in vitro* have been described [75]. Several authors have proposed that such structures can form at the single-strand 3′ overhang present at telomeres and that the formation of G4 DNA at this site inhibits telomerase action [76,77]. Indeed, a large amount of work has been done to identify compounds that inhibit telomerase by favoring the formation or stabilization of G4 telomeric DNA [78]. In principle, G4 DNA could arise at any place where single-strand G-rich DNA is exposed. At telomeres, this could be at the 3′ end of the chromosome but also at the base of the T loop [24] or, indeed, anywhere along the track of telomere repeats during replication, repair and recombination. Specialized helicases, such as DOG-1, RTEL, WRN and BLM (and, perhaps, also BACH1 [79] – a protein even more similar to DOG-1 than RTEL and DDX11, a DNA helicase recently implicated in human telomere-length regulation [80]), could be required (with or without additional nucleases) to resolve G4-DNA arising at telomeric and genomic sites [81]. The BLM and WRN helicases are known to resolve G4-DNA *in vitro* [82,83] and both molecules have been implicated in telomere-length regulation [21,84]. For telomere repeats, the successful outcome of recombination-based repair of G4-DNA lesions could be complicated by the repeat nature of the target sequence and by the possible involvement of the single-strand overhang in the recombination reaction [20,52,85,86].

## Concluding remarks

The notion that telomere loss in somatic cells primarily results from the ‘end-replication problem’ has become increasingly untenable. Instead, telomere loss seems to reflect DNA losses at every chromosome end, which are inherent to replication and processing of telomeric DNA, in addition to sporadic losses of variable length, which are related to the repair of stalled replication forks, damage to telomeric DNA and T-loop deletion. Sporadic telomere-loss events are expected to affect only a minority of telomeres at any given cell cycle, yet such events could be the most important factor in the overall rate of telomere attrition in cells. Because sporadic telomere-loss events are not readily detectable using methods that measure the average telomere length, novel methods that can measure events at single telomeres are needed to advance studies in this area. Such studies will no doubt yield many more surprises regarding the mechanisms of telomere loss in eukaryotic cells.

## Acknowledgements

I apologize to colleagues whose work could not be cited owing to space limitations. I thank anonymous reviewers, Gerry Krystal and the members of my laboratory for helpful comments and discussions. Work in my laboratory is supported by grants from the National Institutes of Health (AI29524), the Canadian Institute of Health Research (MOP38075) and the Terry Fox Foundation.

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