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Competing interests statement
The authors declare that they have no competing financial interests.

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OPINION

T-loops and the origin of telomeres

Titia de Lange

Most eukaryotes stabilize the ends of their linear chromosomes with a telomerase-based system. Telomerase maintains specific repetitive sequences, which protect chromosome ends with the help of telomere-binding proteins. How did this elaborate system evolve? Here, I propose that telomere function was originally mediated by t-loops, which could have been generated by prokaryotic DNA-replication factors. These early telomeres would have required only the presence of a few repeats at chromosome ends. Telomerase could have been a later innovation with specific advantages for telomere function and regulation.

At the time of their switch to linear genomes, eukaryotes must have evolved a mechanism to manage their chromosome ends. Chromosome ends create two major problems. The DNA-replication machineries of all cells, regardless of their genome organization, use short RNA primers to initiate DNA synthesis. Removal of the terminal primer at the end of lagging-strand synthesis leaves a small gap that cannot be filled in. If left unfixed, this gap leads to the loss of terminal sequences. This is known as the end-replication problem.

The second problem is that cells must distinguish their natural chromosome ends from sites of DNA damage, so that checkpoint activation and inappropriate repair are avoided. Present-day telomeres solve both problems.

The protection and replication functions are currently provided by a telomere system that comprises the telomere-specific reverse transcriptase known as telomerase, an array of telomeric repeats that are created by telomerase, and telomere-specific proteins that bind to the telomeric repeats. The telomeric proteins protect chromosome ends from being recognized as sites of DNA damage and also regulate telomere-length maintenance by telomerase.

The complexity of the telomerase-based telomere system has raised the question of how it evolved. Here, I discuss the possibility that eukaryotes originally existed without the benefits of telomerase or telomeric-DNA-binding proteins. I propose that these first eukaryotes could have stabilized their chromosome ends using the t-loop structure. If the ends contained a few terminal repeats, t-loops could have been generated by factors that were involved in recombination-dependent replication (RDR), a form of DNA replication that was already in existence before eukaryotes evolved. The presence of t-loops at present-day telomeres and their association with proteins that have evolved from RDR factors might be remnants of the original telomere system. Furthermore, the relative ease with which many eukaryotes can maintain telomeres without telomerase might reflect this ancient system of chromosome-end replication. This proposal ends with a discussion of the advantages of the telomerase-based system that could explain the emergence of this almost universal mechanism for telomere maintenance.

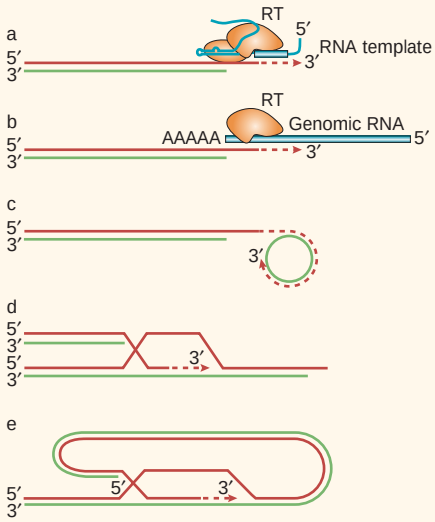

Figure 1 | **Solutions to the end-replication problem. a** | In eukaryotic chromosomes, the maintenance of terminal sequences is primarily facilitated by telomerase, which extends the 3′ terminus using reverse transcriptase (RT in the figure) and an RNA template. **b** | Dipteran insects solve the end-replication problem by retrotransposition, a pathway that is analogous to the telomerase pathway in that a reverse transcriptase uses the 3′ end of the chromosome as a primer for DNA synthesis using an RNA template. **c** | Experiments in telomerase-deficient *Kluyveromyces lactis* have provided evidence for rolling-circle replication in which the 3′ end is extended on an extrachromosomal circular template as a means of extension of chromosome ends. **d** | In *Saccharomyces cerevisiae* strains that lack telomerase, telomeric sequences can be maintained with a break-induced replication (BIR)/recombination-dependent replication (RDR)-like pathway in which one telomere uses another telomere as a template for extension. **e** | T-loop formation using terminal repeats and extension of the invaded 3′ end might be another mechanism of telomere maintenance. The figure only shows the elongation of the 3′ ends. For each pathway, elongation of the 5′ end will require further (lagging-strand) DNA synthesis, which could take place concomitantly with 3′-end extension or in the next round of DNA replication. The blue boxes in parts **a** and **b** signify the sequence in the telomerase RNA and in the retrotransposon RNA, respectively, that will be added to the chromosome end by reverse transcription.

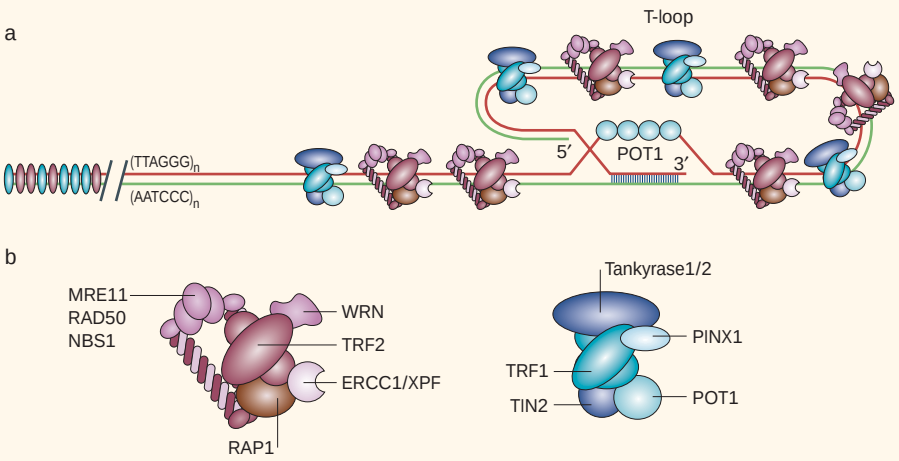

Figure 2 | **Proposed structure of the human telomeric complex. a** | Human telomeres are comprised of a 2–30-kb array of duplex TTAGGG repeats, ending in a 100–200-nucleotide 3′ protrusion of single-stranded TTAGGG repeats. This DNA can exist as a t-loop in which the 3′ overhang invades the duplex-repeat array forming a displacement (D) loop of TTAGGG repeats. Other configurations cannot be excluded. POT1 binds to the single-stranded TTAGGG-repeat DNA. Two double-stranded TTAGGG-repeat-binding factors (TRFs), TRF1 and TRF2, are associated with the duplex repeats. **b** | TRF1 and TRF2 each recruit other proteins to the telomere (such as the tankyrases and RAP1). Some aspects of the complexes depicted have not been shown to exist *in vivo*. For example, it is possible that TRF1 and TRF2 form multiple complexes, and some of the protein interactions that are depicted have not been firmly established *in vivo*. The primary function of the TRF2-containing complex (left) is to protect chromosome ends. The TRF1-containing complex (right) has a role in the regulation of telomerase-mediated telomere maintenance. In addition to the proteins shown, Ku70/80, DNA-PK_{cs} and the Bloom helicase might be bound to telomeres. The human telomeric complex is reviewed in REFS 15,43. ERCC1/XPF is a nucleotide-excision-repair endonuclease; the MRE11 complex is composed of MRE11, RAD50 and NBS1; PINX1, PIN2-interacting factor-1; POT1, protection of telomeres-1; TIN2, TRF1-interacting factor-2; WRN, Werner syndrome helicase.

Telomerase: who needs it?

Most eukaryotes use telomerase to compensate for the loss of chromosome-terminal sequences[1] (reviewed in REFS 2,3). The reverse-transcriptase component of telomerase is conserved and has been identified in vertebrates, invertebrates, plants, fungi and many unicellular organisms, including ciliates, kinetoplastids and even Giardia. The success of the telomerase-based system is further illustrated by the striking conservation of its product, the telomeric-repeat DNA. The majority of eukaryotes have a telomerase that synthesizes TTAGGG repeat arrays, making this hexamer one of the most pervasive nucleic-acid sequences in nature. Some of the telomeric repeats are slightly different; for example, plants have TTTAGGG repeats, but only a few eukaryotes (mostly budding yeasts, such as the well-studied *Saccharomyces cerevisiae*) have telomeric repeats that depart substantially from the TTAGGG motif.

Despite its prevalence, telomerase is not the only way terminal sequences are maintained. Many different ways to circumvent the loss of terminal sequences have surfaced from studies of viral, prokaryotic and eukaryotic genomes. Poxvirus has a covalently-closed hairpin at each end of its double-stranded DNA (dsDNA) genome (reviewed in REF. 4). Controlled nicking of the hairpin provides the 3′ OH group that is necessary for DNA replication. A similar strategy is used by linear plasmids in the Lyme disease spirochete *Borrelia burgdorferi* (reviewed in REF. 4). A complication of this replication strategy is that it generates a circular dimer, which requires a specialized reaction for conversion into monomers. Retroviruses prevent losing their chromosome ends by having their reverse transcriptase execute an elaborate terminal jump (reviewed in REF. 5) and adenoviruses fix their end-replication problem with a proteinaceous primer, known as terminal protein, that is covalently attached to the 5′ ends of its genome[6].

Alternative solutions to the end-replication problem also occur in eukaryotic genomes (FIG. 1). An extreme example is seen in dipteran insects[7], an order that includes the model organism *Drosophila melanogaster*. Although most insects have telomerase-made TTAGG-repeat telomeres, the dipterans have lost this system. The widespread occurrence of flies and mosquitoes suggests that life without telomerase has been possible, if not good, for at least 200 million years. The fruitfly uses retrotransposition to keep its chromosome ends intact. *D. melanogaster* chromosome ends carry a medley of two retrotransposons, HetA and TART. Occasionally, a new HetA or TART element will jump onto a chromosome end, using the 3′ chromosome terminus as a primer to reverse transcribe its RNA genome. While haphazard, this system of balancing the loss of terminal sequences clearly works well. Interestingly, telomerase loss has also occurred in several species of Coleoptera (beetles)[8], an order of insects that is closely related to the diptera.

Genetic disruption of the telomerase pathway has revealed the ease with which eukaryotes get by without this enzyme. *S. cerevisiae* strains lacking telomerase lose their telomeres gradually and eventually perish due to chromosome instability[9]. But survivors arise readily and are so frequent that spontaneous mutations cannot explain their occurrence[10]. They can use two homologous-recombination pathways to elongate their telomeres. One pathway is dependent on Rad50 (a component of the Mre11 complex, see below) and involves recombination between tracts of telomeric repeats; the second pathway is governed by the recombination protein Rad51 and creates arrays of sub-telomeric tandem repeats (reviewed in REF. 11).

Analogous experiments in another budding yeast, *Kluyveromyces lactis*, have revealed yet another mode of telomerase-independent telomere maintenance. Here, small extrachromosomal circles can provide the template for DNA-repeat addition to the 3′ chromosome end. Through a rolling-circle mode of DNA replication, long arrays of terminal repeats can be added in a single step[12].

Even human cells are quite good at maintaining their telomeres without telomerase. Many human cell lines that were isolated by *in vitro* immortalization have escaped from the telomere crisis without the use of telomerase. The mechanism of telomere maintenance in such cell lines is referred to as alternative lengthening of telomeres (ALT)[13]. ALT is also observed in a subset of human tumours (reviewed in REF. 14). The molecular mechanism(s) of ALT is not yet fully understood, but possible mechanisms include rolling-circle replication, telomere–telomere recombination and t-loop-mediated extension (FIG. 1).

End protection: no need for telomerase

The ability of telomeres to dodge the DNA-damage response is a consequence of their association with a set of proteins that protect

chromosome ends. Some of these protecting factors bind along the duplex array of telomeric repeats; others bind to the single-stranded part of the telomeric DNA. Loss of these factors leads to telomere uncapping, a dysfunctional state that induces a DNA-damage response and results in inappropriate processing of chromosome ends by DNA-repair enzymes such as nucleases and ligases.

An example of a protecting factor is TRF2, a small dimeric protein that binds to the double-stranded TTAGGG repeat arrays of mammalian telomeres (FIG. 2; reviewed in REF. 15). Loss of TRF2 results in activation of the ATM checkpoint kinase and induction of a p53-dependent cell-cycle arrest. The uncapped telomeres are also attacked by an endonuclease that cleaves off the telomeric overhang and they can become fused together by DNA ligase IV. TRF2 must somehow prevent these disastrous events and recruits a large complex of other factors to telomeres to accomplish this task. Another example of a telomere-protection factor is the *S. cerevisiae* single-stranded DNA (ssDNA)-binding protein Cdc13, which protects telomeres against exonucleolytic attack and prevents the activation of a DNA-damage checkpoint by chromosome ends (reviewed in REF. 16). Telomeres of the fission yeast *Schizosaccharomyces pombe* are protected by a TRF2-like factor, Taz1, and a Cdc13-related protein, Pot1 (reviewed in REF. 17).

Since each of these factors is recruited to telomeres through direct binding to the telomeric-repeat array, telomere protection depends on the maintenance of their recognition sequences at chromosome ends. So, although telomerase is generally required for the synthesis of telomeric repeats, the enzyme is not needed for telomere protection *per se* as long as there is enough telomeric DNA[18,19].

End protection by t-loops?

How do telomeric proteins protect chromosome ends? Some early models simply envisioned the formation of a proteinaceous cap over the vulnerable telomere termini. This protection strategy probably operates in the macronucleus (the vegetative nucleus) of hypotrichous ciliates[20].

A new model proposes that telomeres are protected by forming a specific configuration — the telomeric loop, or t-loop[21]. T-loops are created through the strand invasion of the 3′ telomeric overhang into the duplex part of the telomere (FIG. 2). They were discovered by electron-microscopic analysis of purified, protein-free telomeric DNA from human and mouse cells but are now also known to occur in many other eukaryotes (BOX 1). The strand invasion of the 3′ overhang displaces the G-rich strand of the duplex telomeric repeat array (FIG. 2). This displacement loop (D-loop) can be detected at the base of the t-loop through coating with the ssDNA-binding protein (SSB) from *Escherichia coli*. The amount of SSB that can be loaded onto isolated human t-loops is consistent with a D-loop of ~150 nucleotides, which suggests that most of the 3′ overhang is tucked into the duplex part of the telomere. It is also possible that a short stretch (<100 nucleotides) of the 5′ strand partakes in the strand invasion, creating a structure that is involved in homologous recombination and is known as a Holliday junction. The size of the t-loop is highly variable and might not be important for telomere function. More likely, the key function of the t-loop is the sequestration of the 3′ end.

T-loops seem to be a good strategy to protect chromosome ends from fusion. It is unlikely that DNA ligase IV could fuse telomeres in the t-loop configuration and the strand invasion of the 3′ overhang will stave off nucleolytic attack. So, some of the main threats to telomeres would be taken care of by simply changing the configuration of the telomeric DNA into the t-loop structure.

The way in which t-loops might allow cells to distinguish chromosome ends from sites of DNA damage is less clear. As detailed below, t-loops resemble a strand-invasion intermediate in homologous recombination (FIG. 1) and are also very similar to a presumed intermediate in replication-restart events (FIG. 3). Perhaps these intermediates in DNA repair and replication are ignored by the DNA-damage-surveillance machinery and t-loops escape detection that way. It is also possible that present-day t-loops are masked from DNA-damage sensors through coating with telomeric proteins.

How are t-loops formed? *In vitro*, TRF2 has the remarkable ability to create t-loops when provided with a short stretch of duplex telomeric DNA that ends in a 3′ overhang[21,22]. The frequency of t-loop formation by TRF2 is low, however. So, *in vivo*, TRF2 is likely to be assisted by other factors in the process of t-loop formation (FIG. 3). Human telomeres contain several recombination and repair proteins that could promote the strand invasion of the 3′ telomeric overhang. One of these is the MRE11 complex, composed of MRE11, RAD50 and NBS1 (Xrs2 in *S. cerevisiae*). In *S. cerevisiae*, this trio has a role in homologous recombination, a reaction that resembles t-loop formation (reviewed in REF. 23). The MRE11 complex is brought to the telomere by TRF2, which is consistent with a

Box 1 | T-loops across evolution: who's out of the loop?

T-loops are a conserved feature of telomere structure. They have been found, not only in vertebrates, but also in the germline DNA (found in the micronucleus) of the hypotrichous ciliate *Oxytricha fallax*[44]. They also occur in the flagellate protozoan *Trypanosoma brucei*. Although trypanosome telomeres are as long as human telomeres, their t-loops are tiny, often less than 1 kb in length (REF. 45). Extremely large t-loops, up to 50 kb in size, are seen in *Pisum sativum* (peas)[46]. Key pieces of information on t-loops are still missing, including what percentage of telomeres carry this structure, how t-loops vary through the cell cycle, and their functional significance. We also have no information yet on t-loops in *S. cerevisiae* and *S. pombe*. This is mainly because of technical reasons. First, there is the crosslinking problem — in order for t-loops to be detected, one must introduce inter-strand crosslinks, so that branch migration will not resolve the structure during the isolation of the telomeric DNA. The crosslinking step is performed on isolated nuclei (when the telomeres are still in their native configuration) and the crosslinking agents used are psoralen and UV light. Unfortunately, the T•A steps (as in TTAGGG) that are the substrate for psoralen are missing in the telomeric DNA of *S. cerevisiae*. The second problem has to do with the purification of telomeric DNA. For mammalian, plant and trypanosome telomeres, isolation of the telomeric fragments is based on a simple trick. The genomic DNA is reduced to very small fragments (~1 kb) by digestion with several frequently cutting restriction enzymes[21]. Under these conditions, the telomeres, which lack most restriction-enzyme recognition sites, remain large (often ~10 kb) and can be separated from the bulk of the genomic DNA on a sizing column. This trick does not work for organisms like *S. pombe* and *S. cerevisiae*, which have telomeres in the 1-kb size range. The only setting where t-loops are known to be absent is found in the macronucleus of hypotrichous ciliates. Although the micronucleus of these ciliates might have t-loops, their macronuclear telomeres do not. The macronucleus contains highly amplified gene-sized DNA molecules at a remarkable copy number of 2×10^7 per nucleus and an average size of just a few kilobases. Rather than containing long telomeres with t-loops, these DNA molecules are capped by ultrashort telomeres with termini that are hidden inside a tenaciously bound protein complex[47].

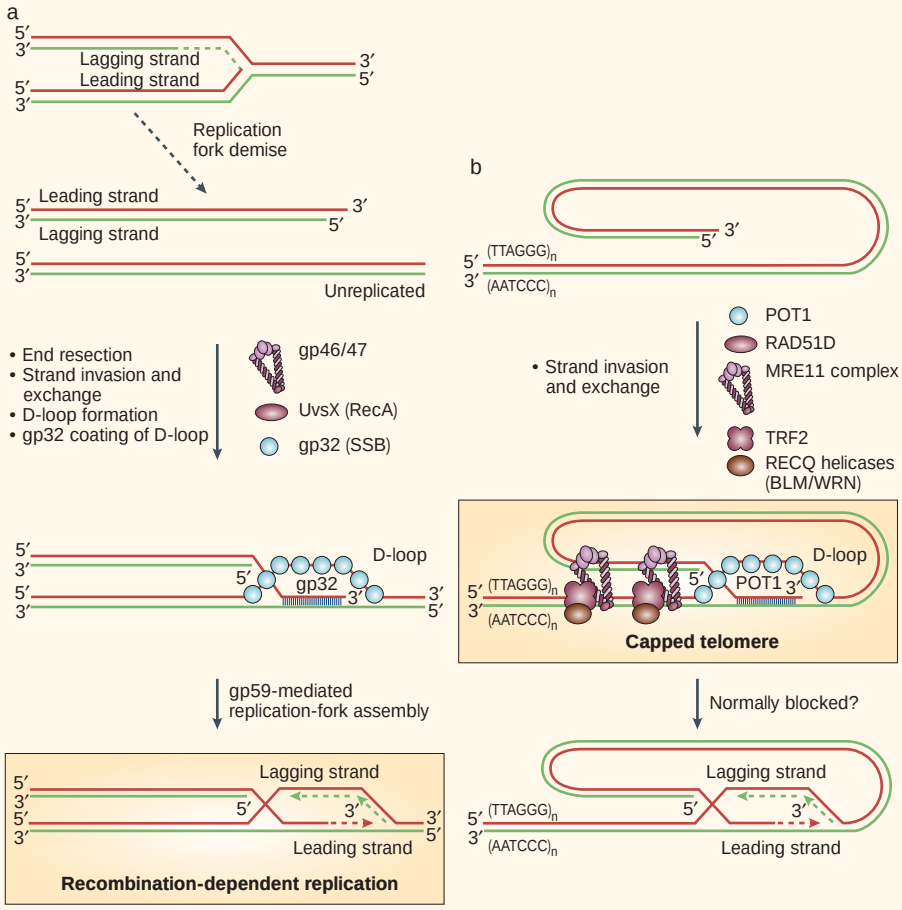

Figure 3 | **T-loop formation resembles initiation of RDR. a** | Recombination-dependent replication (RDR). After the demise of a replication fork, RDR can reinitiate replication. In RDR of phage T4, a 3′ overhang is formed by end-resection involving gp46/47, and coated with the single-stranded-DNA-binding protein gp32. Strand invasion and exchange takes place after loading of the strand-exchange factor UvsX. A D-loop is formed and is coated with gp32. gp59 mediates the assembly of the replication fork and replication is continued. Only a subset of RDR factors from T4 are shown (see TABLE 1 for further details and *Escherichia coli* orthologue). Similar steps might be required for t-loop formation. **b** | Capped-telomere formation. A 3′ overhang has to be generated and a strand invasion/exchange step will take place to form a D-loop. The relationship between a subset of telomeric proteins and some of the RDR factors is depicted (see TABLE 1 for further comparison). In RDR, D-loop formation is followed by assembly of a replication fork and semi-conservative replication (or bubble-migration synthesis). Presumably, this latter step is blocked at telomeres resulting in a stable t-loop state (capped telomere). Telomerase-independent telomere elongation could result from the loss of factors that block RDR at telomeres.

role in establishing the protective t-loop state. Additional components of the TRF2 complex might be the Werner syndrome (WRN) and Bloom syndrome (BLM) helicases[24], which could facilitate the unwinding step that is involved in t-loop formation. Furthermore, this family of helicases has the ability to disrupt G•G (Hoogsteen) base pairs[25,26], a feature that might be necessary to remove G•G base-paired structures from the 3′ TTAGGG repeat overhang, so that it can anneal to the CCCTAA repeat strand of the duplex telomeric tract.

If telomeres indeed employ homologous-recombination factors for the formation of t-loops, there is a risk that the reaction would go too far. If a Holliday junction is formed, its resolution could lead to deletion of the t-loop, resulting in a much shorter telomere. Interestingly, sudden large deletions occasionally happen at over-elongated yeast telomeres (known as telomere rapid deletions (TRDs)) and a t-loop-like structure has been proposed as an intermediate (reviewed in REF. 27). Clearly then, the homologous-recombination factors at telomeres must be highly controlled and it is expected that telomere-specific components in the telomeric complex govern this regulation.

A link between t-loops and RDR

Several factors that are known to be associated with present-day telomeres, including components of the MRE11 complex and the single-stranded telomeric proteins POT1 and Cdc13, are distantly related to proteins that play a role in RDR in prokaryotes (TABLE 1). RDR was first discovered in bacteriophages[28], but more recently it has become clear that RDR is also crucial for replication of the bacterial-host genome (reviewed in REFS 29,30). RDR is necessary for the reinitiation of a broken replication fork after replication has encountered a lesion. Such lesions are so frequent that replication of the *E. coli* genome often requires an RDR event to be completed. So, the processing of broken forks by RDR represents an ancient pathway for the repair of DNA ends that was in existence before the evolution of eukaryotes.

The early steps in RDR resemble the reaction that is required for t-loop formation (FIG. 3). In T4-phage replication, a 3′ overhang is generated with the aid of gp46/47 and is coated with the ssDNA-binding protein gp32 (which is related to SSB). The strand-exchange factor UvsX (related to bacterial RecA) mediates strand invasion in homologous sequences, thereby creating a D-loop. If a replication fork is established at this site (requiring several specific loading steps of the replication machinery), semi-conservative replication can take place. An alternative pathway is bubble-migration synthesis in which the 3′ end is extended while the D-loop migrates forward. The latter pathway would require further lagging-strand synthesis to render the DNA double stranded.

At least some of the proteins that are proposed to promote t-loop formation in mammals are evolutionarily related to RDR factors. The telomeric protein POT1 shares an OB-fold (a structural motif often used for binding to ssDNA) with the T4 protein gp32 and *E. coli* SSB. MRE11 and RAD50, which are recruited to telomeres by TRF2, have evolved from the T4 RDR proteins gp46 and gp47. Furthermore, vertebrate RAD51 and its related paralogues are the eukaryotic versions of the strand-exchange factors UvsX and RecA. Interestingly, a RAD51 paralogue was recently implicated in telomere biology in chicken cells[31]. Finally, UvsX loading seems to be facilitated by UvsY, a protein that is functionally related to the RAD52 family of recombination proteins. A mammalian member of the RAD52 family, RAD54, has been implicated in telomere function[32].

According to the t-loop–RDR comparison, one could speculate that t-loop formation involves the generation of a 3′ overhang,

Table 1 | Relationship between T4 RDR factors and mammalian telomeric proteins

Function in T4 RDR	T4	*Escherichia coli* orthologue	Eukaryotic orthologues or functional homologues	Role at mammalian telomeres?
ssDNA binding	gp32	SSB	RPA (DNA replication) POT1, Cdc13 (telomeres)	Unknown Yes (POT1)
Strand exchange	UvsX	RecA	RAD51 family	Yes (RAD51D)
Loading of UvsX	UvsY	RecO/RecR	RAD52 family	Yes (RAD54)
End resection	gp46/47	SbcCD	MRE11/RAD50/NBS1(Xsr2)	Yes

POT1, protection of telomeres-1; RDR, recombination-dependent replication; RPA, replication protein A; ssDNA, single-stranded DNA, SSB, single-stranded-DNA-binding protein

which is then coated by POT1. The next step would involve strand exchange, and one of the RAD51 paralogues could be a player in this reaction. These early steps would also require the MRE11 complex and RAD54. TRF2 could orchestrate these events through its inherent ability to promote the t-loop configuration and would also serve to bring (some of) the other factors to the telomere. The t-loop structure that is now established resembles RDR before capture of the replication machinery or bubble-migration synthesis (FIG. 3).

The t-loop-evolution model

The similarity between present-day telomeres in their t-loop configuration and the initiation of RDR indicates that there might be a simple scenario for telomere evolution. In this model, the first linear chromosomes had two or more repeats at their ends that are remodelled by RDR enzymes into a t-loop (FIG. 4). It is easy to see how these t-loops could have solved the end-replication problem using the enzymes involved in RDR. Capture of the lagging- and leading-strand replication machinery at the base of the t-loop could have elongated chromosome ends by semi-conservative DNA replication. Alternatively, bubble-migration synthesis could have extended just the 3′ end. The product of the latter reaction is identical to that of telomerase and would require further DNA synthesis to create the complementary strand. Although haphazard and unregulated, any mechanism of extension of the 3′ end of the t-loop could have solved the end-replication problem.

The t-loops would also have the ability to protect the ends from nucleases and ligases. Furthermore, chromosome ends bearing t-loops might not have been detected as sites of DNA damage. The prokaryotic DNA-damage response (the SOS response) is triggered by RecA bound to ssDNA. If the first chromosome ends had very short 3′ overhangs, the amount of bound RecA might have been too little for the activation of the SOS response. In this way, the ends of the first linear chromosomes would have achieved a protected state.

The t-loop-evolution model is attractive because there is no need to postulate the emergence of new protein functions when the first linear chromosomes were created. The newly formed eukaryote would have dealt with its chromosome ends using a pre-existing major pathway for the repair and processing of DNA ends. The only requirement for the generation of a linear chromosome is the (accidental) formation of a few terminal repeats.

Neither the size nor the sequence of these early terminal repeats would have been important. Indeed, as each end is replicated by a *cis*-acting process, the two ends of a linear chromosome could have contained entirely different sequences. One amusing possibility, given the close ties between telomerase and

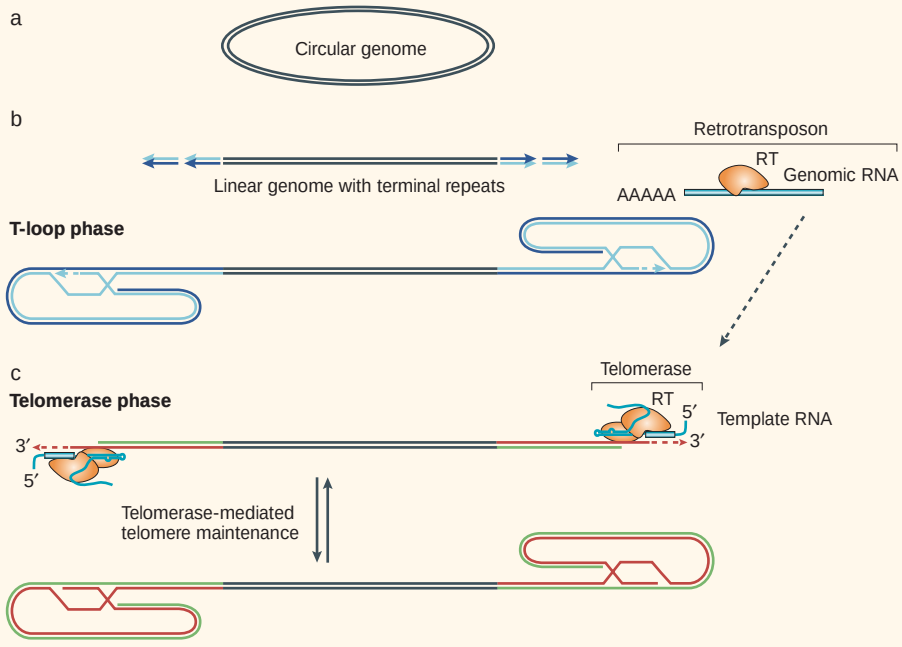

Figure 4 | **The t-loop-evolution model.** With a few notable exceptions, prokaryotes lack chromosome ends (**a**), and the first linear chromosomes (**b**) are proposed to have been capped and maintained by t-loops. The only requirement for this early form of telomere function is the presence of a few terminal repeats at each chromosome end. The length and sequence of the repeats is not important and can be different at each terminus. Using the prokaryotic recombination-dependent-replication (RDR) pathway, these repeats form a t-loop at each end, providing a mechanism for extension of the terminus. The t-loop structure is also proposed to protect the ends from nucleases and ligases. The SOS DNA-damage response is presumed not to be activated by t-loops. In present-day eukaryotes (**c**), telomere maintenance is primarily executed through the telomerase pathway. The telomerase reverse transcriptase (RT in the figure) could have evolved from pre-existing retrotransposon reverse transcriptases. T-loops might still be formed in some (but not all; see BOX 1) eukaryotes, providing telomere protection, and potentially regulating telomerase access to the telomere terminus. These t-loops are not used for telomere replication (except in unusual circumstances). The advent of telomerase has the advantage that all telomeres contain the same repeat sequence allowing emergence of specific *trans*-acting factors that can facilitate telomere protection and regulation of telomere length.

retrotransposons, is that the first terminal repeats were created by retrotransposition.

The relative ease by which eukaryotes can come up with telomerase-independent systems of telomere maintenance might be testimony to their past use of the t-loop-replication strategy. Some of the yeast strains that survive telomerase deletion have been proposed to use break-induced replication (BIR)[33], a eukaryotic replication mechanism akin to RDR. Indeed, the reliance of these survivors on Rad52/Rad50 or Rad52/Rad51 is in keeping with the idea that BIR or RDR is at work (TABLE 1). In addition, some of the human ALT cell lines might simply have found a way to return to the t-loop-replication strategy.

So, why is t-loop extension not happening all the time? In eukaryotes that use telomerase for telomere maintenance and have t-loops at their chromosome ends, regulatory steps might be in place to avoid inappropriate t-loop replication. Perhaps ALT cell lines lack factors that normally keep t-loops from entering an RDR (or BIR)-like pathway.

There is one linear genome, discovered in the mitochondria of a ciliate, that might represent a 'living fossil' of the original t-loop-replication and -capping strategy. Unlike most mitochondrial DNAs, the genome of *Tetrahymena* sp mitochondria is linear. Early electron-microscopy studies and later sequencing of the termini showed that the ends of this chromosome contain tandem direct repeats of about 50 base pairs (REFS 34,35). Remarkably, the terminal repeats diverge extremely fast and show no sequence similarity in related *Tetrahymena* sp[36]. In extreme cases, the left and right ends of the mitochondrial DNA have different repeats. These findings are consistent with a t-loop-replication mechanism, which allows repeats to diverge rapidly. Indeed, electron-microscopy spreads indicate that the *Tetrahymena* sp mitochondrial DNA can form t-loop-like structures *in vitro*[34]. It would be of interest to examine crosslinked *Tetrahymena* sp mitochondrial DNA by electron microscopy to establish whether the ends occur as t-loops *in vivo*.

The age of telomerase

The telomerase reverse transcriptase TERT is related to the enzyme that is used by many retrotransposons, including retroviruses[37]. The telomerases are most closely related to the reverse transcriptases of non-long-terminal-repeat (non-LTR) elements, which date back to the origin of eukaryotes. They also have functional similarity to non-LTR reverse transcriptases, since both use a DNA 3′ end as a primer. On the basis of this relationship as well as other arguments, Eickbush has proposed a scenario in which telomerase evolved from a non-LTR retro-element[38]. The t-loop-evolution model explains how eukaryotes could have survived before telomerase emerged. In this way, the telomerase-based telomere system could have evolved at a later stage (although still very early) from the non-LTR retrotransposons that were already invading the eukaryotic genomes (FIG. 4). The view that telomerase evolved from a retrotransposon (rather than the other way around) is consistent with the presence of reverse transcriptases in prokaryotes.

The telomerase-based telomere system brought enormous advantages. First, unlike the t-loop-replication strategy, telomerase can create new telomeres where none existed before. In doing so, telomerase can heal broken chromosomes and speed up genome evolution by permitting chromosome fragmentation and other rearrangements. Furthermore, telomerase has the advantage of homogenizing telomeric sequences. Whereas t-loop replication can lead to rapid divergence of the telomeric sequences, telomerase ensures that all telomeres are created equally. This is a crucial change in the system because it allows the use of sequence-specific *trans*-acting factors. With the emergence of telomere-binding factors, cells can connect events at telomeres to other pathways, resulting in the precise regulation of telomere transactions that is seen in present-day eukaryotes. In other words, telomerase allows a more bureaucratic approach to telomeres in which large 'committees' of protein networks decide on events at chromosome ends.

Once telomerase is firmly in control at telomeres, there might be a need to suppress the original t-loop-replication mechanism, as it could lead to unregulated telomere extension. Again, a regulatory machinery that allows t-loop formation but keeps RDR as well as homologous recombination under control seems a likely solution. Here too, the logic of the present-day telomere might be that its telomerase-made repeats recruit specific regulatory factors to keep the t-loop from reverting to its old RDR mode. With the advent of telomerase, some eukaryotes could even have avoided the hazards of t-loops by switching to an entirely different system. One example might be the telomeres in the macronucleus (vegetative nucleus) of hypotrichous ciliates, which are kept at such a short length (<40 bp) that t-loop formation is impossible (BOX 1). Decades ago, these short telomeres contributed to the birth of telomere molecular biology[39–41], and, more recently, they were instrumental for the identification of the telomerase reverse-transcriptase gene[42]. In a twist of fate, the t-loop-evolution model would suggest that these ultra-short, protein-capped structures might be among the most highly evolved of present-day telomeres.

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Competing interests statement

The author declares that she has no competing financial interests.

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CORRECTION

TRAFFIC JAMS AFFECT PLANT DEVELOPMENT AND SIGNAL TRANSDUCTION

Marci Surpin and Natasha Raikhel

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The authors wish to correct an error in the annotation of the bibliography. This version differs from the previous version, in which reference 51 was incorrectly annotated as 'This paper highlighted the importance of the endodermal tissue in mediating the gravitropic response, and provided a detailed description of vacuolar defects in two gravitropic-response mutants.' The authors intended to publish reference 51 without further annotation; and the annotation was intended for reference 52. This comment has now been removed.

The authors of this article apologize to the authors of reference 52 for the error. The online versions of this article have been corrected.