# On the origin of telomeres: a glimpse at the pretelomerase world

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#### Summary

Chromosomes may be either circular or linear, the latter being prone to erosion caused by incomplete replication, degradation and inappropriate repair. Despite these problems, the linear form of DNA is frequently found in viruses, bacteria, eukaryotic nuclei and organelles. The high incidence of linear chromosomes and/or genomes evokes why and how they emerged in evolution. Here we suggest that the primordial terminal structures (telomeres) of linear chromosomes in eukaryotic nuclei were derived from selfish element(s), which caused the linearization of ancestral circular genome. The telomeres were then essential in solving the emerged problems. Molecular fossils of such elements were recently identified in phylogenetically distant genomes and were shown to generate terminal arrays of tandem repeats. These arrays might mediate the formation of higher order structures at chromosomal termini that stabilize the linear chromosomal form by fulfilling essential telomeric functions. *BioEssays* 28:182–190, 2006. © 2006 Wiley Periodicals, Inc.

Introduction

Telomeres are DNA-protein complexes at the ends of linear chromosomes that are essential for (i) solving the end-replication problem, (ii) stabilization of the termini,

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Funding agency: Our work is supported by grants from the Howard Hughes Medical Institute (55000327), Fogarty International Research Collaboration Award (1-R03-TW05654-01), the Slovak grant agencies VEGA (1/0006/03 and 1/2331/05) and APVT (20-001604).

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Published online in Wiley InterScience (www.interscience.wiley.com).

Abbreviations: ALT, alternative lengthening of telomeres; bp, base pair; ERC, extrachromosomal rDNA circle; mtDNA, mitochondrial DNA; RC, rolling-circle; rDNA, ribosomal DNA; RTE, recombinational telomere elongation; t-circle, telomeric circle; t-loop, telomeric loop; TERT, telomerase reverse transcriptase; TRD, telomeric rapid deletion. (iii) protection from DNA repair machinery and subsequent end-to-end fusion, (iv) regulation of gene expression, and (v) macromolecular interactions in eukaryotic nuclei. Importantly, telomeres and pathways for their maintenance are directly implicated in complex biological processes such as cellular senescence and tumorigenesis.<sup>(1,2)</sup>

Sequencing of the first telomere by Blackburn and Gall<sup>(3)</sup> and the discovery of telomerase,<sup>(4,5)</sup> which adds de novo sequence onto chromosome ends thereby counteracting replicative sequence loss, were major breakthroughs in the field. These discoveries gradually attracted the attention of a broad scientific and pharmacological audience due to their potential clinical impact. The number of PubMed entries containing 'telomere' or 'telomerase' as keywords keeps growing dramatically (1,365 articles in 2004 versus 77 in 1990) illustrating the fact that telomere research remains at the cutting edge of contemporary cell biology.

Although telomerase-mediated synthesis is by far the most extensively studied mechanism of telomere replication, it is only one of several ways of maintaining the ends of linear chromosomal DNA.<sup>(6)</sup> The increasing interest in telomeraseindependent systems is not only due to their presence in a variety of tumor cells, but also because detailed information might be instrumental in our understanding of why and how natural selection favored linear chromosomes over their circular counterparts in nuclei of eukaryotic cells.

A few years ago, we proposed that the linear DNA genomes found in mitochondria of phylogenetically distant organisms<sup>(7)</sup> provide a unique opportunity to address problems concerning the origin of telomeric structures and telomere-maintenance pathways. This seemingly ambitious statement can be justified by the number of telomeric structures that have evolved in mitochondria possibly reflecting different end-replication strategies.<sup>(7,8)</sup> Since some of these structures essentially display the same features as their nuclear counterparts,<sup>(8,9)</sup> we suggest that mitochondrial telomeres and their replication strategies represent an evolutionary paradigm for the emergence of linear chromosomes in the nuclei of early eukaryotes (i.e. in pre-telomerase era).

Based on our studies on mitochondrial telomeres, we hypothesize that a selfish element replicating via a rolling-circle (RC) mechanism could generate terminal arrays of tandem repeats at the chromosomal termini of early eukaryotes. The expansion of telomeric repeats was followed by the formation of higher-order structures mediating the essential roles of telomeres. Molecular fossils of the key players of this scenario, i.e. telomeric circles (t-circles) and telomeric loops (t-loops) seem to represent conserved features of telomeres.<sup>(9,10)</sup> Primordial telomeric structures then recruited the telomerase, which provided a more robust way of dealing with the chromosomal ends and out-competed evolutionary ancient mechanisms.

In the first part of this article, we briefly review examples of terminal structures and their replication mechanisms. The different and shared features of these systems are then employed to discuss the evolutionary origin of telomeres in early eukaryotes.

# Strategies of telomere maintenance: multiple ways of dealing with chromosomal ends

Structurally different telomeres found in phylogeneticaly distant species may be considered as independent, successful evolutionary attempts to stabilize and replicate the linear chromosomal form. A range of terminal structures is utilized by the linear DNA genomes of viruses and plasmids as well as the linear chromosomes of bacteria, eukaryotic nuclei and organelles. Clearly, linear DNA molecules employ a wide repertoire of mechanisms for the maintenance of their ends (Fig. 1, Table 1).

#### How to back-up telomerase?

Telomeric DNA consisting of tandem arrays of short G-rich repeats and single-stranded 3' overhang that is elongated through the activity of telomerase is the most common solution to the end-replication problem in eukaryotic nuclei (Fig. 1A).<sup>(2)</sup> However, due to the essential roles of telomeres in replication and stabilization of linear chromosomes, their maintenance is backed-up by several mechanisms. In some instances, the back-up system could take over the primary role of telomerase leading to its loss in the corresponding branch of the phylogenetic tree. This might be the case in the fruitfly *Drosophila melanogaster* which utilizes telomeric retrotransposons.<sup>(11)</sup> Yet, in most cases, the telomerase or in hierarchical order.

#### **Recombinational telomere elongation (RTE)**

In the absence of telomerase, the replication of telomeres often relies on recombination-dependent mechanisms. The



**Figure 1.** Telomeres can be maintained by a wide variety of mechanisms. **A:** Most of the eukaryotic nuclear telomeres are replicated by telomerase, a ribonucleoprotein complex elongating the 3' single-stranded telomeric overhang using a region of its RNA subunit as a template.<sup>(4,5)</sup> **B:** Interchromosomal non-reciprocal recombination between two arrays of telomeric repeats can result in elongation of one chromosomal end in both natural and artificial telomerase-deficient systems.<sup>(98,99)</sup> **C:** In a t-loop, the 3' single-stranded overhang invades the double-stranded region of a telomere and thus may serve as a primer for DNA polymerase resulting in elongation of the G-rich strand without an assistance of telomerase.<sup>(31)</sup> **D:** Intrachromosomal recombination between telomeric repeats leads to generation of t-circles identified in yeast mitochondria and nuclei of several organisms including mammalian ALT cells. Their replication via rolling-circle results in amplification of telomeric sequences that can be spread by gene conversion to chromosomal telomeric regions.<sup>(50,52,54–56)</sup> **E:** Telomeres of several viruses, mitochondrial DNA and specific yeast mutants consist of telomeric palindromes containing a hairpin loop enabling replication via an intermediate resolved by a terminal resolvase.<sup>(79,80,92,100)</sup> **F:** A primer for DNA polymerase acting on the ends of linear DNA genome can be provided by hydroxylamino acids of terminal proteins covalently attached to the 5' end of the molecule (found in some viruses, plasmids and bacterial chromosomes).<sup>(93–97)</sup> **G:** The erosion of the terminal sequences can be compensated by transposition of mobile retroelements as in the case of *D. melanogaster*.<sup>(11,101)</sup>

Telomeric structure	Examples	References
Terminal hairpins/palindromes	Poxvirus, linear chromosomes in <i>Borrelia</i> , linear mtDNA of yeasts and protozoa, yeast <i>tlc1</i> <i>rad52 exo1</i> mutants	58,79,80,92
Terminal proteins covalently attached to the 5' ends	Adenovirus, linear chromosomes and plasmids in <i>Streptomyces</i> , linear plasmids in cytoplasm and mitochondria of fungi	93–97
Terminal arrays of tandem repeats	Eukaryotic nuclear chromosomes, linear mtDNA in protozoans and yeasts	2,28,29
Telomeric loops (t-loops)	Nuclear chromosomes in protozoan, plant and mammalian species, linear mtDNA of <i>Candida parapsilosis</i>	31,35–38
Telomeric circles (t-circles)	Yeast mitochondria and nuclei, mammalian nuclei	50,52,54-56

repetitive nature of telomeric regions makes these loci prone to intra- and interchromosomal recombination, often resulting in telomere elongation (Fig. 1B). The main molecular principles of RTE are derived from studies in Saccharomyces cerevisiae and Kluyveromyces lactis mutants lacking telomerase. These strains encounter progressive telomere shortening accompanied by a growth senescence followed by the emergence of two types of rare survivors whose telomeres are lengthened by RAD52-dependent recombination.(12)

RTE is of great clinical importance for a subset of human cancers. Although telomerase appears responsible for telomere maintenance in most cases, ~5-20% have no detectable telomerase and are thought to maintain telomeres using ALT (alternative lengthening of telomeres).<sup>(13,14)</sup> There are some types of human cancers without detectable ALT or telomerase activity,<sup>(15,16)</sup> possibly because these tumors may not need any telomere maintenance mechanism due to specific features of their biology.<sup>(17)</sup> The evidence supporting recombinational telomere maintenance in ALT cells is that these cells are capable of copying a targeted DNA tag from one telomere into other chromosomal ends.<sup>(18)</sup> Recombination between the telomeric repeats in ALT cells may lead to dramatic changes in the length of individual telomeres similar to telomere-rapid-deletion (TRD) in yeast.<sup>(19,20)</sup> Recent experiments demonstrated that, as in yeast, there may be two independent recombinational back-up systems operating in the absence of telomerase.<sup>(21,22)</sup>

While in yeast and human cells, RTE can be considered as a back-up to telomerase, there are a number of natural telomerase-deficient situations where telomere maintenance relies on recombination. These include chromosomal telomeres of dipteran insects, (23,24) beetles (25) and certain plants (26,27) as well as the linear mitochondrial DNAs (mtDNA) from ciliated protozoans and yeasts that terminate with tandem repeat arrays.<sup>(28,29)</sup> In each of these cases, the lack of telomerase correlates with an absence of canonical short telomeric motifs that are replaced by large and/or complex repetitive elements.

## **Telomeric loops (t-loops)**

It has been suggested that telomeres exist in 'open' states to allow telomerase to access the end of the telomeric array and then 'closed' states that protect chromosome ends from unwanted recombination and mask them from the doublestrand-break repair systems.<sup>(30)</sup> In 1999, using electron microscopy, Jack Grifith and co-workers discovered that mammalian telomeres end in giant duplex loops, which were termed t-loops.<sup>(31)</sup> These structures are presumably formed by an invasion of the 3' single-stranded overhang into the duplex telomeric region and were suggested to hide the natural end of the chromosome. Their formation is mediated by a specific telomere-binding protein TRF2,<sup>(32)</sup> presumably aided by other factors.<sup>(33,34)</sup> T-loops were subsequently found at the termini of micronuclear chromosomes of Oxytricha nova, (35) at the telomeres of Trypanosoma bruce(36) and Pisum sativum, (37) and at the ends of linear mtDNA of the yeast Candida parapsilosis.<sup>(38)</sup> Recently, Nikitina and Woodcock<sup>(39)</sup> described the isolation of t-loops in a chromatinized state from chicken and mouse cells. In addition, the ability of Taz1p, the fission yeast homolog of mammalian TRF1 and TRF2 proteins, to promote the formation of the t-loop-like structure in vitro,<sup>(40)</sup> together with indirect evidence for the presence of t-loops in K. lactis mutants with long telomeres,<sup>(41)</sup> suggest that these structures might also form in the nuclei of corresponding yeast species. It is therefore likely that t-loops represent an evolutionarily conserved characteristic of chromosomal ends<sup>(10)</sup> and that the invading 3' end may serve as a primer for the synthesis of telomeric sequences in situ (Fig. 1C).

### **Telomeric circles (t-circles)**

Telomere instability is often accompanied by the accumulation of extrachromosomal telomeric DNA.<sup>(42)</sup> In cell lines maintaining their telomeres by ALT pathways, the generation of telomeric fragments relies on recombination.<sup>(18,43)</sup> The frequency of homologous recombination is the same for ALT- and telomerase-positive cells indicating that ALT cells have a recombination defect specifically affecting the telomeres.<sup>(44)</sup> In principle, extrachromosomal telomeric fragments can be either linear or circular. The latter (which we termed t-circles) are especially interesting as they have been found in a variety of distantly related organisms.<sup>(9)</sup> The production of t-circles seems to be based on homologous intramolecular recombination between telomeric repeats. Support for this scenario comes from an analogy between telomeres and the tandem repeat nature of ribosomal DNA (rDNA) cluster in S. cerevisiae composed of 100-200 copies. An FOB1-dependent replication block could cause DNA double-strand breaks within the rDNA,<sup>(45,46)</sup> which can be repaired by homologous recombination resulting in the formation of extrachromosomal rDNA circles (ERC).<sup>(47)</sup> Deletion of RAD52 results in the loss of ERCs, thereby implicating recombinational repair processes in their formation.<sup>(48)</sup>

The analogy between t-circle dynamics and ERC is supported by the results of genome sequencing of the social amoeba *Dictyostelium discoideum*.<sup>(49)</sup> Analysis of the *D. discoideum* telomeres did not reveal any canonical telomeric motifs within the terminal regions. Instead, rDNA elements capped by GA-rich repeats seem to be present at the chromosomal tips. Thus it is likely that recombination between telomere-associated rDNA, possibly involving ERC, may be responsible for telomere maintenance in *D. discoideum*.

An important insight into the physiological relevance of t-circles was gained by the studies on yeast with linear mtDNA. Namely, it was found that mitochondria of C. parapsilosis, in addition to linear DNA molecules coding for typical organellar proteins, contain series of circular molecules derived solely from the telomeric repeat motif.<sup>(50)</sup> The presence of the t-circles correlates with the occurrence of a linear form of the mitochondrial genome.<sup>(51)</sup> The t-circles observed in nuclei of several eukaryotes<sup>(9,41)</sup> were shown to be able to promote telomere lengthening.<sup>(52,53)</sup> Moreover, two groups independently demonstrated that human ALT cells have abundant t-circles, pointing to their potential role in promoting telomere replication in the absence of telomerase.<sup>(54,55)</sup> Our recent results indicate that t-circles play an active role in the telomere maintenance, since mitochondrial t-circles amplify the array of telomeric sequence via RC replication machinery (Fig. 1D).<sup>(56)</sup> The amplified telomeric arrays may recombine with the linear DNA molecules to lengthen their termini. The feasibility of such assumptions is substantiated by the observation that artificial telomeric nanocircles can act as efficient templates for the synthesis of long telomeres by conventional DNA polymerase.<sup>(57)</sup>

#### **Telomeric palindromes**

Recently, it became evident that maintenance of nuclear telomeres is (at least in yeast) not limited to telomerase and recombination. Maringele and Lydall<sup>(58)</sup> found that, in the

absence of telomerase, Rad52 and Exo1 nuclease, the degradation of terminal chromosomal sequences is efficiently prevented through the formation of large DNA palindromes mediated by the double-strand-break DNA repair machinery (Fig. 1E). This exciting study demonstrated that the palindrome theory of end-replication proposed by Cavalier-Smith<sup>(59)</sup> and Bateman<sup>(60)</sup> not only applies to viral, prokaryotic and organellar genomes, but also can be activated on any genome that has inverted terminal repeats.

# Why and how linear chromosomes emerged in evolution?

Although linear chromosomes are sporadically found in prokaryotes, organelles and viruses, the chromosomes of eukaryotic nuclei are almost exclusively linear terminating with specific telomeric structures and, in most cases, maintained by telomerase. Circular or ring chromosomes in eukaryotes are rare and appear only occasionally in species normally possessing linear chromosomes. Ubiquitous occurrence of linear chromosomes in eukaryotic nuclei suggests that their emergence can be traced back to early eukaryotes. The linear form of eukaryotic chromosomes was possibly fixed at the time of the emergence of sex.<sup>(61)</sup>

It is generally accepted that the genome of modern eukaryotic cells represents a complex evolutionary mosaic originating from a stable intracellular symbiosis of methanotrophic archaea and *a*-proteobacteria and many of their features were inherited from prokaryotic ancestors. Although a survey of the chromosomal forms occurring among bacteria and archaea is far from complete, up to now only circular chromosomes were identified in archaeal species. In contrast, several bacteria (e.g. Agrobacterium, Borrelia, Coxiella, Streptomyces) possess linear chromosomes.<sup>(62)</sup> These species belong to independent phylogenetic lineages and their chromosomes differ in telomeric structures and therefore deal with the end-replication problem differently. In addition, close relatives of these species harbor circular chromosomes suggesting that linear chromosomes in bacteria emerged relatively recently from a circular ancestor. The molecular form of eukaryotic chromosomes with their specific telomeric structures does not seem to be related to any linear bacterial genome. This implies that linear chromosomes in eukaryotic cells represent evolutionary innovation and raises the question of how linear chromosomes and the primordial pathways for the maintenance of their terminal structures appeared in early eukaryotes.

One possible scenario includes an accidental linearization of originally circular genophore accompanied by the formation of specific terminal structures that stabilized the linear form (Fig. 2A). Another possibility is an invasion of selfish element(s) such as transposons or plasmids that integrated into an ancestral circular genome, forced its conversion toward a linear form and provided the means for stabilization and



replication of its termini (Fig. 2B, C).<sup>(63)</sup> The collisions of a circular chromosome with linear DNA plasmids resulting in its linearization are well documented in the mitochondrial DNAs of maize<sup>(64)</sup> and slime mold<sup>(65)</sup> as well as in the chromosomes of *Streptomyces* (Fig. 2C).<sup>(62,66)</sup> The latter examples illustrate that telomeres can be considered as structural and/or functional modules transferable between different replicons. Importantly, the linearity and/or the presence of telomeres may be preferred by natural selection. To test this hypothesis experimentally one can take advantage of 'circular mutants', whose ancestors originally harbored linear chromosomes.

#### Linear and circular chromosomes in bacteria

Recent analyses revealed linear chromosomal forms in *E. coli* wild-type cells suggesting that chromosome breakage easily occurs under normal growth conditions. The broken chromosomes accumulate in several recombination-deficient mutants indicating that large bacterial chromosomes face the problem of stability.<sup>(67)</sup>

However, the linear chromosomes of *Streptomyces* are also relatively unstable. Chromosome rearrangements often accompanied by elimination of telomeres occur at high frequency and result in circularization of the chromosome. Circular mutants are viable and do not exhibit any significant difference in growth compared to the wild-type cells. Nevertheless, circularized derivatives undergo various rearrangements including amplifications of certain chromosomal segments and seem to be more unstable than corresponding linear chromosomes. Therefore, neither circular nor linear forms ensure stability of the bacterial genome.<sup>(68–70)</sup>

### Schizosaccharomyces pombe telomere mutants

Fission yeast cells lacking telomerase activity escape the senescence and subsequent cell death by circularization of all three chromosomes.<sup>(71)</sup> A similar phenomenon was observed in *tel1 rad3* double mutants.<sup>(72)</sup> Although these circular mutants display only a small defect in mitotic growth, the segregation of chromosomes in meiosis is severely affected. Since sexual reproduction occurs in most eukaryotes, the essential role of telomeres in proper chromosome segregation in meiosis may represent a significant cause of the maintenance of chromosomes in the linear form.<sup>(61,73)</sup>

# Linear and circular mitochondrial genomes in yeasts

Circular mutants were identified in two types of yeast mitochondrial telomeres represented by *W. suaveolens*<sup>(74)</sup> (telomeric hairpins) and *C. parapsilosis*<sup>(51)</sup> (terminal arrays of tandem repeats), respectively. In both cases, mitochondrial telomere mutants harbor a circularized form of the mitochondrial genome that lost a significant portion of the telomeric sequence and fused its termini. In *C. parapsilosis* mutants, the occurrence of the circularized form correlates with the absence of the t-circles. This implies that t-circle-dependent telomere replication may represent the main, or even the only, telomere maintenance pathway in mitochondria of *C. parapsilosis*.<sup>(51,56)</sup> Existence of isogenic strains differing only in the

form of mtDNA enables us to test potential differences in their fitness by co-cultivation experiments in either batch or continuous cultures. In addition, providing that linear mtDNA is converted to a circular genome in the absence of t-circles, the yeast mitochondrial system may be exploited for screening the drugs that eliminate t-circles and thus interfere with the telomere maintenance pathway.

# On the origin of telomere maintenance pathways in eukaryotes: who was on first base?

Although the origin of telomerase may be traced back to the RNA world,<sup>(75,76)</sup> as pointed out by de Lange<sup>(10)</sup> and Fajkus et al.,<sup>(77)</sup> this does not necessarily imply that the enzyme maintained telomeres in ancestral eukaryotes. Rather, different mechanisms to deal with the end-replication problem might have existed. Some of them might have been employed by early eukaryotes, with telomerase being recruited only later to replace these evolutionary earlier attempt(s) at telomere maintenance, nowadays providing the most robust way of maintaining chromosomal termini. Replacement of one mechanism of telomere synthesis by another is exemplified by the recombination-dependent pathway(s) employed in ALT pathways substituting for or operating in parallel with telomerase, such as the generation of large terminal palindromes, maintenance of telomeres via retrotransposons, or interconversion between linear and circular forms of mitochondrial genomes in yeasts. Subterminal sequences of eukaryotic chromosomes were shown to be structurally conserved<sup>(78)</sup> and may be derived from molecular fossils from an earlier (i.e. pre-telomerase) era of telomere maintenance. Thus their analysis provides insights into ancient telomeric sequence(s) and/or structure(s).

Formation of telomeric palindromes seen at nuclear chromosomes of the yeast *tlc1 rad52 exo1* triple mutant,<sup>(58)</sup> linear mitochondrial DNA of *Williopsis* and *Pichia* species,<sup>(79)</sup> and poxviruses<sup>(80)</sup> represents a candidate for primordial pathway of chromosome end-replication, long before telomerase became responsible for telomere maintenance.

Another possibility for the origin of telomeres involves t-loop structures. Demonstration of t-loops in mitochondria<sup>(38)</sup> is in line with the scenario of de Lange<sup>(10)</sup> and others<sup>(31,35–37)</sup> that t-loops represent a general, evolutionarily conserved property of terminal tandem arrays and may explain several telomere-related phenomena such as capping function, masking the ends from DNA repair machinery, providing a solution to the end-replication problem and TRD. However, their formation requires pre-existing and sufficiently long arrays of terminal repeats since short telomeric tracts in yeasts or macronuclei of ciliates seem to be unable to form the t-loops.<sup>(35)</sup> We propose that this step in the evolution of telomeres was provided by t-circles. Their presence in a wide variety of systems<sup>(9)</sup> as well as the demonstration of their active role in the maintenance of mitochondrial telomeres employing a RC replication,<sup>(56)</sup>

indicate that t-circles may mediate generation of long tandem arrays of the telomeric sequence that may recombine with the linear DNA molecules to lengthen the termini. From the evolutionary point of view, it is important to note that RC replication strategy is common among various prokaryotic and eukaryotic replicons. Telomeres might have evolved from a selfish element functionally related to t-circles that integrated into the primitive eukaryotic, presumably circular, genome, forced its conversion toward a linear form and produced amplified tandem repeats at its termini. Expanded telomeric arrays subsequently might have allowed formation of the tloop structures.

Later on, the primordial telomere structures and maintenance pathways were replaced by telomerase. The presence of telomerase-dependent synthesis of telomeric arrays in the major eukaryotic kingdoms such as protozoa, fungi, plants and animals indicates that recruitment of telomerase happened relatively early in the evolution of eukaryotes. The absence of this enzyme in several species indicates that it was later lost, either due to a defect in the pathway or out-competed by alternative mechanisms such as telomeric retrotransposons.

An interesting example of a genome that is either heading to or from a telomerase-based mechanism of telomere maintenance is represented by linear mitochondrial plasmids of *Fusarium oxysporum*. These reverse transcriptase encoding plasmids have a terminal hairpin at one terminus and a telomere-like iteration of a 5 bp sequence at the other terminus. It was demonstrated that telomeric repeats are added during reverse transcription, and the ability to extend loosely associated primers could play a role in repeat formation by mechanisms similar to those associated with telomerase.<sup>(81,82)</sup> Based on these results, it is tempting to add telomerase to the list of selfish elements that have driven the evolution of linear DNA genomes.

### **Multiple lives of telomerase**

The evolutionary success of telomerase in the eukaryotic world might be caused not only by the efficient synthesis of telomeric arrays but also by its extracurricular functions(83,84) that might increase cellular viability. Several lines of evidence support this hypothesis. Expression of antisense RNA directed against the RNA component of telomerase and a dominant-negative catalytically inactive hTERT (human telomerase reverse transcriptase) mutant induced apoptosis independent of telomere shortening. In contrast, an hTERT mutant lacking telomerase enzymatic activity rescued cells with lowered telomerase activity from undergoing apoptosis.<sup>(85)</sup> This indicates that hTERT operates to regulate cell survival over and above the catalytic activity of telomerase on telomeric DNA by distinct interactions beyond the normal telomerase complex. Ectopic expression of telomerase in rats with experimentally induced liver cirrhosis extended the life

span of hepatocytes thus illustrating the potential of telomerase in treatment of patients with degenerative diseases,<sup>(86)</sup> although one must keep in mind that overexpression of telomerase also increases the risk of induction of tumorigenesis.

Some of the additional activities of telomerase emerged later in evolution. For example, TERT in higher eukaryotes, but not in yeast, possesses a specific mitochondrial targeting signal and is imported into mitochondria although its role in the organelle remains obscure.<sup>(87)</sup> More importantly, emergence of multicellular organisms and the split into somatic and germline cells resulted in the tight regulation of the telomerase activity. The repression of telomerase is associated with cell differentiation and represents an anticancer mechanism. Hence, most human somatic cells have little or no telomerase activity and, as a consequence, have a limited replicative capacity. In contrast, the vast majority of human cancers reactivate telomerase and become immortal. Recent studies indicate that additional functions of hTERT not directly associated with replication of chromosomal ends seem to be crucial for tumorigenesis.<sup>(83,84)</sup> The expression of oncogenic H-Ras in immortal cell lines maintaining telomeres by telomerase-independent mechanisms did not result in their transformation. However, subsequent ectopic expression of hTERT in these cells resulted in a tumorigenic phenotype. Importantly, the same outcome was observed after introduction of a mutant hTERT that retained catalytic activity but was incapable of maintaining telomere length.<sup>(88)</sup>

One possibility for addressing the question of the involvement of telomerase in 'nontelomeric' functions might be provided by the circular mutants of *Sch. pombe*. It might be of interest to assess a difference in fitness of the mutants with and without functional telomerase and exposed to various conditions, such as those inducing DNA damage. Although it might not be so simple to execute (it seems that circular chromosomes open frequently and place an additional load on the DNA repair machinery; P. Baumann, personal communication), one could distinguish between "additional load" and role of telomerase in repair by comparing telomerase-deficient strains shortly after telomerase loss—when they still have linear chromosomes.

#### Perspectives

It is clear that various systems (bacteria, viruses, plasmids, organelles and nuclei) containing linear DNA genomes employ different mechanisms of telomere maintenance. We suggest that, while in some cases the system 'retained' the original mechanisms, other systems (such as eukaryotic nuclei) underwent serial additions of alternative mechanisms, nowadays operating either hierarchically or in parallel. This hypothesis may be addressed by different means. It was shown that ALT and telomerase can co-exist within the same cell.<sup>(89,90)</sup> Therefore it would be interesting to see if the t-circles, in addition to their role in telomere lengthening in telomerase-deficient systems, can perturb telomere functioning in cells expressing telomerase.

One approach that may address the above questions could be based on the introduction of telomerase into natural telomerase-deficient cells, like *D. discoideum* or *D. melanogaster*. Would telomerase out-compete the rDNA-based telomeres, retrotransposons or RTE? If yes, what would be the perspectives of the strains with reprogrammed telomeres?

An attractive alternative would be to reconstitute the early phases of telomere evolution by artificial linearization of contemporary circular genomes and follow their fates. For example, mitochondrial t-circles isolated from yeast with linear mitochondrial genomes could be transferred to the mitochondria of species (e.g. S. cerevisiae) with circular mtDNA. If tcircles really represent a selfish DNA element, they should mediate conversion of mtDNA to a linear form and subsequently solve the end-replication problem by taking advantage of the presence of the RC machinery in the mitochondria of S. cerevisiae.<sup>(91)</sup> An even more farfetched experiment would be to introduce mitochondrial t-circles into bacteria or yeast nuclei and see if it is possible to reprogram these systems. Would the reprogrammed strains differ in their relative fitness? How will the original telomeres change during prolonged cultivation? Will the reprogrammed yeast nuclei retain telomerase?

Studies along these lines not only would be instrumental in understanding the evolutionary roots of telomeres, but also may have clinical implications. For example, expression of telomerase delays cell senescence and thus potentially extends life span. However, at the same time, its expression increases the risk of tumorigenesis, probably due to its replication-independent activities. Modulation of ALT pathways may represent distinct means of deliberately affecting the length of telomeric arrays (increase as well as decrease via TRD) thus manipulating the replicative life span without a direct risk of tumor induction.

#### **Acknowledgments**

We wish to thank Ladislav Kovac (Comenius University, Bratislava) for continuous support and helpful comments, Jack Griffith (University of North Carolina, Chapel Hill) for a long-term collaboration, Deepa Subramanian for editorial advice, and members of our laboratory for discussions.

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