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Review

Mouse models to study the role of telomeres in cancer, aging and DNA repair

María A. Blasco*

Department of Immunology and Oncology, National Centre of Biotechnology, E-28049 Madrid, Spain

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Abstract

The chromosome ends have protective structures that distinguish them from broken chromosomes, known as telomeres. The function of telomeres, and that of the cellular activity that synthesises them, telomerase, are proposed to be biological determinants in the processes of cancer and aging. In this review, we will focus on mammalian telomeres and, in particular, on the analysis of different mouse models for proteins that are important for telomere function, such as telomerase and various telomere-binding proteins. These mouse models have allowed the relevance of telomeres and telomerase in tumour development and the aging of the organism to be directly tested.

Keywords: Telomeres; Telomerase; Mouse models; Cancer; Aging; DNA repair

1. Telomeres distinguish chromosome ends from DNA breaks

Telomeres are capping structures at chromosome ends that protect them from undesired rearrangements, and that are composed of tandem repeats of the TTAGGG sequence bound to a complex array of proteins [1—3]. Telomeres are also characterised by having a 3′-overhang of the G-rich strand, known as the G-strand overhang [3]. The current model for chromosome capping is that telomeres form a higher-order chromatin structure that physically hides the 3′-chromosome end from cellular activities (Fig. 1). This protective structure could be provided by the ability of the 3′-overhang to fold back and invade the double-strand region of the telomere forming the so-called T-loop and generating a displacement loop, or D-loop (Fig. 1) [4]. In support of the T-loop model is the fact that telomere-binding proteins and average telomere length, simultaneously influence both telomere function and T-loop formation [3–5]. Nevertheless, alternative telomere structures for chromosome capping have not been ruled out.

Importantly, functional telomeres are proposed to prevent the ends of eukaryotic chromosomes from being recognised as DNA breaks [1,2]. Strong evidence for this comes from the fact that the Ku86 protein, which is central to the non-homologous end-joining (NHEJ) pathway for repair of DNA double-strand breaks (DSB), mediates both end-to-end chromosome fusions and apoptosis triggered by critically short telomeres, suggesting that the NHEJ machinery detects and signals the presence of dysfunctional telomeres as DNA damage and ''repairs'' them leading to end-to-end chromosomal fusions and other chromosomal rearrangements (Fig. 2) [5–7]. Mutation of telomere-binding proteins can also result in dysfunctional telomeres in the absence of significant telomere shortening (Fig. 2) [3–5] (see below).

2. Telomere binding proteins

TRF1 and TRF2 bind to the double-stranded TTAGGG repeats at mammalian telomeres [8–10]. Both TRF1 and TRF2 are found at telomeric T-loops and are negative regulators of telomere length [4,11]. TRF1 function is regulated by TIN2 [12], and by TANK1 (also known as tankyrase) and TANK 2 [13,14]. TANK1 inactivates TRF1 by poly(ADP-ribosyl)ation [13], and causes telomeric elongation when overexpressed [15]. TANK2, in contrast, causes rapid cell death by necrosis when overexpressed [14]. TANK1 and TANK2 have a

* Tel.: +34-915854846; fax: +34-913720493.
E-mail address: mblasco@cnb.uam.es (M.A. Blasco).

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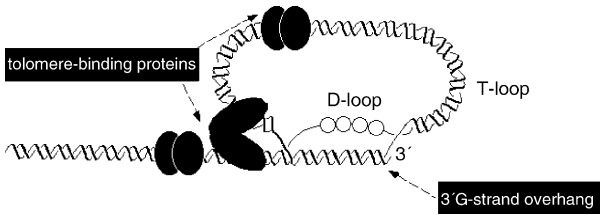

Fig. 1. A putative structure of a capped telomere based on the T-loop model. Both telomere-binding proteins and the G-strand overhang are required to maintain telomere capping.

catalytic domain homologous to that of poly(ADP-ribose) polymerase (PARP-1) [16].

TRF2 has also been shown to stabilise the G-strand overhang, and prevent telomeric fusions [17]. Telomere dysfunction due to *TRF2* mutation can either cause premature senescence (17) or activation of the apoptotic cascade mediated by ataxin telaugiectasia mutated (ATM) and p53 [18]. TRF2 also recruits hRAP1 to human telomeres. hRAP1 is the homologue of yeast RAP1 protein and its overexpression causes telomere elongation [19]. In addition, TRF2 recruits the MRE11 complex to human telomeres [20]. The MRE 11 complex is composed of RAD50, MRE11 and NBS1 and is a key component of the homologous recombination (HR) and NHEJ pathways involved in DNA double-strand break (DSB) repair.

Another NHEJ DNA repair complex found at the telomeres is the DNA dependent protein kinase complex (DNA–PK). DNA–PK is composed of Ku70 and Ku86 proteins, and of DNA–PKcs [21]. Ku proteins interact with TTAGGG repeats [22,23] and with telomeric proteins TRF1 and TRF2 [24,25]. The study of Ku86 and DNA dependent protein tinase catalytic submit (DNA–PKcs) deficient mice indicated that these proteins also have essential roles at the mammalian telomere [7,24,26—29] (see below).

Pot 1 is the only single-stranded DNA telomere-binding protein found to date, and has been shown to

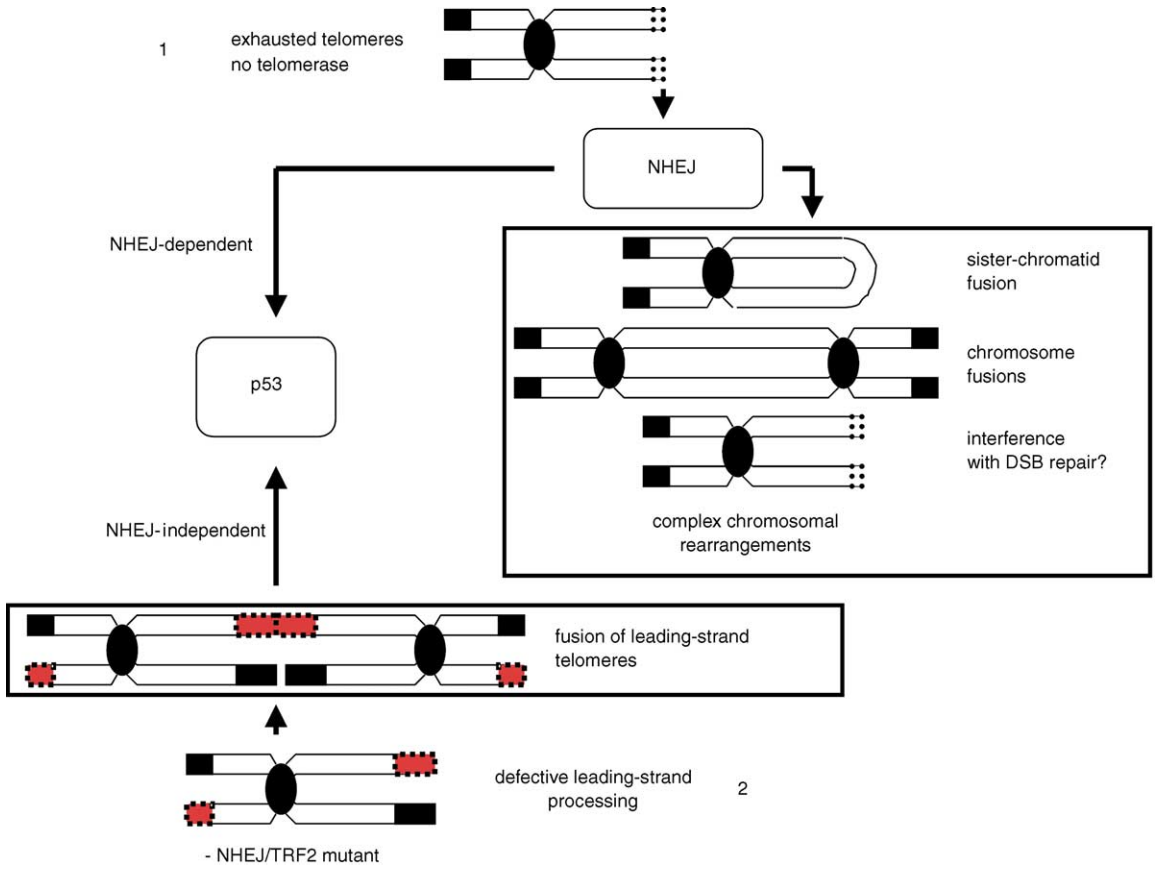

Fig. 2. As telomerase-deficient cells proliferate telomeres are lost from the chromosome ends, eventually resulting in telomere-exhausted chromosomes. Non-homologous end-joining (NHEJ) proteins have been shown to detect, and to "repair" telomere-exhausted ends leading to various types of chromosomal rearrangements. Similarly, telomere-exhausted chromosome ends are regarded as DNA breaks and can interfere with the efficient repair of DNA breaks in the genome, resulting in a higher sensitivity to genotoxic agents. NHEJ activities have also been shown to mediate apoptosis due to critical telomere loss, suggesting that NHEJ can be placed upstream of p53 in signalling telomere-exhausted chromosomes as damaged DNA. In the absence of DNA–PKcs (NHEJ) or TRF2, telomere processing is inappropriate, leading to NHEJ-independent telomere fusions independently of telomerase activity and telomere length. p53 been has also shown to mediate apoptosis due to *TRF2* mutation. DSB, double-strand break.

interact with the G-strand overhang both in yeast and in mammals [30].

3. Other factors that regulate telomere length

The main factor responsible for telomere dysfunction in human somatic cells is accumulation of cell doublings. As cells proliferate, TTAGGG repeats are lost from telomeres [31]. This progressive telomere loss can be prevented if cells have sufficiently high levels of telomerase, a cellular reverse transcriptase that adds TTAGGG repeats onto pre-existing telomeres [32]. Telomerase consists of two essential components, a reverse transcriptase subunit known as Tert (Telomerase Reverse Transcriptase) and an RNA molecule or Terc (Telomerase RNA component), which contains the template for the synthesis of new telomeric repeats [33]. Most normal somatic cells do not have sufficient telomerase and suffer telomere attrition coupled to cell division [31] and this telomere shortening eventually results in TTAGGG-exhausted chromosome ends [6,34,35]. This loss of telomere repeats leads to chromosomal fusions and cell arrest or apoptosis [6,34,35]. Re-introduction of telomerase prevents telomere exhaustion and allows viability both in cultured cells and in the context of a telomerase-deficient mouse [36—38], demonstrating that short telomeres are responsible for the triggering of the adverse phenotypes due to telomerase-deficiency.

Telomerase also prevents TTAGGG exhaustion at telomeres in more than 90% of all human tumours that reactivate telomerase at some point during their formation [39]. Telomerase inhibition in human tumour cell lines leads to telomere shortening and loss of cell viability [40], suggesting that telomerase inhibition could be an effective way to abolish tumour growth by provoking telomere shortening to a critical length. In addition to the role of telomerase in maintaining telomere length, new evidence suggests that telomerase might enhance survival and promote proliferation independently of telomere length [41,42] (see below).

However, some human cell lines and tumours that lack telomerase activity, are able to maintain or elongate their telomeres by alternative mechanisms, which have been termed ALT. ALT-positive cells are characterised by the presence of long and heterogeneous telomeres, as well as by the co-localisation of telomere-binding proteins and PML in the so-called APB bodies (ALT-associated PML bodies) [43,44].

4. The telomerase-deficient mouse: a model for telomere-mediated aging?

A telomerase-deficient mouse model was obtained by the elimination of the gene encoding for the murine *Terc* gene, $Terc^{-/-}$ mice [6,45]. These mice are viable, but only a limited number of generations can be derived before complete loss of viability is observed due to telomere loss and increased end-to-end fusions [6,34,35]. The phenotypes associated with telomere dysfunction included (i) male and female infertility [34,35]; embryonic mortality due to a defective closure of the neural tube [46]; (ii) small size and severe intestinal atrophy [35,47]; (iii) spleen atrophy and reduced proliferation of B and T lymphocytes [34,35]; (iv) impaired germinal centre function [48]; (v) reduced angiogenic potential [49]; (vi) reduced proliferative potential of the bone marrow stem cells [50]. Therefore, telomeres are necessary for maintaining tissue homeostasis in the mouse, and predict that telomere shortening with age in humans may lead to similar disease states.

A recent study has addressed the capability of telomerase to ''repair'' critically short telomeres in late generation telomerase-deficient mice [36]. This study showed that telomerase is able to recognise short telomeres and to elongate them, preventing the occurrence of end-to-end fusions and the appearance of phenotypes in these mice [36]. These findings open the possibility of using telomerase re-introduction as a putative gene therapy for human premature aging syndromes characterised by a faster rate of telomere loss [32], as well as for age-associated pathologies.

5. The telomerase-deficient mouse: a model for telomerase inhibition in human cancer

The telomerase-deficient mouse model has provided strong evidence that short telomeres suppress tumour progression, in agreement with the fact that telomerase activity is upregulated in most human tumours [39,51]. In particular, late generation $Terc^{-/-}$ mice show less skin tumours than wild–type controls upon chemical carcinogenesis of the skin [52]. This tumour suppressor phenotype coincides with p53 upregulation in $Terc^{-/-}$ papilomas, that has been proposed to be sensing short telomeres as damaged DNA and contributing to the cessation of growth (52). In addition, telomerase deficiency in combination with deficiencies in tumour suppressor genes other than *TP53*, significantly reduce carcinogenesis [53–56], suggesting that a telomerase inhibitor may be effective in the cessation of tumour growth. Importantly, the anti-tumour effect of telomerase inhibitors may be enhanced in combination with genotoxic agents, as has been recently suggested [57,58]. In particular, two different studies described that late generation $Terc^{-/-}$ mice are radiosensitive compared with wild-type controls [57,58]. Irradiation of late generation $Terc^{-/-}$ mice resulted in a higher chromosomal damage and greater apoptosis than wild type controls

[57,58]. These results may have important implications for the radiotherapy of cancer, as tumours treated with telomerase inhibitors will suffer a faster rate of telomere loss, which in turn is likely to increase the sensitivity of these tumours to radiotherapy [57].

6. Mouse models for transgenic telomerase overexpression

Telomerase activity is upregulated during mouse tumorigenesis, despite the fact that mice have very long telomeres [59,60]. This finding suggested that telomerase might have additional roles in promoting tumorigenesis that are not mediated by its role in net telomere elongation. In support of this, first generation (G1) telomerase deficient mice, *Terc*$^{-/-}$ mice, which lack telomerase activity, but still have long telomeres, were shown to have less skin tumours than wild-type mice following skin chemical carcinogenesis, indicating a negative impact of telomerase deficiency on tumour growth, even in the presence of sufficiently long telomeres [52].

More recent evidence for an active role of telomerase in promoting tumorigenesis independently of the length of telomeres, comes from the study of mice that overexpress the catalytic component of mouse telomerase in basal keratinocytes, K5-Tert mice [61]. K5-Tert mice show high levels of telomerase activity and long telomeres in the skin [61]. Interestingly, the epidermis of telomerase-transgenic mice is highly responsive to the mitogenic effects of phorbol esters, and shows an approximately 2-fold increase in the rate of would-healing compared with wild-type littermates [61]. K5-Tert mice were also found to be 2-fold more susceptible to developing skin tumours than wild-type mice upon chemical carcinogenesis of the skin [61]. These results indicate that telomerase actively promotes growth in cells that have sufficiently long telomeres and that have been subjected to tumorigenic insults. Interestingly, when these mice were left to age, they showed a decreased viability compared with the corresponding wild-type controls, which was coincidental with a significant increase in spontaneous tumours in tissues with transgenic telomerase expression [62]. In addition, the detrimental effects of transgenic telomerase expression were aggravated when in a *TP53*$^{+/-}$ genetic background, suggesting cooperation between high telomerase expression and *TP53* mutation in spontaneous tumorigenesis in the mouse [62]. Similarly, mice with transgenic telomerase expression under a *β-actin* constitutive promoter have an increased incidence of spontaneous mammary epithelial tumours as they age [63]. In summary, high transgenic telomerase expression seems to cooperate both with age and *TP53* mutations in promoting tumorigenesis in the mouse.

These findings suggest that telomerase activation at early stages of tumour growth may actively promote tumour growth and survival, even if telomeres are still sufficiently long, and that telomerase activation could favour tumorigenesis by at least at two different mechanisms: by signalling proliferation and promoting growth independently of telomere length, and by rescuing tumour cells with critically short telomeres.

7. Severe telomere dysfunction in Ku86-deficient mice

The analysis of Ku86-deficient mice revealed that Ku86-deficiency results in telomeric fusions [24,26,27], which are characterised by showing long TTAGGG segments at the fusion point [27]. This suggested that these fusions are not the result of telomere shortening below a minimum length, but rather by the loss of the telomere capping structure. Ku86 deficiency also resulted in significant telomere lengthening [27], suggesting that Ku86 impairs the access of elongating activities to the telomere, in agreement with a role of Ku86 in maintaining a telomeric capping structure (i.e., T-loops). The study of doubly deficient mice for telomerase and Ku86, demonstrated that Ku86 was specifically impairing the access of telomerase to the telomeres [7]. Interestingly, Ku86-deficient mice show similar phenotypes to those of late generation telomerase-deficient mice such as infertility, small size and decreased viability with age [7,64,65]. Furthermore, Ku86 deficiency can suppress tumour growth in a wild-type *TP53* background [66], similar to that reported for late generation telomerase-deficient mice [52, 54–56]. Hence, the role of Ku86 protein in aging, cellular proliferation and transformation could be mediated by its essential function at the telomere.

Besides its role in telomere capping, Ku86 has been also shown to be essential in signalling and processing critically short telomeres as damaged DNA, in agreement with its role in NHEJ of double-strand DNA breaks (Fig. 2). In particular, the study of doubly deficient telomerase and Ku86 mice showed that Ku86 deficiency rescued both end-to-end chromosomal fusions and apoptosis due to critically short telomeres [7]. These findings suggest that NHEJ can be placed above p53 in the detection and signalling of TTAGGG-exhausted telomeres (Fig. 2) [7].

8. Differential roles of Ku86 and DNA–PKcs at the mammalian telomere

Similar to Ku86 deficiency, DNA–PKcs absence results in end-to-end fusions with TTAGGG repeats at the fusion point, indicating telomere dysfunction in the absence of telomere shortening (28). Interestingly, the frequency of end-to-end fusions detected in DNA–PKcs$^{-/-}$ cells is significantly lower than in Ku86$^{-/-}$

cells, suggesting that Ku86 deficiency has a more dramatic effect on telomere end-capping than DNA–PKcs deficiency (28). Furthermore, in contrast to Ku86 deficiency, DNA–PKcs deficiency *per se* did not affect telomere length [28]. The fact that the telomeric phenotypes of DNA–PKcs$^{-/-}$ cells are milder than those of Ku86$^{-/-}$ cells, is consistent with the greater severity of aging phenotypes in the Ku86$^{-/-}$ mouse than in the DNA–PKcs-deficient mice [5].

A recent report has shed some light into the possible mechanism by which DNA–PKcs could be contributing to telomere capping. In particular, mutation of either TRF2 or DNA–PKcs, lead to end-to-end chromosomal fusions always involving telomeres produced by leading-strand synthesis [29]. These findings suggest that both proteins share a similar role at the mammalian telomere. Moreover, the fact that the fusions always involved the leading-strand of telomeres led to the proposal that these proteins could be involved in the post-replicative processing of telomeres, hence for the generation of a proper telomere capping structure [29] (Fig. 2). A similar role for Ku86 is likely although this has not been formally demonstrated.

9. Two mayor ways leading to telomere dysfunction in mammalian cells

The current view is that both a minimal length of telomeric repeats and the telomere binding proteins are necessary for proper telomere function (Figs. 1 and 2). First, telomere shortening due to lack of telomerase, results in TTAGGG-exhausted telomeres, thus disrupting the telomere structure and exposing the end to NHEJ DNA repair activities (Fig. 2). As a consequence of this, short telomeres result in NHEJ-mediated end-to-end chromatid-type and chromosome-type fusions, as well as loss of cell viability [7] (Fig. 2). In addition, critically short telomeres are likely to interfere with the efficient repair of DSB in the genome, thus resulting in a higher sensitivity to ionising radiation and to other genotoxic agents, as recently shown in Refs. [57,58]. Second, mutation of telomere binding proteins, such as DNA–PKcs and TRF2, results in defective processing of telomeres produced by leading-strand synthesis, thus leading to loss of telomere capping and to telomere fusions in the absence of significant telomere shortening. These fusions are not likely to be mediated by NHEJ, since they occur in the absence of DNA–PKcs activity (Fig. 2). Similarly, these fusions are independent of telomere length and of telomerase activity, as they occur in telomerase-proficient cells (Fig. 2).

Importantly, unravelling the functional interactions at the mammalian telomere will allow the consequences of telomere dysfunction for aging and cancer to be established, as well as allowing the rational design of therapeutic strategies.

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