Springer Semin Immunopathol (2002) 24:75–85
DOI 10.1007/s00281-001-0096-1

Springer Seminars
in Immunopathology

Immunosenescence phenotypes in the telomerase knockout mouse

María A. Blasco

Department of Immunology and Oncology, Centro Nacional de Biotecnología-CSIC,
Campus Cantoblanco, 28049, Madrid, Spain

Abstract. Increasing generations of the telomerase knockout mouse, Terc$^{-/-}$, show severe telomere dysfunction characterized by critically short telomeres and end-to-end chromosomal fusions. These mice also suffer from various age-related diseases affecting highly proliferative tissues. Among these pathologies are a reduced proliferative capacity of B and T cells, as well as a reduction of germinal center reactivity upon immunization. Both immune system defects are landmarks of immunosenescence. The study of the telomerase-deficient mouse model supports the notion that telomere shortening with age contributes to immunological dysfunction in the elderly.

What are telomeres?

Telomeres are large nucleoprotein complexes at chromosome ends that are composed of G-rich tandem repeats of the sequence TTAGGG in vertebrates and associated proteins [7, 16]. In addition, telomeres are characterized by having a 3′ G-rich overhang (G-strand overhang) [47, 69], which is likely to originate as a direct consequence of the so-called "end replication problem" [53]. The G-strand overhang can fold back and invade the duplex telomeric repeats, displacing one strand and hybridizing to its complementary sequence [25]. This higher-order telomere structure has been named the "T-loop" (Fig. 1). The T-loop model provides a mechanism for the sequestering of the G-strand overhang, which could otherwise activate DNA damage checkpoints and DNA repair enzymes [14, 24]. The formation of T-loops has been associated to the binding of telomeric proteins TRF1 and TRF2 [25] (see below). More recently, a novel telomeric protein, Pot 1, has been shown to interact with the single-stranded G-strand overhang both in *S. pombe* and in humans [4].

The first TTAGGG-binding protein identified was TRF1, a negative regulator of telomere length [15]. TRF1 function is regulated by TIN2 [41], and by two proteins highly homologous to each other, TANK1 (also known as tankyrase) and TANK 2 [39, 60]. Another TTAGGG-repeat binding protein is TRF2 [6, 11]. Like TRF1 and TIN2, TRF2 is also a negative regulator of telomere length [62]. In addition, TRF2 has unique functions at the telomere such as stabilizing the G-strand overhang, and pre-

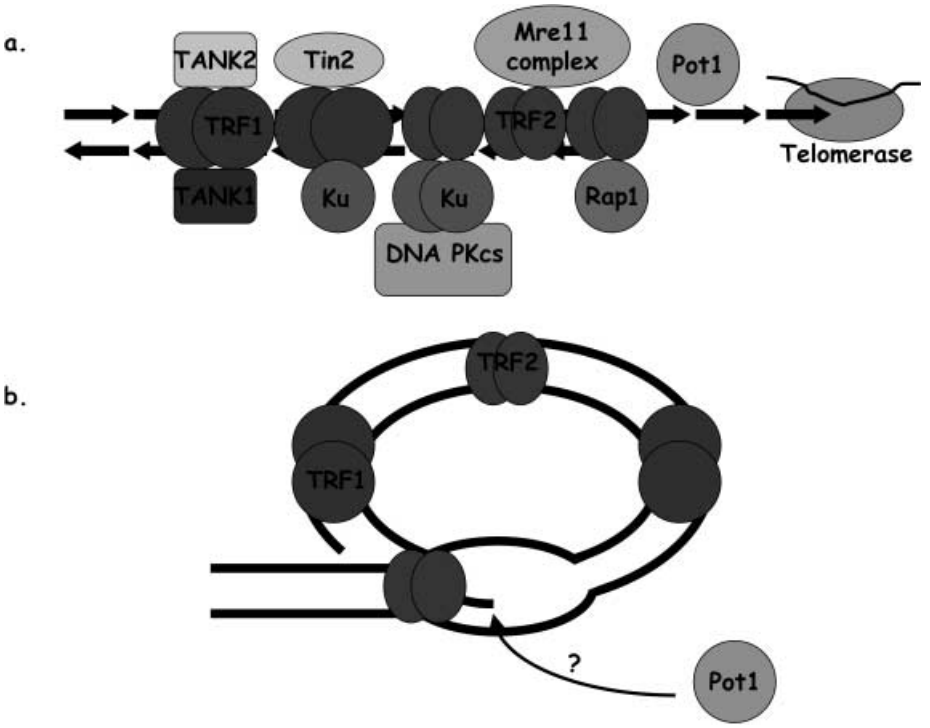

Fig. 1. a Different proteins found at the mammalian telomere. **b** Mammalian telomeres can form a higher order structure known as the T-loop, which has been seen associated with telomeric proteins TRF1 and TRF2

venting telomeric fusions [65]. Furthermore, over-expression of a TRF2 dominant negative mutant causes premature senescence [65] and apoptosis [38]. TRF1 and TRF2 are found at telomeric T-loops and their effects on telomere length and end-protection could be mediated by this property [25]. TRF2 has been shown to recruit hRAP1 and the MRE11 complex to human telomeres [43, 72]. Ku proteins also interact with TTAGGG repeats [5, 34] and with telomere binding proteins [35]. Ku70 and Ku85 together with DNA-PKcs form an enzyme called DNA-dependent protein kinase (DNA-PK), which is involved in DNA double-strand break (DSB) repair by non-homologous end-joining (NHEJ) and in V(D)J recombination [61]. The study of Ku86- and DNA-PKcs-deficient mice has demonstrated that these proteins also have a role at the mammalian telomere [3, 22, 35, 57]. In addition, the study of human premature aging syndromes has contributed to the identification of other proteins that affect telomeric function in mammals, such as Ataxia telangectaxia, Bloom and Werner [26].

Telomerase: the cellular enzyme that makes telomeres

Telomerase synthesizes telomeres de novo, hence preventing telomere shortening due to the end-replication problem in those cells where it is expressed at sufficiently high levels [2]. Telomerase consists of two essential components, a reverse transcrip-

tase known as telomerase reverse transcriptase (Tert) and an RNA molecule or telomerase RNA component (Terc), which contains the template for the synthesis of new telomeric repeats [14, 52]. Telomerase activity regulation is relevant both for cancer and aging. On one hand, telomerase activity is up-regulated in the vast majority of human tumors compared to normal somatic tissues [58], and its inhibition in human tumor cell lines leads to telomere shortening and loss of cell viability [73], suggesting that telomerase inhibition could be an effective way to abolish tumor growth by provoking telomere shortening to a critical length. In this regard, mice deficient for telomerase activity and that show critically short telomeres are resistant to carcinogenesis in a p53 wild-type genetic background [1, 20, 23, 55].

On the other hand, loss of telomeric sequences with increasing age has been proposed to be a biological determinant in the process of aging. As an example, telomere length in human fibroblasts and peripheral blood mononuclear cells declines proportionally to donor age at a rate of 50–200 bp/year [19, 28, 66]. This telomere loss is thought to occur due to incomplete DNA replication (end-replication problem) [2, 53]. Importantly, the introduction of a constitutively expressed telomerase catalytic subunit into cells with a limited life-span is sufficient to stabilize their telomeres and to extend their life-span, apparently without inducing changes associated with neoplasic transformation [10, 37, 48]. However, the recent construction of mice that overexpress constitutive levels of telomerase activity in the skin, has suggested that high levels of telomerase in adult tissues can favor tumorigenesis [21].

Interestingly, telomerase-independent telomere elongation or alternative lengthening of telomeres (ALT) has been described in immortal human cell lines that do not have detectable telomerase activity [12, 13], as well as in cell lines derived from telomerase-deficient mice [27]. ALT seems to involve homologous recombination between different telomeres [17].

Finally, the exact mechanisms that regulate the access of telomerase or ALT activities to the telomere are still unknown. However, it has been proposed that T-loops, as well as different telomere binding proteins regulate this process (see above) (Fig. 1).

Telomerase and the immune system

Mammals are protected from antigenic and pathogenic stresses by an effective immune system response. The antibody-mediated immune response is achieved by a process that occurs primarily in the germinal centers (GC) where naive B lymphocytes undergo somatic hypermutation and clonal expansion followed by the selection of cells expressing receptors with the highest affinity for the antigen [40, 64]. The generation of long-lived immune memory assures a more effective response and a faster neutralization of the pathogen after a secondary challenge. Telomerase is activated when B cells enter the GC, and is subsequently down-regulated when B cells differentiate to memory B cells [32, 36, 44, 51, 67]. In accordance with this, telomeres are longer in GC B cells than in naive B cells or in memory B cells [67]. During aging, however, there is a marked decline in the reactivity of the immune system, which has been attributed in part to impairment of lymphocyte function coincident with a decrease in the GC reaction [71]. The decline in immune system reactivity could be partly due to the exhaustion of proliferative potential of naive B cells as a consequence of telomere shortening with increasing age [18, 33]. In particular, telo-

mere length has been shown to decrease in peripheral blood mononuclear cells at a rate of 50–200 bp/year of age [19, 66]. It has been proposed, in the past, that this telomere loss is a consequence of the down-regulation of telomerase activity with age [32]. However, more recent analysis showed that age does not significantly alter the capacity of telomerase induction in human lymphocytes [63]. Either way, telomere shortening with age is thought to be responsible for the limited replicative capacity of antigenic-specific T lymphocytes. In support of this hypothesis, human T clones that have been transduced with hTert, and that therefore express high telomerase activity, retain their proliferative potential for more population doublings than the controls transduced with an empty vector [56]. These observations suggest that ectopic expression of the catalytic subunit of telomerase, Tert, is capable of extending the replicative life-span of primary human T lymphocytes. These findings have important implications for gene therapy of diseases associated with immunosenescence.

The telomerase-deficient mouse model

Mice genetically deficient for telomerase activity provide a unique opportunity to understand the role of telomere maintenance in organism viability. Several telomerase-deficient mice have been generated in which expression of either Terc [9, 49], or Tert [45, 50], has been eliminated. Most of the studies described, however, have been carried out on the model first described [9], which was obtained by the elimination of the gene encoding for Terc [8]. $Terc^{-/-}$ mice are viable for only four to six generations, depending on the specific genetic background; however, as telomeres shorten and chromosome fusions accumulate with increasing generations, $Terc^{-/-}$ mice become infertile and no further generations can be derived [9, 30, 42, 54]. In both genetic backgrounds the phenotypes associated to telomere dysfunction include (1) partial embryonic mortality due to a defect in the closure of the neural tube and to increased neuronal apoptosis [29]; (2) small size and severe intestinal atrophy [30, 54]; (3) spleen atrophy and reduced proliferation of B and T lymphocytes upon mitogenic stimulation [30, 42]; (4) impaired GC function upon mice immunization [31]; and (5) a reduced incidence of malignancies except when in a p53-deficient genetic background [1, 20, 23, 55]. Overall, these results support an essential role of telomerase in highly proliferative tissues [42]. Here, we focus on the impact of telomere dysfunction on immunosenescence in the $Terc^{-/-}$ mouse model.

Immune system phenotypes in the telomerase-deficient mouse model

Reduced spleen size

Analysis of late generation $Terc^{-/-}$ mice with the C57BL/6 genetic background revealed diminished spleen size in those animals that showed poor health [30]. Complete flow cytometric analysis of the splenocytes derived from the affected spleens showed lower numbers of cells positive for the B cell marker B220/CD45R, indicating a decrease in the total B cell number in the affected spleens [30].

Decreased follicle numbers in the spleen

The lymphoid area of the spleen, or white pulp, consists of three distinct parts: the marginal zone, the periarteriolar lymphoid sheath (PALS) and the follicles. The folli-

cles are made up of naive B cells and follicular dendritic cells. Spleen follicles are easily visualized by morphological criteria and by immunohistochemistry with naive B cell marker B220. Interestingly, the spleens from late generation Terc$^{-/-}$ mice show a 50% reduction in follicle numbers compared to age-matched wild-type spleens [31]. This reduction in the number of follicles in late generation Terc$^{-/-}$ spleens coincides with a decreased proliferative response of B and T cells upon mitogen stimulation (see below). Since naive B cells are generated in the bone marrow, the lower follicle number in the late generation Terc$^{-/-}$ spleens could also reflect the reduced bone marrow function in the late generation Terc$^{-/-}$ mice [42].

Reduced B and T cell proliferation upon mitogen stimulation

Splenocytes derived from late generation Terc$^{-/-}$ mice with either of the two genetic backgrounds studied show a decreased proliferative response to B (LPS, CD40 ligand), T (anti-CD3 concanavalin A) and B + T cell (ionomycin-PMA)-specific mitogens, indicating a lower proliferative capacity of both T and B cells when telomeres are critically short [30, 42]. Further studies indicated that the decreased proliferative capacity of B and T cells was not accompanied by increased apoptosis or by an abnormal cell cycle profile as determined by flow cytometry [31], suggesting that it was exclusively due to an exhaustion of the proliferative capacity of these cells.

Abnormal blood cell counts

Extensive hematological analysis of late generation Terc$^{-/-}$ mice in the C57BL/6 background showed abnormalities in total lymphocyte and neutrophil numbers compared to wild-type controls [30]. In particular, the late generation Terc$^{-/-}$ mice showed reduced numbers of lymphocytes, concurring with the previously described reduction in spleen B lymphocytes (see above). In those Terc$^{-/-}$ mice that showed reduced lymphocyte counts, there was also a dramatic increase in neutrophils [30]. These increased neutrophil numbers may be compensating for the poor immune response of late generation Terc$^{-/-}$ lymphocytes. Similar increased neutrophil numbers have been described in 27-month-old animals [59]. No significant differences were detected in total leukocyte numbers or in hematocrit between the late generation Terc$^{-/-}$ mice and the corresponding wild-type controls [30]. Interestingly, those mice with aberrant blood cell counts showed symptoms of poor health or died, suggesting a hematological defect as a possible factor contributing to some deaths [31].

Impaired GC formation

After antigenic stimulation, naive B cells undergo extensive proliferation in the follicles forming the GC and generating memory B cells [40]. GC can be identified by immunostaining with peanut agglutinin. As mentioned above, telomerase activity is present in both naive and memory B cells, but is further up-regulated in the GC [32, 36, 51, 67]. Concurring with this, several studies have shown that both components of human telomerase, Terc and Tert, are present in the GC B cells in both humans and mice [31, 44, 70].

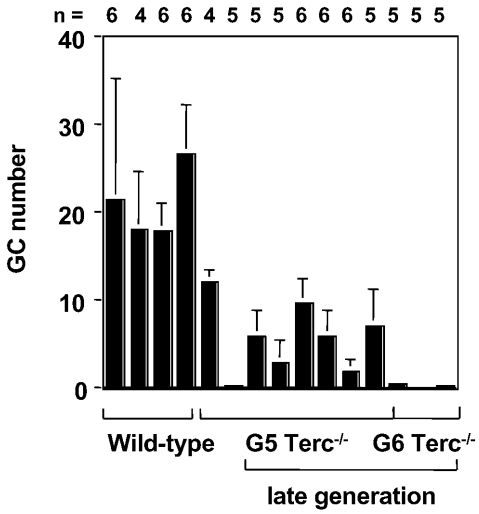

Fig. 2. The average number +SD of GC visualized per spleen section from wild-type, G5 and G6 Terc$^{-/-}$ mice is shown. The total number of spleen sections used for the analysis is indicated at the *top* of the graph (*GC* germinal centers)

In agreement with the idea that telomere maintenance by telomerase plays a role during GC formation, late generation Terc$^{-/-}$ spleens show a dramatic reduction in GC number compared to their wild-type counterparts after immunization with an antigen [31] (Fig. 2). This dramatic decrease in the number of GC in the late generation Terc$^{-/-}$ mice is accompanied by diminished antigen-specific IgM and IgG antibodies in the sera [31]. In contrast, first generation Terc$^{-/-}$ mice, which lack telomerase activity but still have sufficiently long telomeres, show normal GC numbers upon immunization [31], indicating that the reduction in GC formation in late generation Terc$^{-/-}$ mice is caused by telomere exhaustion and not by telomerase deficiency per se. The GC formation defect in late generation Terc$^{-/-}$ mice coincides with diminished proliferation of the splenocytes derived from these mice upon mitogen stimulation [31].

Telomerase is responsible for telomere elongation during the GC immune reaction

Previous studies showed that telomeric terminal restriction fragments (TRF) are longer in GC B cells than in naive or memory B cells [67], indicating that telomeres are elongated during this process. Indeed, it has been suggested that telomerase elongates telomeres during GC formation, allowing the clonal expansion that occurs in the GC to form the memory B cell pool. The telomerase-deficient mouse model constitutes an excellent experimental system to directly test this hypothesis. In wild-type mice, it was observed that telomeres were elongated approximately 5 kb in the splenocytes derived from immunized mice as compared to those from the non-immunized controls [31]. Furthermore, it was possible to conclude that this telomere elongation was mediated by telomerase, since splenocytes from immunized first generation (G1) Terc$^{-/-}$ mice, which lack telomerase activity, showed an average telomere shortening of 7 kb compared to those from non-immunized G1 Terc$^{-/-}$ controls (Fig. 3). These results demonstrated that the telomere elongation detected in wild-type spleens following in vivo immunization is mediated by telomerase activity.

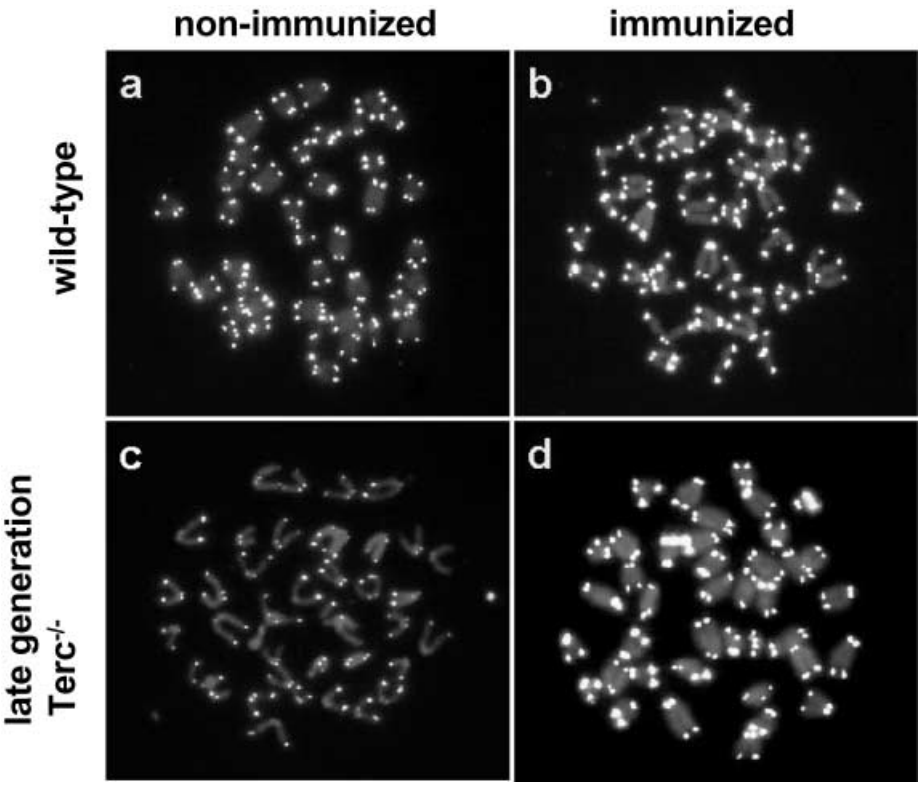

Fig. 3a, b. Quantitative fluorescence in situ hybridization on splenocyte metaphases. **a, b** Metaphases from wild-type splenocytes. **a** A metaphase corresponding to splenocytes from an non-immunized mouse; **b** a metaphase of splenocytes from a wild-type immunized mouse. Notice the higher telomeric fluorescence in splenocytes derived from immunized mice. **c, d** Metaphases from fifth generation $Terc^{-/-}$ splenocytes. **c** A metaphase corresponding to splenocytes from a non-immunized mouse; **b** a metaphase of splenocytes from an immunized mouse. Notice that splenocytes with extremely long telomeres are selected in late generation immunized $Terc^{-/-}$ mice compared to the non-immunized controls, suggesting the activation of alternative lengthening of telomeres in these cells

Alternative lengthening of telomeres in late generation $Terc^{-/-}$ splenocytes

When telomere length was measured in splenocytes derived from non-immunized late generation $Terc^{-/-}$ mice, they were considerably shorter than those of their wild-type counterparts, concurring with the fact that telomeres shorten with increasing generations in $Terc^{-/-}$ mice [9, 30]. Strikingly, splenocytes from immunized late generation $Terc^{-/-}$ mice, showed telomeres which were on average 12 kb longer than those of splenocytes from non-immunized late generation $Terc^{-/-}$ controls. These results suggest that during the splenocyte proliferation triggered by immunization there is a selection of clones which show long telomeres. Since these mice are telomerase deficient, these long telomeres may derive: (1) from a surviving subpopulation of late generation $Terc^{-/-}$ cells that have preserved long telomeres after five generations in mice without telomerase (which appears unlikely), or, alternatively, (2) from activation of still-to-be-defined telomerase-independent telomere rescue mechanisms

in late generation Terc$^{-/-}$ splenocytes. Either way, telomeres appear to be critical in sustaining cell proliferation during the GC reaction. The existence of telomere-rescue mechanisms in late generation Terc$^{-/-}$ mice is supported by previous reports showing that late generation Terc$^{-/-}$ cells in culture can maintain telomeres without telomerase [27]. It is important to note, however, that in contrast to cultured cells, these putative telomere elongation activities in late generation splenocytes are not sufficient to rescue the immune system phenotypes in these mice.

Conclusions

The analysis of the telomerase-deficient mouse model points to immunological defects as possible causes of the poor health and death of these mice. Immunosenescence is regarded as one of the landmarks of human aging. It has been widely reported that the immune system function declines with increasing age; in particular, as humans age, there is a decrease in T cell numbers, as well as impaired responsiveness to mitogens [68]. Furthermore, late generation Terc$^{-/-}$ mice show a dramatic reduction in the number of GC formed following in vivo antigen immunization. This reduced GC reactivity of late generation Terc$^{-/-}$ mice may contribute to immunosenescence in these mice. The study of the telomerase knockout mouse also supports the idea that telomere maintenance by telomerase is responsible for the extensive proliferation undergone by B lymphocytes in the GC during the immune response.

Acknowledgements. Research at the laboratory of M.A.B. is funded by Swiss Bridge Award 2000, by grants PM97-0133 from the Ministry of Science and Technology (MCT), Spain, and by grants FIGH-CT1999-00009, FIGH-CT-1999-00002 and QLG1-1999-01341, from the EU, and by the Department of Immunology and Oncology (DIO). The DIO was founded and is supported by the Spanish Research Council (CSIC) and by the Pharmacia Corporation.

References

1. Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L, DePinho RA (2000) Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. Nature 406:641
2. Autexier C, Greider CW (1996) Telomerase and cancer: revisiting the telomere hypothesis. Trends Biochem 21:387
3. Bailey SM, Meyne J, Chen DJ, Kurimasa A, Li GC, Lehnert BE, Goodwin EH (1999) DNA double-strand break repair proteins are required to cap the ends of mammalian chromosomes. Proc Natl Acad Sci U S A 96:14899
4. Baumann P, Cech TR (2001) Pot1, the putative telomere end-binding protein in fission yeast and humans. Science 292:1171
5. Bianchi A, Lange T de (1999) Ku binds telomeric DNA in vitro. J Biol Chem 274:21223
6. Bilaud T, Brun C, Ancelin K, Koering CE, Laroche T, Gilson E (1997) Telomeric localization of TRF2, a novel human telobox protein. Nat Genet 17:236
7. Blackburn EH (2000) Telomere states and cell fates. Nature 408:53
8. Blasco MA, Funk WD, Villeponteau B, Greider CW (1995) Functional characterization and developmental regulation of mouse telomerase RNA. Science 269:1267
9. Blasco MA, Lee H-W, Hande P, Samper E, Lansdorp P, DePinho R, Greider CW (1997) Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 91:25
10. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu C-P, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE (1998) Extension of life-span by introduction of telomerase into normal human cells. Science 279:349
11. Broccoli D, Smogorzewska A, Chong L, Lange T de (1997) Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2. Nat Genet 17:231

12. Bryan TM, Englezou A, Gupta J, Bacchetti S, Reddel RR (1995) Telomere elongation in immortal human cells without detectable telomerase activity. EMBO J 14:4240
13. Bryan TM, Marusic L, Bacchetti S, Namba M, Reddel RR (1997) The telomere lengthening mechanism in telomerase-negative immortal human cells does not involve the telomerase RNA subunit. Hum Mol Genet 6:921
14. Collins K (2000) Mammalian telomeres and telomerase. Curr Opin Cell Biol 12:378
15. Chong L, Steensel B van, Broccoli D, Erdjument-Bromage H, Hanish J, Tempst P, Lange T de (1995) A human telomeric protein. Science 270:1663
16. De Lange T (2001) Telomere capping: one strand fits all. Science 292:1171
17. Dunham MA, Neumann AA, Fasching CL, Reddel RR (2000) Telomere maintenance by recombination in human cells. Nat Genet 26:447
18. Effros RB, Pawelec G (1997) Replicative senescence of T cells: does Hayflick limit lead to immune exhaustion?. Immunol Today 18:450
19. Frenck RW Jr, Blackburn EH, Shannon KM (1988) The rate of telomere sequence loss in human leukocytes varies with age. Proc Natl Acad Sci USA 95:5607
20. González-Suárez E, Samper E, Flores JM, Blasco MA (2000) Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis. Nat Genet 26:114
21. González-Suárez E, Samper E, Ramirez A, Flores JM, Martin-Caballero J, Jorcano JL, Blasco MA (2001) Increased epidermal tumors and increased skin wound healing in transgenic mice overexpressing the catalytic subunit of telomerase, mTERT, in basal keratinocytes. EMBO J 20:2619
22. Goytisolo FA, Samper E, Edmonson S, Taccioli GE, Blasco MA (2001) The absence of the DNA-dependent protein kinase catalytic subunit in mice results in anaphase bridges and in increased telomeric fusions with normal telomere length and G-strand overhang. Mol Cell Biol 21:3642
23. Greenberg RA, Chin L, Femino A, Lee KH, Gottlieb GJ, Singer RH, Greider CW, DePinho RA (1999) Short dysfunctional telomeres impair tumorigenesis in the INK4a(*2/3) cancer-prone mouse. Cell 97:515
24. Greider CW (1999) Telomeres do D-loop-T-loop. Cell 97:419
25. Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, Lange T de (1999) Mammalian telomeres end in a large duplex loop. Cell 97:503
26. Guarente L (1997) Aging. What makes us tick? Science 275:943
27. Hande P, Samper E, Lansdorp P, Blasco MA (1999) Telomere length dynamics and chromosomal instability in cells derived from telomerase null mice. J.Cell Biol 144:589
28. Harley CB, Futcher AB, Greider CW (1990) Telomeres shorten during aging of human fibroblasts. Nature 345:458
29. Herrera E, Samper E, Blasco MA (1999) Telomere shortening in mTR$^{-/-}$ embryos is associated with failure to close the neural tube. EMBO J 18:1172
30. Herrera E, Samper E, Martín-Caballero J, Flores JM, Lee H-W, Blasco MA (1999) Disease states associated to telomerase deficiency appear earlier in mice with short telomeres. EMBO J 18:2950
31. Herrera E, Martinez AC, Blasco MA (2000) Impaired germinal center reaction in mice with short telomeres. EMBO J 19:472
32. Hiyama K, Hirai Y, Kyoizumi S, Akiyama M, Hiyama E, Piatyszek MA, Shay JW, Ishioka S, Yamakido M (1995) Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. J Immunol 155:3711
33. Hodes RJ (1999) Telomere length, aging, and somatic cell turnover. J Exp Med 190:153
34. Hsu HL, Gilley D, Blackburn EH, Chen DJ (1999) Ku is associated with the telomere in mammals. Proc Natl Acad Sci USA 96:12454
35. Hsu HL, Gilley D, Galande SA, Hande MP, Allen B, Kim SH, Li GC, Campisi J, Kohwi-Shigematsu T, Chen DJ (2000) Ku acts in a unique way at the mammalian telomere to prevent end joining. Genes Dev 14:2807
36. Hu BT, Lee SC, Marin E, Ryan DH, Insel RA (1997) Telomerase is up-regulated in human germinal center B cells in vivo and can be re-expressed in memory B cells activated in vitro. J Immunol 159:1068
37. Jiang X-R, Jimenez G, Chang E, Frolkis M, Kusler B, Sage M, Beeche M, Bodnar A, Wahl GM, Tlsty TD, Chiu C-P (1999) Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype. Nat Genet 21:111
38. Karlseder J, Broccoli D, Dai Y, Hardy S, Lange T de (1999) p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. Science 283:1321

39. Kaminker PG, Kim SH, Taylor RD, Zebarjadian Y, Funk WD, Morin GB, Yaswen P, Campisi J (2001) TANK2, a new TRF1-associated PARP, causes rapid induction of cell death upon overexpression. J Biol Chem: 35891–35899
40. Kelsoe G (1996) Life and death in germinal centers. Immunity 4:107
41. Kim SH, Kaminker P, Campisi J (1999) TIN2, a new regulator of telomere length in human cells. Nat Genet 23:405
42. Lee H-W, Blasco MA, Gottlieb GJ, Horner JW, Greider CW, DePinho RA (1998) Essential role of mouse telomerase in highly proliferative organs. Nature 392:569
43. Li B, Oestreich S, Lange T de (2000) Identification of human Rap1: implications for telomere evolution. Cell 101:471
44. Liu K, Schoonmaker MM, Levine BL, June CH, Hodes RJ, Weng NP (1999) Constitutive and regulated expression of telomerase reverse transcriptase (hTERT) in human lymphocytes. Proc Natl Acad Sci USA 96:5147
45. Liu Y, Snow BE, Hande MP, Yeung D, Erdmann NJ, Wakeham A, Itie A, Siderovski DP, Lansdorp PM, Robinson MO, Harrington L (2000) The telomerase reverse transcriptase is limiting and necessary for telomerase function in vivo. Curr Biol 10:1459
46. Martín-Rivera L, Herrera E, Albar JP, Blasco MA (1998) Expression of mouse telomerase catalytic subunit in embryos and adult tissues. Proc Natl Acad Sci USA 95:10471
47. McElligott R, Wellinger RJ (1997) The terminal DNA structure of mammalian chromosomes. EMBO J 16:3705
48. Morales CP, Holt SE, Ouellette M, Kaur KJ, Yan Y, Wilson KS, White MA, Wright WE, Shay JW (1999) Absence of cancer-associated changes in human fibroblasts immortalized with telomerase. Nat Genet 21:115
49. Niida H, Matsumoto T, Satoh H, Shiwa M, Tokutake Y, Furuici Y, Shinkai Y (1998) Severe growth defect in mouse cells lacking the telomerase RNA component. Nat Genet 19:203
50. Nikaido R, Haruyama T, Watanabe Y, Iwata H, Iida M, Sugimura H, Yamada N, Ishikawa F (1999) Presence of telomeric G-strand tails in the telomerase catalytic subunit TERT knockout mice. Genes Cells 4:563
51. Norrback KF, Dahlenborg K, Carlsson R, Roos G (1996) Telomerase activation in normal B lymphocytes and non-Hodgkin's lymphomas. Blood 88:222
52. Nugent CI, Lundblad V (1998) The telomerase reverse transcriptase: components and regulation. Genes Dev 12:1073
53. Ohki R, Tsurimoto T, Ishikawa F (2001) In vitro reconstitution of the end replication problem. Mol Cell Biol 21:5753
54. Rudolph KL, Chang S, Lee H-W, Blasco M, Gottlieb G, Greider WC, DePinho RA (1999) Longevity, stress response, and cancer in aging telomerase deficient mice. Cell 96:701
55. Rudolph KL, Millard M, Bosenberg MW, DePinho RA (2001) Telomere dysfunction and evolution of intestinal carcinoma in mice and humans. Nat Genet 28:155
56. Rufer N, Migliaccio M, Antonchuk J, Humphries RK, Roosnek E, Lansdorp PM (2001) Transfer of the human telomerase reverse transcriptase (TERT) gene into T lymphocytes results in extension of replicative potential. Blood 98:597
57. Samper E, Goytisolo FA, Slijepcevic P, van Buul PP, Blasco MA (2000) Mammalian Ku86 protein prevents telomeric fusions independently of the length of TTAGGG repeats and the G-strand overhang. EMBO Rep 1:244
58. Shay JW, Bacchetti S (1997) A survey of telomerase activity in human cancer. Eur J Cancer 33:787
59. Smith CA, Andrews CM, Collard JK, Hall DE, Walker AK, Wolfe M (1994) Color atlas of comparative Diagnostic and experimental hematology. Wolfe Publishing, London, UK, p 13–14
60. Smith S, Giriat I, Schmitt A, Lange T de (1998) Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. Science 282:1484
61. Smith GC, Jackson SP (1999) The DNA-dependent protein kinase. Genes Dev 13:916
62. Smogorzewska A, Steensel B van, Bianchi A, Oelmann S, Schaefer MR, Schnapp G, Lange T de (2000) Control of human telomere length by TRF1 and TRF2. Mol Cell Biol 20:1659
63. Son NH, Murray S, Yanovski J, Hodes RJ, Weng N (2000) Lineage-specific telomere shortening and unaltered capacity for telomerase expression in human T and B lymphocytes with age. J Immunol 165:1191
64. Sprent J (1997) Immunological memory. Curr Opin Immunol 9:371
65. Steensel B van, Smogorzewska A, Lange T de (1998) TRF2 protects human telomeres from end-to-end fusions. Cell 92:401

66. Vaziri H, Schachter F, Uchida I (1993) Loss of telomeric DNA during aging of normal and trisomy 21 human lymphocytes. Am J Hum Genet 52:661
67. Weng N-P, Granger L, Hodes RJ (1997) Telomere lengthening and telomerase activation during human B cell differentiation. Proc Natl Acad Sci USA 94:10827
68. Wick G, Grubeck-Loebenstein B (1997) Primary and secondary alterations of immune reactivity in the elderly: impact of dietary factors and disease. Immunol Rev 160:171
69. Wright WE, Tesmer VM, Huffman KE, Levene SD, Shay JW (1997) Normal human chromosomes have long G-rich telomeric overhangs at one end. Genes Dev 11:2801
70. Yashima K, Maitra A, Timmons CF, Rogers BB, Pinar H, Milchgrub S, Wright WE, Shay JW, Gazdar AF (1998) Expression of the RNA component of human telomerase during development and differentiation. Cell Growth Differ 9:805
71. Zheng B, Han S, Takahashi Y, Kelsoe G (1997) Immunosenescence and germinal center reaction. Immunol Rev 160:63
72. Zhu XD, Kuster B, Mann M, Petrini JH, Lange T (2000) Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 and human telomeres. Nat Genet 25:347
73. Zumstein LA, Lundblad V (1999) Telomeres: has cancer's Achilles' heel been exposed? Nat Med. 5: 1129