The EMBO Journal (2005) 24, 1095–1103 | © 2005 European Molecular Biology Organization | All Rights Reserved 0261-4189/05
www.embojournal.org

THE EMBO JOURNAL

EMBO Gold Medal Review 2004

Mice with bad ends: mouse models for the study of telomeres and telomerase in cancer and aging

María A Blasco*

Telomeres and Telomerase Group, Molecular Oncology Program, Spanish National Cancer Centre (CNIO), Madrid, Spain

Telomeres are capping structures at the ends of eukaryotic chromosomes, which consist of repetitive DNA bound to an array of specialized proteins. Telomeres are part of the constitutive heterochromatin and are subjected to epigenetic modifications. The function of telomeres is to prevent chromosome ends from being detected as damaged DNA. Both the length of telomere repeats and the integrity of the telomere-binding proteins are important for telomere protection. Telomere length is regulated by telomerase, by the telomere-binding proteins, as well as by activities that modify the state of the chromatin. Various mouse models with altered levels of telomerase activity, or mutant for different telomere-binding proteins, have been recently generated. Here, I will discuss how these different mouse models have contributed to our understanding on the role of telomeres and telomerase in cancer and aging.

The EMBO Journal (2005) **24,** 1095–1103. doi:10.1038/sj.emboj.7600598; Published online 10 March 2005
Subject Categories: chromatin & transcription; molecular biology of disease
Keywords: aging; cancer; mouse; telomerase; telomeres

The making of a telomerase scientist

The discovery of telomerase has fascinated me since my early days as a PhD student with Margarita Salas at the ‘Severo Ochoa’ Molecular Biology Center in Madrid. It was exactly 20 years ago that telomerase activity was first discovered in the ciliate *Tetrahymena* by Greider and Blackburn (1985). They first named this activity ‘telomere terminal transferase’ for its capacity to elongate telomeric primers in the absence of a DNA template. Soon after they discovered that telomerase was a ribonucleoprotein that used an essential RNA component as a template, and therefore had reverse transcriptase activity (Greider and Blackburn, 1987, 1989). The discovery of telomerase was not simply down to mere chance—its existence was predicted on the basis of DNA duplication, and its importance for cancer and aging soon became clear.

*Corresponding author. Telomeres and Telomerase Group, Molecular Oncology Program, Spanish National Cancer Centre (CNIO), 28029 Madrid, Spain. Tel.: +34 91 732 8031; Fax: +34 91 732 8028; E-mail: mblasco@cnio.es

Received: 14 December 2004; accepted: 4 February 2005; published online: 10 March 2005

Polymerases that replicate ends were not, however, entirely unfamiliar to me. During my PhD studies, I had already studied a DNA polymerase implicated in maintaining the ends of the linear genome of ø29 bacteriophage, through its ability to use a terminal protein as primer. As Margarita probably still recalls from my very first interview, I expressed an early interest in cancer and aging. How could I ever have predicted at that time though that chromosome ends and human diseases were indeed related?

Completion of my PhD studies thankfully coincided with Carol Greider setting up her own research group at the Cold Spring Harbor Laboratory in Long Island, NY (CSHL). The CSHL, as it turned out, had two special connections with telomere biology. Not only was it the home to the majority of McClintock’s (1941) research work, during which time she had described the existence of a special structure at the ends of chromosomes that prevented them from being ‘sticky’, but its then Director, Watson (1972), had predicted that material from the ends of chromosomes was lost every time that a cell divides due to the so-called end-replication problem. Consequently, when Carol accepted my application to work in her group, I was truly confident that I was making the right career choice. I can still recall that my project was ‘to identify the mouse telomerase RNA and to generate a knockout mouse’. Upon reflection, I am still astonished that we actually managed to achieve both objectives in less than 3 years. This of course was only possible thanks to the essential collaboration of many other scientists, especially those working at Carol’s lab, at the Geron Corporation, as well as thanks to Han-Wong Lee who was involved in generating the mice. Without a doubt, the most exhilarating point of my scientific career to date is the discovery that the knockout mouse for the telomerase RNA did not show any detectable telomerase activity. Just as Titia de Lange had pointed out so poignantly, telomerase ‘was not essential for life, nor for sex’, since the mice were viable and fertile as long as their telomeres were long enough. These mice have been the basis of a major part of my scientific contribution and, I hope, of interpreting the role of telomeres and telomerase in cancer and aging.

You can imagine my euphoria when last summer, Frank Gannon, Director of EMBO, called me up to announce that I had been awarded the 2004 EMBO Gold Medal. This award represents a two-fold triumph: not only was my work being consequently acknowledged by a large community of European scientists, but also telomerase was being recognized by EMBO as an interesting and important research subject. Back in 1993, however, when I was applying for fellowships to work with Carol, three main European agencies rejected my applications on the basis that the research subject was ‘still very new and uncertain’, and ‘the mammalian genes were not even cloned’. It is to my great satisfaction

to think that I, along with many other researchers, may have contributed to this complete turn-around in perception.

Telomeric chromatin

Vertebrate telomeres are composed of tandem repeats of the TTAGGG sequence, as well as of a number of associated proteins (Blackburn, 2001; Chan and Blackburn, 2002; de Lange, 2002). Telomeres are also characterized by having a 150–200 nucleotide-long 3′-overhang of the G-rich strand, the so-called G-strand overhang (de Lange, 2002). The length of the double-stranded TTAGGG track varies from ~10 kb at human telomeres to >40 kb in mouse inbreed strains (Zijlmans *et al*, 1997). The current model is that telomeres can form a structure that physically hides the 3′-overhang from cellular activities that may be hazardous for its integrity, such as DNA repair activities and nucleases. The most accepted telomere structure model is based on electron microscopy studies, which suggest that the 3′-overhang can fold back and invade the double-stranded region of the telomere forming the so-called T-loop and generating a displacement loop, or D-loop (Griffith *et al*, 1999; Nikitina and Woodcock, 2004). T-loops have been recently proposed to represent a primordial mechanism for chromosome end protection (de Lange, 2004).

Proteins that bind to the double-stranded TTAGGG region, such as TRF1 and TRF2, or that bind to the single-stranded G-strand overhang, such as Pot 1, have been shown to influence both telomere capping and telomere length (Chong *et al*, 1995; Bilaud *et al*, 1997; Broccoli *et al*, 1997; van Steensel *et al*, 1998; Baumann and Cech, 2001; Loayza and De Lange, 2003). TRF1 and TRF2 have been also visualized at telomeric T-loops (Griffith *et al*, 1999), and demonstrated to be negative regulators of telomere length (Smogorzewska *et al*, 2000). TRF1 function is regulated by TIN2 (Kim *et al*, 1999), and by the poly(ADP-ribose) polymerases TANK1 (also known as tankyrase) and TANK2 (Smith *et al*, 1998; Kaminker *et al*, 2001). In particular, TIN2 is a TANK1 modulator and controls telomere length via the TRF1 protein complex; furthermore, TIN2 can also bind to the TRF2 complex (Kim *et al*, 2004; Ye and de Lange, 2004a; Ye *et al*, 2004b). In addition to its role at telomeres, TANK1 has been recently demonstrated to be essential for separation of sister chromatid telomeres during mitosis, suggesting the existence of a new telomere-specific cohesion which is regulated by poly(ADP-ribosylation) (Dynek and Smith, 2004). Finally, TRF1 interacts with Pot 1, and this interaction has been proposed to convey information from the double-stranded telomere region to the single-stranded 3′-overhang (Loayza and De Lange, 2003). Recently, a new Pot-1 interacting protein (PTOP/PIP1) has been identified and shown to be important for telomere length regulation by the TRF1 complex (Liu *et al*, 2004; Ye *et al*, 2004c). All these findings suggest that TRF1 forms a multi-protein complex, which is involved in telomere length control and that contains at least TRF1, TIN2, the TANK1 and TANK2 tankyrases, Pot-1 and PTOP/PIP1, and may also contain TRF2 through its interaction with TIN2 (Figure 1). Interestingly, the role of TRF1 in the context of the organism seems to go beyond telomeres, since mice with targeted deletion of TRF1 are embryonic lethal in the absence of loss of telomere capping or telomere shortening (Karlseder *et al*, 2003).

TRF2 has been proposed to have a fundamental role in protecting the G-strand overhang from degradation, as well as

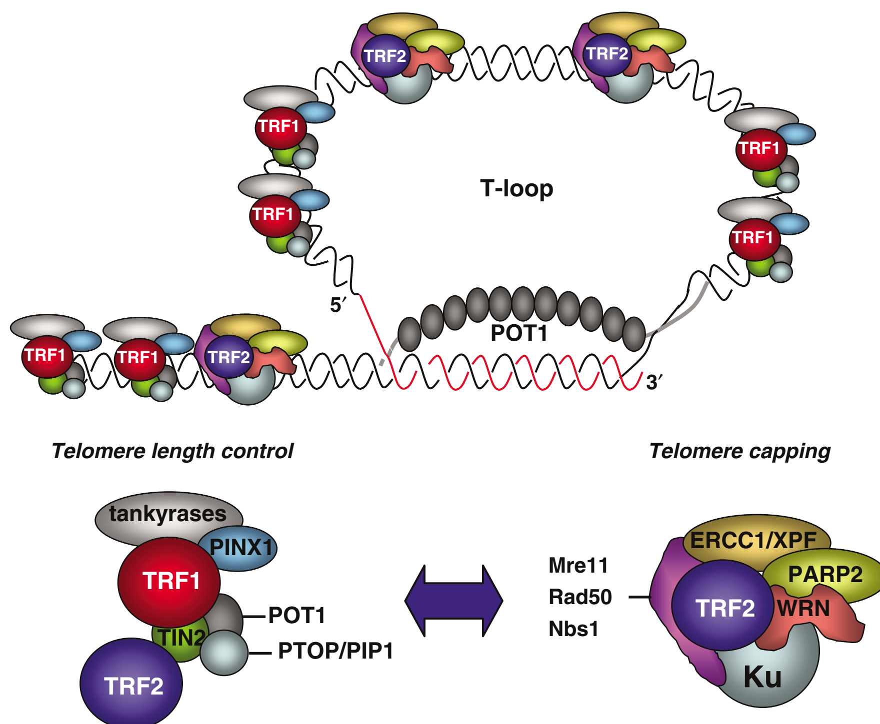

Figure 1 Telomere-binding proteins. Scheme showing the telomere in a T-loop conformation, as well as with different protein complexes found at mammalian telomeres. The TRF1 complex has been shown to influence telomere length, while the TRF2 complex has been shown to influence both telomere length and telomere capping.

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in preventing telomeric fusions (van Steensel *et al*, 1998). TRF2 also recruits a number of proteins to the telomeres, many of which are involved in different DNA repair processes (Figure 1). In particular, TRF2 recruits the MRE11 complex to telomeres (Zhu *et al*, 2000). The MRE11 complex is composed of RAD50, MRE11 and NBS1 and is a key component of the homologous recombination (HR) and non-homologous end-joining pathways (NHEJ) involved in DNA double-strand break (DSB) repair. TRF2 also interacts with other DNA repair proteins such as PARP-2 (Dantzer *et al*, 2004), Ku proteins (Song *et al*, 2000), Werner (Opresko *et al*, 2004), and the nucleotide excision repair complex XPF/ERCC1 (Zhu *et al*, 2003) among others. Interestingly, XPF/ERCC1 has been identified as the exonuclease that resects the 3′-overhang in the absence of functional TRF2 (Zhu *et al*, 2003). In addition, TRF2 has been recently shown to specifically bind to ATM and to block the ATM-dependent DNA damage response, suggesting that TRF2 could be specifically inhibiting ATM activation at telomeres (Karlseder *et al*, 2004). Finally, TRF2 recruits hRAP1 to human telomeres. hRAP1 is the homologue of yeast RAP1 protein and its overexpression causes telomere elongation (Li *et al*, 2000; Li and de Lange, 2003). No mouse models for TRF2 are available to date.

Besides their known role in DNA repair, the different repair proteins present at telomeres also have a fundamental role in telomere metabolism. In particular, the study of Ku86- and DNA-PKcs-deficient mice has indicated that these proteins are also required for telomere protection (reviewed in Smith and Jackson, 1999; Goytisolo and Blasco, 2002). In particular, abrogation of either Ku86 or DNA-PKcs results in telomeric fusions characterized by showing TTAGGG repeats at the fusion point (Bailey *et al*, 1999, 2001; Hsu *et al*, 2000; Samper *et al*, 2000; Gilley *et al*, 2001; Goytisolo *et al*, 2001; Espejel *et al*, 2002a, b). These end-to-end chromosome fusions are not the result of telomere shortening below a minimum length, but rather they are due to loss of telomere capping. In addition, these fusions have been shown to preferentially involve telomeres produced by leading strand synthesis, thus suggesting a role for these proteins in the post-replicative processing of the leading strand telomere, that is, to generate the 3′G-strand overhang (Bailey *et al*, 2001; Jaco *et al*, 2004).

Deficiency in either Ku86 or DNA-PKcs also influences telomere length, in accordance with a role for these proteins in generating or maintaining a proper telomere structure. In particular, both in plants and mice Ku86 acts as a negative regulator of telomerase (Espejel *et al*, 2002a; Riha *et al*, 2002). In contrast, human cells deficient for Ku86 show shorter telomeres and a dramatic loss of viability, suggesting important differences in the role of Ku86 at both human and mouse telomeres (Jaco *et al*, 2004; Myung *et al*, 2004). DNA-PKcs has been shown to cooperate with telomerase in telomere length maintenance, and mice doubly deficient for both activities show an accelerated rate of telomere loss (Espejel *et al*, 2002b). Also in agreement with this notion, single mutant DNA-PKcs mice show decreased telomere length with age, as well as with increasing mouse generations compared to the wild-type controls (Espejel *et al*, 2004). Besides their roles in telomere capping and telomere length regulation, Ku86 and DNA-PKcs have also been shown to be essential in signalling and processing critically short telomeres as damaged DNA (Espejel *et al*, 2002a, b).

In addition to NHEJ, HR also plays a role in telomere biology. In particular, proteins involved in HR-mediated DNA repair, such as Rad54 and Rad51D, are important for telomere capping and telomere length regulation, suggesting that HR has an important role at mammalian telomeres (Jaco *et al*, 2003; Tarsounas *et al*, 2004). Since T-loop structures resemble in part an intermediate of HR, it has been proposed that HR activities may have an important role in the regulation of T-loops at telomeres (de Lange, 2004; Wang *et al*, 2004).

Epigenetic regulation of telomeric chromatin

Human and mouse telomeres show nucleosome arrays, suggesting that they may be subjected to histone modifications (Tommerup *et al*, 1994). Histone modifications include acetylation, methylation and phosphorylation, which in turn generate a repertoire of chromatin structures that can regulate various cellular responses (Jenuwein and Allis, 2001; Lachner *et al*, 2001). In particular, constitutive heterochromatin is found at transcriptionally inactive ('silenced'), repetitive genomic regions, such those of pericentric chromatin, and it is characterized by hypermethylation of DNA, hypoacetylation of histones, and hypermethylation of histones H3 and H4. In particular, H3-K9 trimethylation by the Suv39h histone methyltransferases (HMTases) as well as H4-K20 trimethylation by the Suv4-20h HMTases are two main hallmarks of pericentric heterochromatin (Peters *et al*, 2001, 2003; Schotta *et al*, 2004). First, H3-K9 trimethylation creates a binding site for the heterochromatin protein 1 (HP1) family of proteins (Lachner *et al*, 2001), which mediate heterochromatin formation by recruiting the Suv4-20 HMTases (Schotta *et al*, 2004).

Telomeres have also been shown to be part of the constitutive heterochromatin in yeast and flies (Hecht *et al*, 1995; Savitsky *et al*, 2002; Cenci *et al*, 2003; Perrod and Gasser, 2003). Furthermore, yeast and flies defective for activities that modify the state of chromatin also have abnormal telomere function and telomere length regulation (Savitsky *et al*, 2002; Cenci *et al*, 2003; reviewed in Perrod and Gasser, 2003). In particular case of flies, HP1 mutations show defective telomere capping, as well as increased recombination at telomeres, suggesting that telomere function can be regulated by epigenetic modifications (Fanti *et al*, 1998; Cenci *et al*, 2003). In mice, it has been recently described that telomeres are enriched for trimethylated H3-K9 and for HP1, similar to pericentric chromatin (Garcia-Cao *et al*, 2004). Furthermore, it has been established that the activity of the Suv39h1 and Suv39h2 HMTases is required to maintain both H3-K9 trimethylation and HP1 binding at telomeres (Garcia-Cao *et al*, 2004). These findings suggest that telomeres have the hallmarks of constitutive heterochromatin, and predict that epigenetic errors at telomeres may also alter telomere function. In fact, mice doubly deficient for the Suv39h1 and Suv39h2 HMTases show abnormally elongated telomeres, suggesting that loss of heterochromatic features at telomeres results in a more 'open' chromatin state, which in turn could facilitate the access of telomerase or other telomere-elongating activities to the chromosome end (Garcia-Cao *et al*, 2004) (Figure 2 for model of telomere heterochromatin assembly). Alternatively, loss of heterochromatic features at telomeres may alter the expression of telomere-length regulator genes,

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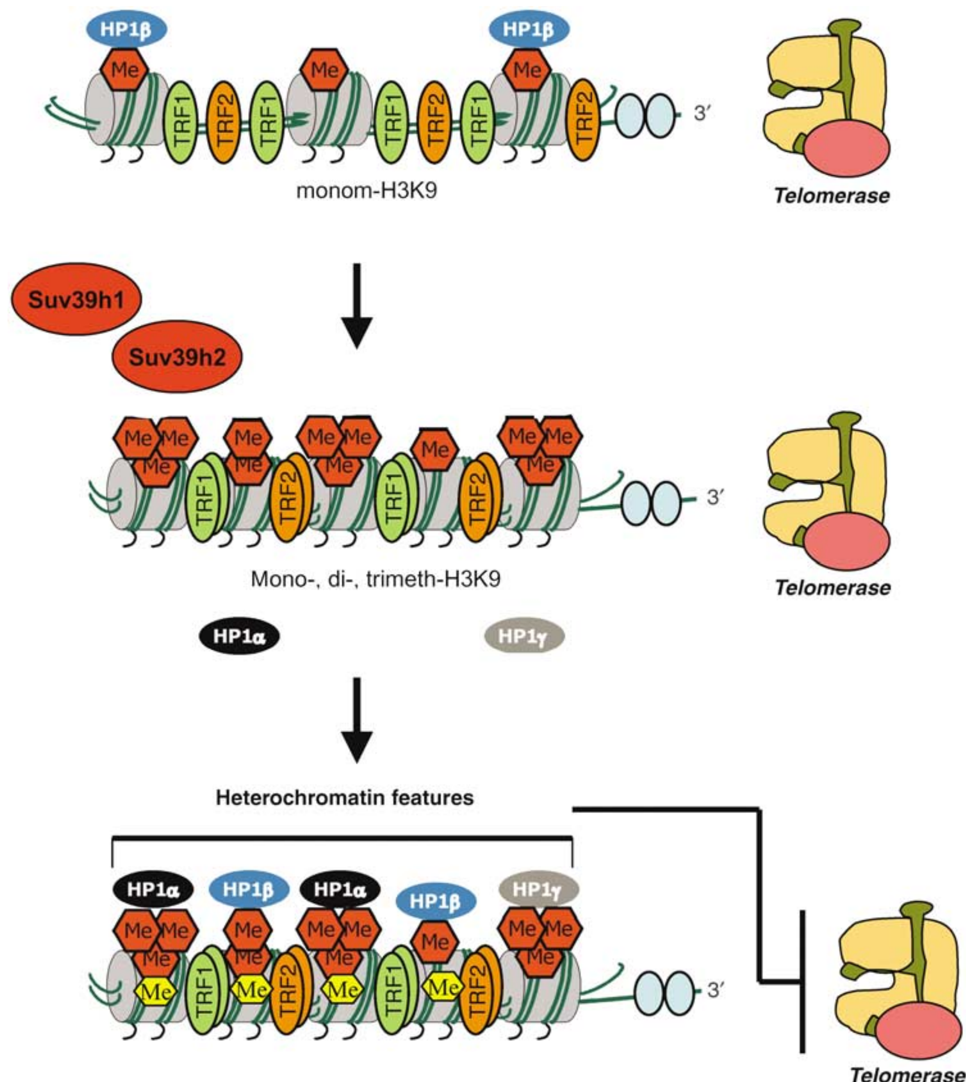

Figure 2 Assembly of telomeric heterochromatin. Mammalian telomeres contain features of the constitutive heterochromatin such as enrichment for H3-K9 di- and trimethylation, as well as binding of the HP1 family of proteins, similar to that previously described for pericentric hetochromatin. The Suv39h1 and Suv39h2 HMTases are required for the di-and trimethylation of H3-K9 at telomeres, which in turn recruits the HP1 proteins. Telomeric chromatin in SUV39DN cells also shows decreased binding of the HP1 proteins. These epigenetic modifications contribute to a 'closed' chromatin state, which may regulate the access of telomerase to the telomeres.

a phenomenon known as 'telomere position effect' (TPE), which in turn is related to the property of telomeric chromatin to repress or silence neighboring genes. This phenomenon has been extensively studied in budding yeast (reviewed in Perrod and Gasser, 2003), but is less well understood in mammals (Baur *et al*, 2001; Koering *et al*, 2002). Similarly, epigenetic modifications could also regulate the binding of specific proteins, such as TRF1 and TRF2, to telomeres. Indeed, a reproducible increase in TRF1 binding per amount of TTAGGG repeats was detected in Suv39h double null telomeres, reflecting on a change in telomere architecture (Garcia-Cao *et al*, 2004).

Finally, these results predict that, besides the Suv39h HMTases, other activities that modify the state of the chromatin may also regulate telomere function (Jenuwein, 2001). In addition, the fact that epigenetic errors can alter telomere length in mammals may explain abnormal re-setting of telomere length in cloned animals (Shiels *et al*, 1999; Lanza *et al*, 2000).

The telomerase enzyme

Telomerase, the cellular reverse transcriptase that adds TTAGGG repeats onto pre-existent telomeres, is the main regulator of telomere length in mammalian cells (Collins and Mitchell, 2002). Telomerase consists of two essential components, a reverse transcriptase subunit 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 (Collins, 2000). Most human normal somatic cells do not have sufficient telomerase and undergo telomere attrition coupled to cell division (Harley *et al*, 1990). Telomere loss in the absence of telomerase activity in telomerase-deficient mice eventually results TTAGGG-exhausted chromosome ends, end-to-end chromosome fusions and loss of cell viability (Blasco *et al*, 1997; Lee *et al*, 1998; Herrera *et al*, 1999a). Re-introduction of telomerase prevents critical shortening of telomeres and allows viability both in cultured cells and in

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the context of the telomerase-deficient mouse (Bodnar *et al*, 1998; Hemann *et al*, 2001b; Samper *et al*, 2001), demonstrating that short telomeres trigger rapid loss of cell viability unless they are rescued by telomerase. In this regard, telomerase may also prevent critical telomere shortening in more than 90% of all human tumors, which reactivate telomerase at some point during their formation (Hiyama and Hiyama, 2002). It has been proposed that telomerase inhibition could be an effective way to abolish tumor growth by provoking telomere shortening to a critical length (Blasco, 2002). In addition, there is evidence that telomerase might enhance survival and promote proliferation independently of telomere length, favoring tumor growth even at stages when telomeres are sufficiently long (Mattson *et al*, 2001; Blasco, 2002) (see below).

Alternative mechanisms of telomere length maintenance

Some human cell lines and tumors that lack telomerase activity, however, are still able to maintain or elongate their telomeres by alternative mechanisms to telomerase, which have been termed alternative lengthening of telomeres (ALT) (Henson *et al*, 2002). ALT-positive cells are characterized by the simultaneous presence of long and short telomeres in the same nucleus, as well as by the co-localization of telomere-binding proteins and PML in the so-called ALT-associated PML bodies (APB) (Bryan *et al*, 1997; Dunham *et al*, 2000). Very little is known, however, on the mechanisms underlying ALT in mammalian cells. In yeast, HR and mismatch repair (MMR) pathways have been involved in telomerase-independent telomere elongation (Lundblad, 2002), suggesting that HR is one of the mechanisms for ALT-mediated rescue of short telomeres.

In the case of mammalian cells, there is increasing evidence that a number of factors can influence telomere length in the absence of significant changes in telomerase activity. Some of these factors are proteins with known roles in HR, such as Rad54, which is central to the HR DNA repair pathway. In particular, mice deficient for Rad54 show a significant loss of telomeric sequences in the absence of changes in telomerase activity (Jaco *et al*, 2003). These mice also show a higher frequency of end-to-end chromosome fusions, indicating a role for Rad54 in telomere capping (Jaco *et al*, 2003). More recently, Rad51D, a Rad51 paralog required for normal levels of genetic recombination, has been also shown to be required for telomere length maintenance and telomere capping (Tarsounas *et al*, 2004). The role of Rad54 and Rad51D in telomere length maintenance may also suggest that HR could be at the basis of the telomerase-independent telomere maintenance mechanisms in mammals.

In addition, activities that modify the state of the telomeric heterochromatin (i.e., Suv39h HMTases) are also likely to influence both telomerase action at telomeres as well as the ALT pathway (Garcia-Cao *et al*, 2004). Similarly, a connection between cell cycle regulators and telomere length has been recently established. In particular, abrogation of p107 and p130, two Rb-family members, results in a massive elongation of telomeres in the absence of changes in telomerase activity, suggesting a connection between cell cycle regulation and telomere length control (García-Cao *et al*, 2002).

The telomerase-deficient mouse model

A telomerase-deficient mouse model has been generated by the elimination of the gene encoding for the murine *Terc* gene, $Terc^{-/-}$ mice (Blasco *et al*, 1995, 1997). These mice are viable, but only a limited number of generations can be derived before loss of viability is observed due to telomere loss and increased end-to-end fusions (Blasco *et al*, 1997; Lee *et al*, 1998). The phenotypes associated to telomere dysfunction in these mice include (i) male and female infertility (Lee *et al*, 1998; Herrera *et al*, 1999a; Hemann *et al*, 2001a); embryonic mortality due to a defective closure of the neural tube (Herrera *et al*, 1999b); (ii) small size and severe intestinal atrophy (Herrera *et al*, 1999a; Rudolph *et al*, 1999); (iii) spleen atrophy and reduced proliferation of B and T lymphocytes (Lee *et al*, 1998; Herrera *et al*, 1999a); (iv) impaired germinal centre function (Herrera *et al*, 2000); (v) reduced angiogenic potential (Franco *et al*, 2002); (vi) reduced proliferative potential of the bone marrow stem cells (Samper *et al*, 2002); (vii) heart dysfunction (Leri *et al*, 2003); (viii) reduced proliferative capacity of adult neural stem cells (Ferron *et al*, 2004). These findings indicate that a minimal telomere length is necessary to maintain tissue homeostasis in the mouse, and predict that telomere shortening with age in humans may also lead to similar disease states, thus contributing to the pathobiology of aging. In this regard, a number of human premature aging syndromes, such as Werner's syndrome and Ataxia telangiectasia, have been modelled in mice only when in combination with telomerase deficiency and short telomeres in the context of the telomerase-deficient mouse model (Wong *et al*, 2003; Chang *et al*, 2004), suggesting that short telomeres are an important component in the pathobiology of these premature-aging diseases as well as possibly in diseases that are aging-related.

Importantly, it has been demonstrated that telomerase can re-elongate critically short telomeres in the context of the late-generation telomerase-deficient mice and prevent their premature aging phenotypes (Samper *et al*, 2001). In particular, telomerase is able to recognize short telomeres and to extend them, preventing the occurrence of end-to-end fusions and the appearance of phenotypes in these mice (Hemann *et al*, 2001b; Samper *et al*, 2001). These findings open the possibility of using telomerase re-introduction as a putative gene therapy for human premature aging syndromes that are characterized by a faster rate of telomere loss such as Dyskeratosis congenita (Collins and Mitchell, 2002), as well as for age-associated pathologies.

Finally, the telomerase-deficient mouse model has provided strong evidence that short telomeres suppress tumor progression, in agreement with the fact that telomerase activity is upregulated in most human tumors (González-Suárez *et al*, 2000). This tumor suppressor phenotype coincides with p53 upregulation in $Terc^{-/-}$ mice (González-Suárez *et al*, 2000). In fact, p53 has been proposed to be sensing short telomeres and contributing to cessation of growth (González-Suárez *et al*, 2000; Leri *et al*, 2003). Telomerase deficiency in combination with deficiencies in tumor suppressor genes other than p53 significantly reduce carcinogenesis (Chin *et al*, 1999; Greenberg *et al*, 1999; Artandi *et al*, 2000; Rudolph *et al*, 2001; Wong *et al*, 2003), suggesting that a telomerase inhibitor may be

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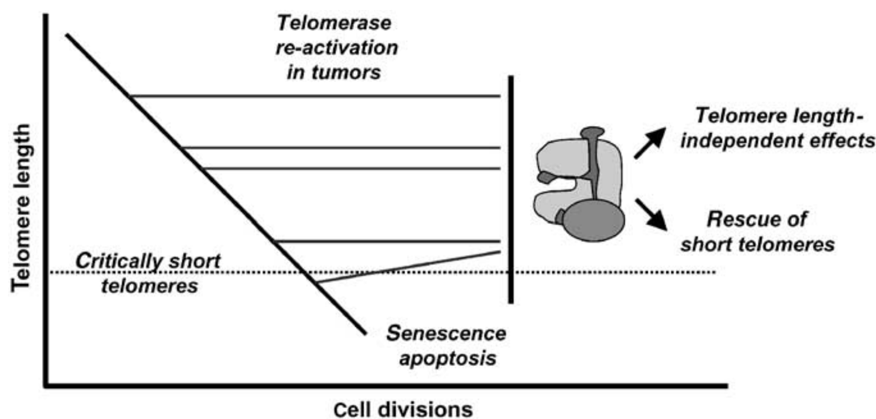

Figure 3 Role of telomerase in tumorigenesis. Telomerase is re-activated in more than 90% of all types of human tumors. Telomerase re-activation in tumors confers a proliferative advantage through two mechanisms: (i) rescue of critically short telomeres and prevention of cell death or cell arrest, (ii) telomere-length independent effects on survival and proliferation.

effective in cessation of tumor growth. Importantly, the antitumor effect of telomerase inhibitors may be enhanced in combination with genotoxic agents, as short telomeres also result in a higher sensitivity to these agents (Goytisolo *et al*, 2000; Wong *et al*, 2000). In particular, critically short telomeres and dysfunctional telomeres have been recently shown to interfere with the proper repair of DSB in the genome, thus increasing chromosomal instability and the sensitivity to genotoxic agents (Latre *et al*, 2003; Bailey *et al*, 2004).

A role for telomerase-promoting growth independent of telomere length

Telomerase activation during human tumor progression is though to be required to rescue critically short telomeres, thus allowing cell viability and tumor growth (Figure 3). Intriguingly, telomerase activity is also upregulated during mouse tumorigenesis, even though mice have much longer telomeres than humans (Blasco *et al*, 1996; Broccoli *et al*, 1996). This fact suggests that telomerase might have additional roles in promoting tumorigenesis, which are not solely mediated by telomere elongation. In support of this notion, first-generation (G1) telomerase-deficient mice, Terc$^{-/-}$ mice, which lack telomerase activity but still have long telomeres, were shown to have less skin tumors than wild-type mice following skin chemical carcinogenesis, indicating a negative impact of telomerase deficiency on tumor growth even in the presence of sufficiently long telomeres (González-Suárez *et al*, 2000).

Additional evidence for a role of telomerase in promoting tumorigenesis independently of telomere length comes from the study of mice that overexpress the catalytic component of mouse telomerase (Martín-Rivera *et al*, 1998) in basal keratinocytes, the so-called K5-Tert mice (González-Suárez *et al*, 2001). K5-Tert mice show high levels of telomerase activity and long telomeres in the skin (González-Suárez *et al*, 2001). K5-Tert mice were found to be more susceptible to developing tumors than wild-type mice upon chemical carcinogenesis of the skin (González-Suárez *et al*, 2001). In addition, when these mice were left to age, they showed a decreased viability during the first year of life compared to the corresponding wild-type controls due to a significant increase in spontaneous tumors (González-Suárez *et al*, 2001, 2002). Mice with transgenic telomerase expression under a β-actin constitutive promoter, or under a thymus-specific promoter (Lck-Tert mice), also showed an increased incidence of spontaneous tumors (Artandi *et al*, 2002; Canela *et al*, 2004). Interestingly, K5-Tert mice that do not die from tumors during the first year of age show an increased survival at older ages, as well as a maximum lifespan extension compared to the wild-type littermates, which is coincidental with increased tissue fitness of the germ line and the kidney (González-Suárez *et al*, 2005). These findings suggest antagonistic roles of Tert in cancer and aging (González-Suárez *et al*, 2005). These findings are in line with data obtained from cultured cells, which also suggest a role for telomerase in enhancing survival and proliferation in the presence of very long telomeres. In particular, the epidermal growth factor receptor (EGFR) is upregulated in cells overexpressing Tert and this upregulation is required to mediate the telomere-length-independent effects of Tert overexpression on cell proliferation (Smith *et al*, 2003).

All together, these findings suggest that telomerase activation at early stages of tumor growth may actively promote tumor growth and survival even if telomeres are still sufficiently long, and that telomerase activation could favor tumorigenesis by at least two different mechanisms: by signaling proliferation and promoting growth independently of telomere length, and by rescuing tumor cells with critically short telomeres (Figure 3).

Acknowledgements

Research in the laboratory of MAB laboratory is funded by the MCYT (SAF2001-1869, GEN2001-4856-C13-08), by the Regional Government of Madrid, CAM (08.1/0054/01), by the European Union (TELOSENS FIGH-CT-2002-00217, INTACT LSHC-CT-2003-506803, ZINCAGE FOOD-CT-2003-506850, RISC-RAD F16R-CT-2003-508842), and the Josef Steiner Cancer Award 2003. MA Blasco's laboratory is funded by the MCyT (SAF2001-1869, GEN2001-4856-C13-08), by the Regional Government of Madrid (08.1/0054/01), European Union (TELOSENS FIGH-CT-2002-00217, INTACT LSHC-CT-2003-506803, ZINCAGE FOOD-CT-2003-506850, RISC-RAD FI6R-CT-2003-508842), and the Josef Steiner Award 2003.

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María A Blasco

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