Mouse models for telomere and telomerase biology

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Abbreviations: SKY, spectral karyotyping; TERC, template RNA component, TERT, telomerase reverse transcriptase

Abstract

Telomeres serve a critical role in maintenance of genomic stability in all eukaryotes, from yeast to human. The maintenance of telomeres is achieved by the telomerase complex, which is largely composed of telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC). A variety of mouse models have provided valuable insights into the relationship between the telomerase complex and telomere dysfunction at the organismal level and helped understand their biological significance in human. Recently, in addition to its role in maintenance of the telomeres, novel functions of the telomerase complex have been emerging. In this review, studies of all gene-targeted or transgenic mouse models so far generated for telomerase and telomere biology are comprehensively described, and potential novel functions of telomerase are briefly discussed.

Keywords: mouse model; telomerase; telomere; transgenic mouse

Telomere and Telomerase

Telomeres are specialized structures that cap the ends of eukaryotic chromosomes and consist of tandem repeats of the TTAGGG sequence that are associated with an array of proteins (Blackburn, 1991). The telomere serves critical biological functions. The telomere is believed to be important in maintaining the genomic stability of the cells, as well

as in allowing complete replication of chromosomes (Greider, 1991). With each cell division, the telomere shortens progressively as a consequence of the end replication problem, which is caused by the inability of the conventional DNA polymerase to fully replicate the 3' end of the lagging strands of the linear DNA molecules. It has been hypothesized that this shortening of the telomere functions as molecular clock in monitoring the replicative history of cells (Wright and Shay, 2002). Maintenance of telomeres ultimately provides a foundation for a stable structure of T-loop and D-loop and makes the capping function possible (Griffith *et al.*, 1999). Lack of telomere maintenance results in improper telomere capping which, in turn, can lead to telomere dysfunction. For instance, the loss of the protective telomeric capping function leads to active degradation of the telomeric G-strand overhangs, and uncapped telomeres are known to be highly recombinogenic (Summarized in Figure 1).

Telomerase is a ribonucleoprotein and consists of two essential components: a template RNA component (TERC) containing the complementary sequence of TTAGGG and a catalytic component (TERT) with reverse transcriptase activity. It is involved in the synthesis of the telomeric sequences, thus compensating for their progressive shortening with each round of DNA replication (Meyerson *et al.*, 1997). In addition to TERT and TERC which are essential for the catalytic activity of the telomerase, telomerase complex is also composed of chaperones, including hsp90 and p23, and proteins that bind to TERC, including TEPI, dyskerin, hStau, and L22. (Summarized in Table 1). However, it is still unclear how these different factors might interact with one another in formation of the telomerase holoenzyme complex, and further studies are needed.

Telomere biology which evolved around yeast and *Tetrahymena* has now prospered with actively ongoing aging and cancer research with the recent emergence of various mouse models of the telomerase complex and telomere-binding proteins. Functional studies on telomere and telomerase through these mouse models can provide valuable information that is directly applicable to human aging and cancer research field. This review will provide a summary of previously reported mouse models for the studies of telomere and telomerase biology and discuss future directions.

Figure 1. Structure of telomere and associated proteins.

Telomere Hypothesis (M1 and M2 hypothesis)

Telomere hypothesis states that cellular senescence is triggered by the shortening of telomere repeats to a critical length due to the loss of telomerase function while cellular immortalization is accompanied by telomerase reactivation. In normal fibroblasts, the progressive shortening of telomeres to a critical length is associated with activation of a growth-arrested senescent state known as mortality stage 1 (M1). The molecular basis of M1 is poorly understood, but a number of studies have suggested that Rb, p53, and p21 are involved in the process (Harley and Villaponteau, 1995). Two models have been proposed for the induction of M1. One model postulates that the loss of telomeric repeats exposes chromosomal ends that are then recognized by the DNA repair machinery that signals an anti-proliferative mechanism that involves Rb and p53 pathways. The other model, although not mutually exclusive with the first one, states that growth-suppressor genes involved in the induction of M1 are buried, and hence silenced, in the heterochromatic telomeric regions of chromosomes. Progressive shortening of the telomeres would lead to a chain of events involving relaxation of the chromatin structure of the subtelomeric heterochromatic region, derepression of senescence genes in-

involved in the negative control of cell cycles, and subsequent proliferative arrest. During the M2 stage, most of human fibroblast cells die from a continuous shortening of telomeres, but a rare population of cells survive by regaining the telomerase activity and thus maintaining the telomere length (Shay and Wright, 2000). These cells are known to be immortalized, and tumor cells are considered to be at this stage.

In support of the telomere hypothesis, studies on telomerase expression have consistently demonstrated the presence of telomerase activity in the majority of various cancerous tissues, as well as in immortalized cells, and the absence of detectable level of telomerase activity in most normal somatic tissues (Kim *et al.*, 1994). The telomerase activity present in most neoplastic lesions appears to be necessary for the sustained proliferation of most advanced cancers. In contrast, most somatic mammalian tissues and primary cells possess low or undetectable telomerase activity, and the telomeres shorten with each cell division *in vivo* and *in vitro* (Harley *et al.*, 1995). After a certain number of cell divisions, known as the Hayflick limit, a critical telomere length is eventually reached, leading to cellular senescence, and this provides a basis for the limited replicative capacity of cultured cells (Hayflick and Moorhead, 1961; Shay and Wright, 2000). Telomere shortening appears to trigger senescence *via* Rb and p53 pathways, since

Table 1. Summary of telomere and telomerase complex binding proteins.

<u>TERC binding proteins</u>	<u>TERT binding proteins</u>
Heat shock proteins	H/ACA snoRNAs (small nucleolar RNA) binding proteins
- hsp90 (Holt <i>et al.</i> , 1999)	- hGAR1 (Dragon <i>et al.</i> , 2000)
- p23 (Holt <i>et al.</i> , 1999)	- Dyskerin (NAP57) (Mitchell <i>et al.</i> , 1999)
Kinesis	- hNOP10 (Pogacic <i>et al.</i> , 2000)
- Akt (Kang <i>et al.</i> , 1999)	- hNHP2 (Pogacic <i>et al.</i> , 2000)
- c-abl (Kharbanda <i>et al.</i> , 2000)	hnRNPs (heterogeneous nuclear ribonucleoproteins)
SMN (Survival of Motor Neuron)	- hnRNP C1 (Ford <i>et al.</i> , 2000)
(Bachand <i>et al.</i> , 2003)	- hnRNP C2 (Ford <i>et al.</i> , 2000)
	La (Ford <i>et al.</i> , 2001)
<u>Telomere binding proteins</u>	hStau (Le <i>et al.</i> , 2002)
TTAGGG repeat factor	L22 (Le <i>et al.</i> , 2002)
- TRF1 (Broccoli <i>et al.</i> , 1997)	TEP1 (Harrington <i>et al.</i> , 1997)
- TRF2 (Broccoli <i>et al.</i> , 1997)	
TIN2 (TRF1 interacting factor) (Kim <i>et al.</i> , 1999)	
hRAP1 (Li <i>et al.</i> , 2000)	
Tankyrase (telomeric poly (ADP-ribose) polymerase)	
- Tank1 (Smith <i>et al.</i> , 1998)	
- Tank2 (Kaminker <i>et al.</i> , 2001)	
PinX1 (Pin2 interacting protein 1) (Zhou <i>et al.</i> , 2001)	
POT1 (Baumann <i>et al.</i> , 2001)	
DNA-PK complex (DNA-PKcs, Ku70:80) (Gottlieb <i>et al.</i> , 1993)	

cell divisions beyond the Hayflick limit can occur after antisense neutralization or inactivation of Rb and p53 by viral oncoproteins (Shay *et al.*, 1991). In primary cells deficient for Rb and p53, continued cellular growth beyond the Hayflick limit results in severe telomere shortening, marked genomic instability, and massive cell death - a period referred to as crisis (Harley *et al.*, 1995). While most cells transiting through crisis die, a rare population of surviving cells surfaces which can now maintain the telomere length either by reactivation of telomerase or by utilization of an alternative pathway, a recombination-based mechanism (Yeager *et al.*, 1999).

Testing the Telomere Hypothesis in Mice

Many questions surrounding the telomere hypothesis still had not been answered until 1996. The most direct method to address these questions in mammals was to develop knockout mouse models of the telomerase complex. At that time, TERT, the catalytic subunit of telomerase complex had not been cloned in mouse. However TERC, the RNA component of

telomerase was available (Blasco *et al.*, 1995). Therefore, mice deficient for TERC were generated by the gene targeting approach in order to examine the consequences of the absence of telomerase activity and subsequent telomere dysfunction.

mTERC-deficient mouse

Requirement for telomerase activity in tumor formation and cell viability had been tested by deleting the mouse telomerase RNA component (mTerc) from the mouse germline (Blasco *et al.*, 1997; Lee *et al.*, 1998). Of note, laboratory mice (*Mus musculus*) possess significantly longer telomeres on average compared to human. mTERC^{-/-} mice lacked detectable telomerase activity and were viable for six generations. Telomeres were shown to shorten at a rate of 4.8±2.4 kb per generation in the mTERC^{-/-} background. Cells from the fourth mTERC^{-/-} generation and onward possessed chromosome ends lacking detectable telomere repeats, and exhibited aneuploidy and chromosomal abnormalities, including end-to-end fusions. However, surprisingly, telomerase-deficient cells could be immortalized in culture, transformed by viral oncogenes, and generated tumors in nude mice

following transformation. These results clearly show that telomerase is essential for telomere length maintenance but is not required for establishment of cell lines, oncogenic transformation, or tumor formation in nude mice (Blasco *et al.*, 1997).

However, late-generation mTERC^{-/-} mice exhibited defective spermatogenesis, with increased programmed cell death (apoptosis) and decreased proliferation in the testis (Lee *et al.*, 1998). The proliferative capacity of hematopoietic cells in the bone marrow and spleen was also compromised. These progressively adverse effects coincided with substantial erosion of telomeres and an increase in the frequency of both fusion and loss of chromosomes. Furthermore, aging mTERC^{-/-} mice showed signs of premature aging in highly proliferative organs and they exhibited a decreased stress response, such as decreased wound healing capacity (Rudolph *et al.*, 1999). The role of telomerase in organ regeneration is further supported by the fact that mTERC^{-/-} mice exhibit defects in liver regeneration and accelerated development of liver cirrhosis in response to chronic liver injury (Rudolph *et al.*, 2000). These findings indicate an essential role for telomerase, and hence telomeres, in the maintenance of genomic integrity and in the long-term viability of organ systems with a high renewal rate.

mTERC^{-/-} mice can be propagated for only a limited number of generations (Lee *et al.*, 1998). In particular, mTERC^{-/-} mice with mixed genetic background were infertile at the sixth generation and showed serious hematopoietic defects. In addition, Herrera *et al.* (1999) found that a subset of mTERC^{-/-} embryos did not develop normally and failed to close the neural tube, preferentially at the forebrain and midbrain. The severity of this defect increased with each generation, with 30% of the mTERC^{-/-} embryos from the fifth generation showing the phenotype. Moreover, mTERC^{-/-} kindred in a pure C57BL/6 background with shorter telomeres were only viable up to the fourth generation and also showed defects in closing of the neural tube. These findings suggest that this particular developmental process is among the most sensitive to telomere loss and chromosomal instability (Herrera *et al.*, 1999).

A recent technical advance such as spectral karyotyping (SKY) made it possible to monitor individual chromosomal abnormality in mTerc deficient cells. Importantly, Hemann *et al.* (2001) found that the loss of telomere function and subsequent chromosomal fusions occurred preferentially on chromosomes with critically short telomeres by SKY analysis. According to this result, a critically short telomere, rather than average telomere shortening, is responsible for the initiation of telomere dysfunction and thus the phenotypes of mTERC^{-/-} mice. Possibly, critically short telomeres have fallen below the threshold of telomere re-

peats required for efficient binding of telomere-associated proteins, such as TRF2 (Broccoli *et al.*, 1997), or the formation of telomere loop structures for telomere capping (Griffith *et al.*, 1999).

mTERT-deficient mouse

A couple of groups have produced mTERT knockout mice (Yuan *et al.*, 1999; Liu *et al.*, 2000a). These mice which showed no telomerase activity lacked any serious abnormalities under standard conditions, suggesting that TERT is primarily responsible for the catalytic activity of telomerase, and is not required for other vital functions (Yuan *et al.*, 1999; Liu *et al.*, 2000a). Telomere shortening was only observed in late generations of mTERT-deficient mice and the G-tails essential for the higher-order telomere were also intact. Although these investigators did not find reproducible differences in telomerase activity between wild-type and heterozygous embryonic stem (ES) cells or tissue, ES cells deficient in one allele of mTert lost telomeric DNA in successive passages (Liu *et al.*, 2000a). These results contrast with mTERC^{-/-} ES cells, in which the average telomeric restriction fragment (TRF) length was virtually unchanged following prolonged culture (Niida *et al.*, 1998). This indicates that TERT is the limiting component for telomere length maintenance. However, the haploinsufficiency phenotype of mTert-deficient ES cells should be examined in various murine genetic backgrounds to explain the discrepancy between mTERC^{+/-} and mTERT^{+/-} ES cells. Importantly, mTERC^{+/-} intercross with a mouse strain with shorter telomere such as *Mus spretus* showed haploinsufficiency, resulting in defects in telomere elongation (Hathcock *et al.*, 2002). These results suggest that the RNA component may be also limiting under certain *in vivo* conditions.

Role of Telomerase Complex-Associated Proteins *in vivo*

Recent discovery of proteins that bind to the telomerase complex has led to generation of knockout mouse models for these proteins in attempt to assess the effects of their deficiency on telomerase and telomere maintenance.

mTEP1-deficient mouse

Mouse TEP1 (mTEP1) is a homologue of *Tetrahymena* telomerase protein p80 and was the first protein identified to be associated with the mammalian telomerase (Harrington *et al.*, 1997). TEP1 was found to be associated with telomerase activity in immortalized-cell extracts from human, mouse and rat (Harrington *et al.*, 1997; Nakayama *et al.*, 1997). The

amino-terminal 900 amino acids of TEP1 have been also shown to interact with telomerase RNA *in vitro* and *in vivo* by an RNA/protein interaction assay (Harrington *et al.*, 1997). Despite its association with telomerase components, mTep1-deficient mice showed no significant change in telomerase activity and telomere length up to seven successive generations (Liu *et al.*, 2000b). One possibility is that mTEP1 may share a redundant role with other telomerase RNA-binding proteins, such as L22, hStau, and dyskerin, with respect to the telomerase function (Mitchell *et al.*, 1999; Le *et al.*, 2000). Another possibility is that only a fraction of the total telomerase complex is associated with TEP1. Along these lines, not all telomerase-associated proteins appear to be restricted to the telomerase complex. Ubiquitous proteins, such as p23 and hsp90, are also associated with at least some fraction of the telomerase complex (Holt *et al.*, 1999; Liu *et al.*, 2000b). Furthermore, TEP1 expression is not restricted to the tissues with telomerase activity, suggesting that TEP1 may serve other cellular functions (Liu *et al.*, 2000b; Kickhoefer *et al.*, 2001).

Dyskerin hypomorphic mutant mouse

Dyskeratosis congenita (DC) is a rare X-linked recessive disease with features of premature aging, as well as nail dystrophy, mucosal leukoplakia, interstitial fibrosis of the lung, and increased susceptibility to cancer (Heiss *et al.*, 1998; Dokal *et al.*, 2000; Knight *et al.*, 2001). DC is caused by mutations in DKC1, a gene coding for dyskerin. Dyskerin is known to be involved in rRNA processing and bind to the RNA component of mammalian telomerase (hTerc and mTerc) via H/ACA motif. Mutations in RNA component of telomerase also have been reported in DC (Vulliamy *et al.*, 2001b). Interestingly, cell lines or tissues derived from DC patients display reduced telomerase activity and relatively shorter telomeres (Mitchell *et al.*, 1999; Vulliamy *et al.*, 2001a). Recently, Ruggero *et al.* (2003) produced a mouse model of DC that had reduced expression of DKC1. These hypomorphic mice reproduced the human DC phenotype within the first two generations. Although reduced mTerc expression and reduced telomerase activity was present within the first two generations, telomere shortening was only observed after four to six generations, closely resembling the phenotype of mTerc knockout mice (Ruggero *et al.*, 2003). These data imply that DC might be caused by defects in both telomere maintenance and RNA processing.

PinX1

Pin2 is the 20 amino acid-deleted isoform of TRF1 and is more abundant than TRF1 in cells (Shen *et*

al., 1997). Pin2/TRF1 is an important ATM kinase substrate involved in mitotic regulation and telomere maintenance (Kishi *et al.*, 2001). Zhou *et al.* (2001) found 6 known genes and 4 unknown genes as Pin2/TRF1 interacting proteins by a yeast two-hybrid system. PinX1, one of the 4 unknown genes was a nuclear protein with 328 amino acid residues. When overexpressed in cells, PinX1 containing C-terminal 74 amino acids induced cellular crisis and reduced telomerase activity. This C-terminal fragment interacted with human TERT (hTERT) *in vivo* and *in vitro*. Notably, stable expression of PinX1 or C-terminal 74 residues in HT1080 cells reduced tumor formation after injection into nude mice. Consistent with these findings, PinX1 depletion by antisense strategy in HT1080 cells produced tumors at a higher frequency after injection into nude mice (Zhou *et al.*, 2001). In this regard, future development of PinX1-deficient mice may help manifest the importance of this molecule as a novel tumor suppressor and a regulator of telomerase and telomere *in vivo*.

DNA Damage Response and Telomerase

Telomere and its maintenance are essential for maintenance of genomic stability, which is well illustrated by the fact that mTerc^{-/-} mice exhibited impaired DNA repair and enhanced sensitivity to ionizing radiation (Wong *et al.*, 2000). The interplay among various genes involved in DNA damage response repair pathways, and telomerase dysfunction has been investigated in different mouse models.

ATM-deficient mouse

ATM, the gene responsible for ataxia telangiectasia (A-T) is a kinase and crucial controller of cellular signaling, orchestrating the responses to DNA double strand breaks (Pandita, 2002). ATM is a mammalian homolog of yeast genes, TEL1, MEC1 and rad3, whose mutations cause defective telomere maintenance (Pandita, 2002). Importantly, A-T cells display higher frequency of chromosome end-to-end fusions and loss of telomeres (Pandita and Dhar, 2000; Pandita, 2002). ATM phosphorylates a number of nuclear proteins, including c-Abl tyrosine kinase, p53, BRCA1, NBS1 and PIN2/TRF1. These target genes are now found to be required for proper maintenance of mammalian telomeres (Pandita, 2002). Notably, telomere shortening in A-T cells is partially rescued by ectopic expression of hTERT (Wood *et al.*, 2001). Human cell culture-based studies have revealed that compromised telomere structures and function, induced by expression of a trans-dominant negative

form of TRF2, elicited ATM- and p53-dependent DNA damage checkpoint responses (Karlseder *et al.*, 1999). Several *Atm* mutant mouse strains have been generated. These mice were viable and displayed most of the pleiotrophic features of the A-T phenotype, including genomic instability, progressive neurological degeneration, premature aging, and increased neoplasia incidence (Barlow *et al.*, 1996; Elson *et al.*, 1996; Xu *et al.*, 1996; Herzog *et al.*, 1998). Indeed, *Atm* deficiency influenced telomere shortening and telomere chromatin structure, especially meiotic telomere distribution (Barlow *et al.*, 1996; Elson *et al.*, 1996; Xu *et al.*, 1996; Herzog *et al.*, 1998; Scherthan *et al.*, 2000).

DNA-PK-deficient mouse

DNA protein kinase (DNA-PK) complex is composed of DNA-PK catalytic subunit (DNA-PKcs) and Ku70:80 heterodimer and is critical for DNA repair by nonhomologous end-joining (NHEJ) (Durocher and Jackson, 2001). It was reported that mice homozygous for the severe combined immunodeficiency (*scid*) mutation have defective DNA-PK catalytic subunit (DNA-PKcs), and the telomeres in a murine *scid* cell lines are significantly longer than those in the corresponding wild-type cell line (Slijepcevic *et al.*, 1997). This data was further confirmed by TRF analysis and quantitative fluorescence *in situ* hybridization (Q-FISH) using *scid* mice of various genetic backgrounds. In line with *in vitro* data, *scid* mice displayed significantly longer telomeres than wild-type mice. However, it should be noted that *scid* mutants might contain other genomic alterations. This notion is supported by the observation that DNA-PKcs-deficient mice generated by gene targeting do not show significant alterations in telomere length (Gilley *et al.*, 2001; Goytisolo *et al.*, 2001), but have a high level of telomere fusions, suggesting DNA-PKcs is critical for telomere capping. Recently, it was shown that DNA-PK complex is physically associated with mammalian telomeric DNA *in vivo* by chromatin immunoprecipitation assay, and these associations are not significantly influenced by DNA-damaging agents (d'Adda di Fagagna *et al.*, 2001). Furthermore, Q-FISH results revealed that mouse embryonic fibroblasts (MEF) or ES cells derived from Ku70- or Ku80-deficient mice have shorter telomeres compared with those of wild-type mice (d'Adda di Fagagna *et al.*, 2001). However, cells from mice deficient for XRCC4/DNA Ligase IV complex, another component of NHEJ, did not show significant telomere loss either by TRF analysis and Q-FISH (d'Adda di Fagagna *et al.*, 2001). These data imply that Ku and possibly DNA-PKcs possess additional specialized roles at telomeres.

Mouse deficient for both Ku86 and mTERC

Espejel *et al.* (2002a) impaired both telomerase-mediated telomere maintenance and Ku86 function by generating double knockout (*TERC*^{-/-}*Ku86*^{-/-}) mice. Previously, they observed reproducible telomere elongations in Ku86-deficient cells compared with wild-type controls (Samper *et al.*, 2000). However, they found that Ku86 deficiency does not result in elongated telomeres in telomerase-deficient MEFs or bone marrow cells by Q-FISH. Interestingly, they found that telomeres of MEFs from different generation of *TERC*^{-/-}*Ku86*^{+/-} and *TERC*^{-/-}*Ku86*^{-/-} did not display significant changes in length. It is plausible that Ku86 negatively regulates telomerase-dependent telomere elongation. End-to-end fusions of chromosome were frequent in both Ku86 and late generations of *mTERC*^{-/-} mice (Blasco *et al.*, 1997; Samper *et al.*, 2000). These fusions through p-arms (Robertsonian-like) were classified into two groups: those with detectable TTAGGG and those without detectable TTAGGG. Late generations of *mTERC*^{-/-} mice showed end-to-end fusions lacking detectable TTAGGG, whereas *Ku86*^{-/-} mice exhibited fusions with detectable TTAGGG. Importantly, MEFs from increasing generation of *TERC*^{-/-}*Ku86*^{-/-} mice exhibited decrease in the frequency of end-to-end fusions without detectable TTAGGG compared with MEFs from mice deficient in *mTerc* alone. These data clearly demonstrated that Ku86 mediates the fusion of chromosomes with critically short telomeres, showing that these fusion events are mediated by NHEJ machinery (Espejel *et al.*, 2002a). In addition, Ku86 deficiency rescued early germ cell apoptosis in the late-generation *mTERC*^{-/-} mice (Lee *et al.*, 1998; Espejel *et al.*, 2002a), indicating a direct or indirect role of Ku86 in apoptosis of these cells elicited by critically short telomeres.

Mouse deficient for both DNA-PK and mTerc

The role of DNA-PKcs in telomere length maintenance has been reported in *scid* mutant mice or DNA-PKcs deficient mice as mentioned above (Slijepcevic *et al.*, 1997; d'Adda di Fagagna *et al.*, 2001; Gilley *et al.*, 2001; Goytisolo *et al.*, 2001). However these studies did not fully demonstrate the role of DNA-PKcs in telomerase-based telomere maintenance. To this end, Espejel *et al.* (2002b) generated mice that are deficient for both *mTERC* and DNA-PKcs. Unexpectedly, doubly deficient mice showed reduced fertility even in the first generation (G1), and were infertile in the second generation. G1 *mTERC*^{-/-}*DNA-PKcs*^{-/-} mice showed a severe reduction in testis size and striking depletion in germ cells, which are the most characteristic phenotypes of *mTERC*^{-/-} mice in a late-generation (Lee *et al.*, 1998). One explanation for this observation is that G1 doubly deficient mice

may have faster shortening of the telomeres, compared with mTERC^{-/-} or DNA-PKcs^{-/-} mice. Of note, the severity of the telomere shortening of G1 DNA-PKcs^{-/-} mTERC^{-/-} testis was equivalent to that of the late generations of mTERC^{-/-}. This faster rate of telomere loss was also evident in different cell types, including MEFs and splenocytes. Therefore, DNA-PKcs activity, but not Ku86, functionally interacts with telomerase in maintaining telomere length. Concomitant with a faster rate of telomere loss, mTERC^{-/-} DNA-PKcs^{-/-} mice also showed an earlier loss of viability than the corresponding telomerase-deficient controls. However, this loss of viability was not mediated by a further increase in end-to-end fusions or apoptosis. Importantly, they found that even short telomeres in the absence of DNA-PKcs activity do not lead to increased end-to-end fusions. Furthermore, apoptosis triggered by telomere shortening also was not increased in mTERC^{-/-} DNA-PKcs^{-/-} mice. In conclusion, DNA-PKcs is required for apoptosis signaling by telomere dysfunction and essential for the fusion of short telomeres.

PARP1-deficient mouse

Poly (ADP-ribose) polymerase (PARP) senses DNA-strand breaks and is involved in the maintenance of genomic stability (Tong *et al.*, 2001). Several groups have produced PARP-1 knockout mice and two of them reported on the role of PARP-1 on telomere maintenance (Wang *et al.*, 1995; De Murcia *et al.*, 1997). PARP1-deficient mice developed by Wang *et al.* were found to have dramatically shorter telomeres and an increased frequency of end-to-end fusions (d'Adda di Fagagna *et al.*, 1999). However, PARP1 knockout mice from De Murcia *et al.* displayed normal telomere length and a slight increase in end-to-end fusions (Robertsonians and dicentrics) compared with wild-types (Samper *et al.*, 2001). Such discrepancy could be attributed to the difference in genetic background of these PARP1-deficient mice. Importantly, PARP1-deficiency in the p53^{-/-} genetic background results in heterogeneity of telomere length, including very long and very short telomere or even uncapped chromosomes compared with those from wild-type, PARP1-deficient or p53-deficient cells (Tong *et al.*, 2001). Together with a recent finding that PARP-1 interacts with telomerase (Cao *et al.*, 2002), these observations raise the possibility of involvement of PARP-1 in the telomere length regulation.

Telomerase: For or against cancer?

The original hypothesis on telomere and telomerase in cancer was that repression of telomerase activity in human cells serves as a tumor suppressor mechanism and that re-activation of telomerase is neces-

sary for immortal growth of tumor cells. In support of this hypothesis, early studies in mTERC^{-/-} mice described suppression of tumor formation in Ink4a deficient mice (Greenberg *et al.*, 1999). However, the following studies in mTERC-deficient mice revealed a more complex phenotype. Despite the inactivation of telomerase, the frequency of tumor development and genomic instability increased in mTERC-deficient mouse models due to increased telomere dysfunction (Blasco *et al.*, 1997; Lee *et al.*, 1998; Chin *et al.*, 1999; Greenberg *et al.*, 1999). Based on this observation, different investigators began to investigate the relationship between telomerase and tumor suppressors using mouse models.

Mouse deficient for both p53 and mTERC

Chin and colleagues observed that telomere shortening and subsequent chromosomal fusions in mTERC^{-/-} mice resulted in activation of p53, leading to growth arrest and/or apoptosis in testis compared with wild-type controls (Chin *et al.*, 1999). The authors explored the interactions between telomere dysfunction and p53 in cells and organs of telomerase-deficient mice by generating mice doubly null for mTERC and p53. Interestingly, reduced fertility of G6 mTERC^{-/-} mice was rescued by p53 deficiency, enabling generation of G7 or G8 mTERC^{-/-} mice under p53-deficient genetic background. In addition, tumorigenic initiation was enhanced in mTERC^{-/-} p53^{-/-} MEFs shown by a transformation assay with Myc/Ras cooperation compared with mTERC^{-/-} p53^{-/-} MEFs cotransfected with mTERC expression vector. However, when these cells were injected into *scid* mice, they did not show any significant differences in tumor formation. Therefore, deletion of p53 significantly attenuated the adverse cellular and organismal effects of telomere dysfunction, but only during the earliest stages of genetic crisis. These findings studies indicate a key role for p53 in cellular response to genetic crisis elicited by telomere dysfunctions.

Mouse deficient for both Ink4a and mTERC

Greenberg *et al.* (1999) have produced successive generation of mice doubly deficient for mTERC and Ink4a tumor suppressor genes. Notably, Ink4a-deficient mice exhibit highly penetrant cancer phenotype, and Ink4a^{-/-} MEFs are readily transformed by cellular oncogenes, possess a high rate of colony formation, and lack a senescent arrest phase in culture (Serrano *et al.*, 1996). Furthermore, tumors from Ink4a-deficient mice (primary solid and lymphoid tumors) showed higher telomerase activity, establishing Ink4a^{-/-} mice as an ideal *in vivo* model for testing the importance of telomerase and telomere functions in the tumorigenic processes. Greenberg *et al.* (1999) employed two-

step DMBA/UVB carcinogenesis protocol to assess the incidence, latency, clinical behavior, and histological grade of primary tumors in the successive generations of mice doubly null for mTERC and Ink4a. As expected, they observed a decrease in tumors and an increase in survival in G4 and G5 mTERC^{-/-}Ink4a^{-/-} mice, suggesting that significant telomere shortening and loss of telomere function in late-generation mTERC^{-/-} mice reduces cancer incidence *in vivo* in mice deficient for the Ink4a allele (Greenberg *et al.*, 1999). In consistent with this finding, the late generation mTERC^{-/-} Ink4a^{-/-} MEFs showed impaired cellular growth, decreased colony formation, and increased resistance to oncogene-induced colony formation. Importantly, these defects were significantly rescued by reintroduction of mTERC alone (Greenberg *et al.*, 1999).

Mouse deficient for both ATM and mTERC

The telomeric phenotype of ATM-deficient mice was further evaluated by crossing with mTERC-deficient mice to study the functional interaction of ATM and telomeres *in vivo* (Wong *et al.*, 2003). Expectedly, these compound mutants showed increased telomere erosion and genomic instability. However, the incidence of T-cell lymphomas associated with the ATM deficiency was reduced in these compounds mice. A generalized proliferation defect was evident in all cell types and tissues examined, and this defect extended to tissue stem/progenitor cell compartments, thereby providing a basis for progressive multi-organ system compromise, accelerated aging and premature death in these compound mice. Thus, ATM deficiency and telomere dysfunction act together to impair cellular and whole-organism viability, supporting the view that aspects of A-T pathophysiology are linked to the functional state of telomeres and the impairment of stem/progenitor cell reserves.

Rb family genes and telomere

The tumor suppressor pathway cdkn2a/Rb1 has long been implicated as a barrier to immortalization. Forced expression of Rb1 in squamous cell carcinomas lines results in decreased telomerase activity (Nguyen and Crowe, 1999). However, how Rb1 negatively regulates telomerase is largely unknown. There have been recent reports that Rb1 family genes, including Rb1 (Retinoblastoma 1), Rbl2 (Retinoblastoma-like 2), and Rbl2 (Retinoblastoma-like 2), have a connection to the telomere length regulation in MEFs. MEFs doubly deficient for Rbl1 and Rbl2 (DKO) or triply deficient for Rbl1, Rbl2 and Rb1 (TKO) possess elongated telomeres compared with those of wild type or Rb1 null cells (Garcia-Cao *et al.*, 2002). Importantly, the telomerase activity in DKO or TKO MEFs did not show significant changes compared with wild-type

MEFs, suggesting that telomere elongation was not due to the increase in telomerase activity (Garcia-Cao *et al.*, 2002). This rapid elongation of telomeres by inactivation of Rb1 family genes could be a possible mechanism for the extended life span of cells elicited by a number of viral oncoproteins such as Simian virus 40 large T antigen, human papilloma virus E7 and adenovirus E1A which interact with Rb (Lee and Cho, 2002). However, further studies are needed to unravel how Rb1 family genes are involved in telomere length regulation.

K5-mTERT transgenic mice

González-Suárez and coworkers generated K5-mTERT transgenic mice in which TERT expression was targeted to basal keratinocytes and various stratified epithelia using bovine keratin 5 (K5) promoter and examined the impact of TERT overexpression on tumorigenesis (González-Suárez *et al.*, 2001). Although stratified epithelia of these transgenic mice were histologically normal, they showed increased susceptibility to tumor development upon administration of chemical carcinogens. Moreover, K5-TERT mice showed increased sensitivity to the mitogenic effects of phorbol esters and accelerated wound healing in skin. In the following study, they demonstrated that K5-TERT mice had a decreased life span compared to wild-type mice, which was associated with a higher incidence of preneoplastic and neoplastic lesions in various tissue types with increasing age (González-Suárez *et al.*, 2002). The increased tumor incidence in K5-TERT mice was seen only at old ages. Increased cancer incidence and the reduced viability of K5-TERT mice were aggravated in a p53^{+/-} genetic background suggesting that TERT cooperates with loss of p53 function in inducing tumorigenesis. Interestingly, the changes observed in these two studies, including the increased spontaneous cancer incidence of K5-TERT mice, were not dependent upon the net telomere length.

pCAGGS-mTERT transgenic mice

Artandi *et al.* generated lines of transgenic mice that constitutively expressed mTERT in a variety of tissues under the control of β -actin promoter and CMV enhancer elements (Artandi *et al.*, 2002). In contrast to the findings of K5-TERT transgenic mice, these mTERT transgenic mice did not show increased sensitivity to the mutagen, DMBA and failed to mount a differential response in the rate of spontaneous tumor formation in intercross with Ink4a-deficient mice, despite the increased TERT expression and telomerase enzymatic activity in various tissues. However, a significant finding was that TERT overexpression promoted development of mammary carcinoma in aging

Table 2. Novel functions of telomerase.

Apoptosis (Protective role)
Protects neural cell death (Mattson <i>et al.</i> , 2001; Lu <i>et al.</i> , 2001)
enhances cardiac muscle cell growth and survival (Oh <i>et al.</i> , 2001)
Tumorigenesis
Enhances tumorigenesis in human ALT cells (Stewart <i>et al.</i> , 2002)
Senescence
Higher expression of telomerase elicits senescence-like growth arrest (Gorbunova <i>et al.</i> , 2003)
Genomic Stability and DNA Repair
Associates with telomeres and enhances genomic stability and DNA repair (Sharma <i>et al.</i> , 2003)
Transcription/Gene Expression
Modulates expression of growth-controlling genes (Smith <i>et al.</i> , 2003)
Modulates DNA-methyltransferase I activity (dnmt1) (Young <i>et al.</i> , 2003)

mice. The authors pointed out that the increased incidence of mammary carcinoma in aging mTERT transgenic mice was evident even in the setting of abundant telomere reserve, which suggests that the mammary tumor-promoting action of telomerase was not dependent on the telomere length. Taken together, studies on K5-mTERT and pCAGGS-mTERT transgenic mice suggest that telomerase is able to promote cell survival and proliferation and contribute to tumorigenesis. It is of interest that changes observed in transgenic models of mouse TERT overexpression were present in the absence of a discernable alternation in the net telomere length, which suggests that telomerase, in addition to its role in telomere maintenance, serve novel roles in maintaining tissue homeostasis.

Future Perspective

Telomerase in a new dimension

Previous studies on telomerase have been limited to its catalytic activity and its role in telomere maintenance and dysfunction. However, recently telomerase has been shown to increase tumorigenic potential *in vivo* in the absence of an increase in telomere length (Artandi *et al.*, 2002; González-Suárez *et al.*, 2002; Stewart *et al.*, 2002), and evidences for novel functions of telomerase have been accumulating. In support of this notion, Oh *et al.* observed that the expression of human TERT in mouse myocardium delayed the cell cycle exit by ventricular myocytes and led to late-onset cardiac hypertrophy (Oh *et al.*, 2001). Moreover, TERT expression conferred protection against cardiac myocyte injury after ischemic injury *in vivo*. A more recent study by the same group demonstrated that TERT overexpression in adult mouse myocardium also provide protection from apo-

ptosis, fibrosis, and systolic dysfunction after mechanical stress (Oh *et al.*, 2003). Additionally, there are a number of reports indicating that TERT plays a cytoprotective role against damaging agents and conditions (Summarized in Table 2). In this regard, our group made a recent observation that transgenic mice that overexpress mTERT were significantly resistant to ischemic brain injury. Among different routes of ischemic neuronal death, including excitotoxicity, oxidative stress, and apoptosis, NMDA receptor-mediated excitotoxicity was attenuated in primary cortical cell cultures from TERT over-expressing mice (Kang *et al.*, manuscript submitted). These findings support the notion that telomerase might have novel and telomere-independent functions.

Although numerous studies on telomerase have examined the effects of telomerase over-expression *in vitro*, few studies have been conducted at the organismal level. In addition to currently available TERT-over-expressing transgenic mice, mTERT-deficient mice have been regenerated by at least two groups (Yuan *et al.*, 1999; Liu *et al.*, 2000a). These mTERT-deficient mice in combination with mTERC-deficient mice should serve as a valuable tool in testing various hypotheses on novel functions of telomerase. For instance, the dependency of newly proposed roles of telomerase on its catalytic activity can be deciphered using these mouse models. Furthermore, combining these tools with TERT over-expressing mice will serve as another useful and effective tool in studying the novel functions of the telomerase during various stress conditions *in vivo* and in understanding the molecular mechanisms behind them.

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