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Review

Cellular senescence, cancer and aging: the telomere connection

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Abstract

Telomeres are the repetitive DNA sequences and specialized proteins that form the distinctive structure that caps the ends of linear chromosomes. Telomeres allow cells to distinguish the chromosome ends from double strand DNA breaks. The telomeric structure prevents the degradation or fusion of chromosome ends, and thus is essential for maintaining the integrity and stability of eukaryotic genomes. In addition, and perhaps less widely appreciated, telomeres may also indirectly influence gene expression. The length, structure and organization of telomeres are regulated by a host of telomere-associated proteins, and can be influenced by basic cellular processes such as cell proliferation, differentiation, and DNA damage. In mammalian cells, telomere length and/or telomere structure have been linked to both cancer and aging. Here, we briefly review what is known about mammalian telomeres and the proteins that associate with them, and discuss the cellular and organismal consequences of telomere dysfunction and the evidence that cells with dysfunctional telomeres can contribute to cancer and aging phenotypes. © 2001 Published by Elsevier Science Inc.

Keywords: Antagonistic pleiotropy; Genomic instability; Immortalization; Telomerase; Telomere binding proteins; Telomere structure; Tumor suppression

1. Telomeres

Telomeres are distinctive DNA-protein structures at the ends of linear chromosomes. Telomeres enable cells to distinguish a chromosome end from a double strand break (DSB) in the genomic DNA. DNA DSBs are potentially catastrophic lesions. If not repaired, DSBs are subject degradation. Even if they are repaired, DSBs can lead to loss of heterozygosity (if repaired by homologous recombination) or chromosomal

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deletions or translocations (if repaired by non-homologous end-joining). Thus, without a distinctive telomeric structure, chromosome ends are at risk for either degradation, recombination or random fusion by cellular DNA repair systems. Unchecked chromosome degradation inevitably results in loss of genetic information and cell death. Recombination at telomeres can lead to an unregulated increase or decrease in telomere length, which in turn can prematurely engage or abrogate the senescence checkpoint (discussed below). Telomere fusion results in dicentric chromosomes, which inevitably break during mitosis, thereby creating additional DSBs and cycles of breakage-fusion-breakage that create genomic instability. Thus, without telomeres, the genetic information contained within linear chromosomes would be either lost or become unstable, leading in either case to loss of viability or mutant phenotypes (Blackburn, 1991; Shore, 1997; Blackburn, 2000; Gasser, 2000).

1.1. Telomeric DNA

Telomeric DNA is generally highly repetitive, with the degree of repetition and length of the telomeric tracts varying considerably among species. Here, unless noted otherwise, we confine our discussion to the telomeres of mammalian species. We will focus on humans, in which telomeres have been proposed to play important roles in both cancer and aging, and mice, which have been a valuable, albeit imperfect, model for human diseases, including cancer and aging.

Mammalian telomeres, like all vertebrate telomeres, are composed of the simple repeated sequence TTAGGG. This sequence is repeated many hundreds to thousands of times at the chromosome ends. Most of the telomeric tract is double stranded DNA, but there is also a short (generally between fifty and a few hundred base pairs) single stranded $3'$ overhang at both chromosome ends (Makarov et al., 1997; reviewed in Greider, 1996; Lingner and Cech, 1998; Wellinger and Sen, 1997).

The length of the TTAGGG tracts (measured in the germ line) varies greatly among mammalian species (Greider, 1996; Coviello-McLaughlin and Prowse, 1997; Zijlmans et al., 1997; Kakuo et al., 1999; Campisi, 2001). For example, in the human germ line, telomeres are 15–20 kb. By contrast, telomeres are much longer and more heterogeneous in laboratory mice (*Mus musculus*), ranging from 30 to $>$50 kb, although the telomeres of a related interfertile mouse species (*Mus spretus*) are slightly shorter than human telomeres. Telomere lengths can also vary, albeit to a much lesser extent, among the somatic cells of a given species. Within an adult organism, telomere lengths vary with the genotype, cell type, and cellular replicative history (Martin et al., 1970; Allsopp et al., 1995; Chang and Harley, 1995; Prowse and Greider, 1995; reviewed in Campisi, 2001).

1.2. Telomere-associated proteins

A number of proteins have been identified that associate with telomeres. Some of these proteins associate exclusively with telomeres, whereas others localize to additional subnuclear or subcellular sites. As is the case with many basic biological processes, much more is known about the telomere-associated proteins of simple eukaryotes, such as yeast, than complex eukaryotes, such as mammals. In recent years, however, substantial

Table 1
Mammalian telomere-associated proteins

Protein	Characteristics	Reference
TRF1	Binds double stranded telomeric DNA; negatively regulates telomere length	Chong et al., 1995
TRF2	Binds double stranded telomeric DNA; negatively regulates telomere length; prevents chromosome end fusion; required for telomeric t loop formation	Broccoli et al., 1997
Tankyrases (TANK1, TANK2)	Interact with TRF1; has poly-ADP[ribose] polymerase activity; positively regulates telomere length by ADP-ribosylation and inactivation of TRF1	Smith et al., 1998; Kaminker et al., 2001
TIN2	Interacts with TRF1; negatively regulates telomere length by modulating TRF1 function	Kim et al., 1999
HRAP1	Interacts with TRF2; positively regulates telomere length	Li et al., 2000
Ku	Interacts with TRF1 and TRF2; DNA end binding component of DNA-dependent protein kinase; required for DSB repair	Hsu et al., 2000; Song et al., 2000
RAD50/MRE11/NBS1	DNA repair complex; fraction associates with telomeres; NBS1 interacts with telomeres during S phase	Zhu et al., 2000

progress has been made in identifying proteins that associate with mammalian telomeres (Table 1).

Two proteins that appear to be exclusively localized to the mammalian telomere are TRF1 and TRF2, both of which directly bind the double stranded telomeric DNA (Chong et al., 1995; Broccoli et al., 1997). Two additional proteins, TIN2 and hRAP1, also appear to localize exclusively to mammalian telomeres, but do so by binding TRF1 (Kim et al., 1999) or TRF2 (Li et al., 2000), respectively. The precise functions of these four proteins are not yet known, but all of them appear to play important roles in regulating the length and/or structure of the telomere.

In addition to proteins that seem to act solely at the telomere, a number of proteins appear to have both telomeric and non-telomeric functions in cells. Recently, a non-classical poly-ADP ribosylase, an enzyme activity commonly associated with DNA repair, was found to interact with TRF1 (Smith et al., 1998). This protein, tankyrase or TANK1, associates with telomeres under some circumstances, but it, and a related TRF1-interacting protein TANK2, is most abundant at the nuclear periphery and in the Golgi (Smith and de Lange, 2000; Chi and Lodish, 2000; Kaminker et al., 2001).

A particularly interesting recent development is the realization that several proteins known to participate in specific DNA repair processes, particularly the repair of DNA double strand breaks, associate with telomeres. One such protein is Ku. Ku is a heterodimer and critical component of DNA-dependent protein kinase (DNA–PK), a trimeric complex that is essential for the repair of double stand breaks by non-homologous end joining. Ku binds and stabilizes the ends of broken DNA, whereupon it recruits the catalytic subunit of DNA–PK (DNA–PKcs) (Smith and Jackson, 1999). Cells from mice deficient in either of the two Ku subunits are genomically unstable owing to frequent telomere–telomere fusions (Bailey et al., 1999; Samper et al., 2000). A similar phenotype is see in cells deficient in DNA–PKcs (Bailey et al., 1999). These findings suggest that

DNA–PK, in addition to its pivotal role in repairing DNA double strand breaks (Smith and Jackson, 1999), has an additional role — to protect the terminal telomeric structure. Consistent with this idea, although the Ku heterodimer is abundant, localized throughout the nucleoplasm, and interacts with a number of nuclear proteins, the 70 kD Ku subunit (Ku70) specifically binds TRF1 (Hsu et al., 2000) and TRF2 (Song et al., 2000). Moreover, a significant fraction of Ku is recruited to the mammalian telomere by virtue of its interaction with TRF1 (Hsu et al., 1999) and possibly TRF2. Similarly, the RAD50/MRE11/NBS1 complex, another trimeric complex that has an important, albeit less well-defined, role in the repair of double strand DNA breaks, may have a dual role in telomere maintenance. This complex also associates with the telomere, at least during S phase, very likely owing to an interaction between NBS1 and TRF2 (Zhu et al., 2000).

Based on the number of telomere-associated proteins that have been identified thus far in simple eukaryotes (Dubrana et al., 2001; Evans and Lundblad, 2000; McEachern et al., 2000), the number of mammalian telomere-associated proteins will undoubtedly expand over the next several years. Nonetheless, from the studies completed thus far in both simple eukaryotes and mammalian cells, two important principles have emerged. First, many telomere-associated proteins function cooperatively to establish and maintain the telomeric structure. Second, telomere structure is likely to be more important than telomere length in determining the fate and phenotypes of cells (Blackburn, 2000).

2. Control of telomere length and structure

2.1. Telomere length

The germ line is thought to maintain telomere lengths within species-specific limits by the balanced action of the enzyme telomerase and a variety telomere-associated proteins. Telomerase is a ribonucleoprotein complex that adds the telomeric repeat sequence directly to the single stranded $3'$ telomeric overhang (Blackburn, 1992; Greider, 1996; Lingner and Cech, 1998; Nugent and Lundblad, 1998; Collins, 2000; McEachern et al., 2000). A number of telomere-associated proteins have been shown to regulate telomere length. In some cases, these proteins facilitate degradation of telomeric DNA, which can occur at the $5'$ end of the telomere. In many cases, however, telomere-associated proteins appear to regulate the telomeric structure, thereby determining whether and how telomerase gains access to the $3'$ overhang (the telomerase substrate) (Blackburn, 2000).

In contrast to the germ line and early embryonic cells, most somatic cells do not express telomerase (Prowse and Greider, 1995; Holt et al., 1997; Kim et al., 1994). The absence of telomerase poses a problem for dividing cells. This problem derives from three features of DNA replication. First, replication is bidirectional (i.e. replication proceeds in both the $3'$ and $5'$ direction from an origin of replication). Second, DNA polymerases are unidirectional (i.e. they polymerize exclusively in $5'$–$3'$ direction). Third, DNA polymerases require a primer, which is supplied as a short, labile tract of RNA. Thus, the biochemistry of DNA replication results in failure to replicate 50–200 bp of $3'$ telomeric DNA with each S phase (Fig. 1). This phenomenon has been termed the end-replication problem (reviewed in Levy et al., 1992; Klapper et al., 2001). Interestingly, there is a $3'$ overhang at both ends

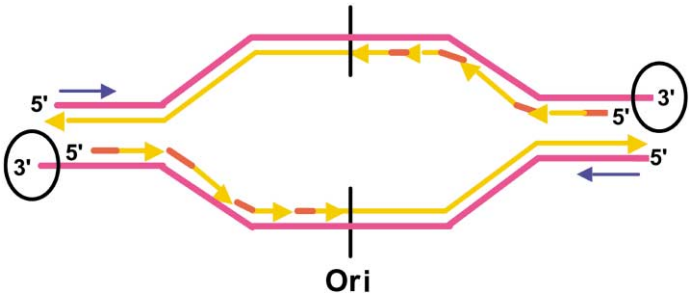

Fig. 1. The end replication problem. Because DNA replication is bidirectional and initiated from a labile primer by a unidirectional DNA polymerase, each round of DNA replication leaves unreplicated 50–200 bp of DNA at the end of each chromosome. In addition, the completely replicated telomere undergoes limited $5' \rightarrow 3'$ exonuclease digestion, creating $3'$ overhangs at both telomere ends.

of mammalian chromosomes (Makarov et al., 1997). This finding suggests that a $5' \rightarrow 3'$ exonuclease specifically trims back the completely synthesized telomere (see Fig. 1). This finding is also consistent with the idea that a single stranded $3'$ tail is essential for establishing a stable telomeric structure (discussed below). Because of the end-replication problem, the telomeres of telomerase-negative cells shorten with each cell cycle (Harley et al., 1990). As discussed below, short telomeres can cause cellular senescence (irreversible growth arrest and altered cell function), cell death or genomic instability, depending on the cell context. Thus, the state of the telomeres can have profound consequences for the phenotype and viability of mammalian cells.

Some adult somatic cells express telomerase, although this is not a widespread phenomenon, particularly among human cells. For example, human T cells transiently express telomerase when activated to proliferate (Buchkovich and Greider, 1996; Weng et al., 1996). In addition, telomerase activity has been detected in human skin, presumably owing to the presence of telomerase-positive stem cells (Harlebachor and Boukamp, 1996). Telomerase activity has also been detected in several mouse tissues (Prowse and Greider, 1995). In human T cells, the telomeres still shortening with each cell division, despite the expression of telomerase, although they do so more slowly than in telomerase-negative cells (reviewed in Effros, 1998). Thus, in some cases, telomerase is not sufficient to prevent the telomere erosion that occurs as a consequence of DNA replication. On the other hand, ectopic expression of telomerase *can* prevent telomere erosion, at least in clones of human fibroblasts, retinal epithelial cells and endothelial cells (Bodnar et al., 1998; Vaziri and Benchimol, 1998; Yang et al., 1999).

What determines whether telomerase will or will not prevent telomere shortening during DNA replication? The answer to this question is not yet known, but at least three possibilities can be envisioned. First, the amount and/or duration of telomerase expression can limit the extent of telomere elongation. Second, the level and activities of telomere-associated proteins can regulate the extent to which telomerase has access to its substrate (the $3'$ overhang). Third, checkpoint proteins (or other regulatory proteins) may determine whether telomerase can act at the telomere. This third possibility is supported by the finding that ectopic expression of telomerase cannot prevent some

human epithelial cells from undergoing replicative senescence (induced by short telomeres) —unless the p16 tumor suppressor is inactivated (Kiyono et al., 1998; Brenner et al., 1998; Dickson et al., 2000), or the cells are maintained under conditions that avoid a p16-induced growth arrest (Ramirez et al., 2001).

2.2. Telomere structure

Despite the evidence that telomere length is important for determining whether cells senesce, die, or survive with genomic instability, there are compelling reasons to believe that telomere structure is more important than the length per se in determining the fate of cells (Blackburn, 2000; de Lange, 2001).

Recently, mammalian (human and mouse) telomeres were shown to end in a large loop resembling a lasso, termed the telomeric t loop. The precise structure of the telomeric t loop is not known. Electron microscopy showed that the t loop circles can be large (several kb) and indirect evidence suggested that the $3'$ overhang is buried in the telomeric duplex DNA (forming, perhaps, triplex DNA) at the junction between the lasso circle and tail (Griffith et al., 1999). The formation of telomeric t loops is critically dependent on the presence of TRF2 (Griffith et al., 1999). In addition, its formation and/or maintenance is very likely facilitated or stabilized by other telomere-associated proteins such as TRF1 and TIN2 (Griffith et al., 1999; Kim et al., 1999).

The telomeric t loop is thought to protect the $3'$ overhang from degradation, and limit the ability of telomerase to access its substrate. It is not yet clear whether the telomeres of all or many species terminate with a t loop structure. However, the recent finding that the telomeres of *Trypanosoma brucei*, a single celled protozoan, are also organized into t loops suggest that t loops may be an evolutionarily conserved telomeric structure (Munoz-Jordan et al., 2001). Interestingly, the *T. brucei* t loops were smaller (approximately 1 kb) than mammalian t loops (up to 25 kb, in mice), despite telomere lengths of 10–20 kb. This finding suggests that the size of the t loop is regulated. Moreover, it suggests that t loops can form on relatively short (1 kb) telomeres.

Disruption of TRF2 function (by expressing a dominant negative TRF2 mutant) causes immortal human tumor cells to die (Karlseder et al., 1999). As discussed below, human tumor cells frequently die if the telomeres erode to critically short lengths (Hahn et al., 1999a; Zhang et al., 1999). Given that TRF2 is essential for t loop formation, which requires a minimal telomere length (Griffith et al., 1999), these findings suggest that tumor cells, which generally lack a normal senescence response (discussed below), tend to die when telomere structure is disrupted. Normal cells, by contrast, tend to arrest growth with a senescent phenotype (discussed below). Interestingly, normal human cells can proliferate with subsenescent telomere lengths, providing telomerase is ectopically expressed (Ouelette et al., 2000). Many telomerase-positive tumor cells also proliferate indefinitely with very short telomeres. These findings suggest that telomerase may act preferentially on the shortest telomeres.

Taken together, the above findings suggest that the cellular response to short telomeres may in actuality be a response to a disrupted telomere structure. According to this hypothesis, recently proposed by Blackburn (Blackburn, 2000), the terminal telomeric structure can exist in either a closed, protected form or an open, unprotected form. Short telomeres

may favor the probability that the terminal structure exists in the open form. Telomerase may recognize, or have access to, only the open form, thereby allowing it to act preferentially on the short telomeres. In the absence of telomerase to extend (and facilitate 'closing') the shortest telomeres, cells may sense the open form as a dysfunctional telomere. The dysfunctional telomere may then signal a senescence arrest or cell death, depending on the integrity of genes that control and implement the senescence/telomere checkpoint. The mechanisms by which dysfunctional telomeres signal the senescence or cell death response are as yet largely unknown.

3. Cellular consequences of telomere dysfunction

3.1. Cellular senescence

Normal cells generally respond to critically short (presumably dysfunctional) telomeres by undergoing cellular senescence — an irreversible arrest of cell proliferation, accompanied by changes in cell function (reviewed in Campisi et al., 1996). At least in mammals, the senescence response very likely evolved to suppress tumorigenesis, acting as a failsafe mechanism to prevent the proliferation of cells at risk for neoplastic transformation. Normal cells undergo a senescence arrest when faced with a variety of potentially oncogenic damage or stimuli. These include dysfunctional telomeres, certain types and levels of DNA damage, perturbations in chromatin, and the expression of certain oncogenes such as mutant RAS (reviewed in Campisi, 1999, 2000).

Cellular senescence causes cells to irreversibly arrest growth with a G1 DNA content owing to the repression of genes required for cell cycle progression (e.g. c-fos), and the upregulation of growth inhibitory genes (e.g. p21 and p16). The senescence response also induces changes in differentiated functions — for example, adoption of a matrix-degrading phenotype by senescent fibroblasts and secretion of an altered profile of steroid hormones by senescent adrenal cortical epithelial cells (reviewed in Campisi et al., 1996; Campisi, 1999, 2000). In contrast to the growth arrest, very little is known about the mechanisms responsible for the functional changes that accompany the senescence response. However, the growth arrest and functional changes are tightly linked, and together define the senescent phenotype.

Human cells senesce when the telomeres (or, more accurately, the terminal restriction fragment) reach an average length of 4–7 kb (reduced from 15–20 kb in the germ line) (Harley et al., 1990). Moreover, ectopic expression of telomerase prevents telomere shortening and senescence in human fibroblasts, retinal epithelial cells, and endothelial cells (Bodnar et al., 1998; Vaziri and Benchimol, 1998; Yang et al., 1999) (Fig. 2). However, telomerase does not prevent human fibroblasts from undergoing senescence in response to mutant RAS (Wei et al., 1999) or DNA damaging agents (Rubio and Campisi, unpublished). Moreover, cells from laboratory mice have substantially longer telomeres than human cells, yet they senesce after fewer divisions, with much longer telomeres, and often despite the presence of (endogenous) telomerase. It has been suggested that mouse cells senesce in culture because they acquire telomere-independent damage inflicted by the culture conditions (Sherr and DePinho, 2000; Wright and Shay, 2000), possibly the

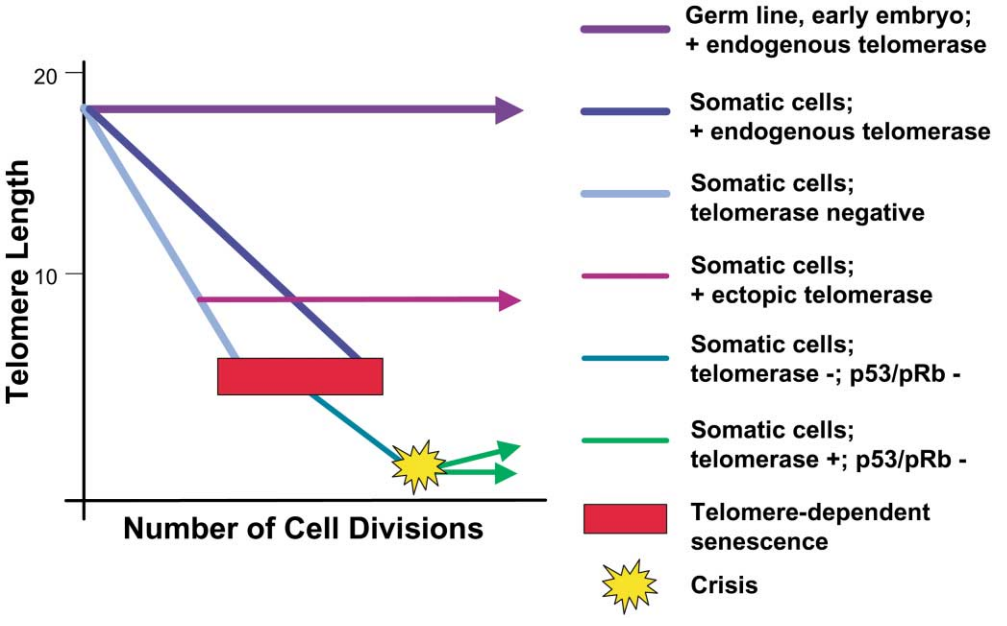

Fig. 2. Telomere-dependent senescence. In the germ line and early embryonic cells, telomerase (endogenous) maintains the terminal telomeric restriction fragment (Telomere Length) at 15–20 kb. In normal somatic cells, whether or not endogenous telomerase is expressed, telomeres shorten until they reach 4–7 kb, whereupon the cells become senescent. Telomere shortening and senescence can be prevented by ectopic expression of telomerase, which can stabilize or even elongate the telomeres. However, telomerase does not prevent senescence induced by telomere-independent stimuli. Telomere-dependent senescence can also be prevented or delayed by inactivation of p53 and/or pRb. However, the telomeres continue to shorten until cells enter crisis. Cells in crisis must activate a telomere stabilization mechanism in order to survive. This common mechanism is induction of endogenous telomerase, which can either stabilize or elongate the telomeres. See text for a detailed explanation.

high oxygen in which most mammalian cells are cultured (Campisi, 2001). Whatever the case, it is clear that telomerase can prevent telomere-dependent (replicative) senescence, but not telomere-independent (cellular) senescence. Interestingly, inactivation of the p53 and pRb tumor suppressor proteins prevents human and mouse cells from undergoing senescence in response to multiple stimuli, including repeated cell division (Shay et al., 1991), DNA damage (Chen et al., 1998), and oncogenic RAS (Serrano et al., 1998).

3.2. Cell death

p53 and pRb are critical regulators of interacting pathways that lead to cell cycle arrest or apoptosis (Kohn, 1999). Mutations that affect key components of either the p53 or pRb pathways partially abrogate the senescence response (reviewed in Lundberg et al., 2000) (Fig. 2). As a result, cells do not respond to the signals that prevent proliferation when the telomeres become critically short or dysfunctional.

If only one of these tumor suppressor pathways is inactive, most human cells eventually arrest growth after a finite number of divisions. For example, human fibroblasts that lack p53 function (owing to expression of the HPV-E6 oncoprotein) and mammary epithelial cells that lack pRb function (owing to loss of p16 expression) eventually cease proliferation. In both cases, however, the population contains many cells in G2 and chromosomal abnormalities (Dulic et al., 2000; Romanov et al., 2001). At least in the case of fibroblasts, the arrested cells are also more prone to die.

When both p53 and pRb are inactive, most human cells proliferate until the telomeres become extremely short, whereupon they enter an unstable state termed crisis. Cells in crisis attempt to proliferate, but because telomere erosion and chromosome instability are so severe, they frequently die. A few cells, however, acquire a mutation or epigenetic event that enables them to stabilize their telomeres (discussed below). Such cells can then proliferate indefinitely, but are at a greatly increased risk for malignant transformation (Fig. 2).

Although most normal cells senesce in response to dysfunctional telomeres, many tumor cells die. Thus, inhibition of telomerase in telomerase-positive tumor cells, in many cases causes eventual cell death (Hahn et al., 1999a; Herbert et al., 1999; Shammas et al., 1999; Zhang et al., 1999). Presumably, these cells die because they lack a normal senescence response, and the telomeres erode until the terminal structure is disrupted. At that point, the cells may undergo apoptosis, or simply lose essential DNA and hence viability. More direct evidence that a disrupted telomere structure can cause tumor cells to die comes from experiments that used a dominant negative form of TRF2. As discussed earlier, TRF2 is required for the formation of telomeric t loops, and the dominant-negative TRF2 induced rapid cell death by apoptosis in human tumor cells (Karlseder et al., 1999). This apoptotic response was strongly p53-dependent. Interestingly, short, presumably near-dysfunctional telomeres also sensitize cells to radiation-induced cell death. This is true not only for transformed cells (McIlrath et al., 2001; Lee et al., 2001), but also for late generation telomerase-deficient mice, which have very short, near-dysfunctional telomeres. In these mice, proliferating normal cells appear to be the radio-sensitive targets (Goytisolo et al., 2000; Wong et al., 2000).

In summary, most normal cells respond to dysfunctional telomeres by mounting a

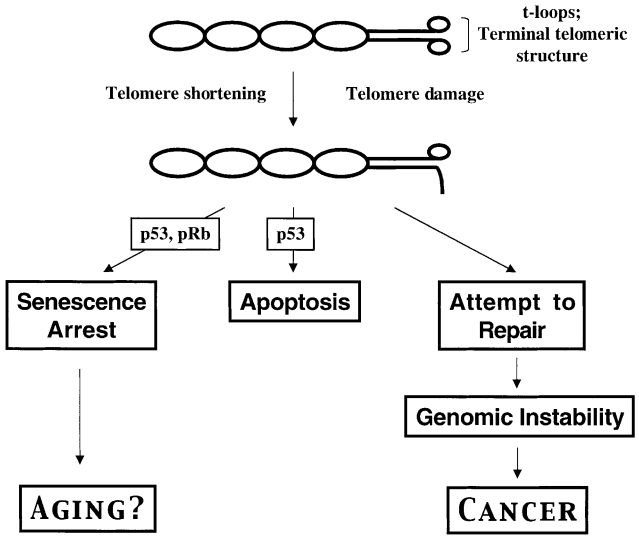

Fig. 3. Cellular consequences of telomere dysfunction. The terminal telomeric structure (t loop) can be disrupted indirectly by telomere shortening or directly by damage. Cells with an intact senescence checkpoint response, which requires p53 and pRb, undergo a senescence arrest. Cells with a defective checkpoint response undergo p53-dependent apoptosis. In the absence of optimal p53 and pRb function, and possibly as yet unidentified factors, telomere fusion leads to genomic instability. Expression of telomerase in such cells greatly predisposes to neoplastic transformation.

senescence response. This response requires the functions of both pRb and p53. If, however, the senescence checkpoint is compromised — for example, by mutations or epigenetic events — p53 ensures that such cells die. In the absence of p53, genomically unstable cells — cells that attempt to repair the damaged telomere by fusing it to another telomere or broken DNA end — may survive (Fig. 3). As discussed below, such cells are at great risk for developing increasingly malignant phenotypes.

3.3. Genomic instability

Chromosomes that lack a protective telomeric structure are highly unstable. They are subject either to degradation or fusion to another chromosome end or double strand DNA break. In the first instance, genetic material is lost. In the second, genetic material can be lost (during mitosis) or rearranged. In addition, chromosome fusion often creates dicentric chromosomes, which break during mitosis, resulting in cycles of further random breakage and fusion. Thus, another possible consequence of telomere dysfunction, particularly in the absence of the senescence checkpoint and p53 function, is genomic instability (Fig. 3).

Genomically unstable cells must find a way to stabilize their telomeres in order to survive. If telomere stabilization does not occur, cells eventually lose genetic material needed for viability. However, because genomic instability greatly increases the frequency of mutations, it favors the chances of acquiring a mutation or epigenetic change that permits telomere stabilization. The most common means by which cells acquire the ability

to stabilize their telomeres is by expressing telomerase. Because most cells express the telomerase RNA component but not the catalytic protein component, TERT, cells most frequently acquire telomerase activity by derepressing TERT (Kim et al., 1994; Chiu and Harley, 1997; Shay and Bacchetti, 1997). There are also telomerase-independent mechanisms that can maintain telomeres (Bryan et al., 1997). These mechanisms very likely use homologous recombination to maintain or even elongate telomeric sequences (Dunham et al., 2000). A small but significant fraction of immortal tumor cells apparently use this mechanism to prevent complete telomere erosion. Needless to say, the genomic instability that occurs when telomeres malfunction in the absence of the normal senescence checkpoint is one of the most striking hallmark of cancer cells (Bishop, 1995; Cahill et al., 1999; Gray and Collins, 2000).

4. Implications for cancer

Several lines of evidence suggest that telomeres contribute to the initiation and progression of malignant tumors in several ways.

First, let us consider telomere dysfunction — whether caused by erosion due to cell proliferation, direct damage, or disruption due to defective telomere-associated proteins. As discussed above, dysfunctional telomeres can have three cellular outcomes — cellular senescence, cell death, or genomic instability. Genomic instability clearly predisposes cells to neoplastic transformation. Cellular senescence and cell death, then, can be considered tumor suppressive responses that prevent the proliferation or survival of cells at risk for developing genomic instability. Cellular senescence may be a double-edged sword in this regard, but this idea will be discussed in the following section.

Consider that cells very likely evolved two lines of defense to prevent the growth of cells at risk for neoplastic transformation. Here, we consider what happens when the risk is one or more dysfunctional telomere. The first defense, used by normal cells, is the senescence response. Cellular senescence prevents the cells with dysfunctional telomeres from proliferating, rendering them incapable of forming a tumor. However, somatic mutations, some of which can inactivate genes required for the senescence response, accumulate throughout life (Dolle et al., 1997, 2000). It is now clear that there is loss of heterozygosity and mutations in tumor suppressor genes, such as *TP53* (which encodes p53), and oncogenes, such as *RAS*, even in relatively young and apparently normal tissue (Deng et al., 1996; Jonason et al., 1996; Cha et al., 1994). Thus, the second defense, which requires only an intact p53 pathway, causes cells with dysfunctional telomeres to die. Eventually, however, cells accumulate mutations in p53 or components of the p53 pathway. Such cells develop genomic instability. These cells are at enormous risk for neoplastic transformation, providing they can acquire a mutation or epigenetic event that stabilizes their telomeres (Fig. 3).

There is strong evidence for this scenario. Mice that lack telomerase owing to germ line inactivation gradually lose telomere length. Because laboratory mice have very long telomeres, it takes 4–6 generations before the telomeres shorten to lengths typical of human cells. Late generation telomerase-deficient mice are moderately cancer-prone, particularly in tissues that are exposed to environmental damage or undergo high cell

turnover (Blasco et al., 1997; Lee et al., 1998). A similar situation may occur in humans with dyskeratosis congenita, a hereditary disease that causes moderate cancer predisposition. The molecular defect underlying this disease was recently shown to affect processing of the telomerase RNA component, rendering affected individuals partially telomerase-deficient (Mitchell et al., 1999). On the other hand, late generation telomerase deficient mice, as well as humans with dyskeratosis congenita suffer from a number of additional pathologies, some of which are associated with aging. These include immune senescence, loss and graying of hair, and impaired wound healing (Rudolph et al., 1999; Herrera et al., 1999; Herrera et al., 2000). Moreover, in some cases, late generation telomerase-deficient mice are moderately resistant to cancer in some tissues and in some genetic backgrounds (Gonzalez-Suarez et al., 2000; Greenberg et al., 1999). However, when telomerase-deficient mice are crossed with p53-null mice, cancer development and progression are markedly accelerated in the doubly deficient offspring (Chin et al., 1999). Moreover, p53 and telomerase deficiency favors the development of epithelial tumors (carcinomas), which are the most common age-associated cancers that develop in humans (Artandi et al., 2001). At least in telomerase-null mice, telomere stabilization occurs by the telomerase-independent mechanism. In both cases, however, cancer is one of the most prominent consequences of the rampant telomere erosion that results from the deficiency in telomerase.

Second, let us consider telomerase and telomere stabilization. Repression of telomerase in most somatic tissues ensures that cells senesce when telomeres become dysfunctional. On the other hand, as discussed earlier, telomerase may act preferentially on short telomeres (Ouelette et al., 2000), thereby reducing their risk for acquiring a dysfunctional structure (Blackburn, 2000). Thus, telomerase might be protective, preventing telomere dysfunction. Because telomerase also prevents cellular senescence, under some circumstances (perhaps under transient or tightly controlled expression) it might retard or prevent age-related pathology, including cancer (discussed below). For example, a TERT transgene targeted to basal keratinocytes in mice, increased telomerase expression in the skin and increased epidermal wound healing (Gonzalez-Suarez et al., 2001). On the other hand, the frequent activation of telomerase by tumor cells suggests that telomerase can ensure the survival of cells with oncogenic mutations and genomic instability.

Two lines of evidence suggest that telomerase is more likely to promote cancer than prevent it. First, somatic expression of telomerase is much more prevalent in the tissues of mice than humans (see Wright and Shay, 2000). However, even when normalized for cell number, mice are more cancer-prone than humans (Miller, 1991). Second, although it is clear that telomerase expression per se does not cause changes associated with neoplastic transformation (Jiang et al., 1999; Morales et al., 1999; Vaziri et al., 1999), telomerase clearly cooperates with potentially oncogenic genetic changes to promote tumorigenesis. This was true for genetically modified human cells, in which telomerase was essential for their ability to form tumors in immunocompromised mice (Hahn et al., 1999b). It was also true for the transgenic mice that constitutively expressed telomerase in the skin owing to a TERT transgene targeted to the basal keratinocytes. Although epidermal wound healing was improved in these mice, the TERT transgene also promoted skin carcinogenesis (Gonzalez-Suarez et al., 2001).

5. Implications for aging

As noted earlier, the senescence response is complex, entailing not only an irreversible growth arrest, but also selected changes in differentiated functions. Among the most striking senescence-associated change in cell function is the secretion of factors that can alter the integrity, function and proliferative homeostasis of tissues (Campisi et al., 1996; Campisi, 2000). This senescence-associated secretory phenotype is particularly striking in fibroblasts, a major component and regulator of the stroma. Senescent fibroblasts secrete extracellular matrix as well as matrix-degrading enzymes, inflammatory cytokines, and growth factors (reviewed in Campisi et al., 1996; Campisi, 2000) — factors that can disrupt tissue homeostasis and structure.

We and others have proposed that the functional changes associated with cellular senescence, particularly factors secreted by senescent stromal cells, may contribute to the decline in tissue function and integrity that is a hallmark of aging (Smith and Pereira-Smith, 1996; Campisi, 1996). Telomere dysfunction, then, may contribute to aging by virtue of its ability to induce cellular senescence (Fig. 3). This being the case, the telomere hypothesis of aging is misnamed because dysfunctional telomeres are but one of several causes of cellular senescence.

As noted above, cellular senescence very likely evolved to protect mammalian organisms from cancer. If, however, cellular senescence also contributes to aging, very likely it is an example of evolutionary antagonistic pleiotropy. This theory predicts that the some traits that were selected to optimize fitness in young adult organisms can have unselected deleterious effects in aged organisms (see Rose, 1991; Kirkwood and Austad, 2000). The growth arrest associated with cellular senescence may be the selected trait, which suppresses tumorigenesis in young organisms. By contrast, the altered functions of senescent cells may be unselected traits that can have deleterious effects. Presumably, these deleterious effects are negligible in young tissues, where senescent cells are rare. However, as organisms age, senescent cells accumulate (Dimri et al., 1995; Mishima et al., 1999; Pendergrass et al., 1999; Paradis et al., 2001). It is possible, then, that as senescent cells accumulate, their altered functions, particularly their secretory phenotype, compromise the physiology and integrity of tissues (Campisi, 1996).

In considering the antagonistic pleiotropy of cellular senescence, we and others have proposed that senescent cells may also contribute to the exponential rise in cancer that occurs with age in many mammalian species (Campisi, 1997, 2000; Rinehart and Torti, 1997; DePinho, 2000). The secretory phenotype of senescent cells can disrupt the tissue microenvironment, which is now recognized as crucial for suppressing the growth and progression of cells with oncogenic mutations (Park et al., 2000). Thus, damage, telomere dysfunction, or errors in mitogenic signaling may cause senescent cells to accumulate, but their influence may become significant and deleterious only later in life when they reach sufficient numbers. Simultaneously, as discussed above, mutations accumulate with age. It is possible, then, that with age, there is an increasing probability that senescent cells and cells with oncogenic mutations occur in close proximity. Senescent cells may then create a microenvironment that promotes the growth and neoplastic progression of the mutant cells. Our recent results indicate that senescent human fibroblasts can indeed stimulate the growth and tumorigenic progression of preneoplastic, but not normal, epithelial cells

(Krtolica et al., in press). Moreover, much of this growth promoting activity can be attributed to the secretory phenotype of the senescent fibroblasts. Thus, despite protecting from cancer in young adults, cellular senescence — driven in part by telomere dysfunction — may promote cancer progression in aged organisms.

6. Summary

Telomeres cap the ends of linear chromosomes and are essential for preserving genomic integrity. The length and structure of telomeres are controlled by a variety of proteins, some of which function exclusively at the telomere, others of which also participate in DNA repair. In the absence of telomerase, telomeres shorten with each cell cycle, but it is likely that cells sense and respond to the integrity of the telomeric t loop or other telomeric structure, rather than telomere length per se. When faced with a dysfunctional telomere, most normal cells engage a checkpoint response, and undergo an irreversible growth arrest termed cellular senescence. In the absence of the senescence checkpoint, cells may die. In the absence of the senescence checkpoint and p53 function, cells with dysfunctional telomeres may continue to proliferate. This inevitably leads to chromosome deletions and fusions, the latter giving rise to additional breaks and fusions and genomic instability. Such cells are then at very high risk for neoplastic transformation, particularly if they activate telomerase or another mechanism to stabilize their chromosomes. Because cellular senescence also entails changes in cell function, the accumulation of senescent cells may contribute to a decline in tissue function and integrity that is seen during aging. This suggests that cellular senescence may be an example of evolutionary antagonistic pleiotropy. In addition to telomere dysfunction, cellular senescence can be induced by telomere-independent events. Thus, the telomere hypothesis of aging is incomplete, as it rests on the hypothesis that senescent cells contribute to aging. Finally, in addition to compromising tissue function and integrity, senescent cells may also promote tumorigenesis by creating an altered tissue microenvironment that facilitates the growth of cells with oncogenic mutations.

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