REVIEW

Telomere dynamics: the means to an end

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Abstract. Telomeres are among the most important structures in eukaryotic cells. Creating the physical ends of linear chromosomes, they play a crucial role in maintaining genome stability, control of cell division, cell growth and senescence. In vertebrates, telomeres consist of G-rich repetitive DNA sequences (TTAGGG)_n and specific proteins, creating a specialized structure called the telosome that through mutual interactions with many other factors in the cell give rise to dynamic regulation of chromosome maintenance. In this review, we survey the structural and mechanistic aspects of telomere length regulation and how these processes lead to alterations in normal and immortal cell growth.

Telomeres are specialized structures found at the ends of linear chromosomes, and over the last decade an increasing body of information has been gathered to create a more complete picture of how telomeres are organized and regulated. Telomeres and telomerase, a reverse transcriptase that extends telomeres, are key players in the initiation of immortalization, as well as the maintenance of the immortal state of cancer cells. Although immortalization of normal cells by spontaneous activation of endogenous telomerase is extremely rare, normal mortal cells may become growth deregulated through mutations of cell cycle control genes such as p53, pRB, p21, p16 and others (Vaziri & Benchimol 1996). Such transformed cells undergo extended lifespan and extreme genome instability in crisis, characterized by massive cell death, through which some of them may become immortal by activation of endogenous telomerase (Hackett & Greider 2002) or through rare activation of alternative recombination-based mechanisms of telomere maintenance (ALT) (Bryan *et al.* 1995). Therefore, knowledge of the molecular structure and function of telomeres, including telomere proteins and their mutual interactions will contribute to our understanding of the processes of cell immortalization and cancer.

TELOMERIC STRUCTURE

Nearly 60 years ago, the first experiments identified specific structures important for genome stability, at the ends of *Drosophila* and maize chromosomes. These protective chromosome ends,

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Figure 1. Telomeres are complex and dynamic structures. The core telomere complex consists of six telomeric DNA binding proteins that interact dynamically with various other protein factors, including DNA repair proteins, DNA processing enzymes, single-stranded DNA binding proteins and in human cells, tankyrase. Tankyrase modifies TRF1 releasing it from telomeric DNA; this contributes to conformational change in the telomeric structure, promotes telomerase access to the 3'-single-stranded telomere end.

named telomeres (for review, see Wai 2004) are composed of specialized DNA/protein complexes. Early analysis of telomeric heterochromatin showed that linear chromosomes end with short tandem repeats consisting of guanine-rich sequences, such as TTAGGG in vertebrates (de Lange *et al.* 1990). Telomeres are dynamic structures and their length varies among organisms or cells of different origin. For example, mice can have telomeres up to 150 kb long, while in humans the length can vary from 3 to 20 kb (Moyzis *et al.* 1988; Harley *et al.* 1990). Electron microscope studies revealed a specific telomere structure in the form of a telomere loop (t-loop) or lariat (Griffith *et al.* 1999; Rubelj & Vondracek 1999). Each telomere ends with a G-rich 3'-single-stranded overhang, at least 12 bp long, which is found in homologous double-stranded telomeric DNA forming a local D-loop (displacement loop) that stabilizes the lariat telomeric structure (Greider 1999, Fig. 1). Because invasion of a 3'-single-stranded overhang takes place at the telomere border region, the radius of the t-loop depends on telomere length. *In vivo* C-rich 5' strands end with exactly the same bases (CCAATC-5'), which indicates modulation by special enzymes (Sfeir *et al.* 2005). These facts implicate the importance of the telomere sequence for the whole DNA-protein superstructure. The whole t-loop entity is held together by several telomere-specific proteins, as well as a number of other ubiquitous proteins involved in DNA processing and structure organization (de Lange 2005). The subtelomeric region adjacent to the t-loop is heterochromatic and contains repetitive, as well as corrupted telomeric sequences and can stretch up to 10–500 kb towards the centromere (Riethman *et al.* 2005). Special histone and DNA modifications (such as acetylation and methylation) in subtelomeric and telomeric

regions can regulate chromatin structure or processes that include replication and homologous recombination (Gonzalo *et al.* 2006).

In normal cells, telomeres shorten with each round of replication, due to the inability of DNA polymerase to synthesize the very end of the DNA lagging strand, as well as due to the presence of 5' exonuclease activity (Makarov *et al.* 1997). It is estimated that each passage through the cell cycle leads to shortening of telomeres by ~50–150 bp (Olovnikov 1973; Makarov *et al.* 1997). Telomere erosion is considered to be a mitotic clock, which regulates the number of divisions before cells enter senescence. In addition to gradual telomere shortening, certain unrepaired DNA damage and epigenetic factors can also contribute to abrupt telomere shortening and sudden and stochastic cell senescence (Smith & Whitney 1980; Rubelj & Vondracek 1999).

As part of the chromosome, telomeres contain histones forming nucleosomes, chromosome structural proteins (Nikitina & Woodcock 2004) and some specific single-stranded DNA binding proteins with a great affinity for single-stranded telomeric sequences (hnRNP A, hnRNP D) (Bandiera *et al.* 2005; Moran-Jones *et al.* 2005). Proteins involved in DNA synthesis, such as helicases and endonucleases are present at the telomere, as well as telomerase, a reverse transcriptase complex involving an hTR RNA component and the hTERT protein catalytic subunit, present in telomerase positive-cells. Telomeres also interact with modulators of specific telomere proteins, which allow access of telomerase, and a number of other proteins involved in damage recognition and repair by homologous/non-homologous recombination (reviewed in de Lange 2005).

TELOMERE DNA BINDING PROTEINS

Six proteins are considered to be strictly telomere specific. As suggested by de Lange (2005), they meet the following criteria; they are specific for telomere DNA, present throughout the cell cycle and have functions restricted to the telomere. They have important roles in protection, elongation and regulation of the telomere. These proteins form a complex named shelterin: TRF1, TRF2, hRap, TIN2, POT1 and TPP (TINT/PTOP/PIP) (Zhong *et al.* 1992; Billaud *et al.* 1997; Broccoli *et al.* 1997; Bianchi & de Lange 1999; Kim *et al.* 1999; Li *et al.* 2000; Loayza & de Lange 2003; Houghtaling *et al.* 2004; Liu *et al.* 2004a,b; Ye & de Lange 2004a; de Lange 2005). Some of these proteins have been characterized as specific telomere sequence-binding proteins, while others were detected in complex with them by gel filtration and mass spectrometry. The whole telomere superstructure seems to be dynamic, switching between an 'open' conformation that allows telomerase to dock onto the telomere and a 'closed' conformation that protects telomeres from telomerase, nucleases or polymerases (Rubelj & Vondracek 1999; Karlseder *et al.* 2002; Colgin & Reddel 2004; Ferenac *et al.* 2005; Hockemeyer *et al.* 2005; Lei *et al.* 2005). Each protein has a specific role in the complex; such as inducing t-loop formation, protecting the telomere from nucleases, checkpoint functions or regulating telomere elongation in telomerase-positive cells (Fig. 1).

TRF1 (telomere repeat binding factor 1) was one of the first proteins identified at telomere sites (Zhong *et al.* 1992). It is expressed continuously throughout the cell cycle, and it binds exclusively to telomeric TTAGGG repeats. TRF1 homodimers can bind, not only neighbouring telomeric sequences, but can also bridge two different DNA molecules or distant regions of the same telomere (Griffith *et al.* 1998; Bianchi & de Lange 1999). TRF1 seems to be the main telomere-binding protein. It binds to telomere DNA as a dimer and probably makes higher-order structures by synaptic pairing with dimers of parallel telomere strands (Bianchi *et al.* 1997; Griffith *et al.*

1998). In addition, a tankyrase-binding motif has been discovered (lacking in mouse TRF1) that is possibly responsible for the regulation of telomere elongation by telomerase (Sbodio & Chi 2002); the amount of TRF1 on telomeres corresponds to the telomere length (Smogorzewska *et al.* 2000). Functional experiments defined this protein as the main regulator of telomere elongation by telomerase. A dominant negative mutant of TRF1 causes telomere elongation in telomerase-positive cells, while TRF1 overexpression causes gradual shortening of telomeres until a new length setting is achieved. Thus, TRF1 is considered to be a negative regulator of telomere length. In telomerase-negative cells, changes in TRF1 levels do not change the rate of telomere shortening, indicating a role for TRF1 in control of telomere elongation by telomerase (Karlseder *et al.* 2002; Karlseder *et al.* 2003).

TRF2 (telomere repeat binding factor 2) is another telomere repeat binding protein, which shares many characteristics with TRF1. Both proteins are expressed throughout the cell cycle and specifically bind telomere sequences. They share some structural similarities, such as domains for dimerization and DNA binding Myb-like motifs. In addition, they both form homodimers and bind DNA through their Myb-like domain. Due to specific binding regions, which differ in each protein, they can not form mutual heterodimers. TRF2 binds DNA four times more weakly than TRF1, due to differences in amino acids responsible for contact with the minor groove of DNA (Hanaoka *et al.* 2005). Structural differences between TRF1 and TRF2 are considered responsible for their different functions on the telomere. While TRF1 is mainly involved in telomere length control, TRF2 is involved in telomere protection (van Steensel *et al.* 1998; Blasco 2005). TRF2 stabilizes the G-rich strand overhang and inhibits telomere–telomere fusions; in addition, TRF2 promotes telomere looping. Griffith *et al.* (1999) have demonstrated that TRF2 can remodel linear telomeric DNA into large duplex loops *in vitro*. TRF2 is considered responsible for loop structure in formation of a tetramer at the loop root (Stansel *et al.* 2001). Although TRF2-mediated DNA looping does not depend on the end structure, a 3'-single-stranded telomeric sequence of at least six nucleotides stabilizes the loop (Yoshimura *et al.* 2004). In overexpression experiments, TRF2 acts as a negative telomere regulator, causing telomere shortening. Inhibition of TRF2 or expression of a dominant negative mutant causes apoptosis to occur and non-homologous end-joining of telomeres. TRF2 was also found at sites of double-stranded breaks at non-telomeric chromosomal locations (Bradshaw *et al.* 2005). In such conditions, it was transiently phosphorylated (Tanaka *et al.* 2005) and its mRNA expression was transiently increased (Klapper *et al.* 2003). These data indicated a possible role for TRF2 in DNA damage response (Bradshaw *et al.* 2005). The most unusual characteristic of TRF2 is its ability to delay senescence when overexpressed and to reduce the senescence setpoint or critical telomere length at which normal cells stop dividing (Karlseder *et al.* 2002).

hRap1 (repressor/activator protein) is a telomere specific protein that acts as a negative regulator of telomere length, although the mechanism by which it does so is still largely unknown. hRap1 is the human homologue of yeast protein scRap1p (Longtine *et al.* 1989), which is the main telomere DNA binding protein (Li *et al.* 2000; Hanaoka *et al.* 2001). In higher eukaryotes, changes in the charge of amino acids in the Myb-like domain, disables hRap1 binding to DNA. As a consequence, hRap1 is connected to the telomere complex through protein–protein interaction with TRF2 (Li *et al.* 2000; Li & de Lange 2003; O'Connor *et al.* 2004). hRap1 has been isolated in complex with TRF2 and proteins involved in homologous and in non-homologous recombination (Rad50, Mre11, Ku 70/86 and PARP), which could be linked to hRap1 *via* TRF2 (O'Connor *et al.* 2004).

TIN2 (TRF1-interacting nuclear factor 2) is one of the most interesting components of the telomeric complex. Its role may be one of providing a scaffold for the binding of other telomeric proteins. TIN2 can simultaneously bind TRF1 and TRF2 *in vitro*. TIN2 is an essential mediator

of TRF1 functions, acting as a negative regulator of telomere length. Mutant TIN2 lacking the N-terminal sequence has caused telomere elongation in telomerase-positive cells (Ye *et al.* 2004b). Transient inhibition of TIN2 led to weakening of telomeric TRF1 signals and TIN2 was found to be a modulator of TRF1 complex ADP-ribosylation (Ye *et al.* 2004c). Another TIN2 interaction is with TPP1 (PIP/PTOP/TINT) that links the TRF1-TIN2-TRF2 complex with POT1 (Liu *et al.* 2004b). These connections give TIN2 a central role in keeping telomeric proteins together: it would connect TRF1 with TRF2 and stabilize TRF2 on telomeres, as well as tether TPP1 to TRF1 and TRF2 (Houghtaling *et al.* 2004). Removal of TIN2 or TRF1 from telomeres leads to TRF2 and Rap1 loss (Ye *et al.* 2004b).

POT1 (protection of telomeres 1) specifically binds to single-stranded telomere overhangs (Lei *et al.* 2003). Its function includes telomere protection and regulation of telomere elongation. POT1 is considered to be a chromosome end-capping protein. Loss of the G-overhang causes a reduction in POT1 binding (Ye & de Lange 2004a). Overexpression of POT1 causes rapid telomere elongation by telomerase, and its loss leads to telomere instability and end fusions (Loayza & de Lange 2003; Veldman *et al.* 2004). Lei *et al.* (2005) analysed its interactions with telomerase *in vitro* and found that, depending on its location on the single-stranded overhang, relative to the DNA 3' end, it could facilitate or inhibit telomerase activity. POT1 was defined as a 'switch' between the two telomere states in which telomerase action is allowed or repressed depending on telomere conformation (Ferenac *et al.* 2005; Hockemeyer *et al.* 2005; Kelleher *et al.* 2005; Lei *et al.* 2005). A third factor would regulate these two states *in vivo*. POT1 deficiency initiates DNA damage checkpoint activation and aberrant homologous recombination at the telomere, and it is supposed that its role is suppression of the telomere DNA damage response (Churikov *et al.* 2006; Wu *et al.* 2006). Additional regulating elements could be differences in POT1 splice variants (Baumann *et al.* 2002), which are found only in mice.

TPP1 (PIP1/PTOP/TINT1) is a POT1 interacting protein previously identified by several groups and given different names (Houghtaling *et al.* 2004; Liu *et al.* 2004a,b; Ye & de Lange 2004a). It was the 'missing link' that helps keep all six proteins together, recruiting POT1 to the TIN2/TRF1 complex (Ye & de Lange 2004a). TPP1 structure reveals two protein-protein interacting domains, a central region that has affinity for POT1, and the C terminus for TIN2 binding. TPP1 acts as negative regulator of telomere length, as its depletion causes telomere elongation (Liu *et al.* 2004b; Ye & de Lange 2004a).

Thus, these six telomeric proteins play an important role in telomere protection on the one hand, while on the other hand, in telomerase-positive cells, they regulate telomerase access to telomeric single-stranded overhangs to allow telomere elongation (such as hRap, POT1, TPP1, TIN2) (Loayza & de Lange 2003; O'Connor *et al.* 2004; Seimiya *et al.* 2004). Different mutations (or the absence of) of one of these proteins (such as POT1, TIN2 or TRF1) would change their compact structure, loosen their connections and allow telomerase activity, probably involving other regulatory proteins (such as tankyrase). Abolishing further proteins or functions in the same complex (such as TRF2) would remove protection from telomeres and lead to end-to-end fusions, apoptotic cell death or senescence (van Steensel & de Lange 1997; Loayza & de Lange 2003; Veldman *et al.* 2004; Ye *et al.* 2004b; de Lange 2005).

TELOMERES AND THE CELL CYCLE

What happens to the telomere during DNA replication in somatic cells is still unknown. Telomere protein-DNA structure should dynamically change in coordination with the cell cycle,

to allow access to the replication machinery. Although by immunostaining, all six specific proteins present on the human telomere have been detected during the whole cell cycle, different modifications and changes in their quantity were observed (Shen *et al.* 1997; Mattern *et al.* 2004). Functional experiments have shown that activity of some of the telomeric proteins could inhibit cell replication.

TRF1 is considered to be the protein responsible for 'closed' telomeric structure. PIN2, an abundant splice variant of TRF1, which can form homodimers as well as heterodimers with TRF1, has shown cell cycle-dependent changes in quantity, increasing at the G₂/M border and decreasing in G₁ (Shen *et al.* 1997). PIN2 interacts with the mitotic kinase NIMA indicating interaction with microtubules (Nakamura *et al.* 2001). Mouse TRF1 has been found on mitotic microtubules, interacting with the spindle checkpoint proteins Mad2 and mitotic kinase Nek2 (Prime & Markie 2005). In *Xenopus*, TRF1 has been found located at telomeres only in mitosis and xTRF1 was regulated by Polo-like kinase phosphorylation (Nishiyama *et al.* 2006). Certain domains responsible for interactions with other proteins are unique to humans, chicken or mouse, indicating different TRF1 regulation (Sbodio & Chi 2002; Nishiyama *et al.* 2006). Although found on human telomeres during the whole cycle, TRF1, TRF2 and POT1 appeared to be highly dynamic with different residence times and quantitative changes that could be the consequence of partial release in certain cell cycle phases (Mattern *et al.* 2004; Wei & Price 2004; Zhang *et al.* 2004; Verdun *et al.* 2005).

These data are in concordance with *in vitro* functional experiments that show that telomere-bound TRF1 and TRF2 significantly stall replication fork progression and inhibit DNA polymerase α at telomeric repeats (Smucker & Turchi 2001; Ohki & Ishikawa 2004). Tankyrase expression has been found to strip a fraction of TRF1 off the telomere (Smith *et al.* 1998).

DNA replication on telomeres is facilitated by the action of helicases, such as WRN and its relative BLM. These 3' to 5' helicases can unwind different DNA substrates, such as Holliday structures or G quadruplexes and alternative DNA structures during recombination (in ALT cells) and replication at telomeric ends. They also have 3' to 5' exonuclease function (Huang *et al.* 1998). In cooperation with telomeric proteins (POT1, TRF1 and TRF2), these helicases unwind telomeres but at the same time they inhibit WRN and BLM exonuclease activity, in order to protect the telomeric 3' tail (Lillard-Wetherell *et al.* 2004; Opresko *et al.* 2004; Opresko *et al.* 2005). Werner syndrome patients (lacking WRN) have accelerated telomere erosion and different length dynamics (Schulz *et al.* 1996; Tahara *et al.* 1997).

Interactions between telomeres and specific cell structural proteins also influence accessibility of the telomere (Fig. 1). TRF1 is considered to be responsible for protein-protein contacts of inter-phase chromosomes with the nuclear envelope (Luderus *et al.* 1996). Human heterochromatin protein 1, a nuclear matrix protein, associates with telomeric DNA (Sharma *et al.* 2003). Kaminker *et al.* (2005) found TIN2 to have different higher-order nuclear organization in cycling and arrested cells, indicating its role in the control of cell proliferation.

TELOMERE LENGTH REGULATION

Normal somatic cells cannot elongate their telomeres and they shorten with each division. Finally, they reach a critical length when the cell stops dividing and starts to become senescent. A number of changes in gene expression and chromatin structure accompany this process (Shelton *et al.* 1999). A cell is disabled from undergoing further divisions as damaged chromosome ends lead to chromosome instability, end fusions and carcinogenetic properties (Bailey &

Murnane 2006). In normal somatic cells, telomeric proteins have a role in end protection. Immortal cells, including germ and cancer cells, exhibit telomerase activity; it catalyses *de novo* synthesis of telomeric repeats at chromosome ends and elongates telomeres (Greider & Blackburn 1985; Kim *et al.* 1994). Small quantities of telomerase can also exist in mortal cells in S phase and play a protective role for telomeres (Masutomi *et al.* 2003). In this context, telomeric proteins take part in the regulation of telomerase access to the telomeres. Additionally, telomerase can be regulated by post-translational modifications, alternative splicing and subcellular localization (Seimiya *et al.* 2000).

In telomerase-positive cells, telomerase access to telomeres is coordinated with cell cycle progression and telomere protein complex structure. It is supposed that telomerase elongates DNA in the S phase (Masutomi *et al.* 2003). During the cell cycle, just a few of the shortest telomeres are presumed to be elongated as they may have a more open structure that increases accessibility of telomerase (Teixeira *et al.* 2004). Regulation of accessibility of the telomeric G-overhang to telomerase is probably dependent on the phase of the cell cycle and telomere length. It also depends on the number and conformation of telomeric proteins; a greater number of TRF1 molecules, in proportion to telomere length, could lead to stronger connections, more compact telomere structure and decreased access of telomerase (de Lange 2005, Fig. 1).

The TRF1 modulating enzyme, tankyrase, releases TRF1 from telomeres and allows access to telomerase. Tankyrase-1 has PARP activity and catalyses the poly(ADP-ribosyl)ation of substrate proteins (Smith *et al.* 1998). Poly ADP-ribosylated TRF1 has diminished affinity for telomeric DNA, thus telomerase is able to access the telomere and to elongate it (Smith *et al.* 1998; Smith & de Lange 2000; Seimiya & Smith 2002). Overexpression of wild-type human tankyrase 1 induces telomere elongation, but mutated tankyrase does not produce this effect; human but not murine TRF1 has a tankyrase-binding motif (Sbodio & Chi 2002; Chiang *et al.* 2006; Hsiao *et al.* 2006). TRF1 released from telomeres is ubiquitinated and may be quickly degraded in the proteasome, in order to prevent its rebinding to telomere DNA (Chang *et al.* 2003; Lee *et al.* 2006). TIN2 protects TRF1 from poly(ADP-ribosyl)ation by tankyrase1, without affecting tankyrase 1 automodification *in vitro* (Ye *et al.* 2004c). Tankyrase has additional non-telomeric functions on other proteins and plays a part in resolution of sister telomere association during progression through mitosis (Dynek & Smith 2004). PARP2 has been found to target TRF2, modifying DNA binding and dimerization domains (Dantzer *et al.* 2004).

Telomere elongation by telomerase, in addition to the affect on telomeric proteins, is also influenced by other mechanisms. Telomerase is tightly regulated at the transcriptional level and its subcellular localization contributes to regulation of the elongation process during the cell cycle. hTR, an RNA telomerase component, ubiquitously expressed, has been found in nucleoplasmic Cajal bodies of inter-phase malignant cells and is transiently concentrated at a few telomeres during S phase (Jady *et al.* 2006). Wong *et al.* (2002) have also found that catalytically active human telomerase localization in the nucleus is dependent on the phase of the cell cycle, it resides in the nucleolus and is sequestered to the nucleoplasm at the expected time of telomere replication. hTERT nuclear export and import is regulated by 14-3-3 kinase (Seimiya *et al.* 2000).

Besides telomerase, there are also various alternative mechanisms of lengthening telomeres (ALT) that employ DNA recombination machinery. These mechanisms may include inter-telomeric homologous recombination, t-loop recombination, rolling-circle extension and extension utilizing extra-chromosomal telomeric repeats (Neumann & Reddel 2002). The mechanisms are still largely unknown, although an increasing number of proteins involved in the process have been described (Dunham *et al.* 2000; Cesare & Griffith 2004). Because ALT is active in only ~15% of tumours and tumour cell lines that have been analysed to date (Shay & Bacchetti 1997), telomerase is the main mechanism that enables immortal cells to undergo continuous divisions.

TELOMERES AND THE DNA DAMAGE RESPONSE

Compact protective structure of the telomere is compromised when telomere DNA has to be 'unpacked' during the process of DNA replication or telomere elongation. In that form, telomeres have many similarities to DNA breaks. Proteins involved in DNA repair and damage signalling are localized on telomeres, but they do not trigger a DNA damage response and/or cell cycle arrest (Wright *et al.* 1999; Zhu *et al.* 2000). Mre11, NBS, ATM, Ku86, DNA-PKcs, Rad50, Rad51 are proteins involved in non-homologous end joining and homologous recombination, that reside on telomeres (Hsu *et al.* 2000; d'Adda di Fagagna *et al.* 2003; Takai *et al.* 2003; de Lange 2005, Fig. 1). Verdun *et al.* (2005) have shown that activated NBS1, Mre11 and ATM are recruited to telomeres in every G₂ phase of the cell cycle in telomerase-negative cells and could have a role in protection and formation of the long G overhang (Herbig *et al.* 2004; Larrivee *et al.* 2004); replication leaves chromosome ends blunt or with short G-tails. POT1 can be partially released leaving telomeres unprotected and accessible to modifying enzymes. Long G-tails required at both ends for t-loop formation are formed by exonuclease erosion at the 5' strands (Makarov *et al.* 1997). TRF2 interacts with the MRN (Mre11, Rad50, NBS1) complex (Stansel *et al.* 2001; Karlseder *et al.* 2002; de Lange 2005), some specific endonucleases and exonucleases that remodel the telomere. One of them is the ERCC1/XPF endonuclease responsible for overhang processing (de Laat *et al.* 1998; Hoeijmakers 2001). Apollo or hSNM1B a 5'→3' exonuclease is another TRF2 interacting enzyme found to cooperate in telomere protection (Lenain *et al.* 2006; van Overbeek & de Lange 2006). Investigations have shown that the telomeric overhang helps form a protective structure and prevents chromosome fusion (Zhu *et al.* 2003; Wang *et al.* 2004; Chai *et al.* 2006; Chiang *et al.* 2006).

In normal cells that do not express telomerase, telomere erosion and subsequent impairment leads to replicative senescence. Dysfunctional telomeres are detected as double-stranded DNA breaks that induce a DNA damage response and activation of the tumour suppressor protein p53. This is accomplished through activation of ATM kinase that phosphorylates p53 directly as well as phosphorylating the Chk2 checkpoint kinase, which promotes additional phosphorylation of p53. These modifications contribute to displacement of mdm2 from p53 allowing its stabilization and activation (Kulju & Lehman 1995; Vaziri *et al.* 1997; Gire *et al.* 2004; Artandi & Attardi 2005). Levels of p21, the main cell cycle inhibitor, increase several times over in senescence, arresting the cell cycle in G₁ phase (Noda *et al.* 1994; Dulic *et al.* 2000). Furthermore, other cyclin-dependent kinase inhibitors (CDKI), such as p16 that controls pRB activity, take part in this process. Long-term increase of p16 leads to chromatin modification and to final irreversibility of senescence (Dai & Enders 2000). Under these conditions, pRb is hypophosphorylated and this contributes to heterochromatinization of DNA and stable cell cycle arrest (Narita *et al.* 2003).

CONCLUSION

In summary, we can say that telomeres are crucial for genome stability and cell growth control in all eukaryotes. They have a complex and dynamic structure that includes numerous specialized proteins involved in interactions with various structures in the cell. As data accumulate, we should gain greater knowledge of their function. This is highly important for attempts to develop therapies against cancer based on telomerase inhibition and subsequent telomere shortening (Corey 2002). In addition, this will lead to greater understanding of the role of these specialized structures, both in normal cell physiology and in processes such as carcinogenesis and ageing.

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