TELOMERES ARE GUARDIANS OF CHROMOSOMAL INTEGRITY

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Telomeres are DNA-protein complexes located at the ends of linear chromosomes in most eukaryotic cells. Telomeres are vital components of chromosome integrity and cell proliferation. They protect internally located genes from degradation due to incomplete DNA replication, and they also mask natural DNA ends from being recognized as DNA breaks. While being guardians of genome integrity, telomeres can also be vulnerable to damage and inappropriate repair. Dysfunction in telomere maintenance can lead to serious illnesses in humans, including cancer. Cancers achieve immortalization through telomere elongation either through the activation of specialized enzyme telomerase or Alternative Lengthening of Telomeres (ALT) pathway. This review highlights the structure and the function of telomeres in yeast and humans, discusses telomere binding proteins and their roles in telomere maintenance, and briefly describes the implications of telomere dysfunction on human health.

I. INTRODUCTION

In the majority of species with linear chromosomes, telomeres, from Greek "telos" for an end and "meros" for a part, protect chromosomal ends from being recognized as breaks and also buffer against gradual DNA loss due to incomplete replication. Telomeres do not contain genes and, therefore, gradual loss of telomeric DNA due to incomplete DNA replication does not lead to cell death. However, in order to perform their functions correctly, telomeres must also be maintained. This maintenance is performed by various telomere-specific binding proteins that protect telomeres against abnormal degradation or damage. Telomere importance in genome protection was noted as early as the 1800s (Gall 1996). However, it was not until Hermann Müller and Barbara McClintock's 1930s discoveries that the importance of telomeres in cell viability was truly appreciated, when it was demonstrated that intact chromosomal ends (telomeres) prevented genomic instability (Murnane 2012). Telomere synthesis is performed by telomerase, a specialized reverse transcriptase that synthesizes telomeres *de novo* using an RNA template (Blackburn and Collins 2011).

Telomeres are typically composed of multiple repeats of 5-8 bp non-coding DNA sequences, although some exceptions exist such as yeast species that can have either degenerate or uniform, 16-31 bp repeats (Oeseburg, de Boer et al. 2010). Telomere length and integrity is tightly regulated by the modulation of telomerase access to telomeres and by telomere associations with telomere-specific binding proteins (also known as "telomere capping") (DeZwaan and Freeman 2010). Telomeres and telomere binding proteins form complex structures which serve as protective telomere "caps" that preserve telomere function and prevent telomeres from being damaged or inappropriately repaired.

Damaged telomeres can undergo repair through an error-prone non-homologous end joining (NHEJ) pathway or homologous recombination (HR), with the latter being a process whereby a telomere can copy a sequence from another telomeric source (Williams and Lustig 2003). Certain human cancers can utilize HR to elongate telomeres

(Nabetani and Ishikawa 2011). This pathway is not understood well, though yeasts are important model organisms for studying the genetics of this mechanism. This review briefly introduces telomeres, their structure and their function.

II. STRUCTURE OF TELOMERES

The first telomeric sequence was obtained from ribosomal DNA isolated from the ciliate protozoan *Tetrahymena* (Blackburn and Gall 1978). In the great majority of eukaryotes, telomeres consist of multiple repeats of a short G-rich DNA sequence. Humans have a 6 bp (TTAGGG) telomeric repeat and so does the human parasite *Trypanosoma brucei* along with agriculturally important pathogens of *Aspergillus* (Blackburn and Challoner 1984, Broun, Ganal et al. 1992, Cohn, McEachern et al. 1998, Kusumoto, Suzuki et al. 2003, Oeseburg, de Boer et al. 2010) (Table 1). However, baker's yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) has the variable telomeric repeat $TG_{2-3}(TG)_{1-6}$, while the milk yeast *K. lactis* has uniform 25 bp (GGTGTACGGATTTGATTAGGTATGT) telomeric repeats (Fulton and Blackburn 1998). Yeast species are excellent models for telomere research because their genomes have been sequenced and they can be easily subjected to a great variety of experimental techniques. *K. lactis* is also uniquely suited for telomere studies because it possesses uniform telomeric repeats that can be easily manipulated and tracked within a cell. The total length of telomeric DNA differs greatly between various representatives of the eukaryotic kingdom, from a dozen bp in the marine ciliate *Euplotes crassus* to several hundred bp in yeast or several thousand bp in humans (Stewart, Chaiken et al. 2012).

Telomeric DNA consists of double-stranded DNA-protein structures terminating in a single-stranded 3' overhang on the G-rich strand. Both the double- and single- stranded telomeric DNA play important roles in telomere capping by providing binding substrates for telomere-specific binding proteins. The 3' overhang is required by telomerase for *de novo* telomere synthesis. This overhang is protected against degradation by the binding of capping proteins and also by formation of a t-loop, a higher-order structure formed when a single-stranded telomeric end loops backwards and strand-invades upstream double-stranded telomeric sequences. t-loop visualization was pioneered by Jack Griffith were (Griffith, Comeau et al. 1999), and, so far, t-loops have been identified in chickens, plants, and protozoans (Cesare, Quinney et al.

Species	Telomere length	Telomere sequence
Protozoa		
Tetrahymena thermophila	120-1000 bp	T_2G_4
Oxytricha	20-28 bp	G_4T_4
Trypanosoma brucei	3-10 kb	T_3AG_3
Yeast		
Saccharomyces cerevisiae	200-300 bp	$TG_{2-3}(TG)_{1-6}$
Vertebrates		
Humans	5-15 kb	T_3AG_3
Mice	up to 150 kb	T_2AG_3
Invertebrates		
Ants	9-13 kb	T_2A_2
Honey bee	< 1kb	T_2A_2
Plants		
Arabidopsis thaliana	2-5 kb	T_3A_3
Tomato	20-50 kb	$T_2(T/A)A_3$

Table 1. Telomere sequence and length vary between species.

2003, Nikitina and Woodcock 2004, Martínez and Blasco 2011).

While telomeres are present in the majority of eukaryotes, quite a few species have evolved non-canonical mechanisms of linear chromosomal end maintenance. For example, *Drosophila melanogaster* and the silk worm *Bombyx mori* maintain their chromosomal ends using gene conversion (DNA copying) and transposition of non-LTR retrotransposons (Fujiwara, Osanai et al. 2005, Rong 2008). The mosquito *Anopheles*, the vector of malaria, contains complex repeats at the chromosomal ends that are maintained by recombination (Roth, Kobeski et al. 1997). In addition, plants in the genera *Cestrum*, *Sessea*, *Vestia* of Solanaceae, and *Allium* (Asparagales) are completely devoid of a predominant telomeric motif. Instead, a complex repeating pattern at the ends of their chromosomes is thought to be produced through recombination between chromosomes (Sykorová, Fajkus et al. 2006).

III. MECHANISMS OF TELOMERE ELONGATION

A. Telomerase origins

Telomerase is a specialized enzyme that uses an RNA template to synthesize telomeric DNA onto chromosome ends de *novo*. An ancient origin of telomerase is supported by a discovery of a putative telomerase catalytic protein subunit in the most primitive eukaryotic species, the parasite *Giardia* (Malik, Burke et al. 2000). Furthermore, the usage of RNA moiety, as a core enzymatic unit for DNA synthesis, suggests that telomerase could be a ribozyme remnant from the RNA-DNA transition where association of early ribozymes with protein particles improved ribozyme function and stability (Pace NR 1985). The human telomerase enzyme can also become an RNA-dependent RNA polymerase, which is presumed to be the oldest form of polymerase enzymes (Nosek and Tomaska 2008). Interestingly, comparison of reverse transcriptase protein coding sequences from *S. cerevisiae*, *Euplotes ardiculatus*, *S. pombe*, human, *Giardia lamblia*, *T. thermophila*, chicken, and *C. elegans* revealed universally conserved motifs. Mutations in any of these motifs lead to either telomere shortening or ablation of telomerase activity across species (Harrington, Zhou et al. 1997, Lingner, Hughes et al. 1997, Nakamura, Morin et al. 1997, Weinrich, Pruzan et al. 1997).

B. Telomerase and telomere binding proteins

Telomerase is responsible for *de novo* telomere synthesis and is preferentially recruited to short telomeres (Teixeira, Arneric et al. 2004). Telomerase alignment with and binding of the 3' overhang is the first step in the telomere synthesis reaction. Telomerase synthesizes multiple copies of the telomeric repeat following a single binding event. Upon completion of one telomeric repeat, telomerase translocates along the telomeric repeat and repeats the synthesis again (Figure 1). Extension of the 3' overhang is coupled with DNA synthesis by DNA polymerase, which synthesizes the DNA strand complementary to the extended 3' overhang. (Podlevsky and Chen 2012).

Telomerase holoenzyme in yeast is composed of RNA (the product of *TLC1* gene in *S. cerevisiae* and *TER1* in *K. lactis*) associated with three protein subunits (Est1, Est2, and Est3) (DeZwaan and Freeman 2010). The catalytic subunit of telomerase (Est2) is found at telomeres throughout the cell cycle, but its peak accumulation occurs in late G1 and S phase (during DNA synthesis) and is dependent on association of the RNA subunit with the end-binding Ku70/80 complex (Stellwagen, Haimberger et al. 2003). The Ku70/80 complex also facilitates telomere-telomere fusions, but it binds telomeric DNA and telomerase in a mutually exclusive manner

(Pfingsten, Goodrich et al. 2012). The reverse transcriptase activity of telomerase is conferred by Est2 protein, but telomerase is

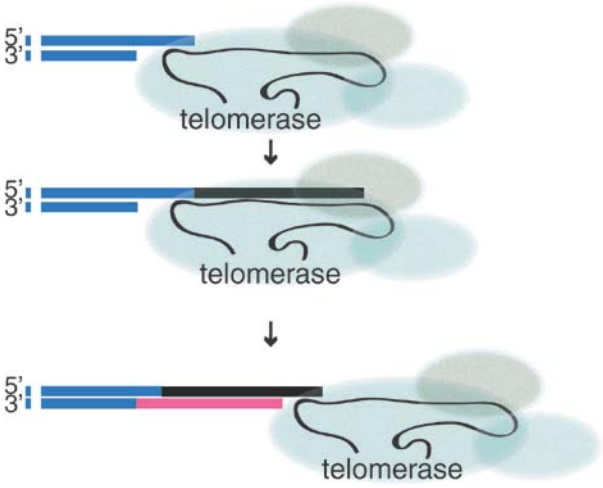

FIGURE 1. Telomere synthesis by telomerase. Telomerase binds telomeric 3' overhang, synthesizes telomeric DNA using RNA template, translocates along the newly synthesized telomeric repeat (in black), and repeats the cycle again. Overall telomere elongation is coupled with the synthesis of DNA that is complementary to the one synthesized by the telomerase enzyme (in red).

recruited to telomeres by Est1's interaction with Cdc13, a telomere-binding protein and a telomerase regulator factor (Lingner, Cech et al. 1997, Bianchi, Negrini et al. 2004, Lee, Mandell et al. 2010). ScEst3 binds Est2 and has been shown to stimulate telomerase activity *in vitro* (Talley, DeZwaan et al. 2011). In addition, Est3 in *Candida* species can also bind telomeric DNA (Yen, Chico et al. 2011). Deletion of any of the *EST* genes or *TLC1/TER1 in vivo* leads to telomere shortening and a growth decline also known as senescence (Lendvay, Morris et al. 1996).

In humans, the active telomerase holoenzyme consists of three major components: TERC (RNA component), TERT (catalytic component), and dyskerin (RNA-binding component). Immunoprecipitation assays identified the presence of two molecules of each component in the active telomerase complex. The proper assembly of this complex requires Cajal bodies and Cajal body-associated proteins (TCAB1, pontin, and reptin). Together, they bind telomerase in a cell cycle-dependent manner and assist in telomerase assembly and its trafficking (Grigoletto, Lestienne et al. 2011, Mason, Schuller et al. 2011).

C. Telomere binding proteins

Telomeres are natural DNA ends of linear chromosomes. They must be differentiated from DNA ends in DNA breaks and must also be protected from inappropriate repair. Telomeres are associated with multiple protein entities. These proteins help to protect telomeres from being recognized as DNA breaks, and they also contribute to the formation of secondary structures, which protect telomeres from degradation.

Telomeres can be subdivided into single-stranded and double-stranded DNA regions, each interacting with telomere-specific binding proteins and having vital functions in telomere function. The essential Repressor Activator Protein 1 (Rap1) is a conserved multifunctional protein found to bind yeast and mammalian telomeres. In yeast, Rap1 binds to double-stranded telomeric DNA and exerts both negative and positive regulation of telomere length (Figure 2). The maximal accumulation of Rap1 at telomeres occurs in S/G2 phase. The degree of telomere saturation by Rap1 molecules has an inverse correlation with telomere length (Williams, Levy et al. 2010).

Investigations into the structure of Rap1 indicate that it contains three domains: a Rap1 C-terminal (RCT) domain, two central MYB domains, and a non-essential N-terminal BRCT domain (Chen, Rai et al. 2011, Fujita, Tanaka et al. 2012). The *S. cerevisiae* RCT domain enables Rap1 interaction with Rap1-associated factors (Rif1 and Rif2) as well as silent information regulator proteins (Sir3 and Sir4). Together with Rif1 and Rif2, Rap1 negatively regulates telomere lengthening

using a mechanism which is not well understood (Levy and Blackburn 2004). Together with Sir3 and Sir4, Rap1 facilitates silencing near telomere regions shown to be important for adaptation and evolution of yeast species (Cockell, Palladino et al. 1995). Mutations within the binding site of Rap1 destabilize telomere length regulation to various degrees in *K. lactis* (Groff-Vindman, Natarajan et al. 2005).

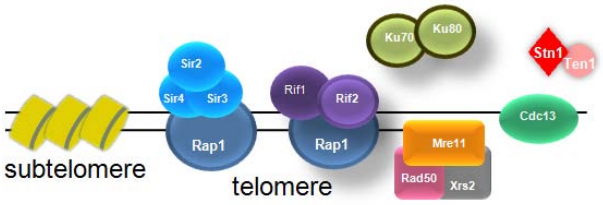

FIGURE 2. Telomere-specific binding proteins in the baker's yeast *S. cerevisiae.*

ScCdc13 is a single-stranded 3' overhang binding protein (Mitton-Fry, Anderson et al. 2004). Just like Rap1, Cdc13 is both a positive and a negative regulator of telomere length (Chandra, Hughes et al. 2001). The functions of Cdc13 have been genetically separated to different protein domains: an N-terminal domain; a recruitment domain (RD) interacting with Est1 (positive telomere length regulator); an OB-fold containing DNA binding domain and a C-terminal domain responsible for negative telomere length regulation (Taggart and Zakian 2003). Positive and negative regulation of telomere length is ensured by Cdc13's binding of Est1 and Stn1, respectively (Gasparyan, Xu et al. 2009). For a long time it was thought that as a positive regulator of telomere length, Cdc13 recruits telomerase by interaction with Est1 upon its phosphorylation by specialized kinase proteins Tel1/Mec1 or specific cyclin-dependent kinase Cdk1 (Li, Makovets et al. 2009). However, an alternative model suggested that Cdc13 may not be a target for phosphorylation by Tel1 and, as such, Tel1 is not required for telomere length regulation (Gao, Toro et al. 2010). These discrepancies may be due to the fact that Cdc13 possesses multiple, and previously unknown, residues that can undergo phosphorylation at different stages of the cell cycle (Wu, DiMaggio et al. 2012). Phosphorylation of Cdc13 by Cdk1 also blocks recruitment of the negative telomere length regulators, Stn1 and Ten1 (Puglisi, Bianchi et al. 2008, Li, Makovets et al. 2009).

Together, Stn1, Cdc13, and Ten1 form an essential capping complex (also referred to as CST complex) and together they participate both in telomere capping and in telomere length regulation (Pennock, Buckley et al. 2001). The N-terminus of Stn1 contains a Ten1 binding domain, while the C-terminus of Stn1 interacts with Pol12, a regulatory subunit of polymerase α-primase (Paschini, Mandell et al. 2010). As a capping protein, Stn1 also prevents repair via mechanism of recombination at telomeres. A mutation in the N-terminal domain of *K. lactis* Stn1 causes telomere instability and telomere hyper-recombination (Xu and McEachern 2012). Components of the CST complex have been found in other species. A human Stn1 homolog, OBFC1, also binds single-stranded telomeric DNA and interacts with another telomere protein TPP1 (Wan, Qin et al. 2009). Ten1 is an essential protein in *S. cerevisiae*; it is unknown whether *K. lactis* has a homolog of Ten1 (Grandin, Damon et al. 2001, Puglisi, Bianchi et al. 2008).

Nucleases and repair proteins are the least expected entities to be found at telomeres in their normal state; however, many repair proteins not only associate but are required for proper telomere function and telomere length maintenance. Telomeres are bound by repair proteins from both the error-prone non-homologous end joining (NHEJ) and precise homologous recombination (HR) repair pathways. In *S. cerevisiae*, the Ku heterodimer, a major factor in DNA repair through NHEJ, is involved in regulation of the 3' overhang length as well as in the positive

regulation of telomere length (Boulton and Jackson 1998). Furthermore, it interacts with telomerase RNA acting as a positive enhancer of its interaction with telomeres (Stellwagen, Haimberger et al. 2003). When Ku is deleted in *S. cerevisiae*, telomeres become vulnerable to shortening and exhibit elongation of their G-rich overhangs (Boulton and Jackson 1996, Gravel, Larrivee et al. 1998, Polotnianka, Li et al. 1998). Contrary to *S. cerevisiae*, loss of Ku80 in *K. lactis* does not affect telomere length maintenance, but causes longer overhangs and more frequent subtelomeric recombination (Carter, Iyer et al. 2007).

The yeast MRX complex consists of Mre11, Rad50, and Xrs2 and is by far the most multifaceted protein complex associated with telomeres. This complex is implicated in DNA damage signaling, DNA replication, DNA repair, meiosis and mitosis, and telomere maintenance (Lamarche, Orazio et al. 2010). The MRX complex participates in DNA resection and DNA repair. Mre11 is a dimer acting as 3'-5' exo- and endonuclease on single- and double-stranded DNA substrates (Paull and Gellert 1999).

Mre11 is a highly conserved and protein and has been implicated in resection of 5' end of DNA breaks during their repair (Reis, Batista et al. 2012). It is possible that the polarity of Mre11 is altered by one of its binding partners. Mre11, however, is not the only nuclease acting at telomeric ends. Exo1 and Dna2 were shown to contribute to telomere resection after initial MRX action (Hopkins and Paull 2008, Farah, Cromie et al. 2009). Mutants that bear a deletion of Mre11 or a double mutation of any of the MRX complex members with ScTel1 exhibit very short telomeres (Ritchie and Petes 2000). In addition, deletion of *mre11* causes compromised maintenance of telomeric overhang, but does not affect extension/resection of overhangs in S-phase-dependent telomere replication (Larrivee, LeBel et al. 2004).

Homologous recombination (HR) is a precise DNA repair mechanism in which a broken DNA end can be repaired by copying lost DNA sequence from another homologous chromosome.

Non-homologous end joining (NHEJ) is an error-prone mechanism of DNA repair whereby two broken DNA ends can be joined together irrespective of the original location on a chromosome

Rad50 belongs to the family of proteins responsible for the structural maintenance of chromosomes (SMC). Rad50 molecules can associate into a structure with affinity for double-stranded DNA and can connect DNA ends ~1200 angstroms apart (Hopfner, Craig et al. 2002). Rad50 is also important for telomerase-dependent telomere elongation and helps to recruit telomerase to telomeres (Hopfner, Craig et al. 2002, Williams, Moncalian et al. 2008).

Xrs2 interacts with Mre11, which is poorly conserved among eukaryotes. NBS1, the human homolog of Xrs2, MRE11, and RAD50 form the MRN complex in humans. NBS1 facilitates interaction with DNA damage signaling factors and it is also responsible for transport of the MRN complex into the nucleus (Lloyd, Chapman et al. 2009, Williams, Dodson et al. 2009). While mutations in MRX are tolerated by yeast, various hypomorphic mutations in higher vertebrates including humans lead to deleterious effects including predisposition to cancer, premature ageing, immunological deficiency, infertility and neurological defects (Lamarche, Orazio et al. 2010).

Surprisingly, the signaling PI-3-kinase-related protein kinases, Tel1 and Mec1, are also found at telomeres but are recruited to telomeres in a mutually exclusive manner (Takata, Kanoh et al. 2004). The significance

of this recruitment pattern is not fully understood, but it appears that ScTel1 and ScMec1 are involved in somewhat redundant pathways of telomere maintenance. Deletion of ScTel1 leads to stably short telomeres without loss of cell viability (Ma and Greider 2009). Tel1 promotes telomerase-dependent telomere elongation, but it also prevents telomere-telomere fusions; its recruitment to telomeres is thought to be promoted by the association with the ScMRX (Mre11/Rad50/Xrs2) complex (Nakada, Matsumoto et al. 2003). The deletion of both ScTel1 and ScMec1 leads to telomere loss and growth senescence similar to that seen in telomerase deletion mutants (Ritchie, Mallory et al. 1999). ScTel1 recruitment

Another class of proteins found to associate with telomeres is RecQ helicases. They are motor proteins that can separate strands of DNA, aiding in several processes including DNA and telomere repair. RecQ helicases are conserved from bacteria to humans and play an important role in

Protein kinases are key regulators of cell function. By adding phosphate groups to target proteins, they modulate the function, activity, and localization of many proteins.

maintenance of genome integrity. Yeast DNA helicase, Sgs1, participates in genomic recombination (Yamagata, Kato et al. 1998), restart of stalled replication forks, suppression of inappropriate (homeologous) recombination (Amin, Chaix et al. 2010). Humans have several RecQ helicases crucial for proper cell functioning. When mutated, these helicases cause serious illnesses, also known as Boom Syndrome and Werner Syndrome, respectively (Ashton and Hickson 2010).

IV. TELOMERES AND HEALTH

A. Senescence

In most human cells telomerase has a low or undetectable level of expression (Blackburn 2005, Cifuentes-Rojas and Shippen 2012). Senescence is a permanent cell cycle arrest that prevents division of abnormal cells. When cells encounter deleterious mutations or other types of damage, senescent cells can initiate apoptosis, a programmed cell death. In human cells, critical shortening of telomeres normally leads to a state of permanent cell cycle arrest also known as replicative senescence. Replicative senescence is induced by activation of p53 and Rb tumor suppression pathways (Shay and Wright 2011). *In vitro*, human senescent cells can exhibit several phenotypes: cell cycle arrest, flattened cell morphology, and resistance to apoptosis (programmed cell death). *In vivo*, senescence can be detected by positive β-galactosidase activity and also by induction of telomere dysfunction-induced foci (Sikora, Arendt et al. 2011). These foci form due to aggregation of DNA damage response/repair proteins at telomeres that are recognized as DNA breaks.

Senescence, or prevention of cell division when DNA mutations have been encountered, is a powerful tumor suppression mechanism that is advantageous to an organism during its peak reproductive period (Prieur and Peeper 2008). At the same time, with the increased life span of an individual, cells are more likely to accumulate deleterious mutations. Therefore, senescent cells can also act as pro-tumorigenic factors (Reddel 2010). Persistent DNA damage signaling triggers secretion of chemokine and cytokine inflammatory factors that generate reactive oxygen species (which can be damaging to DNA overall and, especially, telomeres) and activate p53-dependent cell cycle arrest in the neighboring cells. This phenomenon is also known as Senescence-Associated Secretory Phenotype (SASP) (Coppé, Desprez et al. 2010). People

with a chronic inflammatory condition known as Barrett's esophagus are more prone to develop esophageal cancer possibly due to persistent cell exposure to inflammatory factors and oxidative DNA damage (Risques, Vaughan et al. 2007).

An interesting alternative hypothesis about the role of senescence in eukaryotic cells was proposed by Roger Reddel, who argues that a tumor suppressive quality of senescence is a by-product of an ancient antiviral mechanism. According to this hypothesis, senescence is advantageous as a response to abnormal activation of cell proliferation and cell signaling as a result of viral infection. SASP response in one cell can trigger senescence in neighboring cells that are most likely to be infected by the same virus as well. This hypothesis is supported by the observation that viruses have developed a number of mechanisms designed to overcome senescence, apoptosis (programmed cell death), and immune responses of a host (Reddel 2010).

B. Consequences of telomere dysfunction on human health.

Telomere dysfunction leads to a number of human diseases. Dysfunctions in telomerase cause Zinsser-Cole-Engman syndrome or Dyskeratosis Congenita (DC or DKC), characterized by various somatic abnormalities including but not limited to bone-marrow failure, abnormal skin pigmentation, nail dystrophy, acquired aplastic anemia, pulmonary fibrosis, and liver disease (Calado and Young 2009). DC is a genetically inherited disorder that has several genetic subtypes: X-linked, autosomal dominant, and autosomal recessive. The causes of autosomal recessive DC are unknown. X-linked DC stems from mutations in *DKC1*, a nucleolar protein Dyskerin, which is a part of telomerase catalytic holoenzyme (Kirwan and Dokal 2009). Haplodeficiency (a state when one of the two genes in diploid cells is either mutated or deleted) in human telomerase RNA (TERC) causes the autosomal dominant form of DC. Dyskerin and TERC are crucial for telomerase function. People with X-linked and autosomal dominant DC exhibit short telomeres, high rates of genomic instability, and predisposition to cancer.

Mutations in telomere-binding proteins Mre11 and ATM (a homolog of yeast Tel1 kinase) lead to the onset of premature ageing syndrome known as Ataxia Telangiectasia (AT). Patients with this disorder have short telomeres, high levels of genomic instability, increased rates of tumorigenesis, neurodegeneration, premature ageing, immunodeficiency, and high sensitivity to DNA damage (Armanios and Blackburn 2012). AT patients also have dysfunction in ATM-dependent DNA damage signaling pathways, leading to accumulation of unrepaired DNA lesions that trigger genome instability.

Human RecQ helicases, BLM and WRN, when mutated, cause Bloom's syndrome (congenital telangiectatic erythema) and Werner's syndrome. These diseases are associated with higher rates of chromosomal aberrations, slow growth, and increased chance of cancer. In addition, people with Werner's syndrome exhibit signs of premature ageing (Bohr 2008).

C. Human cancers activate telomere maintenance pathways.

Most human cells, except germ and stem cells, do not have an active telomerase meaning that telomeres cannot be actively extended. In addition, DNA replication has innate inability to precisely reproduce the ends of chromosomes and, as a result, a small portion of telomeric DNA is being lost upon every cell division. This leads to gradual telomere shortening over the life span of an individual and it also contributes to the ageing of an organism. Replicative senescence is a permanent cell cycle arrest in human cells that

prevents abnormal cells from entering division or proliferation. Human cells that bypass senescence undergo significant genetic and epigenetic changes, critical telomere shortening, chromosomal damage, genome rearrangements, and eventually death. Only a few cells, approximately 1 x 10^{-7}, can escape mortality (Reddel 2000Reddel 2000). These survivors exhibit significant genomic alterations coupled with activation of a telomere maintenance pathway. Activation of telomerase occurs in ~85-90% of cancers, including melanomas, lymphomas, neuroblastomas, leukemia, and various carcinomas (Cesare and Reddel 2010, Reddel 2010).

A subset of cancers, prevalently originating from mesenchyme and central nervous system tissues, utilizes an HR-dependent mechanism also called Alternative Lengthening of Telomeres (ALT). ALT cancers comprise about 10-15% of all known cancers and can be very aggressive in invasion of organs. ALT cancers achieve telomere elongation by utilization of the protein factors required for DNA repair in normal cells. ALT cancers exhibit telomeres that are more variable in length than those of telomerase-positive cancers or germ cells (Henson, Cao et al. 2009). The ALT-phenotype is also defined by the presence of extrachromosomal linear and circular telomeric DNA (also known as t-circles) (Wang, Smogorzewska et al. 2004). An accurate determinant of the ALT phenotype is enrichment of cancerous cells for C-stand single-stranded t-circles where thousands of C-strand t-circles can be contained in one ALT cell (Henson, Cao et al. 2009). T-circles can form upon aberrant resolution of t-loops. T-loops is a higher-order structure formed when a single-stranded telomeric end strand-invades upstream telomeric sequences. It has been demonstrated that t-loop stabilization mainly depends on the telomere-binding protein TRF2 and that NBS1- and XRCC3-dependent resolution of t-loops yields t-circles that can be double stranded, partially double-stranded, or single-stranded (Wang, Smogorzewska et al. 2004, Compton, Choi et al. 2007).

Cancer cells achieve immortality through activation of telomere elongation pathways. Most human cancers activate enzyme telomerase. A subset of less understood cancers relies on Alternative Lengthening of Telomere pathway (ALT). This pathway utilizes homologous recombination, a mechanism by which telomeres copy sequence from other telomeres or sources of telomeric sequences.

Circular and linear telomeric DNA is often present in ALT cancer cells and is sequestered in ALT-associated promyelocytic leukemia nuclear bodies (APBs) (Reddel and Bryan 2003). Promyelocytic leukemia nuclear (PML) bodies are regularly found in normal cells, and they were demonstrated to participate in DNA repair, apoptosis, tumor suppression, regulation of transcription, and response to viral infection. PML bodies in ALT cells, however, are distinct from those found in normal cells. In addition to circular and linear telomeric DNA, they also contain DNA repair proteins and telomere-specific binding proteins (Dellaire and Bazett-Jones 2004, Henson and Reddel 2010). Considerable evidence from research in various organisms indicates that t-circles can be used as templates to produce long telomeres. In yeast, t-circles can be produced due to mutations in telomere-binding proteins and destabilization of telomeres and serve as templates in extensive telomere lengthening events (Basenko, Cesare et al. 2010, Xu and McEachern 2012).

V. IN CONCLUSION

Telomeres are essential for proper chromosome maintenance in most eukaryotic species. Various model systems have provided

us with information for in-depth understanding of many aspects of telomere maintenance. We have broadened our knowledge of telomere-protein complexes, telomere-related ageing processes, and telomere dysfunction-related human diseases. Despite the vast amount of accumulated information, telomeres are still elusive in some aspects of their function and remain a topic of intense investigations. In particular, the mechanism of telomere maintenance in cancers is not very well understood and requires detailed investigations.

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