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Telomere Structure, Replication and Length Maintenance

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ABSTRACT: Telomeres are the termini of linear eukaryotic chromosomes consisting of tandem repeats of DNA and proteins that bind to these repeat sequences. Telomeres ensure the complete replication of chromosome ends, impart protection to ends from nucleolytic degradation, end-to-end fusion, and guide the localization of chromosomes within the nucleus. In addition, a combination of genetic, biochemical, and molecular biological approaches have implicated key roles for telomeres in diverse cellular processes such as regulation of gene expression, cell division, cell senescence, and cancer. This review focuses on recent advances in our understanding of the organization of telomeres, telomere replication, proteins that bind telomeric DNA, and the establishment of telomere length equilibrium.

KEY WORDS: $TG_{1–3}$ repeats, subtelomeric elements, G4-quadruplex structures, telomere replication, telomerase RNA, telomere end-binding proteins, telomere position effect, transcriptional silencing, heterochromatin, recombinational repair, aging, cancer.

I. INTRODUCTION

Telomeres, the termini of eukaryotic chromosomes, are nucleoprotein complexes comprised of tandem repeats of DNA and proteins that bind to these repeat sequences. These distinguish the natural ends of chromosomes from random breaks inflicted by ionizing radiation, mechanical damage or chemical mutagens. Unlike telomeres, the random breaks are subjected to nucleolytic degradation and to end-to-end fusion with other chromosomes. Additionally, chromosomes lacking telomeres are frequently lost during cell division. Over a century ago, Rabl (1885) first hypothesized that interphase chromosomes occupy distinct territories and are positioned in a telophase orientation throughout the cell cycle. The twin properties of 'Rabl orientation', centromeres located at one pole and distal telomeres at the opposite pole, appears to be phylogenetically conserved (Fussel, 1987). However, the concept of telomeres as special structures at the ends of chromosomes originated from cytogenetic experiments in *Drosophila melanogaster* (Muller, 1938). At approximately the same time, McClintock (1938, 1941) noted that broken chromosomes in maize would fuse with one another to form dicen-

tric chromosomes. Furthermore, she observed that asymmetric breakage of fused chromosomes led to the loss of chromosomes in some tissues and were subjected to repeated cycles of breakage-fusion-breakage during successive cell divisions (reviewed by Blackburn and Szostak, 1984; Zakian, 1989). As chromosomes experienced bridge-breakage-fusion cycle, McClintock occasionally observed that the ends would heal in a developmentally regulated and tissue-specific manner. These studies revealed that the repair of the broken chromatid was limited to a specific time in the cell cycle. The ends would heal during meiotic anaphase and behaved permanently normal throughout subsequent development, thereby implying the presence of an 'end healing' activity in the zygotic tissue. On the other hand, the breakage cycle continued in the endosperm leading to chromosome instability. These observations led to the notion that at least one function of telomere is to confer stability to chromosomal termini from degradation and prevent end-to-end fusions. Furthermore, the biochemical properties of eukaryotic DNA polymerases in conjunction with the mechanism of semiconservative replication of linear DNA presented a conundrum. It was presumed that some mechanism overcomes the problem of replication of the ends of chromosomes. Molecular analysis has now uncovered how telomerases accomplish complete replication of the ends of linear chromosomes (see below).

In recent years there has been a substantial increase in our understanding of the biological roles of telomeres at the molecular level (reviewed by Blackburn, 1992; Zakian, 1996; Greider, 1996). Telomeres are involved in chromosome replication, nuclear architecture, chromosome stability, gene expression, human tumor formation, aging, cell division, and senescence. A number of excellent reviews of telomere structure and length regulation have appeared in recent years (Zakian, 1996; Greider, 1996; Blackburn and Greider, 1995; Rhodes and Giraldo, 1995; Blackburn, 1992). In this review, we focus on the results of recent studies on the organization of telomeres, protein components associated with telomeres, and telomere length regulation.

II. STRUCTURE OF TELOMERES

A. Telomeric DNA

Although the importance of telomeres for stable maintenance of chromosomes was recognized for over 50 years, studies in this area gained momentum in part due to the isolation and characterization of telomeric sequences of *Tetrahymena thermophila* (Blackburn and Gall, 1978). Subsequently, telomeric DNA sequences have been characterized from a number of organisms, including humans. One of the most extraordinary features of telomeres is the conservation of tandemly repeated DNA sequences among distantly related eukaryotes. Telomeric DNA typically consists of 8 to 26 bp G-rich sequence on the strand extending from 5′ to 3′ toward the terminus. Although the bulk of telomeric DNA is double stranded, the extreme terminus contains a 12 to 16 nucleotide 3′ single-stranded overhang that serves as a template for elongation (reviewed by Zakian, 1996). The primary sequence of telomeric repeats has been determined for a great variety of species and the number of these repeats varies among different organisms (Table 1). The repeats, in most organisms, contain three or more guanines embedded on one strand and an identical number of cytosines on the complementary strand. However, a small but diverse set of organisms such as *Schizosaccharomyces pombe*, *Candida*, and *Paramecium* and some higher plants harbor irregular repeat sequences. Telomeric DNA in *Saccharomyces cerevi-*

TABLE 1
Telomeric Repeat Sequences in Eukaryotic Organisms

Organism	Telomeric repeat*	Ref.
Tetrahymena thermophila	TT**GGGG**	Blackburn and Gall, 1978
Oxytricha nova	TTTT**GGGG**	Klobutcher et al., 1981
Physarum polycephalum	TTA**GGG**	Johnson, 1980
Trypanosoma brucei	**GGG**TTA	van Leeuwin et al., 1996
Saccharomyces cerevisiae	$T(\mathbf{G})_{2\text{-}3}(\mathbf{TG})_{1\text{-}6}$	Shampay et al., 1984 Wang and Zakian, 1990a
Kluyveromyces lactis	**GG**TATG**TGGTGT**	McEachern and Blackburn, 1994
Candida pseudotropicalis	GTTATG**TGGTGT**	McEachern and Blackburn, 1994
Candida albicans	TTCT**TGGTGT**	McEachern and Blackburn, 1994
Candida tropicalis	TCAT**TGGTGT**	McEachern and Blackburn, 1994
Candida glabrata	TG**TGGTGT**	McEachern and Blackburn, 1994
Candida guilliermondi	**TGGTGT**	McEachern and Blackburn, 1994
Schizosaccharomyces pombe	$CA_{O\text{-}2}C_{O\text{-}1}\mathbf{G}_{1\text{-}8}$	Sugawara, 1989
Neurospora crassa	TTA**GGG**	Schecthtman, 1987
Chamydomonas reinhardtii	TTTTA**GGG**	Petracek et al., 1990
Chlorella vulgaris	TTTA**GGG**	Higashiyama et al., 1995
Arabidopsis thaliana	TTTA**GGG**	Richards and Ausubel, 1988
Ascaris lumbricoides	TTA**GG**C	Muller et al., 1991
Bombyx mori	TTA**GG**	Okazaki et al., 1993
Human	TTA**GGG**	Moyzis et al., 1988

[a] The nucleotide sequences are in the 5′ to 3′ direction.

siae is not a precise repeat: the double-stranded region consists of ~300 bp heterogeneous telomeric repeat $TG_{2\text{-}3}(TG)_{1\text{-}6}$ (abbreviated $TG_{1\text{-}3}$ or $C_{1\text{-}3}A$) (Shampay et al., 1984; Wang and Zakian, 1990a). The actual amount of double-stranded DNA comprised of these repeats varies considerably among organisms and between individual telomeres in the same organism. Telomeric repeat sequence $(TTAGGG)_n$ is conserved among vertebrates; however, the actual number of repeats varies from 500 to 3000, and in mouse cells they extend up to 100 kb (Moyzis et al., 1988; Allshire et al., 1988; Meyne et al., 1989; Kipling and Cooke, 1990; de Lange et al., 1990; Lejnine et al., 1995). Furthermore, in humans, telomeres in germ line cells are significantly longer than telomeres in somatic tissue (Cooke and Smith, 1986; de Lange et al., 1990; Hastie et al., 1990). Intriguingly, *Leishmania donovani* and *Trypanosoma cruzi* contain abundant amounts of the unusual base β-glucosylhydroxymethyluracil, instead of thymine, in their $(GGGTTA)_n$ telomeric DNA (van Leeuwin et al., 1996). As this unusual base has also been found in *Diplonema*, a marine flagellate, it has been implicated in the repression of transcription or recombination, or a combination of both (van Leeuwin et al., 1998). As telomeres are dynamic structures, the amount of telomeric DNA varies considerably both at individual chromosomes and in different organisms giving telomeric restriction fragments a characteristic fuzzy appearance in Southern analysis.

The configuration of chromosome ends was first analyzed in hypotrichous ciliates. The double-stranded region is followed by 12 to 16 nucleotide G-rich overhang that persists in part, if not all, of the cell cycle (Klobutcher et al., 1981; Henderson and Blackburn, 1989), and during *de novo* telomere formation in *Euplotes* (Roth and Prescott, 1985; Vermeesch

and Price, 1994). During replication of *S. cerevisiae* telomeres, chromosomal ends acquire a transient S-phase-specific single-stranded extension of the G-rich strand. Most notably, cells deleted for *TLC1* gene acquired S-phase specific overhangs, implicating that telomere formation is promoted by a telomerase-independent mechanism (Wellinger et al., 1993; 1996; Dione and Wellinger, 1996). The nature of mouse and human chromosomal termini have been examined by three independent groups. The chromosomes from normal diploid, telomerase-negative, G0-arrested or transformed cells contain single-stranded termini in the range of 45 to 200 nucleotides, an order of magnitude greater than in yeast and ciliates (Makarov et al., 1997; Wright et al., 1997; McElligott and Wellinger, 1997). However, as in yeast and ciliates, Makarov et al. (1997) observed long G-tails on both the ends, while Wright et al. (1997) detected overhangs on only one end of the chromosome. These observations are particularly significant in light of models of telomere replication and telomere length maintenance (see below).

In several organisms, middle repetitive sequences, also termed as telomere-associated DNA are located immediately internal to the terminal telomeric DNA. In *S. cerevisiae*, middle repetitive sequences are composed of two types of elements, called Y′ and X (Figure 1). Y′ is a conserved element that exists in a long (6.7 kb) and a short (5.2 kb) form and is present in one or more copies proximal to the terminal sequence (Chan and Tye, 1983; Louis and Haber, 1990). The X element, located internal to the Y′ element, is relatively less conserved and ranges from 0.3 through 3 kb (reviewed by Blackburn and Szostak, 1984; Zakian, 1996). The middle repetitive elements, such as the telomeric DNA, are dynamic structures that are subjected to both deletion and amplification apparently involving homologous recombination (reviewed by Greider, 1996; Zakian, 1996).

B. Insect Chromosomes Bear Atypical Telomeres

Most of the examples in the foregoing discussion concern canonical telomeric DNA that consists of tandem repeat sequences with three or more guanine residues. Analysis of telomeric DNA in insect species of the order *Lepidoptera*, *Diptera*, and *Hymenoptera*, using telomere-specific probes and cross-hybridization, has revealed certain unusual features. Intriguingly, dipteran insects lack typical telomeric DNA sequences (Biessmann et al., 1990; Levis et al., 1993; Okazaki et al., 1995). However, *Bombyx mori* bears the simple telomeric repeat $(TTAGG)_n$ which is 6 to 8 kb, but is devoid of one guanine residue with respect to the vertebrate telomeric DNA sequences (Okazaki et al., 1993). In addition, two non-LTR-retrotransposons, TRAS1 and SART1, are integrated in the telomeric region of *B. mori* (Okazaki et al., 1995; Takahashi et al., 1997). Likewise, *D. melanogaster* and *Drosophila yakuba*, which are devoid of canonical telomeric DNA sequences, the chromosome termini bear two retrotransposable elements, HeT-A and TART (Traverse and Pardue, 1988; Biessmann et al., 1990; 1996; Levis et al., 1993; Danilevskaya et al., 1994; 1998). On the other hand, *Chironomus* chromosomes carry large 50- to 200-kb tandem repeats at the termini of seven out of their eight chromosomes (Zhang et al., 1994; Lopez et al., 1996). A notable feature of these elements is that they share considerable homology with those of mammalian interspersed repetitive elements, termed LINEs. As telomerase is undetectable in cell-free extracts of *Drosophila*, telomere length appears to be regulated by insertion of retrotransposons at the termini of chromosomes during successive cycles of DNA replication. Taken together, these observations suggest that telomeric DNA in these organisms is replenished very

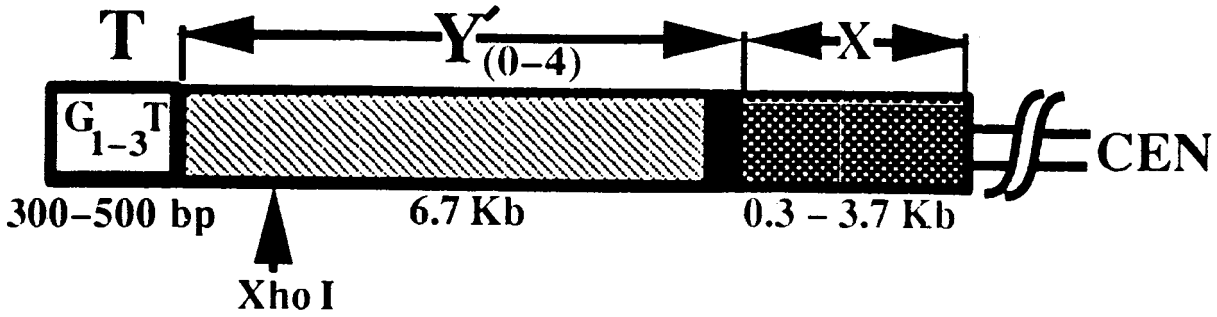

FIGURE 1. Schematic representation of telomere structure of *S. cerevisiae* (Chan and Tye, 1983). X and Y′ are two classes of repetitive elements that occur in varying numbers and are separated by ~100 bp of TG_{1-3} sequence.

differently from those of most other eukaryotes. It has been proposed that maintenance of telomere length in these organisms may involve recombination via gene conversion events (Biessmann and Mason, 1997).

C. Telomeric DNA of Plants

It has long been hoped that the pioneering work of McClintock in the 1930s would help elucidation of the structure and organization of telomeres in plants. However, little additional work was carried out in this area until the telomeric repeats of type $(TTTAGGG)_n$ were isolated and characterized from *Arabidopsis thaliana* (Richards and Ausubel, 1988). Subsequent work showed that the telomeric repeat 5′TTTAGGG is common to chromosomes of several agronomically important species such as maize, tomato, and related plant varieties (Richards and Ausubel, 1988; Ganal et al., 1991; Broun et al., 1992). The telomeric DNA sequence 5′TTTTAGGG of the unicellular green alga *Chlamydomonas reinhardtii* (Petracek et al., 1990) is different from that of higher plants. In contrast, telomeric DNA in a related species, *Chlorella vulgaris* (Higashiyama et al., 1995), is identical to those of higher plants. Additionally, the latter contains multiple copies of *Zepp* retrotransposon at its telomeric and subtelomeric regions (Higashiyama et al., 1997). The canonical telomeric repeats of type $(TTTAGGG)_n$ have not been detected in plants such as *Allium*, rice, and wheat (Ashikawa et al., 1994; Cheung et al., 1994; Pich et al., 1996). Instead, these plants bear a family of highly polymorphic telomere-associated DNA repeats. Furthermore, *in situ* hybridization experiments in wheat and rice have revealed that telomere-associated sequences are present at or near the ends of chromosomes, and are also prevalent at interstitial sites (Mao et al., 1997; Ohmido and Fukui, 1997). Telomerase activity has been detected in the meristematic tissue and in undifferentiated cells of *Arabidopsis thaliana*, soya bean, suspension cultures of carrot, barley embryo, anther, but not in the differentiated tissues (Fitzgerald et al., 1996; Heller et al., 1996). In plants such as *Allium*, rice, and wheat that are devoid of canonical telomeric DNA repeats, exchange of termini between nonhomologous chromosomes has been implicated as an alternative mechanism of telomere replenishment (see below).

D. Structural Organization of Telomeric DNA

Earlier studies have shown that RNA and DNA having as few as four contiguous guanine residues associate into four-stranded right-handed helices *in vitro* (Gellert et al., 1962; Zimmerman et al., 1975). More recently, G-rich sequences located at the immunoglobulin switch regions and the retroviral dimer linkage sequences have been found to form parallel four-stranded structures (Sen and Gilbert, 1988; 1990; Sundquist and Heaply, 1993; Awang and Sen, 1993). Although the chromosome ends of few organisms do not terminate with G-rich single-stranded overhang, the extensive conservation of G-rich sequence among evolutionarily divergent species predicts an important function. The formation of G4-DNA by synthetic oligonucleotides having tandem arrays of G-residues is well established (Sundquist and Klug, 1989; Williamson et al., 1989). The fundamental unit in G4-DNA is the G-tetrad assembled by the association of four guanines, where each guanine serves as both hydrogen bond acceptor and donor in a Hoogsteen base pair generating successive layers of G-quartets (reviewed by Rhodes and Giraldo, 1995).

G-quartets are formed within a single DNA molecule or between two DNA molecules. Although G-strands self-associate to form G-quartets in physiological conditions, their formation and stability is greatly enhanced by K^+ and Na^+ that position in the axial cavity of the helix. A large number of studies using NMR, circular dichroism spectroscopy, and crystal structures have revealed the main features of the G-quartet structure (Kang et al., 1992; Wang and Patel, 1992; Smith and Feigon, 1992; Balagurumoorthy and Brahmachari, 1994). In addition, these studies have also identified three major helical forms of G-quartets, distinguished by parallel or *cis* or *trans* antiparallel strand orientations (reviewed by Rhodes and Giraldo, 1995). Additional evidence for the existence of G-quartets has come from various approaches, including chemical, biochemical, and structural methods (reviewed by Williamson, 1994). If G-quartets were to arise *in vivo*, what could be the biological significance? A possible function for G-quartets has been envisaged, but not experimentally proven yet, in the association of sister chromatids in mitosis or pairing of homologous chromosomes during meiosis (Sen and Gilbert, 1988; Sundquist and Klug, 1989; Williamson et al., 1989).

E. Telomere-Binding Proteins

The kinetics of the formation of G-quartets *in vitro* is a slow process and occurs at high oligonucleotide concentrations (micromolar-to-millimolar). Consequently, it is suggested that their assembly may involve specific proteins (Sen and Gilbert, 1988; Sundquist and Klug, 1989). This notion is supported by the isolation and characterization of a number of telomeric DNA-binding proteins from diverse sources (Table 2). Two groups of telomere-binding proteins have been distinguished based on their specificity for single- or double-stranded telomeric DNA. The most well-characterized telomere end-binding proteins are from the ciliated protozoa, *Oxytricha* and *Euplotes* (Gottschling and Zakian, 1986; Price and Cech, 1987; Price, 1990). In *Oxytricha*, the telomere protein complex is comprised of a heterodimer of α (56 kDa) and β (41 kDa) polypeptides, while in *Euplotes* it consists of a single polypeptide (51 kDa) (Price et al., 1992; Fang et al., 1993). Genetic and molecular biological studies suggested that other organisms such as *S. cerevisiae* and humans also possess telomere-binding factors. Recent work has demonstrated that Est1 protein and Cdc13 protein from *S. cerevisiae* bind specifically to single-stranded $TG_{1\text{-}3}$ sequence *in vitro* (Virta-Pearlman et al., 1996; Lin and Zakian, 1996; Nugent et al., 1996). Both Est1p and Cdc13p show similar substrate specificity. However, Est1p requires a free 3′ end. By genetic analysis it has been shown that Ku protein, previously implicated in nonhomologous DNA end-joining, is required with telomerase and Cdc13p to protect the ends of chromosomes (Nugent et al., 1998; Gravel et al., 1998).

In human cells, de Lange and colleagues have identified telomere-binding proteins that bind preferentially to the TTAGGG repeats *in vitro*. These include Trf1p and Trf2p, which are localized to all telomeres of human metaphase chromosomes throughout the cell cycle (Chong et al., 1995; van Steensel and de Lange, 1997; Broccoli et al., 1997). Significantly, Trf1p and Trf2p appear to play distinct roles at telomeres: Trf1p functions as a suppressor of telomere elongation, while Trf2p contributes to the maintenance of correct structure of telomeres, thereby preventing end-to-end fusion of chromosomes (van Steensel and de Lange, 1997; van Steensel et al., 1998). Similarly, a protein with expected features of an end-binding activity has been detected in the cell-free extracts of *Xenopus*

TABLE 2
List of Telomere-Binding Proteins from Various Organisms

Telomere-binding protein	Organism	Telomere DNA	Ref.
Rap1(Repressor activator protein)	*S. cerevisiae*	Double-stranded promotes formation of G4 DNA	Gilson et al., 1993; Giraldo and Rhodes, 1994; Konig et al., 1996
Tbf1p/TBPα (Telomere-binding factor 1)	*S. cerevisiae*	Double-stranded	Liu and Tye, 1991
Est1p (Ever shortening telomere)	*S. cerevisiae*	Single-stranded	Virta-Perlman et al.. 1996
Cdc13p (Cell division cycle)	*S. cerevisiae*	Single-stranded	Nugent et al., 1996; Lin and Zakian, 1996
G_4P_2 (G4-binding protein)	*S. cerevisiae*	G4 nucleic acid	Frantz and Gilbert, 1995
Single-stranded binding protein	*S. cerevisiae*	Single-stranded	Santori and Donini, 1994
Tel2 protein	*S. cerevisiae*	Double-stranded	Kota and Runge, 1998
TBPα-β (Telomere-binding protein)	*Oxytricha*	Single-stranded; promotes formation of G4 DNA	Fang and Cech, 1993a; 1993b; Raghuraman and Cech, 1989
TBP (Telomere-binding protein)	*E. crassus*	Single-stranded	Price, 1990
rTP (replication Telomere Protein)	*E. crassus*	G-rich DNA	Carlson et al., 1997
TGP (*Tetrahymena* G4-binding Protein)	*T.thermophila*	G4-DNA	Schierer and Henderson, 1994
TEP (Telomere end-binding protein)	*T. thermophila*	Single-stranded	Sheng et al., 1995
GBP (G-strand binding protein)	*C. reinhardtii*	Single-stranded	Petracek et al., 1994
PPT (*Physarum* (*polycephalum* TBP)	*Physarum*	Double-stranded	Coren et al.,1991
XTEF (Xenopus telomeric end factor)	*Xenopus*	Single-stranded	Cardenas et al., 1993
mTRF1 (Telomere repeat binding factor	Mouse	Double-stranded	Zhong et al., 1992
hTRF1p	Human	Double-stranded	Chong et al., 1995
hTRF2p	Human	Double-stranded	Broccoli et al., 1997; van Steensel et al., 1998

TABLE 2A
Non-Telomeric Factors That Recognize Telomeric DNA Sequences

MyoD	Mammals	G4 quadruplex	Walsh and Gaulberto, 1992
Xrn1 nuclease	Mouse	G4 RNA tetraplex	Bashkirov et al., 1997
Histone H1	Rat	G-DNA	Fang and Cech, 1993
α-thrombin	Human	G-DNA	Wang et al., 1993
Macrophage factor	Bovine	G-DNA	Pearson et al.,1993
HeLa hn RNPs A2/B1	Human	Single-stranded	Ishikawa et al., 1993
Kem1p (Karyogamy enhancing mutation)	*S. cerevisiae*	G4 quadruplex	Liu et al., 1993; Liu and Gilbert, 1994
Topoisomerase II	Chicken	Double-stranded; G4 DNA	Chung et al., 1992
Hepatocyte factor QUAD	Rabbit	Single-stranded	Weisman-Shomer and Fry, 1993
qTBP42p	Rat	Single-stranded; G4-DNA	Sarig et al., 1997
G4-DNA resolvase	Human	G4-DNA	Harrington et al., 1997
qTBP25p	Rat	Single-stranded; G4-DNA	Erlitzki and Fry, 1997

and *Tetrahymena* (Cardenas et al., 1993; Sheng et al., 1994; Schierer and Henderson, 1994). It has been suggested that these proteins may cap chromosome termini against exonucleolytic resection and may regulate telomerase activity, either positively or negatively (Gray et al., 1991; Price, 1990; Shippen et al., 1994; Vermeesch and Price, 1994). Consistent with this, proteins from ciliated protozoa have been shown to protect telomeres from exonucleolytic degradation *in vitro* (Gottschling and Zakian, 1986; Price and Cech, 1987).

Among the proteins that bind double-stranded telomeric DNA, Rap1 protein (repressor activator protein 1) of *S. cerevisiae* is the best characterized, both as an activator and repressor of transcription (Shore and Nasmyth, 1987; Buchman et al., 1988a; 1988b). *S. cerevisiae* telomeres contain ~20 sites having the sequence 5′GGTGTGTGGGTGT3′ for DNA-binding of Rap1 protein (Gilson et al., 1993). The minimal DNA-binding domain of Rap1 protein consists of a large portion (residues 361 to 596) located in the center of the molecule (Giraldo and Rhodes, 1994). The crystal structure of DNA-binding motif of Rap1 protein has indicated similarity to the DNA-binding motif of *Kluyeromyces lactis* Rap1p, human telomere-binding protein, and Myb family of transcription factors (Konig et al., 1996; Konig and Rhodes, 1997). More importantly, Rap1p of *S. cerevisiae* and β-subunit of *Oxytricha* telomere-binding proteins have the ability to catalyze the formation of G-quartets *in vitro* (Giraldo and Rhodes, 1994; Fang and Cech, 1993).

Using a combination of conventional and DNA quadruplex-affinity chromatography, a nuclease was purified from the cell-free ex-

tracts of*S. cerevisiae* specific for G4-DNA (Liu et al., 1993; Liu and Gilbert, 1994). A nuclease encoded by *KEM1* gene, also known as *SEP1*/*DST2*/*XRN1*/*RAR5* (Kim et al., 1990; Dykstra et al., 1991; Larimer and Stevens, 1990; Kipling et al., 1991), cleaves single-stranded region 5′ to the G4 quadruplex structure (Liu et al., 1993; Liu and Gilbert, 1994). Furthermore, *sep1*Δ mutants experience higher rates of chromosome loss during mitosis and were arrested at pachytene presumably due to the failure to process G4 structures (Bahler et al., 1994; Tishkoff et al., 1995). Based on these observations it has been conjectured that G4-DNA may arise during the course of chromosome alignment or recombination (Sen and Gilbert, 1988; Kim et al., 1990; Bahler et al., 1994). Using G4-DNA-affinity matrix, Frantz and Gilbert (1995) have purified a protein designated as G4p2, encoded by *MPT4*/*STO1*, from cell-free extracts of *S. cerevisiae* that displays high specificity to nucleic acids harboring G4 quadruplex structures. Recently, mouse exoribonuclease, a homologue of *S. cerevisiae* Xrn1 nuclease, having preference for G4-RNA tetraplex has been reported (Bashkirov et al., 1997). In addition, proteins have been isolated from a variety of sources, including humans that bind single- and/or double-stranded telomeric DNA sequences (Table 2). However, the biochemical function of such recognition by some of these proteins is obscure.

F. Chromatinization of Telomeric DNA and Telomere Position Effects

A number of findings have brought into focus the possibility of the incorporation of $TG_{1–3}$ repeats and subtelomeric DNA into a specialized chromatin structure (Zakian, 1996). The characterization of tightly bound telomere-binding proteins in ciliated protozoa and yeast reinforced the idea that these proteins may confer a putative chromatin structure on telomeric DNA. Nuclease mapping and salt solubility experiments indicate that telomeric DNA repeats in *Oxytricha* and *Tetrahymena* are in a nonnucleosomal structure (Gottschling and Cech, 1984; Price and Cech, 1987; Budarf and Blackburn, 1986). Similarly, in *S. cerevisiae*, nuclease mapping of telomeric region on chromosomal DNA and incubation of protein-DNA complexes with salt suggested a distinctive supramolecular structure (Wright et al., 1992; Wright and Zakian, 1995). Genetic and cytological experiments have established that Rap1 protein is the major structural protein directly associated with telomeres *in vivo* (reviewed by Zakian, 1996; Greider, 1996). Consequently, reconstitution, immunoprecipitation with anti-Rap1p, and crosslinking experiments indicate that Rap1p is complexed with Sir2, Sir4, and Sir3 proteins. These results suggest that Sir2-4 protein complex organizes telomeric DNA into heterochromatin-like structure (Conrad et al., 1990; Wright et al., 1992; Palladino et al., 1993; Strahl-Bolsinger et al., 1997). The protein-DNA complex formed with TG_{1-3} repeats and end-binding proteins is called a telosome to distinguish it from the typical nucleosomal structure (Wright et al., 1992; Zakian, 1996). The nonconventional features of a telosome are that it contains larger amount of DNA than a typical nucleosome and that its proximal boundary has a DNaseI-hypersensitive site. In *S. cerevisiae*, telosomes are resistant to concentrations of ethidium bromide that are sufficient to disrupt the nucleosomal structure but are somewhat less stable to salt (Wright and Zakian, 1995). Interestingly, the nonconventional nucleosomal pattern of telomere is conserved in several other organisms including humans (Budarf and Blackburn, 1986; Tommerup et al., 1994). Nuclease mapping

experiments indicated that subtelomeric repeats X and Y′ were incorporated into nucleosomes. However, these structures differ from the conventional nucleosomes in two ways. First, GATC sites in the telomere proximal DNA are refractory to methylation *in vivo* by *Escherichia coli dam* methylase (Gottschling, 1992). Second, histone octamers within the subtelomeric nucleosomes are hypo-acetylated compared with histones found elsewhere in the genome (Brachmann et al., 1995).

In addition to the maintenance of genomic integrity, telomeres act as silencer elements repressing the expression of genes inserted in their vicinity. In *S. cerevisiae*, biochemical and genetic evidence indicates that genes positioned within or near telomeres suffer transcriptional repression, a phenomenon called telomere position effect (hereafter called TPE) or telomere silencing (Gottschling, 1992). Similarly, an origin of replication positioned proximal to a telomere is subjected to late initiation, whereas the same site located elsewhere in the genome initiates replication early in S-phase (McCarroll and Fangman, 1988; Ferguson and Fangman, 1992). TPE is typically assessed by inserting a reporter gene adjacent to a telomere and assaying for transcriptional repression (Gottschling, 1992). It has been suggested that TPE begins at the telomere and extends continuously inward toward the centromere. Consistent with this, *in vivo* experiments showed that silencing of a reporter gene gradually declines with its distance from the telomere (Renauld et al., 1993). TPE is relieved by mutations in a variety of genes that are known to modify the chromatin structure. The mechanism underlying TPE in yeast and the related phenomenon of position-effect variegation in *D. melanogaster* appears to involve the organization of transcriptionally repressed regions of the genome into heterochromatin-like structure (Levis et al., 1985; Wallrath and Elgin, 1995).

Genetic studies in *S. cerevisiae* have uncovered a surprising and provocative relationship between silencing at telomeres and silencing at the *HML* and *HMR* loci on chromosome III. Specifically, many of the *trans*-acting factors that are involved in transcriptional repression at the *HM* loci (Klar et al., 1979; Rine et al., 1979; Rine and Herskowitz, 1987) also assist in the establishment of TPE (Aparicio et al., 1991). However, unlike TPE, the transcriptional repression at the *HM* loci is extremely stable and must remain repressed for the cells to manifest the appropriate cell type. It has been demonstrated that Rap1p directly binds to specific sites adjacent to the silencer elements flanking the silent mating-type loci (Shore and Nasmyth, 1987; Buchman et al., 1988b). Rap1 protein binding sites are abundant within the telomeric $TG_{1–3}$ repeats as well as in the upstream activating regions of many genes. Several gene products have been implicated in TPE. The best characterized include the silent information regulators Sir2p, Sir3p and Sir4p as well as histone H3 and H4 (Aparicio et al., 1991; Kurtz and Shore, 1991; Liu et al., 1994; Thompson et al., 1993). At present, the biochemical functions of Sir proteins are obscure, but none of them contain typical DNA-binding motifs. However, Sir2p forms a complex with Sir4p, which in turn interacts with Sir3p and finally this complex associates with Rap1p at the carboxyl terminal domain (Chien et al., 1991; Moretti et al., 1994; Strahl-Bolsinger et al., 1997). Consistent with these results, truncation of the carboxy terminus portion of Rap1 protein lead to complete loss of TPE and *HML* silencing (Kyrion et al., 1993). Direct interaction of Sir3p-Sir4p complex with the amino-termini of histone H3 and H4 suggests that TPE is attained by the interaction of Sir proteins with nucleosomal DNA (Hecht et al., 1995). In a wild-type strain, TPE extends ~2 to 4 kb toward centromere, and an increase in the cellular concentration of Sir3p further extends

it as much as 20 kb through packaging of DNA into heterochromatin-like structure. These data indicate that maximal silencing requires the participation of telomere-binding proteins. For example, high-copy number plasmids containing $C_{1\text{-}3}A$ repeat sequences de-repress $C_{1\text{-}3}A$-dependent silencing but have minor effect on TPE (Stavenhagen and Zakian, 1995), while same repeats present on linear plasmids relieved both TPE and $C_{1–3}A$-dependent silencing (Wiley and Zakain, 1995).

Recent genetic studies have suggested that the largest subunit of chromatin assembly factor I encoded by *CAC1*/*RLF2* gene is required for the stable inheritance and maintenance of transcriptionally repressed state of chromatin structure at telomeres (Enomoto et al., 1997; Monson et al., 1997; Enomoto and Berman, 1998). Consequently, *cac1*/*rlf2* mutant displays reduced telomere silencing and mislocalization of Rap1p at the telomeric, but not subtelomeric region. Additionally, loss of Cac1p, or other subunits (Cac2p and Cac3p/Msi1p) of CAF-I complex, led to complete repression at the silent mating loci (Enomoto et al., 1997). Together, these data suggest that Cac1p/Rlf2p promote normal distribution of Rap1p within the nucleus and efficient incorporation of telomeric DNA into nucleosomes.

How might the process of heterochromatinization of telomere and adjacent DNA be accomplished under physiological conditions? Grunstein and his colleagues have proposed that telomeric or internal tracts of $TG_{1–3}$ repeats serve as nucleation centers for the formation of heterochromatin-like structure by Rap1 protein. According to this model, Rap1 protein binds to telomeric repeats where it recruits Sir2p, Sir3p, and Sir4p to the chromosome through interactions with its carboxy-terminal motif. Sir protein complexes polymerize further into adjacent nucleosome through interactions with histone H3 and H4 (Strahl-Bolsinger et al., 1997). Consistent with this model, overexpression of Sir3p or increasing the number of $TG_{1–3}$ repeats culminated in the formation of extended telomeric heterochromatin (Kyrion et al., 1993; Renauld et al., 1993; Stavenhagen and Zakian, 1994; Strahl-Bolsinger et al., 1997). A further link has been provided by recent studies that the C-teminus of Rap1p enhances telomere formation by providing access to telomerase (Ray and Runge, 1998).

Cytological studies have demonstrated that telomeres play a key role in establishing the three-dimensional architecture of the nucleus. Telomeres often associate with each other and are frequently anchored to a region of the nuclear envelope in interphase nucleus (Holmquist and Dancis, 1979). Three-dimensional reconstruction of *Drosophila* salivary gland nuclei showed self-association and polarized arrangement of telomeres under the nuclear envelope throughout prophase and metaphase (Hiraoka et al., 1990). In *S. cerevisiae*, Rap1 protein localizes to the nuclear periphery in wild-type mitotic cells as well as meiotic chromosomes (Klein et al., 1992). Interestigly, mutations in *HDF1* and *HDF2* disrupt the subnuclear localization of telomeres (Laroche et al., 1998). In mammalian cells, telomeres co-fractionate with nuclear matrix, bind to nuclear lamins, and are anchored to the nuclear periphery (de Lange, 1992; Shoeman and Traub, 1990; Markova et al., 1994).

G. Telomeres Facilitate Pairing of Homologous Chromosomes

Recognition between homologous chromosomes and pairing are the first steps in the meiotic process. A possible role for telomeres in the alignment of homologues was deduced from observations of telomere clustering in meiotic cells (Loidl, 1990; Scherthan et al., 1994). In many organisms, early

cytological studies noted that meiotic chromosomes adopt a bouquet-like arrangement with their telomeres anchored to a small region of the nuclear periphery (Dernberg et al., 1995; Scherthan et al., 1996; Bass et al., 1997). Toward this end, *S. pombe* has emerged as an instructive model to elucidate the role of telomeres in meiotic recombination. Visualization of nuclear events during meiotic prophase suggest that telomeres gather adjacent to the spindle pole body (SPB) (Chikashige et al., 1994; Scherthan et al., 1994). Furthermore, the zygotic nuclei oscillate back and forth through the cell, termed 'horsetail' movement, led by SPB (Chikashige et al., 1994; 1997). The ends of chromosomes anchored to the SPB, and the telomere-led movement of chromosomes, is believed to facilitate the alignment of homologues and recombination. Thus, mutations that diminish telomere clustering in *S. pombe* display defects in meiotic recombination (Shimanuki et al., 1997). More recently, two groups have provided compelling evidence that telomeres are vital for pairing of homologues and recombination (Nimmo et al., 1998; Cooper et al., 1998). Earlier studies showed that *S. pombe TAZ1* encodes a telomere-binding protein related to the Myb family of transcription factors (Cooper et al., 1997). A paradoxical feature of *taz1* mutants was the formation of aberrant tetrads with inviable spores. Recent studies have indicated that *taz1* mutants display scattering of telomeres and decreased association with the SPB during meiotic prophase (Nimmo et al., 1998; Cooper et al., 1998). These results are consistent with an important role for Taz1p in telomere clustering, recombination, and segregation of homologous chromosomes. In *S. cerevisiae*, Tam1/Ndj1p, a protein implicated in homologue pairing, is localized to the ends of meiotic chromosomes (Conrad et al., 1997; Chua and Roeder, 1997). Consistent with this, *tam1*/*ndj1* mutants display mispairing between chromosomes and considerable delay in chromosome synapsis.

III. REPLICATION OF TELOMERIC DNA

The problem of completion of replication of a linear chromosome by the conventional mechanisms of DNA replication has been recognized for a number of years (Watson, 1972). Consequently, chromosomes suffer gradual loss of terminal sequences, a phenomenon termed the 'end replication' problem. Although experimental evidence to this puzzle was lacking, several schemes for replication of the ends of chromosomes have been proposed (reviewed by Blackburn and Szostak, 1984). Olovnikov (1973) proposed a alternative mechanism for initiation of replication from the ends of eukaryotic linear chromosomes. An insight into 'end replication' problem has its roots in a series of experiments showing that hypotrichous ciliates fragment and process genomic DNA during their life cycle. During this process, simple repeat sequences of variable length are added *de novo* onto chromosome ends suggesting the involvement of a novel template-independent DNA-processing activity (Boswell et al., 1982; King and Yao, 1983). Similarly, the broken ends of *Oxytricha* chromosomes were also shown to elongate in yeast cells (Pluta et al., 1984; Walmsley et al., 1984). Telomerase activity has been identified in cell-free extracts of a wide variety of organisms (Zahler and Prescott, 1989; Morin, 1989; Shippen-Lentz and Blackburn, 1989; Prowse et al., 1993; Cohn and Blackburn, 1995; Fitzgerald et al., 1996). Subsequently, telomerase has been isolated as a protein-RNA complex, and the latter functions as an internal template for synthesis of telomeric DNA (Greider and Blackburn, 1985; Shippen-Lentz and Blackburn, 1989).

The genes encoding telomerase RNA component and the catalytic subunits of telomerases have been isolated and analyzed from multiple organisms, including humans. In *Tetrahymena*, telomerase contains a tightly bound 159-nucleotide RNA component with several arrays of the sequence 5′CAACCCCAA3′ (Greider and Blackburn, 1989). Additionally, highly purified preparations of *Tetrahymena* telomerase contained two protein subunits of 80 and 95 kDa (Collins et al., 1995). p80 could be cross-linked to telomerase RNA and p95 to telomeric DNA primers in an RNA-dependent manner (Collins et al., 1995). Likewise, *Euplotes aediculatus* telomerase consists of an RNA component and two protein subunits, p123 and p43, that appear to be unrelated to p80 and p95 (Lingner and Cech 1996). Phylogenetic comparisons of telomerase RNA sequences have revealed an overall sequence divergence of ~35% but retain elements of conserved secondary structure (Romero and Blackburn, 1991). The essential role of RNA for telomerase function *in vivo* was ascertained by introducing a mutant telomerase RNA containing base changes in the template motif into *Tetrahymena*. Such cells incorporated altered sequences on to their chromosomal termini and consequently displayed defects in macronuclear division (Yu et al., 1990).

In *S. cerevisiae*, telomerase is associated with an RNA sub unit of ~1.3 kb, encoded by the *TLC1* gene, that is essential for the maintenance of normal telomeres (Singer and Gottschling, 1994; Cohn and Blackburn, 1995; Prescott and Blackburn, 1997a). Genetic studies have identified four *EST* (*E*ver *S*hort *T*elomere) genes, *EST1*, *EST2*, *EST3*, and *EST4* that were essential for the maintenance of telomeres (Lundblad and Szostak, 1989; Lendvay et al., 1996). Cells bearing mutations in any one of these genes exhibit a phenotype indistinguishable from those cells carrying *tlc1*D mutation (Lendvay et al., 1996). Analysis of *EST2* nucleotide sequence revealed that it encodes a protein of ~103 kDa with no significant homology to Est1p (Lingner et al., 1997). Epistasis analysis suggested that all the four genes function in the same pathway as *TLC1*, implicating all the four in the regulation of telomere length. Similarly, *ter1*Δ mutant of *K. lactis*, a homologue of *TLC1*, displayed slow growth and shortened telomeres, but a small percentage of cells quelled their growth defect coincident with massive lengthening of telomeres (McEachern and Blackburn, 1995). Consistent with earlier models (reviewed by Zakian, 1996), *S. cerevisiae* telomerase consists of two RNA subunits in a single telomerase complex and both are required for DNA polymerization (Prescott and Blackburn, 1997b). It has been speculated that this novel property may enable telomerase to function as a structural component of the telomere throughout all or part of the cell cycle.

S. cerevisiae Est1p, 77 kDa, contains single-stranded telomere end-binding activity (Virta-Pearlman et al., 1996). Although the *in vivo* function of Est1p is obscure, the absence of additive or synergetic effect of *est1*/*tlc1* double mutants indicate that both act in the same pathway of telomere replication (Lundblad and Blackburn, 1993; Singer and Gottschling, 1994). However, Est1p appears not to be a component of the holoenzyme as cell-free extracts from *est1*Δ cells retained full levels of telomerase activity (Cohn and Blackburn, 1995). Intriguingly, Est1p coimmunoprecipitated with the *TLC1* RNA, suggesting that Est1p is an integral component of telomerase (Lin and Zakian, 1996; Steiner et al., 1996; Virta-Pearlman et al., 1996).

Human telomerase contains an RNA subunit of ~450 nucleotides with no sequence homology to any of the lower eukaryotic telomerase RNA components (Feng et al., 1995). However, the RNA subunit of mouse telomerase is significantly larger than that

of humans and ciliates (Blasco et al., 1995). Recently, protein components associated with mammalian telomerase activity have been identified. The protein associated with telomerase complex in human (designated as *TP1*), mouse, and rat (*TLP1*) are larger than the *Tetrahymena* 80-kDa subunit (Harrington et al., 1997; Nakayama et al., 1997). Furthermore, proteins from mouse and rat are both 2629 amino acids in length (human *TP1* is 2 amino acids shorter) and produce p240 and p230 that co-purify with telomerase activity (Nakayama et al., 1997). Intriguingly, the pattern of expression of *TLP1* of rat, or *TP1* of human and mice, do not coincide with the activation of telomerase activity (Harrington et al., 1997; Nakayama et al., 1997). Recent studies have identified a second, but bona fide, catalytic subunit of human telomerase, encoded by h*TRT*, that has significant sequence similarity to those of Est2p/p123 (Meyerson et al., 1997; Nakamura et al., 1997; Harrington et al., 1997; Kilian et al., 1997). The expression of hEst2p is tightly associated with the onset of telomerase activity (Meyerson et al., 1997; Nakamura et al., 1997). Taken together, these studies suggest that human telomerase contains both Est2p homologue as catalytic subunit and a set of weakly associated but conserved proteins that may play a structural or regulatory role.

The observation that telomerases are RNA-dependent DNA polymerases that use RNA as the template inspired comparisons between the catalytic subunit and the conventional reverse transcriptases encoded by retroviruses. This formal similarity was realized with the identification and comparison of sequence homology among catalytic protein subunits of telomerase of *S. cerevisiae*, *E. aediculatus*, and *S. pombe*. Significantly, the telomerase catalytic subunits of these organisms bear signature residues of reverse transcriptases (Lingner et al., 1997; Counter et al., 1997; Nakamura et al., 1997). Mutations in the reverse transcriptase motifs of Est2p displayed shortened telomeres, decrease in telomerase activity, and senescent phenotype. Like the yeast and ciliate telomerase proteins, hEst2p is a member of the reverse transcriptase family of enzymes (Meyerson et al., 1997).

The isolation of a gene-encoding mouse telomerase RNA component has opened up experimental approaches to rigorously test the significance of telomerase for genomic stability, cell viability, and tumorigenesis in mammals. Recent data from Greider and DePinho laboratories suggest that mice deleted for telomerase RNA produced progeny that were normal and fertile up to six generations (Blasco et al., 1997). However, FISH analysis of chromosomes revealed progressive telomere attrition and substantial increase in chromosome abnormalities in cells derived from the $mTR^{-/-}$ mouse (Blasco et al., 1997). Additionally, the phenotypic analysis of highly proliferative organs of progeny mice indicated overall clinical abnormalities. These effects coincided with substantial erosion of telomeres, fusion and loss of chromosomes, defective spermatogenesis, and increased apoptosis (Lee et al., 1998). Taken together, these data suggest that telomerase is vital for the maintenance of genomic stability and the long-term viability of cells in actively proliferating organs.

A. Assay of Telomerase Activity

The substrate specificity and elongation processivity of telomerases have been characterized by primer extension assays using single-stranded DNA as the substrate.

The telomerase activity is normally assayed in enriched nuclear extracts by the elongation of a G-strand telomeric oligonucleotide by the addition of radiolabeled precursors. Telomerases isolated from all organisms require appropriate NTPs, telomeric

RNA primer (>12 mer), and are inhibited by pyrophosphate at millimolar concentrations and ionic strength at >100 m*M* (reviewed by Greider, 1996). *In vitro* selection experiments have been developed with a labeled G-strand oligonucleotide as the substrate to assay telomerase activity in human cells (Kim et al., 1994) and in yeast (Lin and Zakian, 1996; Lue and Wang, 1995). Samples are subjected to PCR amplification to obtain an enriched pool of oligonucleotides. The products are assayed for the increase in the size of the labeled oligonucleotide by agarose gel electrophoresis. These results have indicated that telomerase contains at least two binding sites: one that aligns substrate with the RNA template and the second interacts with residues 5′ to the aligned region and functions as an anchor site for high-affinity binding and processive elongation (Harrington and Greider, 1991; Morin, 1989; Collins and Greider, 1993).

B. Synthesis of Cytosine-Rich Strand

To completely replicate telomeres, the complementary strand to the leading G-strand must be synthesized for net elongation of telomeric DNA. Although this could be accomplished by polα/primase of DNA replication apparatus, it is not clear whether G-rich strand could serve as a substrate *in vivo*. In *Oxytricha*, an enzymatic activity has been detected in the cell-free extracts that can initiate C-strand synthesis (Zahler and Prescott, 1989). Furthermore, the physical structure of the ends of newly formed telomeres during development of *Euplotes* was found to be precise (Vermeesch and Price, 1994), suggesting that the synthesis of the complementary C-rich strand appears to be a regulated process. More recently, cell-free extract from mammalian cells has been assayed to test the mechanism of lagging strand synthesis (Reveal et al., 1997). Characterization of the catalytic activity suggest that RNA priming and DNA polymerization by DNA polα/primase complex has a role in telomere maintenance. The exact mechanism for the formation of resected telomeric DNA is unknown, but it probably entails degradation of C-rich strand by strand-specific exonuclease (Wellinger et al., 1996). For example, *MEC3* in conjunction with *RAD24* and *RAD17* degrade the C-rich strand of telomeric and subtelomeric DNA consequent to DNA damage in *S. cerevisiae* (Lydall and Weinert, 1995). Two lines of evidence implicate conventional DNA pol α/primase complex in telomere metabolism. First, *cdc17*^{ts} cells bearing mutations in DNA polα carry elongated telomeres (Carson and Hartwell, 1985; Adams and Holm, 1996). Second, *mec3 pri1* double mutants are synthetically lethal (Longhese et al., 1996). The most straightforward interpretation of all the data is that DNA pol α/primase complex is essential for telomere maintenance.

IV. REGULATION OF TELOMERE LENGTH

Telomeres are dynamic structures whose length is modified in continuously growing normal eukaryotic cells as well as tumor cells (reviewed by Greider, 1996). In most organisms, the telomere length on any given chromosome end is not fixed. Evidence from a number of studies indicate that telomere length is maintained by a balance between processes that lengthen and those that shorten telomeres (McClintock, 1941; Bernards et al., 1983; Haber and Thornburn, 1984; Surosky et al., 1986; Jager and Philipsen, 1989; Larson et al., 1987; Yu and Blackburn, 1991; Muller et al., 1991). Consequently, the maintenance of telomere length is a process of stagger**ing**

complexity and mutations in several genes, including those that are relevant to DNA metabolism affect the equilibrium between elongation and shortening (reviewed by Greider, 1996; Zakian, 1996). Although the complete set of factors that govern telomere length is unknown, telomerase-mediated elongation is the major pathway for replicating chromosome ends. However, a number of studies have suggested that telomere elongation by telomerase is not a universal mechanism. Three other fundamentally different mechanisms of telomere elongation have been established. First, organisms such as *D. melanogaster*, *B. mori*, and *C. vulgaris* maintain a normal length of telomeres by integration of retrotransposons at chromosome termini. Second, elongation by recombination might contribute to net increase in telomeric DNA. Finally, telomere end-binding proteins play a significant role in the maintenance of telomere length. We review the recent evidence supporting each of these mechanisms and their implications.

A. Regulation by Telomerase and Activities Relevant to Repair of Double Strand Breaks

A large number of genes have been identified that are essential for the maintenance of telomere length. Mutations in the template region of *TLC1* of *S. cerevisiae* and telomerase RNA component of *Tetrahymena* caused corresponding base changes in telomeric DNA repeats (Singer and Gottschling, 1994; Yu and Blackburn, 1991; Cohn and Blackburn, 1995). In addition, these mutations caused severe consequences for cells, including aberrant nuclear and cellular divisions and cell death (Yu and Blackburn, 1991; Prescott and Blackburn, 1997b). Similarly, some mutations in telomerase RNA component of *K. lactis* brought about immediate runaway telomere elongation, whereas others caused elongation after a long latent period of growth (McEachern and Blackburn, 1995). Mutations in genes such as *RAP1* (Conrad et al., 1990; Lustig et al., 1990) or *TEL1* and *TEL2* (Lustig and Petes, 1986) alter equilibrium length. Taken together, these results favor a self-regulatory model for the control of normal telomere length that can be altered by changes in telomeric DNA sequence.

S. cerevisiae cells bearing *TEL1* and *TEL2* mutations gradually lose ~200 to 300 bp of telomeric sequences after ~100 generations (Lustig and Petes, 1986). Unlike *TEL1*, *TEL2* is an essential gene and a decrease in the effective cellular concentration of Tel2p resulted in multiple phenotypes, implying that Tel2p has additional functions in the cell as well (Greenwell et al., 1995; Morrow et al., 1995; Runge and Zakian, 1996). Recent data suggest that regulation of telomere length by Tel2p probably involves its binding to telomeric DNA in a sequence-specific manner (Kota and Runge, 1998). On the other hand, *TEL1* is believed to have DNA checkpoint functions. Analysis of nucleotide sequence revealed that it is structurally homologous to *MEC1* and to the catalytic subunit of DNA-dependent protein kinase (DNA-PK_{cs}) (reviewed by Zakian, 1996). In mammalian cells, DNA-PK is a multisubunit complex consisting of a protein kinase catalytic subunit and the DNA targeting component, Ku, a heterodimer of two polypeptides of 70 and 86 kDa (Jackson, 1996). Genetic analysis indicate that *S. cerevisiae* genes *HDF1* and *HDF2* encode components of Ku heterodimer, yKu70p, and yKu80p, respectively (Feldman and Winnacker, 1993; Mages et al., 1996; Milne et al., 1996), but is devoid of DNA-PK_{cs} homologue. Therefore, it is possible that *TEL1* or *MEC1* may encode the presumed catalytic subunit (Greenwell et al., 1995; Sun et al., 1996).

To explore the mechanism of creation of new telomeres, a DSB was introduced at

the *MAT* locus of *S. cerevisiae* (Kramer and Haber, 1993). Consistent with previous studies (Wang and Zakian, 1990), healing of broken chromosomes involved addition of $TG_{1\text{-}3}$ repeats, in a telomerase-dependent manner, only when $(T_2G_4)_{13}$ sequence was present proximal to the break site. Likewise, elimination of a telomere from yeast chromosome led to *RAD9*-mediated cell cycle arrest and caused a dramatic increase in chromosome loss (Haber and Thorburn, 1984; Sandell and Zakian, 1993). These results underscore the notion that telomeres are essential for cells to distinguish broken DNA molecules from intact chromosomes and maintain genomic stability.

How do activities implicated in repair of double-strand breaks (DSBs) distinguish chromosome ends, where end-to-end fusions are not desirable, from DSBs within the chromosome? *S. cerevisiae* cells bearing mutations in *HDF1* and *HDF2* harbor shortened telomeres and are severely impaired for nonhomologous DNA end-joining (Feldmann and Winnacker, 1993; Boulton and Jackson, 1996; 1998; Milne et al., 1996; Porter et al., 1996; Siede et al., 1996; Nugent et al., 1998). Interestingly, cells carrying mutations in *TEL1* display shortened telomeres, raising the formal possibility that Tel1p might interact with Ku heterodimer. According to one study, Tel1p is not a component of Ku-mediated nonhomologous end-joining pathway (Boulton and Jackson, 1998). It has been demonstrated that Ku protein exerts TPE via direct interaction with Sir4p, an essential component of Sir2-Sir3-Sir4 complex (Tsukamoto et al., 1997). Consistent with this, *in vivo* crosslinking experiments have ascertained that telomeric DNA is complexed with Ku80p, suggesting its involvement in the organization of terminal DNA structure on yeast chromosomes (Gravel et al., 1998). Whereas *TEL1* is not required for DSB repair, Ku protein functions in DSB repair, telomere maintenance, and in TPE (Boulton and Jackson, 1996; 1998). Thus, it is possible that specific interaction of yKu70p or yKu80p with Tel1p, or Mec1p, or additional factors implicated in DSB repair is crucial in distinguishing DSBs within the chromosome from those of telomeric ends. Further characterization of telomere-specific factors and their interaction with each other in relation to DSB repair and telomere maintenance may provide clues as to how these two different types of ends are processed.

Mutations in genes that are relevant to general DNA metabolism such as *cdc17* and *cdc44* that encode polymerase α and replication factor C, respectively, cause considerable lengthening of telomeres than the corresponding wild-type strains (Carson and Hartwell, 1987; Adams and Holm, 1996). In addition, *S. cerevisiae* cells bearing *top3*Δ (Kim et al., 1995) or *kem1*Δ (Liu et al., 1995) or *gal11* (Suzuki and Nishizawa, 1994) mutations displayed shortened telomeres.

B. Telomerase-Independent Maintenance of Telomere Length

Loss of telomerase activity has enabled some organisms to invent alternative ways such as integration of transposons at the termini or recombination to maintain normal chromosome ends. For example, in *D. melanogaster*, two non-LTR retrotransposons, HeT-A and TART, transpose preferentially to the ends of chromosomes and function as telomeres (Biessmann et al., 1990; Levis et al., 1993). Telomerase activity is undetectable in *Drosophila*, and, consequently, maintenance of telomere length depends entirely on the frequent transposition of these retrotransposons to the termini of chromosomes. These elements use the 3′-OH of a DNA end as the primer for reverse transcription during the process of transposition

(Finnegan, 1997). In *S. cerevisiae*, the LTR-transposable element Ty5 integrates preferentially in the subtelomeric regions, thereby providing a potential means of protection to the chromosome ends (Zou et al., 1996).

Telomerase-negative, and perhaps even telomerase-proficient, cells may use recombination to replenish telomeric DNA sequences. The most compelling evidence for recombination as an efficient bypass mechanism for telomere length maintenance comes from studies in *S. cerevisiae* (Pluta and Zakian, 1989; Louis and Haber, 1990; Wang and Zakian, 1990; Lundblad and Blackburn, 1993; McEachern and Blackburn, 1996). Yeast cells bearing mutations in the *EST* genes progressively lose telomeric DNA sequences and die after ~50 generations (Lundblad and Szostak, 1989; Lendvay et al., 1996; Lingner et al., 1997). However, among *est1*Δ cells, pseudorevertants arise both by amplification of Y′ elements and elongation of terminal $TG_{1\text{-}3}$ repeats through a *RAD52*-dependent recombination pathway (Lundblad and Blackburn, 1993). Similarly, postsenescence survivors of *K. lactis ter1*Δ mutants arise through a *RAD52*-dependent mechanism (McEachern and Blackburn, 1995; 1996). These mutants harbor lengthened telomeres, probably not involving amplification of subtelomeric sequences, and support robust cell growth in the absence of functionally active telomerase. Additionally, it has been demonstrated that *RAD52*-independent recombination of heterologous T_2G_4 repeats on the opposite ends of an artificial chromosome occurs by gene conversion (Pluta and Zakian, 1989; Wang and Zakian, 1990b). The addition of telomeric DNA repeats might involve a sister chromatid, homologue, or telomere-associated DNA, culminating in the duplication of the entire end of the chromosome (reviewed by Zakian, 1996). The molecular mechanism of this exchange process is not fully understood; however, it is possible that it could entail reciprocal or nonreciprocal recombination.

Recent results have uncovered a role for recombination in the elongation of chromosome ends in *Anopheles gambiae* and in a multiplicity of organisms. Biessmann and colleagues (Roth et al., 1997) have experimentally modified the telomere length by inserting a transgene pUChsneo and subsequently examined the dynamics of chromosome length. They observed that some chromosomes had elongated telomeres by regeneration of a part of the integrated pUChsneo by recombination between the ends of homologous chromosomes. Similar recombinational pathways have been documented in cells from a variety of organisms, including humans. A significant percentage of tumor-derived and immortalized cell lines and primary tumors lacking detectable telomerase activity harbor strikingly long telomeres (Bryan et al., 1995; 1997). In addition, the manifestation of long telomeres in the immediate progeny (up to six generations) of telomerase 'knock out' mice raises the possibility that recombination may contribute to the maintenance of normal telomeres (Blasco et al., 1997). Furthermore, telomere length in *Alliaceae* is regulated primarily by recombination between LTR sequences (Pich et al., 1996). Taken together, these observations reveal that telomerase-negative cells, including human tumor cells, maintain normal lengths of telomeres via telomere-telomere recombination.

C. Regulation of Telomere Length by Recombinational Repair

S. cerevisiae epistasis group mutants, which include *rad50* to *57*, *mre2*, *mre11*, *xrs2*, and *rfa1*, are sensitive to DNA dam-

age inflicted by X-ray (but not UV) and methane methanesulfonate (Game et al., 1980; Malone, 1983; Cao et al., 1990). *RAD52* epistasis mutants can be divided into two subgroups: *rad51*, *–52*,*–53*, *–54*, *–55*, and *–57* display sharply reduced rates of mitotic recombination, whereas *rad50*, *mre11*, and *xrs2* mutants confer hyper-Rec phenotype for both intra- and interchromosomal recombination and are defective in repair of DSBs but proficient in mating-type switching (Malone, 1983; Gottlieb et al., 1989; Alani et al., 1990; Ivanov et al., 1994). *XRS2* and *MRE11* share *RAD50*-like phenotypes and, consequently, their gene products apparently function as a multiprotein complex *in vivo* (Ajimura et al., 1993; Johzuka and Ogawa, 1995). Vegetatively dividing cells of *S. cerevisiae* bearing a mutation in *RAD50* grow significantly more slowly in rich medium and are sensitive to damage inflicted by X-ray and chemical mutagens (Farnet et al., 1988; Resnick and Martin, 1976). Correspondingly, *RAD50* function is essential for the repair of double-strand breaks and chromosome stability. Mutations in the conserved ATP-binding domain confer a nonnull, *rad50S*, phenotype, and these strains have been instrumental in elucidating the role of *RAD50* in the formation and processing of DSBs (Alani et al., 1990; Wu and Lichten, 1994). The steady-state amounts of *RAD50* mRNA and Rad50p in both vegetatively dividing and meiotic cells and the ability of the latter to interact with DNA in an ATP-dependent manner (Raymond and Kleckner, 1993a; 1993b) imply a role for *RAD50* in recombinational repair and maintenance of natural as well as artificially induced DSBs.

Insights into the role of *RAD50* in telomere length regulation originate in phenotypes associated with cells deleted for *RAD50* (Kironmai and Muniyappa, 1997; Boulton and Jackson, 1998). *rad50*Δ mutants exhibited abnormal cell size, appeared to have problems with nuclear division, and displayed phenotypes consistent with defects in S-phase checkpoint control (Kironmai and Muniyappa, 1997). In addition, *rad50*Δ cells divide quickly but harbor shortened telomeres and die after several generations of growth (Figure 2). In agreement with these studies, loss of *RAD50*, or *MRE11* or *XRS2*, which display *RAD50*-like phenotypes, affected telomere length as well as Ku-mediated DSB repair (Boulton and Jackson, 1998; Nugent et al., 1998). Given the evidence that *RAD51* is required for homologous recombination, we examined its involvement in telomere length maintenance. Significantly, the pattern of telomeres in *rad51*Δ strain was indistinguishable from that of wild-type cells (Kironmai and Muniyappa, 1997). Although additional experiments are required, our data suggest that single-stranded overhangs may be acted on by Rad50p and associated proteins for recombinational repair (Figure 3). However, epistasis analysis suggests that Rad50p/Mre11p complex may play a role in maintenance of telomere length via the telomerase-dependent pathway (Nugent et al., 1998). Consistent with reduced 5′ to 3′exonucleolytic activity in *mre11* mutants (Tsubouchi and Ogawa, 1998), Rad50/Mer11 complex is proposed to generate single-stranded DNA overhangs that can be acted on by telomerase (Nugent et al., 1998). In either interpretation the data point to an unmistakable role of *RAD50* in telomere length maintenance and cell growth.

How do DSBs and recombination facilitate elongation of telomeres? In *S. cerevisiae* much of homologous recombination in vegetatively dividing and meiotic cells is initiated by DSBs. Given this scenario, the absence of telomerase and gradual loss of telomeric DNA suggest a model in which the shortened telomeric ends may be presented to the cells as putative DSBs. Consequently, components involved in DNA repair are recruited to the DSBs to initiate recombination and lengthen telomeres.

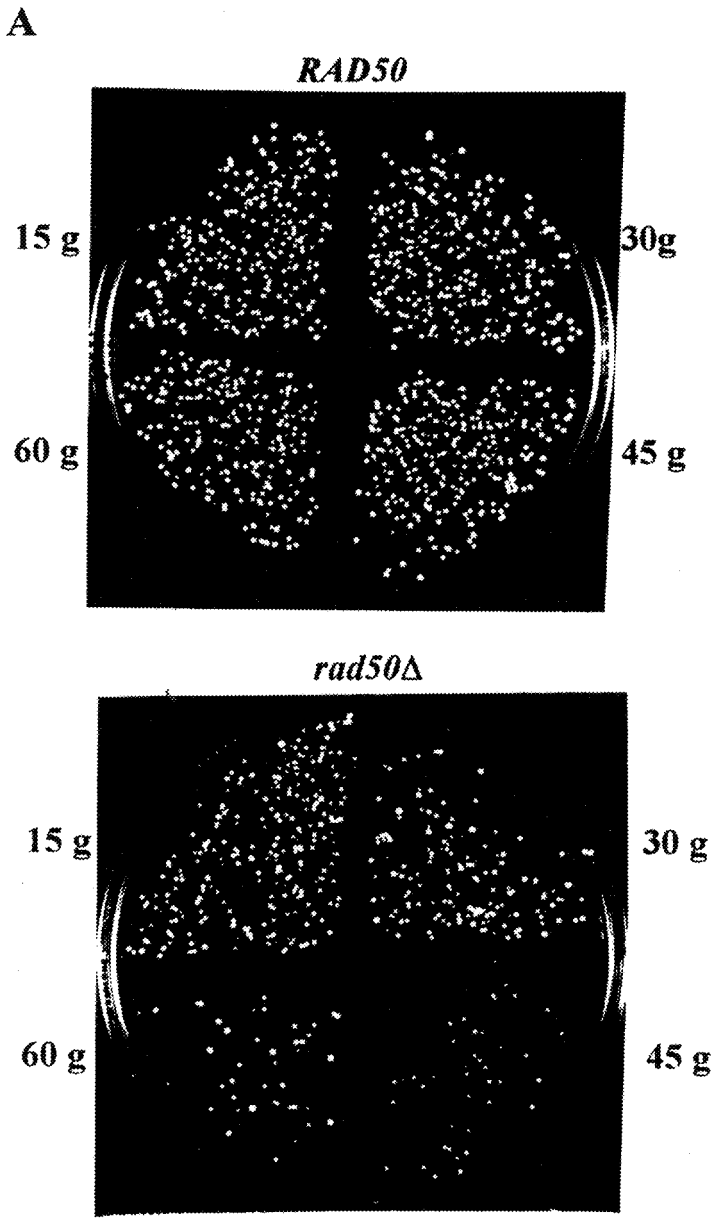

FIGURE 2. Cells carrying *rad50*Δ mutation display cell senescence. Cultures of freshly dissected wild type and mutant spores from a hemizygous *RAD50/rad50*Δ were grown in YPD liquid medium. Equal number of cells from 15, 30, 45, and 60 generations of growth (g) were plated on quadrants of a YPD plate and incubated at 30°C for 48 h. [Adapted with permission from *Genes Cells* (Kironmai and Muniyappa, 1997)]. Copyright (1997) Blackwell Science Limited.

D. Regulation by Telomere End-Binding Proteins

A large number of proteins that bind sequence-specifically to telomeric termini modulate telomerase activity by rendering chromosome ends inaccessible, thereby affect telomere length. Among the proteins that bind double-stranded telomeric DNA sequences, the most studied with respect to regulation of telomere length is Rap1p. At

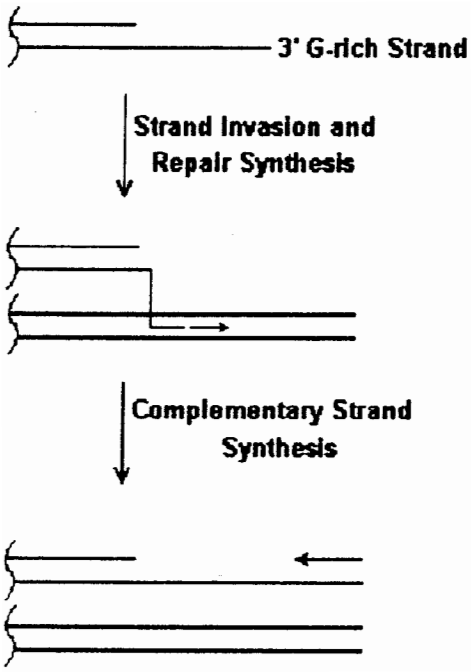

FIGURE 3. A generalized model for elongation of telomeres by recombinational repair. (Modified from Wang and Zakian, 1990b.)

chromosomal termini, Rap1p plays a key role in both telomere length maintenance and TPE. *RAP1* is an essential gene required for the viability of *S. cerevisiae* (Shore and Nasmyth, 1987). Several studies have ascertained that Rap1p has a number of binding sites within the telomeric DNA (Longtine et al., 1989; Gilson et al., 1993) and, consequently, plays a complex role in telomere length regulation (Conrad et al., 1990; Lustig et al., 1990; Sussel and Shore, 1991; Kyrion et al., 1992). For instance, point mutation, truncation, or increasing the cellular concentration of the carboxy terminal portion of Rap1p resulted in a dramatic increase in telomere length compared with wild-type cells (Conrad et al., 1990; Sussel and Shore, 1991; Kyrion et al., 1992). On the other hand, cells bearing *rap1*^{ts} alleles displayed shortened telomeres when cultured in semipermissive temperatures but wild-type telomere lengths when cells were returned to permissive conditions (Conrad et al., 1990; Lustig et al., 1990). Furthermore, sets of *rap1*^{ts} suppressor mutations have been identified. One mutant *sds4-1/gal11* restores telomeres nearly to wild-type length in *rap1*^{ts} strain in nonpermissive conditions (Sussel et al., 1995).

It has been proposed that mature telomeres arise from both *de novo* synthesis and nucleolytic processing activities *in vivo*. Telomere processing was first noted in hypotrichous ciliates, which undergo programmed chromosome breakage during development. Simple repeat sequences that function as telomeres are added onto the ends of DNA molecules that are generated by fragmentation during the course of macronuclear development. Interestingly, in most ciliate species the extent of telomeric DNA sequences are variable (Forney and Blackburn, 1988; Baroin et al., 1987; Herrick et al., 1987), suggesting that varying amounts of processing by endo- and/or exonucleolytic activity produce the mature form of telomere(s). When *Tetrahymena* cells were maintained under conditions of continuous exponential growth, the average telomere length increased but decreased immediately after return to the stationary phase (Larson et al., 1987).

The chromosome termini and the mean length of any telomere varies in different clonal populations of the same strain and among strains of different genetic backgrounds in yeast (Walmsley and Petes, 1985; Shampay and Blackburn, 1988), maize (Burr et al., 1992), *Arabidopsis* (Richards, 1995), and mouse (Kipling and Cooke, 1990; Prowse and Greider, 1995). In *S. cerevisiae*, length heterogeneity at each chromosome end increased with successive rounds of cell division (Shampay and Blackburn, 1988). It is believed that the overall maintenance of telomere length involves an intricate balance among competing factors. Therefore, any model for telomere length regulation must

account for at least two requirements: (1) ensure complete replication of chromosome ends, and (2) generate substrates with 3′ overhang to favor the interaction of telomere-specific DNA-binding proteins. In lower eukaryotes, telomeres contain short 3′ overhangs: ciliated protozoa have 12 to 16 nucleotide G-rich tails throughout their cell cycle (Klobutcher et al., 1981; Pluta et al., 1984; Henderson and Blackburn, 1989), and *S. cerevisiae* has G-tails >30 nucleotides in late S phase (Wellinger et al., 1993). Cells bearing *cdc13* mutation (Garvik et al., 1995) or deficient for telomerase RNA (Wellinger et al., 1996; Dionne and Wellinger, 1996) accumulate long $TG_{1\text{-}3}$ tails in late S-phase of the cell cycle. *In S. cerevisiae*, the binding of Cdc13p to the terminal 5′ telomeric end has been thought to render protection from degradation (Garvik et al., 1995; Nugent et al., 1996; Lin and Zakian, 1996). Likewise, human telomeres from a variety of cells bear 130 to 230 nucleotide protrusions of single-stranded TTAGGG repeats (Makarov et al., 1997; McElligott and Wellinger, 1997; Wright et al., 1997). It has been suggested that the G-tails probably arise due to the degradation of C-rich strand leading to telomere shortening (Wellinger et al., 1996; Makarov et al., 1997). The nature of the enzymatic activities involved and the mechanism of precise trimming of telomeres are obscure. However, two enzymatic activities have been implicated. First, the 3′ cleavage activity of telomerase could in principle participate in telomere processing, but the occurrence of TT3′ *in vivo*, not 3′dG, suggests that it is unlikely (Collins and Greider, 1993). Second, the G4-DNA substrate specificity of yeast Kem1p and mXrn1p, *in situ* localization and *kem1*Δ mutant phenotypes suggest a potential role for these nucleases in the processing of telomeric ends (Liu and Gilbert, 1994; Bashkirov et al., 1997).

It is reasonable to suppose that cells monitor telomere length and use this information to exert negative control on telomerase activity *in vivo*. Failure to do this would result in excessive growth of chromosome termini. Genetic studies in *S. cerevisiae* have provided compelling evidence in support of this notion. Cells bearing *pif1* mutation, a gene that encodes 5′ to 3′ DNA helicase, display multiple phenotypes including loss of expression of genes in the subtelomeric region, a dramatic increase in *de novo* synthesis of telomeres both at the artificially induced and spontaneous chromosome breaks, and lengthening of the existing $TG_{1\text{-}3}$ repeat sequences (Schulz and Zakian, 1994). These results favor the notion that Pif1p inhibits lengthening of telomeres in a wild-type strain.

Negative regulation of telomere lengthening by telomere repeat-binding proteins has been noted in *S. cerevisiae* (*Sc*Rap1p), *K. lactis* (*Kl*Rap1p), *S. pombe* (Taz1p), and human cells (Trf1p) (Marcand et al., 1997; McEachern and Blackburn, 1995; Cooper et al., 1997; van Steensel and de Lange, 1997). Disruption or deletion of genes coding for these proteins causes a massive increase in telomere length in the respective systems. Two human telomeric DNA-binding proteins have been identified: Trf1 and Trf2. Both bind specifically to double-stranded TTAGGG repeats *in vitro* and are localized to telomeres *in vivo* (Chong et al., 1995; Broccoli et al., 1997). Trf1p functions as a negative regulator of telomere length maintenance (van Steensel and de Lange, 1997), whereas Trf2 protects telomeres from end-to-end fusions (van Steensel et al., 1998). Although the telomeric repeat-binding proteins carry no significant sequence homology with each other, but harbor a motif similar to the Myb family of transcription factors (reviewed by Konig and Rhodes, 1997). The general theme that emerges from these reports is that the amount of repeat-binding protein bound to double-stranded telomere repeats serves to suppress telomerase activity by a negative feedback mechanism termed ‘protein-counting’ (Marcand et al., 1997).

Furthermore, negative regulation by Rap1p in *S. cerevisiae* is independent of the orientation of telomeric repeats but proportional to the number of targeted molecules (Marcand et al., 1997).

VI. TELOMERE LENGTH AND TELOMERASE ACTIVITY IN CELLULAR AGING

The original observation that human cells derived from newborns, unlike germ line cells, undergo a limited number of cell divisions by virtue of replicative senescence (Hayflick, 1965) led to the hypothesis that progressive shortening of chromosomes causes cellular senescence (Olovnikov, 1973). Correspondingly, as human and mouse cell in which telomerase is repressed divide, telomere length becomes progressively shorter with successive cell divisions *in vitro* and with age *in vivo* (Allshire et al., 1988; Harley et al., 1990; Hastie et al., 1990; Prowse and Greider, 1995; Harley and Villeponteau, 1995). Furthermore, a significant percentage of tumors contain increased levels of telomerase activity, emphasizing the relationship between telomerase activation and oncogenesis. Likewise, dividing $CD34^+$ $CD38^+$ cells, testis germ line cells (unlike sperm cells), and *Xenopus* embryonic cells contain increased levels of telomerase activity during both M and S phases of the cell cycle (Chiu et al., 1996; Mantell and Greider, 1994; Cooke and Smith, 1986; Holt et al., 1996). These set of findings led to a model in which the replicative capacity of cells is correlated with an increase in telomerase activity and telomere size (reviewed by Harley and Villeponteau, 1995). The fibroblasts derived from patients with Werner's syndrome, the premature aging syndrome, divide fewer times in culture before senescing (Salk et al., 1981), providing a curious link between telomere size and aging. Consistent with this, telomeres from several human cells, including lymphocytes and peripheral blood leukocytes, have been shown to shorten with age (Hastie et al., 1990; Schwartz et al., 1993; Vaziri et al., 1994). Recent studies have demonstrated that the ectopic expression of telomerase delays senescence *in vitro*, generating a phenotype similar to that of cellular immortality (Bodnar et al., 1998; Vaziri and Benchimol, et al., 1998). However, some of the actively proliferating cells such as stem cells harbor shorter telomeres despite containing active telomerase (Vaziri et al., 1994). These findings suggest that shortened telomeres could arise as a consequence of either insufficient telomerase activity or loss of some other integral noncatalytic factor(s) of the telomerase complex (Counter et al., 1995).

A corollary to this casual relationship also exists in yeast wherein cell senescence has been correlated with shortened telomeres. Mutations in *EST* genes or *TLC1* that encode components of telomerase result in gradual attrition of telomeric DNA with replicative age (Lundblad and Szostak, 1989; Singer and Gottschling, 1994; Lendvay et al., 1996; Lingner et al., 1997). Although yeast cultures are immortal, individual mother cells die after ~25 cell divisions (Mortimer and Johnston, 1959), but the length of telomeres in these cells is indistinguishable from the young cultures (d'Mello and Jazwinski, 1991; Kennedy et al., 1995). Yeast cells carrying *sir4* mutations confer extended life span by >30% apparently by preventing the recruitment of SIR proteins to the telomeres (Kennedy et al., 1995).

A. Telomere Length and Telomerase Activity in Cancer Cells

A number of studies point to an important correlation between shortened telomeres

and senescence in humans. For example, telomeres shorten as normal human fibroblasts grow in culture (Harley et al., 1990) and in skin samples of older people when compared with young individuals (Hastie et al., 1990). The relationship between cellular senescence and shortened telomeres posit that immortal cells should contain higher levels of telomerase activity. Correlative support for this hypothesis has been provided by a number of studies showing that primary cells and immortalized cell lines contain increased levels of telomerase activity (reviewed by Greider, 1996). Studies of cell culture models suggest that cells are required to overcome two critical stages, Mortality stage 1 and stage 2 to attain immortality (Allsopp et al., 1992; Holt et al., 1996). M1 is a cell cycle checkpoint and loss of this control leads to M2 stage of extended proliferation (Shay et al., 1991). It has been proposed that immortalization of human cells in culture requires the reactivation of telomerase. For instance, the life span of primary human embryonic kidney cells transformed with SV40 is extended. After continuous passage in culture, cells display a gradual shortening of telomeres, an absence of telomerase activity until they enter a crisis period, and, consequently, most cells die. Immortal clones arise from this crisis at a low frequency that contains stabilized telomeres and increased levels of telomerase activity (Counter et al., 1992). Similar results have also been documented with primary human epithelial cells transformed with human papilloma virus (Counter et al., 1994) or B-lymphocytes with Eppstein-Barr virus (Klingelhutz et al., 1994). Furthermore, increased levels of telomerase activity have been detected in 80 to 90% of all human cancer cells (Kim et al., 1994; Hiyama et al., 1995). These results suggest that immortal cells, including cancer cells, avoid gradual shortening of telomeres by expressing telomerase (reviewed by Autexier and Greider, 1996). Immortalization of human cells in culture occurs concomitantly with high levels of telomerase activity, and stabilization of telomeric ends and leads to cell cycle exit (reviewed by Harley and Villeponteau, 1995).

It is also apparent that up-regulation of telomerase also occurs in >90% of malignant samples compared with samples from adjacent tissue (Kim et al., 1994). However, several studies have demonstrated an inverse correlation between telomere length and telomerase activity in immortalized cell lines and primary tumors (Kim et al., 1994; Counter et al., 1994; Blasco et al., 1996; Bryan et al., 1997). Interestingly, a significant percentage of tumors maintain strikingly long telomeres in the absence of detectable telomerase activity (Bryan et al., 1995; 1997). These data are consistent with genetic evidence in *S. cerevisiae* that alternative mechanisms play a key role in the maintenance of telomere length equilibrium. Whether there is a correlation between telomerase activity and telomere shortening, the selection for telomerase-positive cells in the progression of tumors offers telomerase as a universal target for anti-cancer strategies.

ADDENDUM

Recent evidence has provided insight into the identity of the actual end-binding factor hypothesized in the maintenance of telomere structure (Wiley and Zakian, 1995). New evidence indicates that Ku heterodimer plays a key role, distinct from that of a component of Sir complex, in maintaining the integrity of telomere structure (Gravel et al., 1998; Larouche et al., 1988; Polotnianka et al. (1998) *Curr. Biol.* 8: 831–834).

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