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REVIEW

Polypeptide Components of Telomere Nucleoprotein Complex

A. N. Kuimov

Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119899, Russia; fax: (7-095) 939-3181; E-mail: kuimov@genebee.msu.su

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Abstract—Chromosome telomeres of humans and many model organisms contain a structure called a t-loop, which is maintained by TERF, TINF2, Pot1, and other proteins. Increase in TERF1 concentration prevents telomere elongation by telomerase. Decrease in TERF2 concentration (preventing t-loop formation) is accompanied by blockade of proliferation and appearance of other signs of cellular senescence in experiments. Natural regulation of TERF1 involves tankyrase, ATM protein kinase, and fluctuations of the protein level across a cell cycle. The telomere nucleoprotein complex also interacts with various polypeptide macromolecules (e.g., Sir2, PinX1, Rap1, Ku, Rad50/Mre11/Nbs1) responsible for heterochromatin formation, modulation of telomerase activity, DNA repair, and signaling to other cell compartments about telomere state. Study of structure and functioning of telomere nucleoprotein complex may contribute to elucidation of poorly understood mechanisms of aging and processes of tumor transformation of cells.

Key words: telomeres, telomerase, TERF, tankyrase, Ku, Rad50/Mre11/Nbs1, replicative senescence, immortalization

Impressive progress in studies of telomeres and regulation of their length already produced the discovery of telomerase, the enzyme responsible for synthesis of telomere repeats, and elucidation of the dependence of telomerase expression on cell differentiation. Study of telomeres is logically connected to a complex of important and unsolved problems of modern biology such as malignant growth, aging, regulation of cell proliferation and tissues regeneration, and DNA repair. Several years ago, this journal already reserved a whole issue for discussion of these problems. However, accumulation of new results in this hot area requires new analysis and systematization. During this period (since the special issue of *Biochemistry* (Moscow)), several enzymes involved in control of telomere length (e.g., tankyrase) have been discovered. New information on previously recognized components of the telomere complex (TERF1) and spatial structure of telomeres has appeared.

In this review, I have considered new data on the structure of telomeres and functioning of telomere proteins. E. Blackburn [1] and other invited authors of the special issue of *Biochemistry* (Moscow) (1997, vol. 62, No. 11) focused their attention on primary structure of telomeres and their synthesis. These papers can be recommended as an excellent introduction to the problem. In addition, *Biochemistry* (Moscow) and other international biochemical journals have published several reviews highlighting related problems [2-4].

STRUCTURE OF TELOMERIC DNA AND ITS INTERACTION WITH HUMAN TERF PROTEINS

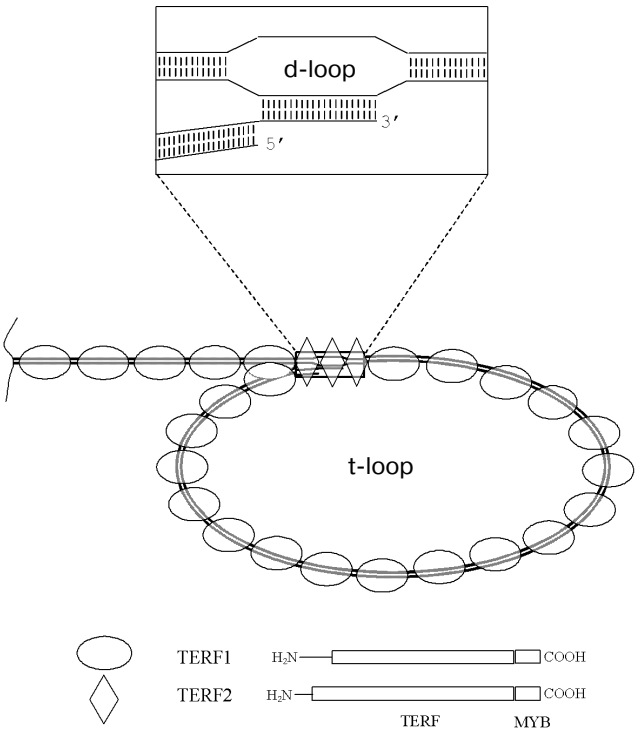
The primary structure of telomeres is highly conservative in most living organisms. In man, nematodes, and protozoa the telomeric structure represents numerous repeats of the following nucleotide sextets: TTAGGG (in man and other vertebrates), TTAGGC (in *Caenorhabditis elegans*), or GGGGTT (in *Tetrahymena thermophila*) (see for review [5]). In many plants, telomeres consist of the nucleotide septet TTTAGGG. However, two important organisms often used as models, yeast and drosophila fruit fly, are exceptions to this general rule. In *Saccharomyces cerevisiae*, relatively short telomeres are composed of G and T nucleotides, which form irregular sequences, whereas telomeres of *Drosophila melanogaster* consist of complexly organized transposons.

In various species, various individuals of the population of any species, and also in various cells of the same organism, on various chromosomes of the same cell (and even on two arms of the same chromosome) the telomere

Abbreviations: DNA-PK) DNA-dependent protein kinase; DSB) double strand break; MAP-kinase) mitogen activated protein kinase; NHEJ) nonhomologous end joining; PAPR) poly(ADP-ribose) polymerase; t-loop) telomere loop.

length may significantly differ [6]. Usually the telomere length varies from a few up to a few tens of kb (kilobase pairs). Interestingly, telomerase preferentially maintains the shortest telomeres, whereas recruitment of this enzyme to rather long telomeres is rather weak [7]. One of two strands of DNA is longer than the complementary C-rich strand and it contains a single-strand overhang (3′-overhang) at the telomere end, which may exceed two hundred nucleotides in length.

TERF1 protein binds to human telomeric DNA duplex. This protein slightly bends DNA so that the latter forms supercoil; one turn of this supercoil includes many hundreds of base pairs. Recently, joint study of scientists from Rockefeller University and University of North Carolina [8] revealed that another protein, TERF2, binds at the 5′-terminus and fixes the telomere end at the tail. This results in formation of giant telomere loops (t-loops), the size of which can be comparable to the size of telomeres (see figure).



Schematic presentation of t-loop and domain structure of telomere specific proteins TERF1 and TERF2. Telomeric DNA duplex is bound to TERF1 protein (see domain structure below) and is bent into a loop (t-loop). TERF2 protein forms a junction on this loop. Both proteins bind DNA via a Myb domain. The second domain of the protein, TERF, is involved in subunit dimerization. It is suggested that in the loop-tail junction the single strand 3′-overhang invades a duplex, partially unwinds the telomeric repeat array, and stabilizes a d-loop [8]

The first report on TERF1 protein (Telomeric Repeat Binding Factor 1) was published in 1992 [9]. During the next five years, two groups independently determined the amino acid sequence of this protein [10, 11]. These groups were working with two polypeptide products of the same gene; these products originated from alternative splicing of RNA. The first product was named TRF or TRF1; another one was named Pin2. Although calculated molecular masses of these protein products were 50 and 48 kD, respectively, their apparent molecular masses determined by electrophoresis were 63 and 61 kD.

The figure schematically shows domain structure of TERF1. In the C-terminus, it has a Myb-related DNA-binding module. Interaction of this protein with nucleic acid requires polypeptide dimerization, which involves the TERF domain [12]. Electron microscopy data demonstrate that TERF1 tetramer binds with high affinity up to 12 telomeric repeats of vertebrate DNA [8], but not to plant or nematode telomeres [10, 13, 14]. TERF1 is also inactive with a single strand DNA located at the end of human telomeres [9]. At saturating concentrations, this protein forms a continuous (10 nm thick) array along the telomere. Each TERF1 molecule bends DNA at 120° [15]. Model experiments revealed that the presence of TERF1 facilitates circularization of a short DNA molecule that consists of telomeric repeats. This suggests involvement of this protein in formation of spatial structure of telomeres. Within DNA sequence, this protein binds to two half-sites 5′-YTAGGGTTR-3′, which may be positioned without constraint on distance or orientation. The DNA-binding domains of TERF1 homodimer are thought to be linked with an extremely flexible polypeptide segment [16].

TERF2 protein, also known as TRF2, was found in late nineties [14, 17, 18]. It shares structural similarity with TERF1, but its TERF domain does not interact with a homologous domain of the second member of this family so that both proteins exist in the cells as homo- but not heterodimers. As in the case of TERF1, alternative splicing yields two variants of TERF2. The calculated molecular mass of protein monomers was lower than that determined by electrophoresis; in the latter case these are 65 and 69 kD.

The telomeres are bound with much less TERF2 than TERF1. The localization of this protein was elucidated by electron microscopy. TERF2 is bound on the loop-tail junction [8]. Its inhibition may result in activation of ATM/p53-dependent pathway of cellular response to DNA damage followed by apoptosis [19], because chromosome ends unprotected by the telomere protein look like DNA double strand breaks (see below).

Telomere loops have been found on chromosomes of HeLa cells, peripheral human leukocytes, primary mouse hepatocytes [8], and also on trypanosome telomeres [20] and micronuclear but not macronuclear chromosomes of

the ciliate *Oxytricha fallax* [21]. In human cells, sizes of t-loops vary from 5 to 40 kb. Although trypanosome telomeres are comparable to those found on human telomeres, their t-loops do not exceed 1 kb. This is perhaps the smallest size of t-loops known to date.

TERF2 binding at the chromosome end requires a single strand site on G-strand of telomeric DNA, which should include at least one repeat of the TTAGGG sextet [22]. The presence of more than one repeat at the single strand site improves binding. On the other hand, under model conditions TERF2 interacts only with double strand telomeric DNA, but does not bind the single strand. At saturating protein concentrations, at least three, or often about ten dimers cover a telomere. Under certain experimental conditions, TERF2 remodels DNA into aggregates of two and more DNA molecules fused together at their ends.

The presence of the single strand region in the loop-tail junction was confirmed by using *Escherichia coli* single strand binding protein [8]. Taking into consideration the number of protein globules adsorbed on the nucleic acid and monitored by electron microscopy, the length of single strand DNA in the junction corresponds to average length of the single strand region at the telomere end. It is suggested that at the junction the single strand DNA invades the duplex of the tail, causes its partial unwinding and hybridizes antiparallel strand of the tail. As a result the junction looks like a d-loop (displacement loop) or Holliday junction-like structure [23].

PHENOTYPIC CONSEQUENCES OF CHANGES IN ACTIVITY OF TERF PROTEINS

Since the middle of the eighties, telomere length has been associated with phenotypic cellular age. Indeed, due to lack of telomerase in somatic cells (and maybe for some other reasons) the number of telomeric repeats at the human chromosome ends reduces after each mitotic act. Unfortunately, high heterogeneity of telomeres and lack of an accurate method for determination of their length (see for review [4]) allows only rough evaluation of age-related changes observed in numerous studies. For example, in primary fibroblasts cultivated *in vitro* each cell division was accompanied by telomere reduction by 48 $\pm$ 21 bp. The same authors believe that in human fibroblasts *in vivo* the mean value of telomere shortening is about 75 bp per mitosis [24]. Similar results were obtained with mouse fibroblasts [25]. These are average data for a large number of cells that are characterized by different division rate. In peripheral blood cells of young children, the telomere shortening occurs at a rate of more than 1000 bp per year. At ages from 4 to 20 years, the rate of shortening becomes slower, and during the remainder of adult life telomere length shortens at a constant rate of 30-60 bp per year (see review [26]).

During age-related shortening, telomeres reach some critical length (in human fibroblasts this length is 5-7 kb) at which replicative senescence begins. The latter is characterized by inhibition (up to full stop) of proliferation. Artificial telomere elongation by telomerase expression prevents senescence and allows obtaining immortalized cell lines [27, 28]. Spontaneously immortalized tumor cells overcome senescence in the same way: such cells either begin telomerase expression or maintain stable level of telomere length by alternative mechanisms [5, 29]. In some cases, telomerase inhibition caused suppression of growth of tumor cell cultures [30].

Discovery and study of telomeric proteins has provided additional information on the role of telomeres in the control of cellular senescence.

Functional studies of TERF proteins revealed that sharp increase in TERF1 concentration (during its overexpression in cell culture by viral promoter) caused premature entry into mitosis followed by subsequent cell death [31]. Controlled expression by tetracycline-dependent promoter allowed cells to survive; however, it resulted in marked telomere shortening during several cell cycles [32]. Under similar conditions, TERF1 inhibition in telomerase-positive cell line was accompanied by slow telomere elongation. Evidently, TERF1 promotes t-loop formation, which prevents telomere elongation due to telomerase activity. It was also shown that *in vitro* TERF1 inhibits telomere C-strand DNA synthesis [33].

Experimental manipulations with TERF2 protein concentrations revealed completely different consequences. TERF2 inhibition in dominant negative deletion mutants caused immediate arrest of proliferation, morphological changes in structure of model fibrosarcoma cells characteristic of senescence, and also induction of senescence-associated molecular markers [34]. Ends of chromosomes of such cells often fused and formed circular structures, but they did not lose telomeric repeats (as occurs during chromosome aberrations in tumor cells). Restriction analysis revealed regions of tandem telomeric repeats that were two times longer than those at telomere ends and presumably were products of end-joining telomere ligation. Besides, ends of "surviving" telomeres lost single strand 3′-overhangs, in spite of unchanged telomerase activity, which synthesizes one of the strands. Thus, telomeres themselves do not protect chromosomes against nucleases, fusion, and subsequent breaks during mitosis. This is the function of TERF2 protein.

Increase of TERF2 concentration in primary fibroblast culture was accompanied by some increase of telomere shortening rate during senescence of the cellular population [19]. However, this effect did not result in premature senescence; moreover, during experiments cells continued to proliferate, while growth of control culture stopped. In control cells, the threshold of telomere length for senile inhibition of proliferation was 6-7 kb. In the experimental cell culture that overexpressed TERF2, this

parameter was 2-2.7 kb lower and these cells passed through 15 additional mitotic divisions beyond the normal senescence setpoint. In cells with critically short telomeres, senile or mutant by p53 and pRb cells, overexpression of TERF2 prevented fusion or breaks of chromosomes. These data suggest that induction of cellular senescence is determined by telomere condition, which is related to the protective function of TERF2 protein rather than to telomere length. For example, senile telomere may be too short for masking chromosome ends within loop-tail junction of t-loops, but increase in TERF2 concentration increases probability for formation of normal t-loop.

Some viral oncoproteins help somatic cells to overcome proliferation barriers. Such viral immortalization involves damage to two proteins, p53 and pRb, required for normal replicative senescence (see for review [35]). In a hypophosphorylated state pRb binds and inhibits transcription factor E2F, required for G1/S transition, and therefore it controls the cell cycle. The genome guard p53, and another protein, p16INK4, suppress signaling pathways that regulate pRb phosphorylation and E2F release.

Experiments with primary fibroblast culture revealed that TERF2 inhibition caused not only appearance of phenotypic changes typical for senescence observed on fibrosarcoma cells [34], but also characteristic genetic changes [36]. Particularly, expression of the dominant negative TERF2 allele caused fusion of chromosomes, formation of more than two centrosomes, and tetraploid karyotype. TERF2 inhibition is accompanied by activation of p53, hypophosphorylation of pRb, increase in p16 concentration, and decrease in cyclin A; these are typical signs of cellular senescence. Simultaneous inactivation of p53 and pRb by T-antigen of SV40 virus (or by other treatments) prevented senescence of experimental cells induced by TERF2 inhibition. These results demonstrate that senescence-inducing proteins, p53 and pRb, may be activated by telomeres (when they are not protected by TERF2 protein).

Interestingly, p53 exhibits higher affinity to the single strand 3′-overhang, to the loop-tail junction of t-loops; it even increases probability of t-loop formation in the presence of TERF2 [23].

PARTNERS AND NATURAL REGULATORS OF HUMAN TERF PROTEINS

Specification of telomere composition of the nucleoprotein complex and elucidation of the role of telomeric proteins in induction of cellular senescence allows the investigation of natural regulators of telomere state. Besides telomerase, there are several putative proteins, which may play similar regulatory roles.

Although the effect of TERF2 on telomeres is clearly demonstrated under experimental conditions, its modulators are poorly characterized. Recently hRap1 protein has been identified as a TERF2 partner; it preferentially binds to telomeres [37], but may also interact with other sites of chromosomes. In contrast, TERF1 activity gently influencing telomere length is known to be regulated *in vivo* in several ways.

For example, TERF1 can be poly(ADP-ribosyl)ated, which causes its dissociation from DNA. This posttranslational modification involves tankyrase (TRF1-interacting ankyrin-related ADP-ribose polymerase, EC 2.4.2.30). The enzyme was originally recognized by means of yeast two-hybrid screen with TERF1 as a bait [38]. Inactive tankyrase is transported into the nucleus together with the telomeric factor. After its activation, both tankyrase and TERF1 function as acceptors for ADP-ribosylation. The following dissociation of the nucleoprotein complex opens up the telomeres to telomerase and other enzymes, and so tankyrase is considered as a positive regulator of telomerase.

Two tankyrase isoenzymes have been identified in humans and other vertebrates [39, 40], denominated as TNKS (or tankyrase 1) and TNKL (or tankyrase 2). In non-modified state, they slightly differ in molecular mass (142 and 127 kD). Besides a catalytic domain (which is homologous to the corresponding PARP domain) tankyrase has a large ankyrin domain of 24 ankyrin repeats and also a SAM module. The latter two are involved in protein–protein interactions. In contrast to tankyrase 2, tankyrase 1 has an additional N-terminal domain with unknown function. Both isoenzymes lack nuclear localization signal and therefore the major proportion of them is localized in the cytoplasm [41-44]. In the cytoplasm, tankyrase is phosphorylated and activated by MAP kinase [42]. (It remains unclear whether activated enzyme is translocated into the nucleus or a nuclear pool of tankyrase is activated by MAP kinase; the latter is known to be transferred from cytoplasm into the nucleus.) Since MAP kinase is regulated by insulin and growth factors via Ras-MAPK signaling pathway, it is plausible to suggest that using tankyrase an organism "puts" telomeres of all cells under hormonal control.

If tankyrase inhibits TERF1 *in vivo*, increased TERF1 activity may be attributed to increase in protein concentration, which is observed at later stages of the cell cycle before mitosis [11]. Since up-regulation of TERF1 under experimental conditions induces premature mitosis [31], this protein is suggested to participate in packaging of mitotic chromosomes. When delay of mitosis is required (e.g., for DNA repair) this protein may be phosphorylated by ATM protein kinase; this kinase also activates p53 and induces apoptosis and cellular senescence via a p53-dependent mechanism. ATM (Ataxia-Telangiectasia Mutated) mutation is a cause of a serious inherited disease known as ataxia-telangiectasia. Some abnormal properties of cells with defective ATM gene may be normalized by TERF1 inhibition. This includes main-

tenance of normal length of telomeres, which are subjected to accelerated shortening in the mutant cells [45].

Recently another telomeric protein interacting with TERF1 has been recognized [46]. This protein of molecular mass of ~40 kD was named TINF2 or TIN2 (TRF1-interacting nuclear factor 2). It shares some structural resemblance with TERF protein: it has a Myb-like DNA binding domain at the C-terminus. This factor (as well as TERF1) promotes telomere shortening and it acts as negative regulator of telomerase activity [47]. Whereas in control culture of immortalized fibroblasts mean telomere length reduced slightly from 6400 to 5700 bp over 43 population doublings, overexpression of TINF2 caused more pronounced telomere shortening (to 5000 bp). Inhibition of TINF2 by expression of its dominant negative mutant resulted in telomere elongation up to 9000 bp. It is suggested that TINF2 modulates TERF1 function.

The mechanism of TERF1 action on telomeres involves not only DNA supercoiling and facilitation of subsequent t-loop formation. The interaction of TERF1 with numerous regulatory proteins can result in their concentrating near telomeres. For example, TERF1 binds potent telomerase inhibitor, PinX1 protein [48]. In contrast to other telomerase modulators influencing substrate availability (i.e., telomeres), PinX1 acts directly on telomerase.

Very recently, another telomere protein, Pot1, which binds at the telomeric single strand overhang, has also been recognized [49]. It is possible that the function of this 71 kD protein consists in protection of single strand DNA, which is especially susceptible to nucleases, irradiation, and various chemical agents. This putative protective role explains the name of this protein, its name being POT (Protection Of Telomeres). Pot1 interacts with TERF1. Expression of dominant negative mutant, which does not bind telomeres, as well as expression of mutant TERF1, inhibits activity of endogenous TERF1; this is accompanied by telomere elongation [50]. Since the concentration of TERF1 is proportional to telomere length, it is suggested that affecting Pot1 binding, TERF1 transmits information about telomere length to the telomere terminus.

It is possible that the list of various components of telomere nucleoprotein complex (also known as the telosome) is not limited to the proteins considered in this section. In the next section more data will be provided indicating the existence of some (unstudied) telomeric proteins and proteins associated with telomeres in structural and/or functional manner.

TELOMERE PROTEINS OF MODEL ORGANISMS

All the above mentioned proteins and their regulatory effects have been discovered during the last five to seven years in humans. Selection of human cells as the research object was closely related to particular research interests in laboratories making major contribution to progress in this field. It should be also noted that human fibroblasts and oncogenic viruses causing immortalization of the infected cells are classical models for studies of cellular or replicative senescence (see for review [35, 51]). However, besides experiments with human cells, other organisms have also been employed as models. It is noteworthy that only a small proportion of telomeric proteins of other eukaryotes have well characterized homologs in the human genome.

The finding of human proteins of the TERF family was soon followed by discovery of homologous protein Taz1 in *Schizosaccharomyces pombe* [37] (see table). TERF-like telomeric proteins possessing a Myb-like DNA-binding domain were also found in tobacco *Nicotiana glutinosa* [52] and some other plants [53]. The telomere binding domain typical for this protein family was denominated as the telobox [54]. Besides the above-mentioned proteins, this telobox was also found in telomeric protein Tbf1 from *S. cerevisiae*. Except for the DNA-binding region, the latter does not share any homology with human TERFs. This unique structure was also found in human TINF2 (see previous section). It has the telobox, but except for mouse, other model organisms (including yeast) lack its homologs.

Myb-like DNA-binding domain was also found in hRap1 protein (which binds at telomeres via TERF2) and its yeast homolog scRap1 [37]. However, these proteins have two such modules per subunit and their similarity with the "referent" telobox is not sufficient for joining these Rap proteins into one class with other telomeric proteins [54]. Yeast Rap1 (Repressor-Activator Protein) has been studied for many years (see review [3]). Binding of this protein at telomeres recruits proteins of Sir (Silent Information Regulator) and Rif (Rap Interacting Factor) families. Sir proteins regulate genome activity and heterochromatin formation, whereas Rif proteins control telomere length. The yeast *Saccharomyces* differs from *Schizosaccharomyces* by the absence of TERF telomeric protein family. In baker's yeast scRap protein binds directly to DNA, whereas its homologs in *S. pombe* or in human cells bind to DNA via spTaz1 and TERF2 protein, respectively.

Yeast [55, 56] and mammalian Sir proteins have evolved significantly. One of yeast members of this family, Sir2, has at least seven homologs in the human genome [56-58]. Sir2 binding at yeast telomeres causes heterochromatin formation and suppression of telomere-adjacent genes via interaction with Sir3 and Sir4. Homologs of these yeast proteins have not been found in man yet. Human homologs of yeast Rif are also still uncharacterized. The human genome has presumably functional genes encoding Rif-like proteins (see the table), but their function requires further investigation.

Homologs of telomere-associated proteins

Human telomere-associated protein	Homologs found in other organisms*	References
TERF1 TERF2	Taz1 (*Schizosaccharomyces pombe*)	[37]
TINF2	AAH30347 (*Mus musculus*)	
—	Tbf1 (*Saccharomyces cerevisiae*)	[54]
Pot1	Pot1 (*Schizosaccharomyces pombe*)	[49]
—	Cdc13 (*Saccharomyces cerevisiae*)	[66]
Rap1	Rap1 (*Saccharomyces cerevisiae*)	[37]
TNKS	NP_651410 (*Drosophila melanogaster*)	
TNKL	AAN40683 (*Caenorhabditis elegans*)	
SIRT1-7	Sir2 (*Saccharomyces cerevisiae*) Hst1–4 (*Saccharomyces cerevisiae*)	[56-59,144]
—	Sir3 (*Saccharomyces cerevisiae*)	
—	Sir4 (*Saccharomyces cerevisiae*)	
BAB14313	Rif1 (*Schizosaccharomyces pombe*)	[145]
ALL-1/HRX/MLL	Set1 (*Saccharomyces cerevisiae*) COMPASS (*Saccharomyces cerevisiae*):	[61] [62]
ASH2L	Cps60	
YAR003	Cps50	
YPL138	Cps40	
YKL018	Cps35	
YBR175	Cps30	
YDR469	Cps25	
Dot1L	Dot1 (*Saccharomyces cerevisiae*)	[146]
ATM	Tel1 (*Schizosaccharomyces pombe*)	[147]
Clk2	Tel2 (*Saccharomyces cerevisiae*) Clk2 (*Caenorhabditis elegans*)	[131] [132]
Ku70	Hdf1 (*Saccharomyces cerevisiae*)	[75]
Ku80	Hdf2 (*Saccharomyces cerevisiae*)	
PinX1	PinX1 (*Saccharomyces cerevisiae*)	[48]
Rad50	Rad50 (*Saccharomyces cerevisiae*)	[89]
Mre11	Mre11 (*Saccharomyces cerevisiae*)	[67]
Nbs1	AAP56684 *(Mycoplasma gallisepticum*)	[101,102]
—	Xrs2 (*Saccharomyces cerevisiae*)	

* In the absence of functional characteristics, the reference on the number of homologous sequences in the Genbank database is given.

Sir proteins exhibit catalytic activity; they deacetylate histones [59]. Histone deacetylases are also involved in regulation of genome activity, including the telomere region [60]. Other enzymes involved in posttranslational modification of histones also regulate genome activity. These include yeast methyl transferases, such as Set1 and Dot1, which have homologs in man. Set1/COMPASS is a multi-protein complex that consists of at least seven various components [61, 62]. One of them (YAR003) is involved into Rb regulation; however, these proteins still require detailed functional characterization. Dot1 is a methyl transferase lacking the Set domain [63]. Its activity is regulated by binding of yeast Sir3 at telomeres [64].

Tankyrase is a TERF1 regulator. This enzyme was not found in yeast; however, homologs of human genes encoding tankyrase have been recognized in genomes of drosophila, nematode *C. elegans* (see table), and in other animals [44]. Apparently, evolution of tankyrases occurred within the animal kingdom; invertebrate genomes have only one tankyrase homolog, whereas vertebrate genomes have two such genes.

S. pombe has an ortholog of human Pot1 protein [49]. Like many other telomeric proteins of baker's yeast, Cdc13 protein of *S. cerevisiae*, which binds at the telomeric single strand overhang, is not homologous to functionally related protein found in humans and *S. pombe*. Nevertheless, its 3-dimensional structure has some resemblance to Pot1 protein [65, 66].

This analysis clearly demonstrates that data on the structure of telomeric nucleoprotein complex in humans and in model organisms (especially *S. cerevisiae*) should be carefully extrapolated. Evolution of budding yeast went in a different direction than in unicellular ancestor of *Metazoa*. Nevertheless, the presence of genes encoding homologous proteins in man and other organisms may provide useful information during model studies.

TELOMERES AND REPAIR SYSTEMS

Evidently, mechanisms underlying telomere protection and the DNA repair system are interrelated. Nevertheless, both DNA repair and telomere functioning require further investigations because many important problems remain to be clarified in this field.

Double strand breaks (DSB) of DNA are provoked by various unfavorable factors such as ionizing radiation. DSB is also considered as an important process in restarting broken replication forks, which affect normal chromosome duplication in S-phase (see reviews [67-69]). Two competitive pathways of DSB repair are recognized: 1) pairing of homologous chromosomes followed by recombination and refilling of damaged strands; 2) nonhomologous DNA end joining (NHEJ). The first pathway predominates in S and G2 phases of the cell cycle and in actively dividing (e.g., yeast) cells, whereas the second one predominates in resting or rarely dividing (e.g., mammalian) cells (see for review [70]). Breaks of genomic DNA may stimulate significantly (up to several orders of magnitude) homologous recombination in mammalian cells and this way the homologous repair was found to account for up to 50% of observed repair events [71].

When telomere ends are not protected by t-loops, they are identical to DSB and so they should be repaired. For example, they can be fused with formation of circular chromosomes and chromosomes with two or more centromeres via the NHEJ mechanism. The latter is observed in cells with critically short telomeres. Irrespectively to repair mechanisms, both DSB and unprotected telomeres activate ATM protein kinase. This results in activation of p53 (see above) followed by blockade of proliferation and induction of mechanisms responsible for cellular senescence and apoptosis. During t-loop formation, invasion of telomeric single strand overhang into duplex of the tail imitates homologous recombination; under certain conditions, this may stimulate synthesis of a new strand of DNA.

Human and yeast cell telomeric nucleoprotein complex always contains Ku protein; this is a component of the NHEJ pathway [72-74]. Human Ku protein is a heterodimer consisting of subunits of 69 and 83 kD designated Ku70 and Ku80 (or Ku86). Homologous yeast proteins are named HDF1 (or yKu70) and HDF2 (or yKu80). Ku protein was discovered in the eighties as autoantigen in patients with various autoimmune diseases; the autoantibodies were used for cloning [75]. Ku protein binds both single and double strand breaks not only in telomeric but also in any other DNA sequence. It is suggested that this protein prevents subsequent nucleic acid degradation. NHEJ is often accompanied by deletion; although deficit of Ku protein cannot affect repair level, it is accompanied by an increase in products containing deletions [76].

In mammals, binding of Ku protein to DNA may be followed by formation of DNA-dependent protein kinase (DNA-PK). The latter consists of two Ku subunits and one catalytic subunit (DNA-PKcs) that is activated upon binding of Ku. Mutations of the catalytic subunit were observed in *scid* mice; this mutation causes suppression of V(D)J recombination in these animals and increased sensitivity of cells to ionizing radiation [77]. Subsequently, DNA-PK activity stimulates NHEJ. Yeast cells lack DNA-PKcs; nevertheless, Ku protein participates in DNA repair, which therefore involves other mechanisms.

Participation of Ku protein in telomere functioning has been noted recently in several laboratories [78-81] (see also a review on yeast Ku proteins [3]). There are also data indicating that inactivation of genes encoding Ku subunits caused shortened lifespan and early onset of senescence in mice [82-84]. Similar results were obtained using human cell cultures [85].

The involvement of Ku in both DSB repair and telomere protection, including protection against fusions, is an apparent contradiction, because the two mechanisms serve opposing goals. It should be also noted that the role of DNA-PK in V(D)J recombination also differs from its role in NHEJ. In the first case, DNA sites subjected to recombination are preliminarily approached by a specific protein complex before cleavage, whereas in the latter case breaks occur randomly due to effects of external factors. It is suggested that in all cases Ku plays protecting functions, whereas other processes involve proteins which are specific for these particular processes (either TERF or repair and recombination systems). For example, on telomeres Ku probably inhibits degradation and recombination of telomeric repeats. Lack of functional Ku caused rearrangements of telomere-associated sequences near chromosome ends while normally such recombination is suppressed [86]. Knockout of the gene encoding Ku80 in mice resulted in inhibition of proliferation and early onset of cellular senescence [87]. However, deficit of Ku did not prevent telomere fusion. In yeast double mutation of Ku and telomerase allowed only mutant cells with circular chromosomes to escape senescence and survive. However, researchers do not have a common viewpoint on the role of Ku in the telosome.

Besides chromosome circularization, other ways of rescuing replicative senescence in the absence of telomerase also exist. A significant proportion of tumor cells lack telomerase; however, they bypass senescence by using an alternative mechanism of maintenance of telomere length (ALT). It is suggested that at least some ALT mechanisms involve homologous telomere recombination [88].

After binding of Ku at DSB and activation of DNA-PK, the repair process involves many proteins including the Rad50/Mre11/Nbs1 complex. Besides NHEJ, these proteins can also act in homologous recombination and maintenance of telomere integrity.

Rad50 (Radiation mutant 50) protein was initially studied in yeast; however, in the nineties its human homolog was also found [89]. Rad50 is a rod-like molecule that shares some resemblance with myosin. The molecular mass of both yeast and human orthologs is 153 kD. The protein has two DNA-binding domains exhibiting ATPase activity, which are located at the N- and C-termini. These domains represent the most conservative amino acid sequences of the polypeptide chain; in man and yeast they share more than 50% identity. Catalytic domains are linked via long coiled coils forming an acute angle at a Zn-binding motif, located in the center of molecule, so that DNA-binding domains are closely co-localized [90]. The Zn-binding hinge domain is required for dimerization of Rad50 and so the dimer of two rod-like proteins may bridge two DNA molecules located at a distance up to 1200 Å [91].

Mre11 (Meiotic recombination 11) is a nuclease that was originally studied in yeast [92]. Later its homologs were found in all kingdoms including animals. In the process of repair, Mre11 degrades hairpins and other incorrect structures of DNA molecules formed for example in broken replication forks. During homologous recombination, nuclease activity is required for pairing of two DNA molecules, which is mediated by 3′-terminal single strand sequences of several hundred nucleotides. The complex Rad50/Mre11, where Mre11 binds ATPase domains of Rad50, exhibits endonuclease and also 3′- to 5′-exonuclease activity [93]. Mre11 seems to be not the only nuclease involved in catalytic recombination [67, 94-98]. Some mutations in Mre11, which block interaction with Rad50, are accompanied by telomere shortening without impairments in repair processes [99]; other mutations decrease catalytic activity of this protein but do not influence involvement of this protein in telomere protection [100]. Therefore, the functions of this protein in repair and maintenance of telomere structure are not identical.

The third component of this complex is not homologous in yeast and man. In *S. cerevisiae*, this is Xrs2 protein, whereas the human component is nibrin or Nbs1, the protein encoded by the gene mutated in Nijmegen breakage syndrome. Mutation in this protein is accompanied at the cellular level by changes similar to those induced by ATM mutations. (However, clinical manifestations differ in these inherited diseases.) In both disorders, mutant cells are characterized by increased sensitivity to ionizing radiation, chromosome fragility, lack of p53 induction in response to genome damage, and some other signs. It is suggested that Nbs1 and ATM are molecules involved in signal transduction within the same signaling pathway [101-103]. Nbs1 modulates catalytic activity of Rad50 and Mre11; it also potentiates ATP-dependent DNA unwinding [96].

Concluding the review, it is noteworthy to remark that telomere functioning needs participation of many proteins; some of them are not identified yet, others require better characterization. Most of the proteins described here have been identified just recently. Some of these proteins still do not have known homologs in other organisms, including humans. Mechanisms responsible for functioning of protein assemblies and signaling pathways are poorly understood.

Sometimes it is hard to understand whether a certain protein is important for telomeres, does it play any role in their protection, replication, repair, and signal transduction to other subcellular structures about telomere state, or alternatively it does not exhibit specificity to telomeres and its DNA-binding activity is not related to specific nucleotide sequence like the telomeric one. For example, proteins involved in repair processes were found not only in telomeres. These include Ku and Rad50/Mre11/Nbs1

complex, which are always present in telosomes and in cases of necessity they are translocated to other DNA sites. This group of proteins also includes UP1 [104-106], which maintains structure of telomeres and some other G-rich sites of DNA.

Telomere-associated proteins may be subdivided by functional characteristics into the following groups.

1. Proteins maintaining spatial structure of telomeres. These include proteins of TERF family and other telobox containing proteins.

2. Proteins involved in formation of telomeric heterochromatin and regulation of telomere-adjacent genes by their association with heterochromatin. This group includes Rap, Sir, Rif, and also histone acetylases, methyl transferases, etc.

3. Proteins regulating telomere replication and association with telomerase. This group includes tankyrase and also proteins involved in direct regulation of telomerase (e.g., PinX1, Stn1/Cdc13/Ten1 complex in yeast [3, 107-110]).

4. Proteins of DNA repair within telomeres and possibly negative regulation of their length (i.e., nucleases). This group includes Rad50/Mre11/Nbs1 and Ku.

5. Signaling molecules "informing" other subcellular compartments about protection or damage of telomeres and also initiating cellular senescence and apoptosis. This group includes ATM protein kinase, p53, p16INK4, pRb, and other proteins of signaling pathways regulating cell proliferation and death.

Of course, the suggested mechanistic classification of telomeric and telomere-associated proteins into the above-mentioned groups does not rule out their possible participation in several various mechanisms that are still under investigation.

Besides basic aspects of studies of telomeres and biochemical mechanisms related to these structures, there is a whole branch of applied studies that investigate regulation of telomeric proteins. The cellular senescence is only one of such problems.

It should be emphasized that replicative or cellular senescence is not necessarily related to aging of multicellular organisms, as well as a molecular mechanism of the aging may not implicate telomere shortening. For example, knockout of telomerase RNA and consequent block of the enzyme activity in mice can produce up to six generations of animals with sequentially shortening telomeres [111]. Normal telomeres of mouse chromosomes are extremely long and may reach up to 150 kb. Experimentally observed reduction in their length by 4.8 ± 2.4 kb per generation was not accompanied by serious changes in telomere structure. Evidently, the change of generations in this species is not related to telomere shortening.

In humans, there are at least two kinds of pathologically rapid aging with known molecular mechanisms. Werner syndrome or progeria of the adult is caused by mutation in the gene encoding helicase [112]; this impairs DNA replication and cell proliferation. Patients die from senile disorders at age 35-50. Hutchinson–Gilford syndrome or premature senility syndrome is related to mutation of lamin A [113]; this complicates proliferation due to dysfunction of the nuclear membrane. This results in premature aging, and patients often die before the age of 20. In both these diseases, genetic abnormalities cause pathological slowing of tissue renewal that does not originate from telomere shortening.

Nevertheless, normal aging in man is apparently related to exhaustion of the Hayflick limit [51]. In senile people, telomere length reaches the limit of 1-2 kb required for formation of minimal t-loop. If this is true, the telomeric mechanism of aging may be the cause of natural change of generations in humans.

The idea of phenotypic difference between mortal soma and immortal germ-line was initially suggested by A. Weismann [114]. Later L. Hayflick experimentally confirmed this hypothesis at the cellular level [51]. In the seventies A. M. Olovnikov postulated a molecular mechanism of telomere under-replication [115]. This mechanism still represent a classical standard of causal link between DNA state and proliferative potential of cells. Indeed, during individual development most human and animal cells lose their ability to express telomerase [2, 116, 117] and, consequently, their telomeres may only shorten. At certain limit, protection of linear chromosome ends becomes impossible, and telomeres initiate proliferation block and cell death via ATM/p53- and p16/pRb-dependent signaling pathways.

Although the outline of this mechanism looks rather simple, it still requires further investigation because results of studies continue to raise new questions. For example, stem cells, which are responsible for maintenance of tissue regeneration, as well as germ cells express telomerase. Some somatic cells capable for clonal expansion (e.g., lymphocytes) also express telomerase under certain conditions [118-126]. Nevertheless, in lymphocytes telomere length reduces as in other somatic cells [26, 127-130]. This suggests that lifespan and aging are regulated not only by telomerase.

The search for genes related to regulation of aging revealed several protein products such as Clk2. A corresponding gene was found in *C. elegans*; mutation in this gene increased lifespan of this nematode. A homologous gene was also known as *Tel2* in yeast. Both homologs encode proteins regulating telomere length; however, the molecular mechanisms of their effects remain unclear [131, 132].

Mutations in the *daf-2* gene of *C. elegans* and its homolog, *Inr*, in drosophila increased lifespan of these model animals by several-fold and 85%, respectively (for review, see [133]). Both genes encode receptors of insulin-like ligands. The phenomenon of increased lifespan caused by caloric restriction has been known for a

long time; however, only identification of insulin- and/or insulin-like growth factor receptors as important components of the mechanism underlying this phenomenon initiated its study on the molecular level. It is suggested that animals have a specific neuroendocrine system regulating life cycle. This system increases lifespan of an individual under conditions of limited food availability.

The possible role of telomeres in its functioning requires detailed study. Some components of this system appear in unicellular organisms. For example, lifespan of maternal cells of budding yeast depends on the presence of nutrients. Diet-dependent regulation of the life cycle somehow involves Sir2 [134-136]. It should be also noted that in animals insulin and growth factor receptors can control telomeres via tankyrase [42]. Short telomeres may also signal about their state to the signaling pathway, which begins at growth factor receptors and leads to tankyrase. It was shown that p53 regulates signal transduction from these receptors via an adapter protein, p66Shc [137, 138]. Mutations in genes encoding p53 and Shc may influence lifespan in mice [139-141].

Studies of molecular mechanisms of aging continue. There are several hypotheses on their functioning (see reviews [133, 142]). In contrast to apoptosis, a known and rather well studied mechanism of programmed cell death, the term phenoptosis was coined for the hypothetical mechanism of natural death of the whole organism [142, 143].

Cancer is another important medical problem that is also related to telomere functioning. Cellular senescence is considered as one of the barriers preventing development of malignant tumors (see review [88]). Malignant transformation of somatic cells results in appearance of some properties (which are not typical to normal cells), including unlimited and unregulated clonal expansion. Such cells either express telomerase or telomere length is maintained at a stable level by an alternative mechanism. A significant proportion of known oncoproteins and tumor suppressors are either involved in regulation of telomeres or in signaling about their state. These include p53, pRb, ATM, Nbs1, and also Ras and other oncoproteins of the Ras-MAPK signaling pathway, which controls tankyrase and mediates mitogenic activity of Src and other tyrosine kinase receptors.

Applied and basic aspects of studies of telomere nucleoprotein complex are very important, and we surely will witness many new discoveries in this intriguing field in the near future.

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