Human Ku70/80 Associates Physically with Telomerase through Interaction with hTERT*

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Telomere length maintenance, an activity essential for chromosome stability and genome integrity, is regulated by telomerase- and telomere-associated factors. The DNA repair protein Ku (a heterodimer of Ku70 and Ku80 subunits) associates with mammalian telomeres and contributes to telomere maintenance. Here, we analyzed the physical association of Ku with human telomerase both in vivo and in vitro. Antibodies specific to human Ku proteins precipitated human telomerase in extracts from tumor cells, as well as from telomerase-immortalized normal cells, regardless of the presence of DNA-dependent protein kinase catalytic subunit. The same Ku antibodies also precipitated in vitro reconstituted telomerase, suggesting that this association does not require telomeric DNA. Moreover, Ku associated with the in vitro translated catalytic subunit of telomerase (hTERT) in the absence of telomerase RNA (hTR) or telomeric DNA. The results presented here are the first to report that Ku associates with hTERT, and this interaction may function to regulate the access of telomerase to telomeric DNA ends.

Telomeres are distinct DNA-protein structures that protect eukaryotic chromosome ends from degradation and inappropriate recombination or fusion. Telomeres shorten every time a cell divides because of incomplete DNA replication and DNA end processing. When telomere length reaches a critical point, cells stop dividing and undergo replicative senescence (reviewed in Refs. 1–3). Telomerase, an unusual reverse transcriptase, prevents telomere shortening by using its integral RNA component as a template to add telomeric DNA repeats to chromosome 3′ ends (reviewed in Ref. 4). Most human somatic cells do not have telomerase activity and have a defined life span (5), whereas most cancer cells have an indefinite proliferative capacity and maintain their telomere length by up-regulating telomerase (6). Telomere length regulation has been proposed to be relevant for both cancer and aging (reviewed in Refs. 6 and 7).

The human telomerase complex is a ribonucleoprotein containing an integral RNA (hTR), a reverse transcriptase protein subunit (hTERT), and several associated proteins (reviewed in Refs. 8 and 9). In human cells, several proteins associated with telomerase activity have been identified. The “foldsome” proteins hsp90 and p23 interact with hTERT and are involved in assembly of telomerase activity (10). Many hTR-binding proteins (dykserin (11), L22 (12), hStau (12), heterogeneous nuclear ribonucleoproteins C1 and C2 (13, 14), the La autoantigen (15), hGAR1 (16), hNOP10 (17), and hNHP2 (17)) (reviewed in Ref. 9) are each associated with telomerase activity in cell extracts. Another telomerase-associated protein, TEP1, associates with both hTR and hTERT but is not essential for telomerase activity or telomere length maintenance in vivo (18–20). It is likely that additional telomerase-associated proteins remain to be identified, because native human telomerase has an estimated mass of over 1000 kDa (21, 22).

The ability of telomerase to elongate telomeres is regulated by many factors. In mammals, the telomere-binding protein TRF1/Pin2, TRF2, tankyrase, Tin2, and heterogeneous nuclear ribonucleoproteins such as A1 affect telomere maintenance (22–23). The Pin2/TRF1-interacting protein PinX1 binds to hTERT and inhibits telomerase activity (29).

Many of the proteins involved in DNA double-strand break repair, such as the RAD50-Mre11 complex (30–32) and Ku (33, 34), have been found to associate with telomeres. Ku is a heterodimeric protein composed of an ~70-kDa subunit (Ku70) and an ~80-kDa subunit (Ku80 or Ku86). It is one of the most abundant proteins in human cells and is involved in multiple important cellular metabolic processes such as non-homologous end joining, V(D)J recombination of immunoglobulins and T-cell receptor genes, transcriptional regulation, DNA replication, regulation of heat shock-induced responses, and regulation of telomere maintenance (reviewed in Ref. 35). Ku is associated physically with mammalian telomeric DNA (33, 34) and appears to bind to the telomere-repeat-binding proteins TRF1 (36) and TRF2 (37). Mouse cells lacking Ku protein display moderate telomere elongation and show a high rate of telomere-telomere fusion events (36, 38, 39), indicating that Ku may act to protect telomere ends from fusion events. Furthermore, Ku80 deficiency leads to telomere elongation in normal mice but not in a telomerase-deficient background (40), suggesting that Ku may be a negative regulator of telomerase-mediated telomere elongation. The precise mechanism of Ku action at the telomere is still unclear.

We report here that Ku associates physically with human telomerase. Immunoprecipitation with Ku-specific antibodies followed by the telomeric repeat amplification protocol (TRAP) assay demonstrated the association of Ku with telomerase both in vivo and in vitro. We also detected the association of Ku with

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The abbreviations used are: TRAP, telomeric repeat amplification protocol; HA, hemagglutinin; ERK, extracellular signal-regulated kinase; RRL, rabbit reticulocyte lysate; RF, release factor; DNA-PKcs, DNA-dependent protein kinase catalytic subunit.
Ku Associates with Telomerase

**EXPERIMENTAL PROCEDURES**

*Cell Lines and Culture Conditions*—The human lung carcinoma cell line H1299 (ATCC CRL-5803), BJ fibroblasts infected with an hTERT-containing retrovirus (41), and the human colon carcinoma cell line HCT116 and its Ku86+/−/− derivative cells (42) were cultured at 37 °C under 5% CO2 in a 4:1 mixture of Dulbecco’s modified Eagle’s medium and medium 199 supplemented with 10% fetal calf serum (HyClone, Logan, UT) and 50 μg/mL of gentamycin (Sigma). The human malignant salivary gland cell line M05Bj4 (43) was cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (HyClone).

*Antibodies*—Monoclonal antibodies used included anti-HA (12CA5; Roche Molecular Biochemicals), anti-Ku70 (NS310; Covance Laboratories Inc., Princeton, NJ and Ab-5; NeoMarkers, Fremont, CA), anti-Ku80 (Ab-2 and Ab-7; NeoMarkers, Fremont, CA), and anti-actin (Santa Cruz Biotechnology, Santa Cruz, CA). Polyclonal anti-hTERT (TKP-1) was a gift from Tg Pautista (Columbia Medical Center, New York, NY). Monoclonal anti-p23 (J33) was provided by David O. Toft (Mayo Graduate School, Rochester, MN). Polyclonal anti-ERK1 was a gift from Michael A. White (University of Texas Southwestern Medical Center at Dallas). Normal mouse IgG was purchased from Santa Cruz Biotechnology.

**Construction of Plasmids and in Vitro Synthesis of Human Ku70 and Ku80**—Full-length human Ku70 cDNA and Ku80 cDNA were cloned into pBluescript/KS(+) vector (Stratagene, La Jolla, CA) by PCR cloning. Primers used to amplify Ku70 were 5′-CCG GTA TCC ACC ATG GAC TAC AAA GAC GAT GAC GAG AAG TCA GGA GGG GAG TCA TAT TA-3′ and 5′-TTC TCC CCC GGC GGG GCA GTC GCT GAA GGT CGT-3′. The PCR reaction for amplifying Ku70 DNA was performed at 94 °C for 2 min, 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min followed by extension at 72 °C for 10 min. The DNA product was gel-purified, subjected to BamHI and Smal digestion (sites are underlined), and then cloned into BamHI- and Smal-digested pBluescript/ KS(+) vector. Primers used to amplify Ku80 were 5′-AGA TCT CGG CAT GAT GGT GCG GTC GGG GAA TAA G-3′ and 5′-TCT AAG GAA TTC CCT ATC TGA TCT GGA AAT C-3′. The PCR reaction for amplifying Ku80 DNA was performed at 94 °C for 2 min, 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2.5 min followed by extension at 72 °C for 10 min. The DNA product was gel-purified, subjected to BamHI and EcoRI digestion (sites are underlined), and then cloned into BamHI- and EcoRI-digested pBluescript/ KS(+) vector. The cloned sequences were subjected to DNA sequencing to ensure that no mutations were introduced. Human Ku70 and Ku80 proteins were synthesized in the rabbit reticulocyte lysate (RRL) system (Promega, Madison, WI) in the presence of [35S]methionine following the protocols provided by manufacture.

*In Vitro Synthesis and Analysis of hTERT—hTERT was synthesized in the RRL system (Promega, Madison, WI) in the presence of [35S]methionine as described previously (44). Telomerase activity was reconstituted in the RRL system as described previously (10), and activity in all samples was determined by TRAP (10, 15, 44).

*TRAP Analysis—*Non-radioactive TRAP analysis was performed as described (15) except that 25 cycles of PCR (95 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s) were used to amplify telomerase-extended product. Products were separated on 10% polyacrylamide gels and visualized by autoradiography. For radioactive TRAP analysis, the TRAP-eze telomerase detection kit (Intergen, Purchase, NY), which includes a 36-bp internal standard to allow quantitation of activity, was used. After telomerase extension for 20 min at room temperature, extended products were amplified by a two-step PCR (94 °C for 30 s and 60 °C for 30 s) for 24 cycles. Products were separated on 10% polyacrylamide gels and visualized by autoradiography. Quantitative estimates of telomerase activity were calculated by determining the ratio of the 36-bp internal standard to the 6-bp telomerase-specific ladder.

**Immunoprecipitations**—Immunoprecipitation from cell lysates was performed as described (10) with minor modifications. Cells were suspended in lysis buffer (0.01% Nonidet P-40, 10 mM Tris, pH 7.6, 50 mM KC1, 0.5 mM EDTA, 50 mM dithiothreitol, and 2 mM phenylmethylsulfonyl fluoride) and then sonicated at 50 J/watt-s for three 5-s pulses. Lysates were spun at 13,000 rpm for 15 min, and the resulting supernatants were used for immunoprecipitation. Approximately 10 μg of each antibody was precoupled to 20 μl of 50% slurry of protein A/G–agarose beads (Santa Cruz Biotechnology) by incubating for 1 h at 4 °C with constant rotation. Antibody-coated beads were washed three times with lysis buffer prior to use in immunoprecipitation reactions. Lysates corresponding to 500,000 cells in 5% bovine serum albumin were added to the antibody beads and rotated for 1 h at 4 °C. Immunoprecipitations were then washed four times with the lysis buffer (350 μl for 10 min with rotation at 4 °C). The agarose bead pellets were resuspended in a final volume of 25 μl with lysis buffer, and aliquots were removed for TRAP assays. For immunoblotting assays after immunoprecipitation, 5 μl of agarose beads was heated at 80 °C for 10 min in SDS sample buffer (boiling in SDS sample buffer caused most of the hTERT protein to remain at the origin, presumably because of aggregative precipitation). The agarose bead pellets were resuspended in a final volume of 15 μl with lysis buffer, and aliquots were removed for TRAP assays. To detect precipitated proteins, washed pellets were heated to 80 °C for 10 min in SDS sample buffer, and the beads were removed by brief centrifugation. The supernatant was loaded immediately on SDS-PAGE gel (7.5%). Dried gels were exposed to a PhosphorImager screen (Molecular Dynamics) for 24 h.

**RESULTS**

*Ku70 and Ku80 Associate Specifically with Telomerase in Both Human Tumor Cells and Telomerase-immortalized Cells*—The human lung carcinoma cell line H1299 is telomerase-positive. To test whether Ku associates with telomerase in vivo, we used monoclonal antibodies against Ku70 and Ku80 to pull down telomerase from H1299 cell extracts. Following immunoprecipitation, the precipitates were subjected to TRAP analysis. As shown in Fig. 1A, Ku70 and Ku80 monoclonal antibodies (α-Ku70 (NS310); see lane 7 and α-Ku80 (Ab-7); see lane 10) precipitated telomerase activity, measured as TRAP activity, similar to an hTERT antibody, indicating that Ku70 and Ku80 may associate with telomerase in vivo. An antibody to p23, which has been shown to associate with telomerase (10), was used as a positive control (Fig. 1A, lane 11). Neither mouse IgG nor control antibodies to ribosomal release factor (RF) precipitated telomerase (Fig. 1A, lanes 4 and 6).

Other anti-Ku antibodies, α-Ku70 (Ab-5) and α-Ku80 (Ab-2), were unable to immunoprecipitate telomerase from H1299 cell extracts (Fig. 1A, lanes 8 and 9). Immunoprecipitation followed by immunoblotting analyses showed that Ab-5 (an antibody that does not associate with telomerase) and NS310 (an antibody that does associate with telomerase) precipitated Ku70 equally (Fig. 1B), and Ab-2 and Ab-7 precipitated Ku80 equally (data not shown). This suggests that if the interaction with telomerase in cell extracts is because of nonspecific attachment of the very abundant Ku proteins (45), it is localized to specific domains that are blocked by the Ab-5 antibody of Ku70 and the Ab-2 antibody to Ku80.

Ku is essential for human somatic tissue culture cells, and Ku−/− cells undergo massive apoptosis after limited rounds of cell divisions (42). The human colon cancer cell HCT116 derivative with a targeted disruption of one copy of Ku80 contains only 20–50% as much Ku80 and Ku70 protein as the parental
Ku Associates with Telomerase

We then tested whether Ku70 and Ku80 associate with telomerase in vivo. To explore the possibility that the anti-Ku70 and anti-Ku80 antibodies may bind nonspecifically to telomerase, we first tested whether the anti-Ku70 and anti-Ku80 antibodies precipitated less telomerase activity from the Ku80 heterozygous cells. As shown in Fig. 1D, the amount of telomerase activity precipitated by anti-Ku70 (N3H10) and anti-Ku80 (Ab-7) antibodies was reduced dramatically in Ku80 heterozygous cells compared with wild-type parental cells, indicating that the association of Ku with telomerase is likely to be specific.

We then tested whether anti-hTERT antibody could immunoprecipitate Ku proteins. Immunoprecipitation followed by immunoblotting analysis revealed that polyclonal anti-hTERT antibody precipitated Ku70 and Ku80 (Fig. 1E) but did not precipitate other abundant proteins such as ERK-1 (46) and actin (Fig. 1E). Meanwhile, neither Ku70 nor Ku80 antibody precipitated ERK-1 or actin (Fig. 1E). This adds additional evidence that the association of Ku with telomerase is specific.

We then tested whether Ku70 and Ku80 associate with telomerase in telomerase immortalized cells. As shown in Fig. 2, antibodies specific to Ku70 and Ku80 proteins precipitated human telomerase activity in BJ fibroblasts overexpressing hTERT ectopically. Therefore, Ku70 and Ku80 associate with telomerase in both cancer cells and hTERT-expressing normal diploid cells.

**DNA-PKcs Subunit Is Not Essential for the Ku Association with Telomerase in Vivo**—DNA-PKcs, the catalytic subunit of DNA-dependent protein kinase, functions together with Ku, the regulatory subunit of DNA-dependent protein kinase, in DNA double-strand break repair (35). To determine whether DNA-PKcs is essential for the association of Ku70/80 (presumably as the Ku heterodimer) with human telomerase, we used the human glioma cell line MOS9J, which lacks DNA-PKcs but contains a normal Ku protein level (43) in the immunoprecipitation assay. Despite the absence of DNA-PKcs, antibodies specific to Ku70 and Ku80 still precipitated telomerase activity (Fig. 3). Therefore, the DNA-PKcs subunit is not essential for the association of Ku with telomerase.

**Ku70 and Ku80 Associate with Telomerase in Vitro**—Telomerase activity was reconstituted in an RRL using in vitro transcribed and translated HA-tagged hTERT (10). Ku70 and Ku80 proteins were also in transcribed and translated in RRL. As shown in Fig. 4, α-Ku70 (N3H10) and α-Ku80 (Ab-7) precipitated telomerase activity whereas α-Ku70 (Ab-5) and α-Ku80 (Ab-2) did not. This is cells as described under “Experimental Procedures.” The immunoprecipitates were analyzed for telomerase activity by non-radioactive TRAP assays. Aliquots equivalent to 12,500 cells were run from these TRAP assays. The input lane corresponds to activity present in 500 or 1,500 cells. LB, lysis buffer only. ITAS represents the 36-bp internal TRAP assay standards. B, immunoprecipitation of Ku70 protein with different anti-Ku70 antibodies. The precipitates from immunoprecipitation assays using different anti-Ku70 antibodies were subjected to immunoblotting using α-Ku70 N3H10. C, Ku80+/− cells have diminished levels of Ku protein. Whole cell extract was prepared from wild-type HCT116 cells (+/+ ) and Ku80 heterozygous cells (+/− ) and analyzed by immunoblotting for Ku80, Ku70, and actin protein levels. D, the indicated antibodies were used to immunoprecipitate proteins from lysates of HCT116 wild-type and Ku80+/− cells corresponding to 500,000 cells as described under “Experimental Procedures.” The immunoprecipitates were analyzed for telomerase activity by radioactive TRAP assays. Aliquots equivalent to 12,500 cells were run from these TRAP assays. The input lane corresponds to activity present in 1,000 cells. Relative telomerase activity was estimated using ImageQuant by determining the ratio of the 36-bp internal standard to the 6-bp telomerase-specific ladder. E, immunoblotting analysis followed by immunoprecipitation using indicated antibodies. The precipitates were subjected to immunoblotting analysis using anti-Ku70 (N3H10), anti-Ku80 (Ab-7), anti-ERK1, and anti-actin, respectively.

![Fig. 1](http://www.jbc.org/fig/4)

**Fig. 1.** Both Ku70 and Ku80 associate with telomerase in H1299 cells. A, the indicated antibodies were used to immunoprecipitate (IP) proteins from lysates of H1299 cells corresponding to 500,000 wild-type cells (42) (Fig. 1C). To explore the possibility that the anti-Ku70 and anti-Ku80 antibodies may bind nonspecifically to telomerase, we first tested whether the anti-Ku70 and anti-Ku80 antibodies precipitated less telomerase activity from the Ku80 heterozygous cells. As shown in Fig. 1D, the amount of telomerase activity precipitated by anti-Ku70 (N3H10) and anti-Ku80 (Ab-7) antibodies was reduced dramatically in Ku80 heterozygous cells compared with wild-type parental cells, indicating that the association of Ku with telomerase is likely to be specific.

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**DNA-PKcs Subunit Is Not Essential for the Ku Association with Telomerase in Vivo**—DNA-PKcs, the catalytic subunit of DNA-dependent protein kinase, functions together with Ku, the regulatory subunit of DNA-dependent protein kinase, in DNA double-strand break repair (35). To determine whether DNA-PKcs is essential for the association of Ku70/80 (presumably as the Ku heterodimer) with human telomerase, we used the human glioma cell line MOS9J, which lacks DNA-PKcs but contains a normal Ku protein level (43) in the immunoprecipitation assay. Despite the absence of DNA-PKcs, antibodies specific to Ku70 and Ku80 still precipitated telomerase activity (Fig. 3). Therefore, the DNA-PKcs subunit is not essential for the association of Ku with telomerase.

**Ku70 and Ku80 Associate with Telomerase in Vitro**—Telomerase activity was reconstituted in an RRL using in vitro transcribed and translated HA-tagged hTERT (10). Ku70 and Ku80 proteins were also in transcribed and translated in RRL. As shown in Fig. 4, α-Ku70 (N3H10) and α-Ku80 (Ab-7) precipitated telomerase activity whereas α-Ku70 (Ab-5) and α-Ku80 (Ab-2) did not. This is
Fig. 2. Ku70 and Ku80 associate with telomerase in hTERT-expressing BJ fibroblasts. The indicated antibodies were used to immunoprecipitate (IP) proteins from lysates of BJ fibroblasts ectopically overexpressing hTERT and H1299 cells corresponding to 500,000 cells as described under "Experimental Procedures." The immunoprecipitates were assayed for telomerase activity by non-radioactive TRAP. Relative telomerase activity was estimated using ImageQuant by determining the ratio of the 36-bp internal standard to the 6-bp telomerase-specific ladder. The input lane corresponds to activity present in lysate from indicated cell numbers. TRAP assays were performed from lysates of 20,000 cells each. LB, lysis buffer only. ITAS represents the 36-bp internal TRAP assay standards.

Fig. 3. Ku70 and Ku80 associate with telomerase in the DNA-PKcs-deficient cell line MO59J. The indicated antibodies were used to immunoprecipitate (IP) proteins from lysates of MO59J corresponding to 500,000 cells as described under "Experimental Procedures." The immunoprecipitates were assayed for telomerase activity by non-radioactive TRAP. Aliquots equivalent to 20,000 cells were run from these TRAP assays. Relative telomerase activity was estimated using ImageQuant by determining the ratio of the 36-bp internal standard to the 6-bp telomerase-specific ladder. The input lane corresponds to activity present in lysate from indicated cell numbers. TRAP assays were performed from lysates of 20,000 cells each. LB, lysis buffer only. ITAS represents the 36-bp internal TRAP assay standards.

consistent with the in vivo results (Fig. 1A). The positive control, an antibody specific to p23, precipitated telomerase (10) whereas an irrelevant antibody to translation release factor (α-RF) did not.

No telomeric DNA is present in the reconstituted telomerase RRL. The only DNA present in the reconstituted telomerase mixture was the cDNA sequence for HA-tagged hTERT from the in vitro transcription/translation reaction and the hTR DNA template sequence from the in vitro transcription reaction. Although the Ku70/80 heterodimer associates with telomeric DNA (34), the capability of Ku association with the in vitro reconstituted telomerase establishes that the Ku associ-
Ku Associates with Telomerase

of the Ku70/Ku80 bands in the anti-HA and anti-p23 precipitates likely reflects the dilution of labeled Ku70/80 with abundant unlabeled Ku70/80 present in the reticulocyte lysate (data not shown). The inability of anti-Ku70 Ab-5 and anti-Ku80 Ab-2 to precipitate hTERT is consistent with their inability to precipitate telomerase in vivo and in vitro, indicating Ab-5 and Ab-2 interfere with the ability of Ku to associate with hTERT.

DISCUSSION

In this report, we present evidence showing a physical association of the Ku heterodimer with telomerase. The association of Ku with telomerase can be mediated through its association with the telomerase catalytic subunit hTERT. This association is independent of the presence of telomeric DNA and does not require DNA-PKcs. This finding is consistent with previous reports that DNA-PKcs is not required for localization of Ku to telomeric DNA (34).

In yeast, the RNA component of telomerase, TLC1, genetically interacts with Ku telomERICally bound (47). We observed that human Ku associated with the catalytic subunit of telomerase (Fig. 5) in the absence of hTERT. However, these results do not exclude the possibility that human Ku may interact with hTERT. Alternatively, significant differences may exist between yeast and mammalian telomere regulation (reviewed in Ref. 48). In yeast, Ku deficiency results in loss of telomeric repeats, loss of telomere clustering, loss of telomeric silencing, and deregulation of the G-strand overhang (31, 33, 49, 50). However, mouse Ku86 deficiency results in elongated telomeres without affecting the G-strand overhang (39, 40). The yeast Ku interaction with telomeres and/or telomerase may be somewhat different from that of mammalian Ku.

Ku plays a role in telomere homeostasis (reviewed in Ref. 35), but its precise mechanism of action is unclear. Ku mutant mouse cells show a high rate of telomere-telomere fusion events (36, 38, 39), indicating that Ku acts to protect telomere ends from fusion events. This telomere “capping” function for Ku is superficially paradoxical in light of its role elsewhere in promoting non-homologous end joining but probably reflects its role as part of a modified DNA repair complex located at the telomere. Recently, it has been shown that Ku86 deficiency results in telomere elongation in telomerase-positive mice but not in telomerase-negative mice, suggesting Ku is a negative regulator of telomerase (40). However, this may reflect other properties of Ku than its properly regulated role at telomeres. Multicellular organisms should actively inhibit telomerase access to double-strand breaks to prevent the addition of telomeric repeats that would interfere with normal double-strand break repair. The end-fusion that occurs in the Ku80/−/− mouse implies that telomeres are now being recognized as double-strand breaks and in that context Ku may function to inhibit telomerase activity. However, it may have a different role when a DNA repair complex modified properly (that masks rather than signals a DNA end problem) coordinates with the replication machinery to regulate telomerase activity on telomere during the normal cell cycle. The dual function of Ku associating with both telomere and telomerase suggests a distinct role of Ku at the telomere. Although it protects telomere ends from fusion events, it may also regulate the access of telomerase to telomere DNA ends.

Proteins that interact with Ku might contribute to the regulation of telomerase. Telomere-binding proteins, such as TRF1 (23) and TRF2 (28), have been proposed to regulate telomerase at the mammalian telomere. Overexpression of TRF1 or TRF2 results in progressive shortening of telomere length (23, 28). TRF1 and TRF2 only bind to double-stranded regions of telomeric DNA (28, 51), and TRF2 is a key component responsible for the formation of the telomeric t-loop (52). However, we found no association of TRF1 or TRF2 with telomerase activity in vivo (data not shown). Interestingly, Ku binds tightly to TRF1 (36) and TRF2 (37), and it has been proposed to contribute to t-loop formation. It is possible that the association of telomerase with Ku may trap telomerase at the double-stranded region of telomeric DNA and thus reducing the ability of telomerase to access the exposed 3′ overhang.

Although this is the first report demonstrating that Ku associates with hTERT and telomerase, many questions remain on how telomere length is actually regulated by telomere- and telomerase-associated factors. Understanding the factors involved in telomere regulation should lead to a better understanding of cancer and cellular senescence.

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