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REPORT

The La antigen associates with the human telomerase ribonucleoprotein and influences telomere length in vivo

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ABSTRACT

La is an important component of ribonucleoprotein complexes and telomerase is a ribonucleoprotein that compensates for the shortening of the ends of linear DNA by adding telomeric repeats onto the ends of chromosomes by using an integral RNA as the template. We have identified a direct and specific interaction between La and the RNA component of human telomerase. Antibodies specific to La precipitate the human telomerase ribonucleoprotein complex derived from tumor cells, telomerase immortalized normal cells, and in vitro transformed cells. Overexpression of La in both experimentally immortalized human cells and prostate cancer cells results in gradual telomere shortening. Our results demonstrate that La can associate with telomerase and its expression level can influence telomere homeostasis in vivo.

Keywords: aging; autoimmunity; cancer; hTR; RNP; telomere length homeostasis

INTRODUCTION

During normal lagging-strand replication of linear chromosomes, the telomeres are left with a gap between the final RNA priming event and the terminus (Olovnikov, 1973). This gap is thought to cause the end of the lagging strand to shorten with each cell division (Watson, 1972). Processing events probably cause the daughter telomere generated by leading-strand synthesis to shorten as well (Wellinger et al., 1996). When a telomere reaches a sufficiently short length, a growth arrest signal is activated and the cell stops dividing (Allsopp et al., 1992; Wright & Shay, 2000). Certain cancer and in vitro immortalized human cells divide continuously and maintain stable telomere lengths by activating expression of telomerase, a ribonucleoprotein (RNP) that compensates for the shortening of the ends of linear DNA by adding telomeric repeats (Greider, 1996). Forced expression of telomerase is capable of immortalizing normal cells and telomerase inhibition in telomerase-positive immortal and cancer cells can lead to telomere shortening and cell death (Bodnar et al., 1998; Hahn et al., 1999; Herbert et al., 1999).

Human telomerase is partially composed of a catalytic protein component and a 451-base integral RNA that are essential for the assembly of telomerase activity in vitro and in vivo (Weinrich et al., 1997; Bodnar et al., 1998). The 3′ sequence of the human telomerase RNA (hTR) resembles the box H/ACA family of small nucleolar RNAs (snoRNAs; Mitchell et al., 1999; Chen et al., 2000). Although the box H/ACA motif is not required for in vitro assembly of telomerase, it does appear to play a role in proper 3′-end processing and nucleolar targeting in vivo (Narayanan et al., 1999). The 5′ end of the RNA contains the template used for the addition of telomeric sequences to the ends of the chromosomes (Greider & Blackburn, 1987; Feng et al., 1995), as well as a pseudoknot that is likely to be important for telomerase function (Gilley & Blackburn, 1999; Chen et al., 2000). The 5′ end of hTR also contains a 6-base U-rich tract required for a direct interaction with heterogeneous nuclear ribonucleoproteins (hnRNPs) C1 and C2 (Ford et al., 2000). Although several regions of hTR interact with the catalytic protein component of telomerase (Tesmer et al., 1999; Bryan

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et al., 2000; Mitchell & Collins, 2000), it is still unclear if this interaction is mediated by auxiliary proteins, direct contacts, or a combination of the two.

Auxiliary proteins found to associate with the human telomerase RNP particle include snoRNA binding proteins (Mitchell et al., 1999), chaperones (Holt et al., 1999), hStau and L22 (Le et al., 2000), members of the hnRNP family of RNA binding proteins (LaBranche et al., 1998; Eversole & Maizels, 2000; Ford et al., 2000) and TEP1 (Harrington et al., 1997). The snoRNA binding proteins dyskerin and hGAR1 bind the snoRNA motif at the 3′ end of hTR (Mitchell et al., 1999; Dragon et al., 2000). The chaperone proteins are involved in the assembly of telomerase activity (Holt et al., 1999). Members of the hnRNP family of RNA binding proteins interact with telomeric DNA as well as telomerase (Ishikawa et al., 1993; Dallaire et al., 2000). Although progress has been made in identifying factors that bind the hTR, the role most of the auxiliary components play in telomerase function remains unclear.

The La autoantigen has been implicated to be important for many different cellular processes including RNP maturation, internal ribosome-entry-site-mediated translation, mRNA stabilization, and transcription termination (reviewed in Kenan, 1995; Maraia & Intine, 2001). La binds to the 3′ poly U tracts of polymerase III precursor RNAs (Chambers & Keene, 1985) and certain polymerase II transcribed RNAs (Kurilla & Keene, 1983; Madore et al., 1984; Kufel et al., 2000; Xue et al., 2000). Recently, the *Euplotes* telomerase RNP was shown to contain a homolog of the human La protein (Aigner et al., 2000). Because the *Euplotes* telomerase integral RNA is a polymerase III transcript ending in poly(U), it is important to determine whether La can also interact with the human telomerase integral RNA which is a polymerase II transcript containing a snoRNA motif at its 3′ end. Here we demonstrate that the human La protein interacts directly with the human telomerase RNA (hTR) and the telomerase RNP. We further demonstrate that La's expression level can influence telomere length in vivo. This suggests that La may play a functional role in telomere biology in humans and may be a conserved component of telomerase RNPs.

RESULTS AND DISCUSSION

We have previously shown that hnRNP C proteins bind to the hTR when incubated in S100 extracts made from a variety of cells types (Ford et al., 2000). We also consistently observed a 50-kDa protein. We have now identified this 50-kDa protein as the human La autoantigen. Antiserum with specificity to La reproducibly precipitated the 50-kDa protein that crosslinked to hTR when it was incubated in S100 extracts from the immortal lung fibroblast cell line VA13 whereas antibody to hnRNP C or to hnRNP A1 did not (Fig. 1A). Antibody

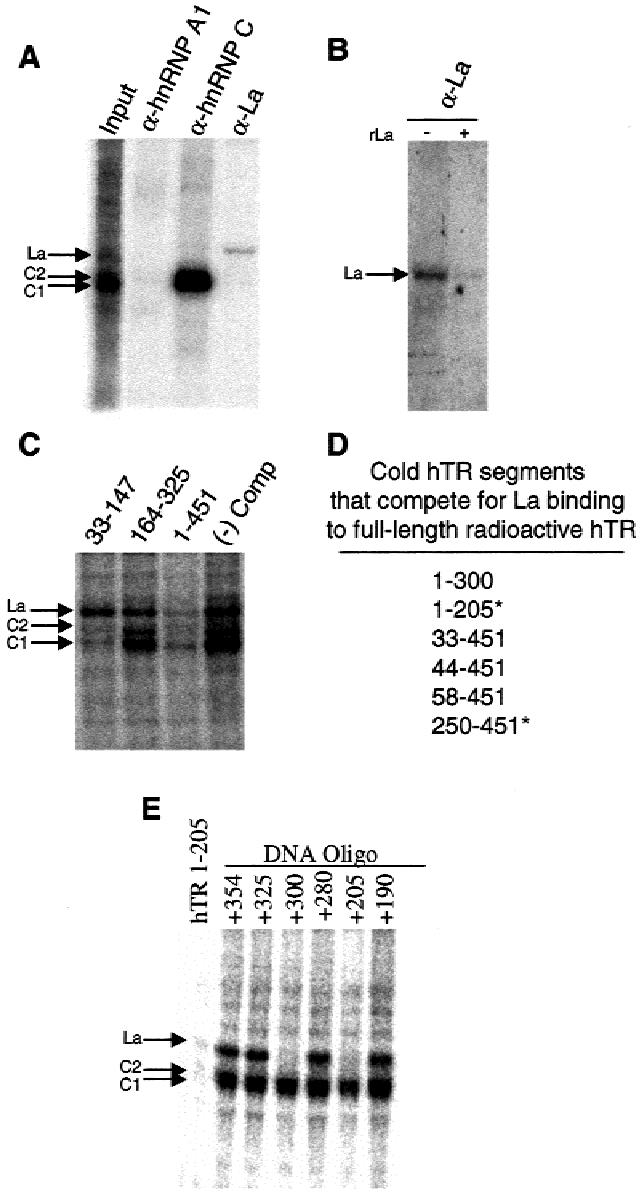

FIGURE 1. The La antigen binds directly and specifically to the human telomerase RNA. **A:** The human telomerase RNA radiolabeled at U residues was incubated in a S100 extract derived from the immortalized human lung fibroblasts cell line, VA13 (which lacks both hTR and hTERT), UV irradiated, digested with RNase, immunoprecipitated with the indicated antibody, run on a 10% acrylamide gel containing SDS, exposed to a Phosphor Screen and visualized using a Storm System (Molecular Dynamics). **B:** Radiolabeled full-length human telomerase RNA was incubated in a S100 extract derived from VA13 cells crosslinked and immunoprecipitated with La specific human serum (GO) in the presence or absence of recombinant La protein. **C:** Radiolabeled full-length human telomerase RNA was incubated in S100 extract derived from VA13 cells and crosslinked in the presence or absence of the cold competitor RNAs listed above each lane. **D:** hTR RNA fragments capable of competing for the binding of S100-extract-derived La to the full-length radiolabeled hTR. **E:** The interaction of La with the telomerase RNA can be competed with oligonucleotides complementary to specific regions of the hTR. The hTR specifically radiolabeled at U residues was incubated in extract and the indicated oligonucleotide or the cold competitor hTR fragment 1–205, exposed to UV irradiation, digested with ribonuclease, run on an acrylamide gel containing SDS, and visualized using autoradiography.

against hnRNP C did immunoprecipitate the 42- and 45-kDa proteins as we have described previously (Ford et al., 2000). Addition of purified recombinant La (rLa) protein prevented the La-specific antibody from precipitating the 50-kDa crosslinked protein (Fig. 1B). This demonstrates that the 50-kDa protein is the La antigen. Purified rLa protein also efficiently bound to the hTR in both crosslinking and RNA band shift experiments, indicating that La can bind directly to the hTR (data not shown). We next analyzed the specificity of the interaction between La and the hTR. Excess cold full-length hTR reduced crosslinking of both La and hnRNPs C1 and C2 to the full-length radiolabeled hTR in S100 extracts (Fig. 1C). However, shorter hTR fragments containing only residues 33–147 or 164–325 did not compete for the ability of La to interact with the radiolabeled full-length hTR (Fig. 1C). Residues 33–147 of the hTR did compete for the binding of hnRNPs C1 and C2, consistent with the previous localization of the binding site for the hnRNP C proteins in that fragment (Fig. 1C). Regions of the hTR that inhibited S100 extract derived La from binding the radiolabeled full-length hTR are listed in Figure 1D. Interestingly, nonoverlapping cold competitor hTR containing residues 1–205 or 250–451 (marked with stars) competed for La's ability to bind the radiolabeled full-length hTR, suggesting that La can contact multiple regions in the hTR. This was confirmed using oligonucleotides that are complementary to specific regions of the 451-base hTR. Oligonucleotides complementary to bases 281–300 (+300) and 187–205 (+205) of the hTR specifically compete for the binding of La to the full-length hTR in a UV crosslinking experiment (Fig. 1E), while oligonucleotides complementary to bases 177–190 (+190), 261–280 (+280), 306–325 (+325), and 337–354 (+354) of hTR do not block La's ability to bind to radiolabeled full-length hTR. In Figure 1E, hTR competitor containing bases 1 to 205 that competes for the binding of both La and hnRNP C proteins to radioactive hTR was used as a positive control. Our results indicate that La interacts directly and specifically with the hTR, and that La may associate with a structural motif in the hTR or that La has two independent binding sites.

Because La can associate with the human telomerase RNA, we tested whether La could also associate with the active human telomerase RNP. Human serum with specificity to La (GO) efficiently and reproducibly precipitated the human telomerase RNP from tumor cells, telomerase immortalized normal cells, and in vitro transformed cells (Fig. 2A; data not shown). Antibody against hnRNP C, which interacts with the telomerase RNP through a direct and sequence specific interaction with the hTR, and the human telomerase catalytic subunit were used as positive controls (Ford et al., 2000). Antibody against the HA epitope and protein G agarose beads (No Ab) were used as negative controls (Fig. 2A). As shown in Figure 2B, recombinant La protein decreased the amount of telomerase precipitated with the human serum (GO) but did not change the recovery of telomerase using antibody to the catalytic protein component of telomerase, hTERT. This indicates that it is the antibody with affinity to the La protein that is important for precipitating telomerase. The residual telomerase activity observed in the +rLa GO precipitation may be due to an exchange between rLa and cellular La bound to telomerase in the extracts. La monoclonal antibodies A1, A2, and SW5 (Chan & Tan, 1987; Pruijn et al., 1995) also precipitate telomerase where nonspecific antibody that recognized a ribosomal release factor does not (Fig. 2C). The relative amount of telomerase activity is listed below the gels in Figure 2A,C. Because human serum and three different monoclonal antibodies reproducibly and specifically immunoprecipitated native telomerase activity (Fig. 2), we conclude that La can associate with the human telomerase RNP particle.

La's ability to influence telomere homeostasis was then examined in intact cells. We infected an SV40 large T-antigen immortalized fibroblast cell line, SW39, and human prostate cancer cells (DU145) with a retrovirus containing the La cDNA. Western blot and immunofluorescence analysis indicated that the infected cells had 25–40% higher La protein levels than control cells (Fig. 4A). Populations of La overexpressing cells were continuously passaged over a 5-month period and analyzed for telomere length. Telomere length in cells overexpressing La shortened at a rate of 19 bp/doubling (Fig. 3A, SW39) and 16 bp/doubling (Fig. 3B, DU145) whereas telomere length in vector-only infected cells did not change (data not shown). Growth rates of both cell types were unchanged, and the cellular distribution of La by immunofluorescence was unaltered in La overexpressing cells (Fig. 4B; data not shown). The fluorescence signal in Figure 4B represents La localization that is mainly nuclear with some faint and dispersed cytoplasmic staining. We also occasionally observed cytoplasmic foci of La; however, it is not clear what this represents. Although only the cellular distribution pattern of La for SW-39 cells is shown (Fig. 4B), we also observed no change in the cellular distribution of La when overexpressed in DU145 cells (data not shown). Therefore, we conclude that La can bind to human telomerase and its expression level can influence telomere length homeostasis. There are several possible explanations for these effects. For example, La may sequester the telomerase RNP particle in a cellular location that prevents it from accessing the telomere or titer out interactions important for recruiting telomerase to the telomere. Excess La could result in the production of an inappropriately processed hTR that gets targeted for rapid degradation or which assembles with telomerase but is unable to function fully on the telomere. Similarly, excess La may block the maturation of a functional telomerase RNP or affect other components that are important for telomere homeostasis.

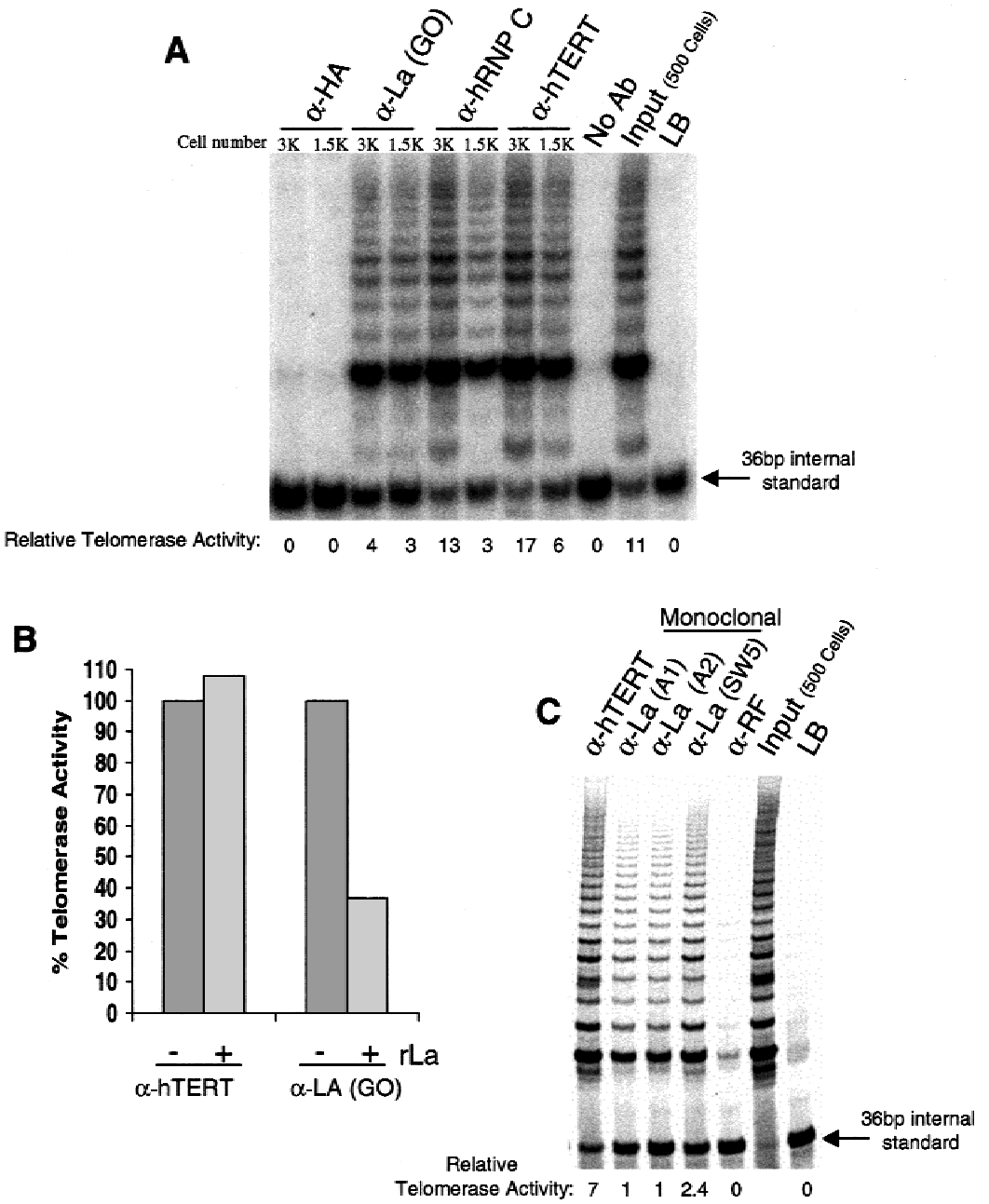

FIGURE 2. The La autoantigen binds to the native human telomerase RNP. **A:** Native human telomerase from H1299 lung cancer cell extracts was precipitated using the listed antibodies that were precoupled to protein G agarose beads. The precipitate from the indicated cell number was analyzed for telomerase activity using the TRAP assay and the relative quantity of telomerase activity was determined using ImageQuant. **B:** Recombinant La protein specifically reduced the amount of telomerase activity precipitated with anti-La human serum. Antibody was coupled to protein G agarose beads in the presence or absence of recombinant La protein (rLa), incubated with H1299 cell extracts, washed, and tested for telomerase activity using the TRAP assay. The graph depicts the relative amount of telomerase activity obtained in relation to the sample lacking recombinant La protein. **C:** Monoclonal antibody to La precipitates telomerase activity from cell extracts. Extracts derived from VA13 cells expressing telomerase (due to the expression of both exogenous hTR and hTERT) were incubated with La monoclonal antibodies A1, A2, and SW5, an antibody against ribosomal release factor (RF), or an antibody against the telomerase catalytic component, washed extensively, and the precipitate from 1000 cell equivalents was analyzed for telomerase activity using the TRAP assay. The relative amount of telomerase activity was determined using ImageQuant.

IMPLICATIONS

Our results directly demonstrate that a change in the expression level of La can influence telomere length. It is possible that changes in the expression of many cellular proteins in various disease states could produce changes in telomere homeostasis that alter the capacity of cells to replicate. In patients with dyskera-

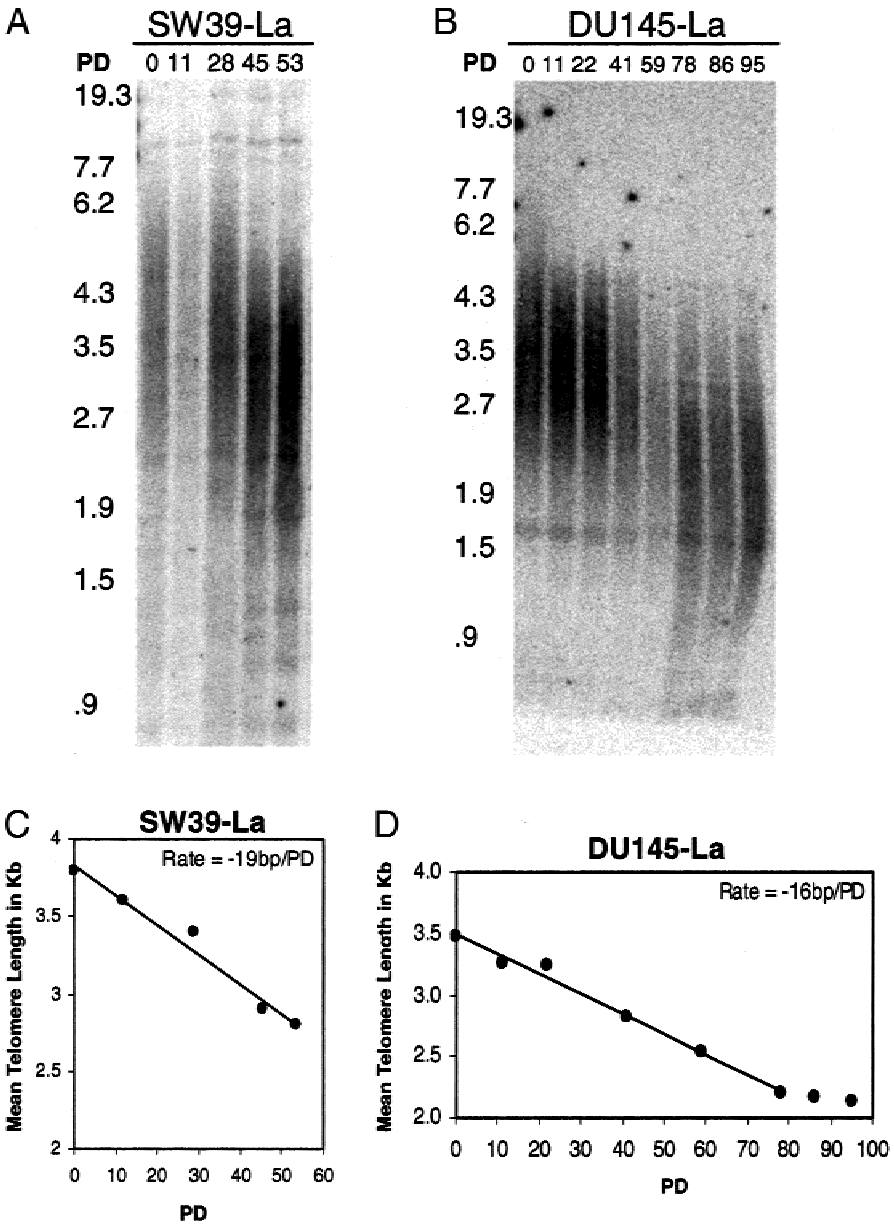

FIGURE 3. La influences telomere length maintenance in vivo. **A** and **B**: Digested genomic DNA of serial passaged telomerase expressing SW39 and DU145 cells overexpressing La (SW39-La and DU145-La) were run on a 0.7% agarose gel, probed with radiolabeled oligonucleotide $(TTAGGG)_4$, exposed to a Phosphor Screen, and visualized using a Storm System (Molecular Dynamics). Numbers to the left of the picture of the TRF gel represent sizes in kilobases and numbers at the top represent population doublings (PD) after retroviral infection. **C** and **D**: The mean telomere lengths were calculated using the program TELORUN. Telomere length in DU145-La cells decreased until a much shorter maintenance length was reached at about PD 80. Telomere length did not change in vector-only infected control cells of DU145 or SW-39 (data not shown).

tosis congenita that harbor a mutation in dyskerin, a component of the human telomerase RNP, telomerase activity levels and telomere length regulation is altered (Mitchell et al., 1999). Because autoantibodies against proteins such as La appear to have the capability to penetrate cells (Deng et al., 2000) it is possible that La function and telomere length is compromised in individuals expressing autoantibodies reactive to La. Autoantibodies produced in patients with a variety of autoimmune diseases could also influence cellular replicative capacity by influencing normal telomere length homeostasis (Yaneva & Arnett, 1989; Jeanclos et al., 1998; Reichlin, 2000; Wallace et al., 2000). Therefore, telomerase-based therapies may in the future be applied to the treatment of autoimmune disorders.

MATERIALS AND METHODS

Plasmids

The La expression vector, pBABEpuro-La (pBp-La), was produced by first inserting a cloning cassette [*Eco*RI-Kozak-*Nco*I-spacer-*Xho*I-Stop codon-*Sal*I, generated by annealing 5′-AATTCACCATGGTCGACTACCTCGAGTAAGTCGACAT-3′ to 5′-TCGAATGTCGACTTACTCGAGGTAGTCGACCATGGTG-3′] into the *Eco*RI and *Xho*I (destroyed after ligation) sites of pBluscript II. The *Nco*I-*Xho*I fragment containing the La cDNA from pET24d-La (kindly provided by Daniel J. Kenan, Duke University) was then inserted into the same sites in the cloning cassette described above. La was then cut out of this vector using *Eco*RI and *Sal*I and cloned into the same sites in the mammalian expression vector

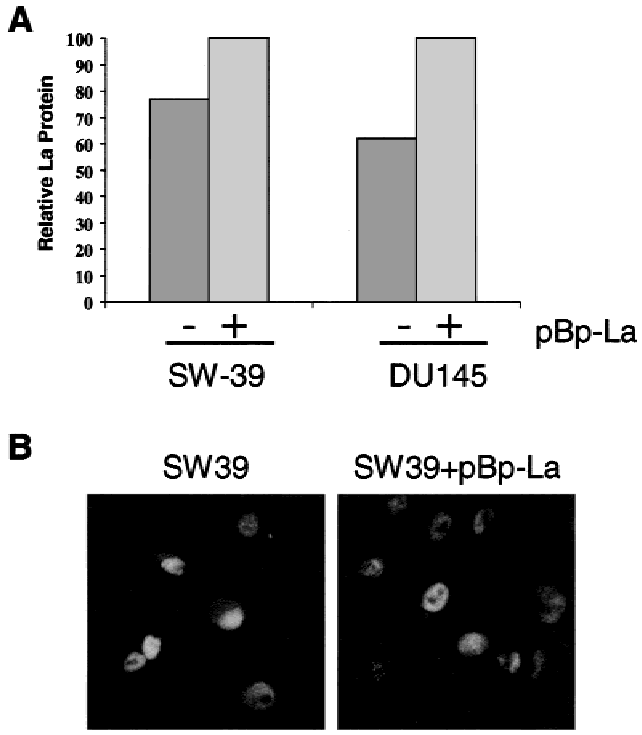

FIGURE 4. Expression level and cellular distribution of La protein. **A**: Graph showing the relative amount of La protein expressed in SW-39 and DU145 cells either containing or lacking the La retroviral expression vector, pBp-La, as determined by western blot analysis or immunofluorescence. Western blot images were acquired using a Molecular Dynamics Storm System and quantitated using ImageQuant while Immunofluorescence images were acquired using a Zeiss Axioskop microscope and quantitated using OpenLab software. **B:** The cellular distribution of La is not altered when overexpressed. Immunofluorescence was performed as described in the Materials and Methods. Image acquisition was done using a Zeiss Axioskop microscope and Adobe Photoshop. The relative intensity of the fluorescence was equalized for this comparison.

pBabepuro to generate pBp-La. The TRC3 plasmid containing the hTR cDNA was linearized with *fsp*I and used for in vitro transcription as previously described (Ford et al., 2000). All other hTR transcription templates were produced by PCR using primers described previously (Tesmer et al., 1999).

TRF and TRAP analysis

Telomere length was determined as described previously (Ouellette et al., 2000). Cell pellets were suspended in 100 mM NaCl, 100 mM EDTA, pH 8.0, and 10 mM Tris, pH 8.0, using 30 μL per million cells. Triton X-100 (1% final concentration) and proteinase K (2 mg/mL final concentration) were added. After digesting at 55 °C for 12 h and inactivating the proteinase K at 70 °C for 30 min, the samples were dialyzed overnight against TE (10 mM Tris, pH 8.0, 0.1 mM EDTA). Following dialysis, 1 μg of the samples was digested with a mixture of six enzymes (*Alu*I, *Cfo*I, *Hae*I, *Hin*fI, *Msp*I, and *Rsa*I) and run on a 0.7% agarose gel overnight at 70 V. The gel was denatured for 20 min in 0.5 M NaOH, 1.5 M NaCl, rinsed for 10 min in distilled water, dried for 1 h at 55 °C, neutralized in 1.5 M NaCl, 0.5 M Tris, and hybridized with ^{32}P-labeled oligonucleotide $(TTAGGG)_4$. After washing with 2× SSC for 15 min and 2 × 10 min in 0.1× SSC + 0.1% SDS, the gel was exposed to a Phosphor screen and analyzed using a Storm System (Molecular Dynamics). The Mean TRF length was calculated using the program TEL-ORUN as described previously (Ouellette et al., 2000).

The TRAP reactions in Figure 2A were performed according to the manufacturer's recommendations (Intergen). Immunoprecipitation/TRAP reactions were performed as previously described (Holt et al., 1999). Briefly, cell lysate was made by resuspending cells in lysis buffer [0.01% NP-40, 10 mM Tris pH 7.5, 50 mM KCl, 5 mM MgCl2, 2 mM DTT, 20% glycerol plus protease inhibitors (CompleteMini EDTA free, Boehringer Mannheim)] at a concentration of 100,000 cells/μL, incubated on ice for 30 min, and sonicated at 50 J/W-s. The lysates were then centrifuged for 30 min at 14,000 rpm and used for immunoprecipitation. Antibodies (5–10 μL concentrated antibody or 50–100 μL of antibody in tissue culture supernatant) were coupled to 20 μL of 50% protein G agarose bead slurry at 4 °C for 1 h and washed extensively with lysis buffer. Cell lysates were mixed with antibody beads (10 μL cell lysates at 50,000–100,000 cells/μL plus 20 μL antibody coated beads), 100 μL cell lysis buffer and 7 μL BSA (50 mg/mL) incubated at 4 °C for 1 h and washed four times with 400 μL of lysis buffer at 4 °C for 1 h. After the beads were washed, they were suspended in lysis buffer and used in the TRAP reaction to detect telomerase activity. In Figure 2B, 20 μg of recombinant La protein was used to saturate La binding sites on the antibody agarose beads before adding them to cell extracts derived from telomerase-expressing VA13 cells. The TRAP shown in Figure 2C was performed as follows. Protein G agarose beads resuspended in lysis buffer as described above were mixed with 5 μL 10× TRAP Reaction Buffer (200 mM Tris-HCL, pH 8.3, 15 mM $MgCl_2$, 630 mM KCl, 0.5% Tween 20, and 10 mM EGTA). One microliter of 50× dNTP mix (2.5 mM each dATP, dTTP, dGTP, and dCTP), 1 μL of 100 ng/μL TS primer (AATCCGTC GAGCAGAGTT) containing a 5′-Cy5 (Indodicarbocyanine) label, 1 μL TRAP-primer mix (RP primer (100 ng/μL, K1 primer 100 ng/μL, and TSK1 template (0.01 amol/μL)), 0.4 μL BSA (50 mg/mL), 0.4 μL of Taq polymerase, and 39.6 μL of DEPC treated water. The mixture was incubated at room temperature for 30 min and subjected to 30 cycles of PCR at 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s. The samples were then mixed with 5 μL of CY-TRAP dye (50% glycerol, 50 mM EDTA, and 0.25% bromophenol blue), run on a 10% polyacrylamide gel, and visualized with the Storm 860 System using red fluorescence (Molecular Dynamics).

Cell growth and retroviral infections

Cells were grown at 37 °C in a 4:1 mixture of Dulbecco's MEM:Medium 199 (GIBCO) plus 10% cosmic calf serum (HyClone Laboratories). To make retroviral vector particles, we transfected 30 μg of vector plasmid into the ecotropic packaging cell line PhoenixE using FUGENE6 (Roche). Two days later, the supernatants from these cells were used to infect the packaging cell line PA317. After 10 days of puromycin (3 μg/mL) selection, the supernatants of the PA317 cells were filtered and used to infect SW39 and DU145 human cells. After the infection, cells were selected using puromycin (750 ng/mL) for 5 days. Overexpression of La was

confirmed using western blot and immunofluorescence analysis (Fig. 4A).

In vitro transcription and UV crosslinking analysis

To synthesize ^{32}P-labeled RNAs, approximately 1 μg of linearized plasmid DNA or 100 ng of purified PCR product was transcribed in vitro using T7 RNA polymerase in the presence of α-^{32}P-UTP as previously described (Ford et al., 2000). Cold competitor RNAs were made using templates generated by PCR of the hTR cDNA of the indicated size with the MEGAscript T7 transcription kit (Ambion).

To make S100 extracts, VA13 cells were harvested and resuspended in 1× PBS at a concentration of 100,000 cells/μL, sonicated with two pulses at 50 J/W-s, centrifuged at 100,000 × *g* for 1 h and stored at −80 °C (Tesmer et al., 1999). Crosslinking experiments were performed as follows. Gel-purified [α-^{32}P]UTP labeled RNAs were incubated at 30 °C in S100 extract for 15 min, irradiated on ice using a G15T8 germicidal light for 10 min, and treated with RNase One, RNase A, and RNase T2 (Ambion) at 37 °C for 15 min to generate crosslinked RNA binding proteins containing small radioactive RNA oligomers. The samples were mixed with protein loading dye, heated to 100 °C for 3 min, and subjected to electrophoresis on 10% acrylamide gels containing SDS. Competition experiment using DNA oligonucleotides were performed by adding 100 μM of the indicated oligonucleotide into the standard reaction mixture and processed as described above. The sequences of the oligonucleotides used for this competition experiment are +190 (GCCAGCAGCT GACA), +205 (GGGAGGGGCGAACGGGC), +280 (CCGG AGAAGCCCCGGGCCGA), +300 (CGCGGTGGCAGTGGG TGCCT), +325 (CCGCGGCTGACAGAGCCCAA), and +354 (CCTGAACCTCGCCCTCGC). Immunoprecipitation of crosslinked proteins was performed as follows. The crosslinked proteins were mixed with antibody and incubated with rotation at 4 °C for 1 h, incubated with protein G agarose beads for 1 h at 4 °C, washed with radioimmunoprecipitation assay buffer five times, resuspended in protein loading dye, and run on a 10% acrylamide gel containing SDS.

Immunofluorescence and western blot analysis

Cells lines used for immunofluorescence were grown on cover slips in a 12-well tissue culture dish, washed briefly with PBS, fixed with 4% paraformaldehyde/PBS for 5 min, and washed 3 times 5 min with PBS. Cells were permeabilized by exposure to 0.1% Triton X-100/PBS for 5 min and washed 3 times 5 min with PBS. The cells were then blocked by addition of 3% BSA in PBS for 1 h and washed briefly with PBS. After incubating with mouse anti-La monoclonal (clone 44 from Transduction Laboratories) at a 1:500 dilution in PBS, the cells were washed briefly with PBS three times, incubated with secondary antibodies (fluorescein-conjugated goat anti-mouse IgG; Jackson ImmunoResearch Laboratories, Inc.) diluted in PBS for 1 h, and washed with PBS three times. The cells were then mounted with Vectashield containing DAPI (Vector Laboratories). Image acquisition was done using a Zeiss Axioskop microscope and Adobe Photoshop. For the purpose of a comparison of the cellular distribution, the intensity of the fluorescence shown in Figure 4B was equalized.

Western blots for La were performed as follows. Several serial dilutions of each cell lysate (cell pellet was lysed in protein loading buffer at 10,000 cells/μL) was run on a 10% acrylamide gel containing SDS and transferred to a Immobilon-P membrane (Millipore) using a Mini Trans-Blot Cell at 200 mA overnight at 4 °C (BioRad). The membrane was then blocked with 5% nonfat dry milk in PBS for 1 h, washed for 25 min in PBS/0.1% Tween 20 at room temperature, incubated with anti-La Ab, washed for 25 min in PBS/0.1% Tween 20, incubated with I^{125}-labeled protein A, washed for 35 min in PBS/0.1% Tween 20, and visualized using the Molecular Dynamics Storm System and quantitated using ImageQuant. For quantitation of La by immunofluorescence, the cells were processed as described above using the exact same image acquisition settings. The fluorescence intensity was quantitated using OpenLab software.

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REFERENCES

Aigner S, Lingner J, Goodrich KJ, Grosshans CA, Shevchenko A, Mann M, Cech TR. 2000. Euplotes telomerase contains an La motif protein produced by apparent translational frameshifting. *EMBO J 19*:6230–6239.

Allsopp RC, Vaziri H, Patterson C, Goldstein S, Younglai EV, Futcher AB, Greider CW, Harley CB. 1992. Telomere length predicts replicative capacity of human fibroblasts. *Proc Natl Acad Sci USA 89*:10114–10118.

Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE. 1998. Extension of life-span by introduction of telomerase into normal human cells. *Science 279*:349–352.

Bryan TM, Goodrich KJ, Cech TR. 2000. Telomerase RNA bound by protein motifs specific to telomerase reverse transcriptase. *Mol Cell 6*:493–499.

Chambers JC, Keene JD. 1985. Isolation and analysis of cDNA clones expressing human lupus La antigen. *Proc Natl Acad Sci USA 82*:2115–2119.

Chan EKL, Tan EM. 1987. Human autoantibody-reactive epitopes of SS-B/La are highly conserved in comparison with epitopes recognized by murine monoclonal antibodies. *J Exp Med 166*: 1627–1640.

Chen JL, Blasco MA, Greider CW. 2000. Secondary structure of vertebrate telomerase RNA. *Cell 100*:503–514.

Dallaire F, Dupuis S, Fiset S, Chabot B. 2000. Heterogeneous nuclear ribonucleoprotein A1 and UP1 protect mammalian telomeric

repeats and modulate telomere replication in vitro. *J Biol Chem 275*:14509–14516.
Deng SX, Hanson E, Sanz I. 2000. In vivo cell penetration and intracellular transport of anti-Sm and anti-La autoantibodies. *Int Immunol 12*:415–423.
Dragon F, Pogacic V, Filipowicz W. 2000. In vitro assembly of human H/ACA small nucleolar RNPs reveals unique features of U17 and telomerase RNAs. *Mol Cell Biol 20*:3037–3048.
Eversole A, Maizels N. 2000. In vitro properties of the conserved mammalian protein hnRNP D suggest a role in telomere maintenance. *Mol Cell Biol 20*:5425–5432.
Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, Chiu CP, Adams RR, Chang E, Allsopp RC, Yu J, Le S, West MD, Harley CB, Andrews WH, Greider CW, Villeponteau B. 1995. The RNA component of human telomerase. *Science 269*:1236–1241.
Ford LP, Suh JM, Wright WE, Shay JW. 2000. Heterogeneous nuclear ribonucleoproteins C1 and C2 associate with the RNA component of human telomerase. *Mol Cell Biol 20*:9084–9091.
Gilley D, Blackburn EH. 1999. The telomerase RNA pseudoknot is critical for the stable assembly of a catalytically active ribonucleoprotein. *Proc Natl Acad Sci USA 96*:6621–6625.
Greider CW. 1996. Telomere length regulation. *Annu Rev Biochem 65*:337–365.
Greider CW, Blackburn EH. 1987. The telomere terminal transferase of *Tetrahymena* is a ribonucleoprotein enzyme with two kinds of primer specificity. *Cell 51*:887–898.
Hahn WC, Stewart SA, Brooks MW, York SG, Eaton E, Kurachi A, Beijersbergen RL, Knoll JH, Meyerson M, Weinberg RA. 1999. Inhibition of telomerase limits the growth of human cancer cells. *Nat Med 5*:1164–1170.
Harrington L, McPhail T, Mar V, Zhou W, Oulton R, Bass MB, Arruda I, Robinson MO. 1997. A mammalian telomerase-associated protein. *Science 275*:973–977.
Herbert B, Pitts AE, Baker SI, Hamilton SE, Wright WE, Shay JW, Corey DR. 1999. Inhibition of human telomerase in immortal human cells leads to progressive telomere shortening and cell death. *Proc Natl Acad Sci USA 96*:14276–14281.
Holt SE, Aisner DL, Baur J, Tesmer VM, Dy M, Ouellette M, Trager JB, Morin GB, Toft DO, Shay JW, Wright WE, White MA. 1999. Functional requirement of p23 and Hsp90 in telomerase complexes. *Genes & Dev 13*:817–826.
Ishikawa F, Matunis MJ, Dreyfuss G, Cech TR. 1993. Nuclear proteins that bind the pre-mRNA 3′ splice site sequence r(UUAG/G) and the human telomeric DNA sequence d(TTAGGG)n. *Mol Cell Biol 13*:4301–4310.
Jeanclos E, Krolewski A, Skurnick J, Kimura M, Aviv H, Warram JH, Aviv A. 1998. Shortened telomere length in white blood cells of patients with IDDM. *Diabetes 47*:482–486.
Kenan DJ. 1995. *RNA recognition by the human La protein and its relevance to transcription, translation and viral infectivity*. Ph.D. thesis, Duke University, Durham, North Carolina.
Kufel J, Allmang C, Chanfreau G, Petfalski E, Lafontaine DL, Tollervey D. 2000. Precursors to the U3 small nucleolar RNA lack small nucleolar RNP proteins but are stabilized by La binding. *Mol Cell Biol 20*:5415–5424.
Kurilla MG, Keene JD. 1983. The leader RNA of vesicular stomatitis virus is bound by a cellular protein reactive with anti-La lupus antibodies. *Cell 34*:837–845.
LaBranche H, Dupuis S, Ben-David Y, Bani MR, Wellinger RJ, Chabot B. 1998. Telomere elongation by hnRNP A1 and a derivative that interacts with telomeric repeats and telomerase. *Nature Genet 19*:199–202.
Le S, Sternglanz R, Greider CW. 2000. Identification of two RNA-binding proteins associated with human telomerase RNA. *Mol Biol Cell 11*:999–1010.
Madore SJ, Wieben ED, Pederson T. 1984. Eukaryotic small ribonucleoproteins. Anti-La human autoantibodies react with U1 RNA–protein complexes. *J Biol Chem 259*:1929–1933.
Maraia RJ, Intine RV. 2001. Recognition of nascent RNA by the human La antigen: Conserved and divergent features of structure and function. *Mol Cell Biol 21*:367–379.
Mitchell JR, Cheng J, Collins K. 1999. A box H/ACA small nucleolar RNA-like domain at the human telomerase RNA 3′ end. *Mol Cell Biol 19*:567–576.
Mitchell JR, Collins K. 2000. Human telomerase activation requires two independent interactions between telomerase RNA and telomerase reverse transcriptase. *Mol Cell 6*:361–371.
Mitchell JR, Wood E, Collins K. 1999. A telomerase component is defective in the human disease dyskeratosis congenita. *Nature 402*:551–555.
Narayanan A, Lukowiak A, Jády BE, Dragon F, Kiss T, Terns RM, Terns MP. 1999. Nucleolar localization signals of Box H/ACA small nucleolar RNAs. *EMBO J 18*:5120–5130.
Olovnikov AM. 1973. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J Theor Biol 41*:181–190.
Ouellette MM, Liao M, Herbert BS, Johnson M, Holt SE, Liss HS, Shay JW, Wright WE. 2000. Subsenescent telomere lengths in fibroblasts immortalized by limiting amounts of telomerase. *J Biol Chem 275*:10072–10076.
Pruijn GJM, Thijssen JPH, Smith PR, Williams DG, Van Venrooij. 1995. Anti-La monoclonal antibodies recognizing epitopes within the RNA-binding domain of the La protein show differential capacities to immunoprecipitate RNA-associated La protein. *Eur J Biochem 232*:611–619.
Reichlin M. 2000. Anti-telomere antibodies in systemic lupus erythematosus. *Lupus 9*:317.
Tesmer VM, Ford LP, Holt SE, Frank BC, Yi X, Aisner DL, Ouellette M, Shay JW, Wright WE. 1999. Two inactive fragments of the integral RNA cooperate to assemble active telomerase with the human protein catalytic subunit (hTERT) in vitro. *Mol Cell Biol 19*:6207–6216.
Wallace DJ, Salonen EM, Avaniss-Aghajani E, Morris R, Metzger AL, Pashinian N. 2000. Anti-telomere antibodies in systemic lupus erythematosus: A new ELISA test for anti-DNA with potential pathogenetic implications. *Lupus 9*:328–332.
Watson JD. 1972. Origin of concatemeric T7 DNA. *Nat New Biol 239*:197–201.
Weinrich SL, Pruzan R, Ma L, Ouellette M, Tesmer VM, Holt SE, Bodnar AG, Lichtsteiner S, Kim NW, Trager JB, Taylor RD, Carlos R, Andrews WH, Wright WE, Shay JW, Harley CB, Morin GB. 1997. Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT. *Nature Genet 17*:498–502.
Wellinger RJ, Ethier K, Labrecque P, Zakian VA. 1996. Evidence for a new step in telomere maintenance. *Cell 85*:423–433.
Wright WE, Shay JW. 2000. Telomere dynamics in cancer progression and prevention: Fundamental differences in human and mouse telomere biology. *Nat Med 6*:849–851.
Xue D, Rubinson DA, Pannone BK, Yoo CJ, Wolin SL. 2000. U snRNP assembly in yeast involves the La protein. *EMBO J 19*:1650–1660.
Yaneva M, Arnett FC. 1989. Antibodies against Ku protein in sera from patients with autoimmune diseases. *Clin Exp Immunol 76*:366–372.