Published in final edited form as:

Gene. 2015 August 15; 568(1): 8–18. doi:10.1016/j.gene.2015.05.013.

Cloning and molecular characterization of telomerase reverse transcriptase (TERT) and telomere length analysis of *Peromyscus leucopus*

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Abstract

Telomerase reverse transcriptase (TERT) is the catalytic subunit of telomerase complex that regulates telomerase activity to maintain telomere length for all animals with linear chromosomes. As the *Mus musculus* (MM) laboratory mouse has very long telomeres compared to humans, a potential alternative animal model for telomere research is the *Peromyscus leucopus* (PL) mouse that has telomere lengths close to the human range and has the wild counterparts for comparison. We report the full *TERT* coding sequence (*pTERT*) from PL mice to use in the telomere research. Comparative analysis with eight other mammalian TERTs revealed a *pTERT* protein considerably homologous to other TERTs and preserved all TERT specific-sequence signatures, yet with some distinctive features. *pTERT* displayed the highest nucleotide and amino acid sequence homology with hamster *TERT*. Unlike human but similar to MM mice, *pTERT* expression was detected in various adult somatic tissues of PL mice, with the highest expression in testes. Four different captive stocks of PL mice and wild-captured PL mice each displayed group-specific average telomere lengths, with the longest and shortest telomeres in inbred and outbred stock mice, respectively. *pTERT* showed considerable numbers of synonymous and nonsynonymous mutations. A *pTERT* proximal promoter region cloned was homologous among PL and MM mice and rat, but with species-specific features. From PL mice, we further cloned and characterized

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Conflicts of Interest: The authors declare no conflicts of interest.

Author Contributions

Xin Zhao, Sachiko Kajigaya, Yasutaka Ueda, Jichun Chen, Robert C. Lacy, and Neal S. Young participated in the design of this project. Xin Zhao, Yasutaka Ueda, Glen Alaks, Marie J Desierto, Sachiko Kajigaya, and Jichun Chen conducted the experiments. Xin Zhao, Yasutaka Ueda, and Sachiko Kajigaya analyzed the data, interpreted the results, and drafted the manuscript. NS. Young, Robert C. Lacy, Danielle M. Townsley, and Bogdan Dumitriu were involved in conceptualization, interim discussions, interpretation of results, and editing the manuscript. All authors critically reviewed the manuscript content and agree with the submission of the final manuscript.

ribosomal protein, large, P0 (p*RPLP0*) to use as an internal control for various assays. *Peromyscus* mice have been extensively used for various studies, including human diseases, for which p*TERT* and p*RPLP0* would be useful tools.

Keywords

TERT mutation; ribosomal protein; large; P0 (RPLP0); cloning

1. Introduction

Telomeres are terminal cap structures of eukaryotic chromosomes and composed of double-stranded hexameric tandem repeats (TTAGGG in all vertebrates), protecting chromosomes from DNA degradation, end-to-end fusions, and rearrangements (Greider and Blackburn, 1997). Telomere maintenance is performed in most eukaryotes by synthesizing *de novo* repeats with a ribonucleoprotein enzyme, telomerase. The telomerase catalytic core consists of two major components: a catalytic subunit of telomerase reverse transcriptase (TERT) and an RNA template (*TERC*). Telomerase is highly regulated during development and cell differentiation, and its expression is a defining feature of germ cells and other self-renewing cells (Prowse and Greider, 1995; Greenberg et al., 1998; Armstrong et al., 2000). Rate-limiting in telomerase activity is transcriptional regulation of *TERT* (Bodnar et al., 1998; Wang and Zhu, 2003; Ducrest et al., 2002). TERT is the multi-functional factor, contributing also to various other molecular mechanisms such as neuronal survival/differentiation and promotion of angiogenesis.

Ribosomal protein, large, P0 (RPLP0) is a structural constituent of the ribosome “stalk” (a highly flexible lateral protuberance of the large (60S) ribosomal subunit); and it plays a pivotal role in protein synthesis (Möller and Maassen, 1986). Although many mammalian ribosomal protein genes have multiple functional copies, only a single copy of the *RPLP0* gene is functional. Therefore, in molecular biology, *RPLP0* is frequently utilized as a single-copy “housekeeping” gene reference for normalization of data obtained from quantitative real-time reverse transcription PCR (RT-PCR) (Lyng et al., 2008).

Peromyscus leucopus (PL), the white-footed mouse, belongs to the genus *Peromyscus* which is the most abundant mammalian genus in the US and only distantly relates to the common house mouse and laboratory mouse, *Mus musculus* (MM). PL and its close relative, *Peromyscus maniculatus* (*P. maniculatus*), are the most common *Peromyscus* species in the US. *Peromyscus* mice have come to the public attention as the primary reservoir for hantavirus (Morzunov et al., 1998) and also as carriers of Lyme disease-transmitting ticks (Ubico et al., 1996). *Peromyscus* wild populations have almost complete turnovers on an annual basis, but animals can live many years in captivity with maximum life spans of 5 to 7 years, twice as long as those of the standard laboratory mice, despite their similar physical sizes. Therefore, *Peromyscus* mice are favored over the common mouse and the laboratory rat (rat, *Rattus norvegicus*) in a variety of research fields. They are good models for the studies of aging, mammalian genomics, epidemiology, ecology, physiology, and evolution in addition to infectious and other human diseases (Joyner et al., 1998). For some studies,

Peromyscus mice have distinct advantages as they have the wild counterparts for comparison, for example, to monitor environmental factors. Moreover, for many of the laboratory stocks of *Peromyscus*, the wild populations from which the laboratory stocks were derived are documented and can be re-sampled for comparison.

Here, we report *TERT* (p*TERT*) and *RPLP0* (p*RPLP0*) cDNA cloning from PL mice and their characterization, which should provide useful tools to explore new research fields using PL mice. Further, we perform comparative analysis of the p*TERT* or p*RPLP0* protein with those of several mammalian species, respectively, to identify characteristic features.

2. Materials and methods

2.1. Mice

We used four captive stocks of PL mice and additionally wild PL mice in the current study. Two captive PL mouse stocks at the research facilities of the Chicago Zoological Society (Brookfield, IL, US) were derived from a common genetic stock created from 20 mice captured in 2001 at Volo Bog State Natural Area, Lake County, IL (Lacy et al., 2013) and were maintained in captivity using random (CH-RAN) or minimizing mean kinship (CH-MK) breeding practices over 10 years. We also captured wild PL mice from the same Chicago area (CH-wild) specifically for this study. Two captive stocks of PL mice were obtained from the *Peromyscus* Genetic Stock Center (PGSC) at the University of South Carolina (Columbia, SC, US): the LL stock (SC-LL) mice were derived from 38 founders captured at Linville Falls, NC between 1982 and 1985 with restricted free-breeding (avoid sister-brother mating) while the inbred stock (SC-inbred) mice were derived from 20 breeding pairs captured at Argonne, IL in 1981. All animal studies were approved by the Institutional Animal Care and Use Committees at the Chicago Zoological Society, and at the National Heart, Lung, and Blood Institute, respectively.

2.2. DNA and RNA extraction and cDNA synthesis

Genomic DNAs were extracted from peripheral blood specimens of PL mice using the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA), according to the manufacturer's protocol.

For RNA extraction, brain, heart, liver, lung, spleen, kidney, intestine, skin, testis, thymus, and bone marrow were obtained from euthanized PL mice, submerged in the RNALater RNA stabilization reagent (Qiagen), and subjected to total RNA extraction using the RNeasy Mini kit (Qiagen) with DNase, following the manufacturer's instruction. Extracted total RNA was applied for first-strand cDNA synthesis using the SuperScript III First-Strand Synthesis System SuperMix (Invitrogen, Carlsbad, CA), according to the manufacturer's protocol.

2.3. Cloning of p*TERT* and p*RPLP0* coding sequences and a p*TERT* proximal promoter region sequence

For cloning of a p*TERT* coding sequence, we used a PCR-based method, based on five mammalian *TERT* mRNA sequences from NCBI GenBank (<http://www.ncbi.nlm.nih.gov>)

which were aligned using the ClustalW2 Multiple Sequence Alignment program: *Homo sapiens* (human, NM_198253), *Bos taurus* (cow, NM_001046242), *Canis familiaris* (dog, NM_001031630), rat (NM_053423), and MM mouse (NM_009354). Using the aligned sequences, PCR primers (20 – 24 mer) were designed to obtain partially overlapping PCR products encompassing an entire *pTERT* coding sequence: primers complementary to the perfectly conserved regions; and semi-degenerate primers based on highly conserved regions. Partially overlapping *pTERT* PCR products were generated with individual cDNAs created from four PL mouse testis RNAs (CH-RAN1, CH-RAN2, CH-MK1, and CH-MK2) with various primer sets using the TaKaRa LA Taq polymerase kit (Clontech, Mountain View, CA), in accordance with the manufacturer's instruction.

Using a similar PCR-based strategy and the four cDNAs described above, we achieved cloning of a *pRPLPO* coding sequence. To design PCR primers for the cloning, *RPLPO* mRNA sequences of 12 mammalian species obtained from NCBI GenBank (<http://www.ncbi.nlm.nih.gov>) were aligned using the ClustalW2 Multiple Sequence Alignment programs. mRNA accession numbers of 12 species were as follows: human (NM_001002), *Pan troglodytes* (chimpanzee, XM_509423), *Macaca mulatta* (rhesus monkey, NM_001195428), *Callithrix jacchus* (white-tufted-ear marmoset, XM_002753072), cow (NM_001012682), *Sus scrofa* (pig, NM_001098598), *Equus ferus caballus* (horse, XM_001489330), dog (XM_535894), *Ailuropoda melanoleuca* (giant panda, HQ318034), *Oryctolagus cuniculus* (rabbit, XM_002719794), rat (NM_022402), and MM mouse (NM_007475).

To identify a 5'-*pTERT* proximal promoter region sequence, genomic DNA samples extracted from peripheral blood specimens of four PL mice (CH-RAN1, CH-RAN2, CH-MK1, and CH-MK2) were subjected to cloning using the Universal Genome Walker kit (Clontech), according to the manufacturer's instructions.

2.4. Sequence analysis and alignment

Sequence analysis was performed using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Grand Island, NY) and the 3130xl Genetic Analyzer (Applied Biosystems). Sequences were verified using different PCR primer sets and sequencing primers. To identify the *pTERT* nucleotide sequence, sequence assembly was performed with several overlapping sequences derived from each mouse using SeqMan software and Chromas 2 software to read ambiguous sequences, resulting in an entire *pTERT* sequence for each of the four PL mice. To obtain a *pTERT* consensus coding sequence, *pTERT* sequences from the four PL mice were aligned using the Multaline-Multiple Sequence Alignment program by Florence Corpet or BLAST (blastn nucleotide or blastp protein alignment programs). Similarly, alignment of four *pRPLPO* nucleotide sequences was carried out to obtain a *pRPLPO* consensus coding sequence. *pTERT* or *pRPLPO* consensus nucleotide and amino acid sequences were analyzed by comparing with those of other mammalian species, using the Multaline-Multiple Sequence Alignment software by Florence Corpet or BLAST, followed by manual gap adjustment. In addition to human, cow, dog, rat, and MM mouse, rhesus monkey (NM_001190967), pig (NM_001244300), and *Mesocricetus auratus* (golden hamster, AF149012) were used for *pTERT* homology comparison. *TERT* or *RPLPO*

sequences of various species were tentatively termed as follows: human (*hTERT*), rhesus monkey (*rmTERT*), cow (*cTERT*), pig (*pgTERT*), dog (*dTERT*), rat (*rTERT* or *rRPLP0*), MM mouse (*mTERT* or *mRPLP0*), hamster (*hmTERT*), and PL mouse (*pTERT* or *pRPLP0*).

A *pTERT* consensus promoter region sequence obtained from the four PL mice were compared to the 5'-*TERT* upstream region sequences (~ 480 nucleotides) of rat (NC_005100) and MM mouse (NC_000079) from NCBI GenBank.

pTERT or *pRPLP0* nucleotide and amino acid consensus sequences were deposited in NCBI GenBank: a *pTERT* coding sequence, accession KP857903 (3369 bp, 1122 aa); a *pTERT* proximal promoter plus partial exon 1 sequence, accession KP857904 (514 bp); and a *pRPLP0* coding sequence, accession KP857902 (954 bp, 317 aa).

2.5. Telomere restriction fragment (TRF) length analysis

Telomere length in the PL mice was measured by TRF analysis with genomic DNA (300 ng) using the TeloTAGGG Telomere Length Assay kit (Roche, Nutley, NJ), according to the manufacturer's protocols. For gel electrophoresis, 0.8% and 0.3% gels were made with SeaKem Gold Agarose (Lonza, Walkersville, MD) for analyses of standard TRF lengths (< 21 kb) and long TRF lengths (21 – 50 kb), respectively. As the 0.3% gel was very fragile, it was generated by pouring 0.3% agarose over a 0.8% supporting agarose gel (2 – 3 mm thick). A mean TRF length was calculated using the ImageQuant TL software (GE Healthcare Life Sciences, Pittsburgh, PA) by comparing signals relative to a molecular weight standard. All statistical analyses were performed using the GraphPad PRISM version 6.0 (GraphPad Software, La Jolla, CA). One-way ANOVA was used for analysis of multiple groups and a two-tailed *P* value < .05 was considered statistically significant. Data were represented as mean ± standard error of the mean (SEM).

2.6. Semi-quantitative RT-PCR analysis of *pTERT* expression

pTERT expression levels in various tissues of PL mice were examined by semi-quantitative RT-PCR using cDNAs generated from their RNAs. In brief, cDNA was PCR amplified with adequate primer sets for *pTERT* and an internal control, *pRPLP0*, followed by 1.5% agarose gel electrophoresis analysis. PCR amplification conditions were an initial hold of 95°C for 3 min, 35 cycles of 95°C for 30 sec, 60°C for 50 sec, and 72°C for 1 min, followed by 72°C for 5 min.

3. Results

3.1. Cloning of a *pTERT* coding sequence and homology comparison among four rodent *TERT*s

We applied a PCR-based cloning method to isolate a *pTERT* coding sequence using cDNA samples generated from four PL mouse testes (two CH-RAN and two CH-MK mice) with multiple primers designed by alignment of *TERT* mRNA sequences of five mammalian species. Sequences of PCR products were confirmed and assembled to construct an entire *pTERT* coding sequence for each of the four mice, resulting in almost identical sequences. A *pTERT* consensus open reading frame (ORF) constructed from four *pTERT*s was composed

of 3366 nucleotides which encoded the predicted 1122 amino acids (Fig. 1). A pTERT protein was rich in leucine (13%) with a calculated molecular weight of 127612.9 daltons and a calculated isoelectric point of 10.16.

The pTERT consensus nucleotide and amino acid sequences were homologous to three other rodent TERT sequences, although there was some variation in TERT amino acid numbers (Fig. 1 and Table 1). Conserved amino acid residues among the four rodent TERTs were localized across the entire pTERT sequence, with a less homologous region between amino acid (aa) 182 and aa 289 of pTERT (Fig. 1). Despite marked sequence conservation, pTERT exhibited apparently unique features in its primary structure: one amino acid insertion of aa 261 (Asp) not presented in the eight other mammalian TERTs; and four or three amino acid gaps between aa 270 and aa 271 or between aa 375 and aa 376, respectively, compared to other rodent TERTs. When comparison was performed based on percentage homology of the pTERT nucleotide (amino acid) sequence with other rodent species, homology rates were 88% (82%) with hmTERT, 86% (78%) with mTERT, and 84% (77%) with rTERT, respectively, showing the highest homology of pTERT with hmTERT, although homology rates were slightly lower in amino acids than in nucleotides (Table 1). rTERT and mTERT shared the highest homology in both nucleotide (90%) and amino acid (85%) sequences, and the lowest TERT nucleotide and amino acid sequence homology was between hamster and rat (84%) or between PL mouse and rat (78%), respectively. By homology comparison of pTERT with five other non-rodent TERTs (human, rhesus monkey, cow, pig, and dog) (Table 1), pTERT displayed the highest homology to hTERT in both nucleotide and amino acid sequences. TERT amino acid numbers in the nine mammals exhibited interspecific variation even among the four rodents, with the highest or lowest number of 1132 residues (human and rhesus monkey) or 1122 residues (PL and MM mice) (Table 1). Less preserved regions among species implies that amino acid replacement of the regions might minimally affect structure and functions of the TERT protein, or might confer a competitive advantage for survival in different environments.

3.2. Homology comparison of motifs and regions of pTERT with other mammalian TERTs

The TERT protein is composed of a central catalytic reverse transcriptase (RT) domain with conserved sequence motifs shared with the other reverse transcriptases (RTs; such as in retroviruses and hepadnaviruses) which is flanked by the long N-terminal extension (NTE) and the short C-terminal extension (CTE). Each of them contains various regions and motifs: regions v-I, v-II, v-III, and v-IV, and motifs GQ, CP, QFP, and T in the NTE; motifs 1, 2, 3, A, IFD (a, b, and c), B, C, D, and E in the RT domain; regions v-V, v-VI, and v-VII, and motifs E-1, E-II, E-III, E-IV, and C-DAT in the CTE. pTERT completely preserved several TERT sequence signatures distinguished from other RT sequences: the TERT-specific motif T not present in other RTs; a conserved arginine residue in motif 1; two aspartate residues followed by an aromatic residue in motif C; and tryptophan-x-glycine-x-serine/leucine in motif E (Fig. 2) (Nakamura et al., 1977; Bryan et al., 1998). When sequence homology comparison of TERT regions and motifs was performed among the nine mammalian species, motifs C (82%) and E (82%) were highly conserved among all mammals examined, whereas homology rates of motifs 1, 2, CP, 3b, IFDb, IFDc, and E-II were extremely low (<38%), with the lowest rate (24%) for motif 2 (Fig.2 and

Supplementary Table 1). When TERT regions and motifs were compared among four rodents, conservation rates of individual regions and motifs were increased overall: 100% conservation was observed in motifs E-IV and C-DAT and even for motif 2 homology was 59%.

3.3. pTERT expression and TRF length analyses of PL mice

Semi-quantitative RT-PCR was performed to examine *pTERT* expression levels in 11 normal tissues (brain, heart, liver, lung, spleen, kidney, intestine, skin, testis, thymus, and bone marrow) from PL mice. Figure 3 exhibits two representative results from SC-LL and SC-inbred mice, showing *pTERT* expression in all tissues of both PL mice, with the highest expression in their testes.

To address potential effects of domestication and breeding practices on telomere lengths in PL mice, we examined telomere lengths of peripheral blood leukocytes of a total of 157 PL mice (38 SC-LL, 9 SC-inbred, 34 CH-RAN, 48 CH-MK, and 28 CH-wild). Figure 4 shows TRF length distributions in the five groups and Table 2 summarizes ranges and mean values of both TRF length (kb) and death age (day) of these mice. A striking difference of TRF length was observed between SC-LL (5.39 ± 0.20 kb) and SC-inbred (20.36 ± 0.96 kb) mice. There was no significant difference between male and female within each of the breeding groups. When comparison of TRF length was performed among the three groups (CH-RAN, CH-MK, and CH-wild), CH-MK mice (14.05 ± 0.28 kb) had significantly shorter telomere than did CH-RAN (15.61 ± 0.39 kb) and CH-wild (15.44 ± 0.38 kb) mice, and telomere lengths of CH-RAN and CH-wild mice were similar. It is unclear whether age was a contributor to this telomere difference since CH-MK mice were on average 100 days older than CH-RAN mice.

3.4. Nucleotide and amino acid substitutions of pTERT

We assessed whether telomere lengths in PL mice were associated with *pTERT* amino acid mutations. In addition to the original four mice, *pTERT* sequence analysis was performed 20 more PL (5 SC-LL, 5 SC-inbred, and 10 CH-wild) mice of different origins. Table 3 shows nonsynonymous substitutions in 24 pTERTs by comparing to the pTERT consensus sequence. All of the *pTERT* nucleotide sequences displayed nucleotide variation while individual 24 pTERT proteins exhibited less amino acid differences (Table 3). Five SC-inbred mice had almost the same pTERT sequences with only three non-synonymous substitutions, two of which were also presented in five SC-LL mice. Higher pTERT mutation numbers (4 – 9 sites) were present in five SC-LL mice, especially three of which carried a high density of *pTERT* nonsynonymous and synonymous nucleotide substitutions. Of interest, among the five mouse groups (SC-LL, SC-inbred, CH-RAN, CH-MK, and CH-wild), the overall pTERT mutation prevalence was fairly low in CH-wild mice. In 23 pTERTs, non-synonymous substitution sites found in 24 PL mice, 10 of which were in TERT motifs, such as Gly61Asp in the motif GQ and Thr551Arg in the motif T, while the 13 other were not present in any motifs.

3.5. Cloning of a pTERT proximal promoter sequence and its characteristic features

A 5'-proximal promoter region of pTERT was identified by sequencing PCR products obtained by the PCR-based GenomeWalker method. Alignment of the TERT proximal promoter regions (~ 480 bp) of PL and MM mice and rat revealed high homology among them: PL mouse vs. rat (71%), PL mouse vs. MM mice (73%), and rat vs. MM mouse (77%) (Fig. 5). In the three rodent TERT promoter regions, three GC-boxes (binding sites of several transcription factors such as members of the SP family) were located at similar positions and one E-box (binding sites of several transcription factors including members of the Myc/Mad/Max family) was present at the exact upstream position from the ATG sites (Greenberg et al., 1998) although the TERT promoter regions of all three rodents lacked a typical TATA box, like hTERT. Despite these similarities, a non-canonical E-box (CACCTG), which was present at about -220 bp in the TERT promoter regions of MM and rat, was not found at the corresponding position in pTERT, while MM and rat carried one more non-canonical E-box at different locations. CpG dinucleotides (DNA methylation sites) were found throughout entire proximal promoter regions of the three rodent TERTs, with higher density in the proximal 180-bp regions. However the number of CpG sites in the TERT promoter region was variable among the three rodents, with the highest (33 CpGs) or lowest (20 CpGs) number in PL or MM mouse, respectively.

3.6. Cloning and homology comparison of the pRPLP0 coding sequence

Using a similar PCR-based cloning strategy that was applied to pTERT cloning, a pRPLP0 consensus coding sequence was obtained from cDNA samples of 14 PL mice (2 CH-RAN, 2 CH-MK, 5 SC-LL, and 5 SC-inbred mice). All mice in SC-LL or SC-inbred groups had identical pRPLP0 coding nucleotide sequences, but the sequences were slightly heterogeneous among CH-RAN or CH-MK mice. However, none of the nucleotide point mutations affected deduced amino acid sequences of the 14 PL mice, which had originated from three different geographic sites. A pRPLP0 protein encoded by 951 nucleotides consisted of 317 amino acids and was considerably hydrophobic with a calculated molecular weight of 34244.5 daltons and its calculated isoelectric point of 5.91.

Among 13 mammalian species, eight species including PL mouse had the same amino acid numbers (317 residues), while rhesus monkey, marmoset, cow, pig, and rabbit had the same three extra residues (GCA) at nucleotide positions 879 to 881, resulting in 318 amino acids (Supplementary Table 2). When the RPLP0 nucleotide sequence was compared based on percentage homology among the three rodent species, rates were 92% (PL mouse vs. MM mouse), 92% (PL mouse vs. rat), and 93% (rat vs. MM mouse) (Supplementary Table 2). Despite being more distantly related, PL mouse shared slightly higher RPLP0 nucleotide homology with five other mammals including horse (94%), human/panda (93%), and chimpanzee/marmoset (93%). Nonetheless, PL mouse displayed the highest amino acid sequence homology with MM mouse (99%) among 13 mammals. Although a C-terminal amino acid region (aas 256 – 299) appeared a hotspot of amino acid changes, a highly acidic C-terminal tail of 19 amino acids (EAKEESEEDMDMGFGLFD at aas 300 – 318) was perfectly conserved in all the 13 mammalian species, consistent with the importance of these 19 residues for PRLP0 function(s).

4. Discussion

We cloned p*TERT* and p*RPLP0* in parallel. In the course of collecting data and preparing our manuscript, an entire *TERT* and a partial *RPLP0* coding sequences of *P. maniculatus* and a *RPLP0* partial coding sequence of PL mouse were deposited in GenBank (Accession number NW_006990005, XM_006970856, and KF935254, respectively). When the p*TERT* consensus nucleotide and amino acid sequences were compared to those of *P. maniculatus bairdii* *TERT* reported, their homology rates were 98.7% and 98.3%, respectively. The partial *RPLP0* nucleotide and amino acid sequences of *P. maniculatus* and PL mice published were 100% identical to the corresponding cloned p*RPLP0* sequence in our current work, respectively. Collectively, our report is the first cloning of the entire *TERT* and *RPLP0* coding sequences from PL mouse of genus *Peromyscus* and describes detailed characteristic features of p*TERT* and p*RPLP0* genes.

PL and MM mice, hamster, and rat are members of the largest mammalian order, Rodentia, in which PL mouse/hamster or rat/MM mouse belong to the rodent family Cricetidae or Muridae, respectively. Although rat and MM mouse are members of the same subfamily Murinae, PL mouse and hamster belong to different subfamilies, Neotominae and Cricetinae, respectively. Thus, despite being phenotypically similar, PL mouse is distantly related to hamster, and much more distantly related to rat and MM mouse phylogenetically. More rapid nucleotide mutation and higher chromosomal rearrangement rates are seen in rat and particularly MM mouse genomes than in those of other mammals, resulting in greater karyotypic divergence between rat and MM mouse than between more distantly related species like human vs. dog (Stanyon et al., 1999; Adkins et al., 2001; Steppan et al., 2004). However, DNA sequence mutation and chromosome rearrangements are believed to occur by independent mechanisms. Comparative genomic mapping as well as cytogenetic data and ancestral karyotype reconstructions in *P. maniculatus* have revealed that the genomic organization of *Peromyscus* and rat is more similar to each other than either is to MM mouse, regardless of the much more recent common ancestor shared by rat and MM mouse (Dawson et al., 1999; Murphy et al., 2005; Ramsdell et al., 2008). A genome rearrangement appears to have occurred later in MM mouse than in rat following their evolutionary separation. In our work, PL mouse displayed the highest *TERT* nucleotide and amino acid sequence homology to hamster, and *Peromyscus* is phylogenetically much closer to hamster than to rat and MM mouse. However, MM mouse and rat (which belong to the same subfamily) shared the highest homology in both nucleotide and amino acid sequences than PL mouse and hamster (which belong to two different subfamilies). Thus, the *TERT* homology rate appeared to reflect evolutionary distance. With respect to the *TERT* amino acid number, phylogenetic discordance was observed, such that PL mice shared the same 1122 residues with MM mice but not with hamster in which *TERT* was longer by additional six residues. It is curious that p*TERT* displayed the highest homology to h*TERT* among the five non-rodent *TERT*s examined.

Telomeres are maintained within species-specific lengths, and telomere length variation occurs within a preset range, even if little is known of underlying genetic and molecular mechanisms controlling variation. A genome wide screen of interspecific crosses using MM mice (which have considerably longer and hypervariable telomeres) and *Mus spretus* mice

(with shorter telomeres ranging from 5 – 15 kb) has implicated an unidentified gene(s) that mapped to distal chromosome 2 as a regulator(s) of differential telomere lengths (Zhu L et al., 1998). Wild-trapped MM mice and several strains recently derived from wild mice have considerably shorter telomere lengths than do various strains of established inbred and outbred mice (< 25 kb, with most TRFs < 20 kb) (Hemann et al., 2000). Similarly, wild-derived *Peromyscus* mouse strains have quite short telomeres (average TRF ~ 12 kb), despite their longevity (Hemann et al., 2000). Among three different *Peromyscus* inbred strains derived from corresponding *Peromyscus* outbred lines, there are significantly lengthened telomeres, but with strain-specific different telomere lengths (Manning et al., 2002), suggesting that inbreeding resulted in telomere elongation, which is presumably genetically determined by multiple segregating loci. Our inbred PL mouse data (SC-inbred) support and expand the conclusions from studies of MM and *Peromyscus* mice, in which inbreeding is accompanied by increased telomere lengths. In contrast to SC-inbred mice, SC-LL (outbred) mice had remarkably short telomeres. However, extensive telomere length differences were not observed between two captive PL mouse stocks (CH-RAN and CH-MK), but with a slightly shorter average telomere length in the CH-MK stock. These two stock mice originated from a common genetic stock to preclude possible founder effects (Lacy et al., 2013; Malo et al., 2010). In the MK breeding protocol, mean kinship is minimized in order to maximize gene diversity, by pairing breeders with the lowest average kinship while, in the RAN protocol, individuals are assigned to pairing randomly without management of gene diversity or active selection for specific traits. Despite these different strategies, inbreeding in small, closed stocks is inevitable and the MK protocol only slows inbreeding relative to RAN. As was described previously, telomere length diversification of PL mice as well as of MM mice might be due to the inheritance of alleles with shorter or longer telomere lengths caused by cumulative effects of gene mutations or minor deletions, which would occur within small, isolated breeding colonies of PL mice.

In MM mouse somatic tissues, modest or detectable levels of *mTERT* expression are observed, albeit at lower levels than in embryonic tissues and in tumor cells, and telomerase activity is easily detectable in colon, liver, ovary, and testis, but undetectable in brain, heart, stomach, and muscle (Prowse et al., 1995; Chadeneau et al., 1995). We detected *pTERT* expression in all PL mouse tissues screened, with the highest level in testes. Although telomerase activity was not measured in this work, correlation between *TERT* expression level and telomerase activity is typical, and thus telomerase was likely present in most PL mouse tissues.

Previous studies have reported that different *TERT* expression profiles in various tissues in individual species depend on species-specific cis-regulation of *TERT* transcription rather than trans-regulation (Horikawa et al., 2005; Takakura et al., 2005; Fujiki et al., 2010). By comparison of the *mTERT* promoter region to its human counterpart, considerable differences are observed in their sequences, transcription factor binding sites, and distribution of CpG islands (Pericuesta et al., 2006). There are fundamental differences in the *TERT* expression profile and the telomerase regulation between human and MM mouse (Horikawa et al., 2005; Takakura et al., 2005), which likely contribute to different characteristics between human and mouse, such as much longer telomeres and absence of

telomere-dependent proliferative senescence in mice. We found high homology and structural similarity among the 480-bp proximal promoter regions of the three rodent *TERT*s (PL and MM mice and rat), such as the presence of three GC-boxes and one E-box (E-box: Greenberg RA et al., 1998), although non-canonical E-boxes (CACCTG) present in MM mouse and rat were not identified at the relative positions in PL mouse. We also could not identify an NFAT5 binding site, which is present in the MM mouse promoter region, in either PL mouse or rat promoter region at the corresponding MM mouse promoter site (Fujiki et al., 2010). The *pTERT* promoter region carried the highest number of CpG among the three rodents examined. Currently, whether methylation regulates *TERT* expression is still unclear. Collectively, our results suggest that different telomere regulatory mechanisms exist even among phylogenetically close rodent species.

In most human adult somatic tissues, telomerase is suppressed (undetectable or extremely low levels) in contrast to MM mouse as well as other mammalian species. In addition, it has been also reported that, even in MM mice, *TERT* expression is highly regulated by transcriptional factors bound to cis-elements (such as two GC-boxes and an E-box) in the *mTERT* promoter region (Greenberg et al., 1998; Nozawa et al., 2001) and that a stringent *mTERT* repression system exists even in adult mouse tissues under physiological conditions (Pericuesta et al., 2006). Another layer of telomerase regulatory mechanism, maybe the condensed native chromatin environment of the *hTERT* locus, is central for silencing *hTERT* during cell differentiation. In contrast, the *mTERT* locus is not embedded in such a condensed chromatin structure (Wang et al., 2009). We observed *pTERT* expression in various tissues, similar to *mTERT*. Therefore, it is plausible that the *pTERT* gene locus was not surrounded by the condensed chromatin environment, as was observed in the *mTERT* locus.

hTERT mutations are associated with a variety of human diseases in which many of the patients' chromosomes carry short telomeres. Almost all mutations in evolutionally conserved *hTERT* regions cause catalytic defects (Huard et al., 2003; Lee et al., 2003; Moriarty et al., 2004), providing evidence that conserved sites are naturally selected. We identified six *pTERT* mutations localized at the conserved residues among nine mammalian species. However, it is unclear whether these mutations affect telomerase activity. *pTERT* functions likely were unaffected by amino acid substitutions at nine mutation sites, including G61D and T267A, as amino acid residues acquired by substitution were present as wild-type residues at the corresponding sites of other mammalian species. Experimental verification is required to address the impact of the identified mutations on telomere length in the PL mice.

Regardless of non-synonymous or synonymous mutations, the frequency of *pTERT* mutation was higher, compared to that of the *hTERT* in our own and others' data. PL mice in the Chicago area have exhibited rapid changes in mitochondrial DNA and concordant morphological evolution over the past 30 years (Pergams et al., 2003; Pergams & Lacy 2008). One explanation is that immigrants from genetically distinct neighboring populations invaded and replaced the regional population over a short time period, which appears to have been facilitated by large environmental changes caused by humans. It is plausible that high *pTERT* variation of the PL mice in the Chicago area was caused by sequential haplotype replacements from invading populations.

The RPLP0 amino acid sequence was extremely homologous overall among the 13 mammalian species, showing remarkable interspecific conservation of RPLP0 during the course of mammalian evolution, as previously described (Wool et al., 1991; Krowczynska et al., 1989). Among the three rodent species, PL mouse displayed the highest homology in both *RPLP0* nucleotide and amino acid sequences to 10 non-rodent species, showing *pRPLP0* was more conserved compared to *rRPLP0* and *mRPLP0*. Rat and especially MM mouse genomes are known to display higher nucleotide mutations and chromosomal rearrangements. The extreme C-terminal 22-amino acid sequence common to three ribosomal proteins (RPLP0, RPLP1 and RPLP2) contains a major epitope motif (Mahler et al., 2003), which is recognized by autoantibodies in 12 – 16% of patients with systemic lupus erythematosus (Elkon et al., 1986). Additionally, RPLP0 overexpression is observed in various cancer patients, such as endometrioid, ovarian, breast, lung, or colon cancer (Chang et al., 2008; Artero-Castro et al., 2011). RPLP0 is important in clinical diagnosis and for evaluation of diseases (Rayno & Reichlin, 2000). Accordingly, *pRPLP0* sequence data should be helpful in investigating RPLP0 cellular functions and animal models of RPLP0-associated human diseases and useful in designing primers and probes for quantitative real-time RT-PCR as a reference “housekeeping” gene for normalization of results.

In conclusion, we report the first cloning of the entire *pTERT* (including its proximal promoter region) and *pRPLP0* coding sequences from PL mice of genus *Peromyscus*. Comparative sequencing analysis revealed that *pTERT* and *pRPLP0* coding sequences were highly homologous to those of eight and 12 other mammalian species examined, respectively. Further, we analyzed *pTERT* expression, telomere lengths of PL mice, and *pTERT* mutations. *Peromyscus* mice have been extensively used for various studies, including human diseases, for which *pTERT* and *pRPLP0* would be useful.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the Intramural Research Program of National Heart, Lung, and Blood Institute.

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Abbreviations list

TERT	Telomerase reverse transcriptase
PL	<i>Peromyscus leucopus</i>
pTERT	<i>Peromyscus leucopus</i> TERT
MM	<i>Mus musculus</i>
RPLP0	Ribosomal protein, large, P0
TERC	An RNA template of telomerase reverse transcriptase
RT-PCR	Quantitative real-time reverse transcription PCR
P. maniculatus	<i>Peromyscus maniculatus</i>
Rat	<i>Rattus norvegicus</i>
pRPLP0	<i>Peromyscus leucopus</i> RPLP0
CH-RAN	Random mean kinship
CH-MK	Minimizing mean kinship
CH-wild	Wild <i>Peromyscus leucopus</i>
PGSC	<i>Peromyscus</i> Genetic Stock Center
SC-LL	South Carolina LL stock mice
SC-inbred	South Carolina inbred stock mice
hTERT	Human TERT
rmTERT	rhesus monkey TERT
cTERT	Cow TERT
pgTERT	Pig TERT
dTERT	Dog TERT
rTERT	Rat TERT
rRPLP0	Rat RPLP0

mTERT	<i>Mus musculus</i> TERT
mRPLP0	<i>Mus musculus</i> RPLP0
hmTERT	Hamster TERT
TRF	Telomere restriction fragment
ORF	Open reading frame
pI	Calculated isoelectric point
aa	Amino acid
RT	Reverse transcriptase
NTE	N-terminal extension
CTE	C-terminal extension

Highlights

- The first cloning of the entire p*TERT* coding sequence from *Peromyscus leucopus* (PL).
- The first cloning of the entire p*RPLP0* coding sequence from PL mice.
- We reported characteristic features of p*TERT* and p*RPLP0* sequences.
- We analyzed p*TERT* expression, p*TERT* mutations, and telomere lengths of PL mice.
- p*TERT* and p*RPLP0* would be useful for various studies using *Peromyscus* mice.

		Motif GQ	
hTERT	VPWDARPPPAAPSFRRQVSCIKELVARVLQRLCERGAKNVLAFGFALLDGAAGGPPPEAFITTSVRSYLPNTVTDTALRGSGANGLLLRVGDVVLVHLLARCALFVLVAPSCAYQVCGGPLY	176	
rmTERT	VPWDARPPPAAPSFRRQVSCIKELVARVLQRLCERGAKNVLAFGFALLDGAAGGPPPEAFITTSVRSYLPNTVTDTALRGSGANGLLLRVGDVVLVHLLARCALFVLVAPSCAYQVCGGPLY	176	
dTERT	VPWGAARPPPAAPCFRRQVSCIKELVARVQRLCERGAKNVLAFGFALLDGAAGGPPPEAFITTSVRSYLPNTVTDTALRGSGANGLLLRVGDVVLVHLLARCALFVLVAPSCAYQVCGGPLY	176	
cTERT	VPWDAQPPPAAPSFRRQVSCIKELVARVQRLCERGAKNVLAFGFALLDGAAGGPPPEAFITTSVRSYLPNTVTDTALRGSGANGLLLRVGDVVLVHLLARCALFVLVAPSCAYQVCGGPLY	176	
pgTERT	VPWDAQPPPAAPSFRRQVSCIKELVARVQRLCERGAKNVLAFGFALLDGAAGGPPPEAFITTSVRSYLPNTVTDTALRGSGANGLLLRVGDVVLVHLLARCALFVLVAPSCAYQVCGGPLY	176	
hmTERT	VPWDSQPPPAADLSFHQVSSSLKELVARVQRLCERGERNVLAFTFGFALLNGAQGGPPPMFTTTSVRSYLPNTVTSLRVSGAWMLLLNRVGDVLLVLLARCALFVLVAPSCAYQVCGGPLY	176	
pTERT	VPWGSQPPPAAGLSFHQVSSSLKELVARVQRLCEKGERNVLAFTFGFALLNGAQGGPPPMFTTTSVRSYLPNTVTSLRVSGAWMLLLNRVGDVLLVLLARCALFVLVAPSCAYQVCGGPLY	176	
rTERT	VPWGSQPPPAADLSFHQVSSSLKELVARVQRLCERGERNVLAFTFGFALLNGAQGGPPPMFTTTSVRSYLPNTVTSLRVSGAWMLLLNRVGDVLLVLLARCALFVLVAPSCAYQVCGGPLY	176	
mTERT	MHWGSGQPPPAADLSFHQVSSSLKELVARVQRLCERNERNVLAFTFGFALLNGAQGGPPPMFTTTSVRSYLPNTVTSLRVSGAWMLLLNRVGDVLLVLLARCALFVLVAPSCAYQVCGGPLY	176	
* * * * *		* * * * *	
		Motif CP	
hTERT	LGNHAQCQPYGVLLKTHCFLRA	417	
rmTERT	LGNHAQCQPYGALLKTHCFLRA	417	
dTERT	LGNHARCQPYRALLRTHCFLRA	401	
cTERT	LGNHARSQPYGALLRAHCFLRA	405	
pgTERT	LGNHARCQPYKALLRAHCFLRA	409	
hmTERT	LGNHARCQPYVALLRASHCRFT	425	
pTERT	LVMNHRCSQYVLLKSHCRFRA	419	
rTERT	LMNHAKCQYVRLASHCRFT	422	
mTERT	LVMNHCQYVALLRASHCRFT	422	
* * *		* * *	
		Motif QFP	
hTERT	TDPRRLVQLLRQHSSPWQVYGFVRACLRRLVFPGLWGSRRHNERRFLRNTKKFISLGKHKAKLSLQELTWKMSVRDCAWLR	521	
rmTERT	TDPRRLVQLLRQHSSPWQVYGFVRACLRRLVFPGLWGSRRHNERRFLRNTKKFISLGKHKAKLSLQELTWKMSVRDCAWLR	521	
dTERT	TDSTRVLVQLLRQHSSPWQVYAFRACLRRLVFPGLWGSRRHNERRFLRNVKKFISLGKHKAKLSLQELTWKMSVRDCWLR	510	
cTERT	ANSGRVLVQLLRQHSSPWQVYGLLRACLRRLVFPGLWGSRRHNERRFLRNVKKFISLGKHKAKLSLQELTWKMSVRDCWLR	514	
pgTERT	SASRRLVQLLRQHSSPWQVYRLLRACLRRLVFPGLWGSRRHNERRFLRNVKKFISLGKHKAKLSLQELTWKMSVRDCWLR	519	
hmTERT	TSPQRLMLNLLRLHSSPWQVYGLQACVGLLRACLRRLVFPGLWGSRRHNERRFLRNVKKFISLGKHKAKLSLQELTWKMSVRDCWLR	515	
pTERT	TSPSHLTLRLHSSPWQVYGLRACLRRLVFPGLWGSRRHNERRFLRNVKKFISLGKHKAKLSLQELTWKMSVRDCWLR	508	
rTERT	TSPSHLTLRLHSSPWQVYGLRACLRRLVFPGLWGSRRHNERRFLRNVKKFISLGKHKAKLSLQELTWKMSVRDCWLR	511	
mTERT	TSPHMLDLRLHSSPWQVYGLRACLRRLVFPGLWGSRRHNERRFLRNVKKFISLGKHKAKLSLQELTWKMSVRDCWLR	511	
* * *		* * *	
		Motif T	
hTERT	WLMS-YVUVLLRSFFYVTTETTFQKNRLFFYRKSVWSKLQSIGIRQHLK	594	
rmTERT	WLMS-YVUVLLRSFFYVTTETTFQKNRLFFYRKSVWSKLQSIGIRQHLK	594	
dTERT	LVDGHIYVUVLLRSFFYVTTETTFQKNRLFFYRKSVWSKLQSIGIRQLFN	584	
cTERT	WLMS-YVUVLLRSFFYVTTETTFQKNRLFFYRKSVWSKLQSIGIRQHLK	587	
pgTERT	WLMS-YVUVLLRSFFYVTTETTFQKNRLFFYRKSVWSKLQSIGIRQHLK	592	
hmTERT	WLMS-YVUVLLRSFFYVTTETTFQKNRLFFYRKSVWSKLQSIGIRQHLK	588	
pTERT	WLMS-YVUVLLRSFFYVTTETTFQKNRLFFYRKSVWSKLQSIGIRQHLK	581	
rTERT	WLMS-YVUVLLRSFFYVTTETTFQKNRLFFYRKSVWSKLQSIGIRQHLK	584	
mTERT	WLMS-YVUVLLRSFFYVTTETTFQKNRLFFYRKSVWSKLQSIGIRQHLK	584	
* * *		* * *	
		Motif 1	
hTERT	EVRQREARPAALLTSRLRFIPKPDG	629	
rmTERT	EVRQREARPAALLTSRLRFIPKPDG	629	
dTERT	EVRQREARPAALLTSRLRFIPKPDG	619	
cTERT	EVRQREARPAALLTSRLRFIPKPDG	622	
pgTERT	EVRQREARPAALLTSRLRFIPKPDG	627	
hmTERT	EVRQREARPAALLTSRLRFIPKPDG	623	
pTERT	EVRQREARPAALLTSRLRFIPKPDG	616	
rTERT	EVRQREARPAALLTSRLRFIPKPDG	619	
mTERT	EVRQREARPAALLTSRLRFIPKPDG	619	
* * *		* * *	
		Motif 2	
hTERT	LAPIVNMMDYVVGARTFRREKRAERLTSRV	658	
rmTERT	LAPIVNMMDYVVGARTFRREKRAERLTSRV	658	
dTERT	LAPIVNMMDYVVGARTFRREKRAERLTSRV	648	
cTERT	LAPIVNMMDYVVGARTFRREKRAERLTSRV	651	
pgTERT	LAPIVNMMDYVVGARTFRREKRAERLTSRV	656	
hmTERT	LAPIVNMMDYVVGARTFRREKRAERLTSRV	652	
pTERT	LAPIVNMMDYVVGARTFRREKRAERLTSRV	645	
rTERT	LAPIVNMMDYVVGARTFRREKRAERLTSRV	648	
mTERT	LAPIVNMMDYVVGARTFRREKRAERLTSRV	648	
* * *		* * *	
		Motif 3a	
hTERT	RLTSRVKALFVSVLNYER	669	
rmTERT	RLTSRVKALFVSVLNYER	669	
dTERT	RLTSRVKALFVSVLNYER	659	
cTERT	RLTSRVKALFVSVLNYER	662	
pgTERT	RLTSRVKALFVSVLNYER	667	
hmTERT	RLTSRVKALFVSVLNYER	663	
pTERT	RLTSRVKALFVSVLNYER	656	
rTERT	RLTSRVKALFVSVLNYER	659	
mTERT	RLTSRVKALFVSVLNYER	659	
* * *		* * *	
		Motif 3b	
hTERT	ARRPGLLGASVVLGDDI	686	
rmTERT	ARRPGLLGASVVLGDDI	686	
dTERT	ARRPGLLGASVVLGDDI	676	
cTERT	ARRPGLLGASVVLGDDI	679	
pgTERT	ARRPGLLGASVVLGDDI	684	
hmTERT	ARRPGLLGASVVLGDDI	679	
pTERT	ARRPGLLGASVVLGDDI	673	
rTERT	ARRPGLLGASVVLGDDI	676	
mTERT	ARRPGLLGASVVLGDDI	676	
* * *		* * *	
		Motif 3c	
hTERT	HRAWRTFVLRLVR	698	
rmTERT	HRAWRTFVLRLVR	698	
dTERT	HRAWRTFVLRLVR	698	
cTERT	HRAWRTFVLRLVR	691	
pgTERT	HRAWRTFVLRLVR	696	
hmTERT	HRAWRTFVLRLVR	691	
pTERT	HRAWRTFVLRLVR	685	
rTERT	HRAWRTFVLRLVR	688	
mTERT	HRAWRTFVLRLVR	688	
* * *		* * *	
		Motif A	
hTERT	PPPELVFVKKVDVGTGAYDTIPQDRLETVIASIINKP	735	
rmTERT	PPPELVFVKKVDVGTGAYDTIPQDRLETVIASIINKP	735	
dTERT	PPPELVFVKKVDVGTGAYDTIPQDRLETVIASIINKP	725	
cTERT	PPPELVFVKKVDVGTGAYDTIPQDRLETVIASIINKP	728	
pgTERT	PPPELVFVKKVDVGTGAYDTIPQDRLETVIASIINKP	733	
hmTERT	PPPELVFVKKVDVGTGAYDTIPQDRLETVIASIINKP	728	
pTERT	PPPELVFVKKVDVGTGAYDTIPQDRLETVIASIINKP	722	
rTERT	PPPELVFVKKVDVGTGAYDTIPQDRLETVIASIINKP	725	
mTERT	PPPELVFVKKVDVGTGAYDTIPQDRLETVIASIINKP	725	
* * *		* * *	
		Motif IFDa	
hTERT	QDRLETVIASI	731	
rmTERT	QDRLETVIASI	731	
dTERT	QDRLETVIASI	724	
cTERT	QDRLETVIASI	729	
pgTERT	QDRLETVIASI	729	
hmTERT	QDRLETVIASI	724	
pTERT	QDRLETVIASI	718	
rTERT	QDRLETVIASI	721	
mTERT	QDRLETVIASI	721	
* * *		* * *	
		Motif IFDb	
hTERT	IIKFPQ-NTYCVRRYAVVQKAAGHVRAKFKSHVSTLTDLPQYMRQFVAHLQE	802	
rmTERT	IIKFPQ-NTYCVRRYAVVQKAAGHVRAKFKSHVSTLTDLPQYMRQFVAHLQE	802	
dTERT	IIKFPQ-NTYCVRRYAVVQKAAGHVRAKFKSHVSTLTDLPQYMRQFVAHLQE	793	
cTERT	IIKFPQ-NTYCVRRYAVVQKAAGHVRAKFKSHVSTLTDLPQYMRQFVAHLQE	795	
pgTERT	IIKFPQ-NTYCVRRYAVVQKAAGHVRAKFKSHVSTLTDLPQYMRQFVAHLQE	801	
hmTERT	IIKFPQ-NTYCVRRYAVVQKAAGHVRAKFKSHVSTLTDLPQYMRQFVAHLQE	798	
pTERT	IIKFPQ-NTYCVRRYAVVQKAAGHVRAKFKSHVSTLTDLPQYMRQFVAHLQE	792	
rTERT	IIKFPQ-NTYCVRRYAVVQKAAGHVRAKFKSHVSTLTDLPQYMRQFVAHLQE	795	
mTERT	IIKFPQ-NTYCVRRYAVVQKAAGHVRAKFKSHVSTLTDLPQYMRQFVAHLQE	795	
* * *		* * *	
		Motif IFDc	
hTERT	SGLFDFVFLRFMC	814	
rmTERT	SGLFDFVFLRFMC	814	
dTERT	SGLFDFVFLRFMC	805	
cTERT	SGLFDFVFLRFMC	807	
pgTERT	SGLFDFVFLRFMC	813	
hmTERT	SGLFDFVFLRFMC	810	
pTERT	SGLFDFVFLRFMC	804	
rTERT	SGLFDFVFLRFMC	807	
mTERT	SGLFDFVFLRFMC	807	
* * *		* * *	
		Motif B	
hTERT	KSYVQCQGGIPQGSILSTLLCSLCYGD MENKLFAGI	857	
rmTERT	KSYVQCQGGIPQGSILSTLLCSLCYGD MENKLFAGI	857	
dTERT	KSYVQCQGGIPQGSILSTLLCSLCYGD MENKLFAGI	848	
cTERT	KSYVQCQGGIPQGSILSTLLCSLCYGD MENKLFAGI	850	
pgTERT	KSYVQCQGGIPQGSILSTLLCSLCYGD MENKLFAGI	856	
hmTERT	KSYVQCQGGIPQGSILSTLLCSLCYGD MENKLFAGI	853	
pTERT	KSYVQCQGGIPQGSILSTLLCSLCYGD MENKLFAGI	847	
rTERT	KSYVQCQGGIPQGSILSTLLCSLCYGD MENKLFAGI	850	
* * *		* * *	
		Motif C	
hTERT	LLRLVDDFLLVTPHLTH	879	
rmTERT	LLRLVDDFLLVTPHLTH	879	
dTERT	LLRLVDDFLLVTPHLTH	870	
cTERT	LLRLVDDFLLVTPHLTH	872	
pgTERT	LLRLVDDFLLVTPHLTH	878	
hmTERT	LLRLVDDFLLVTPHLTH	875	
pTERT	LLRLVDDFLLVTPHLTH	869	
rTERT	LLRLVDDFLLVTPHLTH	872	
* * *		* * *	
		Motif D	
hTERT	AKTFLRLTLVRGVPEYGCVMNLKRTVV	905	
rmTERT	AKTFLRLTLVRGVPEYGCVMNLKRTVV	905	
dTERT	AKTFLRLTLVRGVPEYGCVMNLKRTVV	896	
cTERT	AKTFLRLTLVRGVPEYGCVMNLKRTVV	898	
pgTERT	AKTFLRLTLVRGVPEYGCVMNLKRTVV	904	
hmTERT	AKTFLRLTLVRGVPEYGCVMNLKRTVV	901	
pTERT	AKTFLRLTLVRGVPEYGCVMNLKRTVV	895	
rTERT	AKTFLRLTLVRGVPEYGCVMNLKRTVV	898	
mTERT	AKTFLRLTLVRGVPEYGCVMNLKRTVV	898	
* * *		* * *	

Fig. 2. Alignment of TERT regions and motifs among nine mammalian species

Nine mammalian TERT sequences were aligned, and their regions and motifs were defined based on previous reports (Nakamura et al., 1997; Bryan et al., 1998; Xia et al., 2000; Kuramoto et al., 2001; Banik et al., 2002; Delany and Daniels, 2004). The PL mouse sequence is depicted in bold letters. Conserved amino acid residues among nine mammalian species are indicated with asterisks (*). Gaps are illustrated with dashes colored in gray. Numbers on the right represent positions of amino acid residues in individual sequences.

Fig. 3. pTERT mRNA expression in various normal tissues

Semi-quantitative RT-PCR was performed to detect pTERT mRNA expression in 11 normal tissues of PL mice. Briefly, cDNA samples generated from total RNAs of individual tissues were subjected to PCR amplification using adequate primers which were designed based on pTERT sequence data, followed by agarose gel electrophoresis. pRPLP0 was used as an internal control to ensure RNA integrity. Two representative data obtained from SC-inbred and SC-LL mice are shown. PCR product sizes of pTERT and pRPLP0 are indicated on the right.

Fig. 4. Telomere length variation among five PL mouse groups

Peripheral blood DNAs were obtained from four captive stocks of PL mice (38 SC-LL, 9 SC-inbred, 34 CH-RAN, and 48 CH-MK mice) and wild-captured mice (28 CH-wild mice). TRF analysis was performed with genomic DNA (300 ng) using the TeloTAGGG Telomere Length Assay kit to assess telomere lengths of PL mice, according to the manufacturer's protocol. A mean TRF length was calculated using the ImageQuant TL software. Horizontal bars in each group represent a mean value and a standard error of the mean (mean $\pm$ SEM). Statistically significant differences were observed between any of the two groups, except for between CH-RAN and CH-wild groups. A two-tailed $*P$ value $< .05$ was considered statistically significant.

Fig. 5. Sequence alignment of *TERT* proximal promoter regions of the three rodents

Shown are *TERT* sequences upstream of ATG translation initiation sites of PL and MM mice and rat aligned using Clustal W2. Numbers on the left indicate nucleotide positions in which the first nucleotides upstream of the ATGs are indicated as -1. GC-boxes, canonical E-boxes, and non-canonical E-boxes (▼) are highlighted in underlined bold letters. CpG dinucleotide sites at which DNA methylation typically occur are marked in gray. Conserved nucleotide residues among three rodent species are marked with asterisks (*).

Table 1

TERT nucleotide or amino acid sequence homology rates among nine mammalian species

Total residue #		Name of Species	Identical nucleotide or amino acid residues (%)			
nt	aa		Human			
3399	1132	Human	100 (100.0)	R. monkey		
3399	1132	Rhesus monkey	96.1 (95.8)	100 (100.0)	Cow	
3378	1125	Cow	76.1 (69.9)	76.1 (70.1)	100 (100.0)	Pig
3396	1131	Pig	77.6 (71.5)	77.3 (71.3)	80.8 (76.2)	100 (100.0)
3372	1123	Dog	76.3 (70.2)	76.3 (70.4)	77.7 (72.8)	100 (100.0)
3387	1128	Hamster	69.9 (63.5)	69.5 (63.3)	67.7 (60.5)	100 (100.0)
3378	1125	Rat	69.0 (62.3)	68.6 (61.7)	67.9 (58.8)	100 (100.0)
3369	1122	MM mouse	68.9 (61.8)	68.3 (61.3)	67.3 (58.2)	100 (100.0)
3369	1122	PL mouse	70.1 (62.8)	69.7 (62.1)	67.9 (59.8)	100 (100.0)

Percentage homology is calculated as the number of identical nucleotide or amino acid residues divided by the total number of nucleotide or amino acid residues, respectively, of the two species being compared. The total numbers of nucleotide (nt, including a termination codon) and amino acid (aa) residues of *TERT* ORFs of individual species are represented in two columns at the left side of 'Name of species'. Under individual percentage numbers of nucleotide sequence homology rates, those of amino acid sequence homology rates are indicated within parentheses.

Table 2

Characteristics of 24 PL mice used for TRF length analysis

PL mouse group	SC-LL		SC-inbred		CH-RAN		CH-MK		CH-wild	
	Male (n = 18)	Female (n = 20)	Male (n = 6)	Female (n = 3)	Male (n = 15)	Female (n = 19)	Male (n = 22)	Female (n = 26)	Male (n = 19)	Female (n = 9)
TRF (kb)	Range	3.7 – 10.0 (3.7 – 10.0)	14.8 – 22.9 (14.8 – 22.9)	19.6 – 21.6 (14.8 – 22.9)	13.2 – 19.9 (11.0 – 20.2)	11.0 – 20.2 (11.0 – 20.2)	10.1 – 18.3 (10.1 – 18.3)	11.2 – 16.4 (10.1 – 18.3)	11.3 – 19.1 (11.3 – 19.3)	12.2 – 19.3 (11.3 – 19.3)
	Mean ± SEM	5.29 ± 0.37 (5.39 ± 0.20)	20.08 ± 1.43	20.92 ± 0.64	15.87 ± 0.52 (15.61 ± 0.39)	15.42 ± 0.58	14.56 ± 0.50 (14.05 ± 0.28)	13.62 ± 0.28	15.63 ± 0.43 (15.44 ± 0.38)	15.03 ± 0.78
Death age (Day)	Range	91 – 176 (60 – 242)	191 – 333 (191 – 333)	246 – 299 (191 – 333)	111 – 112 (87 – 112)	87 – 107	210 – 275 (210 – 275)	225 – 275	N/A (N/A)	N/A
	Mean ± SEM	123.3 ± 8.1 (140.5 ± 9.44)	265.0 ± 26.1	281.3 ± 17.7 (270.4 ± 17.83)	111.6 ± 0.13 (102.0 ± 1.75)	94.4 ± 1.60	236.6 ± 4.2 (247.4 ± 2.97)	256.6 ± 3.3	N/A (N/A)	N/A

TRF lengths (kb) or death ages of male, female, or male plus female (in parenthesis) in five PL mouse groups are indicated with ranges and mean ± SEM. N/A: not applicable because ages of wild-captured mice were unknown.

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Table 3

Nonsynonymous substitutions of pTert in 24 PL mice

Substituted position			SC-LL					SC-inbred					CH-RAN		CH-MK		CH-wild									
Nucleotide	Amino acid	Motif name ^a	1	2	3	4	5	1	2	3	4	5	1	2	1	2	3	4	5	6	7	8	9	10		
182 G/A	Gly61Asp	Motif GQ											+		+									+		
182 A/A		Motif GQ	+	+		+	+																			
695 G/A	*Arg232Gln												+		+											
695 A/A															+											
733–734 A/CT	*Lys245Leu		+	+	+	+	+	+	+	+	+	+														
799 A/G	Thr267Ala																	+	+							
962 A/G	Gln321Arg				+											+										
1115 G/A	Gly372Asp																				+					
1279 G/A	Ala427Thr			+			+																			
1283 C/T	Ser428Leu			+		+																				
1292 G/A	Ser431Asn	Motif QFP														+										
1541 A/G	Asn514Ser						+																			
1652 G/G	*Thr551Arg	Motif T							+																	
1690 C/T	*Arg564Cys	Motif T							+																	
1757 A/G	Gln586Arg								+				+		+			+	+		+		+	+		
1757 G/G			+	+				+		+	+	+			+											
1784 G/A	Arg595Gln	Motif I												+	+											
2065 C/A	Pro689Thr	Motif A											+													
2263 T/G	Ser755Ala					+	+							+												
2263 G/G																										
2317 G/T	Gly773Cys		+	+															+							
2338 G/A	*Val780Ile												+	+	+						+		+	+		
2338 A/A																+										
2681 T/C	Met894Thr	Motif D											+	+	+			+						+		
2776 G/A	Asp926Asn	Motif E-I		+		+									+	+		+								
2876 A/G	Iys959Arg	Motif E-I		+	+	+	+						+	+	+	+		+						+		

Substituted position		SC-LL					SC-inbred					CH-RAN					CH-MK					CH-wild				
Nucleotide	Amino acid	Motif name ^a	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10				
3178 G/A	Ala1060Thr	Motif E-III																								
3276 G/T	Gln1092His		+				+																			
		TRF (kb)	4	4.1	5.1	3.9	4.4	16.4	14.8	22.4	22	22	14.9	13.4	17	16.6	14.9	14.5	14.7	14.8	19.1	17.1				

pTert sequence analysis were performed in a total of 24 PL mice: 5, 5, 2, 2, and 10 mice in SC-LL, SC-inbred, CH-RAN, CH-MK, and CH-wild groups, respectively.

^aMotifs which carried substituted amino acid residues.

^{*}These substitutions were occurred at the conserved residues among nine mammalian species. PL mice with substituted residues are marked with “+”. The last row of Table shows TRF lengths (kb) of individual PL mice. For example, heterozygous and homozygous substitutions are shown like 182 G/A and 182 A/A, respectively.