Molecular cloning and characterization of AtTERT, a telomerase reverse transcriptase homolog in *Arabidopsis thaliana*

Keiko Oguchi, Hongtu Liu, Katsunori Tamura, Hideo Takahashi*

Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0032, Japan

Received 15 June 1999; received in revised form 4 August 1999

Abstract On the basis of its predicted homology to human telomerase reverse transcriptase (hTERT), a cDNA for *Arabidopsis thaliana* TERT (AtTERT) has now been isolated from cultured cells. The cDNA contains an open reading frame of 3372 bp, encoding a protein with a predicted size of 131 kDa and isoelectric point of 9.9. The AtTERT protein contains the conserved reverse transcriptase motifs 1, 2 and A–E as well as the TERT-specific T motif. Reverse transcription-polymerase chain reaction analysis and an assay of telomerase activity revealed that both *AtTERT* mRNA and telomerase activity are abundant in shoot apical meristems but are not detectable in rosette leaves.

Key words: Telomerase; Telomere; Reverse transcriptase; *Arabidopsis thaliana*

1. Introduction

Telomeres are elements present at the ends of linear eukaryotic chromosomes that are essential for their stabilization. They consist of simple tandem repeat sequences, such as $(TTAGGG)_n$ in mammals, that decrease in number with every conventional cell division, finally resulting in cell senescence and death. However, this problem of chromosome end replication can be overcome by telomerase, a ribonucleoprotein complex that mediates the elongation of telomere repeat sequences by catalyzing the reverse transcription of a template region within its RNA component. Telomerase activity has been detected in primitive eukaryotes, such as yeast and ciliates, as well as in immortalized and germ-line cells, but not in most normal somatic cells of humans [1], suggesting that telomerase-mediated maintenance of telomere length is associated with the cell division capacity.

Telomerase reverse transcriptases (TERTs) have been identified as the catalytic subunits of telomerase in various eukaryotes, including human [2,3], mouse [4], *Saccharomyces cerevisiae* [5], *Schizosaccharomyces pombe* [2], *Euplotes aediculatus* [6], *Tetrahymena thermophila* [7,8] and *Oxytricha trifallax* [8]. Given that telomerase activity has been reconstituted with the combination of the TERT (hTERT) and the RNA component of human telomerase in vitro [9–11], these two molecules are thought to constitute a minimal core unit for expression of such activity. Overexpression of the *hTERT* gene in normal human cells results in an increase in telomerase activity and a consequent lengthening of both the telomeric DNA and cellular life span [12,13]. Point mutations in or deletion of the yeast *TERT* gene result in gradual telomere shortening and aberrant cell morphogenesis [2]. The expression of the *hTERT* gene associated with cell immortality is controlled predominantly at the transcriptional level [2,3] and the abundance of mouse *TERT* (*mTERT*) mRNA is regulated during both cell differentiation and proliferation [4]. Taken together, these observations indicate that expression of the structurally conserved catalytic subunit of telomerase is an important determinant of telomerase activity.

Compared to TERT proteins in yeast, ciliates and mammals, all of which have been extensively studied, little information is available on plant telomerases. Plant telomerase activity has been detected in certain tissues and cells, including the barley embryo, immature seeds of *Arabidopsis thaliana* [14] and cultured tobacco BY-2 cells [15], with the use of the telomeric repeat amplification protocol (TRAP) assay. Telomerase activity is abundant in undifferentiated cells and meristematic tissues but is low or absent in non-proliferating tissues and mature organs [16,17]. Moreover, both telomere length and telomerase activity are developmentally regulated in *Melandrium album* [18]. These observations suggest that plant telomeres are maintained by a telomerase-mediated mechanism. However, neither TERT nor the telomerase RNA component have been identified in any plant species to date.

Our preliminary observation that suspension cultures of *A. thaliana* cells show both a high proliferative capacity and a high level of telomerase activity suggested that these cells express a *TERT* gene homolog. With the help of the DNA sequence available from the *Arabidopsis* genome project, we have now isolated a cDNA from cultured *A. thaliana* cells that encodes a protein homologous to hTERT. We also show that the extent of expression of this *A. thaliana* gene correlates with telomerase activity in plant tissues.

2. Materials and methods

2.1. Plant materials and growth conditions

Suspension cultures of *A. thaliana* ecotype Col-0 [19] cells were grown on a rotary shaker (120 rpm) at 22°C in modified Murashige-Skoog salt medium [20] supplemented with 2,4-dichlorophenoxy acetic acid (Wako, Osaka, Japan) at 1 mg/l and 2.5 mM KH_2PO_4. The cells were subcultured once a week. Intact *A. thaliana* plants were grown on potted vermiculite in a glasshouse at 23°C with light for 14 h.

2.2. Molecular cloning and sequence analysis

With the use of the BLAST program, we searched the GenBank

*Corresponding author. Fax: (81) (3) 5841-8476.
E-mail: htakaha@imcbns.iam.u-tokyo.ac.jp

Abbreviations: TERT, telomerase reverse transcriptase; TRAP, telomerase repeat amplification protocol; RT, reverse transcriptase; PCR, polymerase chain reaction; poly(A)$^+$, polyadenylated

PII: S0014-5793(99)01083-2

Arabidopsis database with the amino acid sequence of hTERT. A bacterial artificial chromosome clone (T1704TRD) was identified that contained a 625 bp genomic sequence (accession number B27802) encoding an amino acid sequence that showed substantial similarity to reverse transcriptase (RT) motifs C, D and E of hTERT. To clone a cDNA fragment corresponding to the T1704TRD genomic clone, we prepared forward (T1704TRD-f, 5′-GCGAGCTCTTACTGAGATTTATTGATGAC-3′) and reverse (T1704TRD-r, 5′-GCTCTAGACAACAGAAAGATCTGATAGATGTT-3′) oligonucleotide primers for polymerase chain reaction (PCR) amplification with cDNA derived from *A. thaliana* cultured cells. Restriction enzyme sites that were added to the 5′-end of each primer are underlined (GAGCTC, *Sac*I; TCTAGA, *Xba*I). The resulting 441 bp amplification product was digested with *Sac*I and *Xba*I and cloned into the pUC118 vector. For preparation of a T1704TRD DNA probe, the fragment was excised and labelled with digoxigenin by a PCR-based method (Boehringer Mannheim) with the same two primers as above.

Polyadenylated (poly(A)$^+$) RNA was extracted from 7 day cultures of *A. thaliana* cells with the use of a QuickPrep Micro mRNA purification kit (Amersham Pharmacia Biotech). A cDNA library was prepared from the poly(A)$^+$ RNA with the use of a ZAP Express cDNA synthesis kit and Gigapack cloning kit (Stratagene) and was then screened with the T1704TRD probe by plaque hybridization. Three independent positive clones were isolated and the corresponding inserts were excised and incorporated into the phagemid vector in vivo. The resulting plasmids were sequenced on both strands with the use of an Applied Biosystems model 373S DNA sequencer (Perkin-Elmer Japan, Chiba, Japan).

Multiple sequence alignments were performed with the Clustal V program. All parameters were default except for during the analysis of motif 2, which was divided into two fragments at the period shown in Fig. 1 and each fragment was analyzed separately with gap penalties of 50. For construction of the phylogenetic tree, the amino acid sequences of the conserved motifs T, 1, 2 and A–E of each TERT homolog were joined and aligned with the use of the Clustal W program and the neighbor-joining method was then applied [21].

2.3. Southern hybridization analysis

For Southern hybridization, genomic DNA was isolated from cultured *A. thaliana* cells with the use of a Plasmid Midi kit (QIAGEN). Genomic DNA was also prepared from tomato, rice and tobacco (BY-2 cultured cells) with the use of the CTAB method [22]. Genomic DNA (10 μg) was digested with *Eco*RI, *Xba*I, *Bgl*II or *Xho*I and the resulting fragments were fractionated by electrophoresis on a 1.0% agarose gel and then transferred to a Hybond-N$^+$ nylon membrane (Amersham Pharmacia Biotech) by capillary transfer. Hybridization was performed with the digoxigenin-labelled T1704TRD probe under high- or low-stringency conditions and signals were detected by chemiluminescence (Boehringer Mannheim).

2.4. Reverse transcription and PCR analysis

Poly(A)$^+$ RNA was extracted from 7 day cultures of *A. thaliana* cells, as well as from shoot segments containing apical meristems and from rosette leaves of 6 week old plants, with the use of a QuickPrep Micro mRNA purification kit. Reverse transcription and subsequent PCR were performed with an RT-PCR High kit (TOYOBO, Osaka, Japan). Complementary DNA was synthesized from 100 ng of poly(A)$^+$ RNA with 100 ng of oligo(dT) primer in a 20 μl reaction volume, with serial incubations for 10 min at 30°C, 60 min at 42°C and 5 min at 99°C. With 1 μl of the reverse transcription product as template, PCR amplification was then performed with the T1704TRD primers described above in a total volume of 20 μl. The amplification protocol comprised 40 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s. To allow for variations in the amount of input RNA and in the efficiency of reverse transcription, we also amplified the *A. thaliana* ubiquitin (*AtUBQ*) gene as a control. A portion (5 μl) of the PCR product was subjected to electrophoresis on a 1.5% agarose gel, which was then stained with GelStar (FMC BioProducts). Signals were scanned and quantified with the use of a FluorImager and ImageQuaNT software (Molecular Dynamics), respectively.

2.5. Telomerase assay

Telomerase activity was assayed by TRAP [23] with minor modifications [28]. Extracts were prepared from 3 day old cultures of *A. thaliana* cells as well as from shoot segments containing apical meristems and from rosette leaves of 6 week old plants. A portion (8 μl) of the PCR product was subjected to electrophoresis on a non-denaturing 10% polyacrylamide gel, which was then stained with GelStar. Ladders of DNA bands were scanned with a FluorImager.

3. Results

3.1. Cloning of the A. thaliana TERT cDNA

To isolate a *TERT* gene homolog from *A. thaliana*, with the use of the BLAST program, we first searched the GenBank *Arabidopsis* database with the entire amino acid sequence of hTERT. A 625 bp genomic sequence (T1704TRD) was thus identified that encoded an amino acid sequence with significant homology to a region of hTERT containing motifs C, D and E (probability, 3.2×10^{-7}). With a primer set based on the nucleotide sequence of T1704TRD, a 441 bp DNA fragment was amplified by reverse transcription and PCR from poly(A)$^+$ RNA isolated from cultured *A. thaliana* cells. This fragment was labelled with digoxigenin and used as a probe to screen an *A. thaliana* cDNA library constructed with mRNA isolated from cultured cells. Three independent clones were obtained, the largest of which contained the initiation codon (ATG) of the open reading frame as revealed by the presence of an in-frame termination codon (TGA) 96 bp upstream. Nucleotide sequence analysis of the three clones revealed an open reading frame of 3372 bp that encodes a protein of 1124 amino acids with a calculated molecular mass of 131 kDa and an isoelectric point of 9.9. These values are similar to those for the seven other known TERTs, which range in size from 103 to 133 kDa (average, 123 kDa) and in isoelectric point from 10.0 to 11.3 (average, 10.4). We therefore designated the encoded protein AtTERT (for *A. thaliana* TERT).

3.2. Structural characterization of AtTERT

Multiple alignment of the amino acid sequences of the various conserved motifs of AtTERT with those of previously characterized TERT proteins is shown in Fig. 1. AtTERT contains all seven RT motifs [24] and the telomerase-specific T motif previously identified. All of these motifs have been considered signature sequences for the identification of TERT family members [2]. In addition, AtTERT contains a conserved Arg residue in motif 1 as well as a conserved aromatic residue (Tyr or Phe) immediately downstream of the two Asp residues in motif C, consistent with telomerase-specific features of RT motifs that distinguish them from the corresponding motifs of retroviral and retrotransposon consensus sequences [2]. AtTERT apparently lacks a CP motif, which is specific to TERTs of ciliated protozoa [8].

The extent of amino acid sequence homology among the conserved motifs of all known TERT proteins and a possible phylogenetic tree based on these amino acid sequences are shown in Table 1 and Fig. 2, respectively. The motifs of AtTERT show a greater sequence identity and similarity to those of hTERT and mTERT than they do to those of TERTs from yeast or ciliates (Table 1). Similarly, AtTERT was clustered with hTERT and mTERT in the same branch of the phylogenetic tree (Fig. 2). The tree contained three clusters of TERT proteins corresponding to those of yeast, ciliates and higher eukaryotes, consistent with the evolutionary relations among these species. Moreover, AtTERT resembles hTERT not only in the conserved motifs (Table 1) but also in the COOH-terminal region (26 and 38% identity and similarity, respectively).

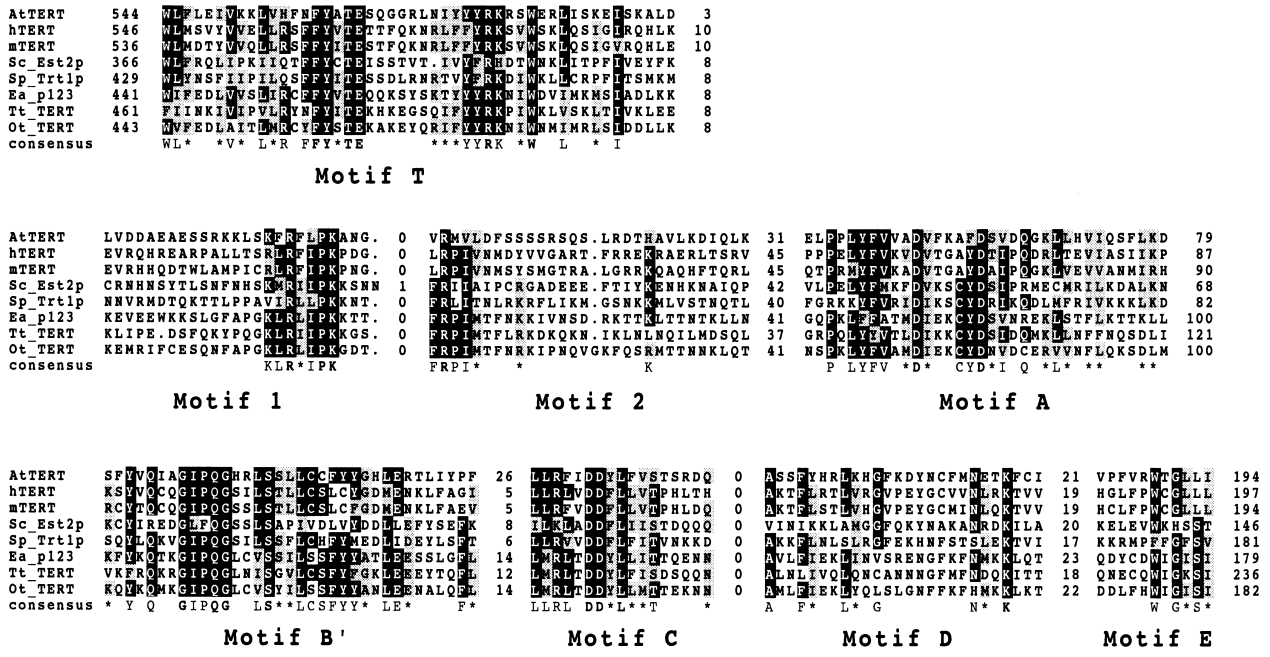

Fig. 1. Multiple sequence alignment of the conserved motifs of the eight TERT proteins identified to date. Organisms represented are *A. thaliana* (AtTERT), *Homo sapiens* (hTERT) [2,3], *Mus musculus* (mTERT) [4], *S. cerevisiae* (Sc_Est2p) [5], *S. pombe* (Sp_Trt1p) [2], *E. aediculatus* (Ea_p123) [6], *T. thermophila* (Tt_TERT) [8] and *O. trifallax* (Ot_TERT) [8]. Boxed residues match the consensus sequence, being present in five or more proteins. Shaded residues represent similar amino acids. The consensus sequence for each motif is also shown, with bold face letters indicating identity among all TERTs and asterisks indicating the presence of similar residues at that position. The numbers of amino acids between motifs and to the ends of each protein are also shown. Periods indicate gaps introduced to optimize alignment. Abbreviation for the amino acids are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp and Y, Tyr. The nucleotide sequence of the *AtTERT* cDNA has been deposited in GenBank under accession number AF135454.

3.3. Southern hybridization analysis of the AtTERT gene

To determine whether the *AtTERT* gene is present in a single copy in the *A. thaliana* genome, we performed Southern hybridization with the T1704TRD probe. The four restriction enzymes *Eco*RI, *Xba*I, *Xho*I and *Bgl*II were chosen for digestion of genomic DNA. There are no restriction sites for *Eco*RI or *Xba*I in the full-length *AtTERT* cDNA or for *Xho*I and *Bgl*II in the T1704TRD region of the cDNA, although there is a unique site for each of *Xho* I and *Bgl* II in the full-length cDNA. A single hybridization signal was detected for each of the digests obtained with these restriction enzymes (Fig. 3), indicating that the *AtTERT* gene is most likely present in the *A. thaliana* genome in a single copy.

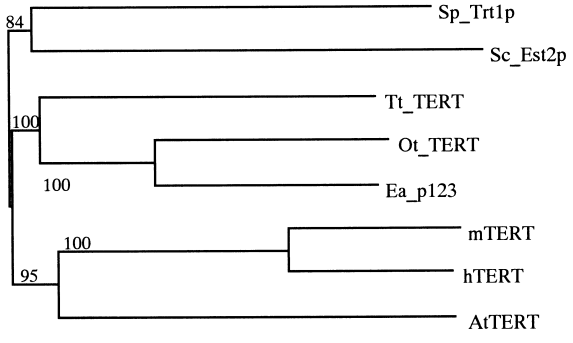

Fig. 2. Phylogenetic tree based on the amino acid sequences of the conserved motifs of TERT proteins. The tree was constructed by the neighbor-joining method [21] after sequence alignment of the conserved motifs (T, 1, 2 and A–E). The number at each node indicates the percentage of 1000 bootstrap replicates for statistical support.

A conserved telomeric repeat sequence and telomerase activity have been detected in various plant species, including tobacco, tomato and rice, suggesting that telomeric maintenance in these species is also mediated by a TERT homolog.

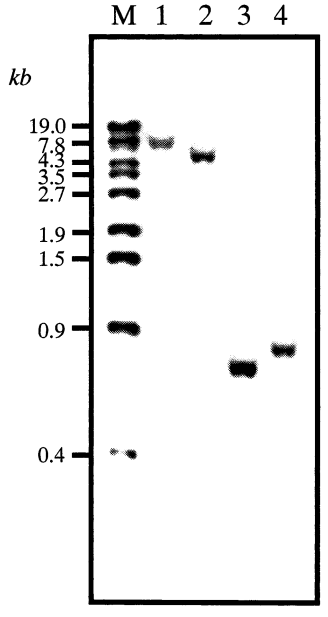

Fig. 3. Southern blot analysis of the *AtTERT* gene. Southern blot analysis of *A. thaliana* genomic DNA with the digoxigenin-labelled T1704TRD probe. Genomic DNA was completely digested with *Eco*RI (lane 1), *Xba*I (lane 2), *Bgl*II (lane 3) or *Xho*I (lane 4). Lane M, molecular mass markers (*Eco*T14I-digested λ phage DNA), the sizes of which are indicated in kb.

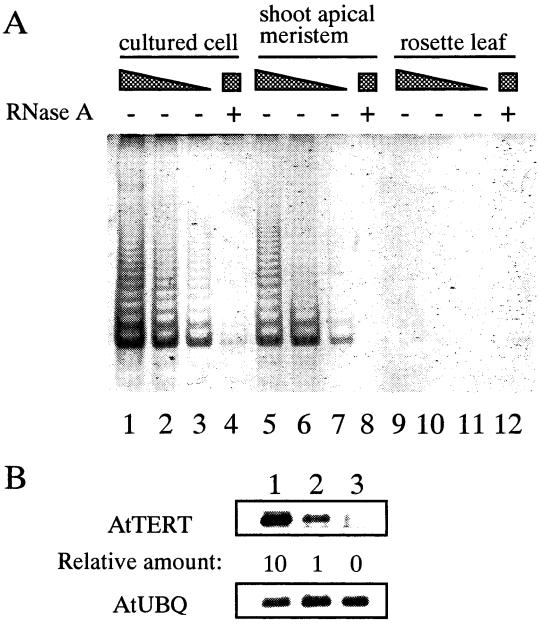

Fig. 4. Telomerase activity (A) and the abundance of *AtTERT* mRNA (B) in *A. thaliana* cells and tissues. (A) TRAP assay of telomerase activity in cultured cells (lanes 1–4), shoot apical meristems (lanes 5–8) and rosette leaves (lanes 9–12). The elongation reactions were performed with 500 ng (lanes 1, 5 and 9), 50 ng (lanes 2, 6 and 10) or 5 ng (lanes 3, 7 and 11) of protein or with 500 ng of protein plus 500 ng of RNase A (lanes 4, 8 and 12). (B) Reverse transcription and PCR analysis of *AtTERT* mRNA in cultured cells (lane 1), shoot apical meristems (lane 2) and rosette leaves (lane 3). Values shown represent the amount of *AtTERT* mRNA in each sample relative to that in shoot apical meristems. Amplification of the *AtUBQ* gene was performed as a control.

To investigate this possibility, we subjected genomic DNA from tomato, rice and cultured tobacco BY-2 cells to Southern hybridization analysis with the T1704TRD probe. Unexpectedly, no hybridization signal was detected with digests of these plant genomes prepared with either *Eco*RI or *Xba*I (data not shown). The same result was obtained when hybridization was performed with the full-length *AtTERT* cDNA as probe, even under low-stringency conditions (data not shown).

3.4. Expression of the AtTERT gene and telomerase activity in A. thaliana cells and tissues

Telomerase activity in human and mouse cells is thought to be controlled predominantly by transcriptional regulation of the *TERT* gene [2–4]. To examine the relation between expression of the *AtTERT* gene and telomerase activity in *A. thaliana*, we measured telomerase activity and the abundance of *AtTERT* mRNA in cultured cells, shoot apical meristems and rosette leaves. With the use of the TRAP assay, we detected marked telomerase activity in cultured cells and in shoot apical meristems (Fig. 4A), both of which possess a proliferative capacity.

The telomerase activity was proportional to the protein concentration and was abolished by the addition of RNase A to the elongation reaction. In contrast, telomerase activity was not detected in rosette leaves (Fig. 4A). Reverse transcription and PCR analysis revealed the presence of *AtTERT* mRNA in cultured cells and shoot apical meristems but not in rosette leaves (Fig. 4B), consistent with the pattern of expression of telomerase activity. Both telomerase activity and the abundance of *AtTERT* mRNA were about 10-fold greater in cultured cells than in shoot apical meristems (Fig. 4), probably reflecting a small proportion of apical meristematic tissue in the shoot segment samples. These results suggest that transcription of the *AtTERT* gene is essential for the expression of telomerase activity in *Arabidopsis*.

4. Discussion

In the present study, we have identified a TERT homolog in the higher plant *A. thaliana*. In vitro reconstitution experiments have shown that the core catalytic unit of telomerase from other organisms consists of the TERT protein in combination with the telomerase RNA [9–11]. Although the RNA component of a plant telomerase has not been identified, it is likely that AtTERT and the corresponding telomerase RNA subunit also constitute an active core enzyme that is essential for catalysis of telomere formation. The identification of AtTERT reinforces the conclusion that the TERT catalytic subunit is a phylogenetically conserved component of telomerase throughout evolution. Moreover, the amino acid sequence of AtTERT resembles those of TERT proteins of higher eukaryotes more closely than it does those of TERTs from lower eukaryotes, consistent with the overall evolutionary relations among eukaryotes.

The human telomerase RNA subunit is most abundant in telomerase-positive cells, but is also present in many telomerase-negative cells. In contrast, the abundance of *hTERT* mRNA is much more closely related to the telomerase activity of a cell, indicating that the expression of telomerase activity is largely determined at the level of transcriptional regulation of the *hTERT* gene. Alternative splicing of the primary *hTERT* transcript has also been shown to contribute to the regulation of telomerase activity during fetal cell development [25]. However, the amount of *hTERT* mRNA in human lymphocytes does not correspond precisely to the level of telomer-

Table 1
Amino acid sequence homology among the eight known TERT proteins

Identity/similarity	AtTERT	hTERT	mTERT	Sc_Est2p	Sp_Trt1p	Ea_p123	Tt_TERT	Ot_TERT
AtTERT		48.4	51.6	36.6	42.0	43.9	42.5	42.5
hTERT	33.8		80.9	40.5	46.5	44.7	39.9	42.1
mTERT	35.6	72.4		39.2	46.5	43.0	42.5	42.5
Sc_Est2p	24.2	26.0	23.3		43.0	40.4	38.6	39.5
Sp_Trt1p	26.5	31.0	31.4	29.4		43.4	41.7	41.7
Ea_p123	29.4	28.1	25.9	24.6	27.6		59.5	74.3
Tt_TERT	28.9	25.4	28.1	22.4	27.2	43.6		59.0
Ot_TERT	27.6	27.6	24.6	23.2	26.8	63.3	42.7	

Sequence identity and similarity among conserved motifs (T, 1, 2 and A–E) of the indicated proteins are shown below and above the diagonal, respectively.

ase activity, which is regulated during cell development, differentiation and activation [26]. Moreover, hTERT possesses two putative phosphorylation sites for the protein kinase Akt, which increases human telomerase activity through phosphorylation [27]. These results thus suggest that telomerase activity is regulated at multiple steps subsequent to transcription of the *TERT* gene, including regulation at both post-transcriptional and post-translational levels. It is possible that telomerase activity in *A. thaliana*, at least in meristematic tissues and cultured cells, is controlled predominantly by transcriptional regulation of the *AtTERT* gene. Alternative splicing of *AtTERT* RNA was not detected in the present study and no phosphorylation sites for protein kinases are apparent in the primary structure of the AtTERT protein. The mechanisms by which telomerase activity is regulated in *A. thaliana* thus require further investigation.

Studies of the mechanisms underlying maintenance of telomere length and regulation of telomerase activity in plants have been hampered by the lack of a molecular characterization of a plant telomerase. Previous studies have shown that plant telomerases are regulated precisely during the cell division cycle [28] as well as during development and differentiation [18]. The molecular cloning of the AtTERT subunit should now facilitate molecular approaches to determining the roles of and defining the regulatory mechanisms of plant telomerase. In other organisms, several factors have been identified as components of the telomerase holoenzyme or regulators of telomerase activity, including p80 homologs [29,30], telomeric repeat binding factors in humans [31] and Est1p in fission yeast [32]. The cloning of the *AtTERT* gene may also facilitate identification of such telomerase regulatory proteins in plants.

Acknowledgements: The authors thank M. Umeda for providing the *A. thaliana* cultured cells and H. Yoshikawa, K. Tanaka, K. Kanamaru and K. Nimura for helpful discussions. This work was supported by grants-in-aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

References

[1] Nugent, C.I. and Lundblad, V. (1998) Genes Dev. 12, 1073–1085.
[2] Nakamura, T.M., Morin, G.B., Chapman, K.B., Weinrich, S.L., Andrews, W.H., Lingner, J., Harley, C.B. and Cech, T.R. (1997) Science 277, 955–959.
[3] Meyerson, M., Counter, C.M., Eaton, E.N., Ellisen, L.W., Steiner, P., Caddle, S.D., Ziaugra, L., Beijersbergen, R.L., Davidoff, M.J., Liu, Q., Bacchetti, S., Haber, D.A. and Weinberg, R.A. (1997) Cell 90, 785–795.
[4] Greenberg, R.A., Allsopp, R.C., Chin, L., Morin, G.B. and DePinho, R.A. (1998) Oncogene 16, 1723–1730.
[5] Lendvay, T.S., Morris, D.K., Sah, J., Balasubramanian, B. and Lundblad, V. (1996) Genetics 144, 1399–1412.
[6] Lingner, J., Hughes, T.R., Shevchenko, A., Mann, M., Lundblad, V. and Cech, T.R. (1997) Science 276, 561–567.
[7] Collins, K. and Gandhi, L. (1998) Proc. Natl. Acad. Sci. USA 95, 8485–8490.
[8] Bryan, T.M., Sperger, J.M., Chapman, K.B. and Cech, T.R. (1998) Proc. Natl. Acad. Sci. USA 95, 8479–8484.
[9] Weinrich, S.L., Pruzan, R., Ma, L., Ouellete, M., Tesmer, V.M., Holt, S.E., Bodnar, A.G., Lichtsteiner, S., Kim, N.W., Trager, J.B., Taylor, R.D., Carlos, R., Andrews, W.H., Wright, W.E., Shay, J.W., Harley, C.B. and Morin, G.B. (1997) Nat. Genet. 17, 498–502.
[10] Beattie, T.L., Zhou, W., Robinson, M.O. and Harrington, L. (1998) Curr. Biol. 8, 177–180.
[11] Holt, S.E., Aisner, D.L., Baur, J., Tesmer, V.M., Dy, M., Ouellette, M., Trager, J.B., Morin, G.B., Toft, D.O., Shay, J.W., Wright, W.E. and White, M.A. (1999) Genes Dev. 13, 817–826.
[12] Bodner, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.-P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S. and Wright, W.E. (1998) Science 279, 349–352.
[13] Vaziri, H. and Benchimol, S. (1998) Curr. Biol. 16, 279–282.
[14] Heller, K., Kilian, A., Piatyszek, M.A. and Kleinhofs, A. (1996) Mol. Gen. Genet. 252, 342–345.
[15] Fajkus, J., Kovarik, A. and Kralovics, R. (1996) FEBS Lett. 391, 307–309.
[16] Fitzgerald, M.S., McKnight, T.D. and Shippen, D.E. (1996) Proc. Natl. Acad. Sci. USA 93, 14422–14427.
[17] Killan, A., Heller, K. and Kleinhofs, A. (1998) Plant Mol. Biol. 37, 621–628.
[18] Riha, K., Fajkus, J., Siroky, J. and Vyskot, B. (1998) Plant Cell 10, 1691–1698.
[19] Umeda, M., Bhalerao, R.P., Schell, J., Uchimiya, H. and Koncz, C. (1998) Proc. Natl. Acad. Sci. USA 95, 5021–5026.
[20] Murashige, T. and Skoog, F. (1962) Physiol. Plant 15, 473–497.
[21] Saitou, N. and Nei, M. (1987) Mol. Biol. Evol. 4, 406–425.
[22] Rogers, S.O. and Bendich, A.J. (1985) Plant Mol. Biol. 5, 69–76.
[23] Kim, N.W., Piatyszek, M.A., Prowse, K.R., Harley, C.B., West, M.D., Ho, P.L.C., Coviello, G.M., Wright, W.E., Weinrich, S.L. and Shay, J.W. (1994) Science 266, 2011–2015.
[24] Xiong, Y. and Eickbush, T.H. (1990) EMBO J. 9, 3353–3362.
[25] Ulaner, G.A., Hu, J.-F., Vu, T.H., Giudice, L.C. and Hoffman, A.R. (1998) Cancer Res. 58, 4168–4172.
[26] Liu, K., Schoonmaker, M.M., Levine, B.L., June, C.H., Hodes, R.J. and Weng, N. (1999) Proc. Natl. Acad. Sci. USA 96, 5147–5152.
[27] Kang, S.S., Kwon, T., Kwon, D.Y. and Do, S.I. (1999) J. Biol. Chem. 274, 13085–13090.
[28] Tamura, K., Liu, H. and Takahashi, H. (1999) J. Biol. Chem. 274, 20997–21002.
[29] Collins, K., Kobayashi, R. and Greider, C.W. (1995) Cell 81, 677–686.
[30] Nakayama, J., Saito, M., Nakamura, H., Matsuura, A. and Ishikawa, F. (1997) Cell 88, 875–884.
[31] van Steensel, B., Smogorzewska, A. and de Lange, T. (1998) Cell 92, 401–413.
[32] Lin, J.-J. and Zakian, V.A. (1995) Cell 81, 1127–1135.