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A Possible mRNA Splicing Mechanism for Regulation of Telomerase Activity in Rice (*Oryza sativa* L.)

Hee Chung, Rie Hishinuma, Sugihiro Ando, and Shingo Sakai*
Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

The mechanism of telomerase regulation is far better understood in mammalian systems than it is in plant systems. In this study, we investigated the possibility that regulation of telomerase activity occurs at the post-transcriptional level in rice plants (*Oryza sativa*). Specifically, we used RT-PCR with primers based on the rice telomerase reverse transcriptase (*OsTERT*) cDNA sequence to detect alternative mRNA splicing products. Five types of DNA product that were shorter than predicted by the cDNA sequence were observed for all the tested cells and tissues. Sequencing of these products indicated that they were alternatively spliced *OsTERT* fragments lacking some portions of the full-length sequence. These results suggest that regulation of telomerase activity occurs at the post-transcriptional level in rice cells and tissues. The relative amounts of full-length *OsTERT* mRNA and the shorter, alternatively spliced mRNAs were estimated by quantitative RT-Southern analysis. No correlation was found between levels of telomerase activity and accumulation of full-length *OsTERT* mRNA in the various cell and tissue types, indicating that splicing of *OsTERT* mRNAs may be important in telomerase regulation in rice. Further studies are necessary to determine whether the spliced *OsTERT* mRNAs are translated into proteins and if these putative proteins affect telomerase activity.

Keywords: alternative splicing, *OsTERT*, post-transcription, rice, telomerase

Telomeres are specialized ribonucleoprotein structures that are present at the ends of chromosomes. They consist of tandem repeats of short, guanine-rich nucleotide sequences in association with a complex array of proteins, including the enzyme telomerase. In vertebrate species, these telomeric repeats consist of the sequence TTAGGG (Meyne et al., 1989), whereas in the plant *Arabidopsis thaliana*, they consist of the sequence TTTAGGG (Richards and Ausubel, 1988; Kipling, 1995).

The primary function of telomeres is to protect chromosomes from degradation by exonucleases and from end-to-end fusion (Henderson and Blackburn, 1989; EcEachern et al., 2000). During DNA replication, however, the 3′-ends of parental strands are not completely replicated, leading to shortening of the telomeric DNA sequences with each round of replication (Olovnikov, 1973). The loss of telomeric repeats owing to the "end replication problem" diminishes the protective capacity of the telomeres, leading to increased chromosomal degradation or fusion during cellular division (Kim et al., 2002). Shortening of telomeres beyond a certain point is closely linked to cellular senescence and death (Allsop et al., 1992). Various mechanisms have evolved to overcome the end replication problem. The enzyme telomerase plays an important role in many of these mechanisms (Kipling, 1995).

The telomerase enzyme is a ribonucleoprotein complex that catalyzes the addition of telomeric repeats to the 3′-end of telomeric DNA, using its RNA components as a template (Greider and Blackburn, 1987). Telomerase activity was first detected in *Tetrahymena thermophila* and yeast (Greider and Blackburn, 1985) and in human HeLa cells (Morin, 1989). Interestingly, the level of telomerase activity in human cancer cells does not correlate with the expression of its protein component, telomerase reverse transcriptase (TERT) (Ulaner et al., 1998, 2000, 2001; Yi et al., 2000), indicating that transcription of the human TERT gene (*hTERT*) is not the limiting factor in synthesis or activation of human telomerase. This phenomenon may be a result of alternative mRNA splicing that is reported to occur during hTERT synthesis (Ulaner et al., 1998; Yi et al., 2000).

In plants, telomerase activity has been detected in many proliferative tissues, including soybean root tips; cauliflower floral buds (Fitzgerald et al., 1996); embryos, anthers, and carpels of barley (Heller et al., 1996); cultured cells and the apical meristem of *A. thaliana* (Oguchi et al., 1999); and suspension-cultured BY-2 tobacco cells (Tamura et al., 1999). In contrast to human cancer cells, *A. thaliana* cells and

*Corresponding author; fax +81-298-534579
e-mail ssakai@sakura.cc.tsukuba.ac.jp

tissues exhibit a correlative relationship between telomerase activity and *A. thaliana* TERT gene (*AtTERT*) expression (Oguchi et al., 1999). Telomerase activity has not been detected in non-proliferative plant tissues, such as the leaves and stems of barley and soybean (Fitzgerald et al., 1996; Heller et al., 1996).

Although the correlation between *AtTERT* expression and telomerase activity in *A. thaliana* indicates that transcription of *AtTERT* is sufficient for telomerase synthesis or activation, a similar relationship does not appear to exist in rice plants. In the previous report (Chung et al., 2003), we found that rice telomerase activity had no correlative relationship with *OsTERT* accumulation level, and also isolated a full-length *OsTERT* cDNA by the combination of RT-PCR and fusion PCR based on the sequence reported in the NCBI data bank. Although we detected several types of cDNA fragments that were shorter than the expected normal fragments, during the previous RT-PCR study, the sequence of shorter fragments were not analyzed as non-specific bands. However, it was found that our results were very similar to those of Heller-Uszynska et al. (2002) by analyzing the sequence of each shorter cDNA fragments. Therefore, in this paper, we report a possibility of alternative splicing of *OsTERT* mRNA as reported by Heller-Uszynska et al. (2002) and Oguchi et al. (2004). Furthermore, in this study, we investigated whether an alternative splicing mechanism was occurred in various rice tissues and different growth stage of suspension-cultured cells, and the relationship of the accumulation level of *OsTERT* full-length and telomerase activity.

The rice tissues studied in this work included fully expanded blades from 3-month-old rice plants as well as five seedling tissues: leaf sheath including apical meristem, third blade, leaf sheath including young blades, root tip, and root without root tips. Suspension-cultured cells of leaf sheath including apical meristem were also tested. These cells and tissues were analyzed for the presence of spliced *O. sativa* TERT (*OsTERT*) mRNAs to examine whether a post-transcriptional regulatory mechanism for telomerase activity, such as alternative splicing, exists in rice.

MATERIALS AND METHODS

Preparation of Suspension-Cultured Cells and Rice Seedlings

Suspensions of rice cells (*O. sativa* L. cv. Nipponbare) were cultured in N6 medium (Chu et al., 1975) containing 2% (w/v) sucrose and 0.2 mg L^{-1} 2,4-D. The cells were cultured at 25°C in the dark with continuous shaking at 120 strokes per min and were subcultured every 4 d into 100 ml of fresh medium. The cells were collected on a membrane filter and the fresh weight was measured every other day for 8 d. Rice plants (*O. sativa* L. cv. Nipponbare) were grown on moist vermiculite at 28°C under a 16 h-light/8 h-dark cycle in a growth chamber for 3 months. The five types of tissues such as the leaf sheath including the apical meristem, the leaf sheath including the young blades, root tip, and root without root tips were prepared from 3-week-old plants. Expanded fourth or fifth blades about 20 cm long were also prepared from 3-month-old plants. These excised tissues and cells were immediately frozen in liquid nitrogen and stored at -80°C until they were processed for extraction.

Isolation of RNA

Total RNA was extracted from different tissues excised from rice plants and suspension-cultured cells, as described by Prescott and Martin (1987). The RNA was purified by successive precipitation in lithium chloride and ethanol. The purified RNA was treated with DNase (Promega, USA) to remove any contaminating DNA before use in RT-PCR.

Cloning of *OsTERT* cDNA Fragments

Four primer pairs were designed based on the *OsTERT* cDNA sequence in the NCBI databank (accession # AF 288216) (Table 1). Each lower primer was also used in the first single-strand cDNA synthesis. The mRNA was denatured at 65°C for 10 min, and the first cDNA strand was synthesized using the Superscript II preamplification system (GIBCO BRL, USA) at 50°C for 1 h. The PCR parameters were: 94°C for 1 min followed by 35 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 1 min. The first cDNA strand was obtained using primer pair 1 (upper and lower primer 1). The second cDNA fragment was amplified using primer pair 2 with an initial incubation at 95°C for 1 min, followed by 35 cycles of 95°C for 30 sec, 57°C for 30 sec, and 72°C for 90 sec. The third and fourth cDNA fragments were amplified using primer pairs 3 and 4, respectively, with initial incubations at 95°C for 1 min, followed by 35 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 2 min.

The PCR products were analyzed by electrophore-

Table 1. Oligonucleotide primers used in this study.

Pimer	Sequence (5'-3')	Spanning position
Upper 1	CCT CGC CAT CGA CCG TCC ATC CAG GTC A	155-182
Lower 1	CCC CGA TTC TGT GCA AAA GAA TAC TCC	868-894
Upper2	GCC AGG CTC GTT CGG GAG ATG ATG AAG T	735-762
Lower 2	TGA GAT TTT TAA TTG ATT TGG GCA GCC A	1962-1989
Upper 3	TGT CCC CAA GTT GGG CCC TCC AGT T	1659-1683
Lower 3	GTC CAC CAC CGT AAA AAC AAC TAG TCC C	3112-3139
Upper 4	CTT CAG GAT TTT GCG CGG TGA ACC TA	2612-2637
Lower 4	ATG AGG ACC ACA CAC GTT TTG TTT GGC CA	4321-4349
Lower 5	GCT TCG CAA AGG CTA CGG TTG CAT T	3843-3867

Spanning position in the OsTERT cDNA sequence reported in the NCBI data bank (AF288216).

sis on 1.5% agarose gels and cloned into pCR™ II (Invitrogen, USA) as described in the TA Cloning Instruction Manual from Invitrogen, after adding dATP. All four cDNA fragments were obtained and ligated by fusion PCR using a KOD-Plus-PCR Kit (Toyobo, Japan). Quantitative analysis of *OsTERT* mRNA accumulation in the tested cells and tissues was performed using 25 PCR cycles.

Preparation of the cDNA Probe

One μg of total RNA treated with DNase (Promega) was reverse-transcribed with a GeneAmp RNA PCR Kit (Perkin Elmer Japan, Japan). The cDNA fragments were amplified using upper primer 4 (Table 1), based on the amino acid sequence FRILRGEPR, and lower primer 5 (Table 1), based on the amino acid sequence RSVSDANVS. The PCR parameters were 35 cycles of 94°C for 1 min, 58°C for 30 sec, and 72°C for 2 min. The PCR product was electrophoresed on a 1.5% agarose gel and was recovered from the gel with a Mermaid Kit (BIO 101, USA) and cloned into pCR™II as described above. The cloned RT-PCR product was amplified in *Escherichia coli* (INVaF'), cleaved from the pCR™II vector using *Eco*RI, and subjected to agarose gel electrophoresis. Because the cloned insert contained an *Eco*RI cleavage site, the cDNA fragment corresponding to nucleotides 3033-3868 was recovered and labeled with [α-^{32}P]-dCTP by random-priming with a Multiprime™ DNA Labeling System (Amersham Pharmacia Biotech, USA) for use as a probe.

Northern Blot Analysis

Poly(A)RNA was purified from the total RNA obtained from 3-month-old blades of rice plants and from 2-day-old suspension-cultured rice cells with the mRNA Purification Kit (Amersham Pharmacia Biotech, UK). Approximately 4 μg of poly(A)RNA was subjected to electrophoresis in each lane of a 1.2% agarose gel containing 0.66 M formaldehyde. The RNA was then transferred to a GeneScreen Plus membrane (DuPont, USA) by capillary action with 10× SSC as recommended by the manufacturer. After baking at 80°C for 30 min, the membrane was pre-incubated in 1 M NaCl, 1% SDS, 10% (w/v) dextran sulfate (sodium salt) solution at 60°C for 1 h. The denatured [α-^{32}P]-labeled probe and salmon-sperm DNA were then added to the pre-hybridization solution, and the membrane was incubated at 60°C for 18 h. Post-hybridization washes were performed once for 2 min with 2× SSC at room temperature and twice for 15 min with 2× SSC containing 1% SDS at 60°C. The washed membrane was visualized using a Bio-Imaging Analyzer (BAS 5000; Fuji Photo Film, Japan). The membranes were then stripped by boiling in 1× SSC, 0.1% SDS and rehybridized with histone H3 cDNA fragments.

Sequencing Analysis

The cDNA cloned into the pCR™II vector was sequenced with an automated DNA sequencer (model 377; Perkin-Elmer Japan, Japan) by the dideoxy sequencing method (Sanger et al., 1977) using a Taq Dye Primer Cycle Sequencing Kit (Perkin-Elmer Japan). The nucleotide and amino acid sequences were analyzed with GENETYX-MAC software, version 10.1.1 (Software Development, Japan). Databases were searched with NCBI BLAST (National Center for Biotechnology Information; Basic Local Alignment Search Tool System 2.0).

Preparation of cDNA Probes for Detecting OsTERT cDNA Fragments

Quantitative RT-Southern analysis was used to quantitate full-length *OsTERT* mRNA accumulation.

Cloned full-length *OsTERT* cDNAs were digested with *Eco*RI or with *Hin*dIII, and the four resulting DNA fragments were separated by gel electrophoresis and recovered. These fragments were labeled with [α-^{32}P]-dCTP by the random-priming method with a Multiple™ DNA Labeling System (Amersham Pharmacia Biotech).

RT-Southern Analysis of OsTERT mRNA Accumulation

Total RNA was isolated from different tissues of rice plants and from suspension-cultured cells, and each sample was treated with DNase (Promega) to eliminate residual genomic DNA contamination. RT-PCR was performed with a GeneAmp RNA PCR Kit (Perkin Elmer Japan) using 1 μg of total RNA with the PCR parameters described previously. The PCR products were separated by 1.5% agarose gel electrophoresis and transferred onto a GeneScreen Plus membrane (DuPont, USA). After baking at 80°C for 30 min, the membrane was pre-incubated in 1 M NaCl, 1% SDS, 10% (w/v) dextran sulfate (sodium salt) solution at 65°C for 1 h. The denatured [^{32}P]-labeled probe and salmon sperm DNA were then added to the pre-hybridization solution, and the membrane was incubated at 65°C for 18 h. Post-hybridization washes were performed twice for 5 min each with 2× SSC at room temperature and twice for 15 min with 2× SC, 1% SDS at 65°C. The washed membrane was subjected to autoradiography with an intensifying screen.

RT-PCR was also performed using sense (5′-CATGCTATCCCTCGTCTCGACCT-3′) and antisense (5′-TGGCAATCCACATCTGCTGGAAT-3′) primers for the rice actin gene (*RAc1*) (Li et al., 2000) to ensure that equal amounts of mRNA were present in each lane. All experiments were repeated at least three times.

RESULTS

Spliced *OsTERT* mRNA Is Present in Suspension-Cultured Cells and Seedlings of Rice

Four fragments of the full-length *OsTERT* cDNA were cloned from poly(A)RNA with primers based on the *OsTERT* sequence in the NCBI data bank. The predicted lengths of the resulting cDNA fragments 1-4 were 740, 1255, 1481, and 1738 bp, respectively (Fig. 1A). However, amplification of the four cDNA fragments by RT-PCR yielded five fragments that were shorter than expected (Fig. 1B and C). These unexpected fragments were detected at 2, 4, 6, and 8 d in suspension-cultured cells (Fig. 1B). They were also observed in 3-month-old blade tissue and in five seedling tissue types: leaf sheath including apical meristem, leaf sheath including young blades, third blade, root tip, and root without root tips (Fig. 1C). These unexpected five types of cDNA fragments were considered as non-specific bands and were excluded from determining *OsTERT* sequence in the previous study (Chung et al., 2003). However, the result of no correlation with rice telomerase activity and *OsTERT* mRNA accumulation level indicated that these unexpected bands might be involved in the post-transcriptional level regulation including an alternative splicing mechanism in rice (Heller-Uszynska et al., 2002; Chung et al., 2003; Oguchi et al., 2004).

To confirm this presumption, each of the five unexpected cDNA fragments was cloned and sequenced, respectively. The fragments proved to be products of *OsTERT* mRNA splicing such that each fragment lacked some portion of the predicted *OsTERT* sequence. The four splicing sites were at nucleotides 1068-1541, 1542-1685, 1686-1942, and 3638-3906 of the reported *OsTERT* cDNA sequence, and were designated a, b, g, and d, respectively (Fig. 2A and B). These splicing events occurred at the same positions in all tested tissues and suspension-cultured cells (Fig. 1B and C). It was found that this result was very similar to the previous reports (Heller-Uszynska et al., 2002; Oguchi et al., 2004).

Theoretically, at least eight different mRNAs, including full-length *OsTERT* mRNA, could be produced using these splice sites (Fig. 2A). However, when the g or d sites were spliced, frame shifts created TAA stop codons at nucleotides 1979-1981 and 3922-3924, respectively (Fig. 2A). As a result, this splicing mechanism could theoretically produce at least five different proteins, of 1259, 1173, 440, 388, and 230 aa, *via* a frame shift (Fig. 2C).

Northern Blot Analysis of *OsTERT*

To detect variant mRNAs and to determine the size of the full-length *OsTERT* mRNA transcript, Northern blot analysis was performed. Approximately 4 μg of poly(A)RNA was purified from 2-day-old suspension-cultured cells, which have high telomerase activity, and 3-month-old blades, in which telomerase activity is undetectable. The probe contained the conserved sequence motifs B, C, D, and E that were previously used in genomic Southern blot analysis (Chung et al., 2003). Northern blot analysis revealed weak and dif-

fuse bands of 1,600 to 5,000 bp in both samples (Fig. 3A). When the blots were stripped and reprobed with a histone H3 probe as a control, both samples yielded only a single band, as expected (Fig. 3B). These results indicate that several variant *OsTERT* mRNAs are present in rice.

Correlation between Telomerase Activity and the Ratio of Full-Length *OsTERT* mRNA Accumulation

The variant spliced mRNAs detected in the Northern blot analysis indicated that an alternative splicing mechanism might regulate telomerase activity in rice

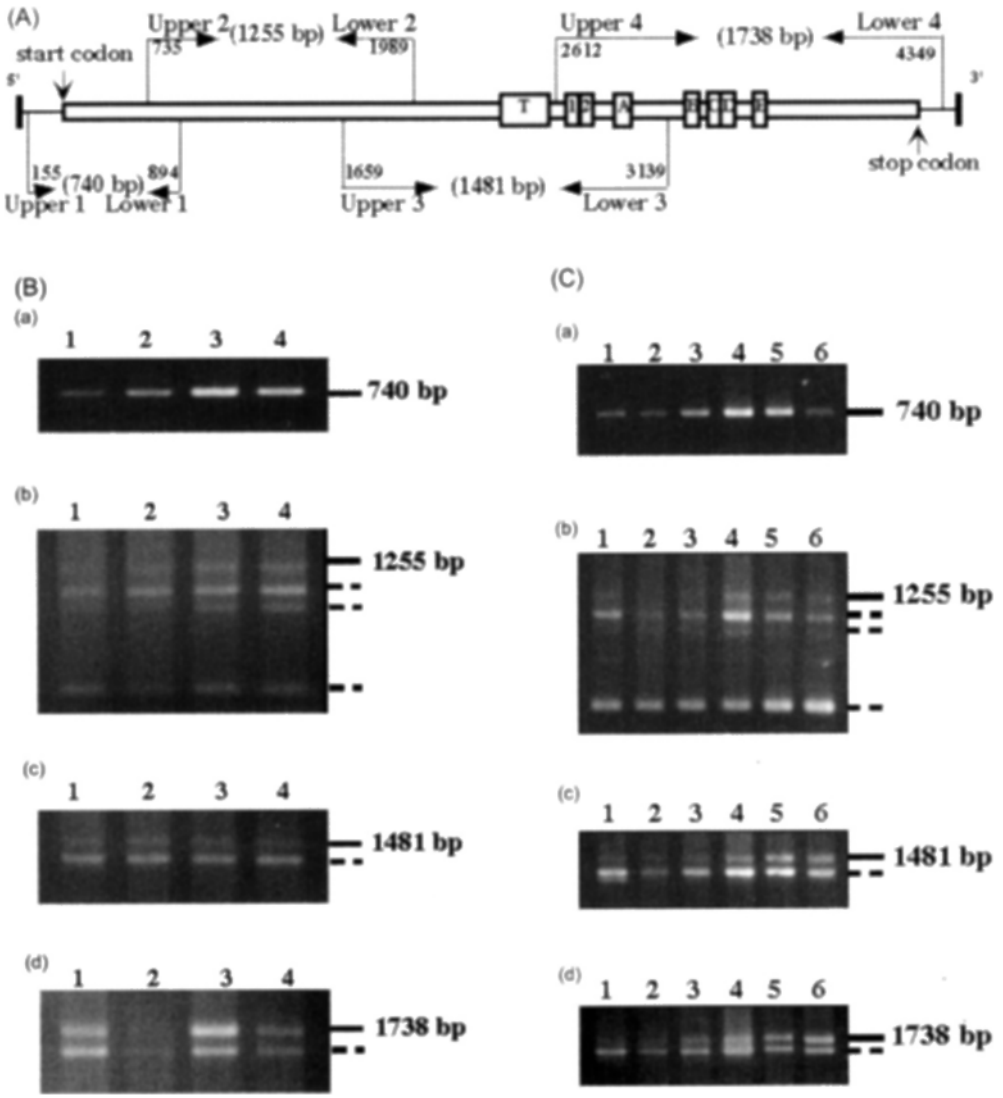

Figure 1. Schematic representation of the *OsTERT* cDNA sequence and RT-PCR-amplified *OsTERT* bands. **(A)** Schematic representation of the *OsTERT* cDNA sequence. The conserved motifs typical of reverse transcriptases are shown as boxes 1, 2, A, B, C, D and E. The T motif is an additional motif conserved in telomerase reverse transcriptases. *OsTERT* cDNA fragments were obtained by RT-PCR using four primer pairs designed using the NCBI databank *OsTERT* sequence (AF288216). The names and spanning positions of the primer pairs are indicated. The expected lengths of the *OsTERT* cDNA fragments were 740, 1255, 1481, and 1738 bp, respectively. The sequences of the primer pairs are shown in Table 1. **(B)** RT-PCR-amplified *OsTERT* bands from 2-, 4-, 6-, and 8-day-old suspension-cultured cells. The expected lengths of the *OsTERT* cDNA fragments obtained with primer pair 1 **(a)**, primer pair 2 **(b)**, primer pair 3 **(c)**, and primer pair 4 **(d)** were 740, 1255, 1481, and 1738 bp, respectively. Unexpected bands are indicated with dotted lines. Lanes 1, 2, 3, and 4: 2-, 4-, 6-, and 8-day-old suspension-cultured cells, respectively. **(C)** RT-PCR-amplified bands from various rice seedling tissues. **(a, b, c, d)** was the same as in Fig. 1(B), respectively. Unexpected bands are indicated with dotted lines. Lane 1, leaf sheath including apical meristem; lane 2, root without root tips; lane 3, leaf sheath including young blade; lane 4, third blade; lane 5, root tip; lane 6, 3-month-old blade.

cells and tissues (Fig. 1). Therefore, we estimated the relative amounts of full-length *OsTERT* mRNA and the five splicing variants by quantitative RT-Southern analysis, using suspension-cultured cells, 3-month-old blades, and five seedling tissue types (Fig. 4). When the proportion of *OsTERT* full-length mRNA accumulation was compared to telomerase activity in these tissues at 2 d and 6 d, no correlative relationship was found (Fig. 5A). Rice seedling leaf sheath including apical meristem had the highest telomerase activity observed for all the tested tissues, whereas 3-month-old blade had no detectable telomerase activity, but their levels of full-length *OsTERT* mRNA accumulation were not significantly different (Fig. 5B). Previous studies in rice have shown that active telomerase can be expressed only from the full-length *OsTERT*

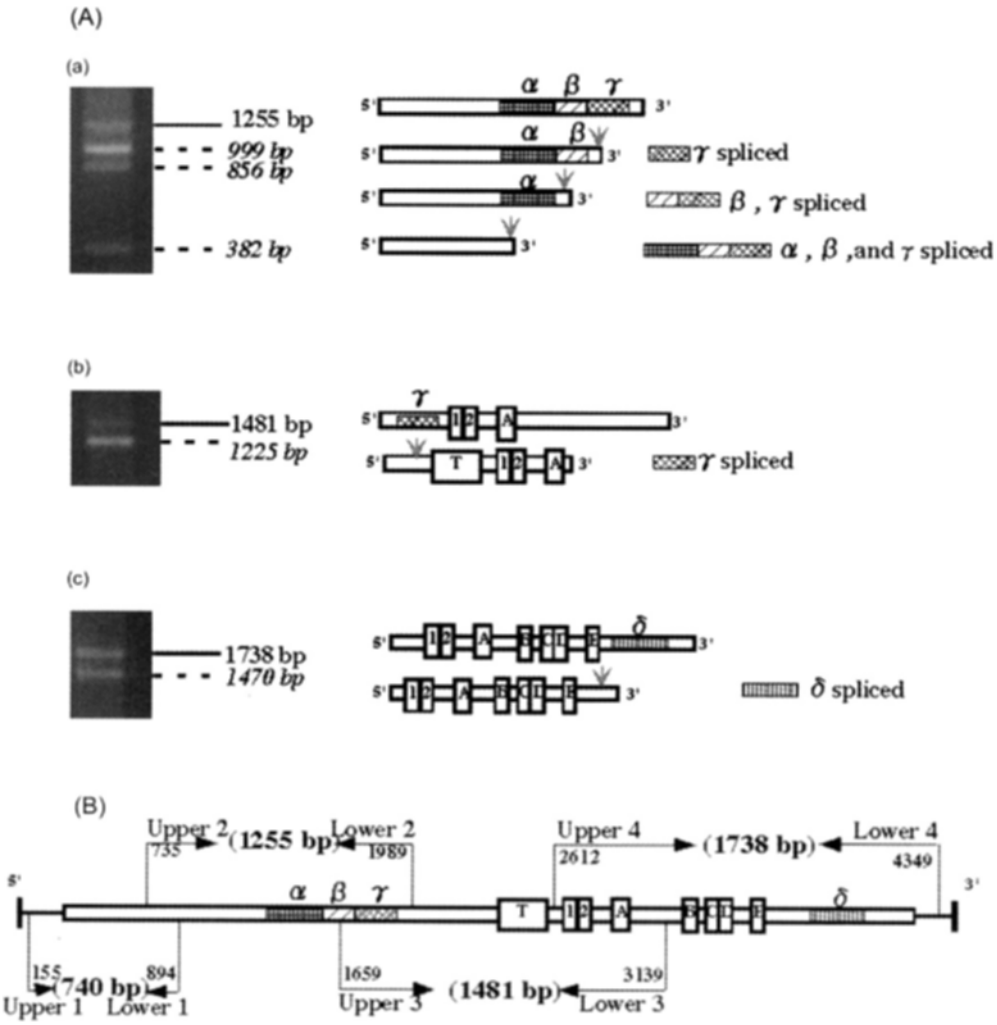

Figure 2. Location of splice sites in the *OsTERT* cDNA sequence and five theoretical OsTERT isoforms. **(A)** The expected length of the *OsTERT* fragment obtained using RT-PCR. Sequence analysis led to the identification of four splice sites, which were designated as a, b, g, and d. The unexpected fragments are indicated with dotted lines. Their lengths were determined by sequence analysis. **(a)** The expected length of the *OsTERT* fragment obtained using RT-PCR with primer pair 2 was 1255 bp. The lengths of the unexpected fragments were 999, 856, and 382 bp, respectively, as shown by sequence analysis. **(b)** The expected length of the *OsTERT* fragment obtained using RT-PCR with primer pair 3 was 1481 bp. The length of the unexpected band was 1225 bp. **(c)** The expected length of the *OsTERT* fragment obtained using RT-PCR with primer pair 4 was 1738 bp. The length of the unexpected band was 1470 bp. The arrows in (**a**), (**b**), and (**c**) indicate the premature stop codons introduced by the splicing events. **(B)** The four spliced sites were determined based on the reported *OsTERT* cDNA sequence. Sequence analysis placed the locations of the splice sites designated as α, β, γ, and δ at nucleotides 473, 143, 256, and 268, respectively, of the *OsTERT* cDNA sequence. **(C)** Five theoretical OsTERT isoforms. Sequence analysis of rice seedlings and suspension-cultured rice cells indicated the presence of an early stop codon in the *OsTERT* splicing products. Five theoretical isoform proteins of 1259, 1173, 440, 388, and 230 aa were encoded by the eight *OsTERT* mRNA variants shown in (**A**).

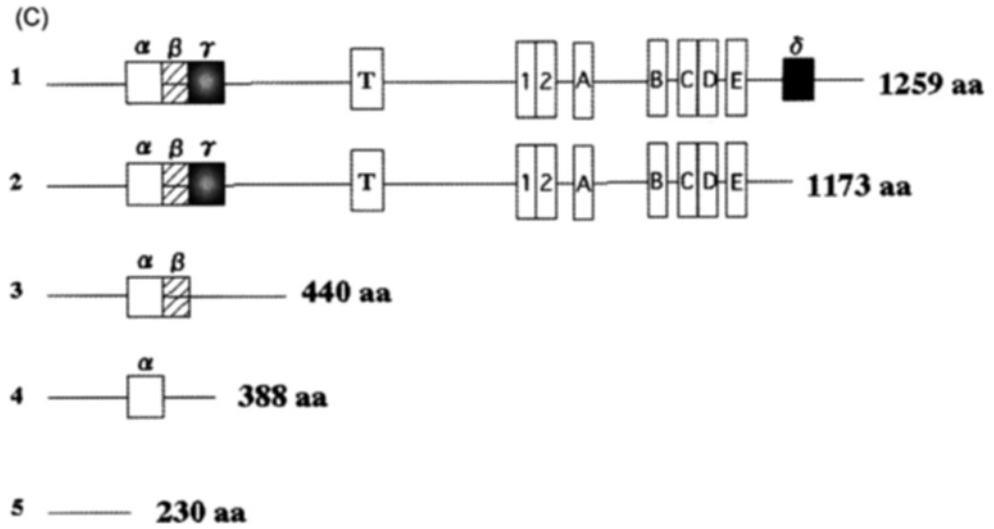

Figure 2. Continued.

mRNA. However, our results show that the level of full-length mRNA transcript accumulation is not the limiting factor regulating telomerase activity, suggesting that the variant *OsTERT* mRNA transcripts may play an important role in regulation.

DISCUSSION

We previously reported that the level of telomerase activity in rice tissues does not correlate with the level of *OsTERT* mRNA accumulation. This result led us to suggest that, in rice, telomerase activity is regulated at the post-transcriptional level (Heller-Uszynska et al., 2002; Chung et al., 2003; Oguchi et al., 2004). In the present study, we explored this possibility by examining the evidence for an alternative splicing mechanism such as that reported in human cancer cells.

RT-PCR was used to amplify four *OsTERT* cDNA fragments (Fig. 1, 2). In all the tested cells and tissues, this amplification yielded five fragments that were shorter than the expected products (Fig. 1 and 2). Sequence analysis showed that these fragments were spliced *OsTERT* mRNA fragments and identified four splice sites in the *OsTERT* cDNA sequence (Fig. 2A and B). These splicing events occurred at very specific positions in the *OsTERT* cDNA sequence and were nonrandom in all the tested cells and tissues. Theoretically, the frame shifts caused by these post-transcriptional splicing events could lead to five different OsTERT isoform proteins (Fig. 2C). In addition, Northern blot analysis suggested that more than one *OsTERT* mRNA transcript was present (Fig. 3), although *OsTERT* exists as a single copy in the rice genome

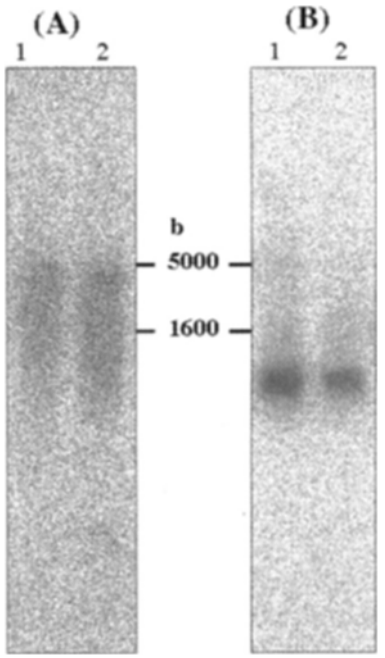

Figure 3. Northern blot analysis of *OsTERT*. To detect variant *OsTERT* mRNAs, Northern blot analysis was performed with purified polyadenylated mRNA from 2-day-old suspension cultured cells (lane 1) and 3-month-old blades (lane 2). **(A)** An *OsTERT* fragment was labeled with [α-^{32}P]-dCTP and used as a probe. **(B)** The membrane shown in (A) was stripped and reprobed with labeled histone H3 cDNA fragment as a control.

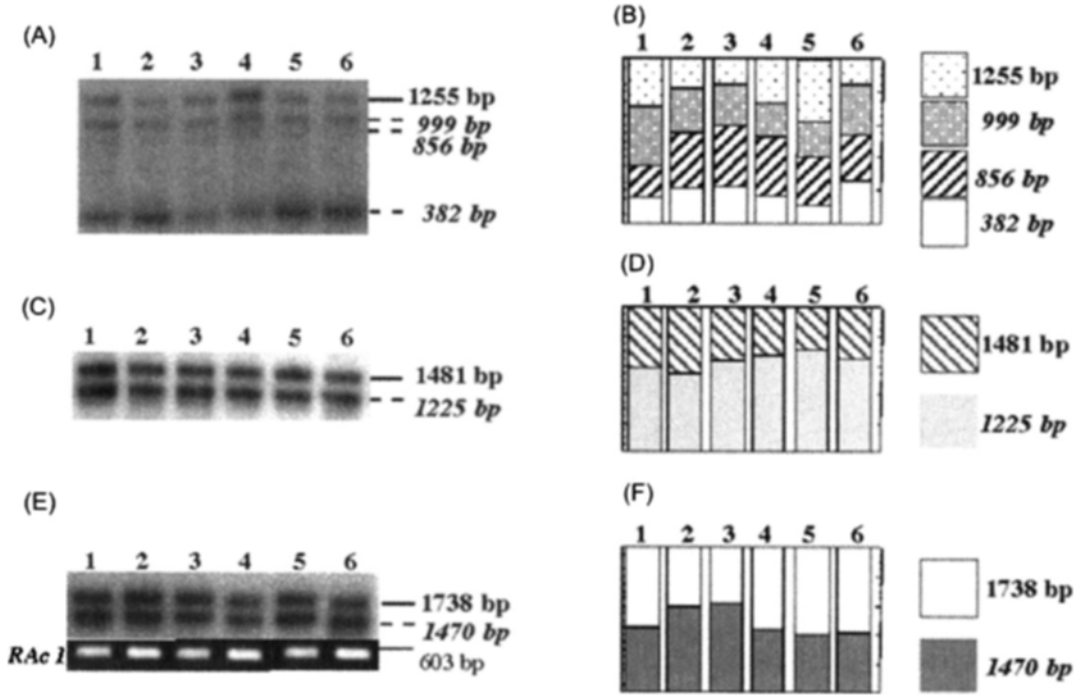

Figure 4. *OsTERT* mRNA levels in suspension-cultured rice cells and in various rice seedling tissues. Levels of *OsTERT* mRNA were determined by quantitative radiometric RT-Southern analysis. **(A)** Four bands were detected spanning nucleotides 735-1989 of the *OsTERT* cDNA. **(C)** Two bands were detected spanning nucleotides 735-1989 of the *OsTERT* cDNA. **(E)** Two bands were detected spanning nucleotides 1659-3139 of the *OsTERT* cDNA. **(B, D, and F)** Typical results of micro-electronic quantitation of the intensity of the ladder bands performed using a BAS5000 Bio-Imaging Analyzer system. The data are the means ± SD of results obtained from three independent experiments. Lane 1, 2-day-old suspension-cultured cells; lane 2, 4-day-old suspension-cultured cells; lane 3, 6-day-old suspension-cultured cells; lane 4, leaf sheath including apical meristem; lane 5, third blade; lane 6, 3-month-old blade.

(Chung et al., 2003).

The above results suggested that the relative amounts of full-length and alternatively spliced *OsTERT* transcripts might be an important factor in regulating telomerase activity in plants. In a previous study, the presence or absence of the full-length *AtTERT* transcript coincided with the presence or absence of telomerase activity in suspension-cultured cells and seedling tissues of *A. thaliana* (Oguchi et al., 1999). However, when we compared levels of telomerase activity and accumulation of full-length *vs.* spliced *OsTERT* mRNAs in rice cells and tissues, no correlative relationship was evident (Fig. 5). In fact, a significant amount of full-length *OsTERT* mRNA was present in 3-month-old blade and in seedling root without root tips despite the absence of detectable telomerase activity. Alternatively spliced *OsTERT* mRNAs were also observed in rice seedling leaf sheath including apical meristem tissue, which exhibits a very high level of telomerase activity (Fig. 1, 5) (Chung et al., 2003). These results suggest that the presence of the full-length *OsTERT* mRNA transcript is insufficient for telomerase activation in rice cells and tissues. Hence, transcriptional regulation does not appear to be the primary regulating mechanism in the synthesis and/or activation of rice telomerase. Instead, a post-transcriptional alternative splicing mechanism may be involved in regulation of telomerase activity in rice.

Alternative mRNA splicing is a common mechanism for increasing the flexibility and complexity of eukaryotic gene expression by the generation of structurally distinct isoenzymes from a single gene (Adams et al., 1996; Yi et al., 2000). For a majority of genes, splicing patterns vary depending on the tissue or the developmental or physiological state of the cell (Woodley and Valcarcel, 2002). Alternative splicing frequently separates sequence elements required for dimerization, cooperativity, transcriptional activation, subcellular localization, or ligand binding, and thus allows them to be substituted, deleted, or modified independently (Modrek and Lee, 2002).

In human cell lines, *hTERT* variants arising from

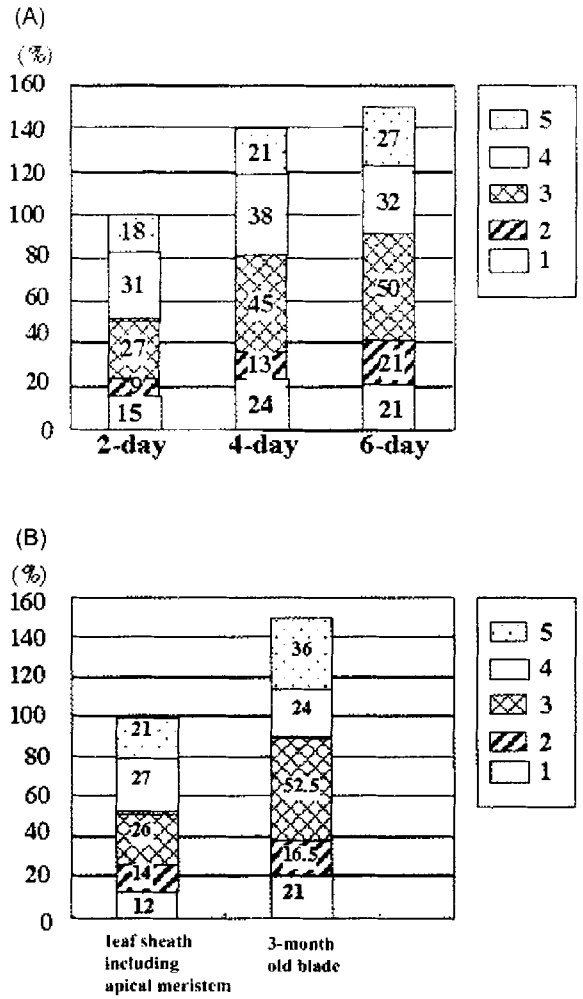

Figure 5. Relative quantities of full-length *vs.* spliced *OsTERT* mRNA in suspension-cultured rice cells and tissues of rice seedlings. The ratio of full-length *OsTERT* mRNA to the five theoretical *OsTERT* mRNA variants was estimated by quantitative RT-southern analysis. **(A)** Two-day-, 4-day-, and 6-day-old suspension-cultured cells. **(B)** Leaf sheath including apical meristem and 3-month-old blade. Telomerase activity levels in these cells and tissues were reported previously (Chung et al, 2003).

alternative splicing may act as endogenous, dominant negative regulators of telomerase activity, perhaps by competing for activating element(s) (Yi et al., 2000; Mergny et al., 2002). Differences in expression of various *hTERT* transcripts have been observed in various human tissues during embryonal development as well as in various normal adult tissues and tumors (Kilian et al., 1997; Ulaner et al., 1998). These findings demonstrate that small changes in amounts or ratios of alternatively spliced products can lead to dramatic changes in cellular metabolism or physiology. The existence of alternative splice sites in the *OsTERT* sequence and the lack of correlation between levels of telomerase activity and full-length *OsTERT* mRNA accumulation suggest that regulation of telomerase activity in rice occurs via a mechanism that is more similar to that of the human, rather than that of the *A. thaliana*, telomerase. Such a mechanism could be one reason that no correlations between levels of telomerase activity and *OsTERT* mRNA accumulation were observed in suspension-cultured cells, 3-month-old blade tissue, or root tissue without root tips from 3-week-old seedlings in the previous study (Chung et al., 2003).

The function of alternative OsTERT splicing products is unclear, although they may encode OsTERT proteins lacking reverse transcriptase activity. In this study, splice sites were concentrated in the N-terminal region of OsTERT (Fig. 2C). In human and yeast telomerases, the N-terminal region is important in binding telomerase-associated proteins and activating elements (Colgin et al., 2000; Klapper et al., 2001; Moriarty et al., 2004) and in ribonucleoprotein formation and oligomerization (Beattie et al., 2001; Moriarty et al., 2002, 2004), leading to the suggestion that the N-terminal region acts in a regulatory capacity (Tesmer et al., 1999; Xia et al., 2000; Beattie et al., 2001). Researchers studying human telomerase have suggested models whereby a functional telomerase complex can be blocked by inhibitory factors (van Steensel and de Lange, 1997; Krauskopf and Blackburn, 1998), but, to our knowledge, no studies examining *in vivo* inhibition of telomerase activity in plants have been reported.

Further examination of the relative accumulation of spliced *OsTERT* transcripts in suspension-cultured cells and/or various tissues of rice seedlings using a more precisely quantitative technique such as real-time PCR (Kotoula et al., 2004) will facilitate our understanding of the role of alternative splicing in regulation of OsTERT activity. Post-translational events including phosphorylation of TERT in plants (Yang et al., 2002; Oguchi et al., 2004) as well as in human (Mergny et al., 2002) could be an important regulation step. However, it has not been identified whether kinase phosphorylates TERT in plants. Other factor, for example, TELOMERSE ACTIVATOR 1 (TAC1) of *A. thaliana* (Ren et al., 2004) and a telomere binding protein NgTRF1 of tobacco (Yang et al., 2004) may also be an important element positively and negatively, respectively, in regulating telomerase activity. However, such a factor has not been reported in rice yet.

Whether alternative transcripts are translated, and whether these putative translation products can asso-

ciate with other components of the telomerase complex is unknown in rice. Alternatively spliced *OsTERT* mRNAs and/or isoform proteins may differ in their abilities to generate active telomerase complexes in rice cells and tissues. The existence of the theoretical isoform proteins or additional interacting proteins regulating telomerase activity remains to be addressed.

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