Sequence-specific DNA Recognition by the Myb-like Domain of Plant Telomeric Protein RTBP1*

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We have identified a rice gene encoding a DNA-binding protein that specifically recognizes the telomeric repeat sequence TTAGGG found in plants. This gene, which we refer to as RTBP1 (rice telomere-binding protein 1), encodes a polypeptide with a predicted molecular mass of 70 kDa. RTBP1 is ubiquitously expressed in various organs and binds DNA with two or more duplex TTAGGG repeats. The predicted protein sequence includes a single domain at the C terminus with extensive homology to Myb-like DNA binding motif. The Myb-like domain of RTBP1 is very closely related to that of other telomere-binding proteins, including TRF1, TRF2, Taz1p, and Tb1p, indicating that DNA-binding domains of telomere-binding proteins are well conserved among evolutionarily distant species. To obtain precise information on the sequence of the DNA binding site recognized by RTBP1, we analyzed the sequence-specific binding properties of the isolated Myb-like domain of RTBP1. The isolated Myb-like domain was capable of sequence-specific DNA binding as a homodimer. Gel retardation analysis with a series of mutated telomere probes revealed that the internal GGGTTT sequence in the two-telomere repeats is critical for binding of Myb-like domain of RTBP1, which is consistent with the model of the TRF1-DNA complex showing that base-specific contacts are made within the sequence GGGTTA. To the best of our knowledge, RTBP1 is the first cloned gene in which the product is able to bind double-stranded telomeric DNA in plants. Because the Myb-like domain appears to be a significant motif for a large class of proteins that bind the duplex telomeric DNA, RTBP1 may play important roles in plant telomere function in vivo.

Telomeres, the specialized nucleoprotein complexes at the ends of linear eukaryotic chromosomes, are essential for the maintenance of chromosome integrity and for protection from exonucleolytic degradation or fusion with other chromosome ends (1, 2). Telomeres in most eukaryotes are composed of tandem repeats of short sequence elements, typically 5 to 8 base pairs in length (3). These repeated sequences are usually rich in G residues on the strand oriented in the 5' to 3' direction toward the end of the chromosome. The G-rich strand is extended beyond the complementary C-rich strand and terminates as a single-stranded 3' overhang in several evolutionarily divergent organisms (4, 5). Recently, it has been shown that the long stretch of mammalian double-stranded telomere DNA bends back on itself, forming a large telomere loop (t-loop), and the 3' G-rich single-stranded overhang at the end of the t-loop invades the double-stranded telomere and produces a displacement loop (d-loop) (6, 7). These t-loops are proposed to mask telomere termini from cellular activities that can act on DNA ends.

The integrity and proper functioning of telomeres seem to be achieved via associations between the telomere repeat sequences and specific binding proteins. Studies of telomere chromatin structure have suggested that telomeres may be packaged into specialized nucleoprotein complexes. Some proteins specifically interact with the single-stranded 3' extension at the extreme termini, which are essential for chromosome capping and telomerase regulation (8–10). The other group of telomere-binding proteins binds specifically to the double-stranded telomeric repeats. The most well characterized proteins include Rap1p, identified from Sacccharomyces cerevisiae. Rap1p, in addition to binding to telomeric repeat sequences and performing important functions in telomere length maintenance (11, 12), is an abundant nuclear protein needed for the expression of a variety of genes and implicated in the establishment of silent transcriptional domains. Taz1p in Schizosaccharomyces pombe, found in a one-hybrid screen using S. pombe telomeric DNA as a target (13), is involved in telomere length regulation, repression of telomere adjacent genes, and the interactions between telomeres and the spindle pole body during the meiotic prophase (14, 15). In mammalian cells, two double-stranded telomere-binding proteins have been identified (16). The binding of TRF1 controls telomere length by inhibiting the action of telomerase (17). TRF2 plays a key role in the protection of chromosome ends from end-to-end fusion (18).

Despite the apparently conserved function, telomere-binding proteins show a limited amino acid sequence similarity. However, they share a domain that resembles the DNA binding motif present in the vertebrate e-Myb family of transcriptional activators (19, 20). Interestingly, whereas the DNA binding domain of the Myb proteins typically consists of three tandem repeats of the Myb motif (21), Rap1p contains two Myb-like domains that bind DNA in a tandem orientation (22). In con-
trast, the DNA binding domains of TRF1, TRF2, and Taz1p contain only a single Myb-like domain at their C terminus (16, 23, 24). Recently, it has been shown that the isolated Myb-like domain of TRF1 binds specifically and with significant affinity to telomeric DNA as a monomer (20). Although with less specificity than the full-length dimer, the isolated Myb-like domain of Taz1p was also capable of sequence-specific DNA binding (23, 24). Taken together, these results indicate that the Myb-like domain is indeed responsible for specific telomeric DNA recognition.

Telomere structure in most plants is very similar to that of other eukaryotes. The plant telomeric DNA sequence (TTTAGGG), was first characterized in Arabidopsis thaliana (25) and was subsequently cloned in several different species (26–28). Compared with the extensive research done in other eukaryotes, many fewer studies of plant telomere-binding proteins have been reported to date. Protein binding to double-stranded telomeric DNA has been found in maize and Arabidopsis cellular extracts (29, 30). Previously, we identified and characterized protein factors that specifically bind to the single-stranded G-rich telomeric repeat in rice and mung bean nuclear extracts (31, 32). However, nothing is known about the physiological role of telomere-binding proteins in plants.

We report here the molecular cloning and characterization of a rice gene encoding a double-stranded telomere-binding protein, designated RTBP1.1 The predicted protein sequence includes a single Myb-like domain at the C terminus. The Myb-like domain of RTBP1 is very closely related to other telomeric proteins, indicating that DNA binding domains of telomere-binding proteins are well conserved among evolutionarily distant species. The isolated Myb-like domain of RTBP1 is capable of sequence-specific DNA binding as a homodimer and recognizes a binding site centered on the sequence GGGTTT. To the best of our knowledge, this is the first cloned gene in which the product is able to bind specifically double-stranded telomeric DNA in plants. Because the Myb-like domain appears to be a significant motif for a large class of proteins that bind the duplex telomeric DNA, RTBP1 may play important roles in plant telomere function in vivo.

**EXPERIMENTAL PROCEDURES**

**Screening of a λ Zap II Rice Seedling cDNA Library**—In the BLAST search for proteins homologous to the Myb repeat, several open reading frames containing a single Myb repeat were found (19). This analysis includes orfR1 from a partial cDNA sequence of rice (GenBank accession no. D23805). Two primers (5′-GGAGGCTTTTCTGTTGCT-GAAG-3′ and 5′-CCCTTAAACGACACACATAGG-3′) were designed to clone orfR1 cDNA. After PCR amplification using the DNA of a rice seedling cDNA library, a product of the expected size (459 base pair fragment) was obtained. To clone the full-length cDNA, the λ Zap II library of rice seedling cDNA was screened using the 459-base pair PCR product as a probe. The full-length cDNA insert containing putative RTBP1 was subcloned into the Bluescript SK plasmid to create pBS-RTBP1 by in vivo excision of pBluescript from the Zap II vector (Stratagene).

**Cloning and Expression of Full-length RTBP1 and the Isolated Myb-like Domain**—The full-length RTBP1 cDNA was PCR-amplified from a pBS-RTBP1 using a set of primers incorporating novel BamHI (at the 5′-end of the upstream primer) and HindIII (at the 5′-end of the downstream primer) sites for ease of cloning. The resulting PCR product was cut with BamHI and HindIII and cloned into the corresponding sites in plasmid pGEX-KG (Amersham Pharmacia Biotech) in order to fuse GST and RTBP1 in frame. To obtain the Myb-like domain of RTBP1, residues 446–633 and 506–615 of RTBP1 were PCR-amplified from a pBS-RTBP1. Primers compatible with cloning into the BamHI and HindIII restriction sites of pGEX-KG were used, and the sequence of the resulting plasmids was confirmed by the dyeoxy sequencing method. The fusion protein GST-RTBP1 was expressed in Escherichia coli BL21 cells by adding 0.1 mM isopropyl-1-thio-β-D-galactopyranoside and purified on glutathione-Sepharose 4B (Amersham Pharmacia Biotech). Correct synthesis was checked by loading the cells directly on SDS-polyacrylamide electrophoresis gels and staining with Coomassie Brilliant Blue. GST protein itself was produced from bacteria carrying an empty pGEX-KG vector. The final protein preparation was dialyzed against TNE buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.5 mM EDTA, 10% glycerol), and stored at −80 °C.

**Southern and Northern blot Analyses**—Rice genomic DNA was prepared from leaves as described by Murray and Thompson (33) and digested with various restriction endonucleases. Digested genomic DNA was separated by electrophoresis in an 0.8% agarose gel and blotted onto a Hybond N+ membrane (Amersham Pharmacia Biotech). Probes were labeled with [α-32P]dCTP using a random-primer kit (Amersham Pharmacia Biotech). Blots were hybridized with an internal fragment of RTBP1 cDNA. For Northern blot analysis, total RNA was isolated from roots, coleoptiles, leaves, and stems according to the manufacturer’s instructions (TRIzol reagent, Life Technologies, Inc.). Twenty mg of total RNA was fractionated by 1%, 2.2% formaldehyde gel electrophoresis and blotted onto a Hybond N+ membrane. Blots were hybridized with an internal fragment of RTBP1 cDNA. To check for equal loading, blots were rehybridized with a 0.9-kilobase pair cDNA fragment of 18 S rRNA.

**Gel Retardation Assays**—DNA probes and competitors for gel retardation assays are described in Table I. To reduce nonspecific DNA-protein binding, purified RTBP1 was preincubated with 0.5 μg of poly(di-dC) and 0.5 μg of nonspecific DNA oligonucleotide in 20 μl of a binding buffer (10 mM Tris-HCl, pH 8.6, 1 mM EDTA, 1 mM dithiothreitol, 0.1 mM NaCl, and 5% glycerol) for 10 min on ice. End-labeled DNA probe (0.25 ng) was then added to the reaction mixtures. After incubation for 10 min on ice, the mixtures were loaded on an 8% nondenaturing polyacrylamide gel. Incubation for 10 min at room temperature gave similar gel retardation results. Before loading, gels were prerun at 10 V/cm for 30 min, and electrophoresis was in 0.5% TBE (54 mM Tris borate, pH 8.3, 1 mM EDTA) for 2.5 h after loading the samples. For the competition experiments, varying amounts of cold competitor molecules were preincubated with nuclear extract before the addition of labeled probe. Binding activity was quantified with a Fuji phosphorimager.

**DNase I Footprinting Analysis**—For DNase I footprinting analysis, the two-telomere repeat oligonucleotide RTR-2 was cloned into the end-filled XhoI of pBend4. The resulting plasmid was digested with XhoI and radioactively labeled by filling using [α-32P]dCTPs and Klenow polymerase. To excise the fragment containing the two-telomere repeat, the 3′-end-labeled fragments were then digested with NheI (for end labeling of the G-rich strand) or EcoRV (for end labeling of the C-rich strand) and were then gel-purified. Binding reactions contained 1 ng of end-labeled fragment, specified amounts of the purified RTBP1 (446–633), 0.5 μg of poly(di-dC), and 0.5 μg of nonspecific oligonucleotide in a total volume of 20 μl of binding buffer (10 mM Tris-HCl, pH 8.6, 1 mM EDTA, 1 mM dithiothreitol, 50 mM NaCl, and 5% glycerol) for 10 min on ice. After incubation for 10 min on ice, DNase I digestions were initiated by adding 1 μl of 40 mM CaCl2 and 1 μl of 50 μg/ml DNase I. Reactions were terminated after 60 s by the addition of 2 μl of 0.5 mM EDTA. Samples were ethanol-precipitated, suspended in sequencing gel loading buffer, and then analyzed on an 8% sequencing gel.

**RESULTS**

**Isolation and Sequence Analysis of RTBP1 cDNA**—The computer search for proteins homologous to the Myb-related motif revealed several open reading frames derived from partial cDNA sequences that contain a single Myb repeat (19). This analysis includes five proteins from plants, the maize Shrunken initiator-binding protein IBP1 (34), the parsley BoxP binding factor BPF1 (35), two open reading frames from rice (orfR1 and orfR2), and one open reading frame from A. thaliana (orfA) (19). To characterize a rice protein derived from partial cDNA sequence (orfR1), we isolated the full-length rice cDNA fragment containing RTBP1 using a combination of cDNA reaction and hybridization strategies. The DNA sequence of the RTBP1 cDNA was determined to be 2031 base pair long. Conceptual translation of the full-length RTBP1 cDNA revealed one long open reading frame encoding a protein of 633 amino acids, with a predicted molecular mass of 70 kDa and a pI of 8.91 (Fig. 1A). The C-terminal region of the protein contains a 55-amino acid stretch of homology with the Myb-related motif from the other telomere-binding proteins such as TRF1, Taz1p, and Tbf1p (13, 16, 36) (Fig. 1B). This presumed helix-turn-helix
cDNA were detected on Northern blots of rice poly(A) 
RTBP1 in the rice genome. Transcripts that hybridized to 
poly(A) band of riched RNA and total RNA from various organs of rice plant. A 
stringency hybridization (data not shown). These results sug-
fragment was visible in any of the digests, even with low 
strongly hybridized band in each digest (Fig. 2
sequence identity) (Fig. 1
strong conservation in their C-terminal Myb-like domains (31%
other three sequences are indicated by bold type
above
TRF1, with the percentage of amino acid identity indicated.

RTBP1 was used in a gel retardation assay with a labeled 
test whether RTBP1 binds to telomeric DNA, the full-length 
like domain of RTBP1 is sufficient to confer specific 
interactions with plant double-stranded telomeric DNA.

mRNA. A, Southern blot analysis of RTBP1 in rice genomic DNA. Rice 
genomic DNA was digested with the indicated restriction enzymes, and 
DNA on the Southern blots was hybridized with the labeled RTBP1
cDNA. B, Northern blot analysis of poly(A)'-enriched RNA and total 
RNA from various rice organs. Total proteins were separated by dena-
turing gel electrophoresis, transferred to a nylon membrane, and hy-ridsed with the labeled RTBP1 cDNA.

RTR-4 (see Table I) containing four plant telomeric DNA re-
peats. RTBP1 gave rise to a discrete DNA-protein complex that 
migrated more slowly than the free probe (Fig. 3A, lanes 2 and 
3). Intensities of shifting bands increased upon the addition of 
increasing amounts of RTBP1. Competition binding experi-
ments showed that a 50-fold excess of cold RTR-4 is enough to 
displace the labeled probe (lane 5), whereas the same excess 
molar amounts of unrelated nonspecific cold competitor did not 
compete at all (lane 7), indicating that RTBP1 is a specific 
telomeric DNA-binding protein.

The C-terminal Myb-like domain of RTBP1 shares 31% 
amino acid sequence identity with that of TRF1, and the iso-
lated Myb-like domain of TRF1 binds specifically to telomeric 
DNA (20). This prompted us to determine whether the isolated 
Myb-like domain of RTBP1 is responsible for the DNA binding 
specificity. Thus, we expressed the C-terminal region of RTBP1 
comprising the Myb-like domain between positions 466 and 633 
as a fusion protein with GST. Gel retardation assay revealed a 
RTR-4 probe binding activity in the bacterial extracts of cells 
induced for the expression of GST-RTBP1-(466–633) (Fig. 3B, 
lane 13), whereas no activity was detected in extracts induced 
for GST alone (lane 12). GST-RTBP1-(466–633) was digested 
with thrombin to isolate the C-terminal region containing the 
entire Myb domain, and its DNA binding property was exam-
ined. This fragment of RTBP1 was found to produce three 
specific complexes, and intensities of slower migrating com-
plexes increased upon the addition of increased amounts of 
RTBP1-(466–633) (lanes 2–5). These complexes were competed 
with cold RTR-4 (lanes 6–8) but not with the unrelated 
nonspecific cold competitor (NS, lanes 9–11), similar to full-
length RTBP1. It is worth noting that the band intensities of 
complexes 2 and 3 decreased more readily than that of complex 
1 with increasing amounts of competitor (lanes 6–8), suggest-
ing that RTBP1-(466–633) binds as a homo-multimer to the 
four-telomere repeat site. The faint bands migrating slower 
length RTBP1. It is worth noting that the band intensities of 
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Myb-like Domain of RTBP1 Binds Telomeric Repeat DNA Site as a Homodimer—Because RTBP1-(466–633) produced three specific complexes for binding to RTR-4, the gel retardation analysis was repeated using a shorter DNA site containing one to three copies of the TTTAGGG repeat to determine the minimal number of telomeric repeats for DNA binding. As shown in Fig. 4A, RTBP1-(466–633) formed a single-shifted complex with the two-repeat sequence (lanes 5 and 6) and two shifted complexes with the three-repeat sequence (lanes 8 and 9), whereas a single-telomere repeat did not exhibit any DNA binding activity (lanes 2 and 3). These results suggest that the binding site of the isolated Myb-like domain of RTBP1 is contained within the two-telomere repeat sequence TTTAGGGTTTAGGG.

Although full-length TRF1 and Taz1p bind to DNA as a preformed homodimer, the isolated Myb-like domains of these proteins were capable of sequence specific binding as a monomer (20, 23). To examine whether the isolated Myb-like domain was active as a monomer, RTBP1-(506–615) was fused to the 28-kDa glutathione S-transferase (GST) protein. Like RTBP1-(466–633), RTBP1-(506–615) contains entire Myb-like domain and was found to produce a single complex with the RTR-2 probe (see below, Fig. 4B). Gel retardation analysis was carried out using GST-RTBP1-(506–615) and RTBP1-(506–615). The two different length proteins were incubated at various concentrations with a DNA binding site containing two copies of the telomeric repeat. Each protein binds to the two-repeat sequences and forms a single complex in which migration is dependent on the size of the protein used (Fig. 4B). When equimolar amounts of the two proteins were used in the incubations (lane 5), a new protein-DNA complex that migrated to an intermediate position was observed. This complex likely corresponds to the binding of one molecule of each length of protein, confirming that two molecules of the Myb-like domain can bind to the two-repeat sequence.

Internal GGGTTT Sequence in the Two-telomere Repeats Is Critical for Binding of the Myb-like Domain of RTBP1—To localize the positions within the telomeric sequence at which DNA-protein interactions occurred, Dnase I footprinting was performed. Uniquely end-labeled fragments containing the two-telomere repeat sequence were incubated with the RTBP1-(466–633) and subsequently digested with limited amounts of Dnase I. The Dnase I footprinting on the G-rich strand revealed that the addition of RTBP1 resulted in protection of the predicted sites in the probe (Fig. 5A). The same result was obtained with the opposite, C-rich strand (Fig. 5B). To further evaluate the sequence specificity of the RTBP1 binding activity at nucleotide level, a series of mutant oligonucleotides were synthesized and assayed for their ability to bind the isolated Myb-like domain of RTBP1. Each double-stranded oligonucleotide contained a single nucleotide transition in the two-telomere repeats (Fig. 6A). Gel retardation assays revealed that nucleoprotein complex was formed between RTBP1-(466–633) and RTR-2, and with M1, M2, M3, M11, M12, M13, and M14, whereas M4 probe was a slightly weaker binder.
ever, M5, M6, M7, M8, M9, and M10 probes were incapable of significant binding (Fig. 6B). The amount of gel shift activity in the complexes was quantified for each probe. The relative amount of shifted complexes was expressed as the ratio of the amount of probe DNA in the complex to the amount of total probe DNA in each reaction (Fig. 6C). These results indicate that the internal GGGTTT sequence in the two-telomere repeats is critical for binding of Myb-like domain of RTBP1.

**DISCUSSION**

In all eukaryotic organisms, the telomere is a well conserved structure that consists of telomeric repeats and telomere binding factors. In addition to the telomeric DNA sequences, a number of proteins play integral roles in telomere structure and function. Therefore, the identification and characterization of the proteins present at the ends of chromosomes will facilitate our understanding about the functions of telomeres. In this report, we describe the molecular cloning and characterization of a gene (*RTBP1*) encoding a rice protein that binds the telomeric repeat sequence found in plants. Despite the appar-

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**Fig. 4.** Gel retardation assay of the Myb-like domain of RTBP1 to the two- and three-telomere repeats. A, gel retardation assays were performed with labeled RTR-1 (lanes 1–3), RTR-2 (lanes 4–6), or RTR-3 (lanes 7–9). Each set of lanes contained 0, 0.2, and 0.4 μg of RTBP1-(466–633). B, two different length proteins, GST-RTBP1-(506–615) and RTBP1-(506–615), were used for binding to labeled RTR-2. The two proteins were mixed in different molar ratios before addition of DNA. The ratios of GST-RTBP1-(506–615) and RTBP1-(506–615) are 0:1, 0.1:0.9, 0.4:0.6, 0.5:0.5, and 1:0. In the schematic representation, the DNA is represented by a line, RTBP1-(506–615) by a black circle, and GST-RTBP1-(506–615) by a gray circle.

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**Fig. 5.** DNase I footprinting of the Myb-like domain of RTBP1 on the two-repeat DNA site. A, DNase I footprint experiments were performed with a XhoI-NheI fragment (G-rich strand) of pBend4 containing the two-telomere repeats. Binding reactions were carried out as described under "Experimental Procedures" with indicated amounts of RTBP1-(466–633). The first two lanes are G+A and C+T chemical sequencing markers. The two-telomere repeats are indicated by vertical brackets on the left. B, the same experiments were performed except that the opposite C-rich strand was analyzed using a XhoI-EcoRV fragment.
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A Mutated gel-shift probes

B Gel retardation assay of the Myb-like domain of RTBP1 to the mutated two-telomere repeats. A, telomeric sequences of mutant repeats used in the experiments. Each double-stranded probe contained a single nucleotide transition in the two-telomere repeats as indicated. B, gel retardation assays were carried out using 0.5 μg of RTBP1-(466–633) and labeled RTR-2 or mutated probes as indicated above each lane. C, the amount of gel shift activity in the complex was quantified for each probe. The relative amount of shifted complex was expressed as the ratios of the amount of probe DNA in the complex to the amount of total probe DNA in each reaction.

Fig. 6. Gel retardation assay of the Myb-like domain of RTBP1 to the mutated two-telomere repeats. A, telomeric sequences of mutant repeats used in the experiments. Each double-stranded probe contained a single nucleotide transition in the two-telomere repeats as indicated. B, gel retardation assays were carried out using 0.5 μg of RTBP1-(466–633) and labeled RTR-2 or mutated probes as indicated above each lane. C, the amount of gel shift activity in the complex was quantified for each probe. The relative amount of shifted complex was expressed as the ratios of the amount of probe DNA in the complex to the amount of total probe DNA in each reaction.

ently conserved function, several telomeric repeat-binding proteins share a limited amino acid sequence similarity. A sequence of ~60 amino acids located in their C termini appears to be critical for DNA binding and exhibits extensive homologies with Myb repeats. An anonymous cDNA sequence encoding a Myb-like motif was reported in the data bases (19). This sequence information was used to isolate the full-length cDNA encoding the rice telomeric protein. The predicted amino acid sequence of RTBP1 includes a single Myb-like domain at its C terminus, which is very closely related to those of other telomeric proteins.

Gel retardation analysis using the full-length RTBP1 and RTR-4 probe gave rise to a single DNA-protein complex, whereas the isolated Myb-like domain was found to produce three specific complexes. These results suggest that the full-length RTBP1 forms higher aggregates, which would lead to strongly cooperative binding. Using gel retardation analysis and DNase I footprinting experiments, we show that the isolated Myb-like domain of RTBP1 binds specifically to the plant telomeric DNA sequences. Telomere binding activity decreased about 9-fold upon changing the plant telomere sequence to that found in human telomere. However, RTBP1 does not exhibit significant binding activity to the C. elegans telomere sequence, indicating that its binding activity is specific to the plant telomere. Although TRF1 and Taz1p specifically bind the G-rich single strand of telomeric DNA with a much lower affinity than the corresponding duplex telomeric DNA (24), RTBP1 did not bind to either single-stranded G-rich or C-rich telomeric DNA, indicating that RTBP1 binding activity is specific to the double-stranded telomeric sequence. Some telomere-binding proteins are resistant to high salt concentrations (e.g., 2 M NaCl or 6 M CaCl2) (8, 37, 38), whereas other proteins are salt-sensitive (39). When the isolated Myb-like domain of RTBP1 was incubated with the RTR-4 probe in the presence of increasing amounts of NaCl concentration, the binding activity gradually decreased upon the addition of increasing salt concentrations, indicating that the binding appears to be salt-sensitive (data not shown).

Gel retardation analysis with a series of mutant oligonucleotides revealed that the internal GGGTTT sequence in the two-telomere repeats is critical for the binding of the isolated Myb-like domain of RTBP1. This result is consistent with the model of the TRF1-DNA complex, showing that base-specific contacts are made within the sequence GGGTTA (20). Their DNA binding sequences are also similar to (G)GGGTGT sequence recognized by the homeodomain-like motifs of the yeast telomere-binding protein RAP1 (40). However, one of the most striking differences of the DNA binding mode between RTBP1 and TRF1 is that the isolated Myb-like domain of RTBP1 binds as a homodimer to the two-telomere repeat site. Although full-length TRF1 binds to DNA as a preformed homodimer, using a large conserved domain near the N terminus, and both Myb-like domains are required for high affinity binding (41), the isolated Myb-like domain of TRF1 binds as a monomer to the two-repeat site (20, 42). This suggests that the DNA binding mode of RTBP1 may be different from that of TRF1.

Plant proteins that specifically bind the duplex TTTAGGG repeat sequences were identified in nuclear extracts of maize and Arabidopsis (29). Computer searches in sequence data bases revealed that two plant proteins have a single Myb-like domain at their C termini and bind a specific DNA sequence related to telomeric repeats (19). The maize initiator-binding protein (IBP1) interacts at the transcription start site of the Shrunken promoter containing an exact plant telomeric repeat AGGGTTT (34), and the parsley BoxP-binding factor (BPF1) binds a series of GT-rich motifs (35). These results suggest that, in addition to their function in transcriptional regulation, these proteins may have a functional role in plant telomeres. Because yeast Rap1p functions both as a structural component of yeast telomeres and a transcriptional regulator (11, 12), the presence of Myb-like domain and the involvement of transcriptional regulation may represent universal characteristics of telomere-binding proteins. This idea is further supported by the fact that a S. pombe telomeric protein, Tel1p, may function as a general transcription factor (24). An analysis of Arabidopsis DNA sequences available in data bases revealed that the sequence AAACCCCTAA, corresponding to 1.3 units of the plant telomeric repeat AAACCC, is preferentially located in the 5' region of the genes (29). Therefore, it is of interest to investigate whether RTBP1 plays a role in transcriptional regulation in addition to binding to telomeric repeat sequences.

The critical question that remains to be answered is whether RTBP1 binds plant telomere in vivo. The homology displayed by RTBP1 to the Myb-like domain of other telomeric proteins suggests that these proteins are functionally related. Proteins
that bind the double-stranded telomeric DNA sequence have been shown to regulate telomere length negatively. Such proteins include Rap1p in budding yeast (43, 44), Tax1p in fission yeast (13), and TRF1 in mammalian cells (16, 45). A second mammalian telomeric protein, TRF2, plays a key role in the protection of chromosome ends from end-to-end fusion (18). Recently, it has been reported that plant cell nuclei contain telomeric proteins that can inhibit telomerase activity by altering the accessibility of telomeric DNA and may thus participate in telomere length regulation (46). Given the evolutionary conservation of telomere sequences and functions, telomeric proteins would also be conserved. Similarly, the ability of RTBP1 to bind specifically the double-stranded plant telomeric repeat sequences in vitro suggests that it may play a role in telomere functions in vivo. Further studies will be required to determine the actual function and physiological relevance of RTBP1 in plant cells.

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