Biochemical and Biophysical Research Communications 372 (2008) 85–90

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Identification and characterization of three telomere repeat-binding factors in rice

Mi Young Byun, Jong-Pil Hong, Woo Taek Kim *

*Department of Biology, College of Life Science and Biotechnology, Yonsei University, Seoul 120-749, Republic of Korea*

ARTICLE INFO

*Article history:*
Received 29 April 2008
Available online 12 May 2008

*Keywords:*
Telomere
Telomere-binding proteins
Myb DNA-binding domain
Single Myb histone protein
Dissociation constant
Rice (*Oryza sativa* L.)

ABSTRACT

Telomeres consist of nucleoprotein complexes that protect chromosome end structures. Here, we describe three *OsTRBF* genes, encoding telomere repeat-binding factors of the single Myb histone family in rice. The predicted proteins contain a Myb DNA-binding motif and a linker histone H1/H5 domain in the N-terminal and central regions, respectively. The *OsTRBF* transcripts were constitutively detected in rice plants grown under greenhouse conditions. Gel retardation assays showed that these OsTRBF proteins bind specifically to the plant double-stranded telomeric sequence, TTTAGGG, with markedly different binding affinities as judged by their respective dissociation constants. Yeast two-hybrid and *in vitro* pull-down assays indicated that both OsTRBF1 and OsTRBF2 interact with one another to form homo- and hetero-complexes, while OsTRBF3 appeared to act as a monomer. Our results suggest that OsTRBFs play combinatory roles in the function and structure of telomeres in rice.



Telomeres, the extreme ends of linear chromosomes, consist of long stretches of G-rich, tandem DNA repeats [1,2]. Telomeres form the specialized nuclear protein complexes in which diverse proteins associate with the telomeric repeat sequences. The non-histone double-stranded telomere-binding proteins (DS-TBPs), such as human TRF1/PIN2 and TRF2 and yeast Rap1 and Taz1, are required for the structure and function of telomeres [3,4]. The human telomere complex is composed of six proteins [5]: three subunits (TRF1, TRF2, and POT1) directly interact with TTAGGG telomere repeats, and three additional subunits (TIN2, TPP1, and Rap1) associate with the complex. This human telomere–protein complex, shelterin, contains DNA remodeling activity and functions to change the telomeric DNA structure, thereby protecting the ends of the chromosomes [5].

DS-TBPs can be divided into two classes according to their structural properties [6,7]. Members of the first group are typified by a single Myb-like DNA-binding domain in the C-terminal region, displaying sequence identity to those proteins of human and yeast. In *Arabidopsis*, there are at least 12 TRF-like proteins, six of which have been shown to bind DS telomeres *in vitro* [8]. Possible *in vivo* functions of DS-TBP NgTRF1 were demonstrated in tobacco BY-2 suspension cells. Both *35S:NgTRF1* and *35S:anti-NgTRF1* transgenic BY-2 cells exhibited marked changes in telomere length and cell viability, suggesting that NgTRF1 is involved in the control of telomere length and stability in tobacco cultured cells [9]. In rice, suppression of *RTBP1* (*RICE TELOMERE BINDING PROTEIN1*) resulted in severe and gradual developmental defects accompanied by genomic instability during four consecutive generations (G1–G4) [10]. These results also indicated that RTBP1 plays an important role in the proper architecture of telomeres.

The second structural class comprises the single Myb histone (SMH) proteins, which have two unique domains, a Myb domain and a linker histone H1/H5 motif in their N-terminal and central regions, respectively. In maize, five *SMH* genes were identified; one of these genes, *Smh1*, encodes a protein that binds to DS telomeric repeats *in vitro* [11]. The *Arabidopsis* genome contains at least six homologous *SMH* genes [12]. AtTRB2 and AtTRB3 both bind to duplex telomeric DNA *in vitro*, forming homo- and heterodimeric complexes with one another and with AtTRB1 [13,14]. Intriguingly, AtTRB1 interacted with AtPOT1, a single-stranded TBP in *Arabidopsis* [14]. These results raise the possibility that SMH DS-TBPs participate in regulating the proper structure and function of telomeres.

As nothing is known about the *in vivo* functions of SMH DS-TBPs, here we identified three *OsTRBF* (*Oryza sativa TELOMERE REPEAT BINDING FACTOR*) genes that belong to the SMH-type DS-TBP homolog in rice. RNA expression studies showed that all three genes were expressed constitutively. Gel retardation assays re-

*Abbreviations:* DS-TBP, double-stranded telomere-binding protein; MBP, maltose binding protein; RT-PCR, reverse transcriptase polymerase chain reaction; SMH, single Myb histone; TRBF, telomere repeat-binding factor.

* Corresponding author. Fax: +82 2 312 5657.
*E-mail address:* wtkim@yonsei.ac.kr (W.T. Kim).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.04.181

vealed that these proteins bind specifically to the plant DS telomeric repeat sequence, TTTAGGG, with markedly different affinities as estimated by their respective dissociation constants. Both OsTRBF1 and OsTRBF2 interacted with one another to form homo- and hetero-complexes, while OsTRBF3 appeared to remain a monomer. These results suggest that OsTRBFs play combinatory roles in the function and structure of telomeres in rice.

## Materials and methods

*Identification of rice OsTRBF genes.* A homolog of maize *SMH* gene was searched in the rice genome (http://signal.salk.edu/cgi-bin/RiceGE) using the BLASTN program with the *E*-value being lower than $10^{-14}$. The *OsTRBF1* was identified as a homolog of *SMH* gene, while *OsTRBF2* and *OsTRBF3* were identified as homologs of *OsTRBF1*.

*cDNA cloning and RT-PCR.* Total RNA was extracted from the rice callus as described previously [15]. To obtain full-length cDNAs for *OsTRBF1, OsTRBF2*, and *OsTRBF3*, first-strand cDNA was synthesized from 10 μg total RNA and amplified by PCR using high-fidelity Ex-Taq polymerase (Takara) as previously described [16]. To examine gene expression levels, total RNAs (5 μg) extracted from various rice organs were reverse-transcribed, and the cDNAs were amplified using gene-specific primers, which are shown in Supplementary Table 1.

*Plasmid construction for protein expression in Escherichia coli.* For recombinant protein expression, full-length cDNA fragments were inserted into the protein expression vectors, pProEx-HTa (Invitrogen) and pMAL-c2x (New England Biolabs). Purification of bacterially expressed proteins was performed as described [17].

*Gel retardation assay.* DNA probes and competitors used in this study are shown in Supplementary Table 1. Gel retardation assays were performed as described [17]. To determine the dissociation constants, gel retardation assays were repeated using a constant amount of probe and various amounts of protein as indicated in Fig. 3. After separation on a 9% non-denatured polyacrylamide gel, both free and bound probes were quantified using a scintillation counter (Beckman). The dissociation constants ($K_d$) were calculated by the method previously established [18]. The $K_d$ was the *x*-intersect of a plot of log [complex]/[free DNA] *versus* log [protein].

*Yeast two-hybrid assay.* Yeast transformations were performed using the MATCHMAKER Two-Hybrid System 3 (Clontech) according to the manufacturer's instructions. The yeast strain AH109 was used in these experiments. For plasmid construction, pGAD T7 was used as the activation domain and pGBK T7 was used as the binding domain. After inoculation, the plates were incubated at 28 °C.

*In vitro pull-down assay.* Recombinant proteins expressed in *E. coli* BL21 (DE3) cells were purified by affinity chromatography using Ni-NTA agarose (Qiagen) for $(His)_6$-tagged proteins and amylose resin (New England Biolabs) for MBP-tagged proteins, respectively, according to the manufacturers' protocols. *In vitro* pull-down and immunoblot analyses were performed as described previously [19]. Blots were visualized using the chemiluminescent, horseradish-peroxidase substrate (Millipore) and then exposed to Kodak BioMax ML film.

## Results and discussion

### *Identification and characterization of three OsTRBF genes in rice plants*

*In vivo* functions of C-terminal Myb DS-TBPs were recently described in tobacco and rice [9,10]. In contrast, although SMH-type DS-TBPs were identified in maize and *Arabidopsis* [11,13,14], their cellular functions are yet to be determined. A database search revealed three putative SMH-type DS-TBP genes in rice, and we designated them as *OsTRBFs* (*Oryza sativa TELOMERE REPEAT BINDING FACTORs*). To gain insight into the function of *OsTRBFs*, we first proceeded to isolate their cDNAs. Total RNA was extracted from rice callus, and full-length cDNAs for *OsTRBF1*, *OsTRBF2*, and *OsTRBF3* were obtained by RT-PCR. The coding regions of *OsTRBF1* (GenBank Accession No. NP_001043442), *OsTRBF2* (GenBank Accession No. NP_001067263), and *OsTRBF3* (GenBank Accession No. NP_001044023) are 900 bp encoding 300 amino acids (32.7 kDa), 891 bp encoding 297 amino acids (32.1 kDa), and 912 bp encoding 304 amino acids (33.0 kDa), respectively (Fig. 1A). OsTRBF1 and OsTRBF2 are 51% identical to each other, while they are 26–29% identical to OsTRBF3. Thus, OsTRBF1 and OsTRBF2 share a higher homology with one another, and OsTRBF3 is a more divergent member of the family. OsTRBF1 and OsTRBF2 are 50–72% identical to the SMH protein from monocot maize (ZmSMH1) [11] and 41–51% identical to those from dicot *Arabidopsis* (AtTRBs) [13,14]. On the other hand, OsTRBF3 is only 28% homologous to ZmSMH1 and AtTRBs. All three OsTRBFs share a relatively low degree of sequence identity (26–30%) with the parsley protein (PcMYB1) [20], consistent with the notion that *SMH* genes comprise a divergent gene family (Fig. 1B and C). As is the case for other SMH proteins, each OsTRBF contains a Myb domain and a linker histone motif in the N-terminal and central regions, respectively. The Myb and linker histone domains are significantly conserved with the corresponding regions in the maize, *Arabidopsis*, and parsley proteins mentioned above (65–90% and 26–79%, respectively). Collectively, this structural conservation suggests that *OsTRBFs* are SMH-type DS-TBP homologs in rice.

To examine the spatial and temporal expression patterns of *OsTRBFs*, we monitored the corresponding mRNA levels in different vegetative and reproductive tissues of rice by RT-PCR using gene-specific primers. As shown in Fig. 1D, substantial levels of *OsTRBF* transcripts were detected at every developmental stage and in every tissue examined in rice plants grown under greenhouse conditions. However, the relative expression patterns of the three mRNAs significantly varied in these tissues, with the *OsTRBF1* transcript exhibiting the lowest expression level. The amounts of the *OsTRBF2* and *OsTRBF3* mRNAs were somewhat similar.

### *OsTRBFs bind specifically to plant DS telomeric sequences in vitro*

To explore whether OsTRBFs can bind telomeric DNA, OsTRBFs were expressed in *E. coli,* and the purified proteins were used in a gel shift assay with $^{32}$P-labeled plant telomeric repeat-4 (PTR-4) that included four plant duplex telomeric DNA repeats, $(TTTAGGG)_4$. All three OsTRBFs gave rise to a single, discrete DNA-protein complex that migrated slower than the free probe, and one additional minor band (Fig. 2A, lanes 10–12). The intensities of these shifted bands increased proportionally upon the addition of increasing amounts (0.5–1 μg) of OsTRBFs. To examine the minimum number of telomeric repeats that are required for OsTRBF binding, we repeated the gel retardation assay using probes (PTR-1, PTR-2, PTR-3, and PTR-4) with different numbers (1–4) of telomeric DNA repeats. Fig. 2A shows that OsTRBFs can only form complexes with probes containing three or more contiguous TTTAGGG repeats. Thus, under these experimental conditions, the minimum length of telomeric DNA bound by OsTRBFs *in vitro* spans at least three repeats, suggesting that OsTRBFs require three TTTAGGG repeats for efficient complex formation.

The DNA-binding capacity of OsTRBFs was next assessed by competition assays, which showed that a 50-fold excess of cold DS PTR-4 was sufficient to displace the labeled probe (Fig. 2B, lane 3). The results revealed that, in the presence of a 100-fold molar excess of human DS telomeric repeats (HTR-4), binding capacities of OsTRBF1, OsTRBF2 and OsTRBF3 to PTR-4 were reduced to

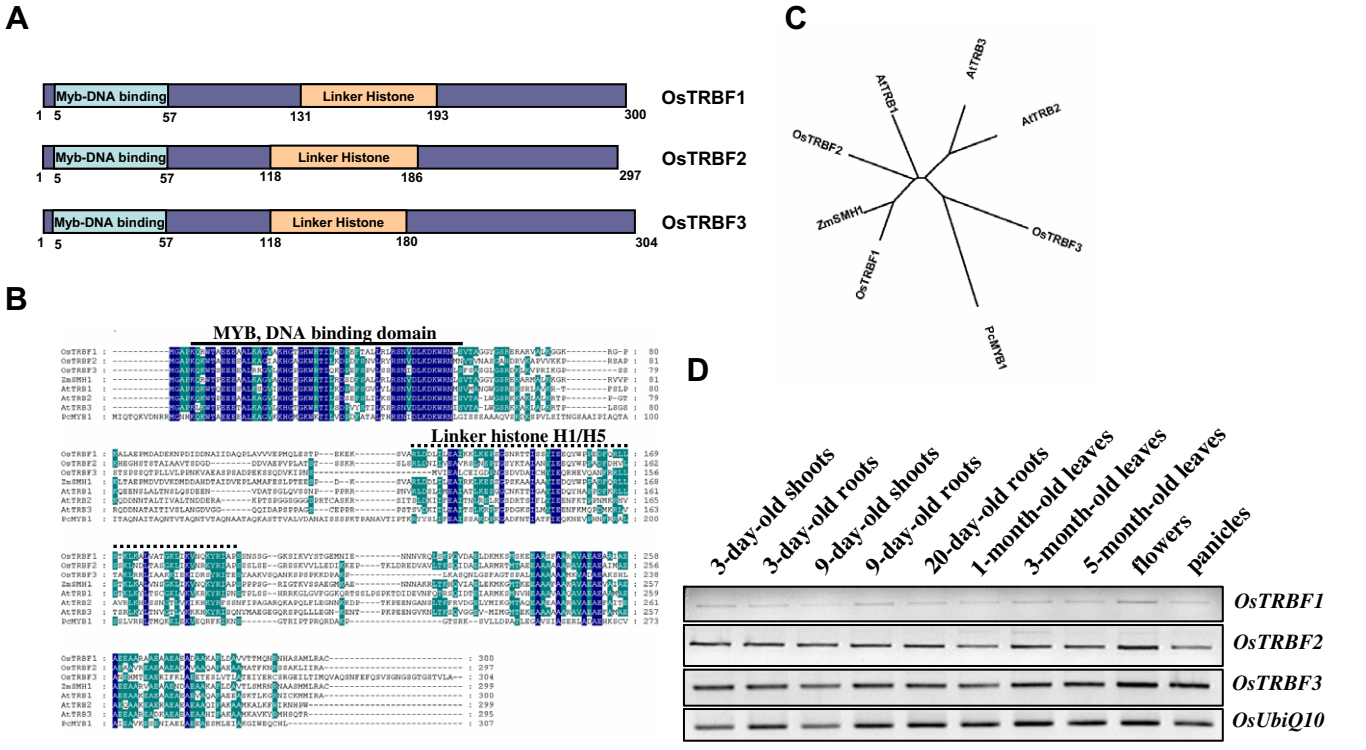



**Fig. 1.** Sequence analysis of OsTRBFs. (A) Schematic structures of OsTRBF1, OsTRBF2, and OsTRBF3 proteins. The N-terminal Myb DNA-binding domain and the central linker histone domain are indicated. (B) Comparison of the derived amino acid sequences of OsTRBFs with the SMH proteins from maize (ZmSMH1), *Arabidopsis* (AtTRBs), and parsley (PcMYB1). Amino acid residues that are conserved in at least five of the eight sequences are shown in green, while amino acids identical in all eight proteins are shown in blue. (C) Phylogenetic relationship of plant SMH family members from rice, maize, *Arabidopsis*, and parsley. (D) Expression profile of *OsTRBF* transcripts. Total RNAs were extracted from various tissues as indicated and analyzed by RT-PCR using gene-specific primers as described in 'Materials and methods'. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

approximately 50%, 10%, and 30% of the original levels, respectively (lanes 5 and 6). This was probably due to the similar sequences of plant (TTTAGGG) and human (TTAGGG) telomeres, implying that the association between OsTRBF1 and PTR-4 was the strongest and most specific. In contrast, *Caenorhabditis elegans* DS telomere sequence (CTR-4), as well as non-specific DNA (NS), failed to compete with PTR-4 (lanes 7–10). Furthermore, single-stranded telomeric repeats did not exert any detectable effects on the DNA-binding capacity of OsTRBFs (Fig. 2B, lanes 11 and 12).

*Determination of the dissociation constants of OsTRBFs to PTR-4*

Since the binding of OsTRBF1 to telomeric repeats appeared to be stronger and more specific than those of OsTRBF2 and OsTRBF3 (Fig. 2), we estimated the *in vitro* DNA-binding affinities of these proteins. The dissociation constant for the binding of each full-length OsTRBF to PTR-4 was calculated from a quantitative gel mobility shift assay as described in 'Materials and methods' (Fig. 3A). The plots in Fig. 3B depict the log of the concentration of the DNA-protein complex divided by the concentration of free DNA probe *versus* the log of the protein concentration; the dissociation constant was the *x*-intersect on the plot. As shown in Fig. 3C, the dissociation constant of OsTRBF1 to PTR-4 was determined to be $3.0 \pm 0.4 \times 10^{-7}$ M, while those of OsTRBF2 and OsTRBF3 were $8.3 \pm 0.1 \times 10^{-7}$ M and $5.7 \pm 0.8 \times 10^{-7}$ M, respectively. These results indicate that OsTRBF1 binds to PTR-4 most strongly and that OsTRBF2 binds most weakly. Thus, these results are also in agreement with those of Fig. 2B, which show that HTR-4 interferes more with the interaction between OsTRBF2 and PTR-4 compared to that of OsTRBF1 and PTR-4. Overall, the gel retardation experiments suggest that bacterially expressed full-length OsTRBFs bind specifically to the plant DS telomeric sequence, but the binding affinities of individual proteins are markedly different.

*Yeast two-hybrid assays suggest homo- and heterodimer formation of OsTRBF1 and OsTRBF2*

The mode of TBP binding to telomeric repeats appears to be dependent on the structure of individual TBPs. For example, Rap1, a DS-TBP in budding yeast, contains two Myb repeats and interacts with telomeric DNA as a monomer [21]. Yeast Taz1 and human TRF1 and TRF2 differ from Rap1 in both amino acid sequence and overall architecture; each binds to DNA as a homodimer via a single Myb DNA-binding domain located at the C-terminus [22–24]. Similarly, *Arabidopsis* TRF-like proteins and tobacco NgTRF1 form dimers to interact with telomeric sequences [8,25,26]. A recent study showed that *Arabidopsis* AtTRB2 and AtTRB3, which belong to the SMH-type DS-TBPs, bind to one another to form homo- and heterodimeric complexes [13,14]. Since OsTRBFs also harbor a single N-terminal Myb-like domain and a single linker histone motif, we considered the possibility that they also bind to telomeric DNA as dimers. To test this possibility, we decided to employ yeast two-hybrid (Y2H) analysis. OsTRBFs were individually fused to the GAL4-binding domain (BD) and co-expressed in yeast with OsTRBFs fused to the GAL4-activation domain (AD) as described in 'Materials and methods'. The results showed that both BD-OsTRBF1 and BD-OsTRBF2 interacted with either AD-OsTRBF1 or AD-OsTRBF2 as evidenced by the selective growth of yeast cells on medium lacking His (Fig. 4B). These results indicate that OsTRBF1 and OsTRBF2 form homo- and hetero-complexes in yeast cells. In contrast, under our experimental conditions, OsTRBF3 did not bind itself. Additionally, neither OsTRBF1

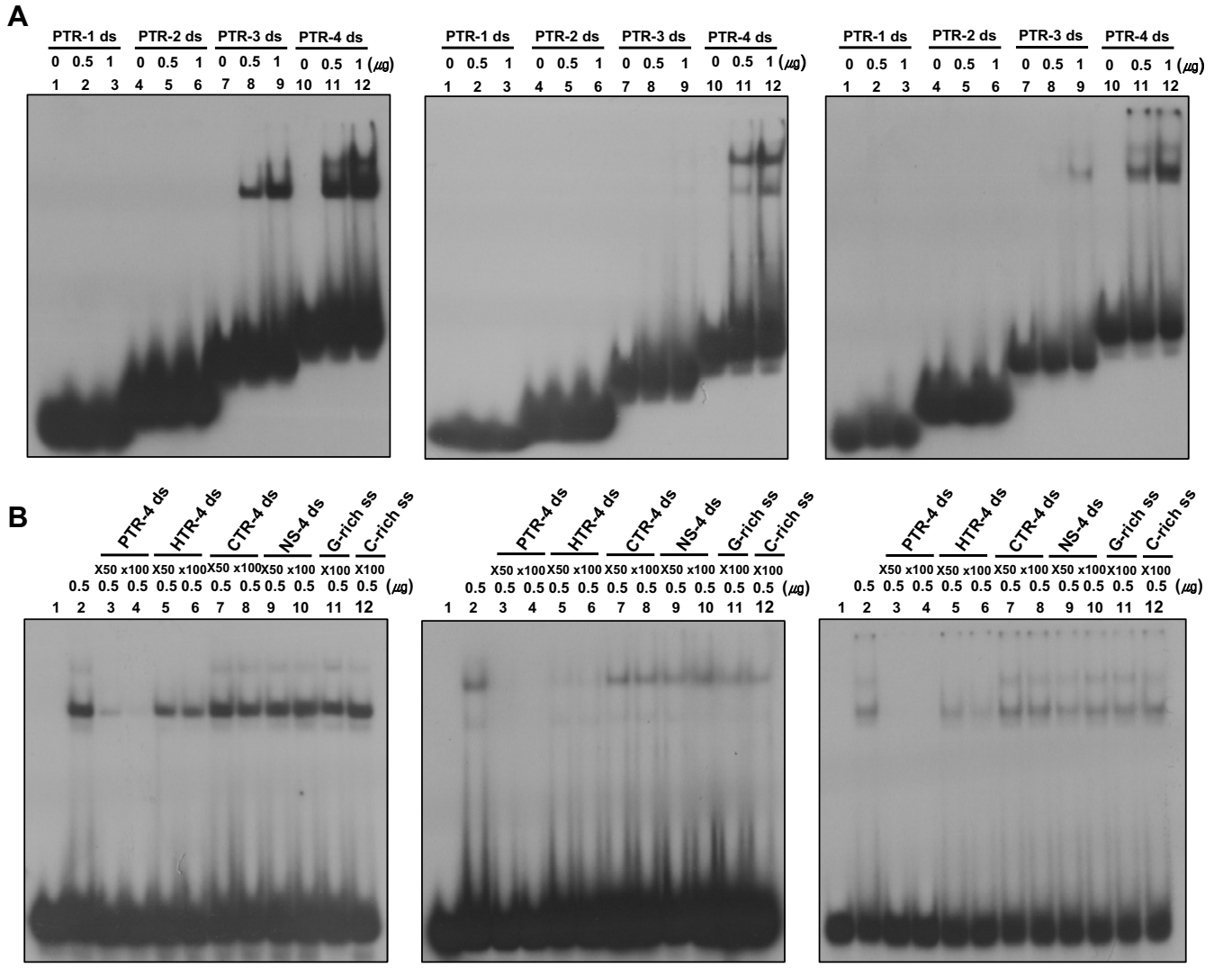


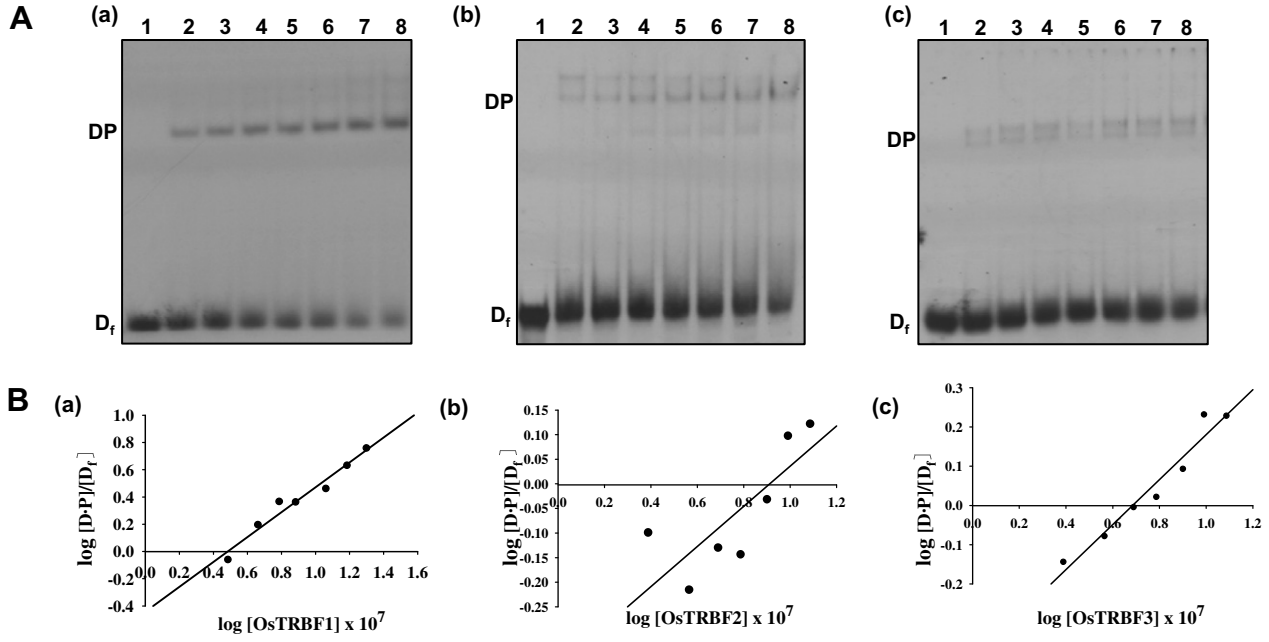

**Fig. 2.** Gel retardation assays showing full-length OsTRBFs binding to plant double-stranded telomeric DNA. (A) Gel retardation assays were performed with radiolabeled PTR-1 (lanes 1–3), PTR-2 (lanes 4–6), PTR-3 (lanes 7–9), or PTR-4 (lanes 10–12). Each set of lanes contained 0, 0.5, or 1.0 μg of bacterially expressed, full-length OsTRBFs, respectively. (B) Sequence-specific binding activity of OsTRBFs to plant double-stranded telomeric DNA. Full-length OsTRBFs (0 or 0.5 μg) were added to each reaction mixture. Lanes 1 and 2, radiolabeled PTR-4 probe; lanes 3 and 4, titration with cold PTR-4 as a competitor; lanes 5–10, titration with cold human (HTR-4), *C. elegans* (CTR-4), and non-specific DNA (NS) as competitors; lanes 11 and 12, titration with cold G-rich (TTTAGGG) and C-rich (AAATCCC) single-stranded DNA as competitors.

nor OsTRBF2 were able to associate with OsTRBF3 (Fig. 4B). As a control for specificity, we repeated the Y2H experiment using two versions of a deletion mutant of OsTRBF1, OsTRBF1$^{1-128}$ (Δ*C* mutant) and OsTRBF1$^{129-300}$ (Δ*N* mutant). As shown in Fig. 4B, OsTRBF1$^{129-300}$ effectively interacted with OsTRBF1 and OsTRBF2, while OsTRBF1$^{1-128}$, which primarily consists of the N-terminal Myb domain, failed to bind OsTRBF1 and OsTRBF2, indicating that the ability to interact resides in the C-terminal region.

*In vitro pull-down assays indicate physical interaction of OsTRBF1 and OsTRBF2*

To further substantiate the interactions of OsTRBF1 and OsTRBF2, we carried out *in vitro* pull-down assays. OsTRBF1 and OsTRBF2 were expressed as MBP- or $(His)_6$-fusion proteins in *E. coli*. Purified fusion proteins were co-incubated in the presence of an amylose-affinity matrix. Bound protein was then eluted from the resin by the addition of 10 mM maltose and immunoblotted with anti-MBP or anti-His antibody. Fig. 4C shows that $(His)_6$-OsTRBF1 and $(His)_6$-OsTRBF2 were effectively pulled down from the amylose resin by either MBP-OsTRBF1 or MBP-OsTRBF2. These results imply that both OsTRBF1 and OsTRBF2 physically interact with one another *in vitro*. As found in the Y2H analysis, OsTRBF1$^{129-300}$, which lacks the N-terminal Myb domain, was able to bind both OsTRBF1 and OsTRBF2 (Supplementary Fig. 1A and B). In contrast, OsTRBF3 did not bind any of the OsTRBFs (Supplementary Fig. 1C–E). A closer inspection of the immunoblots suggests that the interaction between OsTRBF1 and OsTRBF2 was relatively weaker than those of the homo-complexes (Fig. 4C-(c) and Supplementary Fig. 1B). Overall, both the Y2H and *in vitro* pull-down experiments are consistent with the theory that OsTRBF1 and OsTRBF2, which belong to the closely related family, exist as homo- and hetero-complexes, while OsTRBF3, a more divergent member, is a monomer. These results raise the possibility that OsTRBF1 and OsTRBF2 form homo- and hetero-dimers. However, further experiments are required to obtain the exact stoichiometry of interaction to ascertain whether they form dimers or higher complexes.

Thus, the binding mode of OsTRBFs to telomeres correlates with a conservation of their primary structures. In this regard, it should be noted that each OsTRBF binds to PTR-4 with markedly different affinity, with OsTRBF1 being the strongest (Figs. 2 and 3). With this




C

| Protein | $K_d$ |
|---|---|
| OsTRBF1 | 3.0 ± 0.4 x $10^{-7}$ M |
| OsTRBF2 | 8.3 ± 0.1 x $10^{-7}$ M |
| OsTRBF3 | 5.7 ± 0.8 x $10^{-7}$ M |

**Fig. 3.** Determination of the dissociation constants for the full-length OsTRBFs to a four-telomeric repeat. (A) Quantitative binding assay of full-length OsTRBF1 (a), OsTRBF2 (b), and OsTRBF3 (c) to PTR-4. The DNA concentration was $1.5 \times 10^{-9}$ M for OsTRBF1 and $7.5 \times 10^{-8}$ M for OsTRBF2 and OsTRBF3, while the proteins in lanes 1–7 were $3.1 \times 10^{-7}$, $4.6 \times 10^{-7}$, $6.1 \times 10^{-7}$, $7.6 \times 10^{-7}$, $1.2 \times 10^{-6}$, $1.5 \times 10^{-6}$, and $2.0 \times 10^{-6}$ M for OsTRBF1 and $2.4 \times 10^{-7}$, $3.7 \times 10^{-7}$, $4.9 \times 10^{-7}$, $6.1 \times 10^{-7}$, $8.0 \times 10^{-7}$, $9.8 \times 10^{-7}$, and $1.2 \times 10^{-6}$ M for OsTRBF2 and OsTRBF3. (B) Plot of the log [complex]/[free DNA] *versus* the log ([protein] $\times 10^7$) using data from (A). The dissociation constants ($K_d$) of each OsTRBF are the *x*-intersects of each plot. (C) The $K_d$ values of OsTRBF1, OsTRBF2, and OsTRBF3 to PTR-4. The experiment was carried out three times independently. The values are means ± SD ($n = 3$).

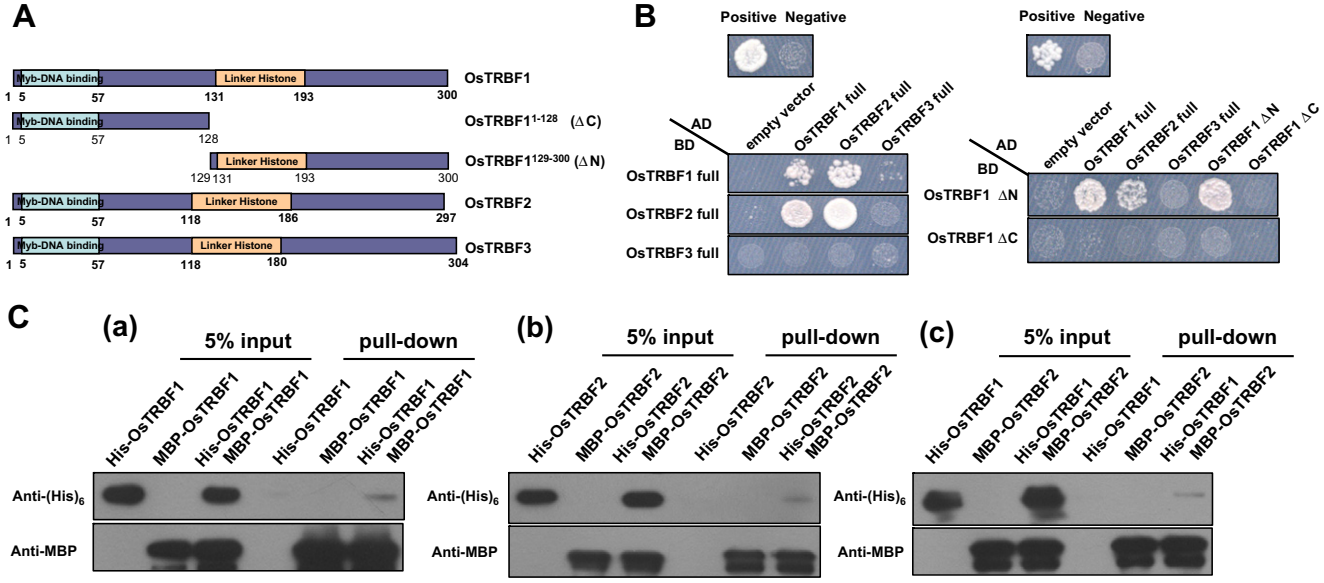



**Fig. 4.** Yeast two-hybrid (Y2H) and *in vitro* pull-down experiments. (A) Schematic structure of the full-length OsTRBF1, OsTRBF2, and OsTRBF3 proteins, and two versions of OsTRBF1 deletion mutants, OsTRBF1$^{1-128}$ (Δ*C* mutant) and OsTRBF1$^{129-300}$ (Δ*N* mutant). (B) Y2H assay indicating the interaction of OsTRBF1 and OsTRBF2. Proteins with a deletion of Δ*N* or Δ*C* were also examined. Yeasts are shown after 3 days on media lacking His for determination of protein–protein interactions. (C) *In vitro* pull-down assay. OsTRBF1 and OsTRBF2 were expressed as MBP- or $(His)_6$-fusion proteins in *E. coli*. The purified fusion proteins were co-incubated as indicated in the presence of an amylose-affinity matrix. The bound protein was then eluted from the resin by 10 mM maltose and immunoblotted with either anti-MBP or anti-His antibody.

in mind, we speculate that OsTRBF1 is a major SMH-type DS-TBP, while both OsTRBF2 and OsTRBF3 function as supplementary proteins in telomeres. These combinatory roles of three different DS-TBPs would permit the plant to fine-tune its cellular responses to protect the structures of the extreme ends of chromosomes. However, we cannot rule out the possibility that the more divergent OsTRBF3, which is a monomer and has a lower affinity for telomeres, may have additional roles, perhaps as a transcription factor. This idea is consistent with the fact that the SMH families comprise a large gene family with subsets of genes that have distinct cellular functions [12]; the parsley SMH protein, PcMYB1, for example, acts as a transcription factor [20]. Further experiments are now required to define more precisely the cellular and physiological functions of individual OsTRBFs in rice plants.

## Acknowledgments

This work was supported by grants to W.T.K. from the Plant Diversity Research Center (21st Century Frontier Research Program funded by the Ministry of Science and Technology of Korea) and the BioGreen 21 Program (funded by Rural Development Administration of Korea). M.Y.B. was a recipient of Brain Korea 21 graduate student scholarship.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.04.181.

## References

[1] E.H. Blackburn, Structure and function of telomeres, Nature 350 (1991) 569–573.
[2] C.W. Greider, Telomere length regulation, Annu. Rev. Biochem. 65 (1996) 337–365.
[3] D. Shore, Telomeric chromatin: replicating and wrapping up chromosome ends, Curr. Opin. Genet. Dev. 11 (2001) 189–198.
[4] E.H. Blackburn, Switching and signaling at the telomere, Cell 106 (2001) 661–673.
[5] T. de Lange, Shelterin: the protein complex that shapes and safeguards human telomeres, Genes Dev. 19 (2005) 2100–2110.
[6] M. Kuchar, Plant telomere-binding proteins, Biol. Plant. 50 (2006) 1–7.
[7] B. Zellinger, K. Riha, Composition of plant telomeres, Biochim. Biophys. Acta 1769 (2007) 399–409.
[8] Z.N. Karamysheva, Y.V. Surovtseva, L. Vespa, E.V. Shakirov, D.E. Shippen, A C-terminal Myb-extension domain defines a novel family of double-strand telomeric DNA binding proteins in *Arabidopsis*, J. Biol. Chem. 279 (2004) 47799–47807.
[9] S.W. Yang, S.K. Kim, W.T. Kim, Perturbation of *NgTRF1* expression induces apoptosis-like cell death in tobacco BY-2 cells and implicates NgTRF1 in the control of telomere length and stability, Plant Cell 16 (2004) 3370–3385.
[10] J.-P. Hong, M.Y. Byun, D.-H. Koo, K. An, J.-W. Bang, I.K. Chung, G. An, W.T. Kim, Suppression of *RICE TELOMERE BINDING PROTEIN 1* results in severe and gradual developmental defects accompanied by genome instability in rice (*Oryza sativa* L.), Plant Cell 19 (2007) 1770–1781.
[11] C.O. Marian, S.J. Bordoli, M. Goltz, R.A. Santarella, L.P. Jackson, O. Danilevskaya, M. Beckstette, R. Meeley, H.W. Bass, The maize *single myb histone 1* gene, *Smh1*, belongs to a novel gene family and encodes a protein that binds telomere DNA repeats in vitro, Plant Physiol. 133 (2003) 1336–1350.
[12] A. Jerzmanowski, SWI/SNF chromatin remodeling and linker histones in plants, Biochim. Biophys. Acta 1769 (2007) 330–345.
[13] P. Schrumpfova, M. Kuchar, G. Mikova, L. Skrisovska, T. Kubicarova, J. Fajkus, Characterization of two *Arabidopsis thaliana* myb-like proteins showing affinity to telomeric DNA sequence, Genome 47 (2004) 316–324.
[14] M. Kuchar, J. Fajkus, Interactions of putative telomere-binding proteins in *Arabidopsis thaliana*: identification of functional TRF2 homolog in plants, FEBS Lett. 578 (2004) 311–315.
[15] W. Park, H.B. Kim, W.T. Kim, P.B. Park, G. An, S. Choe, Rice *bending lamina 2* (*bla2*) mutants are defective in a cytochrome P450 (CYP734A6) gene predicted to mediate brassinosteroid catabolism, J. Plant Biol. 49 (2006) 469–476.
[16] S.W. Yang, E.S. Jin, I.K. Chung, W.T. Kim, Cell cycle-dependent regulation of telomerase activity by auxin, ABA and protein phosphorylation in tobacco BY-2 suspension culture cells, Plant J. 29 (2002) 617–626.
[17] S.W. Yang, D.H. Kim, J.J. Lee, Y.J. Chun, J.-H. Lee, Y.J. Kim, I.K. Chung, W.T. Kim, Expression of the telomeric repeat binding factor gene *NgTRF1* is closely coordinated with the cell division program in tobacco BY-2 suspension culture cells, J. Biol. Chem. 278 (2003) 21395–21407.
[18] S. Chung, L. Jiang, S. Cheng, H. Furneaux, Purification and properties of HuD, a neuronal RNA-binding protein, J. Biol. Chem. 271 (1996) 11518–11524.
[19] J.-H. Lee, X.W. Deng, W.T. Kim, Possible role of light in the maintenance of EIN3/EIL1 stability in *Arabidopsis* seedlings, Biochem. Biophys. Res. Commun. 350 (2006) 484–491.
[20] M. Feldbrugge, M. Sprenger, K. Hahlbrock, B. Weisshaar, *PcMYB1*, a novel plant protein containing a DNA-binding domain with one MYB repeat, interacts *in vivo* with a light-regulatory promoter unit, Plant J. 11 (1997) 1079–1093.
[21] P. Konig, R. Giraldo, L. Chapman, D. Rhodes, The crystal structure of the DNA-binding domain of yeast RAP1 in complex with telomeric DNA, Cell 85 (1996) 125–136.
[22] A. Bianchi, S. Smith, L. Chong, P. Elias, T. de Lange, TRF1 is a dimer and bends telomeric DNA, EMBO J. 16 (1997) 1785–1794.
[23] D. Broccoli, A. Smogorzewska, L. Chong, T. de Lange, Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2, Nat. Genet. 17 (1997) 231–235.
[24] K.G. Spink, R.J. Evans, A. Chambers, Sequence-specific binding of Taz1p dimers to fission yeast telomeric DNA, Nucleic Acids Res. 28 (2000) 527–533.
[25] S.C. Sue, H.H. Hsiao, B.C. Chung, Y.H. Cheng, K.L. Hsueh, C.M. Chen, C.H. Ho, T.H. Huang, Solution structure of the *Arabidopsis thaliana* telomeric repeat-binding protein DNA binding domain: a new fold with an additional C-terminal helix, J. Mol. Biol. 356 (2006) 72–85.
[26] S.G. Ko, S.-H. Jun, H. Bae, J.-S. Byun, W. Han, H. Park, S.W. Yang, S.Y. Park, W.T. Kim, W. Lee, H.-S. Cho, Structure of the DNA binding domain of NgTRF1 reveals unique features of plant telomere-binding proteins, Nucleic Acids Res. (2008), in press, doi:10.1093/nar/gkn030.