

Short Communication

Two Alternatively Spliced Transcripts Generated from *OsMUS81*, a Rice Homolog of Yeast *MUS81*, Are Up-Regulated by DNA-Damaging Treatments

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OsMUS81, a rice homolog of the yeast *MUS81* endonuclease gene, produced two alternative transcripts, *OsMUS81α* and *OsMUS81β*. *OsMus81α* contained a Helix–hairpin–Helix (HhH) motif at the N- and C-termini, and a conserved XPF-like motif in the center, while the *OsMus81β* isoform lacked the second HhH motif by alternative splicing of a cryptic intron generating a truncated protein. The two transcripts were induced after DNA-damaging treatments such as high intensity light, UV-C and γ -radiation. The yeast two-hybrid assay detected a strong interaction between *OsMus81* and *OsRad54* recombinational repair proteins. These findings suggest that *OsMus81* functions in maintaining genome integrity through homologous recombination.

Keywords: Alternative splicing — DNA repair — Homologous recombination — *MUS81* — Rice — XPF endonuclease superfamily.

Abbreviations: HhH, Helix–hairpin–Helix; HJ, Holliday junction; HR, homologous recombination; MMR, mismatch repair; MMS, methylmethane sulfonate; NER, nucleotide excision repair; ORF, open reading frame; RF, replication fork; RT-PCR, reverse transcription–PCR.

The cDNA sequences for *Arabidopsis MUS81* and rice *RAD54* reported in this paper have been submitted to DDBJ under accession numbers AB177892 and AB240577, respectively.

Since plants cannot escape any changes in the environment where they grow, plant cells undergoing DNA replication, transcription and translation are always exposed to various mutagens such as UV radiation from the sunlight, reactive oxygen species (ROS), natural and

artificial chemicals, and ionizing radiation that harm the DNA strands. Additionally, programmed double-strand breaks (DSBs) are formed during meiosis. To restart DNA replication, maintain proper gene expression and genome integrity and produce progeny, specific damage to the DNA strands and spontaneous or programmed DSBs must be repaired by one of the appropriate mechanisms such as nucleotide excision repair (NER), mismatch repair (MMR), non-homologous end-joining (NHEJ) or homologous recombination (HR). To date, various genes involved in the repair processes have been isolated in plants (reviewed in Kimura and Sakaguchi 2006). Among them, nucleases perform crucial roles in various DNA repair processes. For example, Mus81, a member of the xeroderma pigmentosum complementation group E (XPF) endonuclease superfamily, functions in the processing of recombination intermediates (e.g. D-loop structure). Mus81 was initially identified by its interaction with the recombination repair protein Rad54 from budding yeast (Interthal and Heyer 2000) and the replication checkpoint serine/threonine kinase Cds1 in fission yeast (Boddy et al. 2000). Mus81 forms a heterodimeric complex with Mms4 from *Saccharomyces cerevisiae* and Emel from *Schizosaccharomyces pombe* (reviewed in Heyer et al. 2003, Hollingsworth and Brill 2004). The Mus81–Mms4/Emel complex shares similarities with the yeast Rad1–Rad10 and human XPF–ERCC1 endonucleases involved in NER (Enzlin and Schärer 2002). In the two yeast species, *mus81* mutant cells show sensitivity against DNA-damaging agents [strong to methylmethane sulfonate (MMS) and weak to UV], suggesting a role for Mus81 in the DNA damage response (Boddy et al. 2000, Interthal and Heyer 2000). Further analyses demonstrated

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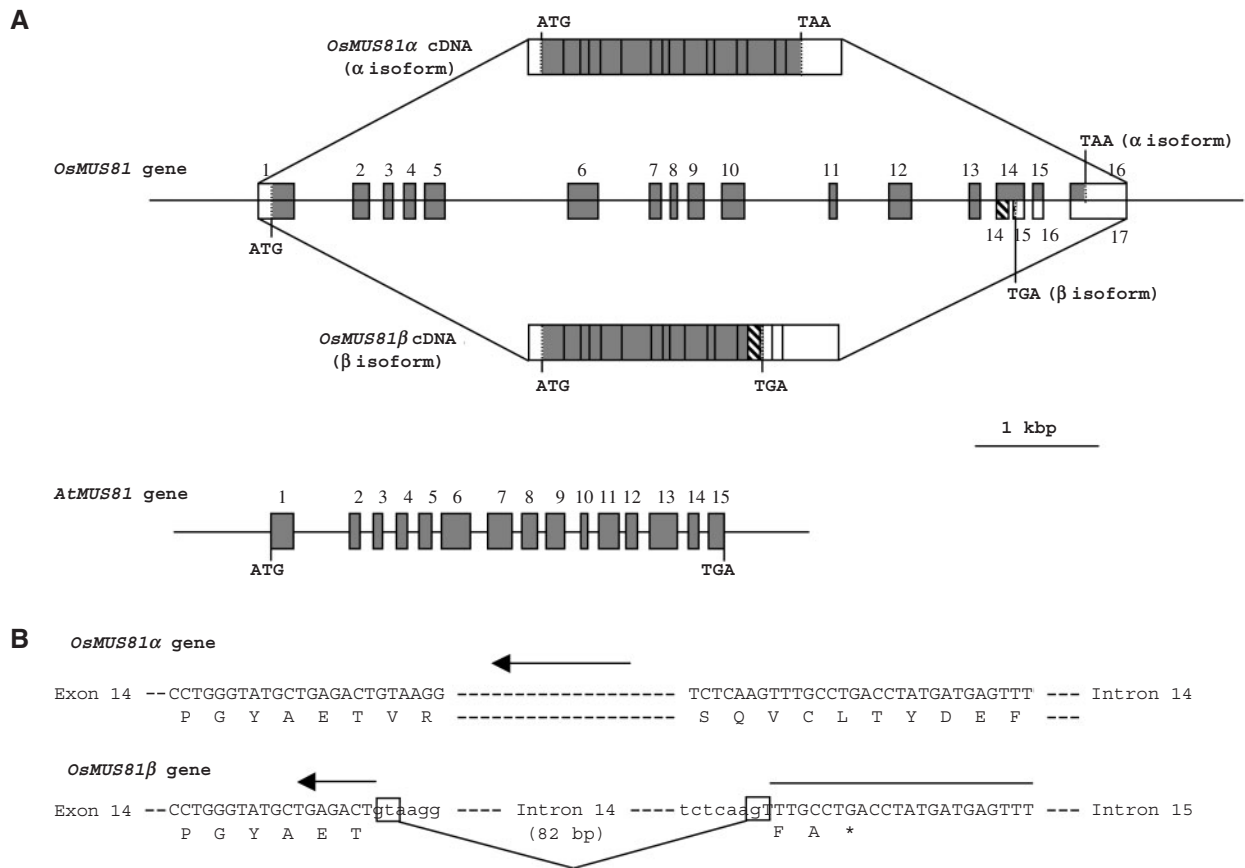


Fig. 1 Gene structures of plant *MUS81* genes. (A) Schematic representation of *OsMUS81* (*OsMUS81α* and *-β*) and *AtMUS81*. Exons and introns are represented as boxes and the intervening lines, respectively. Shaded boxes, coding exons; slashed regions, exons 14 and a part of 15 in *OsMUS81β* cDNA; white boxes, non-coding exons. Scale bar: 1 kbp. (B) Genomic DNA sequences in the 14th exon–intron junctions of two splicing variants, *OsMUS81α* and *-β*. The nucleotides and their corresponding amino acid (indicated with single-letter code) sequences are shown. Slice donor (gt) and acceptor (ag) sites are shown with boxes and a stop codon (TGA) with an asterisk. Each arrow indicates a gene-specific RT–PCR primer for *OsMUS81α* and *OsMUS81β*.

that Mus81–Mms4/Eme1 is involved in the processing of DNA junctions at stalled replication forks (RFs) and the generation of meiotic crossovers through the resolution of recombination intermediates before the formation of X-shaped structures known as Holliday junctions (HJs) (Heyer et al. 2003, Hollingsworth and Brill 2004).

Other than the first report on two T-DNA insertion lines of *AtMUS81* (Hartung et al. 2006) that was published while this manuscript was in preparation, little is known about the *MUS81* gene structure and function in plants. Therefore, we characterized *OsMUS81*, a rice homolog of *MUS81*. Initially, we searched the DNA database with the *S. cerevisiae* Mus81 (ScMus81) amino acid sequence as a query. The search hit a full-length cDNA sequence (accession No. AK111411) encoding a rice *MUS81* homolog (designated *OsMUS81*) located on chromosome 1 (accession No. AP003259, gene ID: P0466H10.31). Interestingly, our analyses with reverse transcription–PCR

(RT–PCR) and DNA sequencing revealed two *OsMUS81* cDNA isoforms apparently arising from alternative splicing of its transcript. The two transcripts could potentially produce two different proteins designated as long (*OsMus81α*) and short (*OsMus81β*) forms (Fig. 1A, B). Genomic Southern blot analysis showed that *OsMus81* is present as a single-copy gene (see Supplementary Fig. S1) and consequently that the splicing variants are derived from a single *MUS81* gene. The *OsMUS81α* cDNA (2,441 bp) included 121 and 333 bp of 5'- and 3'-untranslated regions (UTRs), respectively, comprising 16 exons encoding an open reading frame (ORF) of 660 amino acids (approximately 73.0 kDa). The second splice variant, *OsMUS81β*, consisted of 17 exons caused by an additional splicing of a cryptic intron within exon 14 in *OsMUS81α*. This alternative splicing generated a novel stop codon in the 15th exon of *OsMUS81β* (Fig. 1A, B) encoding a truncated protein of 542 amino acids (60.0 kDa). Such splice variants

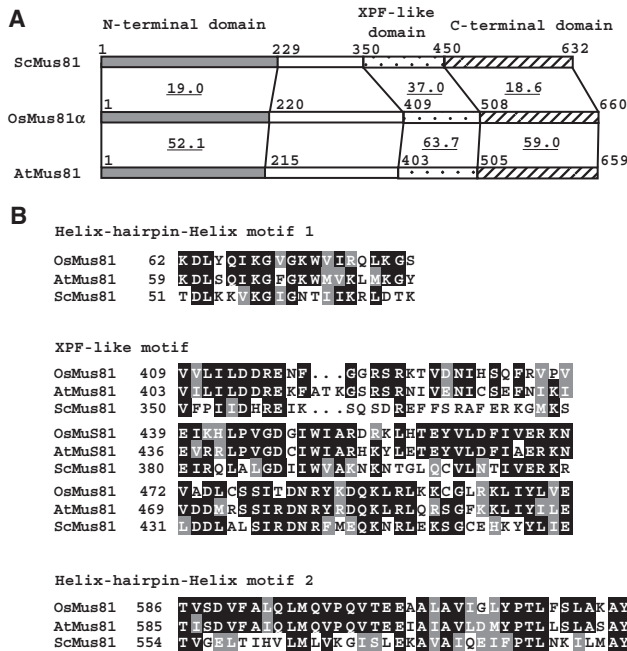


Fig. 2 Comparison of MUS81 proteins. (A) A schematic alignment of Mus81 proteins from budding yeast (*Saccharomyces cerevisiae*), rice and *Arabidopsis*. Each protein was divided into an N-terminal domain (shaded box), XPF endonuclease-like domain (dotted box) and C-terminal domain (hatched box). Each underlined number indicates amino acid identity (%) between the particular domain from OsMus81 α and one of the other two proteins. (B) Alignments of Helix-hairpin-Helix (HhH) motifs in both the N- and C-terminal regions and the XPF-like motif in Mus81 from rice, *Arabidopsis* and budding yeast (*S. cerevisiae*). The black boxes represent conserved amino acids in at least two sequences, and the gray boxes show similar amino acids.

have been identified in various plant DNA repair genes such as *AtXRCC3* and *AtRAD51C* (Osakabe et al. 2002), and *AtRAD1* (Vonarx et al. 2002). In addition, our *OsMUS81 α* cDNA sequence was identical to a corresponding sequence in the rice genome (gene ID: P0466H10.31) and showed a single base mismatch with the AK111411 sequence that replaced proline (OsMus81 α) with serine (AK111411) at position 269. It is unlikely that the substitution affects the OsMus81 functions, because the change is located in the variable region between the N-terminal and XPF-like functional domains (Fig. 2A). We also analyzed the primary structure of the *MUS81* homolog in *Arabidopsis* (ecotype Columbia) using RT-PCR. Cloning and sequencing of the PCR products revealed that *AtMUS81* consisted of 15 exons and 14 introns, and contained an ORF of 659 amino acids (74.1 kDa) (Fig. 1A), and that the 'AtMUS81-like' sequence in the database (AL161577) was incorrect due to misprediction of the exon-intron junctions.

Phylogenetic analysis identified an intimate relationship between OsMus81 α and AtMus81, revealing a plant

subgroup in the Mus81 family (Supplementary Fig. S2). Comparison of OsMus81 α with AtMus81 and ScMus81 indicated that the three proteins share homologies in three domains: the N-terminal, XPF-like and C-terminal domains (Fig. 2A, B). The XPF-like domain contains an endonuclease motif sharing significant homology with other XPF superfamily members (Heyer et al. 2003). The N- and C-terminal domains possessed Helix-hairpin-Helix (HhH) motifs (Interthal and Heyer 2000; Fig. 2A). OsMus81 β lacked the second HhH motif in the C-terminal region (Fig. 2A). Fu and Xiao (2003) reported that 100 amino acids in the C-terminus of ScMus81 were required for association with Mms4. Taken together, it is suggested that OsMus81 β lacks the putative interaction domain with Mms4. Since Mus81 forms an obligate heterodimer with Mms4/Eme1 to exert its function (Heyer et al. 2003; Hollingsworth and Brill 2004), it appears unlikely that this splice variant is functional.

Interthal and Heyer (2000) identified Mus81 as an interacting protein with Rad54 in budding yeast. This suggested that Mus81 could act in later stages of the HR pathway in cooperation with the Rad54 motor protein. To investigate such an interaction in rice, yeast two-hybrid experiments were conducted using full-length cDNAs for *OsMUS81 α* , *OsMUS81 β* and *OsRAD54*. The *OsRAD54* cDNA encodes an ORF with 980 amino acids (the detailed characterization will be published elsewhere). Strong β -galactosidase activities were detected in the strains bearing the LexA-OsRad54p (BD) fusion and the B42-OsMus81 α p or B42-OsMus81 β p (AD) fusion proteins (Fig. 3). Interestingly, the OsRad54-OsMus81 β interaction was stronger than the OsRad54-OsMus81 α interaction. This may be caused by a possible self-association between the two B42-OsMus81 α p molecules through their HhH domains, and such an interaction may not efficiently induce transcriptional activation of *LacZ*. A similar phenomenon was observed using the budding yeast proteins (Interthal and Heyer 2000). The interaction between the XPF-like domain of OsMus81 and the C-terminal region of OsRad54 was detected (Fig. 3), as well as that of yeast (Interthal and Heyer 2000). Much weaker interactions were observed between the yeast Rad54 C-terminus and the OsMus81 α and - β proteins (Fig. 3) compared with the cognate ScRad54-ScMus81 interaction. The reduced interaction may be the reason why OsMus81 α could not compensate for the loss of function of yeast *MUS81* (Supplementary Fig. S3). These results suggest that rice Mus81 has a function similar to yeast Mus81 by interacting with rice Rad54 protein in vivo in HR.

Since Northern hybridization gave no positive signals for *OsMUS81* transcripts using the total RNA preparations from various tissues, we performed RT-PCR analyses to quantitate tissue-specific *OsMUS81* transcripts. Both the

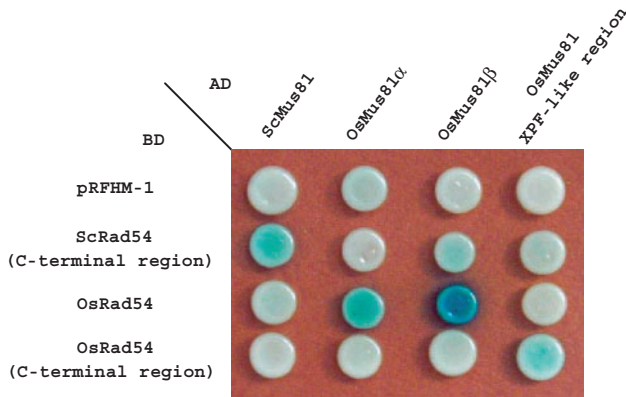


Fig. 3 Interaction between *OsMUS81* and *OsRAD54* using the yeast two-hybrid system. A yeast strain EGY48 carrying the *LacZ* reporter plasmid pSH18-34 was co-transformed with the appropriate combinations of DNA-binding domain (BD) and activation domain (AD) fusion constructs. For AD, *OsMUS81α* (amino acid position: 1–660) and *OsMUS81β* (1–542) lacking HhH motif 2, and truncated constructs for the XPF-like region of *OsMus81* (376–590) and *ScMus81* (220–557) were used. For BD, the C-terminal region of *OsRad54* (253–980), the *OsRad54* entire coding sequence (1–980), the C-terminal portion of *ScRad54* (291–898) (Interthal and Heyer 2000) and *D. melanogaster bicoid* (pRFHM-1) as a negative control were fused to the LexA operators.

OsMUS81α and $-β$ transcripts were detectable by semi-quantitative RT-PCR using purified mRNAs prepared from various tissues (Fig. 4A). Although higher expression levels were detected for *OsMUS81α* than for $-β$ in various rice tissues, similar tissue-specific accumulation patterns for the two transcripts were observed: low in shoots and roots from etiolated seedlings, and panicles after meiosis (10–15 cm); moderate in young panicles (3–4 cm) under differentiation of floral organs before and during meiosis; and high in mature leaves and calli (Fig. 4A, left panel). These results suggested that the *OsMUS81α* and $-β$ expression levels were rather low, and that the transcripts were preferentially accumulated in actively dividing tissues under illumination and mature green leaves. Among the tissues tested, mature green leaves were sampled in the daytime from rice plants grown in a greenhouse with exposure to strong daylight. Rice calli were grown under continuous white light. Young shoots and roots were grown in darkness, and immature panicles before heading were covered with leaf sheaths which blocked strong illumination. The highest expression levels of *OsMUS81α* and $-β$ in mature leaves implied plant response to DNA damage caused by sunlight.

To examine whether *OsMUS81α* and $-β$ transcripts are induced in rice calli by $γ$ -radiation, semi-quantitative RT-PCR was carried out. A Southern hybridization profile following RT-PCR showed that both transcripts were significantly up-regulated by 100 Gy of $γ$ -radiation (Fig. 4A, right panel). Induction by $γ$ -rays was also

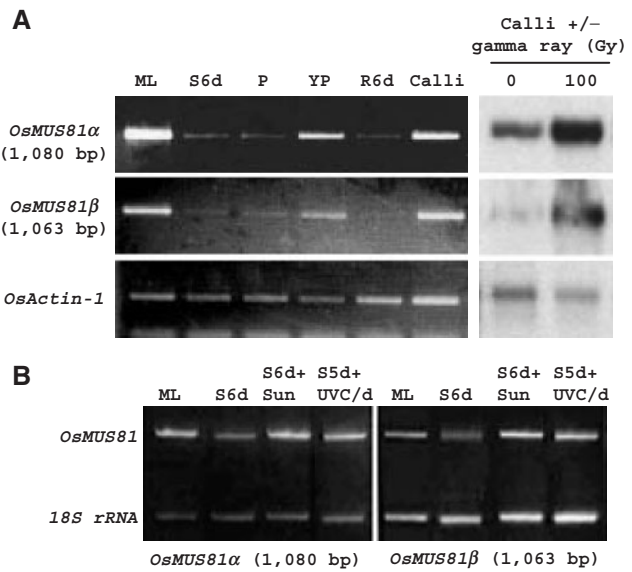


Fig. 4 Tissue- and radiation-specific expression of *OsMUS81α* and $-β$. (A) Left panel (from left to right): ML, mature leaves; S6d, etiolated shoots from 6-d-old dark-grown seedlings; P, panicles (10–15 cm) before heading; YP, young panicles (3–4 cm); R6d, roots from 6-d-old etiolated seedlings; and Calli, cultured cells 14 d after transfer to new N6D agar medium under continuous white light ($60 \mu\text{mol m}^{-2} \text{s}^{-1}$). RT-PCRs for *OsMUS81* genes were performed with 35 cycles (25 cycles for *OsActin-1*). Right panel: RT-PCR products detected by Southern hybridization. Total RNAs were prepared from Nipponbare calli 6 h after exposure to gamma radiation (100 Gy for 2 h) and from untreated calli. The electrophoresed and blotted products were hybridized with gene-specific probes. (B) Relative quantitative RT-PCR with QuantumRNA™ 18S internal standards. PCR was performed with a 1:9 ratio of 18S rDNA primers:competimers and first-strand cDNAs as follows (from left to right): mature leaves from greenhouse-grown rice plants; 6-d-old etiolated shoots (S6d); S6d followed by exposure to daylight in the greenhouse for 6 h (S6d+Sun); and 5-d-old seedlings with exposure to UV-C ($10 \text{ J m}^{-2} \text{ s}^{-1}$, 10 kJ m^{-2} in total) followed by 24 h in darkness (S5d+UVC/d). The PCR condition was 35 cycles for *OsMUS81α* and *OsMUS81β*.

observed in other recombinational repair genes including *RAD54* and two *RAD51* (*OsRAD51A-1* and -2) genes from rice (N. Mimida et al., unpublished results) and with *RAD51* (Klimyuk and Jones 1997, Doutriaux et al. 1998, Osakabe et al. 2002) and *RAD54* (Osakabe et al. 2006). Moreover, the amplification profile with the ‘relative quantitative RT-PCR’ system showed that both *OsMUS81* transcripts were dramatically up-regulated by radiation of etiolated rice seedlings with sunlight and UV-C. The relative expression levels of *OsMUS81α* were found to be much higher than those of *OsMUS81β* (Fig. 4B). These findings suggest a role for *OsMUS81* in the DNA damage response. The high level expression of *OsMUS81α* and $-β$ in mature leaves (Fig. 4A) was also confirmed in this experiment (Fig. 4B).

Rice leaves receive strong UV radiation through sunlight, which is harmful to the maintenance of genome integrity (Hidema and Kumagai 2006). The budding yeast *mus81* mutant exhibits sensitivity to UV (Intherthal and Heyer 2000). The human hereditary syndrome, xeroderma pigmentosum (XP), which is characterized by sensitivity to sunlight (Enzlin and Schärer 2002), results in NER deficiency. In NER, the XPF-ERCC1 endonuclease complex repairs DNA cross-linking damage caused by compounds such as mitomycin C and cisplatin (Sijbers et al. 1996). Recently, ERCC1 has been shown to function in HR, and the ERCC1-XPF complex plays important roles in various DNA repair processes (Niedernhofer et al. 2001). XPF-ERCC1 shares similarity with Mus81-Mms4 in their structures (reviewed in Heyer et al. 2003). Mouse Emel/Mms4 is expressed at a high level in skin. Mouse embryonic stem cells with a loss-of-function mutation showed sensitivity to DNA cross-linking damage and spontaneous genomic instability (Abraham et al. 2003, Dendouga et al. 2005). Mus81-Emel/Mms4 is not expected to function in NER of UV damage, but is probably critical for the recovery of RFs stalled by UV and other types of DNA damage. Interestingly, rice oligo microarray analysis revealed that various MMR genes were more actively expressed in mature leaves rather than shoot apices, in which most of the excision repair genes were more actively expressed (Kimura et al. 2004). The MMR genes play important roles in repairing mispaired bases that arise during DNA replication and recombination, and maintaining genomic stability (Surtees et al. 2004, Kimura and Sakaguchi 2006). The observation by Kimura et al. (2004) may suggest that OsMus81 functions in association with the MMR system.

The RecQ helicase family is conserved from bacteria to human, and shows ATP-dependent 3'-5' DNA helicase activity to unwind the DNA duplex. RecQ helicases play important roles in the maintenance of genome stability through the regulation of DNA repair and recombination pathways. Bloom's syndrome is a hereditary cancer-predisposition disorder caused by mutations in a RecQ helicase gene, *BLM* (Hickson 2003). Interestingly, BLM stimulates Mus81 endonuclease activity by enhancing binding of Mus81 to stalled RFs in human cells (Zhang et al. 2005).

Hartung et al. (2006) have reported the role of *Arabidopsis MUS81*. Two T-DNA insertion lines of *AtMUS81* were sensitive to MMS and mitomycin C, and showed synthetic lethality when combined with a mutation in the *Atrecq4A* helicase gene, indicative of two alternative repair pathways for RF arrest. In budding yeast, the *mus81* mutant also exhibited lethality in combination with mutation in the *SGS1* helicase gene (Fabre et al. 2002). Despite the high degree of homology among the helicase

domains of AtRecQ4A, Sgs1 and BLM, and hyper-recombination phenotypes caused by individual mutations (Gangloff et al. 1994, Hickson 2003, Bagherieh-Najjar et al. 2005), AtRecQ4A and Sgs1 may have different roles from BLM that displays direct interaction with Mus81 (Zhang et al. 2005). Because seven *RecQ* homologs have been found in both Arabidopsis and rice genomes (Hartung and Puchta 2006, Saotome et al. 2006), future studies may identify the RecQ homolog(s) interacting with Mus81 (Zhang et al. 2005) and 5' flap endonuclease/5'-3' exonuclease (FEN-1) (Sharma et al. 2005). Moreover, BLM activity to process HJs was stimulated by the MSH2/6 mismatch repair complex (Yang et al. 2004). It has also been demonstrated that mouse Mus81 is required for genome stability (McPherson et al. 2004, Dendouga et al. 2005).

We characterized the expression pattern of rice *RAD54* (*OsRAD54*). *OsRAD54* was expressed in various tissues at low levels: relatively higher in young panicles and calli, and much lower in mature leaves. *OsRAD54* expression was induced by γ -radiation (N. Mimida et al. unpublished results). Therefore, the expression pattern of *OsMUS81* shares some similarity with that of *OsRAD54*, exhibiting strong expression in panicles and induction by radiation, while displaying the distinct difference in mature rice leaves that rarely proliferate. When the interaction between the two gene products (Fig. 3) is considered, OsMus81 is likely to function in meiotic HR in cooperation with Rad54.

In conclusion, our findings imply that rice Mus81 functions in both meiosis and mitosis in cooperating with recombinational and mismatch DNA repair activities, respectively. Since the relationship between Mus81 and Rad54 is poorly understood even in yeast, it would be meaningful to elucidate the function(s) in plants. In addition, an Mms4/Emel homolog in higher plants, the partner of Mus81, needs to be isolated and characterized to define the functions of the Mus81-Mms4/Emel endonuclease complex in HR and other DNA repair pathways. These studies would help us to understand mechanisms in plants to protect them from various genome stresses and to keep their genome integrity while sustaining normal plant growth.

Materials and Methods

Yeast two-hybrid experiments were performed with the DupLEX-A™ yeast two-hybrid system (OriGene Technologies). The following rice cDNA clones were used in this test: *OsRAD54*, *OsMUS81 α* and - β lacking the second HhH motif, and truncated constructs bearing a C-terminal region of *OsRAD54* and the XPF-like motif of *OsMUS81*. Each cDNA was subcloned in-frame into the plasmid pJG4-5 carrying the B42 transcription activation domain (AD) and the plasmid pEG202 carrying the LexA DNA-binding domain (BD).

A yeast strain EGY48 (*MAT α trp1 his3 ura3 leu2::6LexAop-LEU2*) was transformed with the reporter plasmid pSH18-34 containing the *GAL1-LacZ* gene under the control of eight LexA operators. EGY48 carrying pSH18-34 was co-transformed with the appropriate combination of pEG202- (BD) and pJG4-5-derived (AD) fusion plasmids. To assay the interaction between bait and prey, the resulting yeast transformants were tested for β -galactosidase activity qualitatively on selective agar medium, Minimal SD Base (Difco), -His-Trp-Ura DO supplement (Clontech) containing 0.7% sodium phosphate (dibasic) + 0.3% sodium phosphate (monobasic), 2% galactose + 1% raffinose as a carbon source and 0.2 mM 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal).

To analyze tissue-specific expression patterns of *OsMUS81 α* and - β , total RNAs were extracted from different tissues of rice (cv. Nipponbare) with an RNeasy Plant Mini Kit and purified with an OligotexTM-dT30<Super> mRNA Purification Kit (TAKARA BIO INC.). Rice calli were γ -irradiated with 100 Gy (50 Gy h⁻¹) using a ⁶⁰Co source. Irradiated calli were incubated for 6 h at 30°C under continuous light (60 μ mol m⁻² s⁻¹). Reverse transcription reactions were carried out in a 20 μ l reaction mixture containing 2 μ g of mRNA and ReverTra Ace reverse transcriptase (Toyobo). The subsequent PCR was performed using 0.2 μ l of reverse transcription product as a template, KOD-Plus-polymerase (Toyobo) and a pair of cDNA-specific primers for *OsMUS81 α* (OsMUS81-FE, 5'-GTAACCCTGCAAAGTATATGATAACTG AAG-3'; and OsMUS81-R α E, 5'-ATTCGGTTATTGAATGAG TAAGGTTGCC-3'), *OsMUS81 β* (OsMUS81-FE and OsMUS81-R β E, 5'-AAACTCATCATAGGTCAGGCAAAAAGTCTCA-3') and rice *Actin-1* (Actin-F1, 5'-TGGAAGTGGTATGGTCAAG GCTGGGTTCCCGGA-3'; and Actin-R1, 5'-CCAGAGTCCA ACACAATACCTTGGGTACGACCACTGGC-3') (accession No. X16280) as a control.

Relative quantitative RT-PCR with QuantumRNATM 18S Internal Standards (Ambion) was carried out to semi-quantitate the two *OsMUS81* transcripts for radiation-specific expression.

Supplementary material

Supplementary material mentioned in the article is available to online subscribers at the journal website www.pcp.oxfordjournals.org.

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References

Abraham, J., Lemmers, B., Hande, M.P., Moynahan, M.E., Chahwan, C., et al. (2003) Emel is involved in DNA damage processing and

- maintenance of genomic stability in mammalian cells. *EMBO J.* 22: 6137–6147.
- Bagherieh-Najjar, M.B., de Vries, O.M., Hille, J. and Dijkwel, P.P. (2005) Arabidopsis RecQ1A suppresses homologous recombination and modulates DNA damage responses. *Plant J.* 43: 789–798.
- Boddy, M.N., Lopez-Girona, A., Shanahan, P., Interthal, H., Heyer, W.D. and Russell, P. (2000) Damage tolerance protein Mus81 associates with the FHA1 domain of checkpoint kinase Cds1. *Mol. Cell. Biol.* 20: 8758–8766.
- Dendouga, N., Gao, H., Moechars, D., Janicot, M., Vialard, J. and McGowan, C.H. (2005) Disruption of murine Mus81 increases genomic instability and DNA damage sensitivity but does not promote tumorigenesis. *Mol. Cell. Biol.* 25: 7569–7579.
- Doutriaux, M.P., Couteau, F., Bergounioux, C. and White, C. (1998) Isolation and characterisation of the *RAD51* and *DMC1* homologs from *Arabidopsis thaliana*. *Mol. Genet.* 257: 283–291.
- Enzlin, J.H. and Schärer, O.D. (2002) The active site of the DNA repair endonuclease XPF-ERCC1 forms a highly conserved nuclease motif. *EMBO J.* 21: 2045–2053.
- Fabre, F., Chan, A., Heyer, W.D. and Gangloff, S. (2002) Alternative pathways involving Sgs1/Top3, Mus81/Mms4, and Srs2 prevent formation of toxic recombination intermediates from single-stranded gaps created by DNA replication. *Proc. Natl Acad. Sci. USA* 99: 16887–92.
- Fu, Y. and Xiao, W. (2003) Functional domains required for the *Saccharomyces cerevisiae* Mus81-Mms4 endonuclease complex formation and nuclear localization. *DNA Repair* 2: 1435–1447.
- Gangloff, S., McDonald, J.P., Bendixen, C., Arthur, L. and Rothstein, R. (1994) The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. *Mol. Cell. Biol.* 14: 8391–8398.
- Hartung, F. and Puchta, H. (2006) The RecQ gene family in plants. *J. Plant Physiol.* 163: 287–296.
- Hartung, F., Suer, S., Bergmann, T. and Puchta, H. (2006) The role of AtMUS81 in DNA repair and its genetic interaction with the helicase AtRecQ4A. *Nucleic Acids Res.* 34: 4438–4448.
- Heyer, W.D., Ehmsen, K.T. and Solinger, J.A. (2003) Holliday junctions in the eukaryotic nucleus: resolution in sight? *Trends Biochem. Sci.* 28: 548–557.
- Hickson, I.D. (2003) RecQ helicases: caretakers of the genome. *Nat. Rev. Cancer* 3: 169–178.
- Hidema, J. and Kumagai, T. (2006) Sensitivity of rice to ultraviolet-B radiation. *Ann. Bot.* 97: 933–42.
- Hollingsworth, N.M. and Brill, S.J. (2004) The Mus81 solution to resolution: generating meiotic crossovers without Holliday junctions. *Genes Dev.* 18: 117–125.
- Interthal, H. and Heyer, W.D. (2000) *MUS81* encodes a novel helix-hairpin-helix protein involved in the response to UV- and methylation-induced DNA damage in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 263: 812–827.
- Kimura, S., Tahira, Y., Ishibashi, T., Mori, Y., Mori, T., Hashimoto, J. and Sakaguchi, K. (2004) DNA repair in higher plants; photoreactivation is the major DNA repair pathway in non-proliferating cells while excision repair (nucleotide excision repair and base excision repair) is active in proliferating cells. *Nucleic Acids Res.* 32: 2760–2767.
- Kimura, S. and Sakaguchi, K. (2006) DNA repair in plants. *Chem. Rev.* 106: 753–66.
- Klimyuk, V.I. and Jones, J.D. (1997) *AtDMC1*, the *Arabidopsis* homologue of the yeast *DMC1* gene: characterization, transposon-induced allelic variation and meiosis-associated expression. *Plant J.* 11: 1–14.
- McPherson, J.P., Lemmers, B., Chahwan, R., Pamidi, A., Migon, E., et al. (2004) Involvement of mammalian Mus81 in genome integrity and tumor suppression. *Science* 304: 1822–1826.
- Niedernhofer, L.J., Essers, J., Weeda, G., Beverloo, B., de Wit, J., Muijtens, M., Odijk, H., Hoeijmakers, J.H.J. and Kanaar, R. (2001) The structure-specific endonuclease ERCC1-XPF is required for targeted gene replacement in embryonic stem cells. *EMBO J.* 20: 6540–6549.
- Osakabe, K., Yoshioka, T., Ichikawa, H. and Toki, S. (2002) Molecular cloning and characterization of *RAD51*-like genes from *Arabidopsis thaliana*. *Plant Mol. Biol.* 50: 71–81.

- Osakabe, K., Abe, K., Yoshioka, T., Osakabe, Y., Todoriki, T., Ichikawa, H., Hohn, B. and Toki, S. (2006) Isolation and characterization of the *RAD54* gene from *Arabidopsis thaliana*. *Plant J.* 48: 827–842.
- Saotome, A., Kimura, K., Mori, Y., Uchiyama, Y., Morohashi, K. and Sakaguchi, K. (2006) Characterization of four RecQ homologues from rice (*Oryza sativa* L. cv. Nipponbare). *Biochem. Biophys. Res. Commun.* 345: 1283–1291.
- Sharma, S., Sommers, J.A., Gary, R.K., Friedrich-Heineken, E., Hübscher, U. and Brosh, R.M., Jr. (2005) The interaction site of Flap endonuclease-1 with WRN helicase suggests a coordination of WRN and PCNA. *Nucleic Acids Res.* 33: 6769–6781.
- Sijbers, A.M., de Laat, W.L., Ariza, R.R., Biggerstaff, M., Wei, Y.F., et al. (1996) Xeroderma pigmentosum group F caused by a defect in a structure-specific DNA repair endonuclease. *Cell* 86: 811–822.
- Surtees, J.A., Argueso, J.L. and Alani, E. (2004) Mismatch repair proteins: key regulators of genetic recombination. *Cytogenet. Genome Res.* 107: 146–159.
- Vonarx, E.J., Howlett, N.G., Schiestl, R.H. and Kunz, B.A. (2002) Detection of *Arabidopsis thaliana AtRAD1* cDNA variants and assessment of function by expression in a yeast *rad1* mutant. *Gene* 296: 1–9.
- Yang, Q., Zhang, R., Wang, X.W., Linke, S.P., Sengupta, S., et al. (2004) The mismatch DNA repair heterodimer, hMSH2/6, regulates BLM helicase. *Oncogene* 23: 3749–3756.
- Zhang, R., Sengupta, S., Yang, Q., Linke, S.P., Yanaihara, N., Bradsher, J., Blais, V., McGowan, C.H. and Harris, C.C. (2005) BLM helicase facilitates Mus81 endonuclease activity in human cells. *Cancer Res.* 65: 2526–31.

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