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Biochemical and Biophysical Research Communications 329 (2005) 668–672
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www.elsevier.com/locate/ybbrc

The expression of the rice (*Oryza sativa* L.) homologue of *Snm1* is induced by DNA damages

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Received 20 January 2005

Abstract

We isolated and characterized the rice homologue of the DNA repair gene *Snm1* (*OsSnm1*). The length of the cDNA was 1862 bp; the open reading frame encoded a predicted product of 485 amino acid residues with a molecular mass of 53.2 kDa. The OsSnm1 protein contained the conserved β-lactamase domain in its internal region. *OsSnm1* was expressed in all rice organs. The expression was induced by MMS, H_2O_2, and mitomycin C, but not by UV. Transient expression of an OsSnm1/GFP fusion protein in onion epidermal cells revealed the localization of OsSnm1 to the nucleus. These results suggest that *OsSnm1* is involved not only in the repair of DNA interstrand crosslinks, but also in various other DNA repair pathways.

Keywords: Rice (*Oryza sativa* L.); DNA repair; Interstrand crosslinks; Snm1

Living organisms have to protect the integrity of their genomes from a wide range of genotoxic stresses. Numerous environmental mutagenic agents such as UV-light, chemical mutagens, fungal and bacterial toxins, and ionizing radiation can cause damage to DNA. Recently, we have isolated and characterized several plant genes related to DNA repair [1–11]. However, in general, little is known about plant DNA repair in comparison with animals or yeasts, and there is clearly a need to fill this gap [12–16].

Interstrand crosslinks (ICLs) are a highly toxic form of DNA damage [17,18]. ICLs can be induced by bifunctional alkylating agents such as nitrogen mustard and mitomycin C. ICLs block transcription, replication, and segregation of DNA, and eventually lead to cell death [19] which causes undesirable effects on the growth and yield of commercially cultivated plants. Therefore, the repair of ICLs is essential for the growth and survival of plants, and an understanding of how ICLs are repaired is important.

Numerous genes are involved in the repair of ICLs [17,18,20]. The ICL repair involves proteins that also act in nucleotide excision repair (NER) [18,20] and homologous recombination (HR) [20,21]. *Snm1* (sensitive to nitrogen mustard) is thought to function specifically in ICL repair; it was first identified in yeast by screening for mutants sensitive to the bifunctional alkylating agent nitrogen mustard [22–24]. Yeast *snm1* mutants respond strongly to agents that cause ICLs, but they are only moderately sensitive to monofunctional alkylating agents and UV [22,24,25]. Snm1 proteins are nuclear proteins containing a conserved β-lactamase domain [19,26]. Yeast Snm1 has 5′-exonuclease activity that is required for Snm1 to be functional in ICL repair [27]. These results suggested that Snm1 is involved in the processing of intermediates of the ICL repair mechanism. Human Snm1 colocalized and physically

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doi:10.1016/j.bbrc.2005.01.161

associated with 53BP1 before and after UV damage [28]. Mammalian Snm1 is a component of a mitotic stress checkpoint that negatively targets the APC (anaphase-promoting complex) prior to chromosome condensation [29]. These results indicated novel functions of Snm1 proteins.

One way to understand the mechanisms of ICL repair in plant is to study the plant homologue of *Snm1*. Recently, the *Arabidopsis* homologue of *Snm1* (*AtSNM1*) was identified and characterized [30]. Interestingly, *AtSNM1*-deficient mutants were not hypersensitive to mitomycin C (MMC), but showed a moderate sensitivity to bleomycin and H_2O_2. The mutants exhibited a delayed repair of oxidative DNA damage and did not show an enhancement of the frequency of somatic homologous recombination on exposure to H_2O_2. These results suggested the existence in plants of Snm1-dependent recombinational repair processes of oxidatively induced DNA damage [30].

To further elucidate the mechanism of ICL repair in plants, we have isolated and characterized a rice (*Oryza sativa* L.) homologue of *Snm1* (*OsSnm1*).

Materials and methods

Plant materials. Rice plants (*Oryza sativa* L. cv. Nipponbare) were grown in a growth cabinet under a 16 h day/8 h night light regime at 28 °C. A suspension culture of rice cells (*Oryza sativa* L. cv. Nipponbare) was maintained as described previously [31].

Identification and cloning of OsSnm1. The rice full-length cDNA database, KOME (Knowledge-based Oryza Molecular biological Encyclopedia) [32], was searched using the Blastp program to identify rice homologues of *Snm1*. The full-length cDNA clone J033149H08 (Accession No. AK103868) was found to have significant homology with yeast *Snm1* and was designated *OsSnm1* (*Oryza sativa Snm1*). *OsSnm1* cDNA was provided by the Rice Genome Resource Center (http://www.rgrc.dna.affrc.go.jp/index.html.en). Sequence and phylogenetic analyses were performed using Genetyx-MAC ver. 12 (Genetyx cooperation).

Expression analysis. Expression patterns of *OsSnm1* were examined by RT-PCR analysis. Total RNA was isolated from rice plants or cultured rice cells using the RNeasy Plant Mini Kit (Qiagen). RT-PCR analysis was performed using SuperScript One-Step RT-PCR with Platinum *Taq* (Invitorgen). RNA isolation and RT-PCR were performed according to the manufacturer's protocols. The following primers were used for RT-PCR amplification: Snm1-F, 5′-CGAGGA GTCC, GGGAAGAACA-3′; Snm1-R, 5′-AACCAAGCGA, GCAGT TAGGG-3′.

Subcellular localization of OsSnm1. The subcellular localization of OsSnm1 was determined by transient expression and visualization of a GFP/OsSnm1 fusion protein in onion epidermal cells. The coding sequence of *OsSnm1* was amplified and cloned into CaMV35S-sGFP (S65T)-nos3′ [33] to fuse OsSnm1 to the C-terminus of GFP. For transient expression in onion epidermal cells, 1.0 μm gold particles were coated with 5 μg of the plasmid DNA, and were then shot into the tissue using a Biolistic PSD-1000/He Particle Delivery System (Bio-Rad) according to the manufacturer's recommendations. The conditions of bombardment were: vacuum of 27 in. of Hg, helium pressure of 1100 psi, and 900 dpi rupture discs. After bombardment, tissues were incubated at room temperature for 12 h. GFP fluorescence in the bombarded onion epidermal cells was observed by fluorescence microscopy.

Results and discussion

Identification and molecular cloning of OsSnm1

We searched the rice full-length cDNA database, KOME, for rice homologues of Snm1 and found that clone J033149H08 (Accession No. AK103868) shared significant homology with yeast Snm1. We designated the clone *OsSnm1* (*Oryza sativa Snm1*). The length of the cDNA was 1862 bp, and the open reading frame of the cDNA encoded a predicted product of 485 amino acids with a molecular mass of 53.2 kDa. The chromosomal location and the structure of the *OsSnm1* gene were determined using rice genome sequence data (http://rgp.dna.affrc.go.jp/index.html). *OsSnm1* was mapped to chromosome 4, and 10 exons and nine introns were identified (Fig. 1).

Snm1 possesses a β-lactamase domain which is essential for the nuclease activity and which is required for the repair of double strand breaks that result from ICL removal [19,27]. OsSnm1 also has such a β-lactamase domain in its internal region (Fig. 2). In contrast to OsSnm1, Artemis, one of the human Snm1-related proteins, has a β-lactamase domain in the N-terminal region.

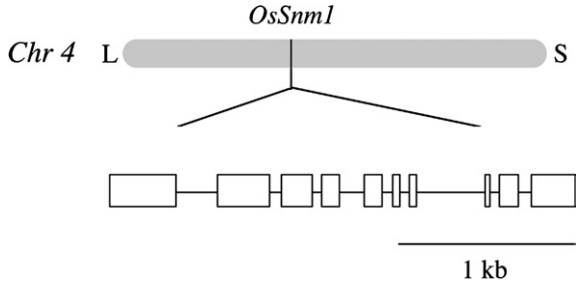

Fig. 1. Chromosome localization and genomic structure of the *OsSnm1* gene. *OsSnm1* is localized in chromosome 4. Boxed areas are exons, lines represent introns.

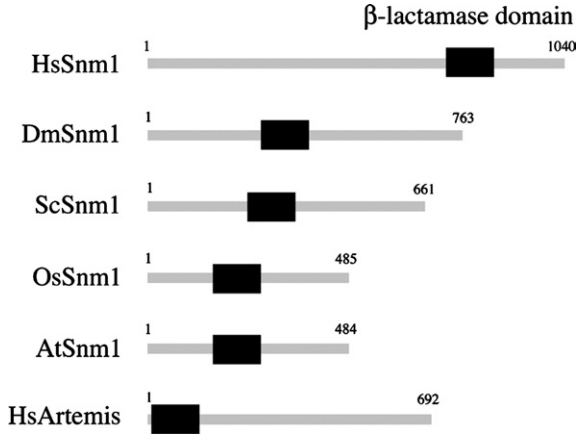

Fig. 2. Position of the β-lactamase domain (black boxes) in several homologous Snm1 proteins.