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# Alternative splicing of the rice *OsMET1* genes encoding maintenance DNA methyltransferase

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## Summary

While the *Arabidopsis* genome carries one copy of the *methyltransferase 1* (*MET1*) gene for DNA methyltransferase, which is mainly responsible for maintaining CpG methylation, the rice genome bears two copies of the *MET1* genes, *OsMET1a* and *OsMET1b*. The transcripts of *OsMET1b* accumulate more abundantly than those of *OsMET1a* in all of the tissues examined, and both genes actively transcribed at the callus, imbibed embryo, root, meristem, young panicle, anther, pistil, and endosperm, all of which contain actively dividing cells. The *OsMET1a* transcripts contain two 5′-untranslated exons and alternatively spliced 3′-terminal exons. The alternatively spliced transcripts consist of 14, 15, or 16 exons, and all of them encode a putative protein of 1527 amino acids. While the 3′-terminal exon of *OsMET1b* is unique, alternative splicing occurs in the 5′-terminal regions, which comprise either exons containing 5′-untranslated regions or an exon bearing the initiation codon. Depending upon alternative usage of 5′ exons by alternative splicing, the *OsMET1b* transcripts comprise 11, 12, 13, or 14 exons, and the former two and the latter two longer transcripts encode putative proteins of 1486 and 1529 amino acids, respectively. Moreover, the 5′ splicing patterns of *OsMET1b* can vary in different tissues. These findings are discussed with respect to the possible regulation of the *OsMET1* genes.


*Abbreviations:* Dnmt1, DNA methyltransferase 1; eEF1α, eukaryotic elongation factor 1α; MET1, methyltransferase 1; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription-polymerase chain reaction; UTR, untranslated region.

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## Introduction

DNA methylation in plants plays two fundamental roles, defending against invasive DNA elements, such as transposons, and regulating gene expression, and these biological roles have been extensively studied in *Arabidopsis* (Finnegan and Kovac, 2000; Chan et al., 2005). Among various plant DNA methyltransferases, the first enzyme identified and characterized was *Arabidopsis* methyltransferase 1 (MET1) (Finnegan and Dennis, 1993), which is thought to be orthologous to the mammalian DNA methyltransferase 1 (Dnmt1) enzyme and mainly responsible for maintaining CpG methylation (Chan et al., 2005). To characterize the *MET1* gene function in *Arabidopsis*, transgenic plants with an antisense construct were generated (Finnegan et al., 1996; Ronemus et al., 1996). Subsequently, both missense mutants (Kankel et al., 2003; Xiao et al., 2003) and nonsense mutants (Xiao et al., 2003, 2006) induced by ethyl methanesulfonate (EMS) mutagenesis as well as T-DNA insertion mutants (Saze et al., 2003) were isolated and characterized. Among these mutants, the *met1* null mutants display severe distinctive traits, including the highly frequent occurrence of developmental abnormalities (Saze et al., 2003; Xiao et al., 2003, 2006), and CpG methylation maintained by MET1 plays a critical role as a coordinator of epigenetic memory, which accomplishes stable transgenerational inheritance (Mathieu et al., 2007). While *Arabidopsis* carries only one copy of the *MET1* gene, rice bears two *MET1* copies, *OsMET1-1* (AF462029) and *OsMET1-2* (BK001405), on chromosomes 3 and 7, respectively (Teerawanichpan et al., 2004). Based on the comparison of the rice genomic sequence with partial reverse transcription-polymerase chain reaction (RT-PCR) and 3′ rapid amplification of cDNA ends (RACE) analyses, Teerawanichpan et al. (2004) have assigned that *OsMET1-1* and *OsMET1-2* comprise 12 and 11 exons (Figure 1) and encode putative proteins of 1522 and 1497 amino acids, respectively, and that the accumulation of the *OsMET1-2* mRNA is abundant in callus, young (10-d old) root, and inflorescence, whereas only the *OsMET1-1* mRNA was slightly accumulated in young leaf, in which virtually no *OsMET1-2* transcripts were detectable.

We are developing a rice gene targeting system by homologous recombination (Terada et al., 2002, 2007; Iida et al., 2007) and planning to generate rice knock-in mutants having the *GUS* reporter gene fused to the endogenous promoters of the *OsMET1* genes (Johzuka-Hisatomi et al., 2008). To accomplish these ends, it is prerequisite to determine the precise transcriptional and translational initiation sites of the *OsMET1* genes in order to fuse the reporter gene to appropriate sequences at their 5′ regions. Reinvestigation of the *OsMET1* transcripts revealed that transcriptional initiation sites and 5′-untranslated regions (UTRs), as well as the exons containing the translation initiation codon of the *OsMET1* genes, are all different from those previously reported by Teerawanichpan et al. (2004). Consequently, the sizes of the predicted OsMET1 proteins and their N-terminal amino acid sequences also differ slightly from those reported previously. Furthermore, we found that alternative splicing takes place in both *OsMET1* genes and appears to be regulated in a tissue-specific manner. To our knowledge, this is the first report on the occurrence of alternative splicing in plant *MET1* genes.

### Materials and methods

#### Plant materials

Rice (*Oryza sativa* L. subspecies *japonica* cv. Nipponbare) seeds were surface-sterilized in sodium hypochlorite (2.5% v/v) for 30 min, rinsed thoroughly with sterile distilled water, and then germinated on a 1/2 MS medium (Murashige and Skoog, 1962) at 25 °C for 2 weeks, transferred to soil, and grown to maturity in a greenhouse at 27 °C. Young shoot and root samples were collected at 2 weeks after germination, adult tissue samples at 1 month, and floral tissue samples immediately before flowering. Callus was induced from mature seeds and maintained as described previously (Terada et al., 2002).

#### Nucleic acid procedures

General nucleic acid procedures, including plant DNA and RNA preparation, PCR and RT-PCR amplification, 5′- and 3′-RACE analyses, and DNA sequencing analysis, were performed as described before (Terada et al., 2007), and the sequences of primers used are listed in Table 1. For RT-PCR analysis, first-strand cDNAs were synthesized using SuperScript III reverse transcriptase (Invitrogen, USA), and LA Taq polymerase (Takara Bio, Japan) was used for the subsequent PCR amplification with appropriate primers (Table 1): initial denaturation (94 °C for 1 min) and 28–35 cycles of denaturation (94 °C for 30 s), annealing (60–63 °C for 30 s), extension (72 °C for 1 min), and final extension (72 °C for 7 min). For the preparation of nearly full-length *OsMET1* cDNAs, 42 cycles of the subsequent PCR amplification were performed in the same way, except for annealing (58 °C for 30 s) and extension (72 °C for 10 min). To compare the accumulation of the two *OsMET1* transcripts, a mixture of RT-PCR-amplified fragments with primers cMF and cMR (Table 1) was digested with either *Nsb*I or *Msp*I. The PCR reactions used were as follows: initial denaturation (94 °C for 1 min) and 36 cycles of denaturation (94 °C for 30 s), annealing (55 °C for 30 s), extension (72 °C for 1 min), and