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RICE (*Oryza sativa*)

NOPMANEE WIRIYAWUTIKORN : CLONING AND CHARACTERIZATION
OF THE cDNA ENCODING RICE DNA METHYLTRANSFERASE. THESIS
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DNA methylation is a specific post-replicative modification of DNA. The methylated DNA is found in diverse organisms. Especially in higher eukaryotes, it has been implicated in the regulation of a number of cellular processes in both vertebrates and plants. The most abundant modified base in DNA of higher eukaryotes is 5-methylcytosine. The level of methylated cytosine in plants can be as high as 30% of total cytosine residues. This important phenomenon is catalyzed by C-5 cytosine methyltransferase (C-5 DNA MTase). We have attempted to isolate and characterize the cDNA encoding this enzyme from rice (*Oryza sativa* ssp. *indica* cv. RD 23), a monocotyledonous plant. C-5 DNA MTase activity was previously reported by our laboratory to be greatest at 10 days in shoots of rice seedlings. The cDNA library was prepared from shoots of 10-day-old seedlings using λ ZAP II. The degenerated primers were designed in the conserved regions of C-5 DNA MTase from *Arabidopsis thaliana*, and PCR was performed using rice cDNA as template. The 274 bp-PCR product was used as a probe to screen for the full length cDNA clone. Three positive clones were excised from λ ZAP II and further sequenced. One putative clone, named pRM1, showed the sequence homology to eukaryotic C-5 DNA MTase cDNA. The 4,985 bp assembled nucleic acid sequence contains an open reading frame of 4,503 bp encoding a protein of 1,501 amino acids, with molecular weight of 167 kDa. Like other eukaryotic C-5 DNA MTases, the inferred protein of rice C-5 DNA MTase has a presumed regulatory N-terminal region linked to a catalytic C-terminal domain by Lysine-Glycine linker. The C-terminal domain has eight conserved motifs found in other C-5 DNA MTase. Using the pRM1 as a probe, we identified a transcript of 4.98 kb. Southern blot analysis of rice genomic DNA with the above probe indicated the presence of a single gene. Northern blot analysis was performed to study the expression of C-5 DNA MTase in rice. The transcripts of C-5 DNA MTase gene was found to be developmental specific and tissue specific. The knowledge gained about the molecular basis of DNA methyltransferase will allow further study of the function and regulation of this gene in rice.