04-1-46

Molecular Characterization of a Gene for Monocot RING-Zn Finger Protein of Rice

Sang-Gu Kang*, Minkyeong Kim

School of Biotechnology and Institute of Biotechnology, Yeungnam University, 214 Dae-dong, Gyeongsan 712-749, Gyeongbook, Korea

Objectives

To characterize for molecular functions of a rice (*Oryza sativa* L.) cDNA RAP19.

Materials and Methods

Plant gene cloning, nucleotide sequencing, Southern blot analysis, Northern blot analysis, and protein expression and purification were performed .

Results and Discussions

Rice cDNA RAP19 contained a putative RING-H2 zinc-finger protein, named OsRH2ZF. In the amino terminus of the deduced amino acid sequences of OsRH2ZF cDNA contained 394 amino acids with a RING-finger (Really Interesting New Gene) domain, which is specialized type of Zn-finger of 40 residues that binds two atoms of zinc. The domain can be conformed Zn-fingers of the cross-brace motif type, C3H2C3 (RING-H2 finger). The function of RING-H2 zinc-finger protein of plant is unclear. The genomic locus of OsRH2ZF is consisted with 9 exons on the chromosome 11. The gene expression of RAP19 (OsRH2ZF) is high expressed in leaf and up-regulated in flowers in meiosis and gradually increased in 5 days after pollination, suggesting that OsRH2ZF may related to the functions in gene expression of reproductive stages. Therefore, we examined the function of protein OSRH2ZF. RAP 19 (OsRH2ZF) were cloned into the *Escherichia coli* expression vector pGEX-2T (Pharmacies), creating C-terminal fusions with glutathione-*S*-transferase (GST). The constructs were expressed in the host strain BL21(DE3) grown in Luria broth. Cells were grown at 25°C or 37°C, and expression of the recombinant proteins was induced at an D_{600} of between 0.6 and 0.8, by the addition of isopropyl thio- - d-galactoside (0.1-0.4m). Protein about 43.3 kDa for RH2F fused with 24 kDa for GST were purified using glutathione affinity chromatography. OsRH2ZF was no nucleotide binding activity Electropheric Mobility Shift Assay using the purified protein, indicating that RH2ZF functions for some protein ubiquitination but not for gene transcription.

Corresponding author: Sang-Gu Kang, TEL: 053-810-2986, E-mail: kangsg@yu.ac.kr