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Knock-out mutations of *Arabidopsis SmD3-b* induce pleotropic phenotypes through altered transcript splicing

A.M. Swaraz[a], Young-Doo Park[b], Yoonkang Hur[a,*]

[a] *Department of Biological Sciences, Chungnam National University, Daejeon 305-764, Republic of Korea*
[b] *Department of Horticultural Biotechnology, Kyung Hee University, Yongin 446-701, Republic of Korea*

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ABSTRACT

SmD3 is a core protein of small nuclear ribonucleoprotein (snRNP) essential for splicing of primary transcripts. To elucidate function of SmD3 protein in plants, phenotypes and gene expression of *SmD3* knock-out and overexpressing mutants in *Arabidopsis* have been analyzed. *smd3-a* knock-out mutant or *SmD3-a* and *SmD3-b* overexpressors did not show phenotypic alteration. Knock-out of *SmD3-b* resulted in the pleotropic phenotypes of delayed flowering time and completion of life cycle, reduced root growth, partially defective leaf venation, abnormal numbers of trichome branches, and changed numbers of floral organs. Microarray data revealed that the *smd3-b* mutant had altered expression of genes related to the above phenotypes, indirectly suggesting that changed splicing of these genes may cause the observed phenotypes. Splicing of selected genes was either totally blocked or reduced in the *smd3-b* mutant, indicating the important role of SmD3-b in the process. A double knock-out mutant of *smd3-a* and *smd3-b* could not be generated, indicating possible redundant function of these two genes. All data indicate that SmD3-b may be major component of the spliceosomal snRNP in *Arabidopsis*, but the function of SmD3-a may be redundant.

1. Introduction

The introns, or the non-coding sequences, from pre-mRNA of a eukaryotic gene must be precisely removed by splicing to form functional mRNA. This process is a prerequisite to expressing intronic eukaryotic genes [1,2]. In *Arabidopsis* plants, introns are found in 79% of nuclear genes, 18% of chloroplast genes, and 12% of mitochondrial genes, while over 90% of vertebrate genes contain introns [3,4]. Splicing occurs cotranscriptionally by two-step transesterification reactions that occur at a 5′ splice site, a 3′ splice site, and the branch point of the pre-mRNA. These reactions are catalyzed by the spliceosome, which is a multi-megadalton complex composed of uridine-rich small nuclear ribonucleoproteins (UsnRNPs) and nearly 300 non-snRNP proteins [2,5–9].

The spliceosomal UsnRNPs are composed of one or two small nuclear RNAs (snRNA), Sm or Like-sm (Sm/Lsm) core proteins assembled by 7 heteromeric proteins (B, D1, D2, D3, E, F and G for Sm; Lsm1/8, Lsm2, Lms3, Lsm4, Lsm5, Lsm6 and Lsm7 for Lsm) and several snRNP associated proteins [2,10–15]. The Sm/Lsm proteins contain a conserved bipartite 'Sm fold' with two motifs, the Sm motif 1 and the Sm motif 2, and a common fold with an α-helix at the N-terminal [16–18]. The Sm/Lsm proteins assemble into characteristic heteromeric rings with a central pore and the presence of this ring is crucial for the U snRNA to bind with it in its Sm/Lsm site [19]. Among Sm/Lsm proteins, SmD1, SmD3, and SmB have long and positively charged C-terminal tails with glycine–arginine–glycine (GRG) and arginine–glycine (RG) motifs. These three proteins make direct contact with the 5′-splice site of pre-mRNA [20–22]. This information suggests that the SmD3 protein could function in pre-mRNA splicing as a core of UsnRNP.

Consensus sequences for splicing and spliceosome structure led to the suggestion that the pre-mRNA splicing mechanism in plants would be similar to that in animals [5,23,24]. However, the precise function of plant splicing factors is poorly understood because no *in vitro* splicing assay for plant cell extracts is currently available and because few mutations in the genes encoding spliceosomal proteins, such as *Lsm5* (*sad1*), stabilized1-1 (*sta1-1*), *Atsmu-1* and *Atsmu-2* mutants of *Arabidopsis*, have been identified [25–27]. The *sad1*, *sta1-1*, *Atsmu-1*, and *Atsmu-2* mutants had altered stress responses, morphological defects, retarded growth, and aberrant cotyledons and seeds, which indicated the pleotropic effects of the mutations on plant growth and development.

We have elucidated the function of SmD3 protein in plants by analyzing the phenotypes, transcriptome profiles, and splicing assays of selected genes. Knock-out of *SmD3-b* resulted in

* Corresponding author at: Department of Biological Sciences, College of Systems Biology, Chungnam National University, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, Republic of Korea. Tel.: +82 42 821 6279, fax: +82 41 822 9690.
E-mail address: ykhur@cnu.ac.kr (Y. Hur).

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pleotropic phenotype changes at both vegetative and reproductive stages and in altered splicing of some genes.

2. Materials and methods

2.1. Plant materials and growth conditions

The wild-type strain used was *Arabidopsis thaliana* (L.) Heynh var. Columbia (Col-0), and mutant strains were derived from it. Plants were grown with $140 \pm 2\ \mu mol/m^2/sec$ light intensity at $22 \pm 0.5\ ^\circ C$ with a long day cycle that had 16 h of light. Seeds were sown in 60 mm × 60 mm pots in potting soil, stratified for 3 days at 4 °C, and then placed in the growth room. The plants were then kept under a lid of transparent polythene for 5–6 days to increase humidity and support equal germination. In case of plate culture seeds were sterilized with 50% bleach with 0.1% Triton X-100 (Sigma, USA), stratified for 3 days at 4 °C, and sown in square dishes that were 125 mm × 125 mm × 20 mm or 100 mm × 100 mm × 20 mm. The dishes contained half-strength MS media (Duchefa Biochemie, The Netherlands) supplemented with 1% sucrose and 0.8% phyto agar. All the seed stocks, including both wild type and T-DNA insertional mutants, were collected from ABRC (http://www.arabidopsis.org).

2.2. Selection of homozygotes for T- DNA insertional mutants

Homozygous T-DNA insertional mutant lines for *SmD3-a* (SALK_025193) and *SmD3-b* (SALK_030487, SALK_006410, SALK_006417) were verified through genomic DNA polymerase chain reaction (PCR). We used the common T-DNA left border (LBa1) sequence 5′-TGGTTCACGTAGTGGGCCATCG and these gene-specific primers: SALK_025193 with LP: 5′-CTACCTATGCTGAAGCCGCTCGG-3′ and RP: 5′-GCTCGAGAACATCACCTATACGGCC-3′; and SALK_006410 and SALK_006417 with LP: 5′-CCTAGAATGCGTGATCCTGCCAGTG-3′ and RP: 5′-CAGGAACCGAACCTCTACACTCCATC-3′. The *SmD3* tran-

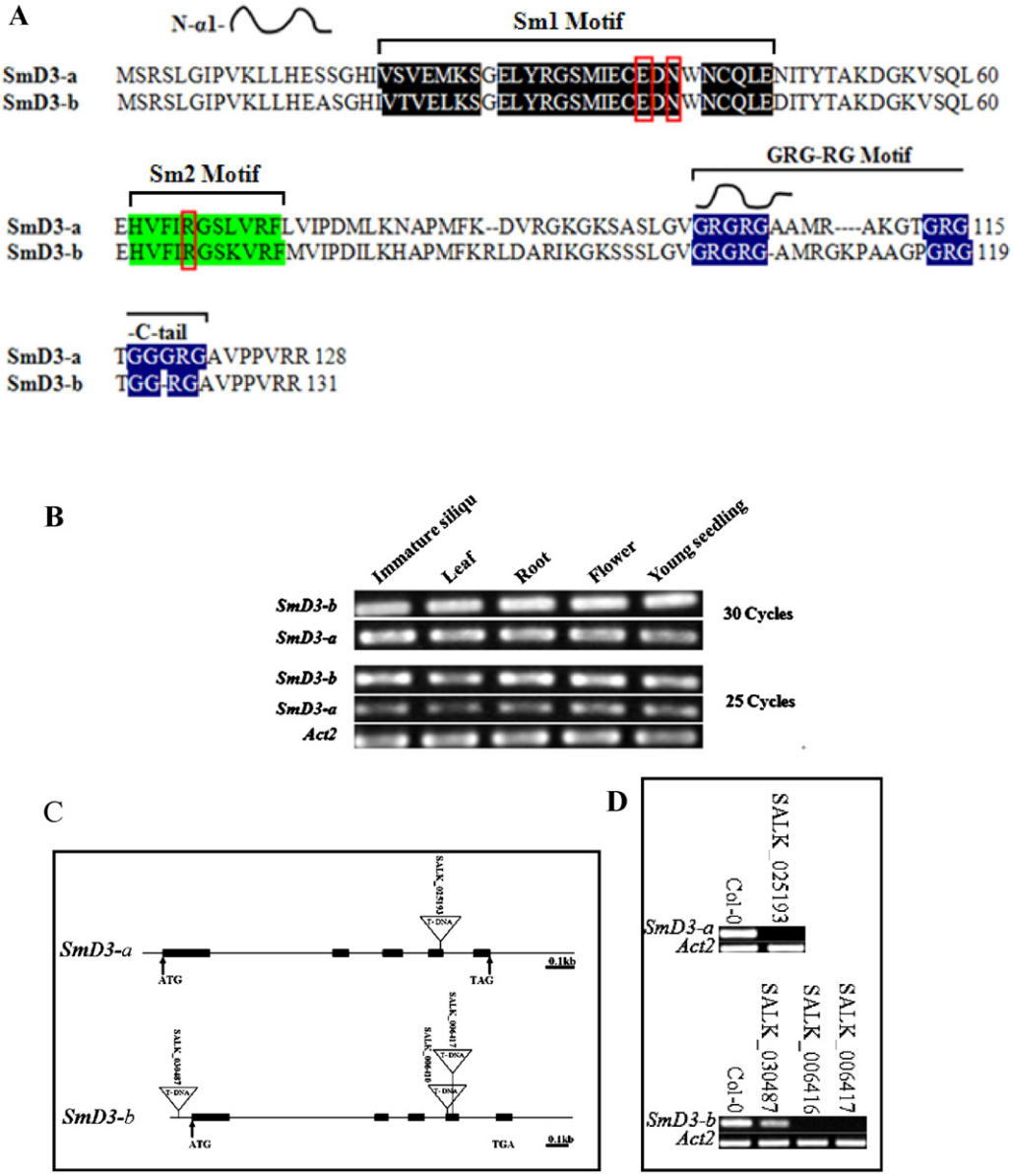

Fig. 1. Structure and expression of the T-DNA insertional mutants of *SmD3* genes. (A) Sequence alignment of two *Arabidopsis* SmD3 proteins shows the N-terminal α-helix, the Sm1 and Sm2 motif, the GRG–RG consensus C-terminal tail, and the RNA binding motif (boxed sequence). (B) Transcript levels of *SmD3* genes in various plant tissues as detected by semi-quantitative RT-PCR. (C) Positions of T-DNA insertions on SmD3 genes. (D) Transcript levels of the T-DNA insertional mutants.