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Alternative Splicing of an *Arabidopsis* Gene, *At2g24600*, Encoding Ankyrin-Repeat Protein

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Abstract**—**In *Arabidopsis*, several genes encoding proteins with ankyrin repeats and transmembrane domains (AtANKTM) have been identified as mediators of biotic and abiotic stress responses. It has been known that the expression of an *AtANKTM* gene, *At2g24600*, is induced in response to abiotic stress and that there are four splicing variants derived from this locus. In this study, by RT-PCR and sequencing analysis, an unknown splicing variant of the *At2g24600* transcript was identified. Based on differences in the predicted amino acid sequences, the five splicing variants are divided into three groups. The three predicted proteins are highly homologous, yet have different numbers of ankyrin repeats and transmembrane domains. It is generally considered that ankyrin repeats mediate protein-protein interaction and that the number of transmembrane domains affects membrane topology of proteins. The protein variants derived from the *At2g24600* locus may have different molecular functions each other.

Keywords**—**Alternative splicing, ankyrin repeats, transmembrane domains, *Arabidopsis*.

I. Introduction

ANKYRIN-REPEAT domains are present in a great variety of proteins in prokaryotes, eukaryotes and some viruses and often mediate protein–protein interactions [1]. Proteins containing ankyrin repeats are involved in diverse cellular functions.

In plants, ankyrin-repeat proteins are involved in plant responses to biotic and abiotic stresses. Because of the importance of ankyrin-repeat proteins in plants, genome-wide localization, phylogenetic relationships and expression profiles have been analyzed in *Arabidopsis* [2] and rice [3]. In the *Arabidopsis* genome, 105 genes encoding ankyrin-repeat proteins have been identified. Becerra et al. [2] classified these genes in 16 groups based on their structural similarity. The most abundant group contains 37 genes encoding proteins with ankyrin repeats and transmembrane domains (named the AtANKTM family), and four of these genes, *ACD6*, *BDA1*, *ITN1* and *DRA1* have been functionally characterized as mediators of stress responses so far [4]-[9].

ACD6 and BDA1 proteins are proposed to act as a plasma membrane (PM)-localized signaling components that control defense responses against pathogens [4]-[6]. We previously demonstrated that ITN1 protein was also localized to the PM and that this protein negatively regulated plant tolerance to salt stress. ITN1 functions as a PM anchor of a nuclear protein RTV1 and partially inhibits the nuclear transport of RTV1, although possible effects of ITN1-RTV1 interaction on salt tolerance remain unclear [7], [8]. These findings raise a possibility that each member of the AtANKTM family may function as signaling components in responses to various environmental factors through interaction with (or release of) their respective partners.

Recently, we identified an AtANKTM member, *DRA1*, which negatively affects plant drought tolerance [9]. An *Arabidopsis* loss-of-function mutant of *DRA1* showed enhanced tolerance to drought stress. In wild-type plants, the expression of *DRA1* was rapidly suppressed through alternative splicing in response to drought treatment.

Alternative splicing is a mechanism for the regulation of gene expression that is widespread in higher eukaryotes [10]. The functional meanings of alternative splicing are 1) the insertion of premature stop codons in transcripts of target genes, resulting in suppression of the gene or translation of truncated proteins, 2) the accumulation of pre-mature transcripts, which can be rapidly converted to the full-length, fully functional transcripts by changing the splicing pattern when necessary or 3) the production of proteins with diverse domain rearrangements from the same gene.

A human TRPV4 channel comprised of ankyrin repeats and transmembrane domains has five isoforms generated by alternative splicing [11]. In these, variants lacking a part of ankyrin domains did not oligomerize and did not produce functional channels. Thus, rearrangement of ankyrin repeats by alternative splicing is an important process for the expression of TRPV4.

In this study, a member of AtANKTM gene, *At2g24600*, was identified as a gene whose expression was regulated through alternative splicing.

II. Materials and Methods

A. Plant Materials and Growth Conditions

Arabidopsis thaliana used in this study was the Columbia wild type. Plants were routinely grown at 22°C under continuous white light on solid MS medium [12] containing 1% w/v sucrose and 0.5% w/v gellan gum.

B. RT-PCR Analysis

Total RNAs were isolated from shoots using RNeasy Plant Mini Kit (QIAGEN) and reverse transcribed using Reverscript I (Wako) according to the manufacturer's instructions. We used 0.4μl of the reverse transcription reactions as templates in 10μl PCR reactions. RT-PCR was performed using specific primers

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for *At2g24600* full-length coding sequence (5'-CACCATGCATCCGATCTTCGATGC-3' and 5'-ATAGGTGAAATAGCCTGACC-3'). PCR programs were as follows: for *At2g24600* full-length coding sequence, 94°C for 2 min, then 35 cycles of 94°C for 30 sec, 53°C for 30 sec and 72°C for 2min.

C. Sequence Analysis

DNA fragments amplified by RT-PCR were inserted into pENTR/D-TOPO (Invitrogen) and sequenced. The nucleotide sequences were aligned using ApE software (http://biologylabs.utah.edu/jorgensen/wayned/ape/).

III. Results and Discussion

A. PCR Cloning of At2g24600

In the *Arabidopsis* genome, 37 genes encoding proteins with ankyrin repeats and transmembrane domains (named the AtANKTM family) have been identified [2]. Each member of the family may function as signaling components in responses to various environmental factors, such as *ACD6*, *BDA1*, *ITN1* and *DRA1* [4]-[9]. Transcriptome data indicated that the expression of an *AtANKTM* gene, *At2g24600*, was induced in response to cold, drought and salt treatments [13]. To understand the possible involvement of *At2g24600* in plant tolerance to abiotic stress, we attempted to generate transgenic *Arabidopsis* overexpressing this gene. The full-length coding sequence of the gene was amplified by RT-PCR using RNAs extracted from *Arabidopsis* seedlings. In this experiment, two types of PCR fragments were detected (Fig. 1), suggesting the possibility that at least two splicing variants of *At2g24600* were expressed in *Arabidopsis* seedlings.

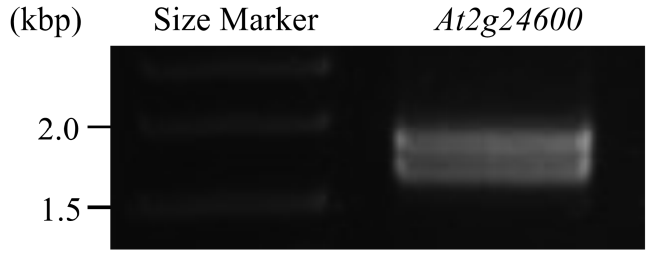

Fig. 1 RT-PCR cloning of the *At2g24600* full-length coding sequence

B. Alternative Splicing of At2g24600

According to TAIR (http://www.arabidopsis.org/), four splicing variants derived from the *At2g24600* locus have been identified (*At2g24600.1*~*.4*in Fig. 2). Especially, in respect to predicted coding regions, these splicing variants are classified into two groups. The *At2g24600.1* and *.2* transcripts belong to the first group, in which all predicted introns are spliced out. The differences between these variants exist in 3' UTR region. Therefore, the *At2g24600.1* and *.2* are likely to encode identical isoforms. On the other hand, in the *At2g24600.3* and *.4* transcripts, the predicted 3rd intron was not spliced out. The differences between the *At2g24600.3* and *.4* also exist in 3' UTR region.

Based on the SMART protein domain prediction program (http://smart.embl-heidelberg.de), the *At2g24600.1* and *.2* encode an AtANKTM protein which contains seven ankyrin repeats at the N-terminal region and three transmembrane domains at the C-terminal region (Fig. 2 (a)). The retaining of the predicted 3rd intron in the *At2g24600.3* and *.4* transcripts results in an in-frame addition of a transmembrane domain to the C-terminal region (Fig. 2 (b)).

To investigate whether two types of PCR fragments detected in Fig. 1 were derived from splicing variants already known, these fragments were sequenced. The upper fragment was identical to the *At2g24600.3* and *.4* coding sequence. On the other hand, the lower fragment was derived from a novel splicing variant of *At2g24600*, in which the predicted 3rdintron was retained and a 120bp region in the predicted 1st exon was spliced out (Figs. 2 (c) and 3). This variant was named as *At2g24600.5*. The novel splice junction in the 1st exon did not contain GT/AG consensus sequence. The deletion of the 120bp region in the *At2g24600.5* transcripts results in an in-frame deletion of an ankyrin repeat motif from the N-terminal region.

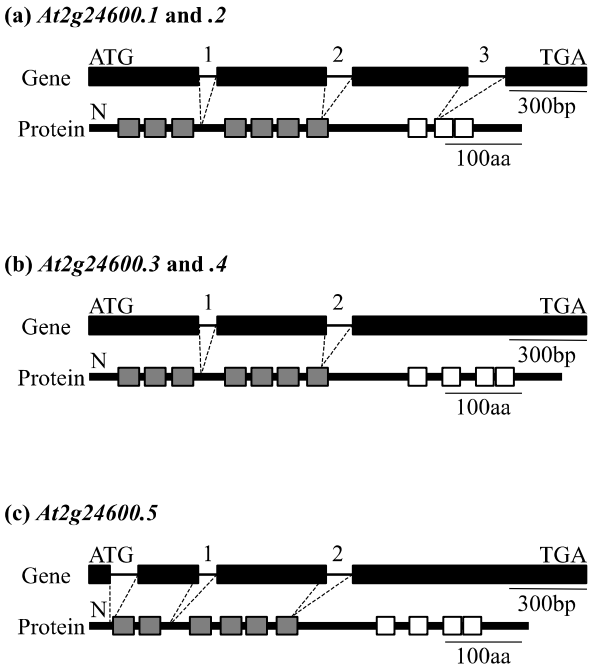

Fig. 2 The structures of the *At2g24600* gene (upper) and the predicted At2g24600 protein (lower). Based on differences in the predicted amino acid sequences, five *At2g24600* splicing variants are divided into three groups (a, b and c). In the gene structure, black boxes indicate exons and lines indicate introns. Introns assigned same numbers are identical sequences. In the protein structure, gray boxes indicate ankyrin repeats and white boxes indicate transmembrane domains. Broken lines between the two structures connect the exons to their encoded protein regions. The "aa" is an abbreviation for "amino acids"

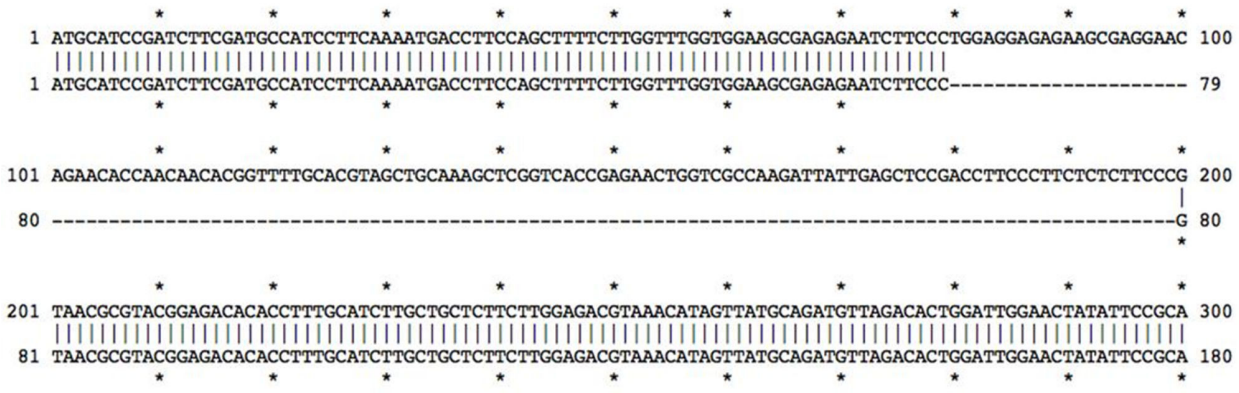

Fig. 3 The partial nucleotide sequences in the predicted 1st exon of the *At2g24600.1, .2, .3 and .4* (upper) and the *At2g24600.5* (lower)

Taken together, it is possible that the *At2g24600* locus encodes at least three protein isoforms. AtANKTM proteins are composed of ankyrin repeats domains and transmembrane domains. Ankyrin repeats mediate protein-protein interactions. Therefore, differences in the number of ankyrin repeats among At2g24600 protein variants may affect oligomerization and function of them such as a human TRPV4 channel [11]. The number of transmembrane domains is involved in determining the membrane topology of proteins, suggesting that At2g24600 protein variants may have different membrane topologies each other. It is possible that these structural differences result in different molecular functions.

A detailed study is required on the modes of action of *At2g24600*. The expression analysis, the phenol typical analysis of over expressing plants and the searching interacting partners will provide valuable information regarding the precise roles of AtANKTM family proteins.

REFERENCES

[1] S. G. Sedgwick, and S. J. Smerdon, "The Ankyrin Repeat: A Diversity of Interactions on a Common Structural Framework," *Trends Biochem. Sci.*, vol. 24, pp. 311–316, 1999.

[2] C. Becerra, T. Jahrmann, P. Puigdomènech, and C. M. Vicient, "Ankyrin Repeat-Containing Proteins in Arabidopsis: Characterization of a Novel and Abundant Group of Genes Coding Ankyrin-Transmembrane Proteins," *Gene*, vol. 340, pp. 111–121, 2004.

[3] J. Huang, X. Zhao, H. Yu, Y. Ouyang, L. Wang, and Q. Zhang, "The Ankyrin Repeat Gene Family in rice. Genome-wide identification, Classification and Expression Profiling," *Plant Mol. Biol.*, vol. 71, pp. 207–226, 2009.

[4] H. Lu, D. N. Rate, J. T. Song, and J. T. Greenberg, "ACD6, a Novel Ankyrin Protein, Is a Regulator and an Effector of Salicylic Acid Signaling in the Arabidopsis Defense Response," *Plant Cell*, vol. 15, pp. 2408–2420, 2003.

[5] H. Lu, Y. Liu, and J. T. Greenberg, "Structure–Function Analysis of the Plasma Membrane-Localized *Arabidopsis* Defense Component ACD6," *Plant J.*, vol. 44, pp. 798–809, 2005.

[6] Y. Yang, Y. Zhang, P. Ding, K. Johnson, X. Li, and Y. Zhang, "The Ankyrin-Repeat Transmembrane Protein BDA1 Functions Downstream of the Receptor-Like Protein SNC2 to Regulate Plant Immunity," *Plant Physiol.*, vol. 159, pp. 1857–1865, 2012.

[7] H. Sakamoto, O. Matsuda, and K. Iba, "ITN1, a Novel Gene Encoding an Ankyrin-Repeat Protein that Affects the ABA-Mediated Production of reactive oxygen species and is involved in Salt-Stress Tolerance in *Arabidopsis thaliana*," *Plant J.*, vol. 56, pp. 411–422, 2008.

[8] H. Sakamoto, K. Sakata, K. Kusumi, M. Kojima, H. Sakakibara, and K. Iba, "Interaction between a Plasma Membrane-Localized Ankyrin-Repeat Protein ITN1 and a Nuclear Protein RTV1," *Biochem. Biophys. Res. Commun.*, vol. 423, pp. 392–397, 2012.

[9] H. Sakamoto, Y. Nakagawara, and S. Oguri, "The Expression of a Novel Gene Encoding an Ankyrin-Repeat Protein, DRA1, is Regulated by Drought-Responsive Alternative Splicing," *Int. J. Biol. Life Sci. Eng.*,vol. 7, pp. 81-84, 2013.

[10] A.M. Mastrangelo, D. Marone, G. Laidò, A. M. De Leonardis, and P. De Vita, "Alternative Splicing: Enhancing Ability to Cope with Stress via Transcriptome Plasticity," *Plant Sci.*, vol. 185–186, pp. 40–49, 2012.

[11] M. Arniges, J. M. Fernández-Fernández, N. Albrecht, M. Schaefer, and M. A. Valverde, "Human TRPV4 Channel Splice Variants Revealed a Key Role of Ankyrin Domains in Multimerization and Trafficking," J. Biol. Chem., vol. 281, pp. 1580-1586, 2006.

[12] T. Murashige, and F. Skoog, "A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Culture," *Physiol. Plant.*, vol. 15, pp. 473–497, 1962.

[13] A. Matsui, J. Ishida, T. Morosawa, Y. Mochizuki, E. Kaminuma, T.A. Endo, M. Okamoto, E. Nambara, M. Nakajima, M. Kawashima, M. Satou, J. M. Kim, N. Kobayashi, T. Toyoda, K. Shinozaki, and M. Seki, "Arabidopsis Transcriptome Analysis under Drought, Cold, High-Salinity and ABA Treatment Conditions Using a Tiling Array," Plant Cell Physiol., vol. 49, pp. 1135-1149, 2008.