Identification of target genes of the DREB1A transcription factor controlling abiotic-stress-responsive gene expression using a full-length cDNA microarray

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Key words : abiotic stress, transcription factor, transgenic plant, stress tolerance, microarray

Objectives

Crop productivity is greatly affected by abiotic stresses such as drought, high salinity, and low temperature. Genetic engineering possesses high potential to improve the stress tolerance of crops through the use of gene transfer technology. A *cis*-acting promoter element DRE plays an important role in regulating gene expression in response to these stresses. We have reported that the *Arabidopsis* transcription factor DREB1A binds to DRE and controls expression of many stress tolerance genes. Overexpression of DREB1A in transgenic *Arabidopsis* activates the expression of target stress tolerance genes and results in improved stress tolerance. To understand how overexpression of DREB1A in transgenic plants increases stress tolerance, cDNA microarray analysis was employed to identify the DREB1A target genes.

Results

First, a cDNA microarray using 1,300 full-length *Arabidopsis* cDNAs was prepared. mRNAs prepared from transgenic *Arabidopsis* plants that overexpress DREB1A under the control of the CaMV 35S promoter (35S:DREB1A), and wild-type control plants were used for the preparation of Cy3-labeled and Cy5-labeled cDNA probes, respectively. These cDNA probes were then hybridized with the cDNA microarray (Fig. 1). Twelve genes were identified as target genes of DREB1A. On the basis of RNA gel blot and microarray analyses, six of them were identified as novel drought- and cold-inducible genes that are controlled by DREB1A (Fig. 2). These target genes contained DRE in their promoter regions.

We comprehensively analyzed further novel target genes using a 7,000 full-length cDNA microarray. More than 40 genes were identified as target genes of DREB1A and confirmed by RNA gel blot and promoter analyses. These target genes encoded enzymes required for the biosynthesis of osmoprotectants such as proline and sugar, membrane proteins, LEA proteins, detoxification enzymes, chaperones and transcription factors. These results indicated that overexpression of the DREB1A proteins in transgenic plants activated more than 40 stress tolerance genes and resulted in improved stress tolerance.

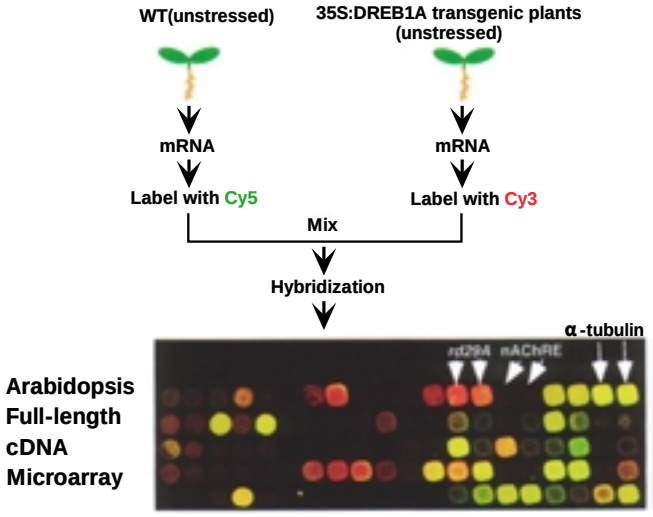

Fig. 1. Strategy for identification of DREB1A target genes. mRNAs from 35S:DREB1A transgenic plants and wild-type(WT) unstressed plants were used for the preparation of Cy3-labeled and Cy5-labeled cDNA probes, respectively. These cDNA probes were mixed and hybridized with the cDNA microarray. In this study, we used the α-tubulin gene as an internal control.

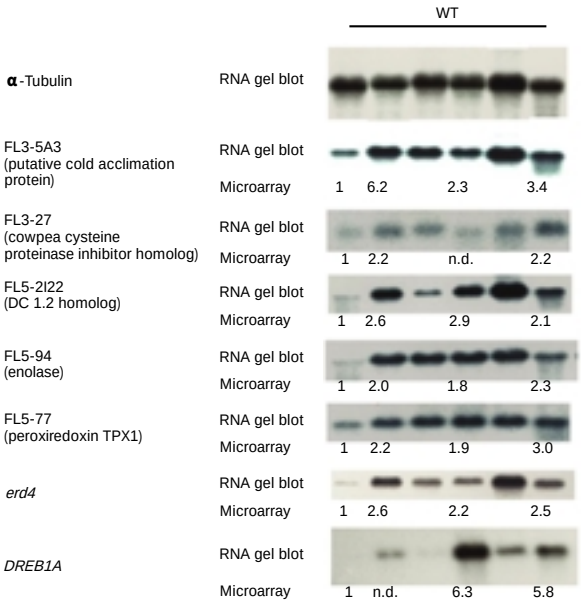

Fig. 2. Comparison of cDNA microarray and RNA gel blot analyses for the identification of new DREB1A target genes controlled by the DREB1A gene.

References

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