Molecular Studies on Stress-Responsive Gene Expression in *Arabidopsis* and Improvement of Stress Tolerance in Crop Plants by Regulon Biotechnology

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Abstract

Molecular studies have shown that several genes with various functions are induced by environmental stresses such as drought, high-salinity and low temperature in plants. Most of the dehydration responsive genes are induced by the plant hormone abscisic acid (ABA), but others are not. Expression analyses of dehydration-responsive genes have provided at least four independent regulatory systems (regulons) for gene expression in a model plant *Arabidopsis thaliana*. The cis-acting elements in the promoters of some genes that have a typical stress-inducible expression profile and the transcription factors that affect the expression of these genes have been analyzed. Transcription factors that bind to a DRE/CRT (dehydration-responsive element / C-repeat) cis-acting element were isolated and termed DREB1/CFB (DRE-binding protein 1/ C-repeat binding factor) and DREB2 (DRE-binding protein 2). Overexpression of DREB1/CFB in transgenic *Arabidopsis* plants increased tolerance to freezing, drought and high salt concentrations. The DREB1/CFB genes have been successfully used to improve abiotic stress tolerance in a number of different crop plants. Studies on the other transcription factors associated with stress response are in progress. We collaborate with many research groups to improve stress tolerant crop plants utilizing regulon biotechnology. We hope the results of these collaborative studies will contribute to the sustainable food production in developing countries and help to prevent the global-scale environmental damage.

Discipline: Biotechnology

Additional key words: DREB1, environmental stress, transcription factors, transgenic plants

Introduction

As plants are sessile organisms, they are directly exposed to environmental stresses such as drought, high salinity and low temperature. Plants respond to environmental stress, and the transduced signals cause expression of numerous genes associated with stress tolerance. A number of genes have been described that respond to environmental stresses such as drought, high salinity and low temperature in plants1,13,33,47,48,60. We isolated more than 60 independent cDNAs for dehydration inducible genes using molecular techniques such as differential screening in a model plant *Arabidopsis thaliana*33,47,48. Recently, 299 drought-inducible genes, 54 cold-inducible genes, and 213 high-salinity-stress-inducible genes were identified using a cDNA microarray containing around 7,000 independent *Arabidopsis* full-length cDNA groups46,48. Functions of their gene products have been predicted from sequence homology with known proteins. Genes induced during dehydration stress conditions are thought to function not only in protecting cells from dehydration by the production of important metabolic proteins (functional proteins) but also in the regulation of genes for signal transduction in the dehydration stress response (regulatory proteins). The functional proteins contain water channel proteins, chaperons, proteases, LEA (Late Embryogenesis Abundant) proteins, and enzymes for the synthesis of osmo-protectants (compatible solutes; sugars, proline, etc.). The regulatory proteins contain transcription factors, protein kinases, and enzymes for phosphoinositide (PI) turn-

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However, and enzymes for the synthesis of the plant hormone abscisic acid (ABA). So far, various kinds of functional proteins such as enzymes for the synthesis of osmoprotectants were overexpressed in plants to improve the stress tolerance. However, it seems that the engineering of one enzyme is not enough as many kinds of stress responses are necessary for plants to survive in severe stress conditions.

In plants, one transcription factor can control the expression of many target genes through the specific binding of the transcription factor to the cis-acting element in the promoters of the target genes. Such kind of a transcription unit is called a “regulon”. Northern analysis of dehydration-inducible genes revealed that there appear to be at least four independent regulons in Arabidopsis (Fig. 1). They are (1) DREB regulon, (2) NAC (NAM, ATAF1, 2, and CUC2) and ZF-HD (zinc-finger homeodomain) regulon, (3) AREB/ABF (ABA-responsive element binding protein / ABA-responsive element binding factor) regulon, and (4) MYC (myeloblastosis oncogene) and MYB (myeloblastosis oncogene) regulon. The DREB regulon and the NAC and ZF-HD regulon are ABA-independent. The AREB/ABF regulon and the MYC and MYB regulon are ABA-dependent. Regulon biotechnology, by controlling the expression of the regulon system, is expected to improve the tolerance against stresses in plants.

**DREB regulon involved in ABA-independent gene expression**

1. **Isolation of DREB1/CFB regulon and DREB2 regulon**
   The promoter of an Arabidopsis drought-, high-salinity- and cold-inducible gene RD29A (responsive to dehydration 29A) encoding a LEA-like protein has been

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**Fig. 1. Regulatory network of gene expression in response to drought, high salinity and cold stresses: specificity and crosstalk of gene networks**

Cis-acting elements that are involved in stress-responsive transcription are shown in boxes. Transcription factors that control stress-inducible gene expression are shown in circles or ovals. Small circles indicate the modification of transcription factors in response to stress signals for their activation, such as phosphorylation. Dotted lines indicate possible regulation. Double arrow lines indicate possible cross talk. ABF: ABRE-binding factor, ABRE: ABA-responsive element, AREB: ABRE-binding protein, CBF: C-repeat-binding factor, CRT: C-repeat, DRE: dehydration-responsive element, DREB: DRE-binding protein, ERD: early responsive to dehydration, ICE: inducer of CBF expression, MYBR: MYB recognition site, MYCR: MYC recognition site, NACR: NAC recognition site, RD: responsive to dehydration, ZF-HD: zinc finger homeodomain protein.
found to contain two major cis-acting elements, the ABA-responsive element (ABRE) and the dehydration-responsive element (DRE)/C-repeat (CRT), that are involved in stress-inducible gene expression. DRE/CRT (CCGAC) is a cis-acting element that functions in ABA-independent gene expression in response to abiotic stress (Fig. 1). Transcription factors belonging to the AP2/ERF (APETALA2 /ethylene-responsive element binding factor) family that bind to DRE/CRT have been isolated and termed DREB1/CBF and DREB2. The conserved DNA-binding motif of DREB1/CBF and DREB2 is A/GCCGAC. The DREB1/CBF genes are quickly and transiently induced by cold stress, and their products activate the expression of target stress-inducible genes. The DREB2 genes are induced by dehydration, leading to the expression of various genes that are involved in drought-stress tolerance.

2. Improved stress tolerance of transgenic plants overexpressing DREB1/CBF

Overexpression of DREB1A/CBF3 in transgenic Arabidopsis plants showed increased tolerance to freezing, drought and high salt concentrations, suggesting that the DREB1A/CBF3 proteins function without modification of the proteins in the development of stress tolerance. Many candidates for the DREB1A/CBF3 target genes have been identified using microarray, most of these target genes contain DRE- or DRE-related CCGAC core motif sequences in their promoter regions. We analyzed the expression of these candidate genes using RNA gel blot and identified more than 40 genes as DREB1A downstream genes. Many of the products of these genes were proteins known to function against stress and were probably responsible for the stress tolerance of the transgenic plants. The downstream genes also included genes for transcription factors involved in further regulation of signal transduction and gene expression in response to stress.

The overexpression of the DREB1/CBF gene results in multiple biochemical changes associated with cold acclimation: DREB1A/CBF3-expressing plants had elevated levels of proline (Pro) and total soluble sugars, including sucrose, raffinose, glucose, and fructose. Plants overexpressing DREB1A/CBF3 also had elevated P5CS (for delta(1)-pyrroline-5-carboxylate synthase) transcript levels suggesting that the increase in Pro levels resulted, at least in part, from increased expression of the key Pro biosynthetic enzyme P5CS. These results lead us to propose that DREB1A/CBF3 integrates the activation of multiple components of the cold acclimation response.

Dwarfism is observed in transgenic Arabidopsis overexpressing DREB1A/CBF3, DREB1B/CBF1, DREB1C/CBF2 or DREB1D/CBF4. The development of dwarf phenotypes was also found in transgenic tomato overexpressing Arabidopsis DREB1A/CBF1, and it was prevented by exogenous application of gibberellin (GA). These suggest that an inhibition of GA biosynthesis is a function common to the DREB1/CBF genes. However, microarray analysis did not detect the changes in transcript levels of GA-related genes in transgenic Arabidopsis overexpressing DREB1A/CBF3, DREB1A/CBF1, or DREB1/CBF2. Recently, DREB1F is reported to be involved in the regulation of GA biosynthesis and stress tolerance. It is not clear yet whether other DREB1/CBF proteins are related to GA synthesis or not.

In contrast to the DREB1/CBF genes, overexpression of DREB2 in transgenic plants does not improve stress tolerance, suggesting that DREB2 proteins require posttranslational activation. The DREB2 protein is expressed under normal growth conditions and is activated in the early stage of the osmotic stress response through posttranslational modification (Fig. 1).

3. Regulation of the expression of DREB1/CBF regulon

The ICE1 (inducer of CBF expression 1) gene was identified through the map-based cloning of the Arabidopsis ice1 mutation, which affected the expression of the DREB1A/CBF3 promoter-LUC (luciferase) transgene. ICE1 encodes a MYC-type bHLH (basic helix-loop-helix) transcription factor that regulates the expression of DREB1A/CBF3 but not of other DREB1/CBF genes (Fig. 1). Overexpression of ICE1 in transgenic plants resulted in improved freezing tolerance, supporting an important role for ICE1 in the cold-stress response. Molecular analysis of the DREB1/CBF2 promoter has identified multiple cis-acting elements that are involved in cold-inducible gene expression (Imura et al., unpublished data). The DNA-binding protein has been cloned and shown to be a MYC-type bHLH transcription factor that is different from ICE1 (Imura et al., unpublished data). These results suggest the redundant involvement of MYC-type bHLH transcription factors in the up-regulation of the DREB1/CBF genes. A cold signal is necessary for the activation of the ICE proteins but the mechanism of this signal remains to be solved. Analysis of the cbf2 mutant, in which the DREB1/CBF2 gene was disrupted, indicated that DREB1/CBF2 is a negative regulator of DREB1A/CBF3 and DREB1B/CBF1 expression and plays a central role in stress tolerance in Arabidopsis. These data suggest that the regulation of the expression of the DREB1/CBF genes might be more complex than previously thought.
NAC and ZF-HD regulon involved in ABA-independent gene expression

The ERD1 (early responsive to dehydration 1) gene encoding a Clp (caseinolytic protease) protease regulatory subunit responds to dehydration and high salinity before the accumulation of ABA, suggesting the existence of an ABA-independent pathway in the dehydration stress response\textsuperscript{39}. Analysis of the ERD1 promoter identified two novel cis-acting elements that are involved in induction by dehydration stress\textsuperscript{39}. Base substitution analysis showed that a 14-bp rps1-like region (CACTAAATCGTCAC) and a CATGTG motif are necessary for the induction of the ERD1 gene in dehydrated plants (Fig. 1). Recently, we isolated three cDNA clones encoding proteins that bind to the 63-bp promoter region of ERD1, which contains the CATGTG motif\textsuperscript{52} (Fig. 1). These three cDNA clones encode proteins which belong to the NAC transcription factor family including RD26. Microarray analysis of transgenic plants overexpressing the NAC genes revealed that several drought inducible genes were up-regulated in the transgenic plants, and the plants showed significantly increased drought tolerance. However, ERD1 was not up-regulated in the transgenic plants. We recently isolated zinc-finger homeodomain (ZF-HD) transcription factors containing a homeodomain that can bind to the rps1 site I-like sequence using the yeast one-hybrid system. Overexpression of both NAC and ZF-HD proteins activated the expression of ERD1 under unstressed normal growth conditions in the transgenic Arabidopsis plants.

AREB/ABF regulon involved in ABA-dependent gene expression

AREB (ABA-responsive elements: ACGTGG/TC) is a major cis-acting element in ABA-responsive gene expression (Fig. 1). Two AREB motifs are important in the ABA-responsive expression of the Arabidopsis gene RD29B encoding a LEA-like protein\textsuperscript{53}. The bZIP (basic leucine zipper) transcription factors ABRE-binding protein (AREB)/ABRE-binding factor (ABF) can bind to ABRE and activate ABA-dependent gene expression\textsuperscript{53}. Activation of the AREB1 and AREB2 proteins has been shown to require an ABA-mediated modification\textsuperscript{53}, which is probably ABA-dependent phosphorylation (Fig. 1). Overexpression of ABF3 or AREB2/ABF4 caused ABA hypersensitivity, reduced transpiration rate and enhanced drought tolerance of the transgenic plants\textsuperscript{58}. The AREB1/ABF2 is reported to be an essential component of glucose signaling and its overexpression affects multiple stress tolerance including drought, salt and heat\textsuperscript{22}.

MYC and MYB regulon involved in ABA-dependent gene expression

The induction of the Arabidopsis drought-inducible gene RD22 encoding a protein having a homology to an unidentified seed protein is mediated by ABA, and this gene requires protein biosynthesis for its ABA-dependent expression\textsuperscript{1}. A MYC transcription factor, AtMYC2 (Arabidopsis thaliana MYC 2), and a MYB transcription factor, AtMYB2 (Arabidopsis thaliana MYB 2), have been shown to bind cis-elements, MYCR (MYC-recognition site: CANNTG) and MYBR (MYB-recognition site: C/TAACNA/G) in the RD22 promoter and cooperatively activate RD22\textsuperscript{3} (Fig. 1). These MYC and MYB proteins are synthesized after the accumulation of endogenous ABA, indicating that their role is in a late stage of the stress responses. Microarray analysis of MYC- and MYB-overexpressing transgenic plants revealed target genes for MYC and MYB, such as the alcohol dehydrogenase gene and ABA- or jasmonic-acid (JA)-inducible genes\textsuperscript{2}. Overexpression of both AtMYC2 and AtMYB2 not only caused an ABA-hypersensitive phenotype but also improved the osmotic-stress tolerance of the transgenic plants\textsuperscript{2}.

Recently, AtMYC2 transcription factors function as members of a MYC-based regulatory system conserved in dicotyledonous plants with a key role in JA-induced defense gene activation\textsuperscript{12}. These reports highlight the crosstalk between biotic stress signaling and abiotic stress signaling.

Crosstalk between the DREB regulon and the other regulons

Many drought- and cold-inducible genes contain both DRE/CRT and ABRE motifs in their promoters. These cis-acting elements are thought to function independently. However, precise analysis of these cis-acting elements in the RD29A gene expression revealed that DRE/CRT functions cooperatively with ABRE as a coupling element in ABA-responsive gene expression in response to drought stress\textsuperscript{34}. This indicates that there are interactions between the DREB regulon and the AREB/ABF regulon (Fig. 1).

Recently, an osmotic-stress inducible CBF4/DREB1D gene has been identified\textsuperscript{12}. Genes of the DREB1/CFB family are mainly induced by cold stress, but the drought-inducible gene CBF4/DREB1D functions to provide crosstalk between DREB2 and DREB1/CFB regulatory systems. The drought-inducible expres-
sion of CBF4/DREB1D is controlled by ABA-dependent pathways, suggesting that CBF4/DREB1D may function in the slow response to drought that relies on the accumulation of ABA (Fig. 1). Moreover, ABA induces the DREB1/CFB gene transcription and subsequent induction of cold-regulated genes via the DRE/CRT promoter element. A maize DRE-binding protein, DBF1, has been shown to function as a transcriptional activator of the rab17 (responsive to abscisic acid 17) promoter by ABA. This also suggests the existence in some plants of an ABA-dependent pathway for the regulation of stress-inducible genes that involves DRE/CRT.

Gene expression in recovery process from abiotic stress in Arabidopsis

Microarray analysis has revealed many genes that respond to rehydration after drought stress, indicating their involvement in the process of recovery from abiotic stress. The products of these genes are thought to function not only in recovery from stress but also in cell growth and elongation. The expression and function of the rehydration-inducible ERD5 gene encoding a proline dehydrogenase (ProDH) gene has been precisely analyzed. This gene is involved in the degradation of the proline that accumulates during dehydration. Promoter analysis of the ProDH gene revealed an important cis-acting element, ACTCAT, that is involved in rehydration-inducible gene expression. Many rehydration-inducible gene promoters contain the ACTCAT motif. Recently we showed that the ATB2 subgroup bZIP proteins functions as transcriptional activators in hypoosmolarity-responsive expression of the ProDH gene in Arabidopsis. The molecular information in the process of recovery from abiotic stress may allow us to improve the resilient plants.
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* Superpromoter consists of three copies of the octopine synthese upstream-activating sequence in front of the manopine synthese promoter.
Application of regulon biotechnology to improve stress tolerance in crop plants

The orthologous genes of DREB1/CBF have been found in many crop plants such as canola, broccoli, tomato, alfalfa, wheat, barley, corn, and rice. These indicate that the DREB1/CBF regulon system is ubiquitous in the plant kingdom, and the “DREB technology” with controlling the expression of the DREB1/CBF regulon system is expected to improve the tolerance against stresses in crop plants. So far the DREB1/CBF genes of Arabidopsis have been successfully used to engineer abiotic stress tolerance in a number of different species (Table 1). For example, constitutive overexpression of the Arabidopsis DREB1/CBF genes in canola results in increased freezing tolerance and drought tolerance.

We have isolated rice orthologs for DREB1/CBF and DREB2, four OsDREB1s and one OsDREB2, in the rice genome sequence and they function in stress-inducible gene expression. Overexpression of OsDREB1A in Arabidopsis revealed that this gene has a similar function to that of its Arabidopsis homolog in stress-responsive gene expression and stress tolerance. This indicates that similar transcription factors function in dicotyledons and monocotyledons. A novel DREB1/CBF transcription factor ZmDREB1A was also identified in Zea mays. The ZmDREB1A was involved in cold-responsive gene expression and overexpression of the ZmDREB1A gene in Arabidopsis which resulted in increased drought and freezing tolerance.

However, constitutive overexpression of the DREB1/CBF genes in plants showed dwarf phenotype. To overcome these problems, stress-inducible promoters that have low background expression under normal growth condition have been used in conjunction with the DREB1/CBF genes to achieve increased stress tolerance without the growth retardation. Constitutive overexpression of Arabidopsis DREB1A/CBF3 improved drought- and low-temperature stress tolerance in tobacco. The stress-inducible RD29A promoter minimized the negative effects on the plant growth in tobacco. Furthermore, we detected overexpression of stress-inducible target genes of DREB1A/CBF3 in tobacco. The Arabidopsis DREB1A/CBF3 gene was placed under control of the RD29A promoter and transferred via biolistic transformation into bread wheat. Plants expressing the DREB1A/CBF3 gene demonstrated substantial resistance to water stress in comparison through checks under experimental greenhouse conditions, manifested by a 10-day delay in wilting when water was withheld. These results indicate that a combination of the RD29A promoter and DREB1A is useful for improvement of various kinds of transgenic plants that are tolerant to environmental stress.

Now we collaborate with many research groups to improve stress tolerant crop plants utilizing regulon biotechnology (Fig. 2). We hope the results of these collaborative studies will contribute to the sustainable food production in developing countries and help to prevent the global-scale environmental damage.

References

2. Abe, H. et al. (2003) Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. Plant Cell, 15, 63–78.


