Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways

Kazuo Shinozaki* and Kazuko Yamaguchi-Shinozaki†

Recently, a major transcription system that controls abscisic-acid-independent gene expression in response to dehydration and low temperature has been identified. The system includes the DRE/CRT (dehydration-responsive element/C-repeat) cis-acting element and its DNA-binding protein, D (DRE-binding protein/C-repeat binding factor), which has an AP2 domain. D

DREB1/CBF and DREB2, which are induced by cold and dehydration, respectively, and control the expression of various genes involved in stress tolerance. Recent studies are providing evidence of differences between dehydration-signaling and cold-stress-signaling cascades, and of cross-talk between them.

Drought and high salinity cause plants to produce high levels of ABA; exogenous application of ABA also induces a number of genes that respond to dehydration and cold stress [1]. Nevertheless, the role of ABA in low-temperature-responsive gene expression is not clear. Several reports have described genes that are induced by dehydration and low temperature but that do not respond to exogenous abscisic acid (ABA) treatment [1–4]. It is likely, therefore, that both ABA-independent and ABA-dependent signal transduction cascades exist [1,4]. One of the transcription systems that function independently of ABA in both dehydration- and low-temperature-responsive gene expression has recently been analyzed extensively (for reviews see [1,5,6]).

In 1994, we indentified a cis-acting dehydration-responsive element (DRE) [7]. A similar cis-acting element has also been reported and named C-repeat (CRT) [8] or ‘low-temperature-responsive element’ [9]. The DRE/CRT element is involved in both dehydration- and low-temperature-responsive gene expression. Our short review focuses on roles of the DRE/CRT cis-element and its DNA-binding protein, DREB/CFB (DRF-binding protein/C-repeat binding factor), in the separation of and cross-talk between two stress signals that are involved in stress-induced gene expression in Arabidopsis. We also discuss the role of ABA in dehydration- and cold-induced gene expression. ABA plays important roles in slow and adaptive responses involving dehydration-induced gene expression. However, ABA seems not to be important in cold-induced gene expression and does not accumulate in response to low temperature. ABA does, however, have important roles in slow adaptive processes during dehydration stress.

Similar genes are induced by dehydration and cold stress

A variety of genes are induced by both dehydration and low temperature, and their mRNA levels are subsequently reduced by release from stress conditions. This suggests that similar biochemical processes function in dehydration- and cold-stress responses. Genes induced in plants that are subjected to these stresses are thought to function not only in protecting cells by producing important metabolic proteins and cellular protectants, but also in regulating genes that are involved in transducing the stress response signal [1,2,10,11]. In Arabidopsis, these genes include rd (responsive to dehydration), erd (early responsive to dehydration), cor (cold-regulated), lti (low-temperature induced), and others.

Addresses

*Laboratory of Plant Molecular Biology, Tsukuba Life Science Center, Institute of Physical and Chemical Research (RIKEN), 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan; e-mail: shinozaki@rtc.riken.go.jp
†Biological Resources Division, Japan International Research Center for Agricultural Sciences (JIRCAS), Ministry of Agriculture, Forestry and Fisheries, 2-1 Ohwashi, Tsukuba, Ibaraki 305-8686, Japan; e-mail: sinozaki@rtc.riken.go.jp


1369-5266/00/$ – see front matter © 2000 Elsevier Science Ltd. All rights reserved.

Abbreviations

†ABA: abscisic acid
aba ABA-deficient
abi ABA-responsive
ABRE ABA-responsive element
ATHK1 ARABIDO TWO-_COMPONENT HISTIDINE KINASE
bp base pairs
CaMV cauliflower mosaic virus
CBF C-repeat-binding factor
cor cold-regulated
CRT C-repeat
DRE dehydration-responsive element
DREB DRE-binding protein
erd early responsive to dehydration
EREBP ethylene-responsive element binding protein
HOS HIGH EXPRESSION OF OSMOTICALLY SENSITIVE
kin cold-inducible
lti low-temperature induced
rd responsive to dehydration
sfr sensitivity to freezing

Introduction

Among abiotic environmental stresses, drought and low temperature affect plant growth most seriously. Plants respond to dehydration and low temperature with a number of physiological and developmental changes. Molecular and cellular responses to these stresses have been analyzed extensively at the biochemical level: various kinds of proteins and smaller molecules, including sugars, proline, and glycine betaine, accumulate; in addition, many genes are induced by both dehydration and cold, but some respond either only to drought or only to cold. These observations suggest the existence of several cellular signal transduction pathways between the perception of stress signals and gene expression.

In 1994, we indentified a cis-acting dehydration-responsive element (DRE) [7]. A similar cis-acting element has also been reported and named C-repeat (CRT) [8] or ‘low-temperature-responsive element’ [9]. The DRE/CRT element is involved in both dehydration- and low-temperature-responsive gene expression. Our short review focuses on roles of the DRE/CRT cis-element and its DNA-binding protein, DREB/CFB (DRF-binding protein/C-repeat binding factor), in the separation of and cross-talk between two stress signals that are involved in stress-induced gene expression in Arabidopsis. We also discuss the role of ABA in dehydration- and cold-induced gene expression. ABA plays important roles in slow and adaptive responses involving dehydration-induced gene expression. However, ABA seems not to be important in cold-induced gene expression and does not accumulate in response to low temperature. ABA does, however, have important roles in slow adaptive processes during dehydration stress.

Similar genes are induced by dehydration and cold stress

A variety of genes are induced by both dehydration and low temperature, and their mRNA levels are subsequently reduced by release from stress conditions. This suggests that similar biochemical processes function in dehydration- and cold-stress responses. Genes induced in plants that are subjected to these stresses are thought to function not only in protecting cells by producing important metabolic proteins and cellular protectants, but also in regulating genes that are involved in transducing the stress response signal [1,2,10,11]. In Arabidopsis, these genes include rd (responsive to dehydration), erd (early responsive to dehydration), cor (cold-regulated), lti (low-temperature induced),
and *kin* (cold-inducible). This variety of stress-inducible genes suggests that the responses of plants to dehydration and cold are complex. Some of the stress-inducible genes are overexpressed in transgenic plants that have enhanced stress tolerance, suggesting that their gene products function in stress tolerance [10–13].

**Regulation of gene expression by dehydration and cold stress**

Most dehydration-inducible genes also respond to cold stress, and, conversely, most cold-inducible genes respond to dehydration. Analyses of the expression patterns of genes induced by both dehydration and cold have revealed broad variation in the timing of their induction and differences in their responsiveness to ABA [4]. Many of the genes that are induced by exogenous ABA treatment are also induced by cold or dehydration in ABA-deficient (*aba*) or ABA-insensitive (*abi*) *Arabidopsis* mutants [2]. These observations indicate that these genes are not induced by the accumulation of endogenous ABA, but respond to ABA [1,4]. Several ABA-inducible genes require protein biosynthesis for their induction by ABA [4], which suggests that at least two independent pathways signal the expression of stress-induced genes in response to endogenous ABA production. As shown in Figure 1, at least four independent signal pathways function under drought conditions [4]: two are ABA-independent and two are ABA-dependent. In *DRE/CRT* two ABA-independent pathways are also involved in low-temperature-responsive gene expression [1]. There is a common signal transduction pathway between dehydration and cold stress involving the *DRE/CRT* cis-acting element, and two additional signal transduction pathways may function only in dehydration or in cold response.

**The role of the *cis*-acting element in ABA-independent gene expression**

In *aba* or *abi* mutants, many genes are induced by both dehydration and low temperature; this suggests that these genes do not require ABA for their expression under cold or drought conditions but that they do respond to ABA. Among these genes, the expression of two dehydration- and cold-inducible *Arabidopsis* genes, *rd29A/cor78/cor78* and *cor15a*, has been analyzed in detail (for reviews see [1,5]). The transcription of *rd29A* in *abi1* and *aba1* mutants suggests that cold- and drought-regulated expression does not require ABA. DRE, a 9-base pair (bp) conserved sequence (i.e. TACCGACAT), is an essential cis-acting element for the regulation of *rd29A* induction in the ABA-independent response to dehydration and cold [7]. Similar motifs, called CRT and low-temperature-responsive element, which include the CCGAC motif that forms the core of the DRE sequence, have been found in the promotor region of cold-inducible genes [8,9].

**DRE/CBF between the dehydration and low temperature stress-signaling pathways**

Protein factors that specifically interact with the 9-bp DRE sequence have been detected in nuclear extracts prepared from either dehydrated or adequately watered *Arabidopsis* plants [7]. Stockinger *et al.* [14] first isolated a cDNA clone for a DRE/CRT-binding protein using yeast one-hybrid screening; they named this clone CBF1 (CRT-binding factor 1). In yeast, CBF1 functions as a transcription factor that upregulates DRE/CRT-dependent transcription. It contains a conserved DNA-binding motif (AP2 domain) that is also found in the EREBP (ethylene-responsive element binding protein) family and AP2 protein, which is involved in floral

---

**Figure 1**

![Cellular signal transduction pathways between the initial drought-stress or cold-stress signal and gene expression in *Arabidopsis*. There are at least six signal transduction pathways: two (b,c) are ABA-dependent and four (a,d,e,f) are ABA-independent. Stress-inducible genes *rd29A/cor78/cor78*, *rd29B/lit78*, *rd29B/lit65*, *rd22*, and *erd1* have been used to analyse the regulation of gene expression and the signalling process [5,40]. *abi1*, *abi2*, and *era1* are involved in ABA signaling [41–44]. *hos5* functions in DREB2-related dehydration signaling, and *sfr6*, *hos1*, and *hos2* function in DREB1/CBF-related cold signaling [28–32–34]. *esk1* is involved in responses to cold via a DRE-independent process [28*]. Thin and thick arrows represent the minor and major signalling pathways that are involved in dehydration-responsive gene expression, respectively. Broken arrows represent the signalling pathways that are involved in low temperature stress responses.**
morphogenesis [15,16]. Independently, Liu et al. [17••] isolated five independent cDNAs for DRE/CRT-binding proteins using yeast one-hybrid screening, which they named DREBs (DRE-binding proteins). All of the DREBs also contain a conserved AP2 domain. The five cDNA clones that encode DRE/CRT-binding proteins are classified into two groups, DREB1 and DREB2. The groups contain similar AP2 domains but have low sequence similarity outside that domain. There are three DREB1 proteins that are encoded by genes that lie in tandem on chromosome 4 in the order DREB1B, DREB1A, and DREB1C [18]. DREB1B is identical to CBF1. Gilmour et al. [19] also isolated two CBF1 homologues named CBF2 and CBF3. There are two DREB2 proteins, DREB2A and DREB2B [17••]. Both DREB1A and DREB2A bind specifically to DRE/CRT and function as transcriptional activators in plant protoplasts, as well as in yeasts. Expression of the DREB1A/CFB3 gene and its two homologues (i.e. DREB1B/CFB1 and DREB1C/CFB2) is induced by low-temperature stress, whereas expression of the two DREB2 genes is induced by dehydration. These results suggest that the DREB1 proteins are involved in cold-specific gene expression, whereas the DREB2 proteins function in dehydration-specific gene expression (Figure 2).

The AP2 domain is found in many plant genes, such as EREBP, APETALA2, AINTEGUMENTA and TINY [16]. EREBPs bind to the ethylene-responsive element (i.e. the GCC box, GCCGCC), whereas DREB1/CFB3 bind to the DRE/CRT core sequence, PuCCGAC. DRE/CRT and the G box contain PuCCGNC as a common sequence [15,17••]. Liu et al. [17••] also identified two DREB2A cDNAs driven by the 35S CaMV promoter in transgenic plants causes severe growth retardation under normal growth conditions [17••,21••]. Recently Kasuga et al. [21••] found that the DREB1A cDNA driven by the stress-inducible rd29A promoter was expressed at a low level in unstressed control conditions and at a high level in plants exposed to dehydra-
tion, salt, and cold stresses. The rd29A promoter minimized the negative effects on the growth of the transgenic plants. Moreover, this stress-inducible promoter enhanced tolerance of drought, salt, and freezing to a greater extent than did the CaMV 35S promoter. The rd29A promoter:

DREB1A system is a self-amplifying system that overexpresses DREB1A protein throughout exposure to stress. Greater expression of the DREB1A protein in the rd29A::DREB1A transgenics results in greater expression of the target genes involved in stress tolerance [21••]. This system provides some promise for engineering multi-stress tolerance of transgenic crops because plants such as tobacco, Brassica, and rice, have similar transcription systems to that of Arabidopsis.

ABA in dehydration and low-temperature stress response

In many plants, endogenous ABA levels increase significantly in conditions of drought and high-salinity [2–4]. In Arabidopsis, however, ABA levels increase only transiently in response to low-temperature stress before returning to their basal level [1,22]. Many drought- and cold-stress-inducible genes are induced by exogenous ABA treatment. These genes contain potential ABA-responsive elements (ABREs; PyACGTGGC) in their promoter regions [1,2]. In Arabidopsis, the rd29B (or lti65) gene is induced by dehydration and high salinity, but not by cold stress [23]. rd29B does not contain a DRE/CRT but contains two ABREs in its promoter [7]. It is controlled downstream of the abf1 and abf1 mutations and so endogenous ABA that accumulates in response to dehydration induces its expression. During cold stress, endogenous ABA is not sufficient to induce rd29B. We therefore believe that the ABA-signaling pathway is not important in cold-stress responses. The endogenous ABA accumulation that has been observed during winter may be attributable to the dehydration of plants, which induces DRE/CRT-dependent gene expression of most cor, lti, and rd genes to confer stress tolerance (Figure 3).

The rd29A promoter contains not only DRE but also an ABRE (Figure 2). An ABRE cis-acting element and bZIP transcription factors function in ABA-responsive gene expression [7]. The rd29A gene is therefore controlled by three independent regulatory systems [7,17••]. These results indicate that complex molecular responses to various environmental stresses may be mediated by both complex regulatory systems of gene expression and signal transduction, and by cross-talk between these systems. The bZIP/ABRE system seems to function after the accumulation of endogenous ABA in drought conditions (Figure 3).

The biosynthesis of novel protein factors is necessary for the expression of ABA-inducible genes in one of the two ABA-dependent pathways (Figure 1). The induction of the Arabidopsis drought-inducible gene rd22 is mediated by ABA and requires protein biosynthesis for its ABA-dependent expression [24]. MYC and MYB recognition sequences are essential for the ABA- and drought-responsive expression of rd22, and ABA-inducible MYC and MYB proteins may function cooperatively in the ABA-dependent expression of rd22 [25,26]. This MYC/MYB system may also function in a slow and adaptive stress response process. The different timing of the induction of stress-inducible genes may be explained by the different regulatory systems that function in their promoters, such as DRE/CRT, ABRE, and MYB/MYC (Figure 3).

Genetic analysis of signal transduction in response to dehydration and cold stress

Many Arabidopsis mutants that are either sensitive to or tolerant of freezing have been isolated, and their phenotypes...
have been analyzed in detail. Warren et al. [27] isolated five freezing-sensitive (sensitivity to freezing [sfr]) mutants and mapped their positions on Arabidopsis chromosomes. Knight et al. [28••] analyzed the expression of kin1, cor15a, and cor78/rd29A, which contain DRE/CRT in their promoters. In the sfr6 mutant, unlike wild-type Arabidopsis plants, these genes were not strongly induced in response to osmotic stress and low temperature. In contrast, ATP5CS, CBF1, CBF2, and CBF3, which do not contain DRE/CRT in their promoters, were not affected in the sfr6 mutant. The SFR6 product may be involved upstream of CBF/DREB1 and function as a positive regulator of this element (Figure 1). A transient increase in cellular calcium concentration in responses to dehydration and low temperature seems to stimulate cellular signalling processes. Nevertheless, evidence from calcium measurement in cold conditions suggests that calcium signalling may not be involved in sfr6 signalling.

Xin and Browse [29•] isolated a constitutively freezing-tolerant mutant named eskimo1 that, without cold acclimatization, has greater freezing tolerance than wild-type plants. The molecular mechanism of the stress tolerance of eskimo1 is not known, but proline accumulates in the eskimo1 mutant [29•]. This proline may be involved in stress tolerance as proline functions in osmoprotection, in detoxication of active oxygen, and in protection of proteins and nucleic acids. Recently, Nanjo et al. [30] showed that the accumulation of proline in transgenics with antisense cDNA for proline dehydrogenase provides strong evidence from calcium measurement in cold conditions suggests that calcium signalling may not be involved in sfr6 signalling.

Genetic analysis of Arabidopsis mutants with the rd29A promoter: luciferase transgene suggests complex signaling pathways in drought-, salt-, and cold-stress responses. Ishitani et al. [31] isolated mutants that overexpressed rd29A or repressed it in response to dehydration, high salinity, cold and ABA. They propose that the ABA- and stress-signaling pathways are not independent and that the various stress-signaling pathways, including ABA-independent and ABA-dependent pathways, are not completely independent. Xiong et al. [32] isolated a hos5 mutant that has increased expression of rd29A when under osmotic stress but not when experiencing cold stress. Genetic analysis showed that HO55 is a negative regulator of osmotic-stress-responsive gene expression. Ishitani et al. [33] and Lee et al. [34] isolated hos1 and hos2 mutants, respectively, that enhanced the expression of rd29A, cor47, cor15a, and kin1. Non-acclimatized hos1 and hos2 mutants were less cold-hardy than wild-type plants. HO51 and HO52 are therefore thought to function as negative regulators of a cold-specific signal transduction pathway (Figure 1). Genetic analysis of these mutants and of stress-resistant or stress-sensitive mutants is likely to provide more information on stress-induced signal transduction [35].

Signal perception and signal transduction
Signal transduction pathways involved in the drought-stress response have been studied in yeast and animal systems [5]. Two-component systems seem to function in sensing osmotic stress in plants as well as in bacteria and yeast [36]. Recently, Urao et al. [37•] isolated an Arabidopsis cDNA that encodes a two-component histidine kinase (ATHK1), which functions as an osmosensor in yeast. ATHK1 has a typical histidine kinase domain, a receiver domain, and two transmembrane domains in the amino-terminal domain. ATHK1 might function in signal perception during dehydration stress in Arabidopsis, but sensors for cold stress have not yet been identified.

Many genes encoding factors that are involved in signal-transduction cascades are upregulated by dehydration and cold: mitogen-activated protein (MAP) kinases, calcium-dependent protein kinases, and enzymes involved in phospholipid metabolism, such as phospholipase C and phosphatidyl-4, 5-phosphate 5-kinase (PIP5) kinase [5,38,39]. These signaling factors might be involved in the amplification of stress signals and in the adaptation of plant cells to drought-stress conditions. No direct evidence has, however, been obtained of the functions of these signaling molecules. Transgenic plants that modify the expression of these genes and mutants with disrupted genes will provide more information on the function of their gene products.

Conclusions and perspectives
A major transcription system regulating ABA-independent gene expression in response to dehydration and cold stress includes a DRE/CRT cis-acting element and its DNA-binding protein, DREB/CFB. The DREB/CFB family of proteins contain two subclasses, DREB1/CFB and DREB2, which are induced by cold and dehydration, respectively, to express various genes involved in stress tolerance. Cross-talk between dehydration and cold occurs at the transcriptional level. ABA plays important roles in the dehydration-stress response but not, it seems, in the cold-stress response. Genetic analysis of stress-resistant or stress-sensitive mutants, and mutants with the rd29A promoter: luciferase transgene, should provide more information on signal transduction in response to dehydration and cold stress.

Sequencing of the Arabidopsis genome will be completed by the end of 2000, which means that the structure of all 25,000 Arabidopsis genes will soon be determined. All stress-inducible genes can then be identified by the systematic analysis of gene expression in microarrays. In the next decade, we think it important to develop novel methods to analyze the complex networks that control the stress responses of higher plants. A reverse genetic approach, as well as classical forward genetics, will become more important for understanding not only the functions of stress-inducible genes but also the complex signaling processes of the dehydration- and cold-stress responses.
Acknowledgements
Our work is supported in part by the Program for Promotion of Basic Research Activities for Innovative Biosciences and the Special Coordination Fund of Science and Technology Agency of Japan.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
* of special interest
** of outstanding interest


The first report of the osmosensor histidine kinase ATHK1 in Arabidopsis. ATHK1 has similar structure and functions to those of yeast osmosensor Shin1. This evidence suggests an important role for the two-component system in osmotic stress perception.


