

Minireview

Role of AP2/EREBP transcription factors in gene regulation during abiotic stress

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Received 14 April 2001; accepted 27 April 2001

First published online 10 May 2001

Edited by Gunnar von Heijne

Abstract Crop plants are exposed to many types of abiotic stress during their life cycle. Water deficit derived from drought, low temperature or high salt concentration in the soil, is one of the most common environmental stresses that affects growth and development of plants through alterations in metabolism and gene expression. Adaptation to these conditions may involve passive tolerance or active homeostatic mechanisms for maintaining water balance. Active responses occur at different levels in the plant and may represent a concomitant protection against other types of stress such as pathogen attack. Many morphological and physiological adaptations to water stress are under the control of the plant hormone abscisic acid and involve specific activation of target genes that in one way or another protect cells against water deficit or participate in the regulation of the drought response. Here, we discuss recent advances in our understanding of drought adaptation mediated by specific changes in gene expression and the role of AP2/EREBP nuclear factors in these processes. © 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

Key words: Drought; DRE *cis*-element; AP2/EREBP transcription factor; Abscisic acid

1. Drought signal transduction

Water deficit causes various alterations in plants, such as stomatal closure, decrease of turgor and changes in the composition of the cell wall or plasma membranes, which can act as a signal for triggering adaptation responses. Although relatively little is known about the mechanisms for sensing these changes, it is well established that abscisic acid (ABA) is a major physiological signal that induces the drought responses [1,2]. ABA-dependent signalling systems have been described that seem to mediate adaptation to drought by activation of bZIP proteins which then bind to so-called ABA-responsive regulatory elements (ABREs) in target genes and induce their transcription. Another ABA-dependent pathway requires protein biosynthesis of the MYC and MYB transcription factors, which function cooperatively to regulate the expression of target genes. However, in *Arabidopsis* not all drought responses appear to be mediated by ABA, since a number of

genes are known to be induced by drought, salt and cold in *aba* (ABA-deficient) and *abi* (ABA-insensitive *Arabidopsis* mutants). This suggests the existence of alternative regulatory systems of gene expression during the stress response. Indeed, recent studies have identified an ABA-independent pathway that leads to rapid responses to drought or cold and functions through members of the AP2/EREBP family of transcription factors, which recognise the previously described drought-responsive element (DRE) in target promoters [3]. Although these different pathways are usually considered to function independently from each other, it is certainly possible that some cross-talk exists between them, as supported by recent data discussed below.

2. The DRE

The DRE (5'-TACCGACAT-3') was first identified in the promoter of the drought-responsive gene *rd29A* from *Arabidopsis* [3]. *rd29A* encodes a protein similar to the late embryogenesis abundant (LEA) proteins, which are induced both during the maturation of embryos and by several types of stress in vegetative tissues and probably function as tolerance effectors [4]. The transcription of *rd29A* in *abil* and *abal* mutants suggests that cold- and drought-regulated expression does not require ABA. The DRE element was shown to be involved in the rapid induction of *rd29A* gene expression under conditions of dehydration and high salinity, but not in the response of this gene to ABA. In parallel, a *cis*-element with a similar sequence to the DRE, the C-repeat 5'-TGCGCGAC-3' (containing the core 5'-CCGAC-3') was identified in the promoter of the cold-inducible gene *cor15a* from *Arabidopsis* and shown to have a role in the cold response [5]. This motif was also described in the promoters of three other cold regulated genes, *kin1* and *kin2* [6,7] and *rab18* [8]. Studies of these genes in ABA-deficient and -insensitive mutants showed activation of the genes during drought and cold stress independently of ABA [9,10]. However, recent evidence indicates that some DRE/C-repeat motifs behave differently. The DRE2 *cis*-element in the maize *rab17* promoter appears to be involved in an ABA-dependent response. This site includes the typical core motif and was identified by *in vivo* footprinting analysis of embryos and leaves [11]. Differences in DRE2 occupancy were observed after both drought treatment and ABA induction, and *in vivo* analyses showed that DRE2 is important for activation of the *rab17* promoter in both situations. In addition, the *rab17* promoter contains

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the related DRE1 site, which mediates ABA-dependent regulation in the embryo but appears insensitive to stimulation by drought in vegetative tissues.

3. AP2/EREBP transcription factors

Several DRE-binding proteins have been identified from *Arabidopsis* and other plants that specifically interact with the DRE sequence. The first cDNA clone for DRE-binding protein was cloned using yeast one-hybrid screening; it was named CBF1 (CRT-binding factor1) [12]. Independently, Liu et al., by using the same approach, isolated two conserved groups of genes, DREB1 and DREB2 [13]. Recently, we have isolated two novel DRE-binding factors from maize that interact with the DRE2 element both in yeast and in maize [14]. These two proteins do not appear to be homologues of DREB1 or DREB2. Together, these observations suggest the existence of a complex family of DRE-binding proteins with the potential to mediate distinct responses to abiotic stresses such as drought, salt and cold (Fig. 1).

All DRE-binding proteins identified to date belong to the larger family of AP2/EREBP transcription factors. Several AP2/EREBP transcription factors are implicated in plant development and hormone-dependent gene expression. AP2/EREBP family proteins are unique to plants and share a highly conserved region of about 60 to 70 amino acids (the so-called AP2 domain) with no apparent similarity outside this domain [15]. The AP2 domain is a new type of DNA-binding module that includes two regions: a 20-amino acid-long N-terminal stretch rich in basic and hydrophilic residues (YRG element), and a C-terminal sequence capable of forming an amphipathic α -helix (RAYD element). DNA binding is thought to depend on the YRG element, whereas the RAYD element could contribute to this interaction or mediate protein–protein associations. DRE-binding proteins contain specific residues within their AP2 domain that may determine their ability to bind the DRE/C-repeat element [16].

4. Function of AP2/EREBP transcription factors in the regulation of stress responses

Several lines of evidence support an *in vivo* role of DREB1 and DREB2 factors in the regulation of drought and cold-inducible genes such as *rd29A*. First, both proteins activate transcription of a reporter gene carrying DRE elements in *Arabidopsis* leaf protoplasts. Also, overexpression of DREB1

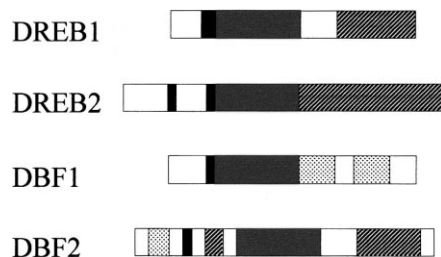


Fig. 1. Schematic representation of DRE-binding proteins from the AP2/EREBP family of plant transcription factors. Grey boxes represent the AP2 domains of each protein. Black boxes represent basic regions that could function as nuclear localisation signals, whereas white ones represent random sequences. Hatched boxes represent serine or serine/threonine abundant regions. Dotted boxes represent acidic and acidic/serine-rich regions respectively.

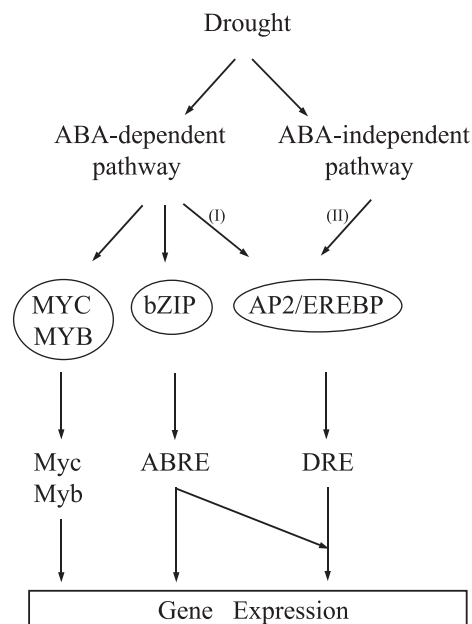


Fig. 2. Schematic representation of drought signal transduction via ABA-dependent and -independent pathways. I, *Zea mays*; II, *Arabidopsis*.

in *Arabidopsis* transgenic plants induced *rd29A* expression in unstressed conditions. Notably, these transgenic plants showed enhanced drought and cold stress resistance. A dwarf phenotype was obtained when the above genes were overexpressed under a strong constitutive promoter. However, no growth alterations were observed when DREB1 gene was overexpressed under the control of a stress-inducible promoter. Normal growth and specific responses to environmental stress are important characteristics for biotechnological applications [13,17,18].

Finally, both DREB1 and DREB2 factors are induced by water stress or cold and their transcripts accumulate at high levels shortly after initiation of the stress treatment. The DREB1 genes are induced by low temperature whereas the DREB2 homologues are induced by drought and high salt stress [13,19,20]. In spite of the increase in endogenous levels of ABA after stress treatments neither of these genes are induced by exogenous ABA, suggesting that their function is independent of this hormone [19].

Interestingly, the *rd29A* promoter also contains ABRE regulatory elements that mediate activation by bZIP proteins during ABA signalling. Recently, six new genes containing both DRE/C-repeat and ABRE motifs in their promoters were identified [21], implying complex regulation of stress-induced genes by ABA-dependent and ABA-independent pathways. Consistent with this idea, one of the maize DRE-binding proteins that we have identified is induced by exogenous ABA [14], arguing that DRE-binding factors may also mediate transcriptional regulation in response to ABA signalling. Future work should help delineate the different signalling pathways and their cross-talk during adaptation of plants to drought and other stresses (Fig. 2).

5. Conclusion and prospects

Our knowledge of the molecular mechanisms underlying the

responses of plants to environmental stresses such as drought is still rather limited, but an increasing number of genes have been identified in recent years that mediate those responses. Some of these genes are induced by stress stimuli and encode products that confer tolerance to adverse conditions, whereas others encode upstream regulators that function within signalling pathways controlling the stress response. DRE-binding proteins are a growing subfamily of AP2/EREBP factors with important roles in directing changes in gene expression during stress. While DREB proteins appear to function mostly in the context of plant responses that are independent of ABA, recent findings support a more complex function in ABA-dependent pathways as well. The challenge now is to understand the relative roles of DRE-binding proteins in different pathways, their coordinated regulation and the interactions with other signalling elements. In the longer term, these studies should assist the manipulation of plants in order to improve their stress tolerance and the productivity of crops. In this context, the increased tolerance to water and cold stress of *Arabidopsis* plants overexpressing DREB1 provides a first approach toward that goal [17,18]. As we learn more about DREB proteins and their regulation, it may be possible to design additional strategies that target specific aspects of plant tolerance to stress.

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