A drought-inducible *myb*-related gene in plants

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Recent studies on plant *myb*-related genes showed that plant *myb* genes constitute a large gene family of more than 20 members and may play wide variety of roles in the regulation of gene expression. One of *myb*-related genes, *Atmyb2*, that is responsive to water stress and abscisic acid (ABA) has been cloned from *Arabidopsis thaliana*. This suggests that the ABA-mediated induction of drought-inducible genes whose expression requires protein synthesis may be regulated by the ATMYB2 protein.

In higher plants, a number of *myb*-related genes have been isolated and extensively characterized by genetic analysis. Among these genes, a maize *C1* gene regulates the expression of structural genes that are involved in the biosynthesis of anthocyanin pigments during seed development and *C1* gene is regulated by plant hormone abscisic acid (ABA) and *VP1* gene.[1] An *Arabidopsis myb*-related gene, *GL1*, is necessary for the formation of leaf trichomes.[2] In addition, many *myb*-related genes have been cloned from various plants by using homology to known *myb* genes. Plant *myb*-related genes are thought to constitute a large family of genes and may play wide variety of roles in the regulation of gene expression. These studies on plant *myb*-related genes led us to examine whether a *myb*-related gene is involved in the ABA-responsive expression of drought-inducible genes in vegetative tissues of a higher plant. We screened a cDNA library prepared from *Arabidopsis* plants that had been dehydrated for 10 hr and cloned a cDNA, designated *Atmyb2*, that encoded an MYB-related protein.[3]

Atmyb2 gene was rapidly induced by dehydration stress. Rehydration of dehydrated Arabidopsis plants caused a decrease in the level of *Atmyb2* mRNA, which indicates that *Atmyb2* responds specifically to drought stress. High-salt conditions and application of exogenous ABA also resulted in the induction of *Atmyb2*, although *Atmyb2* did not respond to cold or heat stresses. Therefore, *Atmyb2* is a gene responsive to water stress such as drought and high-salt stresses, and ABA is involved in the expression of *Atmyb2*.

The putative ATMYB2 protein has several features common to plant homologs of MYB. Figure 1 shows a comparison of the amino acid sequences of the DNA binding domains of plant MYB-related proteins, Drosophila MYB and human c-MYB. ATMYB2, like other plant MYB-related proteins, has two imperfect repeats of 51 to 53 amino acids with conserved tryptophan residues. However, the first tryptophan residue in the second repeat (repeat III) found in animal MYB proteins is replaced by an isoleucine or a phenylalanine residue in plants. The ATMYB2 protein, as well as other plant MYB proteins, lacks repeat I in the DNA binding domain that has been found in human, mouse and Drosophila MYB proteins. Products of viral *myb* onco-genes (v-*myb*) also interact with

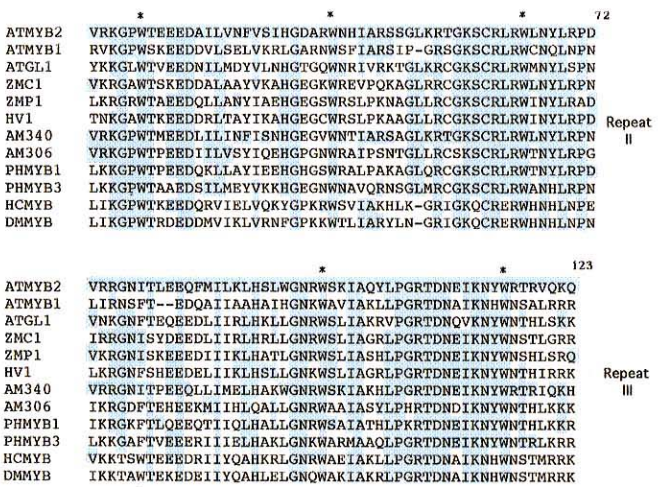

Fig. 1. Comparison of amino acid sequences of the DNA binding domains of MYB-related proteins; maize *C1* (ZMC1), maize P1 (ZMP1), barley Hv1 (HV1), Antirrhinum 340 and 306 (AM340 and AM306, respectively), Petunia MYB1 and 3 (PHMYB1 and PHMYB3, respectively), Arabidopsis GL1 (ATGL1), Arabidopsis ATMYB1 (ATMYB1), Drosophila MYB (DMMYB) and human c-MYB (HCMYB). Shaded boxes indicate amino acid residues identical to those of ATMYB2, and asterisks represent conserved tryptophan residues. Dashes indicate gaps introduced to maximize alignment. Numbers above each repeat refer to amino acid positions in ATMYB2.

DNA in a sequence-specific manner in spite of the absence of repeat I. These findings imply that only repeats II and III are required for a functional DNA binding domain in plant MYB proteins. The c-MYB protein recognizes the conserved DNA sequence PyAACTG and AAC has been proposed as the core sequence of the binding site. The ATMYB2 protein expressed in *E. coli* was shown to bind specifically to the MYB-recognition sequence found in the simian virus 40 enhancer and the maize *Bz-1* promoter.

Since *Atmyb2* is induced by water stress, it seems likely that the ATMYB2 protein functions as a transcription factor that controls the expression of genes that are induced by drought stress or high-salt conditions. We have isolated nine cDNAs whose corresponding genes (*rd*) are induced by dehydration stress and analyzed their expression.[4] One of

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Table 1. Induction of mRNAs for *Atmyb2* and *rd22* by various stimuli.

	Dehydration	Salt[a]	ABA[b]	Cold[c]	Heat[d]
Atmyb2	+	+	+	-	-
rd22	+	+	+	-	-

a. 250 mM NaCl. b. 100 μM ABA. c. 4°C. d. 40°C.

drought-responsive genes in *Arabidopsis*, *rd22*, showed a pattern of gene expression similar to that of *Atmyb2* (Table 1). Both *rd22* and *Atmyb2* are induced by dehydration and high-salt stresses, but not by cold and heat stresses. ABA causes the induction of both *rd22* and *Atmyb2*. Moreover, protein synthesis is necessary for the induction of *rd22*, which suggests the possibility that the expression of *rd22* might be regulated by trans-acting factors whose synthesis *de novo* is induced by dehydration or ABA.[5] The promoter region of *rd22* that is involved in the drought-induced expression contains the sequence of two closely located recognition sites for the transcription factors, MYC and MYB, but not the consensus ABRE sequence.[6] Thus, it appears that the expression of *rd22* is regulated by a novel mechanism. Recently, maize homologs of MYB and MYC, designated *C1* and B, respectively, were shown to act synergistically to activate the promoter of the maize *Bz-1* gene, an anthocyanin-biosynthetic gene. As in the *rd22* promoter, an MYC-recognition sequence is located closely to the MYB-recognition site in the *Bz-1* promoter. Maize R and *C1* proteins, when constitutively expressed in *Arabidopsis*, have been shown to act coordinately to induce production of anthocyanin. We postulated that the ATMYB2 protein induced by ABA might act in cooperation with the MYC protein to activate transcription of *rd22* (Fig. 2).

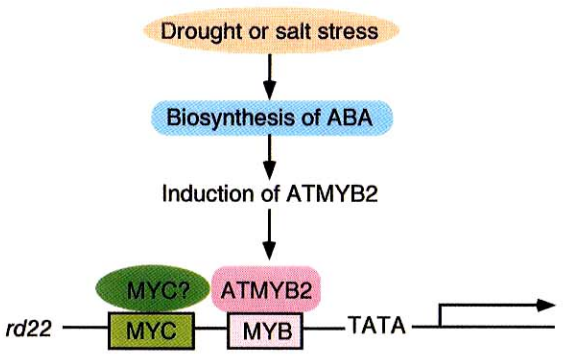

Fig. 2. A model for the mechanism that regulates the dehydration-responsive expression of *rd22*.

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