CONTROL *of* PLANT GENE EXPRESSION

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Chapter
5

Regulation of Gene Expression by Abscisic Acid

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INTRODUCTION

The molecular basis of phytohormone action has been the subject of intense interest to plant physiologists for many years. Most phytohormones mediate distinctly diverse physiological responses in different plant tissues. For example, abscisic acid (ABA) interacts with leaf epidermal guard cells to inhibit stomatal opening, while in embryonic cells ABA inhibits the germination process (see Hetherington and Quatrano, 1991). Several general and important characteristics of these hormonal responses are illustrated in Figure 1. Some responses of plant cells to hormones may not require a direct alteration of gene expression, e.g., the stomatal closure response by ABA (PR_1, Figure 1A). However, most phytohormone response chains ($PR_{2,3}$) include a specific set of RNA transcripts and protein products that are correlated with the unique physiological response, e.g., inhibition of embryo germination by ABA. Such a specific ABA response (PR_2) that results from the activation of a gene set is characterized by tissue-specific gene products (genes 1,2). Other hormones like auxin (H_B) have been shown to elicit expression from a different gene set which results in an auxin-specific physiological response (PR_3). The auxin-stimulated gene set would likely include genes not activated by ABA (genes 5,6), some that may be common to both ABA and auxin (genes 3,4), and perhaps intermediates that do not include direct gene activation but are essential for the physiological response (PR_3). To date, however, series of intermediates in any one phytohormone response chain have not been elucidated.

The expression of hormone-regulated genes is controlled by a variety of processes. One level of control that is operative in many hormone responsive genes is at transcription.

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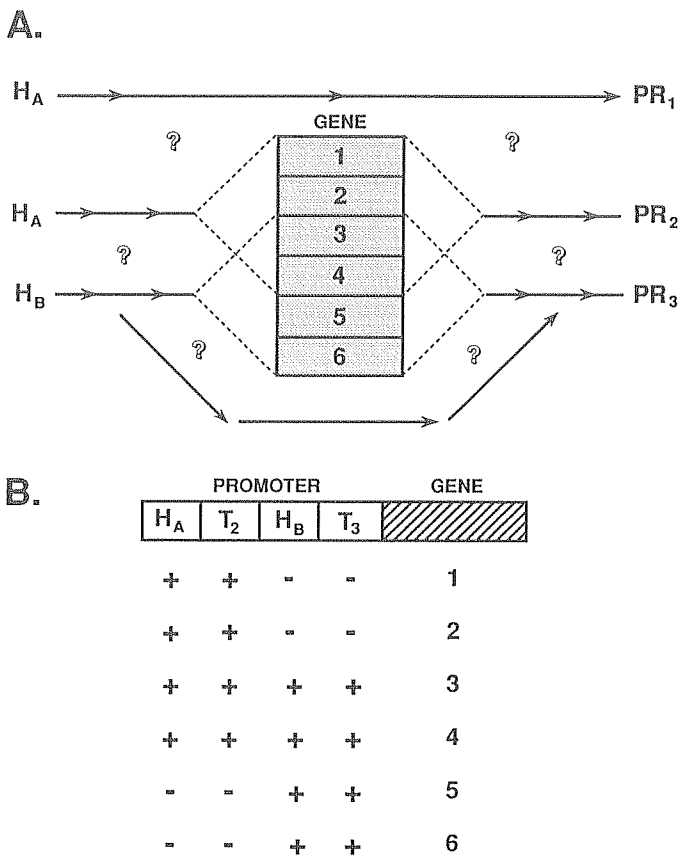

Figure 1. (A.) Diagram of hypothetical hormonal response pathways showing how different hormones ($H_{A,B}$) can elicit physiological responses ($PR_{1,2,3}$) in different tissues. These pathways can result in responses that occur independently of new gene activation (PR_1) or in combination with differential gene activation ($PR_{2,3}$). (B) Highly schematic model of genes 1 through 6 in A, whose tissue-specific (T) and hormone-responsive (H) regulatory sequences in the promoter are differentially utilized (+) to regulate gene expression in tissues ($PR_{2,3}$) under various hormonal ($H_{A,B}$) stimuli.

Regulatory sequences that are required for the specific pattern of expression of these responsive genes are depicted in Figure 1B. In this diagram, the sequence elements directing hormone (H) and tissue (T) specificity of the response genes (1 through 6) are localized in discrete regions of the promoters. In an oversimplication shown in Figure 1B, genes 1 and 2 require the activation (+) of an ABA response element (H_A), as well as a tissue-specific element (T_2). However, other levels of control (e.g., posttranscriptional and translational) may also be operative in these response chains.

The specific focus of this chapter is on ABA and recent results that begin to elucidate its mechanism of action at the level of gene expression. By initially concentrating on this central site in the response chain, we hope to begin deciphering the ABA response pathways in both directions, i.e., to characterize the initial receptor of the ABA signal and intermediates involved in the signal transduction, as well as to determine the role of the products of these genes in the specific physiological response. In particular, we will examine the evidence for (1) ABA-responsive genes, (2) specific regulatory sequences in the promoters of these genes that can confer ABA-responsiveness, and (3) DNA binding proteins that interact with such sequences and may be involved in the regulation of transcription. This

evidence is derived from two of the most well-studied systems in which ABA affects gene expression, seed development (Quatrano, 1987; Hetherington and Quatrano, 1991), and the response to water stress (Skriver and Mundy, 1990; Hetherington and Quatrano, 1991). The role of ABA in the stomatal response has recently been reviewed (Hetherington and Quatrano, 1991) and will not be discussed in this chapter.

ABA-RESPONSIVE GENES

Seed Development

Towards the latter third of seed development in both monocots and dicots, a set of gene products begins to accumulate in embryos that include a variety of proteins such as a lectin (Raikel and Wilkins, 1987), enzyme inhibitor (Mundy et al., 1986), lipid body membrane protein (Hatzopoulos et al., 1990), storage proteins (Finkelstein et al., 1985; Bray and Beachy, 1985; Quatrano, 1986), and a number of functionally uncharacterized proteins that appear at the time embryos acquire the ability to withstand desiccation (Dure et al., 1989). If these embryos are isolated at earlier developmental stages and exposed to exogenous ABA in culture, most of these same proteins are precociously accumulated (Quatrano, 1987; Skriver and Mundy, 1990). In the absence of ABA, these products are not accumulated, embryos bypass maturation, do not acquire desiccation tolerance, and precociously germinate into seedlings. For example, Williamson and Quatrano (1988) showed that a 10-kDa soluble protein in wheat embryos, Em, accumulates during the latter third of grain development. When wheat embryos are removed from the grain at the end of the first third of grain development and cultured in the presence of ABA, Em accumulates. In the absence of ABA, the immature embryos do not accumulate the mRNA or protein product from the Em gene and precociously germinate. Similarly, Bartels et al. (1988) identified a set of proteins that are synthesized or enhanced in barley embryos during the developmental period leading to the acquisition of desiccation tolerance. They found that tolerance to desiccation can be precociously induced by ABA and was accompanied by the accumulation of the same set of proteins that coincide with the developmentally acquired ability to withstand desiccation. Although this special class of proteins has been suggested to function in desiccation tolerance (see Baker et al., 1988; Dure et al., 1989), a direct relationship between a protein(s) and tolerance has yet to be demonstrated. Hence, ABA has been shown to play a critical role in promoting embryogenesis and the acquisition of desiccation tolerance, while preventing precocious germination. All of these physiological responses to ABA are accompanied by changes in the levels of specific mRNA and protein species.

Water Stress

When vegetative plant tissues are exposed to water stress by high osmoticum, NaCl, or desiccation, specific mRNAs are accumulated, some of which are identical to those induced during embryo development. These include the Em gene from wheat (Berge et al., 1989; Morris et al., 1990) and 15 to 25-kDa proteins from barley (Chandler et al., 1988), maize (Gomez et al., 1988), and rice (Mundy and Chua, 1988). The expression of these cereal genes in embryos as well as vegetative tissue subjected to water stress is at least in part due to increases in the level of endogenous ABA (Henson, 1984; Jones et al., 1987). The ABA responsiveness of these genes appears to be tissue-independent, making them good candidates to study the basic mechanism responsible for ABA-dependent gene expres-

sion. Hence, we will focus most of our subsequent discussion on these cereal genes, in particular, the Em gene from wheat.

CELLULAR REGULATION OF EM EXPRESSION

Tissue Independence

Em transcripts not only accumulate in wheat embryos, but also in seedling tissue when incubated in ABA or under conditions of water stress (Berge, 1989; Morris et al., 1990). A similar response is reported for the *rab 21* gene, which is normally expressed in rice embryos but can be induced in root and shoot tissue of seedlings (Mundy and Chua, 1988). Since Em transcripts are induced by ABA in both embryonic and vegetative tissues, suspension cultures have also been examined for their ability to respond to ABA with increasing levels of Em transcripts. Rice suspension cultures, derived from immature embryos, respond to ABA by accumulating endogenous Em-like transcripts. Northern analysis of ABA-treated rice suspension cultures, using the wheat Em cDNA as a probe, detects the accumulation of a mRNA of the same approximate size as that found in ABA-treated wheat embryos, i.e., 700 to 750 nucleotides (Bostock and Quatrano, 1992). Similar results are obtained in rice suspension cultures with the ABA-responsive *rab 21* gene (Mundy and Chua, 1988). Hence, the accumulation and regulation of Em and rab mRNA transcripts in rice suspension cultures by ABA indicates that the response at the transcript level is similar to the response found in embryonic and vegetative tissues.

Multiple-Level Control

The accumulation of Em transcripts in response to ABA is controlled at least in part at the level of transcription (Williamson and Quatrano, 1988). DeLisle and Crouch (1989) have obtained similar results in their studies of the ABA-regulated napin and cruciferin genes in *Brassica*. However, unlike their results, the ABA-induced expression of the Em gene is also controlled at posttranscriptional and/or translational level(s) (Williamson and Quatrano, 1988; Berge et al., 1989). When mature embryos are imbibed in ABA, the Em transcripts that are accumulated in the dry embryo from early stages of embryogenesis are maintained at high levels. In the absence of ABA, these transcripts are rapidly degraded within the first several hours of imbibition (Williamson et al., 1985; Morris et al., 1990). The requirement for ABA to maintain levels of the Em transcript found in mature embryos occurs even in the presence of α-amanitin, at concentrations that specifically inhibit RNA synthesis from polymerase II (Williamson and Quatrano, 1988). As such, ABA may have an effect on the stability/translatability of the Em mRNA.

Posttranscriptional regulation of the ABA response is also seen in vegetative tissue. While transcripts of Em are detected in wheat seedlings (1 to 10 days) when exposed to exogenous ABA, antibodies to Em detect lower levels of Em protein in these tissues (Berge et al., 1989). These results indicate that when comparable levels of Em mRNA are induced specifically by ABA in embryonic and vegetative tissues, the Em protein is detected at higher levels in embryonic tissue. Controls at the level of Em mRNA translatability and/or Em protein stability are likely to be involved. Hence, transcription of the Em gene is regulated in a tissue-independent, ABA-dependent manner, and other levels of control are operative in the regulation of mRNA stability and protein accumulation in nonembryonic tissue.

Interactions with Other Signals

The Em gene is not expressed in response to auxin, cytokinin, salicylic acid, or gibberellic acid (GA), nor does GA reverse the effect of ABA on Em gene expression in a transient assay (Jacobsen and Close, 1991). However, environmental signals that are believed to be transduced, at least in part by changes in ABA levels (e.g., desiccation, temperature extremes, salinity, wounding), can also result in Em gene expression. For example, desiccation of rice suspension cultures to 10% of their initial fresh weight increases endogenous ABA over twofold and is accompanied by an increase in Em mRNA that parallels the increase in ABA levels (Bostock and Quatrano, 1992). Other environmental signals such as exposure to heat shock temperatures, chilling, or UV light, however, neither increase endogenous ABA levels nor induce Em expression in rice suspension cultures (Bostock and Quatrano, 1992).

Em expression has been investigated in rice cell suspension cultures following exposure to various combinations of ABA and osmotic stress. Response-saturating concentrations of either ABA (50 m*M*) or NaCl (0.4 *M*) rapidly induce (by 60 min) the accumulation of Em mRNA, with a maximum accumulation occurring at 12 to 24 h after treatment. At least a part of the salt effect is via changes in endogenous ABA levels. Inhibition of ABA biosynthesis by fluoridone during NaCl treatment reduces the levels of endogenous ABA by fourfold and Em expression by 50%. Treatment with saturating levels of both NaCl and ABA results in a doubling of Em transcript levels over the maximum for each signal alone. Likewise, when a single, saturating level of NaCl is used with increasing levels of ABA, Em transcripts are detected at ABA concentrations that alone did not result in changes in Em expression. This increase in sensitivity of tissue to ABA in the presence of salt may help explain the results of Morris et al. (1990). They found that under conditions of osmotic stress, Em expression is high but ABA levels do not increase. ABA is required for Em expression, however, since if they reduce endogenous levels of ABA with a biosynthetic inhibitor, Em transcripts are not detected. One interpretation of these results might be that the osmotic stress results in a change in the sensitivity to ABA, thereby making the existing levels of ABA sufficient to trigger the response. Data from the rice culture studies suggests that salt interacts synergistically with ABA, in part because of the increased sensitivity of rice cells to ABA. The effect of salt stress on Em gene expression in rice suspension cells appears to operate through two pathways: one is mediated through increases in the level of ABA; the other is via a unique salt response pathway that includes an intermediate that is common to both the salt and ABA response chains (Bostock and Quatrano, 1992).

Regulatory Genes That Modulate/Control the ABA-Responsive Genes

Another powerful approach to understand a hormonal response pathway is to isolate mutants that have an aberrant response to a particular hormone, e.g., hormone-insensitive mutants. Mutants with reduced sensitivity to ABA or altered ABA metabolism have been studied in potato (droopy), pea (wilty), and tomato (fic, sit, not) (see Neill and Horgan, 1985) as well as in *Arabidopsis* (Koornneef et al., 1989). The general characteristics of these mutants have been recently reviewed (Quartrano, 1987; Hetherington and Quatrano, 1991), but for the purposes of this chapter and the focus of our review, the ABA-insensitive viviparous mutant of maize (vp1) will be discussed.

ABA insensitivity in the homozygous vp1 mutant is confined to the embryo tissue. These vp1 embryos bypass the normal maturation and dormancy phases (Robichaud et al., 1980; Neill et al., 1987) and germinate precociously. Additionally, anthocyanin production is blocked in kernels (i.e., they appear yellow) and a number of other enzyme deficiencies have been detected in aleurone tissues (Dooner, 1985). Vp1 cDNA clones have recently

been isolated from both wild type and mutant maize and used to analyze Vp1 expression at the molecular level (McCarty et al., 1989). These studies indicate that Vp1 is required for the transcription or mRNA stability of a number of genes and is likely to be involved in the ABA response pathway. Recent results by Kriz et al. (1990) and Rivin and Grundt (1991) clearly demonstrate that the ABA-responsive 7S globulin gene from maize embryos is not expressed at the mRNA or protein level unless both the Vp1 product and ABA are present. It is possible that the Vp1 gene product is a regulatory protein which interacts with DNA sequences common to both the ABA response and anthocyanin biosynthesis genes. As such, the product of Vp1 could be a component of the ABA response pathway and may "confer tissue specificity to ABA and light-regulated gene expression associated with seed maturation" (McCarty et al., 1989).

MOLECULAR REGULATION OF EM EXPRESSION

Cis Regulatory Sequences

A common strategy used to identify sequences that are involved in the regulation of inducible gene expression involves the construction of chimeric genes. These fusion genes contain putative regulatory sequences from the gene of interest, linked to an easy-to-assay reporter coding sequence, usually one that encodes an enzyme. The regulatory sequences can be derived from different regions of the gene, including the 5′ and 3′ flanking regions, introns, etc. The chimeric constructs are then introduced into cells which are competent to respond to a signal (e.g., a hormone) and tested for inducible expression. The ability of the promoter sequences to confer responsiveness to the fusion is determined by comparing the level of reporter gene activity in the absence and presence of the specific inducer. This strategy was utilized by Marcotte et al. (1988) to identify the first phytohormone response element, an ABA response element, from the promoter of the Em gene.

Using a region of the promoter from an Em genomic clone (Litts et al., 1991), Marcotte et al. (1988, 1989) demonstrated the expected ABA and embryo-specific regulation of a fused reporter gene in a transient expression system using rice protoplasts and in stably transformed tobacco plants. The following sections characterize the transient assay system and summarize the functional dissection of the Em promoter.

Characterization of the Transient Assay System

For many years, functional characterization of plant promoters has been hindered by the lack of a system for rapid analysis. Studies relied on the direct measurement of either mRNA or protein levels in the plant of interest or in transgenic plants. However, these procedures are time consuming and labor intensive. More recently, plant protoplasts have been employed to study the transient expression of introduced genes (e.g., Walker et al., 1987). This type of system is now widely used in plant systems to rapidly identify sequences involved in gene regulation (Marcotte et al. 1988; Huttly and Baulcombe, 1989; Mundy et al., 1990; Jacobsen and Close, 1991). The results from the more rapid transient analyses then serve as a guide for the generation of transgenic plants for detailed *in vivo* analyses.

The first use of a hormone-responsive protoplast transient expression system has been reported by Marcotte et al. (1988) to analyze the Em promoter. In this system, protoplasts are isolated from rice suspension cultures and chimeric genes are introduced into these wall-less cells by polyethyleneglycol-mediated direct DNA uptake. In the study by Marcotte et al. (1988), a 646 bp promoter segment (-554 to $+92$) of the Em gene is linked to the reporter gene encoding the bacterial β-glucuronidase enzyme (GUS) and the cauliflower

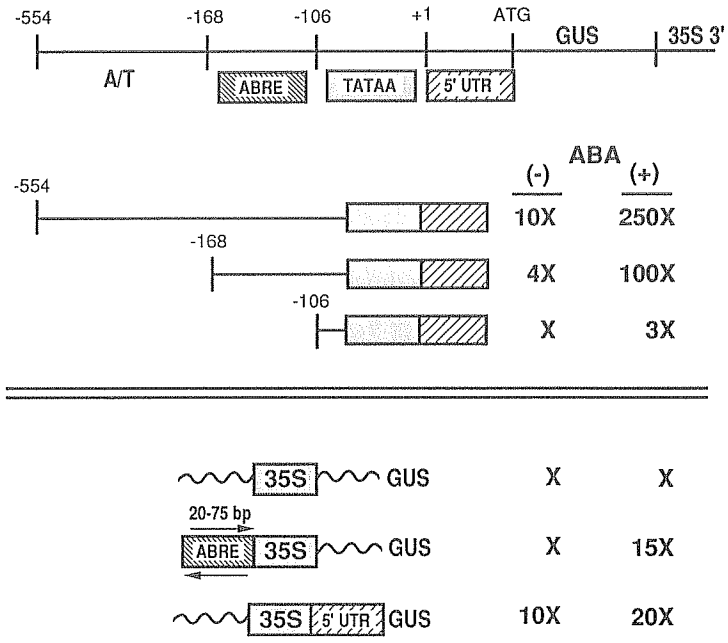

Figure 2. Diagram of the Em chimeric gene construct utilized in the transient assay which includes the Em promoter, the reporter gene GUS, and the 3′ segment of the CaMV 35S gene. Representative deletions and the corresponding GUS activities in the absence (−) or presence (+) of ABA (100 μM) illustrate the functional dissection of the Em promoter into an AT enhancer region, the ABA response element (ABRE), and the 5′ untranslated leader (UTL). Removal of the ABRE and 5′ UTL from the Em promoter and linking them to a minimal CaMV 35S (−90) promoter demonstrates their ability to confer ABA inducibility and enhance expression, respectively (see Marcotte et al., 1989).

mosaic virus (CaMV) 3′ end (Figure 2). After introduction into the protoplasts, the addition of ABA triggers a 15- to 30-fold induction in GUS activity after an 18-h incubation period. The response of the Em promoter-GUS fusion to ABA is rapid, with enzyme activity being detectable at less than 1 h after addition of ABA. The induction level is proportional to the concentration of ABA used and occurs at the same concentrations of ABA that had been previously shown to inhibit precocious germination of cultured wheat embryos (Walker-Simmons, 1987).

The 646-bp truncated Em promoter-GUS construct gives approximately the same level of GUS activity in the transient assay as a longer 1800-bp promoter-GUS fusion, both in the presence and absence of ABA. However, when present in the opposite orientation, the 646-bp promoter of EM is totally inactive with or without ABA (Marcotte et al., 1988). RNase protection experiments demonstrate that protected fragments are detected only in samples that have been treated with ABA. In addition, the transcription start site (at −86 bp from the ATG) is the same in rice protoplasts transiently expressing either the Em-GUS fusion or the wheat Em genomic clone and is identical to the transcriptional start site in wheat embryos (Marcotte et al., 1988).

The ABA response of the Em gene in this system is phytohormone specific since it is not elicited by GA, auxin, kinetin, ethylene, salicylic acid, or by any combination of these hormones. In addition, ABA does not stimulate GUS expression when the reporter gene is linked to the GA-inducible α-amylase promoter (α2GT; Huttly and Baulcombe, 1989) or to the CaMV 35S promoter (Marcotte et al., 1988). Jacobsen and Close (1991) have shown that whereas the Em-GUS fusion does not respond to GA in barley aleurone protoplasts, it

is responsive to ABA in that same system. In addition, the α-amylase promoter not only responds to GA in the barley aleurone protoplast, but its expression is reduced by ABA, the normal response in intact aleurone layers and barley seeds. These results indicate that protoplast transient expression systems from at least two cell types and three species can appropriately regulate the Em promoter in response to ABA in a specific way. Furthermore, these cells are competent not only for directing responses to ABA but also to other stimuli, for example, GA.

A few studies have indicated that results from promoter analyses in transient assays are similar to those from transgenic experiments and that some promoters from monocots can function normally in dicotyledonous plants, such as tobacco (Marcotte et al., 1989; Robert et al., 1989). To extend the above observations and to determine if the 646-bp promoter fragment from wheat could be properly regulated in embryos of developing tobacco seeds, tobacco leaf disks were transformed using *Agrobacterium,* and regenerated transgenic plants were analyzed for GUS expression. No GUS activity was detected in vegetative tissue nor in young seeds from the Em-GUS transformants. However, in mature seeds removed from the Em-GUS transformants, GUS expression was very high and was confined to the embryo. In addition, the expression pattern of this fusion correlates with the developmental rise in ABA levels during tobacco seed development (Yamaguchi-Shinozaki et al., 1991). Control 35S-GUS transformants showed normal expression, and the 646-bp Em promoter fragment in the reverse orientation was totally inactive in all tissues, including embryos.

If immature transgenic tobacco seeds are removed and cultured on ABA, a threefold increase in Em-GUS activity is observed (Marcotte et al., 1989) indicating that the activity is precociously induced, similar to the expression of Em in *in vitro* cultured wheat embryos. This is not always the case as Yamaguchi-Shinozaki et al. (1991) found that a similar length promoter region from the rice *rab-16B* gene fused to GUS, while expressed at high levels in a developmentally appropriate manner in transgenic tobacco seeds, is not precociously induced in transgenic zygotic embryos. They did demonstrate ABA-inducible expression in pollen-derived embryos of tobacco and attribute this to a difference in the transcription machinery between rice and tobacco. Taken together, these observations demonstrate that the results from protoplast transient expression systems can accurately predict the results of transgenic analyses and attest to the utility of this type of system for the rapid functional analysis of regulatory sequences.

Functional Dissection of the Em Promoter

AT-Rich Enhancer Sequences. Using the rice transient expression system, Marcotte et al. (1989) and Guiltinan et al. (1990) have defined the sequences within the 646-bp 5′ Em segment (−554 to +92) that are necessary and sufficient for the ABA response. Marcotte et al. (1989) demonstrate that a GUS fusion construct containing the Em promoter segment from −168 to +92 gives the same approximate 25-fold induction as longer Em promoter fragments in response to ABA, but with a reduced level of GUS expression. When a slightly shorter Em promoter fragment is used, (−106 to +92), the ABA response and expression is essentially abolished (Figure 2). Hence, the observed reduction in absolute levels of expression is proportional to the length of sequences removed and is evident both in the presence and absence of ABA.

Examination of the sequences between −554 and −168 reveals the presence of three regions, 40 to 70 nucleotides each, which contain at least 84% A plus T (AT) residues. Similar AT-rich regions are observed in several other plant genes (Jofuku et al., 1987; Jordano et al., 1989) and have been shown to be associated with high levels of gene

expression (Bustos et al., 1989). As such, the drop in expression levels in the Em-GUS fusions mentioned above has been attributed to the removal of these nonspecific transcriptional enhancer sequences (Marcotte et al., 1989). Other investigators have found a similar phenomenon using a 5′ deletion strategy (Huttly and Baulcombe, 1989; Takahashi et al., 1990; Jacobsen and Close, 1991) for genes which respond to different signals.

Abscisic Acid Response Element (ABRE). Conserved sequence motifs in similarly regulated genes have frequently been taken to imply that those sequences may be intimately involved in the mechanism of regulation, although functional data exists in only a few cases. Results of the deletion analysis of the Em promoter in the transient assay has identified a 62-bp region (−168 to −106) that is likely to contain at least part of a specific ABA response element (ABRE). Comparison of gene sequences from seed and ABA-regulated promoter regions has revealed several conserved sequence motifs in the Em promoter region near the ABRE. Other consensus sequences have been proposed (see Em1a, Em1b, and Em2 in Marcotte et al., 1989; Mundy et al., 1990).

One such conserved sequence, Em1, is found in all ABA-regulated promoters for which sequence data are available. In addition, similar sequences have been found in the promoter regions for genes which respond to a wide variety of environmental stimuli such as light (Schulze-Lefert et al., 1989), wounding (Herrmann et al., 1988), and stresses such as drought (Mundy and Chua, 1988). While the importance of these sequences can not be confidently addressed until further functional and structural analyses of all of these promoters are completed, we will discuss the significance and implications of their presence in a later section.

To test the functionality of the region of the Em promoter containing the sequences Em1a/b and Em2, various oligonucleotides containing these conserved motifs have been synthesized and linked to the 5′ end of a truncated 35S(−90)-GUS fusion (Figure 2). These constructs are then introduced into rice protoplasts to determine if the oligonucleotide sequences are sufficient to confer ABA responsiveness on the 35S promoter. A 75-bp sequence containing Em1a/b and Em2 fused in either orientation to the 35S promoter gives a greater than tenfold induction of GUS activity in the presence of ABA (Guiltinan et al., 1990). A 20-bp oligonucleotide which contains only Em1a is sufficient to confer ABA responsiveness and, as such, constitutes a minimal ABRE (Figure 2). Transient expression analyses demonstrated that mutations at Em1a reduce or eliminate the ABA response (Guiltinan et al., 1990). The results to date strongly suggest that the sequences necessary for the ABA response in the rice protoplast transient expression system reside in the sequences at Em1a. As the sequence requirements for the minimal ABRE are short and the element functions in an essentially orientation-independent manner, the ABRE is characteristic of the hormone response elements found in animal systems. The conserved sequence Em2, found in some ABA-regulated promoters and also in many seed-specific promoters that may or may not be regulated by ABA (e.g., α′ subunit of α-conglycinin), appears not to be essential for the ABA response and, as such, may be involved in the seed-specific regulation of these genes.

Structural analyses of two distinct auxin-regulated genes from soybean have shown conserved sequences in the 5′ promoter regions, but there are no data to address their functionality (Ainley et al., 1988; McClure et al., 1989). Another auxin-responsive promoter, for which there is some functional data, does not contain this conserved sequence (Takahashi et al., 1990). More recently, Conner et al. (1990) compared the promoter sequences of two auxin-regulated genes from *Arabidopsis* and found some regions of high conservation between them, but these conserved sequences from *Arabidopsis* are different from those of soybean. Broglie et al. (1989) have identified a sequence conserved in several

chitinase gene-promoter regions. In this case, they have used a transient assay to demonstrate that a 50-bp oligonucleotide linked 5′ to a CaMV(−90)-GUS fusion is sufficient for ethylene-induced expression. In yet another study, regions have been functionally identified that appear to be responsible for GA-induced expression of α-amylase genes from wheat (Huttly and Baulcombe, 1989) and barley (Jacobsen and Close, 1991). None of these ethylene, GA, or auxin sequences bear any resemblance to the proposed ABRE.

5′ Untranslated Region. Regulation of gene expression at the level of transcription has been quite well studied in a variety of systems. However, other levels of regulation are known to have profound effects on the ultimate level of expression. The results of Williamson and Quatrano (1988) suggested that the Em mRNA may be preferentially stabilized in wheat embryos as compared to other ABA-regulated sequences. As such, this could represent an additional level of control that contributes to the high level of Em expression. To further investigate this phenomenon, and to determine if sequences in the 5′ and/or 3′ untranslated regions (UTR) of the Em gene are involved in the ABA response, chimeric genes have been constructed which contain various combinations of the Em 5′/3′ UTR sequences on either side of GUS. These constructs, utilizing the 35S (−90) promoter, are introduced into rice protoplasts and incubated in the presence or absence of ABA. The 35S promoter-GUS fusion flanked by its own 3′ sequences is approximately 20% as active in the rice protoplasts as much longer "full-length" 35S promoter (with enhancers) and, like the full-length promoter, is not responsive to ABA. Substitution of the Em 3′ end for the 35S 3′ end results in unaltered activities. However, inclusion of the Em 5′ UTR between the −90 35S promoter and the translational initiation codon, ATG, increases GUS activity to double the level of the full-length 35S promoter in the absence of ABA. In the presence of ABA, an additional doubling of GUS activity is observed. Therefore, compared to the truncated 35S promoter alone, inclusion of the Em leader from wheat leads to a 10-fold increase in activity without ABA and a 20-fold increase in the presence of ABA (Figure 2). It is clear from these results that the +6 to +86 segment of the Em promoter has a major effect on GUS expression in rice protoplasts in the absence of ABA and may indicate a component of posttranscriptional regulation involving ABA (Marcotte et al., 1989). Experiments are currently underway to determine the mechanism(s) of this level of regulation.

Summary. The data above indicate that the Em promoter region can be functionally dissected into several components (see Figure 2). The 5′ distal region consists, at least in part, of an AT-rich region that appears to be responsible for quantitative levels of expression through effects on transcription. These sequences would then be analogous to nonspecific enhancer regions. These 5′ distal sequences may also contain additional elements which are intimately involved in Em regulation that only further analyses will identify. The next region of regulatory sequences is the ABRE which is responsible for a dramatic increase in expression in the presence of ABA, probably as a result of an interaction between the ABRE and a specific DNA binding protein (see "*Trans*-Acting Protein Factors"). The specific sequences of the Em gene that have been shown to be involved are defined below. A third component of the Em gene 5′ flanking region, the 5′ UTR, also appears to be involved in the ultimate level of expression from the Em promoter. This effect is thought to be posttranscriptional but has yet to be unambiguously demonstrated. The presence of several of these components which have been either brought together and/or selectively maintained during evolution are the main components that together result in the extremely high levels of expression that are observed with the Em promoter, both in synthetic gene fusion experiments and, naturally, in wheat embroys.

Trans-Acting Protein Factors

The control of gene transcription is mediated by interactions of DNA sequences with proteins (transcription factors) within the nucleus. The modulation of gene expression levels by hormonal, environmental, and developmental signals is, in part, accomplished through changes in these interactions. In some cases, the nature of the complex between a single DNA binding protein and a promoter element determines the functional state of a gene (i.e., induced or repressed). In more complex examples, the interaction of several transcription factors, as well as modifications of these factors by kinases, etc., act in concert to affect the ultimate level of gene expression.

Sequence-specific DNA binding proteins have been implicated in the regulation of gene expression in a variety of systems. A number of these proteins have a characteristic juxtaposition of conserved amino acid sequence motifs linked in tandem: a conserved basic region (b) next to either a leucine heptad repeat (''leucine zipper'') (ZIP), a helix-loop-helix motif (HLH), or a combination of HLH and ZIP. Examples of the bZIP group include Jun and Fos from mammals, GCN4 from yeast, and O2 from maize (Vinson et al., 1989; Gruissem, 1990). Myo D is representative of bHLH (Weintraub et al., 1991), while Myc and USF are examples of the bHLH/ZIP class (Kerkhoff et al., 1991). Other groups of DNA binding proteins have been identified and implicated in the control of transcription, some of which display different conserved protein motifs, for example, the zinc finger and homeobox proteins (Levine and Hoey, 1988; Johnson and McKnight, 1989). The following sections summarize what is known of the proteins that bind the Em promoter and related transcription factors from plants.

Proteins Which Bind the Wheat Em Gene Promoter

Interactions with the AT-Rich Enhancer. The binding of nuclear proteins to the wheat Em promoter can be investigated by electrophoretic mobility shift assay (EMSA). This assay exploits the reduction in mobility of a DNA/protein complex upon electrophoresis relative to an unbound DNA probe. Proteins extracted from crude nuclear preparations are incubated with a radiolabeled DNA probe and applied to a gel. After electrophoresis, the positions of the free and bound probe are revealed by autoradiography. Specificity of the interaction is determined with the addition of unlabeled DNA competitors to the binding reaction. Only a DNA sequence containing the putative binding site should compete for binding and, in excess, should eliminate the autoradiographic signal of the bound complex.

Crude nuclear extracts are prepared by the method of Jensen et al. (1988) from ABA-treated wheat embryos and rice cell suspension cultures. A 300-bp AT-rich fragment (300KS) from the 5′ region of the Em promoter (-559 to -263, see Figure 3) forms a specific complex with proteins in both the rice (R) and wheat (W) extracts (Figure 3, lanes 2 and 3). Unlabeled plasmid (S) containing the AT-rich fragment competes for the binding (lane 4), while nonspecific (NS) plasmid does not (lane 5), veryifying the specificity of this interaction.

DNA footprinting of the 300-bp wheat AT fragment protein complex is performed using the orthophenanthroline-copper ion method (Kuwabara and Sigman, 1987). Three regions of diffuse protection have been identified and summarized in Figure 3. The three protected sequences contain AT motifs similar to those found in protein binding sites from several other plant genes (Jensen et al., 1988; Bustos et al., 1989; Jordano et al., 1989; Datta and Cashmore, 1989).

Previous studies in animal systems have shown that AT-rich sequence elements are frequently bound by high mobility group chromosomal proteins. Purified wheat HMGs

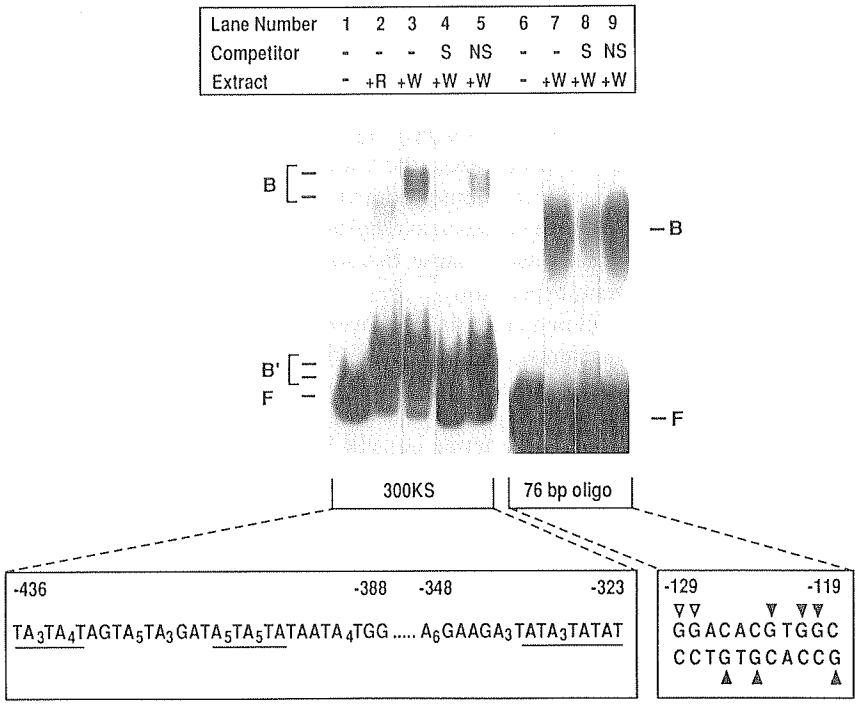

Figure 3. Electrophoretic mobility shift assays using the 300 bp AT-rich fragment (300 KS) and a 76-bp oligonucleotide fragment (ABRE), both sequences from the Em promoter (Marcotte et al., 1989; Guiltinan et al., 1990). Wheat (W) and rice (R) nuclear extracts demonstrate the presence of protein factors that bind to both the 300 KS and ABRE, resulting in a shift in mobility of the radioactively bound (B) fragment compared to the free (F). The binding is eliminated by specific (S) competitions using excess nonradioactive 300 KS (lane 6) and ABRE (lane 8) fragments, but not by a nonspecific (NS) competitor (i.e., an Em coding region sequence). The specific sequences in the 300-bp fragment that are protected by the binding of the specific factor(s) are underlined below the gel, while the sequences protected in the ABRE (i.e., Em1a) are indicated with arrows (see Guiltinan et al., 1990).

interact specifically with the AT-rich fragment of the Em promoter (Pederson et al., 1991), exhibiting a pattern similar to the multiple-banding patterns seen with wheat and rice nuclear extracts (Figure 3). The finding that the AT-rich element can confer quantitative enhancement of Em gene expression levels (Marcotte et al., 1989) is consistent with previous studies showing that HMGs bind preferentially to actively transcribing chromatin (see Pederson et al., 1991).

Interactions with the ABRE. Proteins in nuclear extracts from wheat embryos and rice tissue cultured cells can interact with a 76-bp oligonucleotide containing the ABRE element responsible for ABA-mediated enhancement of gene expressin (Figure 3, lanes 6 through 9; Guiltinan et al., 1990). The specificity of binding is confirmed by adding specific (S) and nonspecific (NS) competitors to the binding reactions (Figure 3, lanes 8 and 9). As expected, the specific competitor (i.e., a DNA fragment containing the ABRE) is capable of competing with the radiolabeled ABRE for binding of the nuclear proteins, while the NS competitor is not. Additionally, a 2-bp mutation of the ABRE (mABRE) eliminates the ability to compete for the binding (Guiltinan et al., 1990). The two base pairs mutated in the mABRE reside within Em1a.

The binding of rice and wheat nuclear proteins to the 76-bp ABRE is more precisely

defined using methylation interference footprinting (Guiltinan et al., 1990). This method involves partially methylating the G residues of a DNA sequence, reacting this methylated DNA probe with a protein extract, and separating the bound DNA probe from the free probe by EMSA. The DNA in each fraction is then isolated, cleaved at methylated G residues with piperidine, and electrophoresed on a DNA sequencing gel. Methylated G residues within the recognition sequence, which interact intimately with a DNA binding protein, interfere with complex formation. This will result in the depletion (or footprint) of the specific fragments in the bound DNA sample, relative to the free sample. Wheat and rice nuclear proteins are footprinted to a small region of the ABRE spanning the consensus motif Em1a (Figure 3b) (Guiltinan et al. 1990). Each G in this motif is either completely or partially reduced in the bound DNA relative to the free DNA. No binding is observed in the Em2 or Em1b boxes.

Isolation of a cDNA Encoding EmBP-1. The presence of a single footprint in the Em1a box (which is eliminated by a 2-bp mutation in the Em1a core CACGTG), together with findings from the transient assay system showing the importance of the Em1a box in ABA mediated enhancement, suggests that this DNA binding protein(s) plays an importnat role in ABA-mediated gene expression.

In order to isolate cDNAs encoding the protein(s) which interact with Em1a, a λgt11 cDNA expression library from wheat embryo RNA has been screened for proteins with the ability to bind an ABRE probe. In this library, random cDNA fragments are expressed inframe with β-galactosidase (β-gal), within phage-infected *E. coli* cells. The library is plated and fusion protein expression is induced after placing a nitrocellulose filter above the cells. After several hours, the filter is removed and incubated with a radiolabeled double-stranded ABRE oligonucleotide. Reacting phage are localized by autoradiography and isolated. Two such clones (λGC12, λGC19) have been obtained from a screen of 120,000 plaques. Protein extracts from lysogens of these clones are assayed for binding to the ABRE by EMSA (Guiltinan et al., 1990) and by southwestern analysis (Figure 4). Lysogen extracts from λGC12 and λGC19 show fusion proteins with higher molecular weights than the λgt11 control or the purified β-gal, and these fusion proteins bind the ABRE probe. The binding affinity of λGC12 appears to be less than that for λGC19. No other proteins in the extracts or in the control samples bind the ABRE (Figure 4).

Subsequently, the specificity of the λGC19 binding has been determined by both EMSA and methylation interference footprinting and is identical to that seen with the nuclear extract from wheat embryo and rice tissue cultured cells (Guiltinan et al., 1990). By these criteria, the binding characteristics of the protein encoded by λGC19 are identical to the protein found *in vivo* which we have designated EmBP-1. The same approach has been successfully used to isolate similar genes for DNA binding proteins from other plants (see "Other Plant bZIP Proteins" below).

The deduced amino acid sequence from the cDNA insert GC19 contains the basic leucine zipper motif (bZip) found in transcription factors from yeast and humans, as well as other plants (see Vinson et al., 1989; Gruissem, 1990). The basic region of this class of proteins is known to determine the specificity of DNA binding and to directly contact the DNA. The leucine zipper regions, which contain leucine heptad repeats, form an amphipathic α-helical structure involved in dimer formation. These dimers have been shown to form homo- and heterodimers with other bZIP proteins (Vinson et al., 1989).

There are seven hybridizing sequences to GC19 in the hexaploid wheat variety Chinese Spring, four in rye, and surprisingly only one in each of thirteen barley cultivars. This apparently highly conserved sequence is also found in gene families in other Graminae (e.g., maize, millet, and rice) and in 21 alien relatives of wheat (e.g., *Aegilops, Agropyron,* and *Dasypyrum*) (Devos et al., 1991).

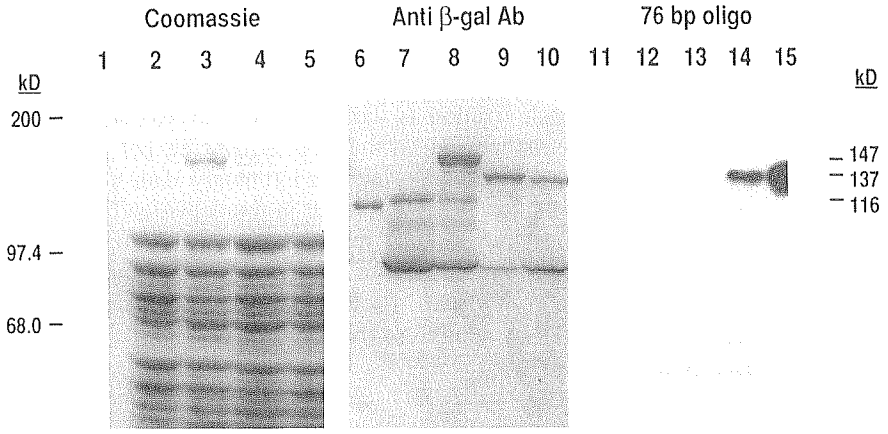

Figure 4. Lysogen extracts from λgt11 clones expressing β-galactosidase (β-gal)-fusion proteins from an expression library constructed with mRNA from wheat embryos treated with ABA (see Guiltinan et al., 1990). Lanes 1, 6, and 11 contained purified β-gal. The remaining lanes represent lysogen extracts from wild-type λgt11 (lanes 2,7,12), a control phage AT10 (lanes 3, 8, 13), and λGC12 (lanes 4, 9, 14) and λGC19 (lanes 5, 10, 15), which were isolated by screening the library with the 76-bp ABRE. The extracts were separated by SDS-polyacrylamide gel electrophoresis and either stained with Coomassie blue (lanes 1 through 5) or electroblotted to a nylon filter (lanes 6 through 15). Immobilized proteins on the filter were reacted with an anti-β-gal antibody (lanes 6 through 10) to detect the positions of the β-gal fusion proteins, while an identical filter (lanes 11 through 15) was incubated with a radiolabeled ABRE probe under the same conditions used for the library screen.

Other Plant bZIP Proteins

In addition to EmBP-1 from wheat, six other plant bZIP proteins have been isolated from wheat (HBP-1), tobacco (TGA-1), maize (O2, OCSBF), *Arabidopsis* (GBF), and celery (CPRF). They all share remarkably similar basic domains with the animal and yeast bZIP proteins and similar but not identical target sequences to plant and bacterial promoters (see Figure 5, Singh et al., 1990; see Figure 4, Weisshaar et al., 1991). The HBP-1 family from wheat encodes at least two proteins (HBP-1a,b) that both bind to a promoter element in the 5′ region of the wheat histone 3 gene (Tabata et al., 1989, 1991). The TGA1 family (TGA1a,b) from tobacco (Katagiri et al., 1989) and the single copy OCSBF from maize (Singh et al., 1990) bind to similar target sequences in *Agrobacterium* (nos, ocs) and CaMV (35S) while the opaque-2 gene product from maize (O2) recognizes sequences in the maize zein promoter (Schmidt et al., 1990). Two additional bZIP protein families each containing three members have been identified from *Arabidopsis* (GBF) by Cashmore (1991) and from celery (CPRF) by Weisshaar et al. (1991).

While the target sequences for each of the cloned plant bZIP proteins appear to have similar core sequences, the signals to which these genes respond can be different. For example, EmBP-1, GBF, and CPRF all bind to the CACGTG motif found within the context of a number of plants (Schulze-Lefert, et al., 1989; Staiger et al., 1989) and yeast (Donald et al., 1990) promoters that are regulated by different signals such as light and ABA. In addition, similar sequence (CANNGT) and transcription factor (bZIP, bHLH) combinations are complexed during activation of certain animal genes (Blackwell and Weintraub, 1990; Blackwood and Eisenman, 1991; Kerkhoff et al., 1991), raising the intriguing possibility of the evolutionary conservation of similar sets of *cis*-elements and protein factors that regulate gene expression (see Guiltinan et al., 1990).

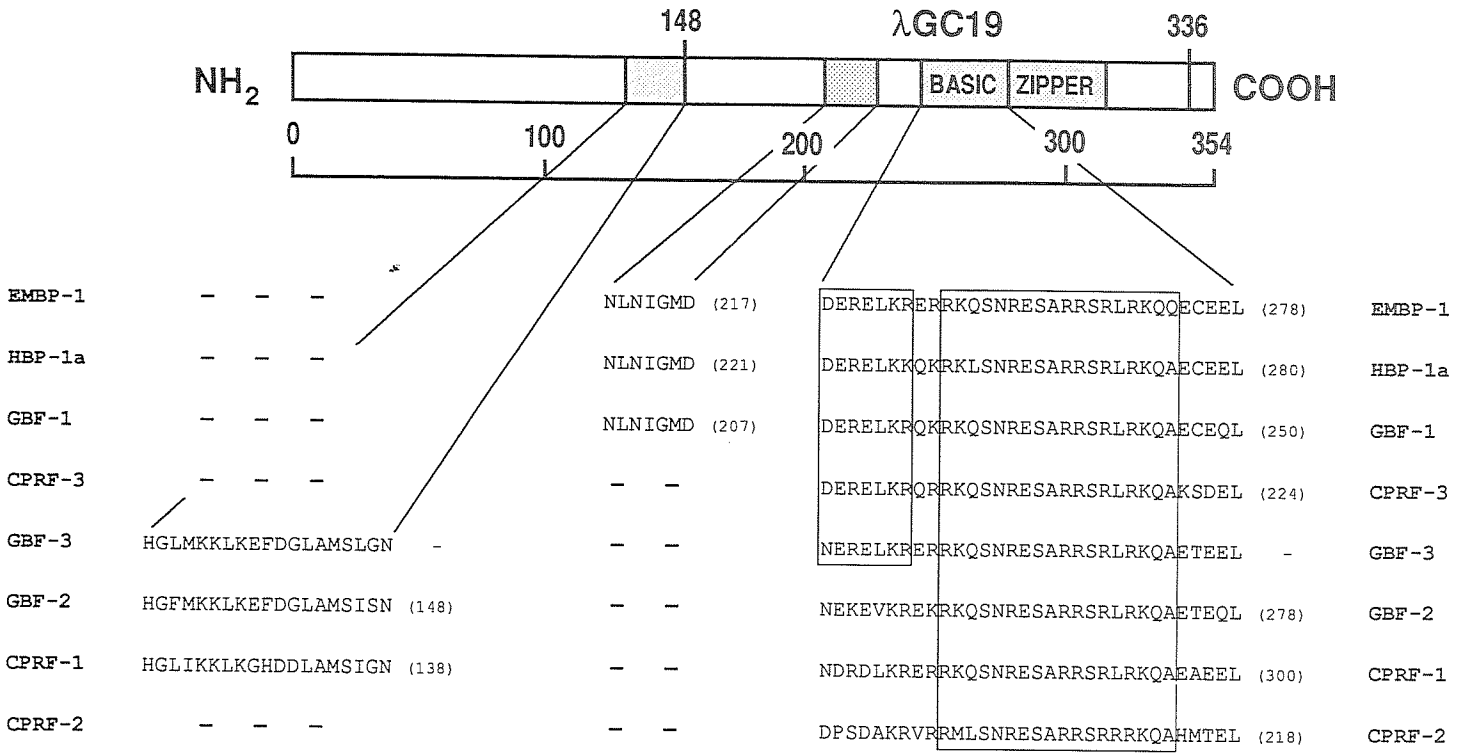

Figure 5. Schematic diagram of the full length cDNA clone of EmBP-1 showing the tandemly arranged basic and leucine zipper regions, as well as the section (amino acids 148 through 336) corresponding to GC19. Below the diagram is listed an abbreviated amino acid sequence of conserved regions (boxed) from several bZIP-P1 proteins: EmBP-1 (Guiltinan et al., 1990) and HBP-1a (Tabata et al., 1989) from wheat, GBF from *Arabidopsis* (Cashmore, 1991), and CPRF from celery (Weisshaar et al., 1991). A high degree of conservation of sequences is shown in the basic regions of all of these plant proteins, but other conserved sequences are found in different areas. These latter segments are not found in all plant bZIP proteins and may be related to functional differences within this class of DNA binding proteins.

TABLE 1.
Target Sequences and Common Core (CACGTG) of Several Plant Genes That are Recognized by Different Members of the bZIP Class of Plant DNA-Binding Proteins

Factor	Target Sequence	Gene
EMBP-1	T T G C C G G A C A C G T G G C G C G A	EM1a
− − −	T Ⓒ G C Ⓣ G Ⓒ A C A C G T G Ⓒ C G C Ⓒ Ⓣ	Em1b
+ + +	G C T C G C G C C A C G T G G G C A T G	Rab 30
+ + +	C G A C A C C G [T] A C G T G G C G C C A	Rab 16A
+ + +	A G A A A T G C C A C G T G G A C G A A	AdH
GBF	T T A T C T T C C A C G T G G C A T T A	rbcS 1A
CPRF	C C T T A T T C C A C G T G G C C A T C	CS (box 2)
HPB-1a,b	C T T T C G G C C A C G T [C] A C C A A T	Histone 3
O2	T G T C A T T C C A C G T [A] G A T G A A	Zein

CONCLUSION

Analyses of the response of the Em gene to ABA have identified several components that must be included in any general model to explain the tissue and chemical specificity of a phytohormone response: (1) a *cis*-response element in the Em promoter, composed of at least 20 bp (ABRE), which has been shown to confer ABA responsiveness to a nonresponsive viral promoter, and (2) a *trans*-acting protein, structurally similar to the bZip transcription factor class, which recognizes and binds to the ABRE for ABA-regulated Em expression. Since there is no evidence yet that the ABA signal interacts directly with this transcriptional complex (i.e., the ABRE and bZIP protein), additional components are likely (i.e., a response coupling element[s]) which could link the ABA signal with activation of this transcriptional complex.

One of the most interesting observations that has emerged from the analyses of Em activation by ABA is that the ABRE and EmBP-1 are not unique to ABA responsive genes or to plants. The nucleotide core sequence characteristic of the ABRE in ABA responsive genes is also found in other plant, yeast, and mammalian promoters. The promoters from yeast and animals that contain the CACGTG core of the ABRE have not been analyzed sufficiently to identify the signal(s) responsible for specifying their expression. However, the same CACGTG core of the ABRE has also been found in a number of plant promoters that are responsive to light but not ABA. Furthermore, the CACGTG core in these light-regulated promoters corresponds to the well-characterized light responsive element, the G-box (Table 1). Similarly, the DNA binding protein that interacts specifically with the ABRE in the Em promoter (i.e., EmBP-1) has a structure similar to other bZIP proteins in plants, animals, and yeast (Gruissem, 1990; Vinson et al., 1989; Blackwood and Eisenman, 1991; Kerkhoff et al., 1991). It now appears that a subset of these bZIP proteins from higher plants (P1) specifically recongizes the .... CACGTG .... motif in the ABRE of ABA-responsive genes (e.g., EmBP-1 from wheat [Guiltinan et al., 1990]), as well as in the G-box of light-responsive genes (e.g., GBF-1, 2, 3 from *Arabidopsis* [Cashmore, 1991] and CPRF-1, 2, 3 from celery [Weisshaar et al., 1991]). These recent observations raise a very interesting question. How does one achieve the specificity of expression from such different signals as light and ABA, when each signal appears to act through response elements which share a common core (CACGTG) and which bind proteins from the same transcription factor family (bZIP-P1)?

Specificity in *Cis*-Regulatory Sequences

Table 1 shows a set of eight sequences (20 nucleotides each) from the promoters of

TABLE 2.
Combinations of Signals, Response Elements, and bZIP Proteins That Interact through a Common Core CACGTG Sequence

Signal	Response Element Subclass	bZIP-P1 Members
Light	y y y y (CACGTG) y y y y (y = rbcS,cs)	GBF (1–3); CPRF (1–3)
ABA	z z z z (CACGTG) z z z z (z = Em, rab)	EMBP-(1–3)
?	x x x x (CACGTG) x x x x (x = adh, histone, zein)	?

various plant genes that contain the common CACGTG core but respond to different signals: Em and rab to ABA, the small subunit of ribulose-bisphosphate carboxylase (rbcS) and chalcone synthase (cs) to light (Schulze-Lefert et al., 1989), alcohol dehydrogenase (adh) (DeLisle and Ferl, 1990), zein (Schmidt, 1990), and histone (Tabata, et al., 1989) to signals other than ABA and light. Obviously, the presence of the core itself in these sequences does not confer specificity of gene activation by the various signals. If there is specificity at this level, it must reside in the sequences flanking the core. One example of specificity at this level is that the response of the Em gene to ABA requires Em1a but not Em1b. We have shown that EmBP-1 protects Em1a but not Em1b in an *in vitro* footprint analysis (see 1b above). Furthermore, Em1a alone is sufficient for the ABA response, and a mutation in Em1a prevents both binding and the ABA response (Guiltinan et al., 1990). Although the core is identical in Em1a,b, there are three nucleotide differences in each of the flanking regions that may account for the differences in recognition by EmBP-1 and expression (Table 1). From this example, it appears that within a 20-nucleotide sequence there may be sufficient information to specify a response. Is there enough specificity at this level to distinguish a different signal response pathway, i.e., ABA from light? Can the 20-nucleotide segment of rbcS or cs substitute for Em1a and bind to EmBP-1? Can either rbcS or cs confer ABA responsiveness on the viral promoter? If the ABA response is associated with only the 20-nucleotide segment of Em1a and not with the same size fragment from rbcS or cs, then sufficient specificity is encoded within Em1a so that each 20-nucleotide segment containing a CACGTG core may be linked to a different signal response pathway. Hence, distinct response element subclasses of 20 nucleotides each may then be identified (Table 2). Alternatively, if Em1a, rbcS, and cs can substitute for each other in their ability to bind to EmBP-1 *in vitro* and confer ABA and/or light responsiveness in a transient assay, additional specificity is conferred by other *cis* sequences and/or at another level to explain the observations *in vivo*.

Specificity in the bZIP and Other Nuclear Proteins

There are two functional regions of the bZIP proteins: a "structural" domain (i.e., the carboxy-terminal portion containing the highly conserved basic region and leucine zipper) that specifies DNA binding to *cis* sequences and heterodimer formation with other bZIP proteins, and a "functional" domain (i.e., the highly variable amino-terminal portion) that can interact with other proteins resulting in functions such as transcriptional activation. From our observations in wheat and those of Weisshaar et al. (1991) in celery and Cashmore (1991) in *Arabidopsis*, there are at least three distinct subsets of the bZIP-P1 protein family in these species (Table 2). Although the structures of these proteins are similar, it is clear from Figure 5 that there are small, but highly conserved, structural differences in the family

members that may give rise to functional specificity. Could each of these family members (and the resulting heterodimers) have a specific binding preference? Such data can be directly obtained for each family member using the technique of selected and amplified binding sites (Blackwell and Weintraub, 1990), as well as by competition experiments using EMSA. Tabata et al. (1991) have recently demonstrated by competition experiments that both HBP-1a and HBP-1b have little if any binding affinity for the G-box (i.e., the CACGTG motif), but each has clear binding preferences to other similar sequences. For the bZIP-P1 class, then, can the rbcs and cs sequences in Table 1 compete with the Em1a sequence for binding sites on EmBP-1? Can the Em1a and cs sequence compete with rbcS for binding sites on GBF-1,2, and/or 3? Are any of these competitions altered when heterodimers between the different members are utilized in this assay? The data from Tabata et al. (1991) seem to indicate that there is binding specificity at the level of the different family members of a bZIP protein. With this specificity for binding demonstrated, if the variable functional domain of each member can be shown to be linked to different response pathways, either directly or via another protein factor(s), the specificity of different signals acting through common elements and factors may be realized.

The basic experimental questions that need to be addressed are does each response element subclass have a greater affinity for a specific bZIP family member or heterodimer? What is the preferred binding site of each member of bZIP? Are the preferred binding sites for each member found in a subset of response elements that respond to the same signal, i.e., light, ABA? Are there additional *cis* elements juxtaposed on the different promoters which might contribute to the specificity, perhaps by binding different protein factors? Are there unique nuclear proteins that bind to specific bZIP family members?

With reference to the last question, Blackwood and Eisenman (1991) recently demonstrated that Max (a bHLH-ZIP) can bind with Myc (another bHLH-ZIP) resulting in a complex that binds to a DNA sequence that neither Max nor Myc alone can recognize. The DNA sequence recognized by the complex, interestingly, contains a CACGTG core. These results also demonstrate that the bHLH-ZIP class of transcription factors can bind to sequences that are similar to those recognized by the bZIP class, suggesting common nucleotide recognition domains in these different classes of DNA-binding proteins.

Model

If specificity of binding/function can be demonstrated in the 20-nucleotide response element as well as by the members of the bZIP family, sufficient diversity may be possible to begin to account for the variety of responses observed. For example, the common core in the light and ABA response elements (CACGTG) is recognized by all members of each protein (i.e., EmBP, GBF, and CPRF) in the bZIP-P1 family. Each member (and any possible heterodimer between members), however, has a *preferred* binding site that recognizes a distinct sequence that borders the core, thereby specifying a response element subclass (Table 2). This specific recognition between a unique member of bZIP-P1 and a subclass of response elements would be governed by the association of the factor with other specific proteins and/or by covalent modification of the factor/associated proteins. Each complex, composed of a unique response element and a protein member of a bZIP-P1 family (and likely other proteins) could now be recognized as the terminal step in a specific signal response pathway (i.e., light or ABA), so that specific gene activation by that signal is triggered.

An example of a system with relevance to this model is one in which one gene responds to two signals through a single response element. Graham and Gilman (1991) have shown that the specificity for two different signals to trigger the response of the same gene operates

at the level of protein-protein interaction at the response element. The mammalian *c-fos* gene contains serum response element (SRE) that is responsible for *c-fos* expression by signals (e.g., phorbol esters) that are transduced via a protein kinase C (PKC) dependent pathway, as well as by other signals (e.g., serum) that operate through a PKC independent pathway. SRE function for all signals (by either pathway) is dependent on the constitutive binding of a serum response factor (SRF). A second protein $p62^{TCF}$ (adapter) forms a ternary complex between the SRE and SRF. The adapter is not required for induction by serum or signals acting through the non-PKC pathway. However, the full ternary complex is required for expression of *c-fos* through the PKC response pathway. Hence, the adapter protein confers PKC responsiveness to SRF, thereby allowing it to respond to a different signal. The analogy of this system to one discussed above would predict that the interaction between the CACGTG core and the bZIP-P1 member is "constitutive" (i.e., SRE/SRF and ABRE/EmBP-1). Whether the linked gene responds to light or ABA depends on the "adapter/linker" protein that recognizes this complex. The nucleotides flanking the CACGTG core of ABRE (or the SRE core) and the unique bZIP-P1 member (or SRF member) bound would then specify the recognition of the adapter/linker. Since it is known that the gene product from the regulatory locus Vp1 of maize is required for the expression of ABA-regulated genes in embryos (see "Regulatory Genes that Modulate/Control the ABA-Responsive Genes" above), it will be interesting to determine if Vp1 plays the role of an adapter/linker protein with ABRE/EmBP-1 to specificy ABA-induced gene expression in embryos.

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