PHYSIOLOGIA PLANTARUM 101: 653–664. 1997

ISSN 0031-9317

Promoter switches specific for abscisic acid (ABA)-induced gene expression in cereals

Qingxi Shen and Tuan-Hua David Ho

Shen, Q. and Ho, T.-H. D. 1997. Promoter switches specific for abscisic acid (ABA)-induced gene expression in cereals. – Physiol. Plant. 101: 653–664.

Abscisic acid (ABA) has been shown to regulate many physiological and developmental processes which are often mediated by the induction and suppression of gene expression. Herein, we review the progress made in the understanding of gene expression regulated by ABA, with the emphasis on *cis*- and *trans*-acting elements controlling gene expression. Promoter sequences containing an ACGT-core have been shown by several groups to be necessary for the ABA-induced gene expression. However, similar ACGT-core-containing sequences are also necessary for responses to a variety of environmental and physiological cues. To address the question what determines the response specificity, we have studied two barley ABA inducible genes and defined the modular nature of ABA response complex (ABRC), the promoter unit necessary and sufficient for ABA induction of gene expression. ABRCs of these genes consist of a 10-bp element with an ACGT-core (ACGT-box) and a coupling element (CE1 or CE3). These ABRCs function in both seed and vegetative tissues. Genetic analysis has led to the cloning of genes, such as maize *Viviparous 1* (*VP1*), involved in the regulation of sensitivity of plants to ABA. In seeds, ABA induction of the ABRC containing CE3, but not the ABRC with CE1, is enhanced in the presence of the transcription regulator, encoded by the *VP1* gene, indicating these two ABRCs are mediated by different ABA signal transduction pathways. Other potential signal transduction components mediating ABA signal transduction pathways are also discussed.

Key words – Abscisic acid (ABA), abscisic acid response complexes (ABRCs), barley, cereals, *cis*-acting promoter sequences, gene expression, signal transduction, *trans*-acting elements.

Q. Shen (present address: Monsanto Company, Mail Zone AA2G, 700 Chesterfield Village Parkway, Chesterfield, MO 63198, USA) and T.-H. D. Ho (corresponding author, e-mail ho@biodec.wustl.edu), Plant Biology Program, Dept of Biology, Division of Biology and Biomedical Sciences, Washington Univ., St Louis, MO 63130, USA.

Introduction

Abscisic acid (ABA) plays a variety of roles in mediating plant development and the adjustment to unfavorable environmental conditions. Specifically, ABA is an important factor regulating seed development and germination. In these processes, the roles of ABA appear to be threefold: regulation of growth and differentiation of the embryo (embryogenesis), enhancement of the synthesis of reserves in storage tissues (cotyledon and/or endosperm), and prevention of precocious germination (Sussex and Dale 1979, Crouch and Sussex 1981). ABA is known to regulate the accumulation of some storage proteins during seed development (Sussex and Dale 1979, Triplett and Quatrano 1982, Bray and Beachy 1985). The onset and maintenance of seed dormancy also relies on the synthesis of ABA (King 1976, Robichaud et al. 1980, Koornneef et al. 1982, Naumann and Dörffling 1982, Karssen et al. 1983, Quatrano 1987).

In vegetative tissues, ABA appears to be involved in the response and adaptation of plants to environmental stresses, especially in drought and cold conditions. It has been well documented that levels of ABA increase in re-

Received 12 May, 1997

sponse to drought (e.g., Wright and Hiron 1969). Plants deficient in ABA production exhibit a 'wilty' phenotype and are very sensitive to drought (Imber and Tal 1970). It is proposed that under water stress, turgor pressure declines and it results in an increase in cytosolic and apoplastic ABA due to de novo synthesis and/or release of the hormone sequestered in organelles of plant cells (Zeevaart and Creelman 1988). The increase of ABA levels leads to: (1) the closure of stomata to avoid water stress, and (2) the induction and accumulation of compatible solutes, such as proline, for water stress tolerance. The combination of the avoidance and tolerance strategies helps plants survive unfavorable conditions. ABA appears to be a 'stress hormone' (Johns 1978) because in addition to drought, other stresses, such as cold and salinity, also cause an increase in ABA content (Chen et al. 1983, Lachno and Baker 1986). Involvement of ABA in cold acclimation has been reported in plants including *Solanum* (Chen et al. 1983), *Nicotiana tabacum* (Bornman and Jansson 1980), and in cultured cells of *Brassica napus* (Johnson-Flanagan et al. 1991), winter wheat and rye (Chen and Gusta 1983). In these studies, an increase in endogenous levels of ABA is observed when plants are exposed to low temperature. In addition, exogenous applications of ABA are shown to increase the cold hardiness in certain plants. These observations suggest the importance of ABA in the adaptation of plants to freezing. However, other reports suggest that the cold acclimation itself, not the increase in endogenous ABA levels, is essential for plant cold hardening (Eamus and Wilson 1983, Mohapatra et al. 1988, Lafuente et al. 1991).

Although some ABA-regulated physiological processes, such as the closure of stomata, do not rely on gene expression, regulation of gene expression plays a major role in many plant responses to ABA. Therefore, analysis of ABA-regulated gene expression appears to be an effective approach to the understanding of the mode of ABA action.

Genes induced by ABA

ABA induces the expression of a variety of genes, including those encoding seed storage proteins (Sussex et al. 1980, Bray and Beachy 1985, Crouch et al. 1985), LEA (late embryogenesis abundant) and RAB (responsive to ABA) proteins in wheat (*Em*), rice (*Rab16A, B, C, D*), barley (*HVA1*, *dehydrins*), rapeseed (*Lea76*), cotton (*LeaD7*, *D11*, *D113*, *D34*, *D29* and *D19*), maize (*Rap*) and carrot (reviewed in Dure et al. 1989). It has been suggested that LEA proteins are neither enzymes nor storage proteins, but may function in protecting proteins and membranes from damages due to loss of water in the cytoplasm during seed desiccation (Dure et al. 1989). In fact, it has been shown that the level of these LEA proteins is closely correlated with desiccation tolerance in both developing and germinating seeds under natural conditions (Blackman et al. 1991). However, a causal relationship between LEA proteins and desiccation tolerance has yet to be demonstrated.

Specific mRNAs and proteins, such as RAB, accumulate in stressed vegetative tissues. Some of these RAB proteins possess conserved, positively charged domains. It was initially suggested that they may bind nucleic acids and hence regulate gene expression (Mundy and Chua 1988). In fact, *pMAH9*, a maize gene induced by ABA and water stress, encodes a protein containing the consensus sequence (RGFGFVXF) of RNA-binding proteins (Gomez et al. 1988, Bandziulis et al. 1989). Recent work has shown that the protein encoded by this gene is indeed an RNA-binding protein, with preference for guanosine-rich and uridine-rich sequences (Ludevid et al. 1992). Therefore, ABA-responsive genes may encode RNA-regulatory proteins capable of altering developmental events in plants. Other ABA-regulated genes include a barley gene encoding GA- and ABA-regulated aldose reductase (Bartels et al. 1991), a *Craterostigma plantagineum* cytosolic glyceraldehyde-3-phosphate dehydrogenase gene (Velasco et al. 1994), a wheat gene encoding L-isoaspartyl protein methyltransferase (Mudgett and Clarke 1994) and a duckweed peroxidase gene (Chaloupkova and Smart 1994).

In aleurone layers of barley seeds, ABA induces dozens of genes. One of these genes, *HVA22*, is unique and shares little homology with any of the reported ABA-inducible genes (Shen et al. 1993). The expression of *HVA22* can be rapidly induced by ABA (Fig. 1A, lanes 6–11) or cycloheximide, a protein synthesis inhibitor (Fig. 1B, lanes 1–6). Addition of both inducers has a synergistic effect on the expression of this gene (Fig. 1B, lanes 7–12). In the absence of cycloheximide, the ABA induction of *HVA22* is transient, with its mRNA level peaking between 4 and 8 h of ABA treatment and declining to the background level later (Fig. 1A, lanes 6–11).

ABA-regulated suppression of gene expression

ABA has been reported to have inhibitory effects on DNA synthesis in *Lemna polyrhiza* (Stewart and Smith 1972), RNA polymerase activity in maize coleoptiles (Bex 1972b), and the synthesis of rRNA and tRNA in a number of plants, including barley (Poulson and Beevers 1970), maize (Bex 1972a), bean (Walton et al. 1970), and radish (Wareing et al. 1968). In addition to inducing the expression of genes such as *HVA22* and *HVA1*, as mentioned above, ABA also suppresses GA induction of α-amylase, nuclease and protease genes which are normally expressed at high level during seed germination (Brown et al. 1988, Khursheed and Rogers 1989, Koehler and Ho 1990).

Mechanism of ABA action

The mechanism of ABA action has been the subject of intense interest to plant biologists for many years. Al-

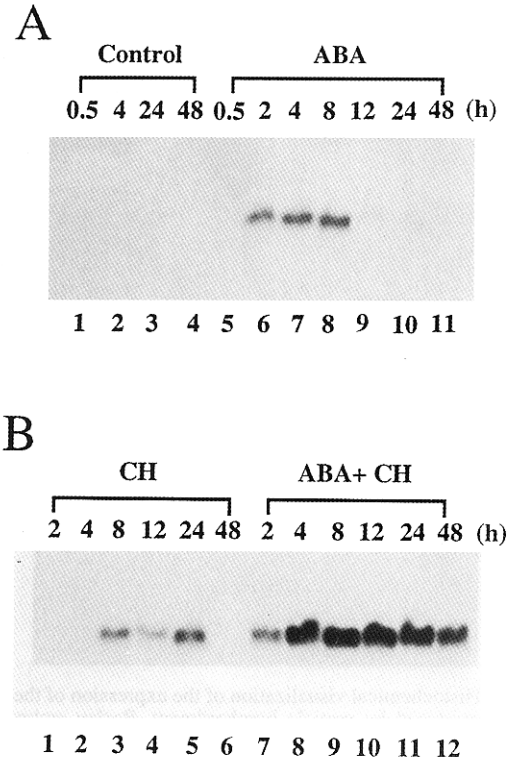

Fig. 1. Northern blot analysis showing the time course of *HVA22* gene expression regulated by ABA and cycloheximide. (A) Treatment with ABA alone. RNA was prepared from barley seed aleurone layers incubated in buffer (20 m*M* Na succinate, pH 5.0, 20 m*M* $CaCl_2$) with 10^{-5} *M* ABA (lanes 5–11) or in buffer alone (lanes 1–4) for the times (h) indicated. (B) Treatments with cycloheximide (CH; 10 μg ml^{-1}) or combination of cycloheximide and ABA. Aleurone layers were incubated either with cycloheximide alone (10 μg ml^{-1}; lanes 1–6) or with cycloheximide plus ABA (lanes 7–12) for times (h) indicated. The RNA blot was probed with the cDNA clone of HVA22. From Shen et al. 1993.

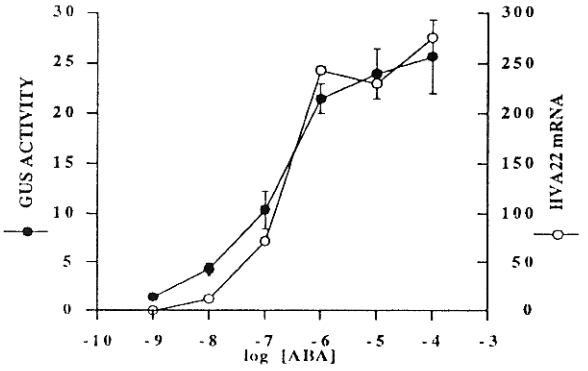

Fig. 2. Dosage response curve of ABA-inducible HVA22 RNA accumulation (○) and dosage response of *GUS* gene expression driven by *HVA22* promoter (●). For the northern analysis, RNA was isolated from mature imbibed barley aleurone layers treated with ABA at concentrations of 10^{-9} to 10^{-4} *M*. Correspondingly, protein extract was prepared from mature barley embryoless half-seeds treated with or without 10^{-9} to 10^{-4} *M* ABA for 24 h, after the seeds having been bombarded with P_{DraII}IGU (*HVA22* promoter/GUS) and the internal control pAMC18 constructs (ubiquitin promoter/luciferase). The ABA induction was expressed as the ratio of normalized GUS activity of the samples treated with ABA over that of those incubated with buffer only. Each point represents the mean of at least six replicas. Each lane of northern blot analysis was loaded with 5 μg of total RNA prepared from the aleurone layers treated without (control) or with 10^{-9} to 10^{-4} *M* ABA. The autoradiography was quantified with a computing densitometer (Model 300A, Molecular Dynamics, CA, USA). From Shen et al. 1993.

though little progress has been made concerning the initial perception of ABA by a putative receptor, there have been quite a few reports about signal transduction pathways, especially *cis*-acting promoter sequences involved in ABA response and DNA-binding proteins interacting with the ABA-responsive *cis*-acting sequence. The prerequisite in analyzing a promoter for ABA-responsive *cis*-acting sequences is to demonstrate that transcription control is the main regulatory mechanism for a given gene. By comparing the level of *HVA22* mRNA and degree of induction of the bacterial β-glucuronidase (GUS) activity from the reporter construct containing the *GUS* coding sequence driven by the ABA-responsive *HVA22* promoter, we have demonstrated that the regulation of *HVA22* promoter is mainly at the level of transcription. As shown in Fig. 2, the level of *HVA22* mRNA is well correlated with the degree of induction of GUS activity. This experiment has shown that the degree of ABA induction obtained from transient studies using an ABA-responsive promoter/reporter gene construct reflects the in vivo effect of ABA on the expression of barley *HVA22* gene.

cis-Acting sequence

To delineate sequences that are important for the ABA response of these genes, several *Rab* and *Lea* genomic clones have been obtained. Sequence comparisons of the 5' upstream sequence of these genes have identified conserved sequences that are potential ABA-responsive DNA elements (Marcotte et al. 1989, Skriver and Mundy 1990). Transient assays have been conducted with protoplasts isolated from rice suspension cultures and chimeric genes with the wheat *Em* gene promoter linking to the coding region of bacterial *GUS* gene. A 260-bp fragment (–168 to +92) of the *Em* gene triggers a 15- to 20-fold increase in *GUS* expression in the presence of ABA (Marcotte et al. 1989). A 75-bp fragment of this gene, when fused in either direction to a truncated 35S promoter, gives a greater than 10-fold induction of GUS activity in the presence of ABA (Guiltinan et al. 1990). In this region, there are three noticeable elements, designated as Em1a (GGACACGTGGC), Em1b (GCACACGTGC) and Em2 (CGAGCAGGC), which are essential for ABA response (Guiltinan et al. 1990). With a similar system, Mundy and Chua (1990) reported that a

promoter fragment between –294 and –52 of the rice *rab-16A* gene was sufficient to confer ABA-dependent expression of the chloramphenicol acetyltransferase reporter gene in rice protoplasts. Sequence comparisons of ABA-inducible genes generate a consensus sequence with an ACGT-core, such as Em1a and Em1b regions in the wheat *Em* gene, which could be essential for ABA responses. Skriver and Mundy (1990) demonstrated that six copies of the sequence GTACGTGGCGC conferred ABA inducibility to a –46 35S minimal promoter (a six-fold induction).

ACGT-core-containing sequences are conserved in all ABA-regulated genes for which sequence data are available (Michel et al. 1993). This is puzzling because these sequences are similar to the consensus G-box motif found in a number of yeast promoters (Donald et al. 1990), plant promoters responsive to visible and UV light (Schulze-Lefert et al. 1989), and in the anaerobically induced *Adh-1* promoter from maize (Delisle and Ferl 1990). This conserved sequence is important for transcription of some of these genes, but none appear to be positively regulated by ABA. Furthermore, a sequence similar to the G-box is also found in the promoters of bacterial (*Agrobacterium* nopaline synthase, nos) and viral (CaMV 35S) genes, yet none of these are known to be directly regulated by ABA. A similar sequence (E-box: GGCCACGTGACC) is also found in the major later promoter of adenovirus and in certain mammalian promoters and can compete with the G-box element for binding to plant nuclear extracts (Guiltinan et al. 1990). For the ease and clarity of presentation, we designated the G-box sequences, which all contain an ACGT-core, as ACGT-boxes. The presence of ACGT-boxes in non-ABA-responsive promoters raises a question: What confers the specificity of ABA response? Is it determined by the flanking sequence of the ACGT-core or another *cis*-acting element?

In order to address this question, we have analyzed the barley *HVA22* and *HVA1* promoters following both the loss- and gain-of-function approaches. Specifically, we performed transient-expression studies of the *GUS* reporter gene driven by the wild type or various mutants of *HVA22* or *HVA1* promoter (Shen et al. 1993, Straub et al. 1994, Shen and Ho 1995; Q. Shen 1993. Thesis, Washington Univ., St Louis, MO, USA). The DNA construct containing the *GUS* coding region driven by the *HVA22* or *HVA1* promoter was delivered into aleurone cells of barley embryoless half-seeds by the particle bombardment technique. To visually demonstrate the rapid and effective transformation of barley aleurone cells with this technique, the bombarded seeds, after 24 h treatment with or without 2×10^{-5} *M* ABA, were stained with X-Gluc (substrate for β-glucuronidase encoded by the *GUS* gene) for 20 h at 37°C. The blue spots on the barley half-seeds in Fig. 3 indicate the locations of *GUS* expression in transformed cells.

Although the GUS staining yields the visual result of *GUS* expression as shown in Fig. 3, a quantitative assay

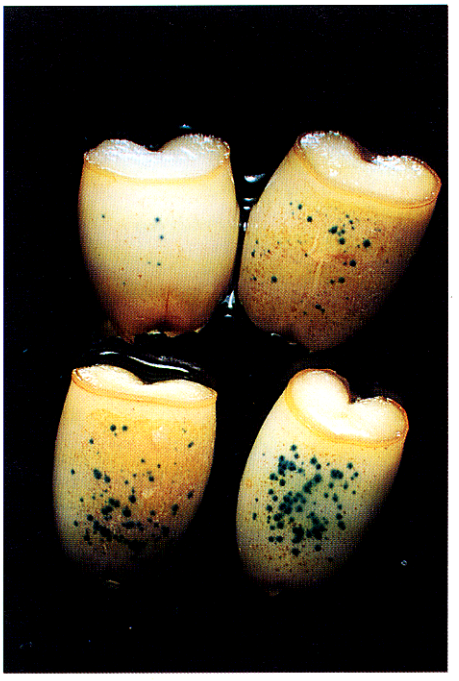

Fig. 3. Histochemical visualization of the expression of the *GUS* gene introduced by particle bombardment. Barley embryoless half-seeds were bombarded with an *HVA22* promoter/*GUS* construct and incubated in the absence (upper two half-seeds), or presence (lower two half-seeds), of 2×10^{-5} *M* ABA. They were stained with X-Gluc, a colorigenic substrate for GUS, for 4 h at 37°C. The blue spots indicate the expression sites of the *GUS* gene.

using a fluorometer is necessary for the promoter study. Because of the inherent variability of bombardment efficiencies, another reporter gene, *LUC*, coding for the firefly luciferase, was included as an internal control. The *LUC* coding sequence is driven by a non-ABA-responsive maize ubiquitin promoter (Bruce et al. 1989). Therefore, the measured GUS activity of one construct could be normalized with luciferase activity from the same shot. This approach has enabled us to define the promoter sequences which are necessary and sufficient for ABA response in two ABA-responsive genes, *HVA22* and *HVA1*.

A short promoter fragment of HVA1 *or* HVA22 *gene confers a high level of ABA induction*

To test whether a sequence containing an ACGT-box is able to confer ABA inducibility, short promoter fragments containing ACGT-boxes were linked to the 5' end of a truncated (–60) barley α-amylase gene (*Amy64*) promoter. While the control (*Amy64* minimal promoter only) construct was not affected by ABA treatment, the addition of the 49-bp ACGT-box (A3) containing fragment in either orientation gave a high level (24- to 38-fold) of induction (Fig. 4, 1C+ and 1C–). Similarly, a 68-bp promoter fragment from the *HVA1* gene, also contain-

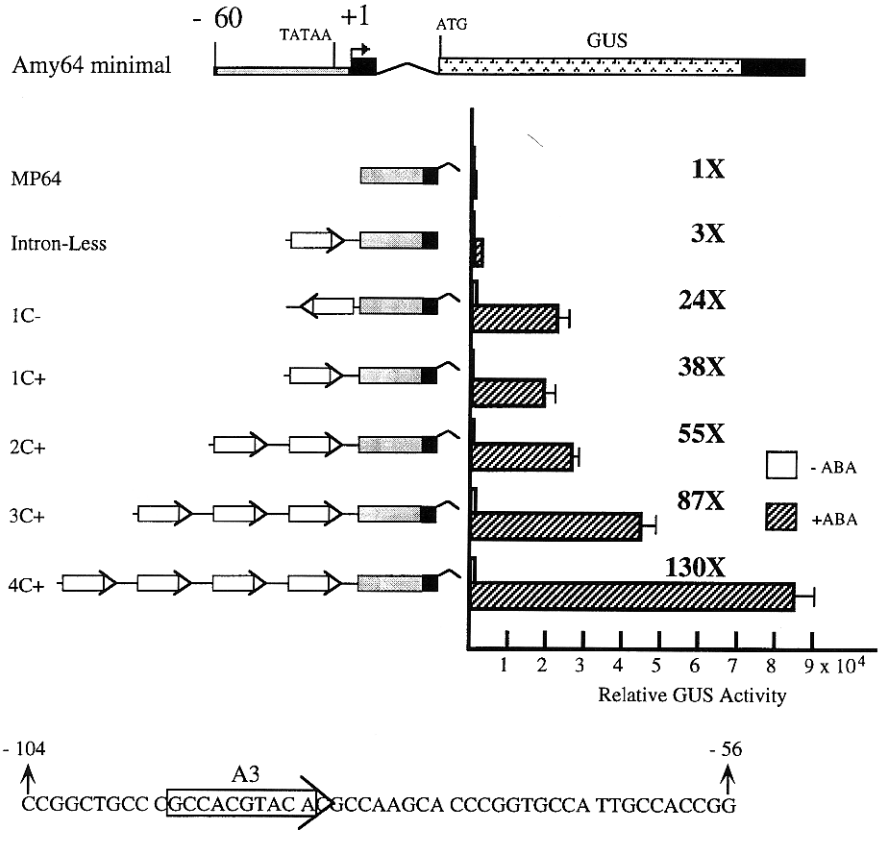

Fig. 4. A 49-bp fragment containing an ACGT-box (A3) confers ABA inducibility to a minimal promoter. The minimal promoter (to –60) and the 5' untranslated region (to +57) from the barley *Amy64* α-amylase gene was fused to the 5' end of *HVA22* intron1-exon2-intron2 fragment (thin black angled line) (Shen and Ho 1995). The 3' region (black bar to the right of the GUS coding sequence) was from the *HVA22* SphI-SphI genomic fragment, including the polyadenylation sequence (AATAAA). This minimal promoter (MP64) is not responsive to gibberellin or ABA. The 49-bp *HVA22* promoter fragment, shown at the bottom, was fused in either positive (i.e. the same as in the native promoter) or negative orientation. The 2C+, 3C+ and 4C+ constructs contain 2, 3, or 4 tandem copies of the 49-bp sequence, respectively. The numbering of the 49-bp fragment is relative to the transcription start site of the *HVA22* gene. Relative GUS activity of each construct is the mean of four replicas. Error bars indicate the standard error of each set of replicas. X indicates fold of increase. From Shen and Ho 1995.

ing an ACGT-box (A2), was able to confer a high level of ABA induction (Shen et al. 1996). These data indicate that all information necessary and sufficient for ABA response is present in these short promoter fragments.

An ACGT-box is necessary but not sufficient for ABA induction

To determine sequences within the 49-bp *HVA22* promoter region governing the ABA responsiveness, a linker-scan analysis was performed with the construct 1C+ (Fig. 5A). The sequence of promoter fragment was replaced at 9- or 10-bp intervals. The most drastic reduction was observed with two mutants, LS-08 and LS-11; the absolute level of GUS activity obtained from these constructs dropped to below 5% of that obtained with the wild type. In addition, the degree of induction decreased from 44-fold in the case of wild type to only 4-fold for these two mutants (Fig. 5A). In construct LS-08 the ACGT-core-containing A3 element was mutated, while in LS-11 the last 9 bp of the 49-bp promoter fragment were replaced. This 9-bp fragment, TGCCACCGG, represents a novel *cis*-acting sequence involving the ABA response and is denoted CE1 (coupling element 1).

Sequences similar to CE1 are present in other ABA-responsive genes such as maize *Rab17* (Vilardell et al. 1990) and rice *Rab16A* (Mundy and Chua 1988). None of these elements has been tested to determine whether they are indeed involved in the ABA responsiveness of those genes. In light of our data described above, it would be definitely worthwhile to investigate whether they also function as coupling elements of ABA response complexes in those genes.

A similar approach was used to study the 68-bp *HVA1* promoter fragment by mutating this sequence at 10- or 12-bp intervals (fragment I to VI, Fig. 5B). The most drastic mutations were in fragments III and IV. When either of these two fragments was replaced with a random

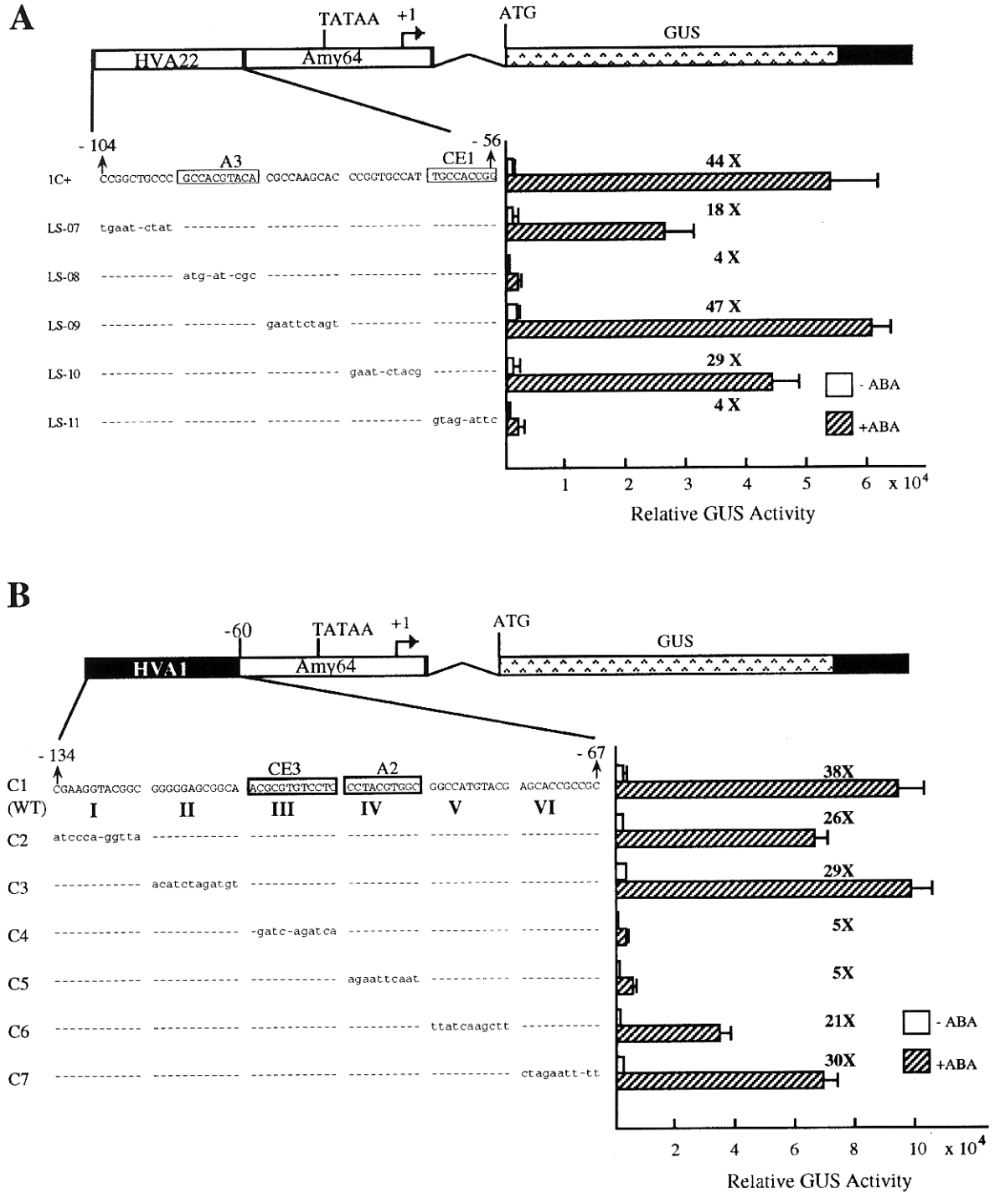

Fig. 5. Linker-scan analyses of the short fragments from *HVA22* and *HVA1* genes define novel coupling elements involved in the ABA response. The numbering of the fragments is relative to the transcription start site of the *HVA22* or *HVA1* gene. (A) The 49-bp *HVA22* promoter sequence was mutated at 9- or 10-bp intervals. (B) The 68-bp *HVA1* promoter sequence was mutated at 10- to 12-bp intervals. These experiments demonstrate that both the ACGT-box and a novel coupling element (either CE1 or CE3) are necessary for ABA response. From Shen and Ho 1995 and Shen et al. 1996.

sequence, the ABA induction dropped from 38- to 5-fold, with the absolute level of GUS activity being less than 10% of that obtained from the wild-type fragment. The negative effect from the fragment IV was expected because it was an ACGT-box (A2). In contrast, fragment III shared no homology with any of the *cis*-acting elements which may be involved in ABA response, including CE1. Hence, we designated fragment III, ACGCGTGTCCTC, in the *HVA1* promoter sequence as CE3 (coupling element 3).

An ACGT-box interacts with either a distal or a proximal coupling element to confer ABA response

It has been shown that to achieve a high level of ABA induction, the ACGT-box sequence in the *HVA22* promoter, i.e. A3, has to interact with a distal element, CE1 (Fig. 6A) and that in *HVA1* the ACGT-box sequence, A2, needs to be coupled with the neighboring CE3 (Fig. 6B). Therefore, we performed an exchange experiment and demonstrated that the two ACGT-boxes, A2 and A3, were fully exchangeable (Shen et al. 1996). Hence, it appears that an ACGT-box can interact with either a distal coupling element (CE1) or a proximal element (CE3) to form a complex and confer a high level of ABA induction. We therefore designate the complex containing A3 and CE1 from the *HVA22* gene ABA response complex 1 or ABRC1 and that from the *HVA1* gene, containing A2 and CE3 ABA response complex 3 or ABRC3 (Fig. 6). It is important to note that ABRCs appear to be the smallest defined promoter units necessary and sufficient for specific ABA-induced gene expression.

Signal response specificity relies on the interaction of an ACGT-box with a coupling element

The 'coupling model' described above has at least partially resolved the puzzle for the involvement of ACGT-boxes in responses to a variety of different environmental and physiological cues. As summarized in Fig. 7, similar to the observation that ABA response relies on the interaction of an ACGT-box (A3, GCCACGTACA or A2, CCTACGTGGC) with a coupling element (CE1, TGCCACCGG or CE3, ACGCGTGTCCTC), the presence of both an ACGT-box (Box II, TCCACGTGGC or

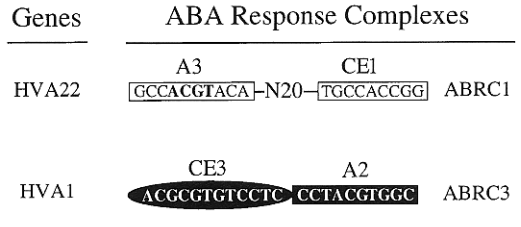

Fig. 6. Modular nature of ABA response complexes in two ABA-responsive barley genes, *HVA1* and *HVA22*. In *HVA22*, an ABA response complex (ABRC1) is composed of an ACGT-box, A3, and a distal coupling element (CE1). In *HVA1*, an ABA response complex (ABRC3) consists of an ACGT-box, A2, and a proximal coupling element (CE3).

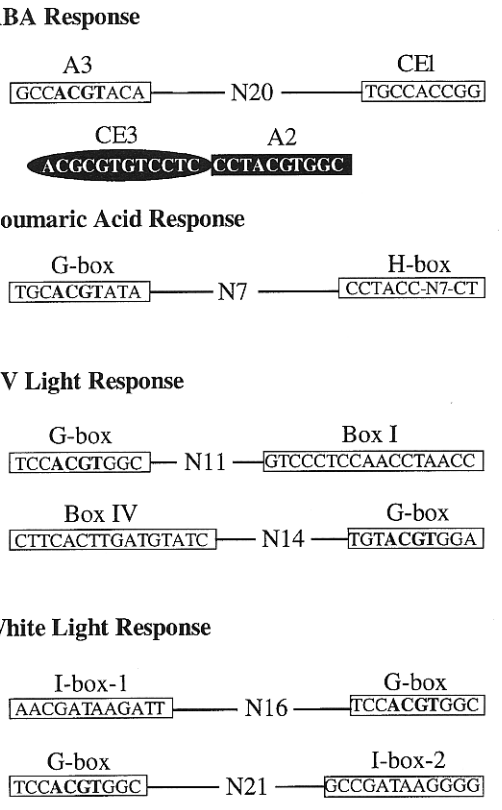

Fig. 7. Schematic model of signal-specific promoter complexes. The ACGT-cores in G-box-like sequences are in boldface letters. The distance between the G-box-like sequence and the coupling sequence is indicated by the number (N) of nucleotides. The coumaric acid response complex is adopted from Loake et al. (1992), UV light response complexes are from Block et al. (1990), and white light response complexes are from Donald and Cashmore (1990). This figure is modified from Shen and Ho 1995.

Box III, TGTACGTGGA) and another element (Box I, GTCCCTCCAACCTAACC or Box IV, CTTCACTTGATGTATC) are necessary for the UV-light response of the chalcone synthase promoter (Schulze-Lefert et al. 1989). Specific point mutations within either Box II or Box I result in a dramatic reduction of light-induced gene expression (Block et al. 1990). Similarly, Donald and Cashmore (1990) have reported that mutations in either G-box (CTTCCACGTGGC, an ACGT-box) or I box (I-1, AACGATAAGATT and I-2, AGCCGATAAGGG) dramatically reduce the expression of the light-responsive *Arabidopsis rbcS-1A* gene. In the case of a chalcone synthase gene, the combination of H-box (CCTACC-N_7-CT) and an ACGT-box (CACGTG) is necessary for the response of this promoter to the phenylpropanoid-pathway intermediate *p*-coumaric acid (Loake et al. 1992). Although ACGT-box sequences in these genes are similar, elements interacting with them, as shown in Fig. 7, are different in complexes involved in the response to ABA (Shen and Ho 1995), coumaric

acid (Loake et al. 1992), UV light (Schulze-Lefert et al. 1989), or white light (Donald et al. 1990). Therefore, it appears that the signal response specificity is at least partially determined by elements coupled with ACGT-boxes (G-boxes) (Fig. 7B).

Construction of ABA switches with different levels of ABA induction and transcription strength

The delineation of ABRC1 and 3 leads to the conclusion that ACGT-boxes in *HVA1* and *HVA22* promoters can confer a high level of ABA response provided that they are coupled with a distal or a proximal coupling element, namely CE1 or CE3. At the same time, several recombinant DNA constructs, based on ABRCs described above, were shown to be able to drive the ABA-induced expression of *GUS* reporter gene at even higher levels. These ABA molecular switches are summarized and their transcription strengths are shown in Fig. 8. One copy of the 49-bp *HVA22* ABRC1 is able to confer more than 30-fold ABA induction and additional copies of ABRC1 added to the reporter construct led to even higher levels of ABA induction (Fig. 8A). The 68-bp ABRC3 of the *HVA1* gene turned out to be stronger than the *HVA22* ABRC1; one copy of this fragment led to 20-fold induction with the absolute level of GUS activity twice as high as that obtained with the *HVA22* ABRC1 (Fig. 8B). Moreover, the presence of two different CEs further enhanced the expression of the construct when the two CEs interacted with the ACGT-box from either the *HVA22* or *HVA1* promoter (Fig. 8C,D).

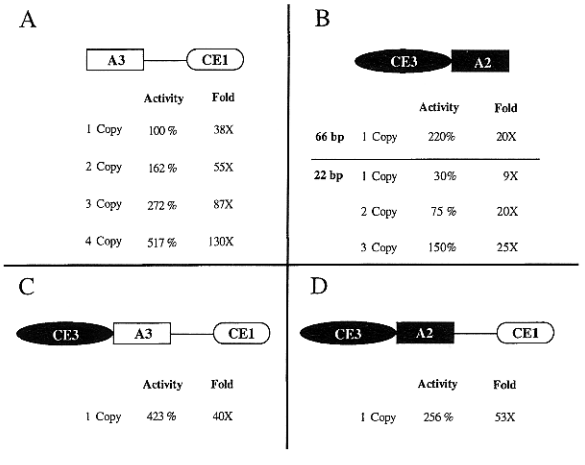

Fig. 8. Versions of DNA molecular switches controlling the expression of ABA-inducible promoters. (A) *HVA22* complex consists of an ACGT-box (A3) and a distal CE1. The normalized GUS activity from the ABA-treated sample of the single copy ABRC1 construct is taken as 100% throughout this figure. 'Fold' stands for fold induction calculated as described (Shen et al. 1996). (B) The ABA response complex in *HVA1* promoter consists of an ACGT-box (A2) and the proximal CE3. (C) and (D) The ternary ABA response complexes consisting of two coupling elements and an ACGT-box. From Shen et al. 1996.

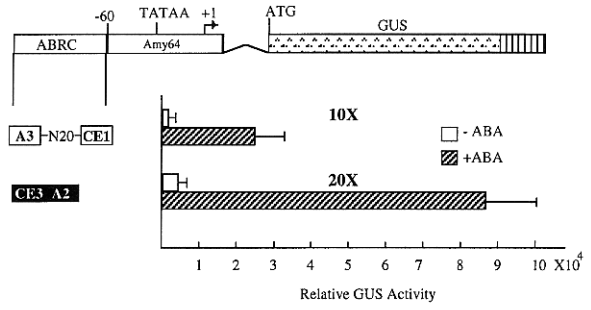

Fig. 9. Both the 49-bp *HVA22* promoter containing ABRC1 and the 68-bp *HVA1* promoter containing ABRC3 are functional in a vegetative tissue. The DNA constructs were bombarded into leaf tissue from six-day-old, greenhouse-grown barley plants and treated with or without 10^{-4} *M* ABA in H_2O at 24°C for 24 h. The relative GUS activity of each construct is the mean of 4 replicates. The error bar indicates the standard error of each set of replicas. X indicates fold induction. From Shen et al. 1996.

ABA molecular switches are also functional in vegetative tissues

All of the data reported above were obtained with aleurone tissues in barley seeds. However, it is known that both *HVA1* and *HVA22* genes are also expressed in vegetative tissues (Hong et al. 1992; Q. Shen and T.-H. D. Ho, unpublished result). To investigate whether the defined ABRCs and other molecular switches also function in the vegetative tissues, we introduced constructs containing either the 49-bp *HVA22* ABRC1 or the 68-bp *HVA1* ABRC3 into six-day-old barley leaf tissues. Results shown in Fig. 9 demonstrated that both ABA switches were able to confer ABA induction in the vegetative tissue. As observed with the aleurone tissue, the *HVA1* ABRC3 was more responsive to ABA than the *HVA22* ABRC1.

DNA-binding proteins

The control of gene transcription is in part dependent on interactions of DNA sequences (*cis*-acting) with proteins (*trans*-acting). Therefore, effort has been made to identify the proteins binding to promoters of ABA-regulated genes. Gel retardation and DNase I footprinting experiments show nuclear factor(s) binding to the conserved sequence motif I (TACGTGGC, an ACGT-box) and motif II (IIa: CGCCGCGCCTGC; IIb: CGC/GCGCGCT) in rice *Rab16A* gene (Mundy et al. 1990). Furthermore, Guiltinan et al. (1990) reported the cloning of a wheat cDNA whose product (EmBP-1) can bind the 8-bp sequence (CACGTGGC), an ACGT-box, in the promoter of the *Em* gene. A 2-bp mutation in this ACGT-box prevented the binding to EmBP-1 (Guiltinan et al. 1990). The EmBP-1 protein contains the basic leucine zipper motif (bZIP) found in many transcription factors in yeast and human (Vinson et al. 1989), as well as in other plants (Fumlaki and Chua 1992). However, nuclear ex-

tracts from both the ABA-treated and non-ABA-treated samples contain proteins capable of binding to this 8-bp ACGT-box which is essential to ABA response. Therefore, the level of EmBP-1 is unlikely to be the key factor regulating ABA-induced gene expression. G-box (ACGT-box)-binding proteins (GBF) have been isolated from *Arabidopsis* and they are also bZIP proteins which can form heterodimers. Different dimers vary in their affinities for a G-box target DNA sequence (Schindler et al. 1992). Recently, an ABA-inducible rice bZIP protein, designated OSBZ8, has been reported (Nakagawa et al. 1996). In addition, a homeodomain-containing leucine zipper (HD-Zip) proteins encoded by the *Arabidopsis ATHB-7* gene is also ABA- and stress-inducible (Söderman et al. 1996).

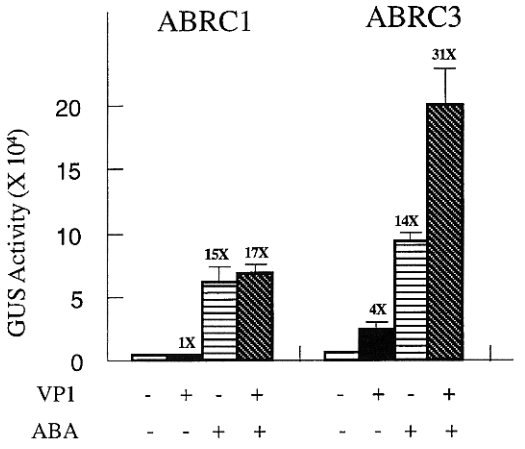

Fig. 10. ABRC3, but not ABRC1, is activated by the maize VP1 transcription regulator. The 35S-Sh-Vp1 construct containing the *VP1* coding sequence driven by the 35S constitutive promoter and a sucrose synthase gene intron was co-bombarded into barley aleurone layers along with the construct containing ABRC1 or ABRC3 at 1:3 ratio (ABRC construct:Vp1 construct). Similar results were obtained at 1:0.2 ratio. Symbols below the bars indicate treatments with (+) or without (–) ABA or the VP1 effector construct. The relative GUS activity of each construct is the mean of four replicates. The error bar indicates the standard error of each set of replicas. X indicates fold induction. From Shen et al. 1996.

ABA signal transduction components

Genetic analyses have led to the cloning of several genes regulating the sensitivity of plants to ABA. One of these genes is maize *Viviparous-1*, or *VP1*, a mutation of which leads to reduced sensitivity to ABA and precocious seed germination. It has been shown that the *VP1* gene encodes a transcription factor involved in ABA-regulated gene expression (McCarty et al. 1991). However, ABA-induced expression of some genes, for instance, maize *Rab28* (Pla et al. 1991) and *Cat1* (Williamson and Scandalios 1992), is *VP1*-independent while others, such as the wheat *Em* gene (McCarty et al. 1991), are *VP1*-dependent. Although the data presented in Fig. 5 demonstrate that ABRC3 is different from ABRC1 in terms of their transcription strengths and structures, we propose that different ABRCs are also mediated by different signal transduction pathways. To test this hypothesis, we co-bombarded the effector construct consisting of the maize *VP1* coding sequence driven by a constitutive 35S promoter (McCarty et al. 1991) along with the reporter construct, C1 (containing ABRC3) or C17 (containing ABRC1). McCarty et al. (1991) have shown that co-expression of *VP1* in maize protoplasts enhanced the ABA response of the wheat *Em* promoter, and the presence of both *VP1* and ABA had a synergistic effect. As shown in the right half of Fig. 10, a similar pattern of *VP1* activation on the ABRC3 of the barley ABA responsive *HVA1 Lea* gene was observed in our system. The co-expression of *VP1* led to a 4-fold induction of ABRC3, compared to the 14-fold induction by ABA. In the presence of ABA and *VP1*, the induction increased to 31-fold, suggesting a synergistic effect of ABA and *VP1* on ABRC3. In contrast, ABRC1 did not appear to respond to *VP1* at all (Fig. 10, left half). In the absence of ABA, *VP1* co-expression failed to activate ABRC1, giving no induction at all (1×). The presence of *VP1* and ABA gave a result (17×) similar to ABA treatment alone (15×). Therefore, *VP1* appears to differentiate ABRC3 from ABRC1 in mediating ABA response.

In addition to *VP1*, other genes governing the sensitivity of plants to ABA have been reported. A mutation of the *Arabidopsis Era1* gene enhanced the sensitivity of plants to ABA. The *Era1* gene has been cloned and shown to encode the β-subunit of a protein farnesyl transferase (Cutler et al. 1996). ABA treatment alters the level of cellular Ca^{2+} concentration (Gilroy and Jones 1992) and an ABA-inducible gene which encodes a novel plant Ca^{2+}-binding protein in rice has been reported (Frandsen et al. 1996).

Protein phosphorylation and dephosphorylation are also likely involved in the ABA-regulated gene expression. An ABA- and stress-inducible gene cloned from wheat embryos encodes a protein with sequence homology to protein kinases, i.e. it contains features of serine/threonine protein kinases, including all 12 conserved regions of the catalytic domain (Anderberg and Walker-Simmons 1992). Recently, an ABA-induced mitogen-activated protein kinase activity has been suggested to be involved in ABA regulation in barley aleurone protoplasts (Knetsch et al. 1996). A dominant mutation in the *Arabidopsis* gene, *Abi1*, encoding a protein phosphatase 2C, abolishes ABA responsiveness (Leung et al. 1994, Meyer et al. 1994).

Perspective

ABA-regulated gene expression has been under intensive studies in the past fifteen years. A lot of progress has been made in the cloning of ABA-regulated genes and the definition of *cis*-acting elements involved in the regulation of ABA response in promoters of these genes.

The discovery of ABRCs demonstrates that an ACGT-box is necessary but not sufficient for ABA response. Instead, a specific ABA response relies on the interaction of two *cis*-acting elements, an ACGT-box and a CE. Although the concept of ABRC is established by working with barley ABA-induced genes, similar ABRCs exist in wheat (R. S. Quatrano, personal communication) and rice (Ono et al. 1996). Conceivably, the combination of an ACGT-box and a CE in forming a promoter unit necessary and sufficient for ABA induction, is universal among all cereal plants.

DNA-binding proteins interacting with ACGT-boxes have been reported. However, CE element-binding proteins have not yet been studied. It is expected that proteins interacting with CE elements will be isolated by techniques such as yeast one-hybrid system and expression library screening. Both protein kinases and phosphatases have been reported to be involved in the regulation of ABA signal transduction pathways. Further studies of these regulatory enzymes would reveal significant new insights into processes mediated by ABA.

Although ABA-suppressible genes have been reported, molecular mechanisms underlying ABA suppression are completely unknown. Approaches similar to those used in the study of ABA induction could be followed in the investigation of *cis*- and *trans*-acting elements involved in ABA suppression of gene expression.

Crosstalks among multiple signals in regulating gene expression appear to be essential in integrating plant responses to complex environmental changes. For instance, the crosstalk between ABA and light has recently been reported (Weatherwax et al. 1996). Finally, although functions of some ABA-regulated genes are known, the role of most ABA-regulated genes, including *LEA* and *RAB*, remains unclear. Both biochemical and genetic approaches should be followed to fully comprehend the role of these genes. Much research effort is needed in this area.

Acknowledgments – We thank current members in T.-H. D. Ho's lab for their critical reading of our manuscript. The original works published by T.H.D.H. and his associates were supported by US National Science Foundation grants (DCB-9006591 and IBN-9408900) and US Department of Agriculture/National Research Initiative grants (91-37100-6625 and 94-37100-0316).

References

Anderberg, R. J. & Walker-Simmons, M. K. 1992. Isolation of a wheat cDNA clone for an abscisic acid-inducible transcript with homology to protein kinase. – Proc. Natl. Acad. Sci. USA 89: 10183–10187.

Bandziulis, R. J., Swanson, M. S. & Dreyfuss, G. 1989. RNA-binding proteins as developmental regulators. – Genes Dev. 3: 431–437.

Bartels, D., Engelhardt, K., Roncarati, R., Schneider, K., Rotter, M. & Salamini, F. 1991. An ABA and GA modulated gene expressed in the barley embryo encodes an aldose reductase related protein. – EMBO J. 10: 1037–1043.

Bex, J. H. M. 1972a. Effects of abscisic acid on nucleic acid metabolism in maize coleoptiles. – Planta 103: 1–10.

– 1972b. Effects of abscisic acid on the soluble RNA polymerase activity in maize coleoptiles. – Planta 103: 11–17.

Blackman, S. A., Wettlaufer, S. H., Obendorf, R. L. & Leopold, A. C. 1991. Maturation protein associated with desiccation tolerance in soybean. – Plant Physiol. 96: 868–874.

Block, A., Dangl, J. L., Hahlbrock, K. & Schulze, L. P. 1990. Functional borders, genetic fine structure and distance requirements of *cis* elements mediating light responsiveness of the parsley chalcone synthase promoter. – Proc. Natl. Acad. Sci. USA 87: 5387–5391.

Bornman, C. H. & Jansson, E. 1980. *Nicotiana tabacum* callus studies. X. ABA increases resistance to cold damage. – Physiol. Plant. 48: 491–493.

Bray, E. A. & Beachy, R. N. 1985. Regulation by ABA of β-conglycinin expression in cultured developing soybean cotyledons. – Plant Physiol. 79: 746–750.

Brown, P. H., Mecham, R. P. & Ho, T.-H. D. 1988. Hormonal regulation of barley nuclease: Investigation using a monoclonal antibody. – Plant Cell Environ. 11: 747–753.

Bruce, W. B., Christensen, A. H., Klein, T., Fromm, M. & Quail, P. H. 1989. Photoregulation of a phytochrome gene promoter from oat transferred into rice by particle bombardment. – Proc. Natl. Acad. Sci. USA 86: 9692–9696.

Chaloupkova, K. & Smart, C. C. 1994. The abscisic acid induction of a novel peroxidase is antagonized by cytokinin in *Spirodela polyrrhiza* L. – Plant Physiol. 105: 497–507.

Chen, H. H., Li, P. H. & Brenner, M. L. 1983. Involvement of abscisic acid in potato cold acclimation. – Plant Physiol. 71: 362–365.

Chen, T. H. H. & Gusta, L. V. 1983. Abscisic acid-induced freezing resistance in cultured plant cells. – Plant Physiol. 73: 71–75.

Crouch, M. L. & Sussex, I. M. 1981. Development and storage-protein synthesis in *Brassica napus* L. embryos in vivo and in vitro. – Planta 153: 64–74.

– , Tenbarge, K., Simon, R., Finkelstein, R., Scofield, S. & Solberg, L. 1984. Storage protein mRNA levels can be regulated by abscisic acid in *Brassica* embryos. – *In* Molecular Form and Function of the Plant Genome (L. Van Vloten-doting, G. S. P. Groot and T. C. Hall, eds), pp. 555–565. Plenum Press, New York, NY. ISBN 0-306-41913-0.

Cutler, S., Ghassemian, M., Bonetta, D., Cooney, S. & McCourt, P. 1996. A protein farnesyl transferase involved in abscisic acid signal transduction in *Arabidopsis*. – Science 273: 1239–1241.

Delisle, A. J. & Ferl, R. J. 1990. Characterization of the *Arabidopsis Adh* G-box binding factor. – Plant Cell 2: 547–557.

Donald, R. G. K., Batschauer, A. & Cashmore, A. R. 1990. The plant G-box promoter sequence activates transcription in *Saccharomyces cerevisiae* and is bound in vitro by a yeast activity similar to GBF, the plant G box binding factor. – EMBO J. 9: 1727–1735.

Dure, L. III, Crouch, M., Harada, J., Ho, T.-H. D., Mundy, J., Quatrano, R. S., Thomas, T. & Sung, Z. R. 1989. Common amino acid sequence domains among the LEA proteins of higher plants. – Plant Mol. Biol. 12: 475–486.

Eamus, D. & Wilson, J. M. 1983. ABA levels and effects in chilled and hardened *Phaselous vulgaris*. – J. Exp. Bot. 34: 1000–1006.

Frandsen, G., Muller, U. F., Nielsen, M., Mundy, J. & Skriver, K. 1996. Novel plant Ca^{2+}-binding protein expressed in response to abscisic acid and osmotic stress. – J. Biol. Chem. 271: 343–348.

Fumlaki, K. & Chua, N. H. 1992. Plant transcription factors: Present knowledge and future challenges. – Trends Genet. 8: 22–27.

Gilroy, S. & Jones, R. L. 1992. Gibberellic acid and abscisic acid coordinately regulate cytoplasmic calcium and secretory activity in barley aleurone protoplasts. – Proc. Natl. Acad. Sci. USA 89: 3591–3595.

Gomez, J., Sanchez-Martinez, D., Stiefel, V., Rigau, J., Puigdo-

menech, P. & Pages, M. 1988. A gene induced by the plant hormone abscisic acid in response to water stress encodes a glycine-rich protein. – Nature 334: 262–264.
Guiltinan, M. J., Marcotte, W. R. Jr & Quatrano, R. S. 1990. A plant leucine zipper protein that recognizes an abscisic acid response element. – Science 250: 267–271.
Hong, B., Barg, R. & Ho, T.-H. D. 1992. Developmental and organ-specific expression of an ABA- and stress-induced protein in barley. – Plant Mol. Biol. 18: 663–674.
Imber, D. & Tal, M. 1970. Phenotypic reversion of *flacca*, a wilty mutant of tomato, by abscisic acid. – Science 169: 592–593.
Johns, H. G. 1978. How plants respond to stress. – Nature 271: 610.
Johnson-Flanagan, A. M., Zhong, H., Thiagarajah, M. R. & Saini, H. S. 1991. Role of abscisic acid in the induction of freezing tolerance in *Brassica napus* suspension-cultured cells. – Plant Physiol. 95: 1044–1048.
Karssen, C. M., Brinkhorst-van der Swan, D. L. C., Breekland, A. E. & Koornneef, M. 1983. Induction of dormancy during seed development by endogenous abscisic acid: Studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* L. Heynh. – Planta 157: 158–165.
Khursheed, B. & Rogers, J. C. 1989. Barley *α*-amylase genes and the thiol protease gene *Aleurain*: Use of a single poly(A) addition signal associated with a conserved pentanucleotide at the cleavage site. – Proc. Natl. Acad. Sci. USA 86: 3987–3991.
King, R. W. 1976. Abscisic acid in developing wheat grains and its relationship to grain growth and maturation. – Planta 132: 43–51.
Knetsch, M. L. W., Wang, M., Snaar-Jagalska, B. E. & Heimovaara-Dijkstra, S. 1996. Abscisic acid induces mitogen-activated protein kinase activation in barley aleurone protoplasts. – Plant Cell 8: 1061–1067.
Koehler, S. M. & Ho, T.-H. D. 1990. Hormonal regulation, processing and secretion of cysteine proteinases in barley aleurone layers. – Plant Cell 2: 769–783.
Koornneef, M., Jorna, M. L., Brinkhorst-van der Swan, D. L. C., & Karssen, C. M. 1982. The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of *Arabidopsis thaliana* L. Heynh. – Theor. Appl. Genet. 61: 385–393.
Lachno, E. R. & Baker, D. A. 1986. Stress induction of abscisic acid in maize roots. – Physiol. Plant. 68: 215–221.
Lafuente, M. T., Belver, A., Guye, M. G. & Saltveit, M. E. 1991. Effect of temperature conditioning on chilling injury of cucumber cotyledons: Possible role of ABA and heat shock proteins. – Plant Physiol. 95: 443–449.
Leung, J., Bouvier-Durand, M., Morris, P. C., Guerrier, D., Chefdor, F. & Giraudat, J. 1994. *Arabidopsis* ABA response gene *ABI1*: Features of a calcium-modulated protein phosphatase. – Science 264: 1448–1452.
Loake, G. J., Faktor, O., Lamb, C. J. & Dixon, R. A. 1992. Combination of H-box (CCTACCN7CT) and G-box (CACGTG) *cis* elements is necessary for feed-forward stimulation of a chalcone synthase promoter by the phenylpropanoid-pathway intermediate *p*-coumaric acid. – Proc. Natl. Acad. Sci. USA 89: 9230–9234.
Ludevid, M. D., Freire, M. A., Gomez, J., Burd, C. G., Albericio, F., Giralt, E., Dreyfuss, G. & Pages, M. 1992. RNA binding characteristics of a 16-kDa glycine-rich protein from maize. – Plant J. 2: 999–1003.
Marcotte, W. R. Jr, Russell, S. H. & Quatrano, R. S. 1989. Abscisic acid-responsive sequences from the *Em* gene of wheat. – Plant Cell 1: 969–976.
McCarty, D. R., Hattori, T., Carson, C. B., Vasil, V., Lazar, M. & Vasil, I. K. 1991. The *Viviparous-1* developmental gene of maize encodes a novel transcriptional activator. – Cell 66: 895–905.
Meyer, K., Leube, M. P. & Grill, E. 1994. A protein phosphatase 2C involved in ABA signal transduction in *Arabidopsis thaliana*. – Science 264: 1452–1455.
Michel, D., Salamini, F., Bartels, D., Dale, P., Baga, M. & Szalay, A. 1993. Analysis of a desiccation and ABA-responsive promoter isolated from the resurrection plant *Craterostigma plantagineum*. – Plant J. 4: 29–40.
Mohapatra, S. S., Poole, R. J. & Dhindsa, R. S. 1988. Abscisic acid-regulated gene expression in relation to freezing tolerance in alfalfa. – Plant Physiol. 87: 468–473.
Mudgett, M. B. & Clarke, S. 1994. Hormonal and environmental responsiveness of a developmentally regulated protein repair L-isoaspartyl methyltransferase in wheat. – J. Biol. Chem. 269: 25605–25612.
Mundy, J. & Chua, N.-H. 1988. Abscisic acid and water-stress induce the expression of a novel rice gene. – EMBO J. 7: 2279–2286.
– , Yamaguchi-Shinozaki, K. & Chua, N.-H. 1990. Nuclear proteins bind conserved elements in the abscisic acid-responsive promoter of a rice *rab* gene. – Proc. Natl. Acad. Sci. USA 87: 1406–1410.
Nakagawa, H., Ohmiya, K. & Hattori, T. 1996. A rice bZIP protein, designated OSBZ8, is rapidly induced by abscisic acid. – Plant J. 9: 217–227.
Naumann, R. & Dörffling, K. 1982. Variation of free and conjugated abscisic acid, phaseic acid, and dihydrophaseic acid levels in ripening barley grains. – Plant Sci. Lett. 27: 111–117.
Ono, A., Izawa, T., Chua, N.-H. & Shimamoto, K. 1996. The rab16B promoter of rice contains two distinct abscisic acid-responsive elements. – Plant Physiol. 112: 483–491.
Pla, M., Gomez, J., Goday, A. & Pages, M. 1991. Regulation of the abscisic acid-responsive gene *rab28* in maize viviparous mutants. – Mol. Gen. Genet. 230: 394–400.
Poulson, R. & Beevers, L. 1970. Effects of growth regulators on ribonucleic acid metabolism of barley leaf segments. – Plant Physiol. 46: 782–785.
Quatrano, R. S. 1987. The role of hormones during seed development. – *In* Plant Hormones and their Role in Plant Growth and Development (P. J. Davies, ed.), pp. 494–514. Martinus Nijhoff, Dordrecht. ISBN 90-247-3497-5.
Robichaud, C. S., Wong, J. & Sussex, I. M. 1980. Control of in vitro growth of viviparous embryo mutants of maize by abscisic acid. – Dev. Genet. 1: 325–330.
Schindler, U., Menkens, A. E., Beckmann, H., Ecker, J. R. & Cashmore, A. R. 1992. Heterodimerization between light-regulated and ubiquitously expressed *Arabidopsis* GBF bZIP proteins. – EMBO J. 11: 1261–1273.
Schulze-Lefert, P., Becker-André, M., Schulz, W., Hahlbrock, K. & Dangl, J. L. 1989. Functional architecture of the light-responsive chalcone synthase promoter from parsley. – Plant Cell 1: 707–714.
Shen, Q. & Ho, T.-H. D. 1995. Functional dissection of an abscisic acid (ABA)-inducible gene reveals two independent ABA-responsive complexes each containing a G-box and a novel *cis*-acting element. – Plant Cell 7: 295–307.
– , Uknes, S. J. & Ho, T.-H. D. 1993. Hormone response complex of a novel abscisic acid and cycloheximide inducible barley gene. – J. Biol. Chem. 268: 23652–23660.
– , Zhang, P. & Ho, T.-H. D. 1996. Modular nature of abscisic acid (ABA) response complexes: Composite promoter units which are necessary and sufficient for ABA induction of gene expression in barley. – Plant Cell 8: 1107–1119.
Skriver, K. & Mundy, J. 1990. Gene expression in response to abscisic acid and osmotic stress. – Plant Cell 2: 503–512.
Söderman, E., Mattsson, J. & Engström, P. 1996. The *Arabidopsis* homeobox gene *ATHB-7* is induced by water deficit and by abscisic acid. – Plant J. 10: 375–381.
Stewart, G. R. & Smith, H. 1972. Effects of abscisic acid on nucleic acid synthesis and the induction of nitrate reductase in *Lemna polyrhiza*. – J. Exp. Bot. 23: 875–885.
Straub, P. F., Shen, Q. & Ho, T.-H. D. 1994. Structure and promoter analysis of an ABA- and stress-regulated barley gene, *HVA1*. – Plant Mol. Biol. 26: 617–630.
Sussex, I. M. & Dale, R. M. K. 1979. Hormonal control of storage protein synthesis in *Phaseolus vulgaris*. – *In* The Plant Seed: Development, Preservation, and Germination (I. Ru-

binstein, R. L. Phillips, E. C. Green and G. B. Gengenbach, eds), pp. 129–141. Academic Press, New York, NY. ISBN 0-12-602050-7.
– , Dale, R. M. K. & Crouch, M. L. 1980. Developmental regulation of seed storage protein synthesis in seeds. – *In* Genome Organization and Expression in Plants (C. J. Leaver, ed.), pp. 283–289. Plenum Press, New York, NY. ISBN 0-306-40340-4.
Triplett, B. A. & Quatrano, R. A. 1982. Timing, localization, and control of wheat germ agglutinin synthesis in developing wheat embryos. – Dev. Biol. 91: 491–496.
Velasco, R., Salamini, F. & Bartels, D. 1994. Dehydration and ABA increase mRNA levels and enzyme activity of cytosolic GAPDH in the resurrection plant *Craterostigma plantagineum*. – Plant Mol. Biol. 26: 541–546.
Vilardell, J., Goday, A., Freire, M. A., Torrent, M., Martínez, M. C., Torné, J. M. & Pagès, M. 1990. Gene sequence, developmental expression, and protein phosphorylation of RAB-17 in maize. – Plant Mol. Biol. 14: 423–432.
Vinson, C. R., Sigler, P. B. & McKnight, S. L. 1989. Scissors-grip model for DNA recognition by a family of leucine zipper proteins. – Science 246: 911–916.
Walton, D. C., Soofi, G. S. & Sondheimer, E. 1970. The effects of abscisic acid on growth and nucleic acid synthesis in excised embryonic bean axes. – Plant Physiol. 45: 37–40.
Wareing, P. F., Good, J. & Manuel, J. 1968. Some possible physiological roles of abscisic acid. – *In* Biochemistry and Physiology of Plant Growth Substances (F. Wightman and G. Setterfield, eds), pp. 1561–1579. Runge Press, Ottawa.
Weatherwax, S. C., Ong, M. S., Degenhardt, J., Bray, E. A. & Tobin, E. M. 1996. The interaction of light and abscisic acid in the regulation of plant gene expression. – Plant Physiol. 111: 363–370.
Williamson, J. D. & Scandalios, J. G. 1992. Differential response of maize catalases to abscisic acid: Vp1 transcriptional activator is not required for abscisic acid-regulated *Cat1* expression. – Proc. Natl. Acad. Sci. USA 89: 8842–8846.
Wright, S. T. C. & Hiron, R. W. P. 1969. (+)-Abscisic acid, the growth inhibitor induced in detached wheat leaves by a period of wilting. – Nature 224: 719–720.
Zeevaart, J. A. D. & Creelman, R. A. 1988. Metabolism and physiology of abscisic acid. – Annu. Rev. Plant Physiol. Plant Mol. Biol. 39: 439–473.

Edited by C. H. Bornman