Bot. Bull. Acad. Sin. (1993) **34:** 103-113

(*Invited review paper*)

Hormone and stress-regulated gene expression in cereal plants[1]

Tuan-Hua David Ho
Department of Biology, Washington University, St. Louis, Missouri 63130, U. S. A.

Key words: Abscisic acid; α-Amylase; Barley aleurone layers; Gibberellin; *Hordeum vulgare.*

Contents

Introduction

Plants are constantly under the influence of their [redacted] internal environment. Among the exter- mental patterns. The external stress signals and the internal hormonal factors are important parts of the regulatory network which ultimately govern the form and function of plants. In this aricle, I will present our efforts to analyze the action of environmental stresses

Table 1. *Hormonal regulation of gene expression in barley aleurone layers*

Gene	Hormone treatment			
	None	GA	ABA	GA+ABA
α-Amylase-high pI	−[a]	+++++	−	−
α-Amylase-low pI	+	+++++	+	+
Endoprotease (30 kD)	+	+++++	−	+++
Endoprotease (37 kD)	−	+++++	−	+
Thio-protease (Aleurain)	−	+++	−	−
Nuclease (RNase+DNase+3'-nucleotidase)	+	+++	−	ND[b]
β-1,3-1,4 glucanase	−	+++	−	ND
Actin	++	++	++	++
Non-differential[c]	++	++	++	++
Alcohol dehydrogenase	+	−	+	+
GA suppressed[c]	+++	−	+++	+
ABA induced p27	+	−	+++	+

[a]See text for details; [b]ND: not determined; [c]Unidentified cDNA clones.

layers of endosperm. After the onset of germination the aleurone layers respond to the hormone gibberellin (GA) from the embryo by synthesizing and secreting several hydrolytic enzymes, including α-amylases (Filner and Varner, 1967), proteases (Jacobsen and Varner, 1967), 1,3;1,4-β-glucanase (Stuart *et al.*, 1986), xylanase (Dashek and Chrispeels, 1977) and nuclease (Brown and Ho, 1987), to the endosperm where these enzymes hydrolyze the stored starch, proteins, cell wall polysaccharides, and remnant DNA and RNA. Another hormone, abscisic acid (ABA), which induces seed dormancy, prevents all the known GA effects in this tissue. The aleurone layers have been considered as a conve-

easily than from the intact cells (Jacobsen and Beach, 1985). Fifth, the antagonism between GA and ABA provides a new dimension to investigate in the role of hormone interactions on plant development. Lastly, genetic mutants with altered sensitivities to these hormones are available (Ho *et al.*, 1980; Lanahan and Ho, 1988). These mutants should be valuable to complement the molecular studies of hormone action.

Effect of Gibberellins on Gene Expression

Both GA and ABA alter the expression of several sets of genes in barley aleurone layers. As

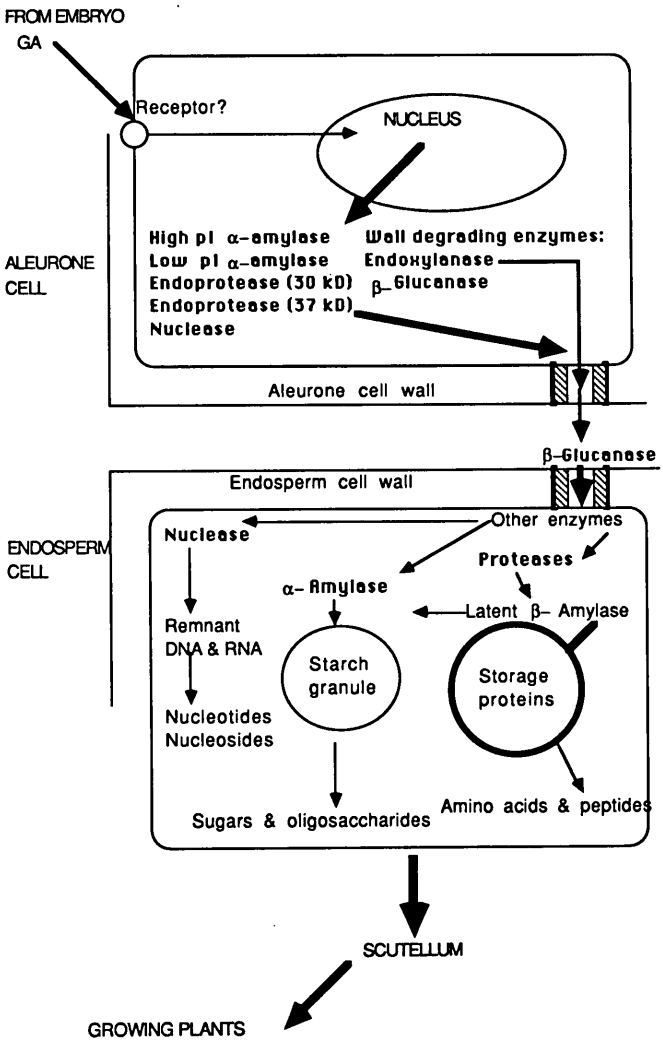

Fig. 1. A diagram showing the effect of GA on the induction of various hydrolytic enzymes in barley aleurone layers and the physiological role of these enzymes in barley endosperm cells.

-amylases together with the preexisting β-amylase hydrolyzes starch granules, proteases hydrolyze storage proteins as well as activating the latent β-amylase that is associated with the storage protein bodies, and nuclease hydrolyzes the remnant nucleic acids (Fig. 1). The processed nutrients are taken up by the scutellum and eventually transported to the growing plants.

Complexity of α-Amylases and their Genes

Because of the abundance of α-amylase the effect of hormones on its synthesis has been most extensively studied. In most of the cereal grains such as barley and rice, α-amylase is composed of two sets of isozymes (high and low pI species) with very similar size (44 kD) but different net charges (Jacobsen and Higgins, 1982; Callis and Ho, 1983). These isozymes can be classified into two groups based on their apparent pI: the high and low pI α-amylases. Using chromosome addition lines Brown and Jacobsen (1982) and Muthukrishnan *et al.* (1983) have demonstrated that the high pI and low pI isozymes are encoded by two sets of structural genes on chromosomes 6 and 1, respectively. Several α-amylase genomic clones from several cereal grains have been isolated and characterized by restriction mapping and sequence analysis (Whittier *et al.*, 1987; Knox *et al.*, 1987; Huang *et al.*, 1990). Sequence comparisons of the promoter regions have revealed interesting conserved sequences (Huang *et al.*, 1990), some of which have later been shown to be important for the expression of α-amylase (see below).

Expression of α-Amylase Genes

The expression of high pI α-amylase is not detectable before the addition of GA_3 to the aleurone layers, yet the low pI α-amylase is expressed at low level in the same tissue. After the onset of hormone treatment the expression of both groups of α-amylase is enhanced within 2 h (Fig. 2). The GA_3-enhanced expression of high pI α-amylase reaches a maximum after about 20 h and then declines (Fig. 2). Very little of this isozyme is still synthesized beyond 30 h after GA_3 treat-

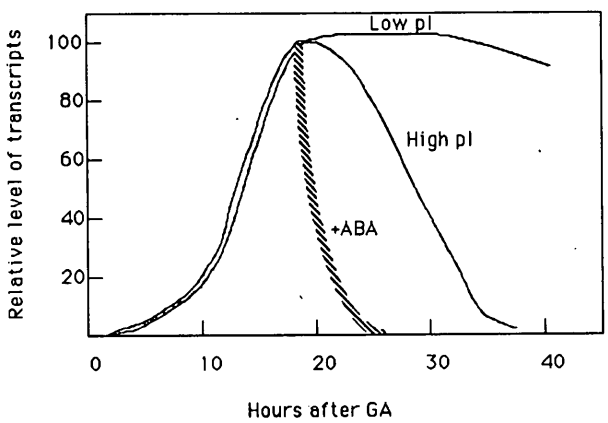

Fig. 2. The timecourse of the static level of α-amylase mRNAs in GA or GA/ABA treated barley aleurone layers. ABA was added 16 h after GA treatment. Data taken from Nolan *et al.* (1987) and Nolan and Ho (1988).

ment. In contrast, the synthesis of low pI α-amylase continues to about 40 h after GA_3 treatment. This differential expression of α-amylase isozymes in GA_3 treated barley aleurone layers can be observed at the protein level by analyzing newly synthesized proteins with native-gel electrophoresis (Nolan *et al.*, 1987). Similar results have been obtained at the RNA level by Northern-gel analysis probed with cDNA specific for the two groups of isozymes (Huang *et al.*, 1984; Chandler *et al.*, 1984; Rogers, 1985; Nolan and Ho, 1988). Since the results of protein analysis match well with those of RNA analysis, it indicates that the regulation of GA_3 induction of α-amylase isozymes is mainly at the level of their mRNA. Jacobsen and Beach (1985) have performed *in vitro* run-on transcription with nuclei isolated from GA_3-treated barley aleurone cells, and showed that GA_3 enhances the rate of transcription of α-amylase genes by about 10-fold, and ABA treatment of aleurone layers reverses this GA_3 effect. More recently, the promoter region of α-amylase genes has been analyzed by introducing α-amylase promoter -reporter (GUS) constructs into barley seeds via particle-bombardment techniques. The expression of the GUS gene in the presence or absence of GA is analyzed so that the degree of GA induction cannot be evaluated. By deleting or modifying α-amylase promoter sequences, one can eventually deduce the regions of the promoter that are essential for GA-mediated α -amylase expression. Using this approach, Lanahan *et al.* (1992) have revealed a GA-response complex (summerized in Fig. 3) which consists of at least four conserved sequences probably working together to achieve the highest level of hormone response. In order to study the protein factors which may interact with the α -amylase promoter sequences, nuclear proteins have been isolated and tested for their ability to bind to α

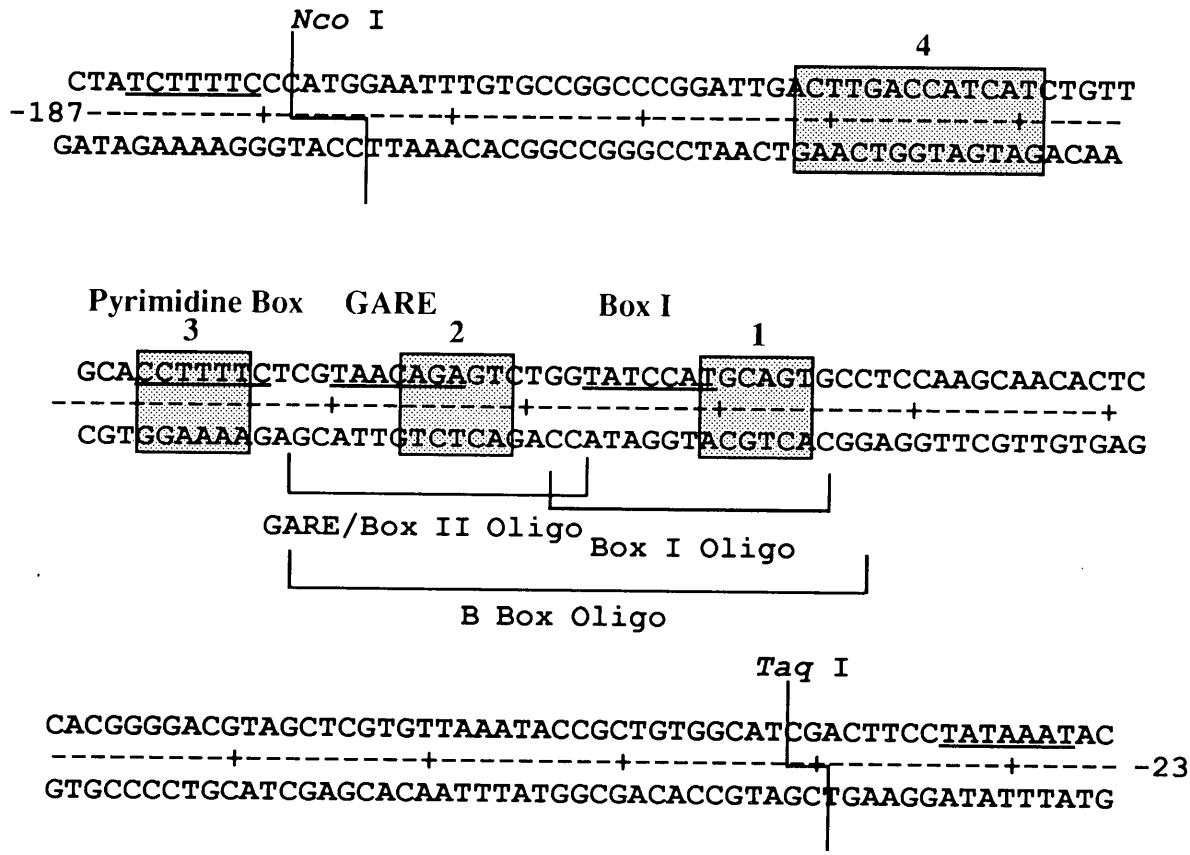

Fig. 3. Barley Amy32b promoter sequences with the important features. The sequence from -187 to -23 of Amy32b is shown. Conserved sequence elements are underlined. Sequences which have been shown to be functionally significant in GA induction by Lanahan *et al.* (1992) are shown in shaded boxes. The extent of sequences found in cloned synthetic promoter fragments are indicated with brackets. These promoter sequences are used in the experiments shown in Fig. 4 and 5.

-amylase promoter fragments. Nuclear proteins are mixed with labeled promoter fragments, and the bound and free DNA fragments are separated by nondenaturing-gel eletrophoresis. To pinpoint the binding site, one can simply treat the DNA with low level of DNase I and determine which part of the DNA fragment is protected against DNase digestion by the binding protein. As shown in Fig. 4, there are three regions which are protected after interacting with the nuclear proteinx. These regions correspond to three of the four elments in the GA-response complex. These DNA-protein interactions appear to be sequence specific; DNA fragments with no sequence homology to the DA -response elements fail to bind to the nuclear proteins. It appears that more than one nuclear protein can interact with the α-amylase promoter, and thus the nuclear proteins can be fractionated to separate the individual DNA-binding proteins. Using a MonoQ column on FPLC, Sutliff *et al.* (1933) have been able to detect two typs of DNA-binding activities, a GA-inducible one and another one which is present in aleurone tissue regardless of whether it has been treated with or without GA (Fig. 5). Using footprinting analysis, Sutliff *et al.* (1933) have shown that the GA-dependent activity binds specifically to one of the four elements in the GA -response complex, which has been named the GA -response element. However, the GA-independent factor appears to be less sequence specific, indicating it may interact with a wide variety of sequences (Fig. 6). How GA activates or induces the expression of the GA -dependent factor remains open. It is also unclear whether the *in vitro* binding data represent what happens in the intact cells.

Regulation of α-amylase synthesis at levels other than transcription also exists in barley aleurone layers. For example, α-amylase mRNA appear to be quite stable. It has been shown that transcription inhibitors such as cordycepin (3'-deoxyadenosine) are very effective in inhibiting the GA_3 induced α-amylase synthesis (Ho and Varner, 1974). However, this inhibitor fails to prevent the continued synthesis of α-amylases when it is added 12 h or more after GA_3 administration, indicating that the α-amylase mRNA are synthesized during the first 12 h of hormone treatment and that the turnover rate of this mRNA is very low (Ho and Varner, 1974). The half-life of this mRNA has been estimated to be longer than 100 h (Ho *et al.*, 1987). However, the stability of α-amylase mRNA does not appear to be an

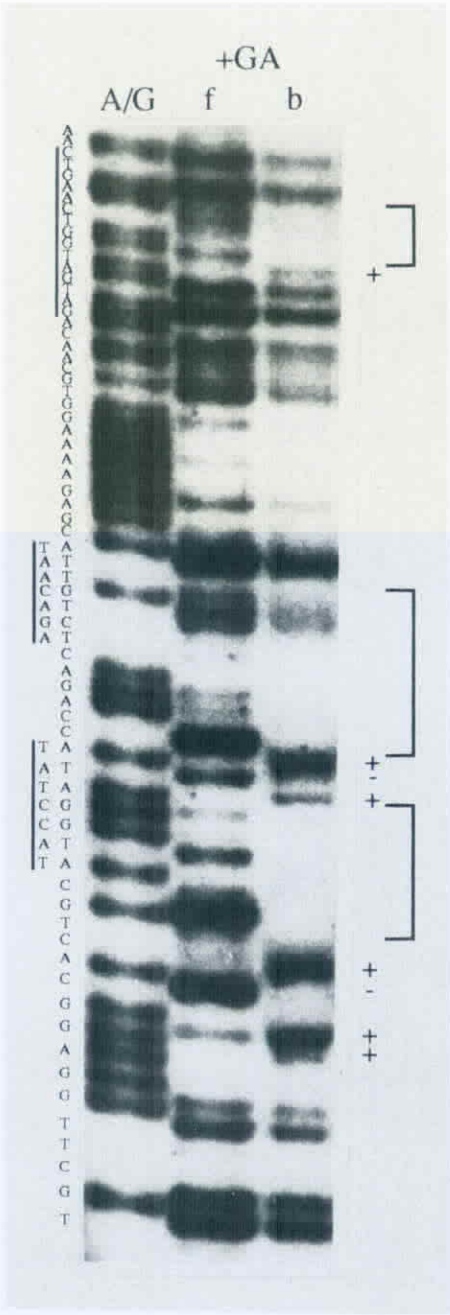

Fig. 4. DNase I footprinting analysis with unfractionated nuclear extract demonstrating the complex nature of DNA/protein interactions. Free and bound indicate DNA isolated from band shift gel following DNase I treatment. A/G is the product of a purine specific sequencing reaction using Amy32b promoter fragment end -labeled on the bottom strand. The sequences highlighted with vertical bars are the major cis-acting elements. The bracketed areas on the right are the major footprints and the "+" denotes new DNase I hypersensitive sites.

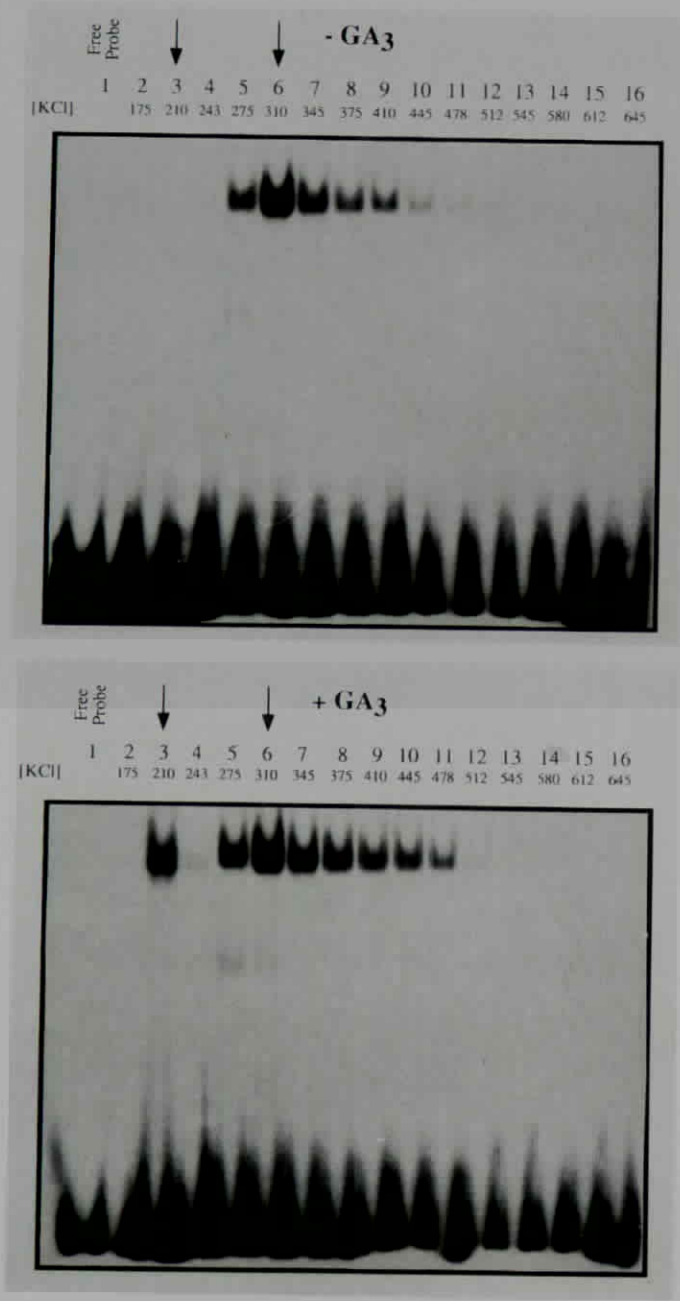

Fig. 5. Band shift assays of FPLC fractions showing the presence of GA-dependent DNA binding activities. Nuclear proteins prepared from aleurone layers incubated in the presence or absence of 2×10^{-6} M GA_3 were fractionated as described in the Methods, and analyzed in mobility shift assays using the B Box probe illustrated in Fig. 3. The signal at the bottom of the gel represents unbound probe. The KCl concentrations in FPLC fractions are in mM. The arrows point to the peak fractions of the GA-dependent (at 210 mM of KCl) and independent (at 310 mM KCl) DNA binding activities.

intrinsic property of this message. Investigating the effect of heat shock in barley aleurone layers, Belanger *et al.* (1986) showed that heat shock treatment (40°C) of barley aleurone layers not only induces the heat shock proteins but also effectively diminishes the synthesis of GA_3 induced α-amylases. Analyzing the levels of α-amylase mRNA by Northern blots probed with specific cDNA, it was observed that the normally stable α-amylase mRNA in heat shocked barley aleurone layers are actively degraded. The timing of the heat shock-induced α-amylase mRNA destruction is closely correlated with another heat stress-induced phenomenon, a fast delamellation of endoplasmic reticulum (ER). Since ER is the site for the synthesis and processing of secretory proteins, we speculate that the association of mRNA encoding secretory proteins with this organelle leads to the stabilization of these mRNA. That is, once ER is altered by heat shock, mRNA normally associated with ER might become unstable and degrade quickly. It is well documented that GA_3 also induces the formation of ER in barley aleurone layers (Jones, 1969; Evans and Varner, 1971), and ER appears to be essential for synthesis and processing of α-amylase (Jones and Jacobsen, 1982). Thus, besides inducing the transcription of α-amylase genes, GA_3 treatment may also stabilize α-amylase mRNA.

Early Events Induced by Gibberellins

Since it takes about 2-4 h of GA_3 treatment to detect the induction of α-amylase genes, it is crucial to investigate the early GA_3 induced events which lead to the later expression of α-amylase. It has been shown that the induction of α-amylase mRNA can be blocked if aleurone layers are treated with the protein synthesis inhibitor cycloheximide or the amino acid analog, aminoethylcysteine during the first 2 h of hormone treatment (Muthukrishnan *et al.*, 1983). This observation indicates that the expression of a (group of) gene(s) during the early hours of GA_3 treatment is necessary for the later expression of α-amylase. To date, this type of early gene has not been further characterized.

Another approach to studying the early events induced by GA_3 is to remove the hormone after a few hours of administration. Chrispeels and Varner (1967) have concluded that GA_3 is continuously required for the synthesis of α-amylase. However, it has been recently observed that GA_3 is only required for a period of time in order to stimulate the continuous synthesis of α-amylase (Lu and Ho, unpublished). When GA_3 is

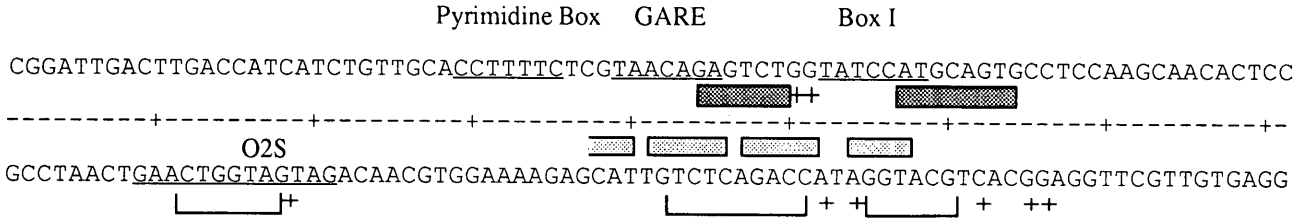

Fig. 6. DNase I footprinting summary showing the difference between the GA-dependent and independent DNA binding activities. Conserved sequence elements are underlined. Sequences footprinted with the unfractionated nuclear extract are indicated by brackets. The dark boxes indicates the footprints produced with the 210 mM KCl fraction and "+" indicate the presence of DNase I hypersensitive sites. Note that the hypersensitive sites generated by the GA-dependent activity are different from those generated by the GA-independent activities.

removed after 4 h (or less) of treatment, aleurone layers produce less α-amylase than tissue that has been continuously treated with GA_3, indicating that early removal of the hormone stops the sequence of events that is triggered by the hormone. On the other hand, removal of the hormone after 8 h has no effect on the continuous synthesis of α-amylase. Thus, the system has somehow reached the "point of no return" after 8 h, and the synthesis of α-amylase is no longer dependent on the presence of hormone molecules. It is not known whether a second messenger is involved in this process. More research effort is certainly needed in characterizing the early changes induced by GA_3.

Effect of ABA on Gene Expression

Suppression of Gibberellin-induced Genes

Abscisic acid is very effective in inhibiting the synthesis of both high and low pI α-amylase isozymes when it is added at the same time as GA_3. However, it has a much more noticeable effect on the synthesis of high pI isozymes than that of low pI isozymes when it is added 20 h after GA_3 (Nolan *et al.*, 1987). Analyzing mRNA sequences on Northern gel blots, Nolan *et al.* (1987) have observed that the ABA inhibition of α-amylase synthesis correlates with the decrease in mRNA sequences for the individual groups of isozymes. Thus, the ABA inhibition on α-amylase synthesis appears to be regulated at the levels of mRNA rather than at the translational level as suggested before (Ho and Varner, 1976; Mozer, 1980). The data of Jacobsen and Beach (1985) also suggest that ABA inhibits the transcription rate of α-amylase genes. Therefore, the major role of ABA in this system is to counter the action of GA_3 at the transcriptional level. These two hormones probably do not compete with each other for a common site of action because it has been shown that high concentrations of GA_3 can not totally alleviate the inhibition of ABA (Chrispeels and Varner, 1966).

Besides acting at the transcription level, ABA also appears to affect the stability of α-amylase mRNA. As mentioned previously, α-amylase mRNA appears to be quite stable after more than 12 h of GA_3 treatment and the mid-course addition of ABA alters the levels of α-amylase mRNA. A treatment with ABA apparently has a much stronger effect on the stability of high pI mRNA than on the low pI mRNA. Within 4 h of ABA treatment, the level of high pI mRNA is reduced to less than 10% of the control (Fig. 2).

Induction of New Proteins by Abscisic Acid

In the course of investigating the action of ABA on α-amylase mRNA stability, Nolan *et al.* (1987) have observed an interesting phenomenon, i.e. the action of ABA itself is dependent on the continuous synthesis of some RNA and proteins. Inhibition of RNA or protein synthesis by cordycepin or cycloheximide blocks the effects of ABA on the decrease of α-amylase mRNA sequences. One can not argue that the effects of these metabolic inhibitors are the consequence of induced cellular toxicity because the α-amylase mRNA sequences are even more abundant in the presence of these compounds. Thus, the action of ABA probably relies on the expression of another gene(s).

In order to study the gene(s) whose expression is crucial to the action of ABA in the inhibiton of α-amylase synthesis, we have analyzed newly synthesized proteins in ABA treated aleurone layers. There are nine ABA induced protein bands on a one-dimensional

gel, and the number increases to 16 when the samples are analyzed by two-dimensional gels (Lin and Ho, 1986). Treatment of aleurone layers also increases the levels of mRNA encoding the ABA-induced proteins, as determined by *in vitro* protein synthesis. ABA concentrations as low as 10^{-8} M are able to induce some of these proteins. The identities of thes ABA-induced proteins are being investigated. An ABA induced protein with a size of 36 kD can be precipitated with antiserum against a barley lectin specific for glucosamine, galactosamine and mannosamine (Patridge *et al.*, 1976; Lin and Ho, 1986). A 21 kD ABA-induced protein appears to be a specific inhibitor to low pI-amylase (Mundy, 1984; Lin, 1987). The most abundant among all of the ABA induced proteins is 29 kD in size and is soluble in 0.1 M HCl (pH 1)(Lin , 1987). The cDNA of a 22 kD ABA-induced protein has recently been cloned and analyzed (Hong *et al.*, 1988). The amino acid sequence from the DNA sequence indicates that this protein is rich in lysine and alanine, and that it contains nine imperfect repeats, each containing 11 amino acids (Fig. 7). Two-dimensional gel electrophoresis indicates that the pI of this protein is higher than 8.5. The expression of this protein can be induced by ABA with a concentration as low 10^{-9} M. The level of mRNA of this 22 kD proteins increases within 30 min of ABA treatment, and reaches a maximum at 8-12 h. Although the exact function of this 27 kD protein remains unknown, its amino acid sequences indicate that it may contain amphiphilic α-helical structures, i.e. hydrophobic amino acids are lined on one side, and hydrophilic amino acids are on another side of the helix. Similar ABA-induced proteins have been observed in several other plants, including cotton, carrot, wheat, and rice (Dure *et al.*, 1989). A few of the ABA induced proteins can be also induced by ABA in developing barley seeds. Although none of the ABA-induced proteins resemble the major barley storage proteins, hordeins, it has not been ruled out that some ABA-induced proteins are less abundant seed storage proteins.

```
 27  Met-Gly-Ala-Thr-Lys-Gln-Lys-Ala-Gly-Gln-Thr  37
 38  Thr-Glu-Ala-Thr-Lys-Gln-Lys-Ala-Gly-Glu-Thr  48
 49  Ala-Glu-Ala-Thr-Lys-Gln-Lys-Thr-Ala-Glu-Thr  59
 60  Ala-Glu-Ala-Ala-Lys-Gln-Lys-Ala-Ala-Glu-Thr  70
 78  Ala-Gln-Ala-Ala-Lys-Asp-Lys-Thr-Tyr-Glu-Thr  88
 89  Ala-Gln-Ala-Ala-Lys-Glu-Arg-Ala-Ala-Gln-Gly  99
111  Thr-Glu-Ala-Ala-Lys-Gln-Lys-Ala-Ala-Glu-Thr 121
122  Thr-Glu-Ala-Ala-Lys-Gln-Lys-Ala-Ala-Glu-Thr 132
133  Thr-Glu-Ala-Ala-Lys-Gln-Lys-Ala-Ser-Asp-Thr 143
```

Fig. 7. Sequences of repeats present in an ABA-induced protein. The numbers denotes the amino acid position at the begining and end of the repeats. Data from Hong *et al.* (1988).

Role of an ABA Metabolite, Phaseic Acid

It has been known for some time that ABA can be metabolized quickly in barley aleurone layers. Besides glucose conjugates of ABA, two metabolites have been identified (Dashek *et al.*, 1979). The first stable metabolite of ABA is phaseic acid (PA) which is very effective in the inhibition of α-amylase synthesis, yet it has little effect on the induction of ABA inducible proteins (Dashek *et al.*, 1979; Ho *et al.*, 1985). Like ABA, when PA is added 12-16 h after GA_3, the synthesis of α-amylase is inhibited. Since the most significant effect of ABA at this stage is to destabilize α-amylase mRNA, PA probably also effects mRNA stability. It has been shown by Uknes and Ho (1986) that the *in vivo* conversion of ABA to PA in barley aleurone layers is enhanced by pretreating the tissue with ABA. Thus, it seems that ABA is capable of enhancing its own metabolism to PA similar to the case of substrate induction of nitrate reductase by nitrate that has been well documented in many plant tissues. On the other hand, the conversion of PA to the next metabolite, dihydrophaseic acid, is not affected by ABA or by PA. Isolated dihydrophaseic acid has little or no effect on the GA_3 -induced synthesis of α-amylase. The ABA enhancement on its own metabolism is unlikely to be a scavenging mechanism that removes excessive amounts of ABA because PA is also biologically effective. An effective scavenging mechanism would have to enhance the metabolism of ABA to dihydrophaseic acid or later metabolites to rid of the biological effect due to ABA.

Mode of Action of ABA

It is apparent that ABA can nullify the effect of GA_3 on the induction of α - amylase. At the transcriptional level, ABA could be involved in the suppression of transcription of α-amylase genes. Since both α-amylase and its mRNA appear to be quite stable, simply preventing the transcription of α-amylase genes can not totally reverse the effect of GA_3. Preexisting α - amylase and its mRNA, which are formed before ABA is added (or synthesized, see discussion later), would continue to function. Therefore, ABA has to exert its regulation at other levels. The effect of ABA on the stability of α-amylase mRNA is probably

mediated by its metabolite, PA, the formation of which is enhanced by ABA. It is also possible that both ABA and PA are active in barley aleurone layers. It is conceivable that ABA induces the monoxygenase responsible for the conversion of ABA to PA. However, evidence supporting this notion is still lacking. To remove the activity of preexisting α-amylase, ABA induces the 21 kD inhibitor which is specific for barley α-amylase. Although cereal grains contains many amylase and protease inhibitors, almost all of them are a means of self-defense against animal enzymes. The ABA-induced 21 kD inhibitor is the only one against the endogenous α-amylase, indicating a role in regulating the physiology of developing and/or germinating seeds. By working at all three levels, i.e. inhibition of transcription of α-amylase genes, destabilizing α-amylase mRNA, and inhibition of α-amylase activity, ABA can effectively perform its physiological role to stop the action of gibberellins.

Stress-induced Synthesis of ABA

Abscisic acid appears to play an essential role in regulating seed development and preventing premature seed germination. The level of ABA has to decrease after seed maturation to allow successful seed germination. Since the research concerning the action of ABA described in this account deals with a tissue in germinating seeds, the aleurone layers, it is crucial to investigate whether the levels of ABA would ever increase again in germinating seeds. It has been reported that water stress can enhance the synthesis of ABA in leaf tissues. Thus, we have also investigated whether aleurone layers under stress would also have elevated levels of ABA. Water or salt stress induces the same group of proteins in barley aleurone layers as ABA does (Lin, 1987). The stress induction of these proteins can be effectively blocked by the ABA biosynthesis inhibitor, fluridone (Lin, 1987). This observation suggests that the stress induction of new proteins is most likely via the elevated synthesis of ABA in the stressed tissue. Furthermore, we have found that water stress (treatment 0.6 M sorbitol) causes a five-fold increase in the level of ABA (from 2.6 to 12 nM), and this increase in ABA level can be effectively blocked by fluridone. Therefore, it is conceivable that after the onset of seed germination, the levels of ABA can increase when a germinating seed encounters stressful conditions. The stress induced ABA will then in turn slow down the post-germination growth of the seedling by down-regulating the production and activity of α-amylase.

Perspective

Plants, like all other organisms, are constantly monitoring their external and internal environment, and making necessary adjustments in response to alterations in the environment. Hormones are probably the most important signals integrating the function of various parts of plants. The physiological role of hormonal regulation in the aleurone layers of cereal grains is well established. Many of the hormone regulated enzymes (and proteins) have been purified and characterized, and they have been used as biochemical markers in the investigation of hormonal action. In recent years, molecular biology techniques have been successfully applied to isolate and characterize hormone responsive genes. With deletion and transformation studies, the putative GA_3 and ABA responsive promotor regions have been defined. Besides the cis-acting sequences, trans-acting elements, such as regulatory proteins bound to the promotor sequences, have also be studied by *in vitro* DNA binding. With this type of approach, one hopes to eventually trace back to the early events that are induced by hormone treatments. The primary action of hormones in aleurone layers is probably the interaction between the hormone molecules and some kind of receptor molecules. Unfortunately, the search for hormone receptors in this system has not been very successful. Recently, new techniques, such as hormone affinity chromatography (Lobler and Klamdt, 1986), photoaffinity labeling (Hornberg and Weiler, 1984), immunochemistry with monoclonal antibodies (Jacobs and Gilbert, 1983), and antiidiotypic antibodies (R. Hooley, Personal Communication) have been applied in several plant systems to study the hormone receptors. Hopefully, these techniques will reveal new insights into the primary action of GA_3 and ABA in aleurone layers.

It is generally accepted that hormone treatments trigger a sequence of events leading to the production of hydrolytic enzymes in aleurone layers. From the biochemical and molecular data, it is possible to postulate this sequence of events. However, the causal relationship among the individual steps can not be firmly established unless genetic analysis is employed. Mutants with altered sensitivity to GA and ABA have been iso-

lated and characterized. For example, a viviparous maize that responds to ABA, and several GA insensitive mutants have been reported (Robichaud *et al.*, 1980; Ho *et al.*, 1980). A more interesting one is the slender barley which functions as if the hormone concentrations are always over-saturating. The aleurone layers of slender mutants have a constitutive synthesis of α-amylase (even in the absence of GA)(Lanahan and Ho, 1988). These mutants should be used to supplement molecular and biochemical studies to further our knowledge of the action of hormones.

Acknowledgements. The research in the author's lab has been supported by grants from the National Science Foundation, the United State Department of Agriculture, and the Anheuser Busch Company.

Literature Cited

Belanger, F. C., M. R. Brodl, and T. H. D. Ho. 1986. Heat shock causes destabilization of specific mRNAs and destruction of endoplasmic reticulum in barley aleurone cells. Proc. Natl. Acad. Sci. **83:** 1354-1358.

Benjavongkulchai, E. and M. S. Spencer. 1986. Purification and characterization of barley aleurone xylanase. Planta **169:** 219-277.

Brodl, M. R. 1987. Effect of heat shock on the synthesis and secretion of plant proteins. Ph.D. thesis, Washington University, St. Louis, MO.

Brown, A. H. D. and J. V. Jacobsen. 1983. Genetic basis and natural variation of α-amylase isozymes in barley. Genet Res. **40:** 315-324.

Brown, P. H. and T-H. D. Ho. 1986. Barley aleurone layers secrete a nuclease in response to gibberellic acid. Purification and partial characterization of the associated ribonuduced synthesis and release of cell-wall degrading endoxylanase by isolated aleurone layers of barley. Planta **134:** 251-256.

Dashek, W. V., B. N. Singh, and D. C. Walton. 1979. Abscisic acid localization and metabolism in barley aleurone layer. Plant Physiol. **42:** 43-48.

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

Evins, W. H. and J. H. Varner. 1971. Hormone-controlled synthesis of endoplasmic reticulum in barley aleurone cells. Proc. Natl. Acad. Sci. USA **68:** 1631-1633.

Filner, P. and J. E. Varner. 1967. A test of *de novo* synthesis of enzymes: density labelling with $H_2{}^{18}O$ of barley α-amylase induced by gibberellic acid. Proc. Natl. Acad. Sci. USA **58:** 1520-1526.

Fincher, G. B., P. A. Lock, M. M. Morgan, K. Lingelbach, R. E. H. Wettenhall, J. F. B. Mercer, A. Brandt, and K. K. Thomsen. 1986. Primary structure of the (1-3:1-4)-β-glucanohydrolase from barley aleurone. Proc. Natl. Acad. Sci. USA **83:** 2081-2085.

Hanson, A. D. and J. V. Jacobsen. 1984. Control of lactate dehydrogenase, lactate glycolysis, and α-amylase by O_2 deficit in barley aleurone layers. Plant Physiol. **75:** 566-572.

Hammerton, R. W. and T. H. D. Ho. 1986. Hormonal regulation of the development of protease and carboxypeptidase activities in barley aleurone layers. Plant Physiol. **80:** 692-697.

Ho, D. T-H. and J. E. Varner. 1974. Hormonal control of messenger ribonucleic acid metabolism in barley aleurone layers. Proc. Natl. Acad. Sci. USA **71:** 4783-4786.

Ho, D. T-H. and J. E. Varner. 1976. Response of barley aleurone layers to abscisic acid. Plant Physiol. **57:** 175-178.

Ho, T. H. D., S-C. Shih, and A. Kleinhofs. 1980. Screening for barley mutants with altered hormone sensitivities in their aleurone layers. Plant Physiol. **66:** 153-157.

220: 1297-1300.

Jacobsen, J. V. and J. E. Varner. 1967. Gibberellic acid-induced synthesis of protease by isolated aleurone layers of barley. Plant Physiol. **42:** 1596-1600.

Jacobsen, J. V. and T. J. V. Higgins. 1982. Characterization of the α-amylase synthesized by aleurone layers of Himalaya barley in response to GA_3. Plant Physiol. **70:** 1647-1653.

Jacobsen, J. V. and L. R. Beach. 1985. Control of transcription of α-amylase and rRNA genes in barley aleurone layer protoplasts by gibberellic acid and abscisic acid. Nature **316:** 275-277.

Jacobsen, J. V., J. A. Zwar, and P. M. Chandler. 1985. Gibberellic acid-responsive protoplasts from mature aleurone of Himalaya Barley. Planta **163:** 430-438.

Jones, R. L. 1969. Gibberellic acid and the fine structure of barley aleurone cells: I. Changes during the lag period of α-amylase synthesis. Planta **87:** 119-133.

Jones, R. L. and J. V. Jacobsen. 1982. The role of the endoplasmic reticulum in the synthesis and transport of α-amylase in barley aleurone layers. Planta **156:** 421-432.

Knox, C. A., B. Sonthayanon, R. C. Chandra, and S. Muthukrishnan. 1987. Structure and organization of two divergent α-amylase genes from barley. Plant Mol. Biol. **9:** 3-17.

Lanahan, M. B. and T. H. D. Ho. 1988. Slender barley: A constitutive gibberellin-respones mutant. Planta **175:** 107-114.

Lin, L. S. and T. H. D. Ho. 1986. Mode of action of abscisic acid in barley aleurone layers: Induction of new proteins by abscisic acid. Plant Physiol. **82:** 289-297.

Lin, L. S. and T. H. D. Ho. 1987. Salt and water stress induced proteins in barley aleurone layers. Plant Physiol. **83S:** 48.

Lobler, M. and D. Klamdt. 1986. Auxin-binding protein from coleoptile membranes of corn (*Zea mays* L.) 1. purification by immunological methods and characterization J. Biol. Chem. **260:** 9848-9853.

Landahan, M. B., T. H. D. Ho, S. Rogers, and J. C. Rogers. 1992. A gibberellin response complex in cereal α-amylase gene promoters. Plant Cell **4:** 203-211.

Mozer, T. J. 1980. Control of protein synthesis in barley aleurone layers by the plant hormones gibberellic acid and abscisic acid. Cell **20:** 479-485.

Mundy, J. 1984. Hormonal regulation of α-amylase inhibitor synthesis in germinating barley. Carsberg Res. Comm. **49:** 439-444.

Muthukrishnan, S., G. R. Chandra, and G. P. Albaugh. 1983. Modulation by abscisic acid and S-2-aminoethyl-L-cysteine of α-amylase mRNA in barley aleurone cells. Plant Mol. Biol. **2:** 249-258.

Muthukrishnan, S., B. S. Gill, M. Swegle, and G. R. Chandra. 1983. Structural genes or α-amylase are located on barley chromosomes 1 and 6. J. Biol. Chem. **259:** 13637-13639.

Nolan, R. C., L. S. Lin, and T. H. D. Ho. 1987. The effect of abscisic acid on the differential expression of α-amylase isozymes in barley aleurone layers. Plant Mol. Biol. **8:** 13-22.

Nolan, R. C. and T. H. D. Ho. 1988. Hormonal regulation of gene expression in barley aleurone layers: induction and suppression of specific genes. Planta **174:** 551-560.

Patridge, J., L. Shannon, and D. Grumpf. 1976. A barley lectin that binds free amino sugars. I. Purification and characterization. Biochim Biophys. Acta **451:** 470-483.

Robichaud, C. S., J. Wong, and I. M. Sussex. 1980. Control of *in vitro* growth of viviparous embryo mutants of maize by abscisic acid. Dev. Genet. **1:** 325-330.

Rogers, J. C. 1985. Two barley α-amylase gene families are regulated differently in aleurone cells. J. Biol. Chem. **260:** 3731-3738.

Rogers, J. C., D. Dean, and G. R. Heck. 1985. Aleurain: A barley thiol protease closely related to mammalian cathepsin H. Proc. Natl. Acad. Sci. USA **82:** 6512-6516.

Storti, R. V., M. P. Scott, A. Rich, and M. L. Pardue. 1980. Translational control of protein synthesis in response to heat shock in *D. melanogaster* cells. Cell **22:** 825-834.

Stuart, I. M., M. L. Loi, and G. B. Fincher. 1986. Development of (1-3,1-4)-β-D-glucan endohydrolase in isolated scutella and aleurone layers of barley (*Hordeum vulgare*). Plant Physiol. **80:** 310-314.

Sutliff, T. D., M. B. Lanahan, A. Anschutz, and T. H. D. Ho. 1993. Gibberellin treatment stimulates nuclear factor binding to the GA response element in a barley α-amylase promoter. Plant Cell, submitted.

Uknes, S. J. and T. H. D. Ho. 1984. Mode of action of abscisic acid in barley aleurone layers: abscisic acid induces its own conversion to phaseic acid. Plant Physiol. **75:** 1126-1132.

Walton, D. C. 1980. Biochemistry and physiology of abscisic acid. Ann. Rev. Plant Physiol. **31:** 453-489.

Whittier, R. F., D. A. Dean, and J. C. Rogers. 1987. Nucleotide sequence analysis of alpha-amylase and thiol protease genes that are hormonally regulated in barley aleurone cells. Nucl. Acids Res. **15:** 2515-2535.