The Journal of the Iowa Academy of Science: JIAS

Volume 98 | Number Article 8

1991

Hormonal Regulation of Gene Expression m the Aleurone Layers of Cereal Grains

Tuan-Hua David Ho
Washington University

Jour. Iowa Acad. Sci. 98(2):72-76, 1991

Hormonal Regulation of Gene Expression in the Aleurone Layers of Cereal Grains

TUAN-HUA DAVID HO

Department of Biology, Washington University, St. Louis, Missouri 63130

The aleurone layers of cereal grains offer a convenient system for studies of the molecular action of two plant hormones, gibberellins (GA) and abscisic acid (ABA). Gibberellins induce the synthesis of α-amylase and several other hydrolytice enzymes. The action of GA is essentially at the transcriptional level, it enhances the level of steady-state levels of α-amylase mRNAs, the rate of transcription of α-amylase genes and the activity of a trans-acting factor which interacts with specific regions of α-amylase genes. Abscisic acid antagonizes the effect of GA by inhibiting the induction of hydrolytic enzymes, yet the effect of ABA itself is dependent on transcriptional and/or translational process. Abscisic acid inhibits the transcription of α-amylase genes, destabilizes α-amylase mRNA and reduces α-amylase activities. Several ABA induced proteins have been studied including an α-amylase inhibitor, a lectin and a basic protein with long repeats. These proteins can also be induced by drought stress, apparently due to the drought-enhanced accumulation of ABA.
INDEX DESCRIPTORS: gibberellin, abscisic acid, α-amylase, barley aleurone layers, *Hordeum vulgare*

I. THE CEREAL ALEURONE LAYERS

The aleurone layers of cereal grains play a crucial role in the mobilization of endosperm nutrients to support the post-germination seedling growth. This tissue is usually one to three cell layers thick and lies directly underneath the seed coat and forms the outermost layers of endosperm. After the onset of germination the aleurone layers respond to the hormone, gibberellins (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, 1986), 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 convenient system to study the mode of action of GA and ABA because of the following advantages: First, in the one cell-thick wheat aleurone layers, the tissue is basically composed of a homogeneous cell population capable of responding to the two hormones. Second, at least for GA, the source (embryo) and the target tissue (aleurone layers) of the hormone can be physically separated by dissecting the dry seeds, thus the target tissue can be treated with known concentrations of exogenously applied hormones. Third, many enzymes and proteins are available which can serve as convenient biochemical markers for the studies of hormone actions (Table 1). Fourth, protoplasts of aleurone cells that still respond to GA can be prepared (Jacobsen et al., 1985). The protoplasts are ideal to study the effect of hormones on secretion. In addition, organelles such as nuclei can be isolated from protoplasts easier than from the intact cells (Jacobsen and Beach, 1985). Fifth, the antagonism between GA and ABA provides a new dimension to investigate 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 on hormone action.

II. EFFECT OF GIBBERELLINS ON GENE EXPRESSION

Both GA and ABA alter the expression of several sets of genes in barley aleurone layers. As summarized in Table 1 and diagramed in Fig. 1, gibberellic acid (GA_3, one of the most active gibberellins in barley aleurone layers) not only induces α-amylase isozymes, two thioproteases, a nuclease and a cell wall-degrading enzyme but also suppresses the expression of several other genes. In contrast, ABA represses the expression of the aforementioned hydrolytic enzymes, yet it induces another set of new proteins (see detailed discussion later).

Table 1. Hormonal Regulation of Gene Expression in Barley Aleurone Layers.

	Hormone Treatment			
Gene	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.

The physiological roles of GA-induced hydrolytic enzymes are well understood. The cell wall-degrading enzymes are responsible for partial degradation of walls of aleurone and endosperm cells, respectively (Fig. 1). It is thought that the passageway created by the cell wall-degrading enzymes facilitates the transport of other hydrolytic enzymes from the site of synthesis, i.e. the aleurone cells, to the site of action, i.e. the endosperm cells. In the endosperm cells, α-amylases together with the preexisting β-amylase hydrolyze 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.

A. Complexity of α-amylase 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 adn low pI species) with very similar size (44 kDa) yet they differ in 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.,

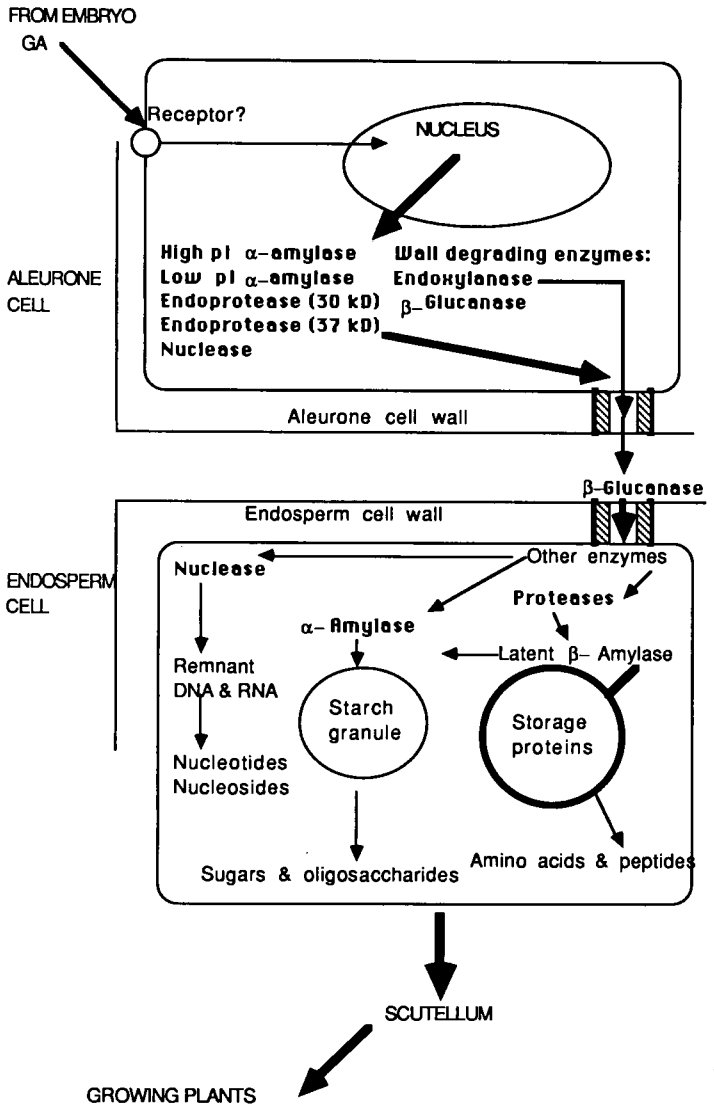

Fig. 1. A diagram showing the effect of GA on the induction of various hydrolytic enzymes in barley aleurone layers and the physiological role of these enzymes in barley endosperm cells.

(1983) have demonstrated that the high pI and low pI isozymes are encoded by a (set of) structural genes on chromosomes 6 and 1, respectively. Several α-amylase genomic clones have been isolated and characterized by restriction mapping and sequence analysis (Whittier et al., 1987; Knox et al., 1987). Four of five genomic clones contain high pI isozyme sequences and one contains the low pI isozyme sequence. The DNA sequences of the two types of α-amylase genes are divergent with the high pI gene containing two introns and the low pI gene containing three introns. Although the putative promotor regions of the two types of α-amylase genes show little sequence homology, both contains several repeats whose function is not yet clear.

B. Expression of α-amylase genes

The expression of high pI α-amylase is not detectable before the addition of GA_3 to 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 two hr (Fig. 2). The GA_3-enhanced expression of high pI α-amylase reaches a maximum around 20 hr and then declines afterwards (Fig. 2). Very little of this isozyme is still synthesized beyond 30 hr of GA_3 treatment. In contrast, the synthesis of low pI α-amylase continues to about 40 hr of 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 the mRNA. Jacobsen and Beach (1985) have performed *in vitro* run-on transcription with nuclei isolated from GA_3-treated barley aleurone cells and shown 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, Ou-Lee et al., (1988) have investigated the factors which interacts with the promoter regions of α-amylase genes in rice aleurone layers. Using the DNA retardation technique (band-shift) they have been able to observe the presence of a trans-acting factor (most likely a protein) whose DNA-binding activity is enhanced in GA-treated tissue. This factor appears to recognize specific sequences in the α-amylase promoter which are protected from nuclease digestion due to its association with the factor. In the nuclease protected region, there is a direct repeat that overlaps with a potential stem-loop structure. Sequences with similar features are present in the promoter region of barley α-amylase genes. Conceivably, through the interactions of its promoter sequence with this factor α-amylase gene transcription is activated.

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) is 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-amylase when it is added 12 hr or later after GA_3 adiminstration indicating that the α-amylase mRNA are synthesized during the first 12 hr of hormone treatment and the turnover rate of this mRNA is very slow (Ho and Varner, 1974). The half-life of these mRNA has been estimated to be longer than 100 hr (Ho et al, 1987). However, the stability of α-amylase mRNA does not appear to be an intrinsic property of this message. Investigating the effect of heat shock in barley aleurone layers, Belanger et al. (1986) showed that heat shock treat (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

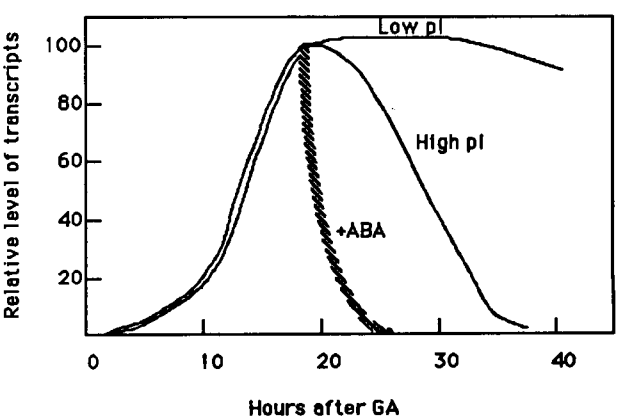

Fig. 2. The timecourse of the steady-state level of α-amylase mRNAs in GA or GA/ABA treated barley aleurone layers. ABA was added 16 hrs after GA treatment. Data taken from Nolan et al., (1987) and Nolan and Ho (1988).

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) (Brodl, 1987). 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 degraded 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-amy-lase genes, GA_3 treatment may also stabilize α-amylase mRNA.

C. Early events induced by gibberellins

Since it takes about 2-4 hr 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 hr 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 genes have not been further characterized.

Another approach to study 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 removed after 4 hr (or shorter) of treatment, aleurone layers produce less α-amylase compared to 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 hormone after 8 hr has no effect on the continuous synthesis of α-amylase. Thus, the system has somehow reached the "point of no return" after 8 hr, 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.

III. EFFECT OF ABA ON GENE EXPRESSION

A. 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 the low pI isozymes when it is added 20 hr longer 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 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 appear to be quite stable after more than 12 hr 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 hr of ABA treatment, the level of high pI mRNA is reduced to less than 10% of the control (Fig. 2).

B. 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 cannot 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 is probably relied 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 inhibition of α-amylase synthesis, we have analyzed newly synthesized proteins in ABA treated aleurone layers. There are nine ABA induced protein bands on an 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 these 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 which is soluble in 0.1M HCl (pH 1) (Lin, 1987). The cDNA of a 27 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 it contains nine imperfect repeats, each contains 11 amino acids (Fig. 3). 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 as 10^{-9}M. The level of mRNA of this 27 kD proteins increases within 30 min of ABA treatment, and reaches a maximum around 8-12 hr. 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-

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

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 also be 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.

C. 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 ABA inducible proteins (Dashek et al., 1979; Ho et al., 1985). Like ABA, when PA is added 12-16 hr 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, the effect of PA is probably also on mRNA stabilty. It has been shown by Uknes and Ho (1984) 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 nor 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 to remove excessive amount 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.

D. 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 cannot totally reverse the effect of GA_3. Preexiting α-amylase and it smRNA, 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, whose formation 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 monooxygenase responsible for the conversion from 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 contain many amylase and protease inhibitors, almost all of them are against animal enzymes as a means of self-defense. The ABA-induce 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.

E. Stress-induced synthesis of ABA

Abscisic acid appears to play an essential role in regulating seed development and prevent 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 0.6M sorbitol) indeed 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.

IV. PERSPECTIVE

Plants, like all other organisms, are constantly monitoring their external and internal environment, and make 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 in 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 isolated and characterize hormone responsive genes. With mutagenesis and transformation studies, it is hoped that the putative GA_3 and ABA responsive promotor regions will be defined in the future. Besides the cis-acting sequences, trans-acting elements, such as regulatory proteins bound to the promotor sequences, can also be studied *in vitro* DNA bindings as demonstrated by Ou-Lee et al., (1988). 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 hormone 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 in the past. 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, this type of techniques would 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 cannot be firmly established unless genetic analysis is employed. Mutants with altered sensitivity to GA and ABA have been isolated and characterized. For example, a viviparous maize fails to respond 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 mutant 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 on the action of hormones.

ACKNOWLEDGEMENT

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

REFERENCES

BELANGER, F.C., M.R. BRODL, 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.

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., J.V. JACOBSEN. 1982. Genetic basis and natural variation of α-amylase isozymes in barley. Genet Res. 40:315-324.

BROWN, P.H., T-H.D. HO. 1986. Barley aleurone layers secrete a nuclease in response to gibberellic acid. Purification and partial characterization of the associated ribonuclease, deoxyribonuclease, and 3′-nucleotidase activities. Plant Physiol. 82:801-806.

CALLIS, J., T.H.D. HO. 1983. Multiple molecular forms of the gibberellin-induced α-amylase from the aleurone layers of barley seeds. Arch. Biochem. Biophys. 224:224-234.

CHANDLER, P.M., J.A. ZWAR, J.V. JACOBSEN, T.J.V. HIGGINS, A.S. INGLIS. 1984. The effects of gibberellic acid and abscisic acid on α-amylase mRNA levels in barley aleurone layers using an α-amylase cDNA clone. Plant Mol. Biol. 3:407-418.

CHRISPEELS, M.J., J.E. VARNER. 1966. Inhibition of gibberellic acid induced formation of α-amylase by abscisin II Nature 212:1066-1067.

CHRISPEELS, M.J., J.E. VARNER. 1967. Gibberellic acid-enhanced synthesis and release of α-amylase and ribonuclease by isolated aleurone layers. Plant Physiol 42:398-406.

DASHEK, W.V., M.J. CHRISPEELS. 1977. Gibberellic acid-induced synthesis and release of cell-wall degrading endoxylanase by isolated aleurone layers of barley. Planta 134:251-256.

DASHEK, W.V., B.N. SINGH, D.C. WALTON. 1979. Abscisic acid localization and metabolism in barley aleurone layers. Plant Physiol. 42:43-48.

DURE, L., M. CROUCH, J. HARADA, T-H.D. HO, J. MUNDY, R. QUATRANO, T. THOMAS, Z.R. SUNG. 1989. Common amino acid sequence domains among the LEA proteins of higher plants. Plant Mol. Biol. 12:475-486.

EVANS, W.H., J.H. VARNER. 1971. Hormone-controlled synthesis of endoplasmic reticulum in barley aleurone cells. Proc. Nat. Acad. Sci., USA 68:1631-1633.

FILNER, P., 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. Nat. Acad. Sci. USA 58:1520-1526.

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

HO, D.T-H., J.E. VARNER. 1976. Response of barley aleurone layers to abscisci acid. Plant Physiol. 57:175-178.

HO, T.H.D., S-C. SHIH, A. KLEINHOFS. 1980. Screening for barley mutants with altered hormone sensitivities in their aleurone layers. Plant Physiol. 66:153-157.

HO, T.H.D., R.C. NOLAN, S.J. UKNES. 1985. On the mode of action of abscisic acid in barley aleurone layers. Curr. Topics Plant Biochem. Physiol. 4:118-125.

HO, T.H.D., R.C. NOLAN, L.-S. LIN, M.R. BRODL, P.H. BROWN. 1987. Regulation of gene expression in barley aleurone layers. In "Molecular biology of Plant Growth Control" eds. Jacobs, M., Fox, E., Alan R. Riss, Inc., p 35-49.

HORNBERG, C., E.W. WEILER. 1984. High-affinity binding sites for abscisic acid on the plasmalemma of *Vicia faba* guard cells. Nature 310:321-324.

HUANG, J-K., M. SWEGLE, A.M. DANDEKAR, S. MUTHUKRISHNAN. 1984. Expression and regulation of α-amylase gene family in barley aleurones. J. Mol. Appl. Genet. 2:579-588.

JACOBS, M., S.F. GILBERT. 1983. Basal localization of the presumptive auxins transport carrier in pea stem cells. Science 220:1297-1300.

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

JACOBSEN, J.V., 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., 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, 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., 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, S. MUTHUKRISHNAN. 1987. Structure and organization of two divergent α-amylase genes from barley. Plant Mol. Biol. 9:Lanahan, M.B., Ho, T.-H.D. (1988). Slender barley:A constitutive gibberellin-response mutant. Planta 175:107-114.

LANAHAN, M.B., T.-H.D. HO. 1988. Slender barley: a constitutive gibberellin-response mutant. Planta 175:107-114.

LIN, L-S. 1987. Mode of action of abscisic acid in barley aleurone layers: induction of proteins by abscisic acid. Ph.D. thesis, University of Illinois, Urbana, IL.

LIN, L-S., 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.

LOBLER, M., 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.

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. Carlsberg Res. Comm. 49:439-444.

MUTHUKRISHNAN, S., G.R. CHANDRA, 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, 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, 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., T.H.D. HO. 1988. Hormonal regulation of gene expression in barley aleurone layers: induction and suppression of specific genes. Planta, in press temperature-dependent membrane transitions. Planta 154:578-586.

OU-LEE, T.-M, R. TURGEON, R. WU. 1988. Interaction of a gibberellin-induced factor with the upstream region of an α-amylase gene in rice aleurone tissue. Proc. Natl. Acad. Sci. USA 85:6366-6369.

PATRIDGE, J., L. SHANNON, 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, 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.

STUART, I.M., M.L. LOI, 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.

UKNES, S.J., 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.

WHITTIER, R.F., D.A. DEAN, 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.