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Hormonal Regulation of Gene Expression

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Abstract. The involvement of plant hormones in the regulation of gene expression is well-recognized. Current research using molecular approaches has resulted in the isolation and characterization of a number of hormone-responsive genes and cDNAs. These genes are proving to be valuable molecular probes to study the mode of action of plant hormones. This review will briefly describe some recent molecular data from selected hormone-responsive plant systems. Results of these studies indicate potential complexity in the regulation of these genes. These results and future challenges are discussed.

The mode of action of plant hormones has been the subject of intensive studies ever since these regulatory molecules were first identified. It has been realized for a long time that plant hormones most likely exert their regulatory roles by affecting gene expression and/or membrane function. Up to about 20 years ago, most of the studies on the effects of hormones on gene expression were restricted to the use of inhibitors of transcription and translation on the activities of hormone-induced enzymes. However, recent advancements in molecular techniques have allowed researchers to routinely isolate and characterize hormone-regulated genes. Consequently, more unequivocal information concerning the action of hormones on gene expression has rapidly accumulated. Several systems have often been used for these types of studies, including the induction of auxin-regulated cell elongation, the regulation of hydrolytic enzyme synthesis by gibberellins (GA) and abscisic acid (ABA) in cereal aleurone layers, ethylene regulation of cell expansion and fruit ripening, and the regulation of seed maturation/dormancy and stress responses by ABA. It is likely that new insights into the mechanisms of hormone-regulated gene expression would be first revealed in these systems because of their convenient and unique features. Moreover, the physiological effects of hormones in these systems have been well documented. Thus, in this review we will highlight the recent discoveries in hormone-regulated gene expression with emphasis placed on results from these particular systems. Although several new regulatory molecules have recently been recognized, such as methyl jasmonate (Staswick 1992), salicylate (Raskin 1992), oligosaccharides derived from cell walls (Ryan and Farmer 1991) and the peptide hormone, systemin (Pearce et al. 1992), their mode of action is not covered in this article.

Regulation of Gene Expression by Auxin

Auxins are thought to be involved in the major growth processes of cell division, cell extension, and differentiation. The role of auxin in cell extension has been studied most extensively, perhaps partly due to the fact that the response is rapid (at least in plant organs that respond to added auxin by undergoing elongation growth) and can be easily measured. Early molecular studies indicated that elongation growth was associated with gene expression events (reviewed by Key 1969). High-resolution, two-dimensional polyacrylamide gel analysis of in vitro translation products enabled investigators to observe rapid and specific changes in gene expression in response to auxin. Many of these changes were detected prior to or concomitant with measurable elongation (reviewed by Guilfoyle 1986). In an effort to investigate auxin-responsive changes in gene expression, several labs have isolated and characterized cDNA clones representing auxin-responsive mRNAs. With the aim of identifying mRNAs specifically associated with cell elongation, several strategies have been used to

construct cDNA libraries (Walker and Key 1982; Theologis et al. 1985; McClure and Guilfoyle 1987). Walker and Key (1982) made a cDNA library to mRNA from the elongating region of untreated, intact soybean seedlings. From this library, clones pJCW1 and pJCW2 were isolated. Libraries made to excised, elongating sections that were incubated in the presence of auxin produced the pIAA clones from pea epicotyl (Theologis et al. 1985) and the SAUR (small auxin-up RNAs) clones from soybean (McClure and Guilfoyle 1987). The cDNAs and, in some cases, the corresponding genes have been characterized. Some of the salient features of the characterization of these clones will be summarized below. To date, there is no evidence that these sequences have a role in elongation growth.

In elongating plant organs, the mRNAs encoded by the cDNA clones are moderately abundant. Incubation of excised organ sections in the absence of auxin will deplete the mRNAs to low or undetectable levels. Addition of auxin to these preincubated sections causes a rapid accumulation of the mRNAs, which can be detected within 15–30 min. In most cases, the mRNA levels continue to rise for several hours. Accumulation of the SAURs, on the other hand, can be detected 2.5 min after auxin addition, and the steady-state level reaches half maximum by 10 min. For all the clones, the mRNA induction appears to be specific for active auxins; nonauxin analogues, other growth regulators, and environmental stresses do not induce these mRNAs. Induction occurs with as little as 10^{-7} M auxin, and maximum levels of induction range from several fold to over 50-fold, depending on the clone. Results of nuclear run-on transcription studies showed that the induction of SAURs by auxin is controlled, at least in part, at the transcriptional level (McClure et al. 1989). The induction response of the pIAA and SAUR sequences was shown to be independent of protein synthesis, although treatment with protein synthesis inhibitors alone caused an increase in mRNA levels (Theologis et al. 1985; McClure and Guilfoyle 1987). For the SAURs, this induction was shown not to occur at the transcriptional level, suggesting that SAUR transcript stabilization may occur in the presence of the inhibitors (Franco et al. 1990).

RNA blot hybridization revealed that pJCW1 and pJCW2 mRNAs were most abundant in the elongation region of the soybean hypocotyl. Similar analyses showed the SAURs predominantly in elongating regions of soybean hypocotyls and epicotyls, and the pIAA mRNAs in the upper region of the third internode of pea seedlings. Tissue print analysis (McClure and Guilfoyle 1989a) and in situ hybridization (Gee et al. 1991) further localized SAUR expression to epidermal and cortical cells. SAUR expression in these cells is symmetrical in a vertically grown seedling. When the seedling is placed horizontally, there is a rapid redistribution of the transcripts, prior to any observable gravitropic bending. SAUR accumulation becomes asymmetric, with the strongest expression associated with the lower, more rapidly elongating side of the gravistimulated hypocotyl (McClure and Guilfoyle 1989b).

Soybean genomic clones for pJCW1 (designated GmAux28) and pJCW2 (GmAux 22; Ainley et al. 1988), and their homologues in *Arabidopsis* (AtAux2-11 and AtAux2-27; Conner et al. 1990) have been isolated and sequenced. The coding regions of the genes are interrupted by several introns. While GmAux28 did not cross-hybridize with GmAux22, the deduced amino acid sequence revealed several regions of high amino acid homology (80%–100%). These same regions of homology are found in the *Arabidopsis* homologues. A computer search did not find homologous proteins in the data bases, and the sequence itself gives no clue as to function.

A cluster of 5 SAUR genes has been located within 7 kbp of soybean genomic DNA, and this fragment has been sequenced (McClure et al. 1989). The genes do not contain introns, are transcribed in opposite orientation to produce small (0.55 kb) RNAs and encode small polypeptides (9–10 kDa). These polypeptides share no homology with proteins of known function. SAUR homologues have been isolated from *Arabidopsis* (G. Hagen, unpublished observations), pea (B. McClure and G. Hagen, unpublished observations) and mungbean (Yamamoto et al. 1992).

Promoter elements involved in tissue- and organ-specific expression and auxin inducibility have been investigated using promoter fragment-reporter gene fusion constructs in transgenic plants. An 800 bp SAUR promoter was shown to be expressed in the cortex and epidermis of elongating regions of tobacco seedlings. The promoter was responsive to exogenous auxin and was asymmetrically activated during gravistimulation (Li et al. 1991). A 617 bp promoter fragment from the AtAux2-11 gene was expressed in regions of elongation and in many cell and tissue types in *Arabidopsis* (Wyatt et al. 1993). This promoter was auxin-inducible and also asymmetrically activated during gravistimulation of seedlings. Deletion of this promoter to 400 bp caused a decrease in expression and auxin inducibility.

An examination of promoter elements in GmAux28 and GmAux22 revealed two homologous sequences at similar distances from the transcrip-

tion start site (Ainley et al. 1988). These sites, however, were not found in the *Arabidopsis* homologues (Conner et al. 1990). Recently, DNase I footprinting and gel mobility shift studies of the protein–DNA binding sites of the GmAux28 gene promoter indicated that these sites did not bind nuclear proteins (Nagao et al. 1993). Two new sequence motifs with similar core sequences were identified as major protein binding sites. Similar sequences were found in the promoters of several auxin-responsive genes. The significance of these sequences to the regulation of these genes by auxin remains to be determined. In the SAUR genes, two highly conserved, closely spaced elements (designated DUE and NDE) were identified in the promoter (McClure et al. 1989). Promoter fragments containing these elements bind nuclear proteins, and their role in SAUR gene regulation is currently under investigation (T. Guilfoyle, unpublished results).

Auxin-responsive genes are valuable molecular tools to study auxin-regulated gene expression events. Current challenges are to define *cis* elements and *trans*-acting factors involved in gene regulation, and determine the function of the auxin-responsive gene products and their role, if any, in auxin-regulated growth responses such as cell extension.

Regulation of Gene Expression by Gibberellins

Gibberellins were first isolated as fungal toxins causing uncontrolled growth of rice plants. Since its isolation from plant tissues, it is well established that GA regulates the physical stature of plants (see review by Phinney and West 1960). Mutants deficient in GA biosynthesis or response display dwarf phenotypes, and normal height could be restored upon application of exogenous GA. Using these genetic mutants, specific details of the GA biosynthesis pathway have been elucidated (see review by Graebe 1987). Using the genomic subtraction technique, a GA biosynthesis gene, GA1, has recently been isolated from *Arabidopsis* (Sun et al. 1992). Although it is known that GA treatment also leads to an increase in wall extensibility, the molecular action of GA in regulating cell elongation has not been studied as extensively as the action of auxin. Several mutants with altered GA sensitivity have been studied. Besides the GA-insensitive dwarfs, slender mutants that have uncontrolled stem elongation have been described in pea (Potts et al. 1985) and barley (Lanahan and Ho 1988). Since the slender mutation is recessive, it has been suggested that the slender gene normally encodes a repressor molecule, and, upon mutation, the repressor activity is lost leading to shoot elongation even in the absence of GA. Recently, an *Arabidopsis* mutant, *SPINDLY* (*SPY*), with phenotype similar to slender has been reported (Jacobsen and Olszewski 1993). To date, only one GA-regulated gene in elongation systems has been isolated and characterized (Shi et al. 1992).

Most of the information concerning GA regulation of gene expression has been obtained with the cereal aleurone cells, which, in response to GA from the germinating embryo, synthesize, and secrete several hydrolytic enzymes for the mobilization of endosperm nutrients. The starch hydrolyzing α-amylases in barley have been most extensively studied. In most of the cereal grains, α-amylase is composed of two sets of isozymes (high and low pI species) with very similar size (44 kDa) yet different net charges (Jacobsen and Higgins 1982). These isozymes can be classified into two groups based on their apparent pI: the high and low pI α-amylases, and their genes have been mapped on chromosomes 6 and 1, respectively (Muthukrishnan et al. 1983). Several α-amylase genomic clones have been isolated and characterized by restriction mapping and sequence analysis (Whittier et al. 1987; Knox et al. 1987; Huttly et al. 1989; Khursheed and Rogers 1988). 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. The α-amylase genes in other systems, most notably in wheat (Baulcombe and Buffard 1983; Knox et al. 1987) and in rice (Huang et al. 1990), have also been characterized.

The expression of high-pI α-amylase in isolated barley aleurone layers is not detectable in the aleurone layers without GA_3 treatment, yet the low-pI α-amylase is expressed at a low level in the same tissue even in the absence of GA_3. After the onset of hormone treatment the expression of both groups of α-amylase is enhanced in 4 h. The GA_3-enhanced expression of high-pI α-amylase reaches a maximum around 20 h and then declines afterwards. Very little of this isozyme is still synthesized beyond 30 h of GA_3 treatment. In contrast, the synthesis of low-pI α-amylase continues to about 40 h 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 cDNAs specific for the two groups of isozymes (Huang et al. 1984; Chandler et al. 1984; Rogers 1985; Nolan and Ho 1988). Jacob-

sen and Beach (1985) have performed in vitro run-on transcription assays 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 tenfold, and ABA treatment of aleurone layers can reverse this GA_3 effect. Using transient expression in oat aleurone protoplasts, Huttly and Baulcome (1989) have been able to delineate the hormone-responsive region in a wheat low-pI α-amylase gene (α-Amy2/54). Their work indicates that in α-Amy2/54, the regions important for high-level hormone-regulated expression lie within 300 bp upstream of the start of transcription. Investigating potential *trans*-acting factors regulating α-amylase gene expression in rice, Ou-Lee et al. (1988) have detected in vitro the interaction between protein factors and promoter regions of a rice low-pI α-amylase gene. Using the DNA retardation (gel mobilityshift) technique, they have observed that the level of activity of a protein factor, which interacts with the α-amylase promoter, is enhanced in GA_3-treated tissue. This DNA-binding factor appears to recognize a specific region in the α-amylase promoter which is protected from exonuclease III digestion. Jacobsen and Close (1991) have shown that a high-pI α-amylase promoter could be deleted to −174 and still retains the ability to respond to GA and ABA. Skriver et al. (1991) demonstrated that six tandomly repeated copies of the sequence 5′-CCGATAACAAACTCCGG-3′ from the promoter of a barley high-pI α-amylase gene could confer proper induction by GA, and suppression by ABA. Lanahan et al. (1992) have further defined the promoter of a low-pI α-amylase gene, there appears to be a GA-response complex (GARC) in this promoter consisting of four regions. Elements in the GARC include a putative opaque 2 binding sequence (Schmidt et al. 1990), a pyrimidine-rich box, a sequence homologous to the GARE suggested by Skriver et al. (1991), and another box (Box 1) which is also highly conserved among different α-amylase genes (Huang et al. 1990). Using transient expression assays in GA-responsive barley aleurone protoplasts, Gubler and Jacobsen (1992) have also concluded that the GARE and Box 1 homolog are essential for GA induction of a high-pI α-amylase gene. Rogers and Rogers (1992) have demonstrated that the putative opaque 2 binding site must be present to allow a single copy of either the GARE or the abscisic acid response element (ABRE, see details later) to mediate their hormonal effect in a barley α-amylase gene promoter. Thus, it appears that a high level of GA response is dependent on the interactions of several defined regions in the α-amylase promoter. This type of complex regulation is probably essential for governing the developmental, tissue-specific, and hormonal regulation of this gene.

To investigate the *trans*-acting factors interacting with the α-amylase promoter, several groups have initiated work on the nuclear proteins that interact with the promoter elements. Using nuclear proteins isolated from wild oat aleurone protoplasts, Rushton et al. (1992) have detected interaction with specific sequences in the wheat α-amylase promoters. Kim et al. (1992) have also detected nuclear protein binding to the pyrimidine box in a rice α-amylase promoter. Sutliff et al. (1993) have fractionated the nuclear proteins from barley aleurone tissue by ion-exchange fast protein liquid chromatography, and assayed for the DNA binding activities among the fractions. They found a GA-dependent binding activity eluting at 210 mM KCl interacting specifically to the GARE and closedly associated Box 1. A second DNA binding activity eluting at 310 mM KCl is present constitutively in nuclear extracts prepared from tissues incubated both in the absence and in the presence of hormone. Proteins in this fraction appear to bind to many DNA sequences in a non-specific manner. Thus, this appears to be the first demonstration in plants that a DNA binding protein(s) capable of interacting with a hormone-responsive element is induced by hormone treatment. However, it is not yet clear whether GA induces the synthesis of this DNA binding protein, or activates a preexisting form of this protein.

Besides α-amylases, proteases are also induced by GA in cereal aleurone tissue. In barley, three groups of cysteine proteinases have been studied, "aleurain," EP (endoprotease) A, and EPB. Aleurain is not secreted but localized mainly in a vacuole-like organelle (Holwerda et al. 1992), and hence is unlikely to be involved in the degradation of endosperm storage proteins. On the other hand, both EP A&B have been shown to be secreted and active in the degradation of the major barley storage proteins, hordeins (Koehler and Ho 1990a,b). The time course and dosage response of GA induction of EPB gene are virtually the same as those of α-amylase induction. However, the promoters of these two types of genes share little homology with each other (Mikkonen and Ho, unpublished observations). The region between −507 and −345 in the EP B promoter appears to be necessary for GA induction (Cercos and Ho, unpublished observations), yet sequences within −300 are necessary for the GA induction of α-amylase genes. Therefore, GA induction of EP B and α-amylase may follow different mechanisms. It is conceivable that EP B and α-amylase genes are turned on by two different DNA binding proteins, both of which are induced or activated by GA at the same time. Despite the lack

of similarities between EP B and α-amylase promoters, these two genes can still be turned on by GA with the same kinetics.

Although the GA receptor in the aleurone tissue has not yet been characterized, Hooley et al. (1991) have demonstrated that immobilized GA linked to Sepharose beads can still induce α-amylase expression in the aleurone protoplasts, but not in intact aleurone cells. Thus, it is likely that the primary action of GA takes place on the outside surface of the aleurone plasma membrane. However, the signal transduction pathway linking the primary action of GA on the plasma membrane and the expression of α-amylase and protease genes is virtually unexplored.

Regulation of Gene Expression by Ethylene

The evidence for the involvement of ethylene in the ripening of climacteric fruit is very clear (Brady and Speirs 1991). In many systems, immature fruit can be induced to ripen earlier than normal by exposure to ethylene. Early events in the ripening process include the respiration climacteric and an increase in ethylene synthesis (Brady and Speirs 1991). Inhibition of ethylene synthesis in ripening fruit using specific inhibitors or removal of ethylene from the environment will delay fruit ripening (Brady and Speirs 1991; Gray et al. 1992). Down-regulation of the ethylene biosynthetic pathway in transgenic tomatoes using antisense RNA technologies (Hamilton et al. 1990; Oeller et al. 1991) and by overexpression of a substrate-degrading enzyme (Klee et al. 1991) also resulted in the repression of fruit ripening.

During fruit ripening, numerous biochemical changes occur that affect both internal and external properties of the organ (e.g., fruit color and texture). Molecular studies have revealed that fruit ripening involves specific changes in gene expression that include the induction of ripening-specific mRNAs and polypeptides (Brady and Speirs 1991; Gray et al. 1992). The control of gene expression during fruit ripening has been studied most extensively in tomato (*Lycopersicon esculentum*). As a model system for climacteric fruit ripening, tomato offers several experimental advantages, such as the availability of ripening mutant lines and the relative ease with which tomato can be transformed (Gray et al. 1992). Ripening tomato fruit cDNA libraries have been constructed in several laboratories, and many ripening-related cDNA clones have been isolated and characterized (see Table 1 in Gray et al. 1992). Many of these cDNA clones represent mRNAs that are absent or present in low levels in unripe fruit, and accumulate during ripening. In some cases, mRNA accumulation appears to be coincident with the observed increase in ethylene synthesis in ripening fruit. Exogenous application of ethylene to unripe fruit has revealed, however, that only a subset of the ripening-related sequences are rapidly induced by ethylene (Gray et al. 1992). Some of the ethylene-inducible, ripening-related tomato sequences have been characterized further and are being used as molecular probes to investigate the mechanism(s) of ethylene action during fruit ripening. Data on these clones will be reviewed briefly below.

Four tomato cDNA clones (E4, E8, E17, J49) were selected from a cDNA library made to ethylene-treated mature green fruit (Lincoln et al. 1987). The mRNA levels for E4 and E8 were rapidly elevated (within 30 min) following ethylene treatment, while the induction kinetics for E17 and J49 were somewhat slower. Both E4 and E8 mRNAs were abundant in ripening fruit and not detected in other organs (Lincoln et al. 1987). Ethylene treatment induced E8 mRNA only in fruits, whereas E4 mRNA was induced in many organs (Lincoln and Fischer 1988a). Both E4 and E8 sequences were induced in ethylene-treated rin (ripening inhibited) tomato mutant fruit (Lincoln and Fischer 1988b). Nuclear run-on transcription assays showed that E4 and E8 gene expression is controlled, at least in part, at the level of transcription by ethylene (Lincoln and Fischer 1988b). Cloning of the E4 and E8 genes and flanking sequences has led to an analysis of *cis*-acting promoter DNA sequences and *trans*-acting nuclear protein factors that are involved in the regulation of these genes during ripening. When a DNA fragment containing the E8 gene plus 5′ (2 kbp) and 3′ (0.5 kbp) flanking sequences was reintroduced into tomato, the E8 gene was shown to be correctly expressed during fruit ripening and was responsive to ethylene (Deikman and Fischer 1988). This indicated that the DNA elements involved in the regulation of the E8 gene were contained within the introduced DNA. A focus on the sequences 5′ to the E8 gene led to studies on the binding of nuclear proteins from ripening fruit to fragments of the E8 promoter. Results of these studies indicated that several regions within 1 kbp of the start of transcription bound specific protein factors (Deikman and Fischer 1988). The binding activity of one of the strongest complexes was competed by a DNA fragment from the E4 promoter, suggesting that both DNA fragments bound the same protein factor. In addition, the DNA binding activity was found to be greater in ripening, mature green fruit than in unripe green fruit (Cordes et al. 1989). Sequence comparison of the region in both E4 and E8 involved in

protein binding revealed a 17 bp element with 11/17 base identity. The location of this element within the 2 promoters is dissimilar (Cordes et al. 1989). To date, however, no DNA element within the promoter of these genes has been identified as an ethylene-response element.

While the predicted polypeptide sequence of E4 was shown to have limited homology to a *Drosophila* sequence (Cordes et al. 1989), the function of E4 has yet to be determined. E4 shares no homology to other ripening-related sequences that have been characterized (Gray et al. 1992). E8 encodes a polypeptide that has homology to the tomato ripening-related sequence pTOM13 (Deikman and Fischer 1988) and is identical in sequence to pTOM99 (Gray et al. 1992). A function for pTOM13 in the ethylene biosynthetic pathway was suggested from studies using antisense pTOM13 constructs in transgenic tomatoes (Hamilton et al. 1990). Subsequently, it has been demonstrated that pTOM13 encodes the ethylene-forming enzyme (EFE; Hamilton et al. 1991; Spanu et al. 1991), which is ACC oxidase (currently designated ACO). This enzyme catalyzes the conversion of ACC (1-amino-cyclopropane-1-carboylic acid) to ethylene (Yang and Hoffman 1984). The level of amino acid identity between E8 and ACO is 34% over 295 residues (Deikman and Fischer 1988). The function of E8 in the ethylene biosynthetic pathway is clearly different from that of ACO. Antisense pTOM13 constructs caused a reduction in ACO and reduced ethylene biosynthesis in transgenic tomatoes (Hamilton et al. 1990), whereas antisense E8 constructs lowered E8 protein levels, but elevated ethylene levels in ripening transgenic tomato fruit (Penarrubia et al. 1992). These results suggest that E8 may have a negative role in ethylene production during fruit ripening (Penarrubia et al. 1992).

In addition to its role in fruit ripening, ethylene is thought to regulate many growth processes. The establishment of the ethylene biosynthetic pathway (Yang and Hoffman 1984) and the recent cloning of several genes encoding key biosynthetic enzymes (Sato et al. 1989; Hamilton et al. 1991) have led to studies designed to perturb endogenous ethylene biosynthesis, in hopes of gaining insight into how ethylene controls growth. Studies to date indicate that transgenic plants with reduced ethylene levels show no phenotypic effects except in ripening fruit (Klee et al. 1991). These studies represent an exciting approach to further elucidate the role of ethylene in growth and development. Continued characterization of ethylene-responsive genes will undoubtedly contribute valuable information towards the current efforts (Kieber et al. 1993) to define the ethylene signal transduction pathway.

Regulation of Stress and ABA-Induced Genes

It has been well documented that ABA exerts its regulatory roles in seed development/dormancy and the plant's response to stress conditions (see review by Zeevaart and Creelman 1988). Various ABA/stress-induced genes and proteins have been isolated and characterized (Skriver and Mundy 1990). Among them are Lea (late embryogenesis abundant) proteins, dehydrins, Rab (response to ABA) proteins, seed storage proteins, and individual enzymes. The expression of *Lea* genes is correlated with the development of desiccation tolerance in embryos. It is suggested that the presence of Lea proteins could stabilize other cellular proteins during desiccation (Dure et al. 1989). The Lea proteins often contain interesting repeats, and are grouped according to their sequence homologies (Dure et al. 1989). In vegetative tissues, ABA levels increase in response to drought, salinity, and cold stress (see review by Zeevaart and Creelman 1988). Elevated levels of ABA induces the expression of Rab and dehydrin genes. Some of the Rab proteins and dehydrins appear to be similar to Lea proteins. A maize Rab contains the consensus sequence of RNA-binding proteins, and it has recently been shown to interact with guanine- and uridine-rich RNAs (Ludevid et al. 1992). Another maize Rab protein with unknown function is phosphorylated by a casein kinase II-like enzyme (Vilardell et al. 1990). In wheat, one of the ABA-induced genes has sequence homology to a protein kinase, indicating that ABA may play a role in regulating protein phosphorylation (Anderberg and Walker-Simmons 1992). An aldose reductase, which is involved in the synthesis of sorbitol, is induced by ABA in barley (Bartels et al. 1991). Since sorbitol is a common osmolyte in animal cells, and probably also in plant cells, the induction of aldose reductase by ABA lends further support to the role of this hormone in drought stress tolerance.

Analyzing the *cis*-acting elements of rice Rab 16 gene promoter, Mundy et al. (1990) have identified that sequences of TACGTGGC, CGCCGCGCC-TGC, and CGC/GCGCGCT located between −292 and −252 are important for ABA response, and are also capable of interacting with nuclear proteins. Guiltinan et al. (1990) reported the cDNA cloning of a plant leucine zipper protein that binds to an 8-bp sequence, CACGTGGC, which is essential for ABA response in the wheat *Lea* gene, *Em*. They have further shown that a two-base mutation in this ABRE (ABA response element) causing a reduction in ABA response lso fails to bind to the leucine zipper protein. However, sequences similar to the ABRE are present in many genes that are not nec-

essarily ABA responsive, hence the specificity of this element to the ABA response is not fully resolved. Comparing the promoter sequences of many ABA-inducible genes, Michel et al. (1993) have suggested that an ACGT core is conserved among all the ABRE and ABRE-like sequences in the promoters of these genes.

Recently, Shen et al. (1993) have performed a thorough analysis of a novel ABA-induced gene, Hv A22, in barley. Although the function of this gene is not clear, the protein encoded by this gene is highly homologous to the product of human *DP1* gene, which is likely involved in colorectal tumorigenesis. The Hv A22 gene product also contains structural features reminiscent of known regulatory proteins. There appears to be at least three elements, two located in the promoter and one in the first intron, which are essential for the high level of ABA induction of barley HV A22 gene. The two promoter elements are both ABRE-type sequences, and deletion of either one of them causes a significant reduction in the ABA response (Shen and Ho, unpublished observations). Furthermore, addition of a 51 bp sequence containing one of the ABREs to a minimal promoter enables the promoter to have a low response to ABA. Thus, it appears that a specialized ABRE probably with its surrounding sequences could promote a low-level response to ABA, yet a high-level response to this hormone appears to be dependent on the interactions of more than one ABRE.

Perspective

With more sophisticated molecular techniques becoming available in recent years, new information about hormonal regulation of gene expression has begun to be unveiled. The studies of *cis*-elements and trans-acting factors involved in the hormonal-regulated gene expression will undoubtedly continue to flourish. However, it has become apparent that the regulatory mechanisms are quite complex. Several elements in a gene promoter as well as multiple DNA-binding proteins need to interact with each other in order to elicit developmentally regulated, tissue-specific, and hormone-induced gene expression. In animal systems, the composite steroid hormone response elements appear to be good examples of this type of regulatory paradigm (Diamond et al. 1990). Therefore, much more work is still needed to sort out the details of the interactions among these regulatory elements. Although regulation of gene expression plays a pivotal role in the action of plant hormones, one should not lose sight of other related processes. The protein products of hormone-regulated genes are presumed to be involved in hormone physiology, yet the function of many hormone-induced genes remains unclear. Recently, progress has been made in the field of hormone-binding proteins, yet little is known concerning how hormone-binding proteins trigger "downstream" events. In fact, the hormone signal transduction pathway remains largely unexplored. The combination of genetic mutation analyses and molecular characterization, as demonstrated in the studies of ethylene, probably constitutes the most rewarding approach toward the elucidation of hormone signal transduction pathways. With research efforts devoted to all of these subfields of hormone research, it is conceivable that we would eventually learn how the interactions between a hormone molecule and its receptor could lead to the alteration of gene expression, which, in turn, elicits the well-documented physiological changes.

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