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Gene Expression in Response to Abscisic Acid and Osmotic Stress

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REVIEW

Abscisic acid (ABA) was discovered in the 1950s to be a phytohormone affecting leaf abscision and bud dormancy. It was soon characterized as a sesquiterpene derived from mevalonate although certain steps of its biosynthesis in plants are still unknown (Li and Walton, 1987; Zeevaart and Creelman, 1988). Continuing work on ABA has shown that it mediates various developmental and physiological processes that affect the agronomic performance of crop plants (Austin et al., 1982; Ramagopal, 1987). These processes include embryo maturation and germination as well as the response of vegetative tissues to osmotic stress (Singh et al., 1987; Zeevaart and Creelman, 1988). ABA levels increase in tissues subjected to osmotic stress by desiccation, salt, or cold (Henson, 1984; Mohapatra et al., 1988). Under these conditions, specific genes are expressed that can also be induced in unstressed tissues by the application of exogenous ABA (Singh et al., 1987; Gomez et al., 1988; Mundy and Chua, 1988). Some of these genes are also expressed during the normal embryogenic program when seeds desiccate and embryos become dormant (Dure et al., 1981). Although different sets of ABA-responsive genes exhibit different patterns of developmental and tissue-specific expression, some of them appear to be part of a general reaction to osmotic stress. This system is a normal part of the embryogenic program but is inducible in vegetative tissues at other times in the plant life cycle. Several ABA-responsive genes have now been isolated (Baker et al., 1988; Gomez et al., 1988; Marcotte et al., 1988; Mundy and Chua, 1988; Vilardell et al., 1990; Yamaguchi-Shinozaki et al., 1990). A major goal of the research discussed below is to understand the role these genes play in osmotic stress and desiccation tolerance.

ABA-responsive genes are also being studied as tools to develop molecular models of ABA action. So far, work on animal hormones has defined two general mechanisms of action: (1) regulation of transcription factors by steroid hormone binding (Beato, 1989), and (2) activation of regulatory factors via "second messenger" pathways (Deutsch et al., 1988). In contrast, models of ABA action are incomplete, in part because ABA receptors have yet to be characterized (Hornberg and Weiler, 1984). However, recent physiological studies implicate second messenger pathways in auxin action (Ettlinger and Lehle, 1988; Jones and Venis, 1989). Other work has shown that the response to ABA is Ca^{2+} dependent, suggesting that second messenger signaling mediates ABA action (Napier et al., 1989; Schroeder and Hedrich, 1989). A goal of current molecular studies is to characterize abscisic acid-responsive DNA elements in the promoters of ABA "target" genes. The characterization of these factors will in turn provide tools with which to dissect earlier steps in the pathway(s) of ABA-induced gene expression.

RESPONSES TO ABA AND DESICCATION STRESS DURING SEED DEVELOPMENT AND GERMINATION

In higher animals embryogeny occurs in maternal tissues that simulate an ancestral aquatic environment. In natural populations mating is timed to produce progeny by ovipary or vivipary at the start of temperate seasons, usually spring. Higher plants, in part because they are sedentary, use a different strategy. Their progeny are dispersed at the end of the growing season and are packaged in a desiccated form to survive a resting or dormant period.

ABA has been implicated in the control of many events during embryogenesis and seed formation including embryo morphogenesis (Quatrano, 1987), storage protein synthesis (Finkelstein et al., 1985), desiccation tolerance (Kermode and Bewley, 1987), and the onset and maintenance of dormancy (Koornneef, 1986). ABA levels peak shortly before or after the onset of seed desiccation (King, 1976; Suzuki et al., 1981). At this time ABA may affect embryonic staging and the onset of dormancy by allowing embryo maturation to proceed but by inhibiting precocious germination (Fong et al., 1983). For example, immature embryos cultured without ABA germinate but application of the hormone prevents this precocity (Quatrano, 1987). Late embryogenesis abundant (*lea*) genes, whose developmental expression may coincide with the rise in endogenous seed ABA, have been described from various species. Experiments with exogenously applied ABA show

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that their expression is indeed responsive to the hormone (Dure et al., 1981).

Figure 1 presents the results of such experiments analyzing gene expression during embryogenesis and germination in rice, a typical monocot. As can be seen, the products of the *lea* genes, whose levels peak toward the end of seed formation and remain constant in resting seeds, normally disappear at the onset of germination. However, their accumulation can be recapitulated during germination by treatment with exogenous ABA. Taken together, these results suggest that the hormone not only halts key steps in germination, but is also capable of reinitiating part of the developmental program that culminates in a dormant seed.

Recent work shows a correlation between the expression of certain *lea*s and the development of desiccation tolerance in embryos (Bartels et al., 1988). Several *lea* genes have been characterized and current work aims to elucidate the functions of their encoded proteins. Table 1 outlines the relationships between these and other ABA-responsive genes according to sequence homologies and physiological characteristics.

Genetic studies indicate that the sensitivity of seeds to ABA modulates dormancy (Robichaud et al., 1980; Koornneef et al., 1989). *Viviparous (nondormant) mutant seeds*, which lack ABA or do not respond to it, neither fully desiccate nor accumulate certain *lea* gene products (Chandler et al., 1988; Pla et al., 1989; Kriz et al., 1990). Agronomic studies conclude that sprouting-resistant seeds are more sensitive to ABA than sprouting-sensitive ones (Walker-Simmons, 1987). Defining the link between ABA and seed dormancy is difficult, in part because maternal ABA and other factors may govern the crucial switch from dormancy to germination (Koornneef et al., 1989). In a similar way, the developmental pathways of root and shoot differentiation are controlled by the relative levels of two hormones, auxin and cytokinin, rather than by the absolute levels of either one (Smigocki and Owens, 1989). Perhaps the maintenance and breaking of dormancy are controlled in a similar fashion by the relative levels of ABA and gibberellic acid (GA) or other growth substances.

During germination in cereals, GA induces the expression of genes necessary for utilization of the stored seed reserves and for seedling growth. At this time the effects of ABA on gene expression are generally antagonistic to those of GA. For example, application of exogenous ABA to germinating cereal seeds sharply reduces levels of GA-responsive hydrolase mRNAs and proteins. This is presumably due to the induction of factors by ABA that inhibit hydrolase transcription and/or translation (Nolan and Ho, 1988; Rogers, 1988). This inhibition may also occur at the level of hydrolase activity: in some cereals, ABA promotes the accumulation of a protein that inhibits germination-specific amylase isozymes (Leah and Mundy, 1989; Table 1).

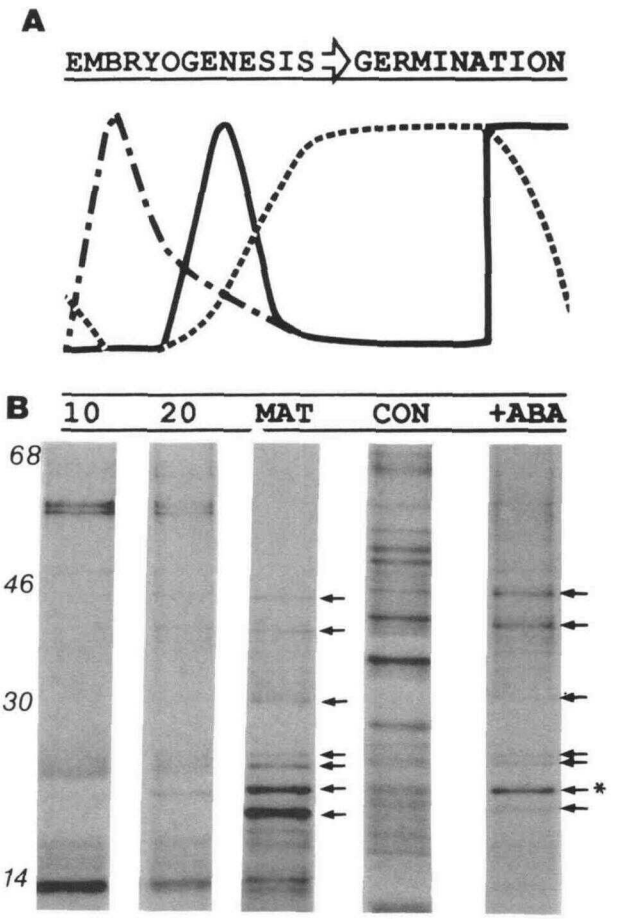

Figure 1. Developmental Expression of *lea* and *rab* Genes during Embryogenesis.

(A) Diagram showing idealized time course of events during seed development and germination. ABA level (——), storage protein synthesis (—— -), and germinability of excised embryos (– – –) are shown. 10 = 10 days after flowering (DAF), 20 = 20 DAF, MAT = mature seed, CON = 4 days control germination, +ABA = 3 days germination, then 1 day with 25 μM ABA.

(B) Translation products of corresponding developing and germinating whole rice seeds. Molecular mass markers are indicated at the left in kilodaltons. ABA-responsive polypeptides are marked among the products from mature and ABA-treated, germinated seeds. They are also discernible among the products from 20-DAF developing seeds. The *rab* 16 polypeptide is marked with an asterisk (*). Data are modified from Mundy and Chua (1988).

RESPONSES TO ABA AND OSMOTIC STRESS DURING VEGETATIVE GROWTH

Plant survival in most environments requires their ability to withstand extremes of osmotic stress caused by drought,

Table 1. ABA-Responsive Genes

Clone Name	Stress Induced	Species	Function	Organ Specific	Reference
pHVA1	?	Barley	?	?	Hong et al. (1988)
pLea76	D	Rape	?	?	Harada et al. (1989)
LEA D7	?	Cotton	?	?	Baker et al. (1988)
RAB	O, D, C	Rice	?	–	Mundy and Chua (1988)
RAB	O, D, C, W	Maize			Vilardell et al. (1990)
LEA D11	?	Cotton	?	?	Baker et al. (1988)
Dehydrin	D	Barley	?	–	Close et al. (1989)
p8B6	D	Radish	?	?	Raynal et al. (1989)
Em	D	Wheat	?	–	Marcotte et al. (1988)
LEA D19	?	Cotton	?	?	Baker et al. (1988)
pN24	O	Tomato	?	–	King et al. (1988)
Osmotin	O	Tobacco	Homology to Pase inhibitor	–	Singh et al. (1989)
LEA D34	?	Cotton	?	?	Baker et al. (1988)
LEA D113	?	Cotton	?	?	Baker et al. (1988)
salT	O, D	Rice	?	St	Claes et al. (1990)
Glb1	?	Maize	7S globulin	Sd	Kriz et al. (1990)
p511	?	Wheat	7S globulin	Sd	Williamson and Quatrano (1988)
Napin	D	Rape	7S globulin	Sd	Finkelstein et al. (1985)
Conglycinin	?	Soybean	7S globulin	Sd	Bray and Beachy (1985)
WGA	O	Wheat	Lectin	–	Cammue et al. (1989)
pMAH9	D, W	Maize	Homology to RNP	–	Gomez et al. (1988) Bandziulis et al. (1989)
ASI	D	Barley	Amylase/Pase inhibitor	Sd	Leah and Mundy (1989)
PI-2	W	Potato	Pase inhibitor	–	Pena-Cortes et al. (1990)
HS 70	O, D, H, W	Maize	Heat shock protein	–	Heikkila et al. (1984)

Homologous genes are bracketed. Data are adapted from compilations of Dure et al. (1989) and Mundy (1989). O = high osmoticum (PEG or salt), D = dessication, C = cold, W = wounding, H = heat, ? = untested or unknown. Sd = seed, St = stem, Pase = protease; ASI = α-amylase/subtilisin inhibitor, WGA = wheat germ agglutinin, RNP = ribonuclear protein; – denotes not organ specific.

salinity, and temperature. They have evolved two major mechanisms for accomplishing this: water stress avoidance and tolerance (Blum, 1988). Avoidance depends primarily upon specialized adaptations in root and shoot architecture and phenology (Paleg and Aspinall, 1981). Long-term changes in photosynthetic chemistry are also used by certain plants to reduce evaporative water loss (Bohnert et al., 1988). Water stress tolerance, on the other hand, involves subtler changes in cellular chemistry. It appears to be the result of the accumulation of compatible solutes and of specific proteins that can be rapidly induced by osmotic stress (Rhodes, 1987).

Current models suggest that osmotic stress is first perceived by cells as plasmalemma perturbations. This is caused by loss in turgor pressure, followed by an increase in cytosolic and apoplastic ABA due to de novo synthesis and/or release of the hormone sequestered in organelles (Zeevaart and Creelman, 1988). Although ion gradient models can explain organellar release, the increase in free ABA is also dependent upon de novo gene expression (Guerrero and Mullet, 1986). It is, therefore, possible that a set of genes rapidly induced by turgor pressure loss regulates ABA levels (Guerrero and Mullet, 1988). Recent work on barley mutants indicates that molybdenum cofactor enzymes, such as aldehyde oxidase, may control ABA accumulation after water stress (Walker-Simmons et al., 1989). The resultant increase in ABA levels then induces the expression of specific genes. These genes are here referred to by the acronym *rab* (responsive to ABA).

Current evidence links changes in ABA levels and the expression of *rab* genes with increased osmotic stress tolerance. For example, glycophyte plants and cells respond to high osmoticum by changes in the composition of cell wall polysaccharides and proteins (Iraki et al., 1989), and by accumulating RAB proteins (Ramagopal, 1987; Singh et al., 1987) and osmoprotectants such as proline (Rhodes et al., 1986). These changes are maintained in cells and plants adapted to high salt (Bressan et al., 1987; Gulick and Dvorak, 1987). However, it is unclear what role ABA plays in regulating the levels of osmoprotectant compounds such as proline. Although applied ABA can induce proline accumulation in turgid leaves and ABA accumula-

tion precedes that of proline in wilting leaves, ABA alone does not control proline accumulation (Stewart and Voetberg, 1987).

ABA is also a component of the resistance response of plants to drought stress (Austin et al., 1982; Quarrie, 1987). *rab* gene products that accumulate in leaves and roots during water deficit may contribute to this (Bray, 1988; Gomez et al., 1988; Mundy and Chua, 1988). Adaptation to cold and freezing tolerance in vegetative tissues may also be mediated by the expression of specific genes (Mohapatra et al., 1988; Schaffer and Fischer, 1988). This acclimation can be accelerated in some crop plants by the application of exogenous ABA. The products of certain *rab* genes apparently accumulate during this adaptive growth phase (Guy et al., 1985; Hahn and Walbot, 1989). Thus, although evidence is sparse to show that ABA mediates cold hardening or freezing tolerance, the adaptive response of plants to lowered water potentials at near-freezing temperatures may in part depend on ABA.

rab genes are also expressed in plant species that are adapted to growth in dry or saline environments. *Mesembryanthemum crystallinum*, a facultative halophyte, expresses certain *rab* genes in response to saline treatments before it switches to Crassulacean acid metabolism to reduce water loss (H. Bohnert, personal communication). The remarkable resurrection plant *Craterostigma plantagineum*, whose foliage can survive complete desiccation, also expresses *rab* genes in response to desiccation. Moreover, desiccation-intolerant calli of this plant can be resurrected after drying by pretreatment with ABA (Bartels et al., 1990).

FUNCTIONS OF ABA- AND OSMOTIC STRESS-RESPONSIVE GENES

Some of the first genes whose expression was shown to be responsive to ABA (napin and β-conglycinin) encode proteins specific to seeds. Their induction upon application of exogenous ABA to developing seeds or cultured embryos is generally slow, and the levels of their gene products increase only a few fold (Bray and Beachy, 1985; Finkelstein et al., 1985). Their patterns of temporal and spatial expression vary in seeds, suggesting that developmental cues other than ABA primarily control their expression. Therefore, it seems unlikely that these proteins are part of the plant's response to osmotic stress.

Recent molecular studies have characterized the rapid induction of *rab* genes by ABA. These novel genes have been isolated by differential screening of cDNA libraries synthesized from mRNAs of hormone-treated tissues. Therefore, they probably encode abundant mRNAs and proteins. They are also expressed during the desiccation phase of embryogenesis and may, therefore, be referred to as *lea* genes (Mundy, 1989; Figure 1).

Table 1 indicates that these and other genes are expressed in various plant organs in response to ABA or osmotic stress. Sequence analysis of novel *lea* and *rab* genes has delineated the three major homology groups bracketed in Table 1 (Dure et al., 1989). The predominant features of the encoded proteins are their hydrophilicity and high content of uncharged and hydroxylated amino acids. Conserved domains are found in each group that have been postulated to be functionally important in desiccation protection (Dure et al., 1989). These models suggest that cellular proteins are stabilized during desiccation via interactions with RAB proteins. The arguments invoked are reminiscent of those on protein stabilization by proline (Csonka, 1989) and by heat shock proteins (Pelham, 1986).

Other conserved, positively charged domains of RAB proteins initially suggested that they may bind nucleic acids (Mundy and Chua, 1988). This appears to be true for the maize protein encoded by pMAH9, which contains a ribonuclear protein consensus sequence (Bandziulis et al., 1989). Recent work has shown that this protein in fact binds single-stranded DNA, poly rU, and poly rG, and remains bound to the latter at high salt concentrations (M. Pages, personal communication). These exciting findings indicate that certain ABA-responsive genes may encode RNA-regulatory proteins capable of altering developmental events in plants. Nonetheless, much work remains to determine the functions of the proteins encoded by the ABA-responsive genes. Knowledge of the precise patterns of expression of these genes at both the protein level and the mRNA level may yield clues. To date, few studies have detailed these patterns or examined the effect of water stress on the stability and translation of *rab* and *lea* mRNAs.

Current data indicate that RAB and LEA proteins are ubiquitous in plants, and it is possible that homologous proteins are expressed during osmotic stress in other organisms (Scherer and Potts, 1989). Several osmoregulatory mechanisms used by plants have been intensively studied in other organisms (Table 2). These include pathways controlling the biosynthesis and transport of compatible solutes such as polyamines and sugar alcohols, and osmoprotectants such as glycine-betaine and proline (Flores et al., 1985; Ostrem et al., 1987). Other pathways modeled in bacteria and mammals have not been studied in plants. For example, the crystallins are abundant structural proteins specific to the lens of the eye. However, recent molecular analysis shows that they evolved from earlier heat shock proteins and stress-inducible enzymes involved in carbohydrate metabolism. Some of these enzymes, such as aldose reductase, catalyze steps in the synthesis of sugar alcohols such as sorbitol (Bedford et al., 1987). Sorbitol is one of several nonmetabolizable sugar alcohols whose levels increase in animal and plant tissues during water stress (Gorham et al., 1981; Seemann et al., 1986). The use of such ancient glycolytic enzymes

Table 2. Osmoregulatory Pathways and Genes in Plants and Other Organisms

Gene or Pathway	Organism	Reference
K^+ transport	Bacteria	Epstein (1986)
Betaine transport	Bacteria	Ramirez et al. (1989)
Betaine biosynthesis	Plants	Hanson et al. (1985)
Proline transport	Bacteria	Csonka (1989)
Proline biosynthesis	Plants	Treichel (1986)
Porins, transport of hydrophilic molecules	Bacteria	Forst and Inouye (1988)
Inositol transport	Mammals	Nakanishi et al. (1989)
Crystallins, structural proteins, and enzymes	Mammals	de Jong et al. (1989)
	Mammals	Carper et al. (1987)
Antifreeze	Bacteria	Yang et al. (1988)
Polyamines	Bacteria	Tabor and Tabor (1985)

as osmoregulators by animals suggests that the same strategy may be employed by plants.

MECHANISMS OF ABA- AND OSMOTIC STRESS-RESPONSIVE GENE EXPRESSION

A goal of studies on gene expression in response to ABA and osmotic stress is to understand how plants sense osmotic changes in their environment and how they transduce this signal to produce changes in specific gene expression. Current models envision a network of turgor pressure responses, including activation of primary turgor pressure-responsive genes, followed by expression of ABA-responsive and other genes. Several turgor-induced cDNAs have been cloned from soybean but their functions are unknown (Guerrero and Mullet, 1988). Studies of gene regulation by osmotic stress in *Escherichia coli* may provide models for understanding plant turgor and osmoresponsive genes. For example, bacterial genes encoding proteins involved in glycine-betaine transport and membrane pore formation are regulated by two different mechanisms, DNA supercoiling (Higgins et al., 1988) and specific osmosensory modulator proteins (Forst and Inouye, 1988). The latter, an inner-membrane, autophosphorylated kinase, is a member of a well-conserved family of bacterial modulators. Similar proteins may transduce osmosensory signals in plants.

Recent studies in plants have begun to outline signal transduction mechanisms connecting osmosensation with changes in gene expression. Biochemical studies indicate that ion channels and active transport are involved in osmoregulation and signaling in plant cells (Schroeder and Hedrich, 1989). Rapid increases in intracellular Ca^{2+} levels after osmotic stress in roots may be mediated by phosphoinositides (Lynch et al., 1989). That such pathways are active in plants is now supported by extensive evidence including Ca^{2+} mobilization studies (Rincon and Boss, 1987), phosphatidylinositol turnover and signaling (Morse et al., 1989), and the cloning of plant protein kinases (Lawton et al., 1989) and calmodulin (Ling and Zielinski, 1989). In theory, then, a transduction pathway involving phosphoinositol second messengers and Ca^{2+} signaling would connect stress sensors to gene activation via protein phosphorylation. It should be mentioned that these messengers may mediate rapid physiological changes in specific cells that do not require gene activation. For example, the ABA-dependent increase in cytosolic Ca^{2+} in guard cells appears to trigger rapid stomatal closure via cation and anion effluxes (McAinsh et al., 1990).

We do not know whether ABA-responsive gene expression is mediated by a cytosolic transduction chain. Evidence from several tissue systems indicates that Ca^{2+} (Napier et al., 1989) and protein phosphorylation (Vilardell et al., 1990) are involved. In contrast, accumulation of specific *rab* transcripts in cultured rice cells is not affected by treatments with Ca^{2+}, a Ca^{2+} ionophore, bromo-cAMP, phorbol ester, or forskolin, molecules which strongly affect second messenger signaling in animal cells (Grega et al., 1987; Mundy et al., 1990). Further experiments employing Ca^{2+}-channel inhibitors and other reagents are needed to examine this question (Graziana et al., 1988).

Current studies aim to understand the events mediating ABA-responsive gene expression by characterizing ABA receptors. Initial experiments used radiolabeled ABA to tag putative receptors by photoaffinity cross-linking (Hornberg and Weiler, 1984). Free ABA was employed because the molecule contains a photoreactive, α-β unsaturated ketone moiety. Although ABA-binding proteins were identified, the results may be confounded by nonspecific binding between ABA, a hydrophobic molecule, and several plasmalemma proteins.

A promising technique, proven in characterizations of animal hormone receptors, involves the use of antiidiotype antibodies (Gaulton and Greene, 1986). This technique has been used in a preliminary characterization of GA-receptors and is being developed for the study of ABA receptors (R. Hooley, personal communication). Studies of hormone-responsive mutants, which have aided the characterization of putative auxin receptors (Hesse et al., 1989; Hicks et al., 1989), may rapidly lead to a characterization of ABA receptors. The maize *viviparous-1* locus, recently isolated via transposon tagging, may encode a regulator of ABA reception or a component of an ABA transduction pathway (McCarty et al., 1989).

Current molecular studies of ABA action aim to delineate the *cis*- and *trans*-acting factors controlling the expression of ABA-responsive genes. This work has begun to delineate ABA-responsive DNA elements in the promoters of ABA-responsive genes. Sequence comparison of the 5′ upstream sequences of several of the *rab* and *lea* genes listed in Table 1 has identified conserved sequences that may be ABA-responsive DNA elements (Marcotte et al.,

1989; Mundy et al., 1990). Transient expression studies have shown that promoter regions of the *Em* and *rab* genes containing these motifs confer ABA responsiveness on reporter genes in cereal protoplasts. In vitro footprinting and gel retardation experiments have also demonstrated nuclear protein(s) binding to motifs I and II of the rice *rab* 16A gene promoter (Mundy et al., 1990). As mentioned in the Introduction, these putative ABA response elements may be used as DNA probes to identify cDNA clones encoding hormone-activated regulatory proteins by screening expression libraries (Katagiri et al., 1989). Further in vitro work with the DNA element and the regulatory protein can elucidate how specific gene transcription is controlled by the hormone (Beato, 1989).

Recent experiments suggest that more than one mechanism determines the level of expression of the various ABA-responsive genes. Differences are most notable between the seed-specific protein genes and those that are inducible in other tissues. Two lines of experimental evidence demonstrate these differences. First, the two ABA-responsive wheat genes, 7S globulin and *Em* (Table 1), respond differently to inhibitors of transcription and translation (Williamson and Quatrano, 1988). Whereas accumulation of the 7S globulin mRNA is inhibited by cycloheximide, accumulation of *Em* mRNA is not. Accumulation of the rice *rab* mRNAs is also independent of de novo protein synthesis (Mundy and Chua, 1988), indicating that they may be induced by the same mechanism as *Em*. Transient expression experiments with constructs containing the *Em* 5′-untranslated region also suggest that mRNA secondary structures contribute to the stability or translational efficiency of this mRNA (Marcotte et al., 1989).

Second, recent work on the wound-inducible expression of the protease inhibitor 2 gene of tomato and potato has shown that application of exogenous ABA induces the systemic response of this gene in vegetative tissues (Pena-Cortes et al., 1990). These experiments, therefore, implicate ABA as the actual mediator of the systemic wound response of the protease inhibitor. Interestingly, inhibitor mRNA is not induced in vegetative tissues by water stress or in seed tissues by ABA itself, treatments which induce the accumulation of *Em* and *rab* mRNAs. These experiments suggest that the different patterns of expression of ABA-responsive genes are due to their different hierarchies of hormonal, developmental, and spatial control elements.

These results raise a fundamental question in plant hormone research: How does the localization of the hormone itself compare with the accumulation of the genes it regulates? More specifically: Are the cells that accumulate ABA tissue specific, or do all cells accumulate ABA? These questions may be addressed by experiments using immunocytochemistry and thaw-mount radiography to measure both endogenous ABA and target gene expression at the cellular level. Such studies may also provide clues as to whether ABA is involved in primary or secondary effects on gene activation.

PROSPECTS

As noted in the Introduction, two major areas of research on gene expression in response to osmotic stress and ABA require continued study. The first involves further experimentation to define functions for the proteins encoded by genes that respond to the hormone and to water stress. This research may lead to an understanding of mechanisms of water stress tolerance at the genetic level and bring these important agronomic traits within the grasp of biotechnologists. For example, structural studies on the novel ABA-responsive proteins may reveal how they act as osmoprotectants (Dure et al., 1989) or regulatory proteins (Bandziulis et al., 1989). Their overexpression in transgenic plants may also provide clues as to their functions and might even produce plants with increased water stress tolerance. In a similar fashion, nucleotide probes for these genes may contribute to selection schemes in breeding programs for drought, salinity, or cold tolerance.

The second area worthy of further study is the mechanism by which plant gene expression is controlled by osmotic stress and ABA. The questions to be answered are not only of basic scientific interest, but also may delineate how dormancy and water stress tolerance are controlled. The recent isolation of rapidly induced, ABA-responsive genes has provided tools with which to test the relevance of animal hormone paradigms or to pioneer new pathways of plant hormone action. A major role will also be played by novel approaches to characterizing receptors, including immunological methods, and the power of genetic systems such as *Arabidopsis*.

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