Plant Physiol. (1993) 103: 1035–1040

Update on Water Deficit

Molecular Responses to Water Deficit[1]

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Water deficit elicits a complex of responses beginning with stress perception, which initiates a signal transduction pathway(s) and is manifested in changes at the cellular, physiological, and developmental levels. The set of responses observed depends upon severity and duration of the stress, plant genotype, developmental stage, and environmental factors providing the stress. Cellular water deficit may result from stresses such as drought, salt, and low temperature. This complexity makes it difficult to uncover the responses to water deficit that enhance stress tolerance. In recent years efforts have turned toward isolation of genes that are induced during water deficit in order to study the function of drought-induced gene products and the pathways that lead to gene induction. Changes in gene expression are fundamental to the responses that occur during water deficit, and they control many of the short- and long-term responses.

Studies on the molecular responses to water deficit have identified multiple changes in gene expression using two-dimensional PAGE, and many genes that are water-deficit-induced have been isolated by differential screening of cDNA libraries. Functions for many of these gene products have been predicted from the deduced amino acid sequence of the genes. Genes expressed during stress are anticipated to promote cellular tolerance of dehydration through protective functions in the cytoplasm, alteration of cellular water potential to promote water uptake, control of ion accumulation, and further regulation of gene expression. Although these studies are promising, it continues to be difficult to ascertain the actual function of drought-induced gene products. Expression of a gene during stress does not guarantee that a gene product promotes the ability of the plant to survive stress. The expression of some genes may result from injury or damage that occurred during stress. Other genes may be induced, but their expression does not alter stress tolerance. Yet others are required for stress tolerance and the accumulation of these gene products is an adaptive response.

Complex regulatory and signaling processes, most of which are not understood, control the expression of genes during water deficit. Multiple stresses may connect into the same or a similar transduction pathway, which is evidenced by the involvement of ABA in the induction of genes induced by a number of different stresses. In addition to induction by stress, the expression of water-deficit-associated genes is controlled with respect to tissue, organ, and developmental stage and may be expressed independently of the stress conditions. For example, some genes expressed during drought stress are also expressed during the maturation and desiccation phases of seed development. The regulation of specific processes will also depend upon the experimental conditions of stress application. Stress conditions that are applied in the laboratory may not accurately represent those that occur in the field. Frequently, laboratory stresses are rapid and severe, whereas stress in the field often develops over an extended period of time (Radin, 1993). These differences must also be evaluated when studying the adaptive value of certain responses. The function of the gene products and the mechanisms of gene expression are intertwined, and both must be understood to fully comprehend the molecular response to water deficit.

GENES EXPRESSED DURING PERIODS OF WATER DEFICIT

The numerous responses to water deficit are controlled by an array of genes with many different functions. As water is lost from the cell, regulatory processes are initiated that adjust cellular metabolism to the new cellular conditions. At the same time, growth inhibition and alterations of developmental pathways will result in changes in gene expression. Many of the water-deficit-induced genes encode gene products predicted to protect cellular function (Fig. 1). Genes that function during changes in metabolism, regulation, signaling, and recognition of stress are also expected to be induced, but fewer of these classes of genes have been identified.

Protection of Cellular Structures

A number of water-deficit-induced gene products are predicted to protect cellular structures from the effects of water loss. These predictions are derived from the deduced amino sequence and expression characteristics. These genes, frequently called *lea*, were first identified as genes that are expressed during the maturation and desiccation phases of seed development (Baker et al., 1988). It has since been recognized that these genes are also expressed in vegetative tissues during periods of water loss resulting from water, osmotic, and low-temperature stress. At least six groups of

[1] This work was supported by the United States Department of Agriculture Competitive Grants, Southwest Consortium on Plant Genetics and Water Resources, and Binational Agricultural Research and Development Fund.

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Abbreviations: ABRE, abscisic acid-response element; LEA, late embryogenesis abundant protein; *lea*, late embryogenesis abundant gene.

lea genes have been identified, based on amino acid sequence similarities among several species (Dure, 1993b). The majority of the *lea* gene products are predominantly hydrophilic, biased in amino acid composition, and lacking in Cys and Trp and are proposed to be located in the cytoplasm. The individual amino acid sequences and predicted protein structures have been used to propose specific functions for each group of LEA proteins (Dure, 1993b). These predicted functions include sequestration of ions, protection of other proteins or membranes, and renaturation of unfolded proteins (Fig. 1).

One of the groups of LEA proteins (D-7 family, or group 3) is predicted to play a role in the sequestration of ions that are concentrated during cellular dehydration. These proteins have an 11-mer amino acid motif with the consensus sequence TAQAAKEKAGE, repeated as many as 13 times (Dure, 1993a). This motif is predicted to form an amphiphilic α-helix. The hydrophobic face may be important in forming a homodimer and the outside charged face may be involved in sequestering ions whose concentration is increased during water deficit (Dure, 1993a). Another group of LEA proteins (D-29 family, or group 5) are also predicted to sequester ions during water loss. This group also has an 11-mer repeat in which each amino acid in the motif has similar chemical properties to group 3, but unlike group 3 a high degree of residue specificity is lacking at each position. Another group of LEA proteins (D-19 family, or group 1) is predicted to have enhanced water-binding capacity. These proteins have a high percentage of charged amino acids and Gly. One member of this group, Em, is approximately 70% random coil, leading to the prediction that it has a high capacity for binding water. Group 4 LEA proteins (D-113 family) may replace water to preserve membrane structure. The amino acid sequence in the N terminus, which is thought to form an α-helix, is conserved. These proteins have little conservation in the C-terminal amino acid sequence, although the C-terminal random coil structure is conserved. At least 30 different genes have been identified as members of *lea* group 2 (*D-11 family*). A conserved 15-mer, EEKKGIMDKIKELPG, occurs at the C terminus and at least once internally in this *lea* family. Possible functions include a chaperone function or one that preserves protein structure (Dure, 1993b).

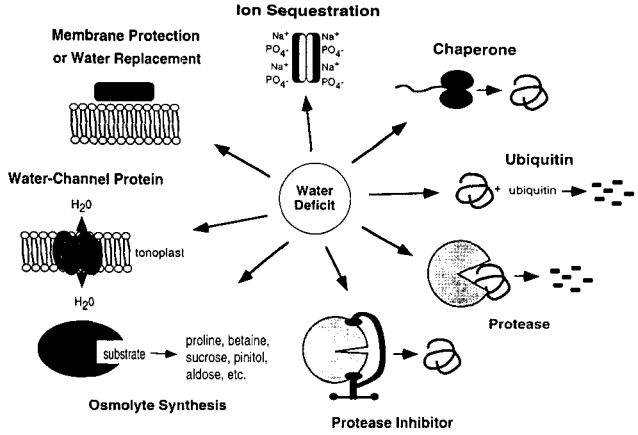

Figure 1. Predicted functions of water-deficit-induced gene products that may act to maintain cellular function during periods of water loss or when osmotic adjustment occurs. Functions include those that are predicted to protect cellular processes by protection of cellular structures and osmotic adjustment.

Genes that are thought to protect cellular structures are induced by stresses that result in water deficit and during periods of development that result in cellular dehydration. A compelling argument can be made that these proteins perform an important function under both of these situations. In addition, similar genes are induced in plants that are desiccation tolerant as well as in plants that tolerate only mild stress. At the present time, it cannot be determined whether these results indicate that these genes are involved in stress tolerance. However, they do indicate that differential expression of these genes does not account for the difference in tolerance between the species. The functions that are predicted for these gene products are those that would be needed during severe stress, when sufficient water is lost to disrupt cellular structure. It is not certain whether under mild stress sufficient water is lost to require these types of protective functions.

Osmotic Adjustment

The maintenance of total water potential during water deficit can be achieved by osmotic adjustment. A reduction in cellular water potential below the external water potential, resulting from a decrease in osmotic potential, allows water to move into the cell. The osmotic potential inside the cell is lowered by the accumulation of osmolytes (compatible solutes) in the cytoplasm. Genes that encode enzymes for steps in the synthesis of these osmolytes have been identified (Fig. 1) and, in some cases, they have been shown to be induced by water deficit. Regulation of osmotic potential and ion compartmentation occurs at the expense of the H^+ electrochemical gradient. Integrated control of the different ATPases of different membranes of the cell and other carriers is required. Salt regulates the 70-kD subunit of the tonoplast H^+-ATPase, a putative ER Ca^{2+}-ATPase, and the plasma membrane H^+-ATPase (Niu et al., 1993, and refs. therein).

Newly discovered proteins that have six putative membrane-spanning domains and a channel-like structure may also play a role in osmotic adjustment. Members of this protein family form water-specific, ion, or solute channels. A plant member of this family, γ-TIP (tonoplast intrinsic protein), forms water-specific channels when expressed in *Xenopus* oocytes (Maurel et al., 1993). Two genes with characteristics of this family are induced by water deficit and may play a role in osmotic adjustment, although tests for function have not been reported (Guerrero et al., 1990; Yamaguchi-Shinozaki et al., 1992). As channel proteins accumulate in the tonoplast during stress, movement of water or solutes from the vacuole to the cytoplasm could be promoted, altering either the water content or the osmotic potential of the cytoplasm. To test this hypothesis, it must be determined whether the drought-induced members of this family are located in the tonoplast and whether they are channels for water, ions, or solutes.

In addition to protection from cellular water deficit, pro-

tection from pathogens may also be required. Two proteins, osmotin and nonspecific lipid transfer proteins, are stress-induced and are thought to play a role in controlling pathogens. Osmotin was first found to accumulate in cells adapted to high concentrations of salt (reviewed in Kononowicz et al., 1993). It has since been shown to be a member of a family of proteins that permeabilizes fungal membranes to protect against fungal attack. Another stress-induced protein is in a family reported to have antifungal activity. Nonspecific lipid transfer proteins are induced by drought (Plant et al., 1991; Torres-Schumann et al., 1992) and low-temperature stress (Hughes et al., 1992). Proteins in this class have been shown to transfer lipids in vitro, and they also inhibit fungal hypha growth (Terras et al., 1992).

Genes thought to play a role in protection and osmotic adjustment have been highlighted. However, genes have also been isolated that may be involved in other diverse functions during water deficit. Genes involved in two types of protein-degrading mechanisms, proteases and ubiquitin, are induced by water deficit. These gene products may be involved in the degradation of proteins that are denatured during cellular water loss. Counteracting these degrading mechanisms are chaperones and protease inhibitors that are also induced by water deficit (Fig. 1). In addition, genes potentially involved in regulation and signaling during periods of water deficit, such as a protein kinase, nuclear proteins, and an RNA-binding protein, have been identified. Transcription factors that recognize DNA elements within drought-induced genes have also been identified. As more genes are isolated and more is learned about the adaptation of plants to stress, our understanding of the functions of water-deficit-induced genes is bound to improve.

DO WATER-DEFICIT-INDUCED GENE PRODUCTS PERFORM AN ADAPTIVE ROLE?

It is critical to determine if plant responses to stress are adaptive or merely a consequence of stress. Responses that are triggered by environmental cues as well as developmental signals are promising as responses that are important for adaptation to stress. However, knowing that these genes are induced is not sufficient to conclude that specific gene products are required for stress tolerance. Therefore, experimental means to evaluate the adaptive nature of specific gene products are required.

A correlation between organ survival and LEA protein accumulation during recovery from severe dehydration has been identified. In wheat seedlings, shoots and scutellum resume growth during recovery from 90% water loss, whereas the roots are killed. Group 3 *lea* transcripts were detected in all organs of the dehydrated seedlings, but the protein accumulated only in shoots and scutellum. Group 3 LEA proteins did not accumulate in roots. There is a correlation between dehydration survival and LEA protein accumulation, but not mRNA accumulation (Reid and Walker-Simmons, 1993). These results support the hypothesis that LEA proteins function in stress tolerance. mRNA accumulation alone certainly cannot be used to demonstrate that a response is adaptive. In addition, these results highlight an important role for posttranscriptional regulation of gene expression during stress.

In combination with physiological studies as described above, specific "mutants" that over- or underexpress a specific gene can be used to determine if a particular gene product is required for stress tolerance. Three LEA-like proteins from *Craterostigma plantagineum* were independently expressed in transgenic tobacco plants. Leaf discs from the transgenic and wild-type plants did not differ in their tolerance to mild stress (Iturriaga et al., 1992). Although the physiological studies above support a role for LEA proteins in stress tolerance, these do not. A number of possibilities that might explain these results must be further investigated: the overexpression of a single protein is not sufficient to alter stress tolerance, protein overexpression must be balanced with accumulation of osmolytes, or the native tobacco genes fulfill the same role as the introduced gene. Of course, it is also possible that these genes do not play a role in the ability of the plants to withstand the rapid and mild experimental stress, or proteins from *C. plantagineum* do not function in tobacco as they do in their native context.

Although improved stress tolerance has not been achieved when native stress-induced genes are overexpressed, transgenic tobacco plants expressing a foreign gene leading to mannitol accumulation do have improved stress tolerance (Tarczynski et al., 1993). Mannitol-1-phosphate dehydrogenase, mtlD, from *Escherichia coli*, catalyzes the synthesis of mannitol-1-phosphate, which is a substrate for general phosphatases in tobacco, resulting in the accumulation of mannitol. After 30 d of exposure to salinity, transgenic plants that produce mannitol had a greater shoot height and reduced weight loss compared with stressed control plants. Therefore, the introduction of a foreign gene into tobacco has been used to emphasize the importance of osmolyte accumulation during stress. The next step is to determine whether increased mannitol content impacts agricultural output when field conditions are unfavorable.

Further studies combining physiological and genetic studies are needed to evaluate the role of water-deficit-induced genes. Comparisons of gene expression between related species or varieties that differ in drought tolerance can be used to determine if specific responses are controlled by the genotype. In addition, it can be determined if drought-induced genes map to loci that segregate for stress tolerance. The power of genetics should be utilized to fully characterize and understand the mechanisms of stress tolerance and the responses to water deficit.

HOW ARE THE MOLECULAR RESPONSES REGULATED?

The molecular response of plants to water deficit defines a very interesting puzzle: how does a physical phenomena, the loss of water from the cell, cause a biochemical response, the induction of specific genes? Or, how does the cell recognize the loss of water and respond to it? The answer to these questions is not known. Currently, it is thought that loss of turgor or change in cell volume resulting from different environmental stresses permits the detection of loss of water at the cellular level. One or both of these changes may

activate stretch-activated channels, alter conformation or juxtaposition of critical proteins, or cause alterations in the cell wall-plasma membrane continuum (Ding and Pickard, 1993), thereby triggering a signal transduction pathway(s) that induces gene expression. Therefore, several different stresses may trigger the same or similar signal transduction pathways. The induction pathway(s) is also poorly understood, although there is evidence that there is more than one pathway. There may be a direct pathway, or additional signals may be generated that, in turn, alter the pattern of gene expression. The plant hormone ABA also accumulates in response to the physical phenomena of loss of water caused by different stresses, and elevation in endogenous ABA content is known to induce certain water-deficit-induced genes. Therefore, ABA accumulation is a step in one of the signal transduction pathways that induces genes during water deficit (Fig. 2). At this time, a convenient way to categorize drought-induced genes is by response to ABA. Sets of genes have been identified that require ABA for expression, are ABA-responsive but may not require ABA for expression, and are not responsive to ABA.

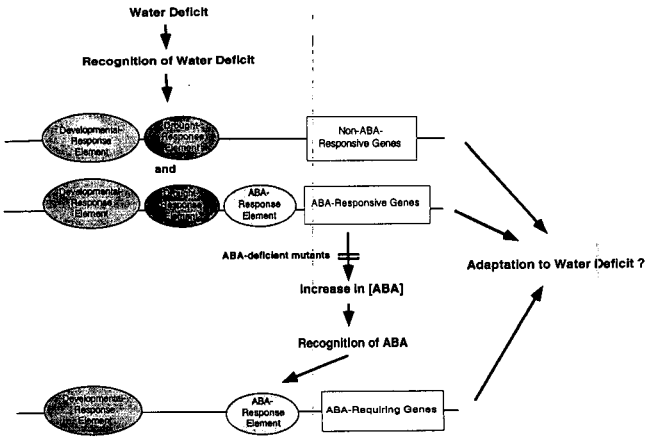

Figure 2. A simplified depiction of the pathway that results in the induction of genes that are ABA-requiring, ABA-responsive, and do not respond to ABA. Upon recognition of water deficit, genes are induced that may or may not be responsive to ABA. The expression of some of these genes will lead to the accumulation of ABA that in turn will induce ABA-requiring genes. ABA-responsive genes may be induced at this time by ABA, or they may have been induced before ABA accumulates by other mechanisms.

ABA Induces Gene Expression during Water Deficit

ABA concentration is altered when there are changes in the environment that result in cellular dehydration; especially well studied are changes that occur during periods of water deficit. ABA is synthesized through the carotenoid biosynthetic pathway. The cleavage of 9′-*cis*-neoxanthin results in the postcleavage intermediate to ABA, xanthoxin. Xanthoxin is oxidized to ABA-aldehyde, which is converted to ABA by ABA-aldehyde oxidase (Parry, 1993). The step in the ABA biosynthetic pathway that is likely to be regulated during drought stress is the cleavage step; the rate of ABA biosynthesis is limited by the production of xanthoxin, not the conversion of xanthoxin to ABA (Parry, 1993). Transcription and translation are required for ABA biosynthesis during stress (Guerrero and Mullet, 1986), indicating that the ABA biosynthetic enzymes or other proteins in the pathway must be synthesized for elevated levels of ABA to accumulate and before ABA-requiring genes can be induced (Fig. 2).

Studies using ABA-deficient mutants have been used to conclusively demonstrate that elevated levels of ABA are required for the expression of specific drought-induced genes. ABA application studies can be used to determine the range of responses that a plant is capable of attaining in response to ABA. However, these types of studies do not provide evidence that the plant responds to changes in the concentration of endogenous ABA. Inhibitors of carotenoid biosynthesis result in a decreased level of ABA (Parry, 1993), and responses of the plant that are reduced by inhibitor application have been used to analyze the role of ABA. But, as with the use of other inhibitors, effects that are not directly caused by the reduction in ABA concentration may also occur, because carotenoid concentration is also reduced after application of these inhibitors. Mutants blocked in the last steps of the ABA biosynthetic pathway, ABA-deficient mutants, are the best tools available with which to examine the role of ABA during plant stress. With single-gene mutants, only a reduction in ABA occurs and pleiotropic effects can be reduced or eliminated. Experiments using mutants blocked in the ABA biosynthesis pathway demonstrated that specific genes require elevated levels of ABA for expression during water and low-temperature stress (Cohen and Bray, 1990; Pla et al., 1991; Lång and Palva, 1992). Several drought-induced genes are not expressed in the ABA-deficient mutant as they are in the wild type. These genes are referred to as ABA-requiring genes in Figure 2.

Although sets of genes can be classified by responses to experimental conditions, the response of each gene to cellular signals is defined by the DNA elements acting within each gene. An ABRE, 5′-C/TACGTGGC-3′, involved in controlling transcription in response to ABA, has been shown to bind a cloned transcription factor, EmBP-1 (Guiltinan et al., 1990). This ABRE has been demonstrated to control ABA-regulated expression in several genes, *Em*, *rab16*, *rab17*, and *rab28*, that are preferentially expressed in seeds (Pla et al., 1993, and refs. therein). This DNA sequence is found in many genes that are expressed during seed development and in response to ABA application. However, it is not found in all genes that are in the ABA-requiring category; for example, *le16*, a gene expressed in wild-type tomato but not in the ABA-deficient mutant, does not contain a consensus ABRE (Plant et al., 1991). Recently, Michel et al. (1993) have shown that DNA regions required for ABA-induced expression in *C. plantagineum* do not contain the ABRE. Therefore, it is expected that there are multiple ABREs with different consensus DNA sequences. In addition, genes that are expressed during drought are also expressed during specific developmental stages and in specific cell types. Therefore, additional elements are required to control tissue- and organ-specific expression and other specific aspects of the expression pattern during water deficit (Fig. 2).

ABA-deficient mutants have been used to define an addi-

tional set of genes, those that are responsive to ABA but do not require ABA for expression (Gilmour and Thomashow, 1991; Nordin et al., 1991; Yamaguchi-Shinozaki and Shinozaki, 1993). These genes are induced by ABA application, but are also induced by water deficit in the ABA-deficient mutant of *Arabidopsis*. Therefore, it has been concluded that these genes do not require elevated levels of endogenous ABA for expression but are ABA-responsive genes (Fig. 2). These results indicate that there are two pathways that can be followed to induce these genes, but it is unknown if the pathways converge or if there are two entirely separate pathways. As promoter analyses are completed on these genes, it must be recognized that there may be multiple mechanisms of gene induction.

ABA applications have also been used to show that there are a number of water-deficit-induced genes that do not respond to ABA application (Guerrero et al., 1990; Yamaguchi-Shinozaki et al., 1992). These genes may be induced directly by the drought stress, or they may be controlled by other signaling mechanisms that may operate during water deficit.

Although the responsiveness of a gene is controlled by its DNA elements, the pathways that lead to the formation of stable transcription complexes are equally important for gene induction. For ABA-requiring and ABA-responsive genes, ABA must be recognized at the cellular level triggering a pathway(s) leading to the formation of transcription complexes and gene induction. Little is known about the mechanism of recognition of the ABA molecule. ABA analogs are being used to identify molecular structures required for gene induction by ABA (Van der Meulen et al., 1993).

After ABA recognition there are many possible pathways that could lead to active transcription complex formation. The pathway taken may be altered by the physiological state of the cell. The sensitivity to ABA is altered by the osmotic potential of the cells; there is increased sensitivity to ABA with increased osmotic stress. In some cases, osmoticum can completely replace exogenous ABA. For *Em* mRNA accumulation in rice cell cultures, increasing concentrations of NaCl increased the accumulation of *Em* mRNA in response to suboptimal concentrations of ABA (Bostock and Quatrano, 1992). Therefore, the cells' response to ABA may be altered by the water potential or water content of the cell. However, another possibility should be considered. Because the cells are induced to accumulate ABA in response to the osmotic stress, it becomes difficult to determine if the newly synthesized ABA is contributing to the induction of genes. ABA that is synthesized in the cell may not be located in the same compartment within the cell as ABA that is applied to the cell (Bray and Zeevaart, 1986). Therefore, the plant may be more sensitive to endogenous ABA than to applied ABA. It is known that high levels of ABA must be applied to elicit a response similar to that stimulated by endogenous ABA concentrations. Further studies with ABA-deficient mutants could be used to resolve this possibility.

FUTURE PERSPECTIVES

Recently progress has been achieved in the isolation of water-deficit-induced genes. Amino acid sequences have been used to predict functions and the genes have been used to follow gene expression. In the coming years, more attention must be paid to the roles of these genes under relevant stress conditions to determine if the expression of these genes is accentuated by the experimental conditions employed. Likewise, the same viewpoint must be used to determine the signal transduction pathways. Pathways that are induced in the laboratory may not be successfully induced in the field.

ACKNOWLEDGMENTS

I appreciate the critical reading of the manuscript by Drs. Mich B. Hein and Julia Bailey-Serres. I apologize to the many authors that I was not able to quote directly.

Received July 15, 1993; accepted September 20, 1993.
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