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Update on Signaling

Gene Expression and Signal Transduction in Water-Stress Response[1]

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Land plants suffer from dehydration or water stress not only under drought and high-salt-concentration conditions but also under low-temperature conditions. They respond and adapt to water stress to survive these environmental stress conditions. Water stress induces various biochemical and physiological responses in plants. Under water-stress conditions plant cells lose water and decrease turgor pressure. The plant hormone ABA increases as a result of water stress, and ABA has important roles in the tolerance of plants to drought, high salinity, and cold. A number of genes that respond to drought, salt, and cold stress at the transcriptional level have recently been described (for review, see Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 1996; Bray, 1997). The mRNAs of water-stress-inducible genes decrease when the plants are released from stress conditions, which is consistent with evidence that shows that these genes respond to water stress or dehydration. The functions of some gene products have been predicted from sequence homology with known proteins and are thought to have a role in protecting the cells from water deficit (Ingram and Bartels, 1996; Bray, 1997).

Expression patterns of dehydration-inducible genes are complex. Some genes respond to water stress very rapidly, whereas others are induced slowly after the accumulation of ABA. Most of the genes that respond to drought, salt, and cold stress are also induced by exogenous application of ABA (for review, see Shinozaki and Yamaguchi-Shinozaki, 1996; Bray et al., 1997). It appears that dehydration triggers the production of ABA, which in turn induces various genes. Several genes that are induced by water stress are not responsive to exogenous ABA treatment. These findings suggest the existence of both ABA-independent and ABA-dependent signal transduction cascades between the initial signal of drought or cold stress and the expression of specific genes (Shinozaki and Yamaguchi-Shinozaki, 1996; Bray et al., 1997). Promoter analysis of drought- and cold-inducible genes has identified several *cis*-acting elements that are involved in ABA-dependent and ABA-independent responses to conditions of water stress.

Details of molecular mechanisms regulating responses of plant genes to water stress remain to be discovered, and there are many questions to be examined at the molecular level. These include the sensing mechanisms of water stress or osmotic stress, modulation of the stress signals to cellular signals, transduction of the cellular signals to the nucleus, transcriptional control of stress-inducible genes, and the function and cooperation of stress-inducible genes allowing water-stress tolerance. This *Update* focuses on recent progress toward understanding the signal transduction cascades leading to expression of water-stress-inducible genes. Possible sensors of osmotic stress in plants are discussed based on our knowledge of yeast and bacterial sensors. A glossary of terms is included to facilitate the reading.

GLOSSARY OF TERMS

Promoter Regulatory Elements

ABRE, ABA-responsive element (PyACGTGGC).
G-box, Ubiquitous regulatory elements (CACGTG).
DRE, Dehydration-responsive element (TACCGACAT).
MYBRS, MYB recognition sequence (PyAACPyPu).
MYCRS, MYC recognition sequence (CANNTG).

Proteins That Bind to Promoter Regulatory Elements

bZIP, A family of transcription factors with basic region and Leu-zipper motif.
MYC, A family of transcription factors with basic-helix loop-helix (bHLH) and Leu-zipper motif.
MYB, A family of transcription factors with Trp cluster motif.
VP1, A maize transcriptional activator that is mutated in the viviparous 1 mutant.

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Proteins of the Signal Transduction Pathways

PLC, Phospholipase C that produces two second messengers, inositol 1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol.
CDPK, Calcium-dependent protein kinase.
MAPK, Mitogen-activated protein kinase.
MAPKK, A protein kinase that phosphorylates MAPK.
MAPKKK, A protein kinase that phosphorylates MAPKK.
RSK, Ribosomal S6 protein kinase.
Two-component His kinase, Bacterial-type sensory kinase.
14-3-3 protein, A signaling molecule acting by kinase modulation and protein-protein interactions.

FUNCTION OF WATER-STRESS-INDUCIBLE GENES

A variety of genes have been reported to respond to water stress in various species, and the functions for many of the proteins they encode have been predicted from sequence homology with known proteins. Genes induced during water-stress conditions are thought to function not only in protecting cells from water deficit by the production of important metabolic proteins but also in the regulation of genes for signal transduction in the water-stress response (Fig. 1). Thus, these gene products are classified into two groups. The first group includes proteins that probably function in stress tolerance: water channel proteins involved in the movement of water through membranes, the enzymes required for the biosynthesis of various osmoprotectants (sugars, Pro, and Gly-betaine), proteins that may protect macromolecules and membranes (LEA protein, osmotin, antifreeze protein, chaperon, and mRNA binding proteins), proteases for protein turnover (thiol proteases, Clp protease, and ubiquitin), the detoxification enzymes (glutathione *S*-transferase, soluble epoxide hydrolase, catalase, superoxide dismutase, and ascorbate peroxidase). Some of the stress-inducible genes that encode proteins, such as a key enzyme for Pro biosynthesis, were overexpressed in transgenic plants to produce a stress-tolerant phenotype of the plants; this indicates that the gene products really function in stress tolerance (Kavi Kishor et al., 1995). The second group contains protein factors involved in further regulation of signal transduction and gene expression that probably function in stress response: protein kinases, transcription factors, PLC, and 14-3-3 proteins. Now it becomes more important to elucidate the role of these regulatory proteins for further understanding of plant responses to water deficit. The possible function of the drought-, high-salinity-, and cold-inducible genes were recently reviewed by Ingram and Bartels (1996).

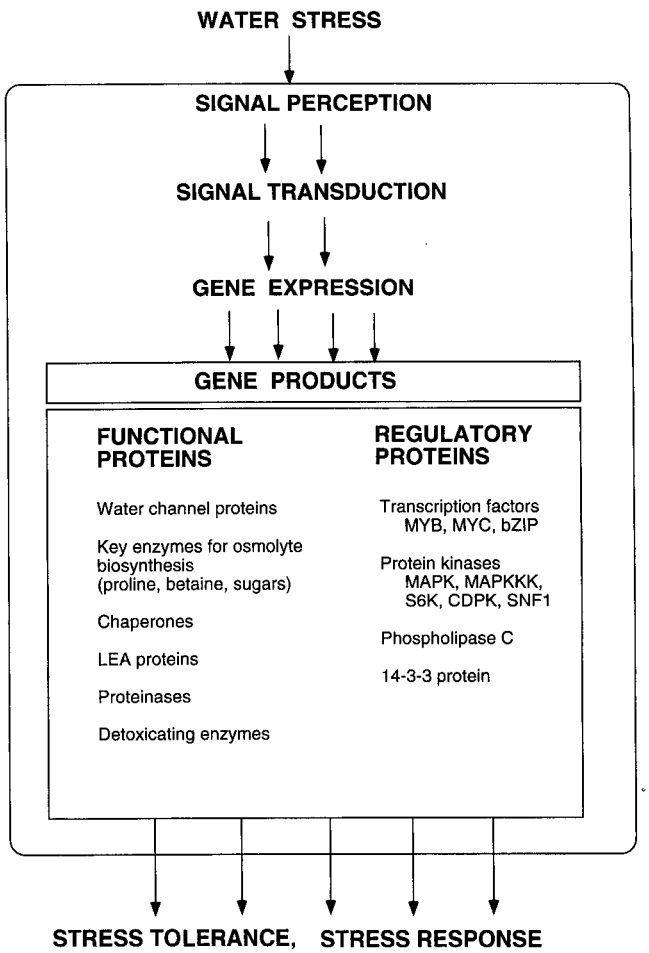

Figure 1. Function of water-stress-inducible gene products in stress tolerance and stress response. The gene products are roughly classified into two groups: functional proteins that are involved in water-stress tolerance and cellular adaptation, and regulatory proteins that may function in gene expression and signal transduction in stress response.

REGULATION OF GENE EXPRESSION BY WATER STRESS

Most water-stress-inducible genes respond to treatment with exogenous ABA, whereas others do not. Analyses of the expression of water-stress-inducible genes by ABA in ABA-deficient (*aba*) or ABA-insensitive (*abi*) Arabidopsis mutants have indicated that some of the stress-inducible genes do not require an accumulation of endogenous ABA under drought or cold conditions (for review, see Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 1996; Bray et al., 1997). Therefore, there are not only ABA-dependent pathways but also ABA-independent pathways involved in the water-stress response. Analysis of the expression of ABA-inducible genes revealed that several genes require protein biosynthesis for their induction by ABA, suggesting that at least two independent pathways exist between the production of endogenous ABA and gene expression during stress.

As shown in Figure 2, it is now hypothesized that at least four independent signal pathways function in the activation of stress-inducible genes under dehydration conditions (Shinozaki and Yamaguchi-Shinozaki, 1996): two are ABA dependent (pathways I and II) and two are ABA independent (pathways III and IV). One of the ABA-independent pathways overlaps with that of the cold response (pathway IV). One of the ABA-dependent pathways

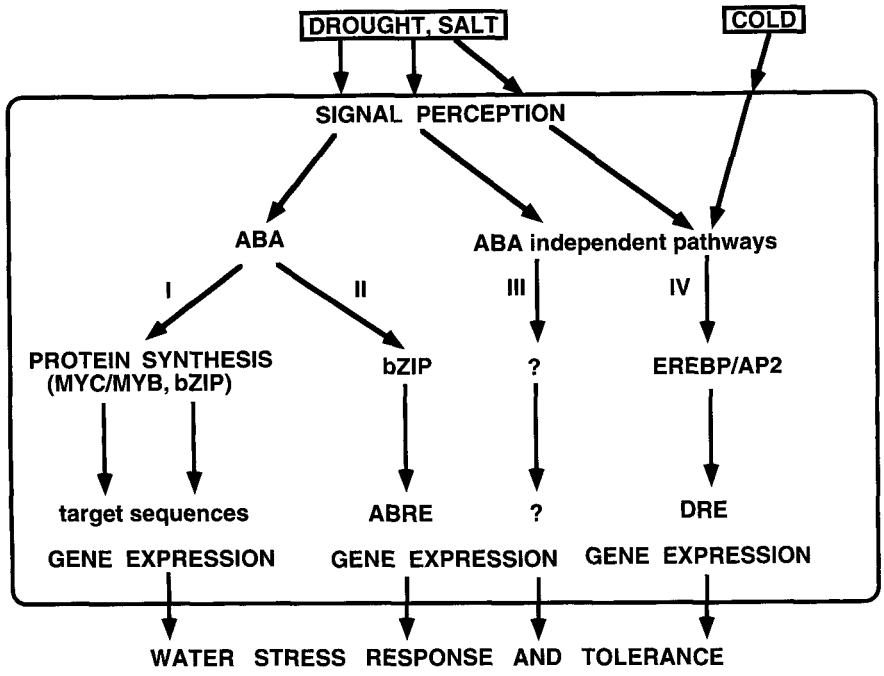

Figure 2. Signal transduction pathways between the perception of a water-stress signal and gene expression. At least four signal transduction pathways exist (I–IV): two are ABA-dependent (I and II) and two are ABA-independent (III and IV). Protein biosynthesis is required in one of the ABA-dependent pathways (I). In another ABA-dependent pathway, ABRE does not require protein biosynthesis (II). In one of the ABA-independent pathways, DRE is involved in the regulation of genes not only by drought and salt but also by cold stress (IV). Another ABA-independent pathway is controlled by drought and salt but not by cold (III).

requires protein biosynthesis (pathway II). Each pathway is discussed separately below.

ABA-Responsive Gene Expression during Water Stress (Pathway II)

Many water-stress-inducible genes are up-regulated by exogenous ABA treatment. The levels of endogenous ABA increase significantly in many plants under drought and high-salinity conditions (Ingram and Bartels, 1996; Bray, 1997). In one of the ABA-dependent pathways (Fig. 2, pathway II), water-stress-inducible genes do not require protein biosynthesis for their expression (for review, see Giraudat et al., 1994; Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 1996). These dehydration-inducible genes contain potential ABREs (PyACGTGGC) in their promoter regions. An ABRE functions as a *cis*-acting DNA element involved in ABA-regulated gene expression. ABREs were first identified in wheat *Em* and rice *rab* genes, and the ABRE-DNA-binding protein EmBP-1 was shown to encode a bZIP protein. The G-box resembles the ABRE motif and functions in the regulation of plant genes in a variety of environmental conditions, such as red light, UV light, anaerobiosis, and wounding. cDNAs for ABRE and G-box-binding proteins have been isolated and have a basic region adjacent to a Leu-zipper motif (bZIP) and constitute a large gene family. Nucleotides around the ACGT core motif have been shown to be involved in determining the binding specificity of bZIP proteins (for review, see Menkens et al., 1995). Furthermore, a coupling element is required to specify the function of the ABRE, constituting an ABA-responsive complex in the regulation of the *HVA22* gene (Shen and Ho, 1995). However, it has not been resolved how ABA activates bZIP proteins to bind to ABRE and initiate transcription of ABA-inducible genes. Further studies are necessary for the precise understanding of the molecular mechanisms of ABA-responsive gene expression that require ABRE as a *cis*-acting element.

There are several *cis*-acting elements other than ABRE that function in ABA-responsive gene expression not only under water-stress conditions but also in seed desiccation. The Sph box and GTGTC motifs regulate ABA- and VP1-dependent expression of the maize *C1* gene, whose product is an MYB-related transcription factor and functions as a controlling element in anthocyanin biosynthesis during seed development (for review, see McCarty, 1995). VP1 encodes a transcriptional activator and is thought to cooperate with bZIP proteins, and the Arabidopsis ABI3 protein has sequence and functional similarity with maize VP1.

ABA-Dependent Gene Expression Requiring Protein Biosynthesis (Pathway I)

In one of the two ABA-dependent pathways (Fig. 2, pathway I), biosynthesis of protein factors is necessary for the expression of water-stress-inducible genes. The induction of an Arabidopsis drought-inducible gene, *rd22*, is mediated by ABA and requires protein biosynthesis for its ABA-dependent expression (Shinozaki and Yamaguchi-Shinozaki, 1996). A 67-bp region of the *rd22* promoter is essential for this ABA-responsive expression and contains several conserved motifs of DNA-binding proteins, such as MYC and MYB, but this region has no ABREs (Iwasaki et al., 1995). A cDNA for a transcription factor MYC homolog, named rd22BP1, was cloned by the DNA-ligand-binding method using the 67-bp DNA as a probe. The *rd22BP1* gene is induced by drought and salt stress. These results suggest that a drought- and salt-inducible MYC homolog might function in the ABA-inducible expression of *rd22* (Abe et al., 1997). The *Atmyb2* gene that encodes a MYB-related

protein is induced by dehydration stress (Urao et al., 1993). High-salt-concentration conditions and application of exogenous ABA also result in the induction of *Atmyb2*, although *Atmyb2* does not respond to cold or heat stress. Recombinant ATMYB2 protein binds to the MYBRS in the 67-bp region of the *rd22* promoter. Therefore, the ATMYB2 protein might also cooperatively function with the rd22BP1 protein as a transcription factor that controls the ABA-dependent expression of the *rd22* gene (Fig. 2, pathway I; Abe et al., 1997).

Several bZIP transcription factors from rice, maize, and Arabidopsis plants (Kusano et al., 1995; Lu et al., 1996; Nakagawa et al., 1996) respond to cold, dehydration, and exogenous ABA treatment. These bZIP proteins bind to G-box-like sequences. These results suggest that ABA-inducible bZIP proteins are also involved in one of the ABA-dependent pathways (Fig. 2, pathway I). Many stress- and ABA-inducible genes encoding various transcription factors have now been reported. These transcription factors are thought to function in the regulation of ABA-inducible genes, which respond to water stress rather slowly after the production of ABA-inducible transcription factors (pathway I).

ABA-Independent Gene Expression during Water Stress (Pathways IV and III)

Several genes are induced by drought, salt, and cold in *aba* (ABA-deficient) or *abi* (ABA-insensitive) Arabidopsis mutants. This suggests that these genes do not require ABA for their expression under cold or drought conditions but do respond to exogenous ABA (for review, see Thomashow, 1994; Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 1996; Bray, 1997). These genes include *rd29A* (*lti78* and *cor78*), *kin1*, *cor6.6* (*kin2*), and *cor47* (*rd17*). Among them, the expression of a drought-inducible gene for *rd29A/lti78/cor78* was extensively analyzed (Yamaguchi-Shinozaki and Shinozaki, 1994). At least two separate regulatory systems function in gene expression during drought and cold stress; one is ABA independent and the other is ABA dependent. A 9-bp conserved sequence, TACCGACAT, termed DRE, is essential for the regulation of the induction of *rd29A* under drought, low-temperature, and high-salt-concentration stress conditions but does not function as an ABRE (Fig. 2, pathway IV). The *rd29A* promoter contains ABRE, which probably functions in ABA-responsive expression. DRE-related motifs have been reported in the promoter regions of many cold- and drought-inducible genes (Thomashow, 1994; Shinozaki and Yamaguchi-Shinozaki, 1996). These results suggest that DRE-related motifs, including C-repeat, which contains a CCGAC core motif, are involved in drought- and cold-responsive but ABA-independent gene expression. Protein factor(s) that specifically interact with the 9-bp DRE sequence were detected in a nuclear extract prepared from either dehydrated or untreated Arabidopsis plants (Yamaguchi-Shinozaki and Shinozaki, 1994). Recently, several independent cDNAs for DRE/C-repeat-binding proteins have been cloned (Stockinger et al., 1997; H. Liu, Q. Abe, K. Yamaguchi-Shinozaki, and K. Shinozaki unpublished data) using the yeast one-hybrid-screening method. All of the DRE/C-repeat-binding proteins contain a conserved DNA-binding motif that has also been reported in EREBP and AP2 proteins (EREBP/AP2 motif) that are involved in ethylene-responsive gene expression and floral morphogenesis, respectively. Analyses of the transcriptional control with these DRE/C-repeat-binding proteins will provide a precise mechanism of the ABA-independent pathway in the water-stress response.

There are several drought-inducible genes that do not respond to either cold or ABA treatment, which suggests that there is a fourth pathway in the dehydration-stress response (Fig. 2, pathway III). These genes include *rd19* and *rd21*, which encode different thiol proteases, and *erd1*, which encodes a Clp protease regulatory subunit (Nakashima et al., 1997). Promoter analysis of these genes will give us more information about the pathway III.

SIGNAL TRANSDUCTION IN RESPONSE TO WATER STRESS

Signal transduction cascades from the sensing of water stress signals to the expression of various genes and the signaling molecules that function in the cascade have not been extensively studied in plants and are attractive research subjects. Stomata closure is well characterized as a model system in the responses of plant cells to water stress (Kearns and Assmann, 1993; Giraudat et al., 1994). During stomata closure, the level of cytoplasmic Ca^{2+} is increased, which suggests that Ca^{2+} functions as a second messenger in the osmotic stress response. In animal cells, IP_3 is involved in the release of Ca^{2+} into the cytoplasm from intracellular stores, and it may play a similar role in plant cells. Ca^{2+} and IP_3 are the most probable candidates as second messengers in water-stress responses in plant cells (Fig. 3; for review, see Coté, 1995). Phosphorylation processes are now thought to have important roles in various signal transduction cascades in plants as well as in yeasts and animals. Various protein kinases have been reported in plants and are thought to function in phosphorylation processes in various signal transduction pathways, including water-stress and ABA responses (Fig. 3; Shinozaki and Yamaguchi-Shinozaki, 1996).

Second Messengers

The turgor pressure of plant cells is subject to feedback in response to changes in external osmotic pressure. The cytoplasmic Ca^{2+} signal transduction pathway is involved in turgor regulation in plant cells (for review, see Coté, 1995; Niu et al., 1995). An increase of cytoplasmic Ca^{2+} serves to stimulate ion transport pathways under hypo-osmotic stress. Stomata closure is induced by the release of Ca^{2+} into the cytoplasm. Phosphoinositide signaling has been implicated in the elevation of cytoplasmic Ca^{2+} in guard cells because artificial elevation of IP_3 in the cytoplasm results in Ca^{2+} mobilization (Blatt et al., 1990; Gilroy et al., 1990). The IP_3 content has been demonstrated to increase following hyperosmotic stress. There are changes in the levels of precursor lipids to IP_3 and in the activity of

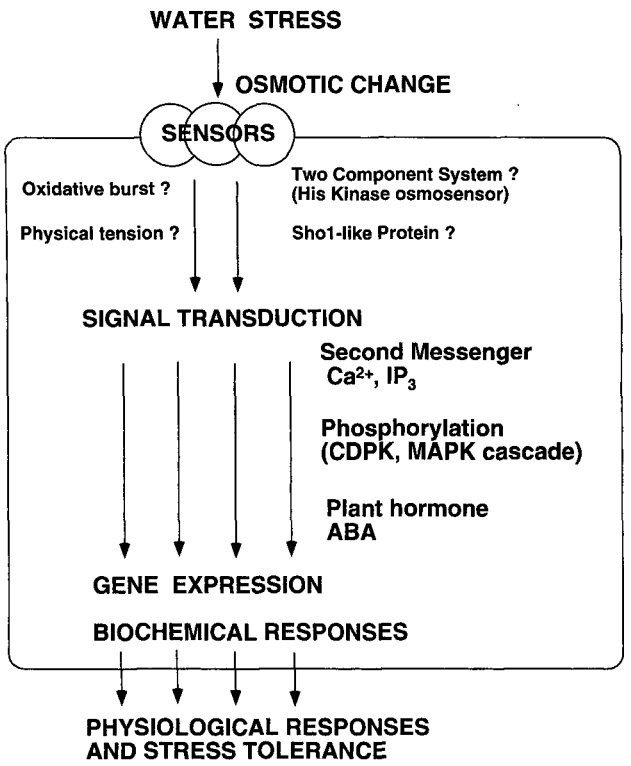

Figure 3. Second messengers and factors involved in the signal perception and the signal transduction in water-stress response. Two-component His kinase is thought to function as an osmosensor in plants. Ca^{2+} and IP_3 are the most probable second messengers of the dehydration signal. The phosphorylation process functions in water-stress and ABA signal transduction pathways. ABA plays important roles in the regulation of gene expression as well as physiological responses during water stress.

enzymes involved in the metabolism of inositol phospholipids after hyperosmotic stress. Phosphatidylinositol 4,5-bisphosphate levels decrease and IP_3 levels increase prior to ABA-induced stomatal closure in guard cells. The competence of vacuoles to respond to IP_3 is enhanced by hyperosmotic stress. These observations provide more evidence for the role of phosphoinositide signaling in osmotic responses.

Cytoplasmic pH is another possible second messenger of ABA signaling in guard cells, and it functions in the Ca^{2+}-independent pathway. ABA evokes an alkalization of the cytoplasm of guard cells, and this has a relationship with the activation of outward-rectifying K^+ channels by ABA (for review, see Giraudat et al., 1994).

Water-Stress-Inducible Genes for Signaling Factors

In higher plants many genes involved in signal transduction pathways, such as those encoding for calmodulins, G-proteins, protein kinases, and transcription factors, are induced by environmental stimuli. The genes for several protein kinases and for PLC are also induced by drought, salt, and cold stress (Shinozaki and Yamaguchi-Shinozaki, 1996).

A cDNA for PLC, AtPLC1, was isolated from dehydrated Arabidopsis (Hirayama et al., 1995). The *AtPLC1* gene is strongly induced by salt and drought and slightly induced by cold at the transcriptional level. Moreover, two genes for the CDPKs, *ATCDPK1* and *ATCDPK2*, have been demonstrated to be rapidly induced by drought and salt stresses in Arabidopsis (Urao et al., 1994). The stress-inducible PLC and CDPKs might function in the signal transduction cascade under water stress (Fig. 3). In animals, PLC digests phosphatidylinositol 4,5-bisphosphate to generate two second messengers, IP_3 and 1,2-diacylglycerol. IP_3 induces the release of Ca^{2+} into the cytoplasm, which in turn causes various responses in the cytoplasm. In plants a similar system may function in the water-stress response. Recently, co-expression of the constitutively active catalytic domain of a stress-inducible CDPK, ATCDPK1, was demonstrated to induce the expression of an ABA-inducible HVA1 promoter-reporter fusion gene in maize protoplasts (Sheen, 1996). The HVA1 promoter is also activated not only by cold, high salt, and ABA treatment but also by Ca^{2+} in protoplasts. These observations also supportthat Ca^{2+} might function as a second messenger and that ATCDPK1 functions as a positive regulator in the signal transduction pathways under water-stress conditions in plants.

MAPK is involved in the signal transduction pathways associated not only with growth-factor-dependent cell proliferation but also with environmental stress responses in yeast and animals. Many genes for protein kinases involved in MAPK cascades have been identified. There are at least four subfamilies of MAPK based on phylogenetic analysis (for review, see Mizoguchi et al., 1997). One of the MAPK genes, ATMPK3, is induced at the mRNA level by drought, low temperature, high salinity, and touch (Mizoguchi et al., 1996). Moreover, two genes for protein kinases involved in the MAPK cascade, MAPKKK (ATMEKK1) and ribosomal S6 kinase (RSK; ATPK19), are induced by similar stresses. Recently, alfalfa MAPK, MMK4, was demonstrated to be activated at posttranslational levels by a variety of stresses, including drought, low temperature, and mechanical stimuli (Jonak et al., 1996). The MMK4 gene is also induced by these stresses at the transcriptional level. These observations indicate that the MAPK cascades might function in the signal transduction pathways in the water-stress response (Fig. 3). In *Saccharomyces cerevisiae*, one of the MAPK cascades (Ssk2/Ssk22, Pbs2, and Hog1) functions in response and adaptation to high osmolarity (for review, see Wurgler-Murphy and Saito, 1997). Furthermore, the mammalian MAPKs p38 and JNK1 can functionally complement yeast *hog1*. These MAPKs are parts of the MAPK cascades that are activated by various stresses, including high osmolarity. There are several other signaling molecules of which genes are up-regulated by water stress (Fig. 1; Shinozaki and Yamaguchi-Shinozaki, 1996). Their roles in the water-stress response have not yet been elucidated.

ABA Signal Transduction

The role of ABA in water-stress signal transduction has been analyzed with ABA-insensitive mutants in various species. Of these, maize VP1 and Arabidopsis *abi1*, *abi2*, and *abi3* have been extensively characterized and their genes cloned. Among them, ABI1 and ABI2 gene products function mainly in vegetative tissues and also participate to some extent in seed development. Because of the wilty phenotypes of *abi1* and *abi2* mutants, ABI1 and ABI2 are thought to have important roles in ABA-dependent signal transduction pathways during water stress (for review, see Giraudat et al., 1994). The *ABI1* and *ABI2* genes have been cloned and shown to encode proteins that are related to type 2C protein Ser/Thr phosphatases (PP2Cs) (Leung et al., 1994, 1997; Meyer et al., 1994). The *ABI1* gene product functions in stomata closure, and the *abi1* plant accordingly has a wilty phenotype (Armstrong et al., 1995). ABI1 was demonstrated to function as a negative regulator in ABA-dependent gene expression in a transient expression experiment in which maize protoplasts were used (Sheen, 1996). By contrast, the dehydration-inducible ATCDPK1 encoding CDPK functions as a positive regulator in this regulation. These results indicate that a protein phosphorylation and dephosphorylation process might be involved in ABA-responsive signaling during water deficit. Recently, ABA was shown to induce a rapid and transient activation of MAPK in barley aleurone protoplasts (Knetsch et al., 1996). Correlation between ABA-induced MAPK activation and ABA-induced gene expression implies that MAPK might be involved in ABA signal transduction (Fig. 3). Another Arabidopsis mutant, *era*, that confers an enhanced response to exogenous ABA, has mutations in the *ERA1* gene, which encodes the β-subunit of farnesyl transferase (Culter et al., 1996). This suggests that a negative regulator of ABA sensitivity may require farnesylation to function.

Under water-stress conditions ABA is synthesized de novo, and this increase in ABA level requires protein biosynthesis. As mentioned above, this process is important for drought-inducible gene expression. Many ABA-deficient mutants that do not produce ABA have been isolated in various plants. Recently, an ABA-deficient tobacco mutant, *aba2*, was isolated by transposon-tagging using the maize Ac transposon (Marin et al., 1996). The ABA2 cDNA encodes a chloroplast-imported protein that exhibits zeaxanthin epoxidase activity, which functions in the first step of the ABA biosynthesis pathway. The tobacco ABA2 gene corresponds to the Arabidopsis ABA1 gene. Recently, Arabidopsis *aba2* and *aba3* mutants were isolated. Molecular analysis of the expression of these genes will aid the study of the regulation of ABA biosynthesis and ABA-dependent gene expression during water stress (Fig. 2).

SIGNAL PERCEPTION AND SENSORS OF WATER STRESS

Water deficits occur not only during drought and under conditions of high salt concentrations but also during cold conditions. They probably also cause the decrease of turgor pressure at the cellular level. A change in the osmotic potential across a plasma membrane, caused by the decrease of turgor pressure, might be a major trigger of the water-stress response at the molecular level. Osmosensors of yeasts have been extensively studied (for review, see Wurgler-Murphy and Saito, 1977), and cloning of osmosensors involved in the signal perception of water stress in plants is in progress based on the knowledge of osmosensors in yeast.

Osmosensors

The "two-component system" is known to be widespread and involved in various signal transduction pathways in bacteria. In *Escherichia coli*, a two-component system is involved in sensing osmotic change and osmotic responses. EnvZ, a two-component His kinase, functions as an "osmosensor," or a "sensory kinase," and monitors mechanical changes of the plasma membrane during osmotic stress (for review, see Wurgler-Murphy and Saito, 1997). EnvZ is activated by autophosphorylation at a His residue under hyperosmotic conditions and then phosphorylates an Asp residue of the OmpR protein, a "response regulator." Phosphorylated OmpR functions as a transcription factor to up-regulate the OmpC gene and down-regulate the OmpF gene. Both genes encode proteins of the bacterial outer membrane, and together these proteins regulate turgor pressure.

In yeast, exposure to high osmolarity activates a MAPK cascade that includes PBS2 (MAPKK) and HOG1 (MAPK) and then activates several genes involved in the biosynthesis of glycerol, which is an important osmoprotectant. Three gene products (Sln1p, Ypd1p, and Ssk1p) that act in an early phase of the hyperosmolarity-stress response encode signaling molecules that constitute a prokaryote-type two-component regulatory system (Posas et al., 1996; for review, see Wurgler-Murphy and Saito, 1997). Sln1p is thought to act as a sensor protein, phosphorylating response regulator proteins Ypd1 and Ssk1p under conditions of high osmolarity. The three protein factors perform a four-step phosphorelay (His-Asp-His-Asp). At high osmolarity phosphorylated Ssk1p activates Ssk2p or Ssk22p (MAPKKKs; Maeda et al., 1995), which results in the activation of Pbs2p (MAPKK) by Ser-Thr phosphorylation. Then, phosphorylated Pbs2p activates Hog1p (MAPK) by Thr-Tyr phosphorylation. A similar osmosensing mechanism might operate in higher plants in response to a water deficit. One of the two-component His kinases might also function as a osmosensor in water-stress response in higher plants because an Arabidopsis SLN1 homolog, ATHK1, was recently shown to complement yeast sln1 mutants and functions as a osmosensor in yeast (T. Urao, K. Yamaguchi-Shinozaki, and K. Shinozaki, unpublished data; Fig. 3). In higher plants another two-component His kinase, ETR1, is a receptor in ethylene signal transduction (Chang, 1996). Two-component His kinases may function as sensors or receptors in various signal transduction pathways in plants.

Another transmembrane osmosensor, Sho1p, has been reported by Maeda et al. (1995). Sho1p contains four closely packed hydrophobic transmembrane peptides. The COOH-

terminal region contains an SH3 domain that modulates various signal transduction pathways. Under conditions of high osmolarity, Sho1p activates the PBS2-HOG1 MAPK cascade. A Sho1p-like membrane protein might be another candidate as an osmosensor in plants (Fig. 3).

Other Cellular Triggers of Water-Stress Responses

Drought stress induces genes for detoxification enzymes, such as ascorbate peroxidase, superoxide dismutase, glutathione *S*-transferases, and soluble epoxide hydrolase (Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 1996). Cold stress also induces similar genes. An oxidative burst might function as one of the triggers of the water-stress responses.

In addition, change in the physical tension of cytoskeletons during water stress might be one of the triggers of osmotic responses. Some of the water-stress-inducible genes are also induced by touch (Mizoguchi et al., 1997). Touch not only induces the release of Ca^{2+} in the cytoplasm but also induces many genes, named touch genes, such as calmodulins, Ca^{2+}-binding proteins, xyloglucan endotransglycosylase, and protein kinases involved in the MAPK cascade. However, the sensing mechanism of oxidative burst or touch have not yet been identified.

CONCLUSIONS AND FUTURE PERSPECTIVES

Many genes that are regulated by water stress have been reported in a variety of plants. Analyses of stress-inducible gene expression have revealed the presence of multiple signal transduction pathways between the perception of water stress and gene expression. This explains the complex stress response observed after exposure of plants to drought, salt, and cold. At least four different transcription factors have been suggested to function in the regulation of dehydration-inducible genes; two are ABA responsive and two are ABA independent. The transcriptional regulatory regions of the dehydration-induced genes have been analyzed to identify several *cis*- and *trans*-acting elements that are involved in the water-stress response. A newly identified DRE *cis* element functions in the regulation of rapidly inducible genes in an ABA-independent manner. ABRE functions in the induction of genes after the accumulation of ABA during water stress. Several genes for transcription factors are induced by water stress and ABA at transcriptional levels, which might be involved in the regulation of slowly induced stress-involved genes. In addition, many genes for factors involved in the signal transduction cascades, such as protein kinases and PLC, are regulated by water-stress signals (Shinozaki and Yamaguchi-Shinozaki 1996; Mizoguchi et al., 1997). These signaling factors might be involved in the amplification of the stress signals and adaptation of plant cells to water-stress conditions. Based on the knowledge of osmosensors in yeasts and bacteria, cloning of homologs of the two-component His kinase as osmosensors in higher plants is in progress.

Molecular analyses of these factors should provide a better understanding of the signal transduction cascades during water stress. Transgenic plants that modify the expression of these genes will give more information about the function of their gene products. Recently, mutants that had a resistant or a sensitive phenotype to water stress were reported. Isolation of these mutant genes will give more information concerning factors involved in the signal transduction cascades and sensors. A combination of genetic and molecular approaches will give more insight into the molecular mechanisms of water-stress responses in plants.

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LITERATURE CITED

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