Bot. Bull. Acad. Sin. (2005) **46:** 1-10

(*Invited review paper*)

Hydrogen peroxide functions as a stress signal in plants

Shu-Hsien HUNG[1], Chih-Wen YU[2], and Chin Ho LIN[3,*]

[1]*Bioindustry Technology Department;* [2]*Molecular Biotechnology Department, Da Yeh University, Taiwan*
[3]*Life Science Department, National Chung Hsing University, Taichung, Taiwan*

Abstract. Plants have evolved complex regulatory mechanisms in adapting to various environmental stresses. One of the consequences of many stresses is an increase in the cellular concentration of reactive oxygen species (ROS), which are subsequently converted to hydrogen peroxide (H_2O_2). An oxidative burst caused by biotic or abiotic stress leads to a disturbance in the cellular redox balance and is highly toxic to cells. Recently, H_2O_2, in addition to being a toxicant, has been regarded as a signaling molecule and a regulator of the expression of some genes in cells. These include genes encoding antioxidants, cell rescue/defense proteins, and signaling proteins such as kinase, phosphatase, and transcription factors. Here, we review the function of H_2O_2 as a signal molecule in the transduction of stress signals to the alteration of expression profiles of target genes, and we summarize the evidence that H_2O_2 acts as a stress signal in plants.

Keywords: Environmental stresses; Hydrogen peroxide (H_2O_2); Reactive oxygen species (ROS); Stress signal.

Abbreviations: ABA, abscisic acid; **AP-1,** activator protein-1; **APX,** ascorbate peroxidase; **AsA,** ascorbic acid; **AtCBL1,** Arabidopsis calcineurin-B-like protein; **BSO,** buthionine sulfoximine; **CAT,** catalase; **CBF1,** C-repeat binding factor; ***COR*,** cold regulated gene; **CRT/DRE,** C-repeat and dehydration-responsive element; **DHAR,** dehydroascorbate reductase; **DREBs,** DRE binding proteins; **γ-ECS,** gamma-glutamylcysteine synthetase; **GPX,** glutathione peroxidase; **GR,** glutathione reductase; **GSH,** glutathione; **GSSG,** glutathione disulfide; **GST,** glutathione S-transferase; **H_2O_2,** hydrogen peroxide; **$HO^{\cdot}$,** hydroxyl radical; **MAPKs,** mitogen-activated protein kinases; **MAPKKs,** mitogen-activated protein kinase kinases; **MAPKKKs,** mitogen-activated protein kinase kinase kinases; **MDHAR,** monodehydroascorbate reductase; **NF-kB,** nuclear factor kB; **NPK-1,** *Nicotiana* protein kinase-1; **$O_2^{\cdot-}$,** superoxide radical; **PCD,** programmed cell death; **POX,** peroxidase; **PP2C,** protein phosphatase 2C; **PTPs,** phosphotyrosine-specific phosphatases; **ROS,** reactive oxygen species; **SOD,** superoxide dismutase; **UV,** ultra-violet.

Contents

Introduction

In nature, plants are exposed to various stresses, which affect their physiology, morphology, and development. Every year, environmental stress causes considerable losses in the productivity of many crops. Among these stresses, the fluctuation of temperature, the water status of soil, and the intensity of light are the most crucial signals affecting plant growth (Boyer, 1982; Trewavas and Malhó, 1997). In addition to external stimuli, a variety of internal signals, such as hormones and nutrient conditions, also modify a plant's metabolism, growth, and development. Hence, rapid and precise perception of and response to various stimuli are important as plants adapt to changing natural environments. However, how a plant perceives environmental changes and how it subsequently triggers signals to activate the physiological response are yet to be explored.

Extensive study on oxidative stress has demonstrated that exposure of plants to adverse environmental conditions induces the overproduction of ROS, such as superoxide radical ($O_2^{\cdot-}$), H_2O_2, and hydroxyl radical ($HO^{\cdot}$) in plant cells (Wise and Naylor, 1987). ROS are highly reactive to

*Corresponding author. Tel: 886-4-22862511; Fax: 886-4-22862511; E-mail: chlin@mail.nchu.edu.tw

membrane lipids, protein, and DNA; they are believed to be the major contributing factors to stress injuries and to cause rapid cellular damage (Hariyadi and Parkin, 1993; O'Kane et al., 1996; Prasad, 1996), particularly when plants are exposed to low temperature. At low temperature, electron transport chains tend to form $O_2^{\cdot-}$, which dismutates to form H_2O_2. In chloroplasts, low temperature limits the light reactions, restricting the supply of $NADP^+$ and promoting the reduction of O_2 by photosystem I. In mitochondria, inhibition of ATP formation or electron flow through cytochrome *b* stimulates $O_2^{\cdot-}$ formation by complex I and by ubiquinone (Elstner, 1991). Therefore, more serious damage is observed when plants are exposed to low temperature in combination with high light intensities, drought, air pollutants (e.g. ozone or sulphur dioxide), ultraviolet light, and herbicides (e.g. paraquat) (Inzé and Van Montagu, 1995).

Plants have evolved both enzymatic and non-enzymatic mechanisms to scavenge the rapidly evolving ROS under low temperature or other stresses. Enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX) and glutathione reductase (GR) (Zhang et al., 1995; Lee and Lee, 2000), and non-enzymatic antioxidants such as tocopherols, ascorbic acid (AsA), and glutathione (GSH) (Wingsle and Hallgren, 1993; Kocsy et al., 1996; Noctor et al., 1998) work in concert to detoxify ROS. Among the antioxidant mechanisms AsA is a key antioxidant for elimination of ROS, especially H_2O_2. The reaction of AsA with H_2O_2 can occur directly or it can be catalysed by APX. APX, a key H_2O_2-scavenging enzyme, is found in the cytosol, chloroplasts, and mitochondria of higher plants (Mittler and Zilinskas, 1991; Patterson and Poulos, 1995; Jimenez et al., 1997; Asada, 1999). The regeneration of AsA is catalysed directly by reduced ferredoxin in photosystem I (Asada, 1999) and by monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and GSH. APX, MDHAR, DHAR, GSH/glutathione disulfide (GSSG), NADPH, and GR together form the AsA–GSH cycle, an H_2O_2-scavenging pathway (Foyer and Halliwell, 1976; Noctor et al., 1998). In accordance with its role in scavenging H_2O_2, APX expression is induced in response to chilling (Mittler and Zilinskas, 1992; Thomsen et al., 1992; Kubo et al., 1995; Karpinski et al., 1997). Glutathione is a crucial antioxidant associated with regenerating AsA in the AsA–GSH cycle, and thus GSH is also involved in the regulation of H_2O_2 concentrations and control of the redox state in plant cells (Kocsy et al., 2000a, b; Kocsy et al., 2001).

Our present understanding about the stress signaling in plants is that the changes of GSH/GSSG, following the synthesis of H_2O_2, constitute an early stress signal leading to the physiological response, usually activation of antioxidant mechanisms or modification of gene expression (Trewavas and Malhó, 1997). It is suggested that GSH and GR are important factors participating in the redox state regulation of plant cells. The ratio of GSH to GSSG decreases under stress conditions. GSH is oxidized for the reduction of excess H_2O_2 in the AsA-GSH cycle (Foyer et al., 1997; Kocsy el al., 2001; Yu et al., 2002) upon stress.

The increase in H_2O_2 level and subsequent changes in the GSH/GSSG ratio during chilling seem to be involved in redox signaling, which activates specific transcription factors and antioxidative enzymes. Indeed, it has been shown that pretreatment of seedlings with H_2O_2 induces chilling tolerance (Yu et al., 2002; Yu et al., 2003). More studies have provided evidence that H_2O_2 itself is a key signal molecule mediating a series of responses. Other studies suggest that H_2O_2 is a signal mediator for programmed cell death (PCD) of plants in response to pathogens, elicitors, and hormones (Levine et al., 1994; Tenhaken et al., 1995; Levine et al., 1996; Desikan et al., 1998; Mittler et al., 1999; Solomon et al., 1999; Bethke and Jones, 2001). Furthermore, a number of studies indicate that H_2O_2 is synthesized in response to exogenous abscisic acid (ABA) and that H_2O_2 mediates, at least in part, ABA responses, including stomatal closure and gene expression (Guan et al., 2000; Pei et al., 2000). Using cDNA microarray technology to carry out a transcriptomic analysis, Desikan et al. (2001a) provided further evidence of H_2O_2 as a central signaling mediator. Their study showed that the expression of some genes is up-regulated by H_2O_2. In addition, some are repressed. Classified by their potential biological functions, these genes include heat shock proteins, heat shock transcription factors, mediators for calcium signal transduction, such as calmodulin, important signaling enzyme protein tyrosine phosphatases (PTPs), a blue copper-binding protein which is an essential catalyst for redox reactions, a mitochondrial uncoupling protein, pyruvate decarboxylase, and a *myb*-related transcription factor. In addition, some of the H_2O_2-sensitive genes could also be involved in plant hormone signaling. It is interesting to note that various genes encoding potential transcription factors were induced by H_2O_2, suggesting that these transcription factors mediate further downstream H_2O_2 responses and finally induce physiological changes to promote adaptation to stresses.

Our knowledge about the scope of stress-induced signaling in plants leading to modification of gene expression has increased in recent years. A number of signaling molecules have been demonstrated to be involved in the stress signaling pathway. Evidence that H_2O_2 serves as a messenger to pass the stress signal to downstream responses in plants is presented below.

The Generation and Removal of H_2O_2

Stress often leads to the production of ROS such as $O_2^{\cdot-}$ and H_2O_2 in plant tissues (Desikan et al., 2003). ROS are highly active molecules that can easily damage membrane and other cellular components. Hence, it is important to remove ROS to avoid chilling or other stress-induced injuries. Anti-oxidation mechanisms of the cell include the enzymatic ROS-scavenging system and non-enzymatic antioxidants. Scavenging mechanisms for ROS involve these enzymes: SOD, CAT, APX, MDHAR, DHAR, glutathione peroxidase (GPX), and GR (Payton et al., 2001).

H_2O_2 can be directly decomposed through CAT. On the other hand, by combining APX with GR, H_2O_2 can also be removed via recurrent oxidation-reduction reactions promoted by GSH, hence preventing cell damage.

Non-enzymatic antioxidants include pigments, vitamin E, tocopherol, AsA, and GSH (Wingsle and Hallgren, 1993). Among these, GSH is most important. Glutathione is a common cellular component. Its biological functions include: (1) adjusting gene performance (May et al., 1998; Wingate et al., 1988); (2) acting as a precursor in the synthesis of phytochelatin; (3) serving as a substrate of glutathione S-transferase (GST), and thus assisting in clearing the harmful components in the cell (Marrs, 1996); (4) relating to cell cycle control (Gyuris et al., 1993; Russo et al., 1995; Shaul et al., 1996; Sanchez-Fernández et al., 1997) ; and (5) playing the key role in the AsA-GSH cycle. This last one is generally considered the most important biological function of GSH (Alscher, 1989; May et al., 1998; Kocsy et al., 2001). Therefore, elevating the total quantity of GSH within a plant becomes the critical step in reducing stress injury (Kocsy et al., 2000a, b; Kocsy et al., 2001).

The fluctuation of the GSH level also could be a good indicator of the redox status within plant cells. Therefore, it is interesting to monitor this fluctuation in stressed plant cells. For example it was observed that mung bean seedlings receiving various stresses—chilling, ozone, and heat shock treatments—showed 2.8, 3.9, and 1.9-fold increases, respectively, in transient GSH level over the level in the control plants. Salt stress and flooding treatments produced moderate GSH increases of 36% and 60%, respectively. Interestingly, the GSH content of drought stressed mung bean plants dropped to 52% of the control level. Using buthionine sulfoximine (BSO) to inhibit reduced GSH synthesis, our previous studies also demonstrated that GSH is the "key" molecule in the acclimation of mung beans to chilling stress (Yu et al., 2002; Yu et al., 2003). Thus, the gene coding for gamma-glutamylcysteine synthetase (γ-ECS) (the rate limiting enzyme in the synthesis of GSH) must be a critical downstream target gene of H_2O_2 triggered signal transduction.

Together, these defensive responses imply that one consequence of many stresses is an increase in the concentration of ROS (Mittler, 2002). ROS are generated via electron transport reactions both in mitochondria and chloroplasts (Wise and Naylor, 1987; Elstner, 1991) and are speedily converted to H_2O_2. In cells, H_2O_2 is also generated via several enzyme-mediated reactions, such as glycollate oxidase during photorespiration, acyl CoA oxidase during β-oxidation, oxalate oxidase that converts oxalate and oxygen to H_2O_2 and CO_2, and NADPH oxidase (a plasma membrane located enzyme) that transfers electrons from NADPH to molecular oxygen to generate $O_2^{\cdot-}$ (subsequently converted to H_2O_2) (Desikan et al., 2003). The effects of various stresses on H_2O_2 generation have not yet been completely revealed, nor is it known which if any of these enzymes is involved in stress-induced H_2O_2 synthesis.

Glutathione was shown to regulate the redox status of cells. Disturbance of cellular redox homeostasis may activate transcription factors and gene expression of those involved in the protection of plants against damage during chilling and cold acclimation (Kocsy et al., 2001). This may be explained by the fact that the stress-induced oxidized state of the cell may lead to the disulfide bridge formation in proteins, including transcription factors. The modification of proteins may lead to conformational change and consequently activate transcription factors, which will in turn control the expression and accumulation of certain antioxidants, as described for *E. coli* (Aslund and Beckwith, 1999).

H_2O_2 as a Signaling Molecule

ROS are extremely reactive molecules that have high affinity to membranes, DNA, or proteins in plant cells. It is not surprising that until recently ROS were still viewed mainly as toxic cellular metabolites in plants. However, increasing lines of evidence support the idea that ROS also function as signaling molecules that mediate responses to various stimuli (Desikan et al., 2004). Among ROS, H_2O_2 seems best suited to play the role of signaling molecule due to its higher stability and longer half-life. If H_2O_2 serves as a stress signal, the fluctuation of H_2O_2 level in plants should spatially and temporally reflect changes in the environment. Indeed, an oxidative burst is a common response to both biotic and abiotic stresses (Desikan et al., 2003). These stresses include pathogens, elicitors, wounding, heat, low temperature, ultra-violet (UV) light, and ozone. For example in winter wheat (*Triticum aestivum* L.) leaf, chilling at 4°C caused the H_2O_2 level to increase to threefold that of control within 1 min (Okuda et al., 1991). Similarly, ROS levels shot up during chilling of non-acclimated maize seedlings.

It is further reported that maize seedlings pretreated with H_2O_2 or menadione, an $O_2^{\cdot-}$-generating compound, acquire additional chilling tolerance as compared with control plants (Prasad et al., 1994). These findings suggest that chilling-induced ROS appears as a message to induce antioxidant systems in cells. In addition to plants, H_2O_2 has been also demonstrated to act as a secondary messenger in mammalian cells, mediated through the activation of transcription activators, such as nuclear factor kB (NF-kB) (Schreck et al., 1991) and AP-1 (activator protein-1; a complex composed of *jun* and *fos* gene products) (Rao et al., 1996). Thus H_2O_2 seems to serve as a common stress signal in organisms.

For H_2O_2 to really be a specific signaling molecule, a mechanism must exist to perceive the elevation of H_2O_2 in cells. H_2O_2 can interact with cysteine residues within proteins. This redox modulation of protein could potentially alter protein conformation, affecting protein activity, and therefore initiating subsequent cellular responses.

The yeast *Saccharomyces cerevisiae* has been a useful model for exploring the eukaryotic response to oxida-

tive stress. The modifications of thiol residues leading to conformational change of protein by H_2O_2 have been demonstrated in vitro for the yeast transcription factor YAP1. Coleman et al. (1999) have shown that controlling the activity of the positive transcriptional regulator YAP1 is a key step in inducing normal tolerance of oxidative stress elicited by the redox-active agents diamide and H_2O_2. A cysteine-rich domain of YAP1 was required for H_2O_2 resistance, which influenced the nuclear localization of YAP1 when it was activated. Two cysteines in this domain are essential for YAP1 oxidation.

Information on how higher organisms perceive the change of the cellular redox state is still limited. Our previous studies showed that H_2O_2 applied to the abaxial surface of a *Phalaenopsis* leaf increased chilling tolerance effectively; however, adaxial surface application was not effective. Although H_2O_2 can freely diffuse across membranes (Bowler et al., 1992), no changes in the leaves' endogenous H_2O_2 levels were observed after exogenous application of H_2O_2 (Yu et al., 2003). These observations suggest that receptors on the plant surface, interacting with H_2O_2 molecules, might initiate the signal for development of chilling tolerance. Alternatively, the difference between the abaxial and adaxial treatment effects might have been due to the easier access of H_2O_2 molecules to receptors located in the mesophyll through the abaxial stomata. In either case, the receptor seems to be isolated from the major pool of endogenous H_2O_2.

H_2O_2 was also clearly demonstrated to play a prominent role in the transduction of ABA signals in Arabidopsis (Meinhard and Grill, 2001; Meinhard et al., 2002) by regulating the activity of phosphatase. In this pathway, Arabidopsis protein phosphatase 2C (PP2C) enzymes ABI1 and ABI2 serve as direct targets for H_2O_2 modification of cysteine residues in vitro (Meinhard and Grill, 2001; Meinhard et al., 2002). ABI1 and ABI2 are type 2C serine/threonine protein-phosphatases that are negative regulators of ABA signaling (Lenug et al., 1997). Upon H_2O_2 challenge in vitro, ABI1 and ABI2 are inactivated rapidly, with an IC_{50} value of 140 μM for ABI1 and 50 μM for ABI2 in the presence of physiological concentrations of GSH (Meinhard and Grill 2001; Meinhard et al., 2002). Based on these findings, ABI1 and ABI2 represent a likely receptor for the H_2O_2 signal in higher plants.

It is reported that, in animals, H_2O_2 activates the transcription factor NF-kB (Schreck et al., 1991). NF-kB usually resides in the cytoplasm of the cell in association with an inhibitor, Ik-B. Addition of H_2O_2 to cells results in the dissociation of NF-kB from Ik-B and translocation of activated NF-kB into the nucleus, thus triggering related gene expression. Other transcriptions affected by exogenous H_2O_2 include AP-1 (Rao et al., 1996), Myb (Myrset et al., 1993), and Ets (Wasylyk and Wasylyk, 1993).

Role of Ca^{2+} in H_2O_2 Signaling Pathway

Cold signal transduction can be defined as a process that starts with the perception of low temperature and ends with the expression of genes encoding proteins that lead to increased chilling tolerance of plants. Ca^{2+} provides favorable features as a secondary messenger and serves as one of the major components in the cellular signaling pathways of many organisms (Sanders et al., 1999). Cytosolic Ca^{2+} signals can be viewed as the result of two opposing factors: influx into and efflux out of the cytosol (Sanders et al., 1999). Under ambient conditions, delicate control is required to maintain homeostasis; and upon instances of stress, monitoring the fluctuation of cytosolic Ca^{2+} concentration, $[Ca^{2+}_{cyt}]$, is necessary for interpreting the signal. Many studies have revealed that transient $[Ca^{2+}_{cyt}]$ fluctuation plays an essential signaling role in many stress responses (Monroy and Dhindsa, 1995; Sanders et al., 1999). Nevertheless, how various stress signals are mediated through Ca^{2+} to subsequently affect the expression of different downstream genes is still unclear. At least we know that many factors—including duration, frequency, and location of the Ca^{2+} signal, as well as interactions with other cellular components—affect the downstream response (McAinsh et al., 1997; Sander et al., 1999).

Cold shock evoked transient increases in $[Ca^{2+}_{cyt}]$ level in tobacco and Arabidopsis (Knight et al., 1991; Knight et al., 1996; Polisensky and Braam, 1996; Lewis et al., 1997). These findings suggested that calcium influx plays a major role in the cold shock response and also that an intracellular calcium source might be involved. Further evidence indicated that H_2O_2 and Ca^{2+} were both involved in a signaling cascade leading to the closure of stomata in Arabidopsis (Pei et al., 2000). In this study, H_2O_2-activated Ca^{2+} channels mediated both the influx of Ca^{2+} in protoplasts and increases in $[Ca^{2+}_{cyt}]$ in intact guard cells (Pei et al., 2000). In addition to serving as a link in a signaling cascade, the fluctuation of $[Ca^{2+}_{cyt}]$ could be one of the mechanisms that lead plants to remember what they have suffered (Knight et al., 1996). This inference comes from the observation that Arabidopsis treated with either sublethal cold or H_2O_2 modifies its calcium signature in response to subsequent cold stress as compared to an untreated control (Knight et al., 1996). Based on these findings, it is proposed that a Ca^{2+} channel may be employed as a possible putative cold sensor in higher plants.

More lines of evidence concerning the relationship between H_2O_2 and Ca^{2+} signals were provided by the study of H_2O_2 homeostasis in Arabidopsis (Yang and Poovaiah, 2002). Yang and Poovaiah (2002) indicated that a close interaction exists between intracellular H_2O_2 and cytosolic Ca^{2+} in response to biotic and abiotic stresses. This study indicated that an increase in cytosolic Ca^{2+} boosted the generation of H_2O_2.

In summary, treatment with H_2O_2 could activate Ca^{2+} channels to elevate $[Ca^{2+}_{cyt}]$ level (Pei et al., 2000). For a certain period of time, this could be autocatalytic, because H_2O_2 production in the oxidative burst requires a continuous Ca^{2+} influx, which activates the plasma membrane-localized NADPH oxidase (Lamb and Dixon, 1997; Xing et al., 1997; Grant et al., 2000; Yang and Poovaiah, 2002). Later, the $[Ca^{2+}_{cyt}]$ elevation activates the calcium sensor

calmodulin and subsequently passes the signal to a downstream target "CAT." This finally down-regulates H_2O_2 levels by stimulating the plant CAT activity. Together, these results provide evidence indicating that Ca^{2+} has dual functions in regulating H_2O_2 homeostasis, which in turn influences redox signaling in response to environmental signals in plants (Yang and Poovaiah, 2002).

The Role of Kinases and Phosphatases in the H_2O_2 Signaling Pathway

Reversible protein phosphorylation and dephosphorylation, catalysed by protein kinases and protein phosphatases (PTPs), respectively, is a common molecular mechanism that passes on information within cells (Rudd and Franklin-Tong, 1999). Substrate specificity for protein phosphatases can be classified into two major groups, serine/threonine phosphatase and tyrosine phosphatase (Luan, 1998). Ca^{2+}-regulated protein phosphatase plays a role in cold signal transduction. The expression of Ca^{2+} binding calcineurin-B-like protein (AtCBL1), an effector of calcium signaling, is highly up-regulated by cold, drought and salt stresses in Arabidopsis (Kudla et al., 1999; Hasegawa et al., 2000). Notably, the expression of receptor-like PTPase, AtPTP1, is transiently down-regulated by cold treatment (Luan, 1998).

Studies of protein kinases have shown that mitogen-activated protein kinases (MAPKs) are activated by H_2O_2 in both animals and plants, which could lead to the modulation of gene expression (Kamata and Hirata, 1999; Zwerger and Hirt, 2001; Torres and Forman, 2003). Whether H_2O_2 has a direct effect on MAPK or activates upstream effectors is unclear (Hancock et al., 2001).

In addition to activating MAPK, H_2O_2 inhibits phosphatase activities, probably by the direct oxidation of cysteine in the active site of these enzymes. Hence, instead of positively transmitting a Ca^{2+} signal, some types of protein phosphatase such as PP2C act as a negative regulator of ABA signaling (Leung et al., 1994; Meyer et al., 1994; Sheen, 1998; Gosti et al., 1999; Merlot et al., 2001). The Arabidopsis with null mutations in *abi1* and *abi2* (genes coding for a PP2C) loses its susceptibility to ABA and shows a diminishing of ABA-induced $[Ca^{2+}_{cyt}]$ elevation (Allen et al., 1999; Murata et al., 2001). The *abi1* mutation has been shown to diminish cold-inducible gene expression, affecting the development of cold-induced freezing tolerance in *abi1*-knockout Arabidopsis (Leung et al., 1994; Meyer et al., 1994; Gosti et al., 1999; Merlot et al., 2001). These observations could explain at least part of the role of H_2O_2 in the ABA-triggered signal transduction pathway. However, the function of PTPase and its interaction with H_2O_2 in cold signal transduction remain to be elucidated.

Besides PTPase, the signal transduction in many eukaryotic cells is also controlled by protein phosphorylation by MAPKs (Stone and Walker, 1995; Gustin et al., 1998). In the MAPK cascade, mitogen-activated protein kinase kinase kinases (MAPKKKs) are the primary signal receiver, activated via phosphorylation by mitogen-activated protein kinase kinases (MAPKKs). In recent years various groups have shown that H_2O_2 is able to activate the MAPK cascade in plants and thus provide the linkage between an upstream H_2O_2 signal and downstream gene expression (Desikan et al., 1999; Grant et al., 2000; Kovtun et al., 2000; Desikan et al., 2001b). Activation of MAPKs by H_2O_2 is a critical step in mediating cellular responses to multiple stresses (Kovtun et al., 2000). The ANP class of MAPKKKs from Arabidopsis can be induced specifically by H_2O_2, and this then activates a specific class of stress-induced MAPKs (Kovtun et al., 2000). These MAPKs activated by ANP1 include AtMPK3 and AtMPK6 of Arabidopsis (Mizoguchi et al., 1996; Ichimura et al., 2000). By sequence comparison, Arabidopsis ANP1 is classified as a *Nicotiana* protein kinase-1 (NPK-1) like protein kinase. Transgenic plants over-expressing ANP1 show extra tolerance to heat shock, freezing, and salt stress (Kovtun et al., 2000). Recently, a related study found that Arabidopsis nucleoside diphosphate kinase 2 (NDPK2) is an important upstream signaling component of the AtMPK3 and AtMPK6-mediated signaling cascade, which fortifies plants against cold, salt, and oxidative stress (Moon et al., 2003). This phenomenon seems to occur because the expression of AtNDPK2 antagonizes the elevation of cytosolic H_2O_2 induced by stresses. Together, all of these data suggest that various stresses induce a generation of cytosolic H_2O_2, which in turn induces a MAPK cascade that subsequently stimulates expression of antioxidant genes, reducing H_2O_2 levels and restoring cellular homeostasis.

Transcriptional Regulation of Gene Expression in Response to H_2O_2

H_2O_2 modulates the expression of various genes, including those encoding antioxidant enzymes and modulators of H_2O_2 production (Neill et al., 2002). However, accessing the enzymes involved in the oxidative scavenging system can only provide pieces of a jigsaw puzzle, and it is difficult to visualize the whole picture of how plants respond to various environmental stimuli. Using cDNA microarray technology, Desikan et al. (2001a) have undertaken a large-scale analysis of the Arabidopsis transcriptome during oxidative stress. Of the transcripts, 113 were induced and 62 were repressed by H_2O_2. A comparison of the microarray results of oxidative stress with other stresses such as wilting, UV irradiation, and elicitor challenge showed overlapping expression of some of these genes (Desikan et al., 2001a). The H_2O_2-induced transcripts encoded proteins with functions such as metabolism, energy, protein destination and transport, cellular organization and biogenesis, cell rescue or defense, and transcription (Desikan et al., 2001a). Among these genes, the genes encoding potential transcription factors should be emphasized due to their capacity for activating the expression of downstream target genes. In Arabidopsis, a transcription factor CBF1 (C-repeat binding factor), binding to C-repeat and dehydration-responsive element (CRT/DRE), was first identified by Stockinger et al. (1997). CBF1

belongs to the APETALA2/EREBP-family of transcription factors. Five additional CBF1 homologous genes, namely DRE binding proteins (DREBs) and two additional CBFs, called CBF2 and CBF3, were subsequently cloned from Arabidopsis (Gilmour et al., 1998; Liu et al., 1998). Tomato seedlings (*Lycopersicon esculentum*) overexpressing an Arabidopsis *CBF1* cDNA showed a higher tolerance for water deficit stress. Subtractive hybridization showed that CAT activity increased and H_2O_2 concentration decreased in the transgenic tomato plants compared with the wild-type plants with or without water deficit stress (Hsieh et al., 2002). According to this result, CBF seems to be able to indirectly regulate the cytosolic redox status by activating downstream genes encoding antioxidant enzymes, for example CAT. Nevertheless, there still is no evidence connecting the H_2O_2 signal with CBF accumulation or activation.

The strongest evidence showing H_2O_2 itself activates transcription factor is provided by the study on yeast transcription factor YAP1 (Coleman et al., 1999). Recently, using a functional selection strategy, Arabidopsis cDNAs were overexpressed in the fission yeast *Schizoaccharomyces prombe*, and the transformants were screened for an enhanced ability to tolerate diamide-induced oxidative stress. The gene selected encodes a transcription factor named OXS2, which is related to oxidative stress tolerance in plants (Robert and David, 2004). This gene belongs to a five-member family of C_3H zinc finger protein genes. OXS2 protein can autoactivate its own promoter, and this correlates with the elevated accumulation of *OXS2* mRNA during oxidative stress (Robert and David, 2004). The regulation mechanism activating OXS2 is similar to that of YAP1. The inactivation of Exportin-mediated export of OXS2 and hence nuclear accumulation of OXS2, and the autoactivation of its own gene, are all caused by oxidative stress and followed by the activation of a stress-induced pathway (Robert and David, 2004). Apart from this study, the direct influence of H_2O_2 on transcription factor activity is, to date, still far from clear.

Conclusions

Currently, research data show that H_2O_2 can play a dual role in cells. During oxidative stress, H_2O_2 is a strong toxic oxidant causing cell damage or even cell death. At the same time it serves conversely as a signaling molecule to activate a rescue/defense system for restoring the redox homeostasis in plant cells. In addition, H_2O_2 is involved in mediating biological processes, including PCD (Desikan et al., 1998), ABA-mediated stomatal closure (Pei et al., 2000), auxin-regulated gravitropic responses (Joo et al., 2001), mechanical wounding response (Orozco-Cardenas et al., 2001), systematically acquired resistance (SAR) (Inzé and Van Montagu, 1995), plant pathogen interaction (Mittler et al., 1999), and some other processes relating to both biotic and abiotic stresses (Desikan et al., 2003).

So far it is still unclear whether H_2O_2 is situated at a common center for the signaling pathways providing responses to various environmental stimuli. Extensive work has indicated that H_2O_2 indeed plays a role in diverse cellular signals, especially signals triggered by stresses. These observations suggest the existence of "cross-talk" between different stresses (Shinozaki and Yamaguchi-Shinozaki, 2000). Hence, elevation of H_2O_2 concentration in cells is a physiological response to many stimuli and results in activation of downstream target genes. However, the same target gene induced by different stimuli always showed a dissimilar expression profile, an observation that needs to be clarified.

Based on current data, a model for an H_2O_2 signaling pathway is proposed here (Figure 1). An H_2O_2 signal may be perceived by a receptor and then result in elevated $[Ca^{2+}_{cyt}]$. Next, Ca^{2+} may activate a signaling protein such as a protein kinase or phosphatase to trigger a cascade that then alters the activity of a transcription factor by phosphorylation or dephosphorylation. In addition, H_2O_2 may activate transcription by directly oxidising H_2O_2-responsive transcription factors via oxidation of thiols of cysteine residues in protein. In either case, having moved into the nucleus, the modified (activated) transcription factor interacts with its corresponding *cis*-acting element on target promoters to regulate gene expression.

In the future, developments in post-genomic technologies will clarify the biological significance of H_2O_2 and provide more clues to how plants sense and respond to environmentally adverse conditions.

Acknowledgements. The authors thank Dr. Terry Murphy of the University of California, Davis for critical reading of the manuscript. The authors are also grateful to the National Science Council of Taiwan for its continuous support of our work on this subject.

Literature Cited

Allen, G.J., K. Kuchitsu, S.P. Chu, Y. Murata, and J.I. Schroeder. 1999. *Arabidopsis abi1-1* and *abi2-1* phosphatase mutations reduce abscisic acid-induced cytoplasmic calcium rises in guard cells. Plant Cell **11:** 1785-1798.

Alscher, R.G. 1989. Biosynthesis and antioxidant function of glutathione in plants. Physiol. Plant. **77:** 457-464.

Asada, K. 1999. The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu. Rev. Plant Physiol. Plant Mol. Biol. **50:** 601-639.

Aslund, F. and J. Beckwith. 1999. The thioredoxin superfamily: redundancy, specificity, and gray-area genomics. J. Bacteriol. **181:** 1375-1379.

Bethke, P.C. and R.L. Jones. 2001. Cell death of barley aleurone protoplasts is mediated by reactive oxygen species. Plant J. **25:** 19-29.

Bowler, C., M.V. Montagu, and D. Inze. 1992. Superoxide dismutase and stress tolerance. Annu. Rev. Plant Physiol. Plant Mol. Biol. **24:** 83-116.

Boyer, J.S. 1982. Plant productivity and environment. Science **218:** 443-448.

Coleman, S.T., E.A. Epping, S.M. Steggerda, and W.S. Moye-Rowley. 1999. Yap1p activates gene transcription in an oxi-

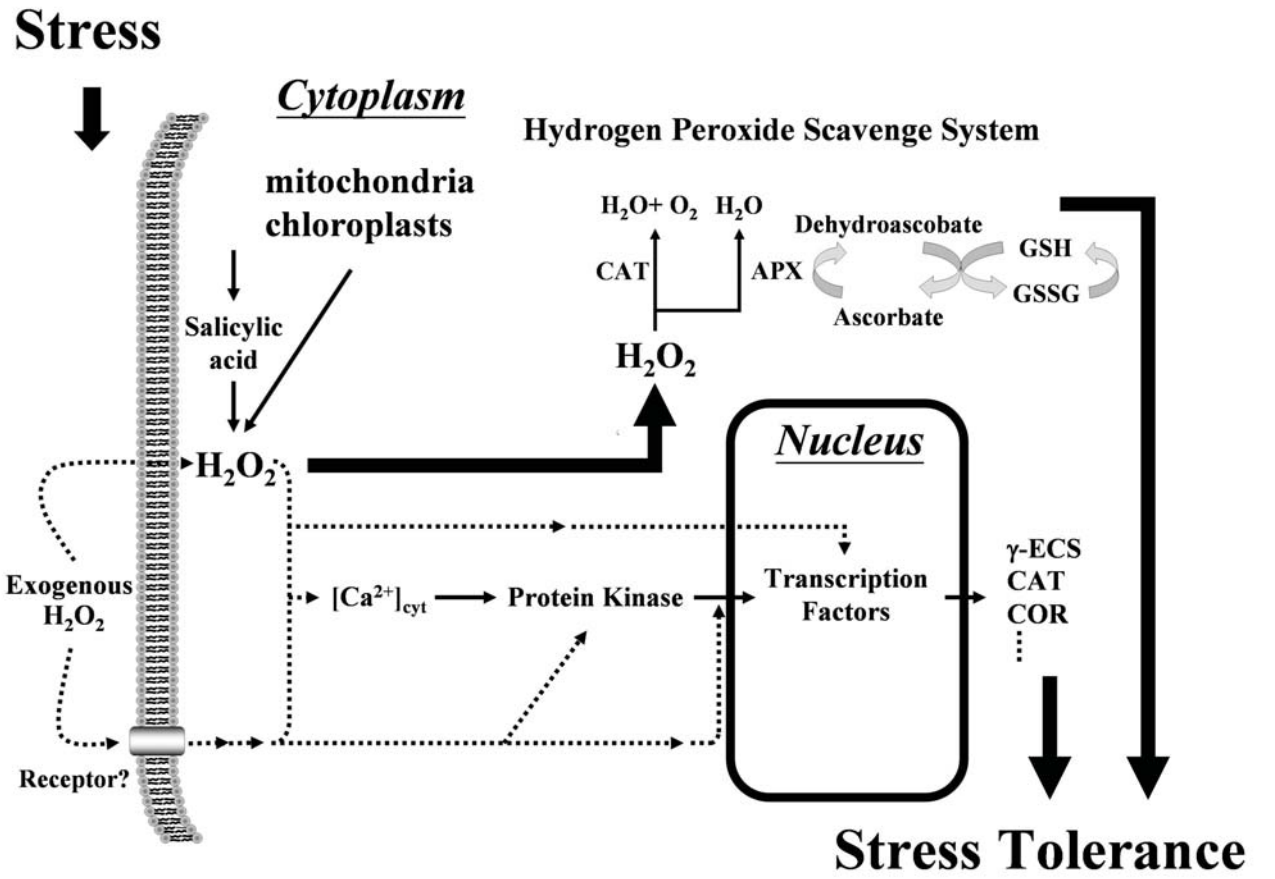

Figure 1. A model for the H_2O_2-induced signaling cascade. See text for details. Abbreviations: APX, ascorbate peroxidase; CAT, catalase; COR, cold regulated gene; γ-ECS, gamma-glutamylcysteine synthetase; GSH, glutathione; GSSG, glutathione disulfide; H_2O_2, hydrogen peroxide.

dant-specific fashion. Mol. Cell. Biol. **19:** 8302-8313.

Desikan, R., A. Clarke, J.T. Hancock, and S.J. Neill. 1999. H_2O_2 activates a MAP kinase-like enzyme in *Arabidopsis thaliana* suspension cultures. J. Exp. Bot. **50:** 1863-1866.

Desikan, R., A. Reynolds, J.T. Hancock, and S.J. Neill. 1998. Harpin and hydrogen peroxide both initiate programmed cell death but have differential effects on gene expression in Arabidopsis suspension cultures. Biochem. J. **330:** 115-120.

Desikan, R., S. A.-H.-Mackerness, J.T. Hancock, and S.J. Neill. 2001a. Regulation of the Arabidopsis transcriptome by oxidative stress. Plant Physiol. **127:** 159-172.

Desikan, R., J.T. Hancock, K. Ichimura, K. Shinozaki, and S.J. Neill. 2001b. Harpin induces activation of the *Arabidopsis* mitogen-activated protein kinase AtMPK4 and AtMPK6. Plant Physiol. **126:** 1579-1587.

Desikan, R., J.T. Hancock, and S.J. Neill. 2003. Oxidative stress signaling. *In* H. Hirt and K. Shinozaki (eds.), Plant responses to abiotic stress: topic in current genetics. Springer-Verlag, Berlin, Heidelberg, New York, pp. 121-148.

Desikan, R., M.K. Cheung, A. Clarke, S. Golding, M. Sagi, R. Fluhr, C. Rock, J. Hancock, and S. Neill. 2004. Hydrogen peroxide is a common signal for darkness- and ABA-induced stomatal closure in *Pisum sativum*. Funct. Plant Biol. **31:** 913-920.

Elstner, E.F. 1991. Mechanisms of oxygen activation in different compartments of plant cells. *In* E.J. Pell and K.L. Steffen (eds.), Active Oxygen/Oxidative Stress and Plant Metabolism. American Society of Plant Physiologists, Rockville, pp. 13-25.

Foyer, C.H. and B. Halliwell. 1976. The presence of glutathione and glutathione reductase in chloroplast: a proposed role in ascorbic acid metabolism. Planta **133:** 21-25.

Foyer, C.H., H. Lopez-Delgado, J.F. Dat, and I.M. Scott. 1997. Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signaling. Physiol. Plant. **100:** 241-254.

Gilmour, S.J., D.G. Zarka, E.J. Stockinger, M.P. Salazar, J.M. Houghton, and M.F. Thomashow. 1998. Low temperature regulation of the *Arabidopsis CBF* family of AP2 transcriptional activators as an early step in cold-induced *COR* gene expression. Plant J. **16:** 433-442.

Gosti, F., N. Beaudoin, C. Serizet, A.A.R. Webb, and N. Vartanian. 1999. ABI1 protein phosphatase 2C is a negative regulator of abscisic acid signaling. Plant Cell **11:** 1897-1909.

Grant, J.J., B.-W. Yun, and G.J. Loake. 2000. Oxidative burst and cognate redox signaling reported by luciferase imaging: identification of a signal network that functions independently of ethylene, SA and Me-JA but is dependent on MAPKK activity. Plant J. **24:** 569-582.

Guan, L.M., J. Zhao, and J.G. Scandalios. 2000. *Cis*-elements and *trans*-factors that regulate expression of the maize *Cat1* antioxidant gene in response to ABA and osmotic stress: H_2O_2 is the likely intermediary signaling molecule for the response. Plant J. **22:** 87-95.

Gustin, M.C., J. Albertyn, M. Alexander, and K. Davenport. 1998. MAP kinase pathways in the yeast Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. **62:** 1264-1300.

Gyuris, J., E. Golemis, H. Chertkov, and R. Brent. 1993. Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. Cell **75:** 791-803.

Hancock, J.T., R. Desikan, and S.J. Neill. 2001. Role of reactive oxygen species in cell signalling pathways. Biochem. Soc. Trans. **29:** 345-350.

Hariyadi, P. and K.L. Parkin. 1993. Chilling-induced oxidative stress in cucumber (*Cucumis sativus* L. *cv. Calypso*) seedlings. J. Plant Physiol. **141:** 733-738.

Hasegawa, P.M., R.A. Bressan, J.-K. Zhu, and H.J. Bohnert. 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol. **51:** 463-499.

Hsieh, T.H., J.T. Lee, Y.Y. Charng, and M.T. Chan. 2002. Tomato plants ectopically expressing *Arabidopsis* CBF1 show enhanced resistance to water deficit stress. Plant Physiol. **130:** 618-626.

Ichimura, K., T. Mizoguchi, R. Yoshida, T. Yuasa, and K. Shinozaki. 2000. Various abiotic stresses rapidly activate *Arabidopsis* MAP kinases AtMPK4 and AtMPK6. Plant J. **24:** 655-665.

Inzé, D. and M. Van Montagu. 1995. Oxidative stress in plants. Curr. Opin. Biotech. **6:** 153-158.

Jimenez, A., J.A. Hernandez, L.A. del-Rio, and F. Sevilla. 1997. Evidence for the presence of the ascorbate–glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiol. **114:** 275-284.

Joo, J.H., Y.S. Bae, and J.S. Lee. 2001. Role of auxin-induced reactive oxygen species in root gravitropism. Plant Physiol. **126:** 1055-1060.

Kamata, H. and H. Hirata. 1999. Redox regulation of cellular signalling. Cell Signal. **11:** 1-14.

Karpinski, S., C. Escobar, B. Karpinska, G. Creissen, and P.M. Mullineaux. 1997. Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in *Arabidopsis* during excess light stress. Plant Cell **9:** 627-640.

Knight, M.R., A.K. Campbell, S.M. Smith, and A.J. Trewavas. 1991. Transgenic plant aequorin reports the effects of cold shock and elicitors on cytoplasmic calcium. Nature **352:** 524-526.

Knight, H., A.J. Trewavas, and M.R. Knight. 1996. Cold calcium signaling in *Arabidopsis* involves two cellular pools and a change in calcium signature after acclimation. Plant Cell **8:** 489-503.

Kocsy, G., M. Brunner, A. Ruegsegger, P. Stamp, and C. Brunold. 1996. Glutathione synthesis in maize genotypes with different sensitivities to chilling. Planta **198:** 365-370.

Kocsy, G., G. Galiba, and C. Brunold. 2001. Role of glutathione in adaptation and signalling during chilling and cold acclimation in plants. Physiol. Plant. **113:** 158-164.

Kocsy, G., P. Ballmoos, M. Suter, A. Ruegsegger, U. Galli, G. Szalai, G. Galiba, and C. Brunold. 2000a. Inhibition of glutathione synthesis reduces chilling tolerance in maize. Planta **211:** 528-536.

Kocsy, G., G. Szalai, A. Vagujfalvi, L. Stehli, G. Orosz, and G. Galiba. 2000b. Genetic study of glutathione accumulation during cold hardening in wheat. Planta **210:** 295-301.

Kovtun, Y., W.-L. Chiu, G. Tena, and J. Sheen. 2000. Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc. Natl. Acad. Sci. USA **97:** 2940-2945.

Kubo, A., H. Saji, K. Tanaka, and N. Kondo. 1995. Expression of Arabidopsis cytosolic ascorbate peroxidase in response to ozone of sulfur dioxide. Plant Mol. Biol. **29:** 479-489.

Kudla, J., Q. Xu, K. Harter, W. Gruissem, and S. Luan. 1999. Genes for calcineurin B-like proteins in *Arabidopsis* are differentially regulated by stress signals. Proc. Natl. Acad. Sci. USA **96:** 4718-4723.

Lamb, C. and R.A. Dixon. 1997. The oxidative burst in plant disease resistance. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48:** 251-275.

Lee, D.H. and C.B. Lee. 2000. Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: in gel enzyme activity assays. Plant Sci. **159:** 75-85.

Leung, J., M. Bourvier-Durant, P-C. Morris, D. Guerrier, F. Chefdor, and J. Giraudad. 1994. *Arabidopsis* ABA response gene *ABI1*: features of a calcium-modulated protein phosphatase. Science **264:** 1448-1452.

Leung, J., S. Merlot, and J. Giraudat. 1997. The *Arabidopsis* abscisic acidinsensitive 2 (ABI2) and ABI1 genes encode homologues protein phosphatases 2C involved in abscisic acid signal transduction. Plant Cell **9:** 759-771.

Levine, A., R.I. Pennell, M.E. Alvarez, R. Palmer, and C. Lamb. 1996. Calcium-mediated apoptosis in a plant hypersensitive disease resistance response. Curr. Biol. **6:** 427-437.

Levine, A., R. Tenhaken, R. Dixon, and C. Lamb. 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell **79:** 583-593.

Lewis, B.D., G. Karlin-Neumann, R.W. Davis, and E.P. Spalding. 1997. Ca^{2+}-activated anion channels and membrane depolarizations induced by blue light and cold in *Arabidopsis* seedlings. Plant Physiol. **114:** 1327-1334.

Liu, Q., M. Kasuga, Y. Sakuma, H. Abe, S. Miura, K. Yamaguchi-Shinozaki, and K. Shinozaki. 1998. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. Plant Cell **10:** 1391-1406.

Luan, S. 1998. Protein phosphatases and signaling cascades in higher plants. Trends Plant Sci. **3:** 271-275.

Marrs, K.A. 1996. The functions and regulation of glutathione S-transferases in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **47:** 127-158.

May, M.J., T. Vernoux, C. Leaver, M. Van Montagu, and D. Inze. 1998. Glutathione homeostasis in plants: implications for environmental sensing and plant development. J. Exp. Bot. **49:** 649-667.

McAinsh, M.R., C. Brownlee, and A.M. Hetherington. 1997. Calcium ions as second messengers in guard cell signal transduction. Physiol. Plant. **100:** 16-29.

Meinhard, M. and E. Grill. 2001. Hydrogen peroxide is a regulator of ABI1, a protein phosphatase 2C from *Arabidopsis*. FEBS Lett. **508:** 443-446.

Meinhard, M., P.L. Rodriguez, and E. Gill. 2002. The sensitivity of ABI2 to hydrogen peroxide links the abscisic acid-response regulator to redox signaling. Planta **214:** 775-782.

Merlot, S., F. Gosti, D. Guerrier, A. Vavasseur, and J. Giraudad. 2001. The ABI1 and ABI2 protein phosphatases 2C act in a network feedback regulatory loop of the abscisic acid signaling pathway. Plant J. **25:** 315-324.

Meyer, K., M.P. Leube, and E. Grill. 1994. A protein phosphatase 2C involved in ABA signaling transduction in *Arabidopsis thaliana*. Science **264:** 1452-1455.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. **7:** 405-410.

Mittler, R., E.H. Herr, B.L. Orvar, W. Van Camp, H. Willekens, D. Inze, and B.E. Ellis. 1999. Transgenic tobacco plants with reduced capability to detoxify reactive oxygen intermediates are hyperresponsive to pathogen infection. Proc. Natl. Acad. Sci. USA **96:** 14165-14170.

Mittler, R. and B.A. Zilinskas. 1991. Purification and characterization of pea cytosolic ascorbate peroxidase. Plant Physiol. **97:** 962-968.

Mittler, R. and B.A. Zilinskas. 1992. Molecular cloning and characterization of a gene encoding pea cytosolic ascorbate peroxidase. J. Biol. Chem. **267:** 21802-21807.

Mizoguchi, T., K. Irie, T. Hirayama, N. Hayashida, K. Yamaguchi-Shinozaki, K. Matsumoto, and K. Shinozaki. 1996. A gene encoding a mitogen activated protein kinase kinase kinase in induced simultaneously with genes for a mitogen-activated protein kinase and S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **93:** 765-769.

Monroy, A.F. and R.S. Dhindsa. 1995. Low temperature signal transduction: induction of cold acclimation-specific genes of alfalfa by calcium at 25ºC. Plant Cell **7:** 321-331.

Moon, H., B. Lee, G. Choi, D. Shin, D.T. Prasad, O. Lee, S.S. Kwak, D.H. Kim, J. Nam, J. Bahk, J.C. Hong, S.Y. Lee, M.J. Cho, C.O. Lim, and D.J. Yun. 2003. NDP kinase 2 interacts with two oxidative stress-activated MAPKs to regulate cellular redox state and enhances multiple stress tolerance in transgenic plants. Proc. Natl. Acad. Sci. USA **100:** 358-363.

Murata, Y., Z.M. Pei, I.C. Mori, and J. Schroeder. 2001. Abscisic acid activation of plasma membrane Ca^{2+} channel in guard cells require cytosolic NAD(P)H and is differentially disrupted upstream and downstream of reactive oxygen species production in *abi1-1* and *abi2-1* protein phosphatase 2C mutants. Plant Cell **13:** 2513-2523.

Myrset, A.H., A. Bostad, N. Jamin, P.N. Lirsac, F. Toma, and O.S. Gabrielsen. 1993. DNA and redox state induced conformational changes in the DNA-binding domain of the Myb oncoprotein. EMBO J. **12:** 4625-4633.

Neill, S., R. Desikan, and J. Hancock. 2002. Hydrogen peroxide signaling. Curr. Opin. Plant Biol. **5:** 388-395.

Noctor, G., A.M. Arisi, L. Jouanin, K.J. Kunert, H. Rennenberg, and C.H. Foyer. 1998. Glutathione: biosynthesis, metabolism and relationship to stress tolerance explored in transformed plants. J. Exp. Bot. **49:** 623-647.

O'Kane, D., V. Gill, P. Boyd, and R. Burdon. 1996. Chilling, oxidative stress and antioxidant responses in *Arabidopsis thaliana* callus. Planta **198**: 371-377.

Okuda, T., Y. Matsuda, A. Yamanaka, and S. Sagisaka. 1991. Abrupt Increase in the Level of Hydrogen Peroxide in Leaves of Winter Wheat Is Caused by Cold Treatment. Plant Physiol. **97:** 1256-1267.

Orozco-Cardenas, M.L., J. Narvaez-Vasquez, and C.A. Ryan. 2001. Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. Plant Cell **13:** 179-191.

Patterson, W.R. and T.L. Poulos. 1995. Crystal structure of recombinant pea cytosolic ascorbate peroxidase. Biochemistry **34:** 4331-4341.

Payton, P., R. Webb, D. Kornyeyev, R. Allen, and A.S. Holaday. 2001. Protecting cotton photosynthesis during moderate chilling at high light intensity by increasing chloroplastic antioxidant enzyme activity. J. Exp. Bot. **52:** 2345-2354.

Pei, Z-M., Y. Murata, G. Benning, S. Thomine, B. Klusener, G. J. Allen, E. Grill, and J.I. Schroeder. 2000. Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature **406:** 731-734.

Polisensky, D.H. and J. Braam. 1996. Cold-shock regulation of the *Arabidopsis TCH* genes and the effects of modulating intracellular calcium levels. Plant Physiol. **111:** 1271-1279.

Prasad, T.K. 1996. Mechanisms of chilling-induced oxidative stress injury and tolerance in developing maize seedlings: changes in antioxidant system, oxidation of proteins and lipids, and protease activities. Plant J. **10:** 1017-1026.

Prasad, T.K., M.D. Anderson, B.A. Martin, and C.R. Stewart. 1994. Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. Plant Cell **6:** 65-74.

Rao, G.N., W.C. Glasgow, T.E. Eling, and M.S. Runge. 1996. Role of hydroperoxyeicosatetraenoic acids in oxidative stress-induced activating protein 1 (AP-1) activity. J. Biol. Chem. **271:** 27760-27764.

Robert, B. and O. David. 2004. Arabidopsis OXS2 is a transcription factor in the oxidative stress response. Meeting Abstract of The 2004 Annual Meeting of the American Society of Plant Biologists. July 24-28, Orlando, Florida USA.

Rudd, J.J. and V.E. Franklin-Tong. 1999. Calcium signaling in plants. Cell Mol. Life. Sci. **55:** 214-232.

Russo, T., N. Zambrano, F. Esposito, R. Ammendola, F. Cimino, M. Fiscella, J. Jackman, M. O'Connor, C.W. Anderson, and E. Apella. 1995. A p53-independent pathway for activation of WAF1/C1P1 expression following oxidative stress. J. Biol. Chem. **270:** 29386-29391.

Sanchez-Fernández, R., M. Fricker, L.B. Corben, N.S. White, N. Sheard, C.J. Leaver, M. Van Montagu, D. Inzé, and M. J. May. 1997. Cell proliferation and hair tip growth in the Arabidopsis root are under mechanistically different forms of redox control. Proc. Natl. Acad. Sci. USA **94:** 2745-2750.

Sanders, D., C. Brownlee, and J.F. Harper. 1999. Communicating with calcium. Plant Cell **11:** 691-706.

Schreck, R., P. Rieber, and P.A. Baeuerle. 1991. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. EMBO J. **10:** 2247-2258.

Shaul, O., V. Mironov, S. Burssens, M.V. Van Montagu, and D. Inzé. 1996. Two Arabidopsis cyclin promoters mediate distinctive transcriptional oscillation in synchronized tobacco 3Y-2 cells. Proc. Natl. Acad. Sci. USA **93:** 4868-4872.

Sheen, J. 1998. Mutational analysis of protein phosphatase 2C involved in abscisic acid signal transduction in higher plants.

Proc. Natl. Acad. Sci. USA **95:** 975-980.

Shinozaki, K. and K. Yamaguchi-Shinozaki. 2000. Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. Curr. Opin. Plant Biol. **3:** 217-223.

Solomon, M., B. Belenghi, M. Delledonne, E. Menachem, and A. Levine. 1999. The involvement of cysteine proteases and protease inhibitor genes in the regulation of programmed cell death in plants. Plant Cell **11:** 431-443.

Stockinger, E.J., S.J. Gilmour, and M.F. Thomashow. 1997. *Arabidopsis thaliana CBF1* encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a *cis*-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc. Natl. Acad. Sci. USA **94:** 1035-1040.

Stone, J.M. and J.C. Walker. 1995. Plant protein kinase families and signal transduction. Plant Physiol. **108:** 451-457.

Tenhaken, R., A. Levine, L.F. Brisson, R.A. Dixon, and C. Lamb. 1995. Function of the oxidative burst in hypersensitive disease resistance. Proc. Natl. Acad. Sci. USA **92:** 4158-4163.

Thomsen, B., H. Drumm-Herrel, and H. Mohr. 1992. Control of the appearance of ascorbate peroxidase (EC 1.11.1.11) in mustard seedling cotyledons by phytochrome and photooxidative treatments. Planta **186:** 600-608.

Torres, M. and H.J. Forman. 2003. Redox signaling and the MAP kinase pathways. Biofactors. **17:** 287-296.

Trewavas, A.J. and R. Malhó. 1997. Signal perception and transduction: the origin of the phenotype. Plant Cell **9:** 1181-1195.

Wasylyk, C. and B. Wasylyk. 1993. Oncogenic conversion of Ets affects redox regulation in-vivo and in-vitro. Nucleic Acids Res. **21:** 523-529.

Wingate, V.P.M., M.A. Lawton, and C.J. Lamb. 1988. Glutathione causes a massive and selective induction of plant defense genes. Plant Physiol. **87:** 206-210.

Wingsle, G. and J.E. Hallgren. 1993. Influence of SO_2 and NO_2 exposure on glutathione, superoxide dismutase and glutathione reductase activities in Scots pine needles. J. Exp. Bot. **44:** 463-470.

Wise, R.R. and A.W. Naylor. 1987. Chilling-enhanced peroxidation: the peroxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. Plant Physiol. **83:** 272-277.

Xing, T., V.J. Higgins, and E. Blumwald. 1997. Race-specific elicitors of cladosporium fulvum promote translocation of cytosolic components of NADPH oxidase to the plasma membrane of tomato cells. Plant Cell **9:** 249-259.

Yang, T. and B.W. Poovaiah. 2002. Hydrogen peroxide homeostasis: activation of plant catalase by calcium/calmodulin. Proc. Natl. Acad. Sci. USA **99:** 4097-4102.

Yu, C.W., T.M. Murphy, W.W. Sung, and C.H. Lin. 2002. H_2O_2 treatment induces glutathione accumulation and chilling tolerance in mung bean. Funct. Plant Biol. **29:** 1081-1087.

Yu, C.W., T.M. Murphy, and C.H. Lin. 2003. Hydrogen peroxide-induced chilling tolerance in mung beans mediated through ABA-independent glutathione accumulation. Funct. Plant Biol. **30:** 955-963.

Zhang, J., S. Cui, J. Li, J. Wei, and M.B. Kirkham. 1995. Protoplasmic factors, antioxidants responses, and chilling resistance in maize. Plant Physiol. Biochem. **33:** 567-575.

Zwerger, K. and H. Hirt. 2001. Recent advances in plant MAP kinase signalling. Biol Chem. **382:** 1123-1131.